

Cancer and Immortality Research Literatures

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Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the related studies.

[Herbert M. 2025;15(1):145-241]. ISSN: 2150-1041 (print); ISSN: 2150-105X (online). <http://www.cancerbio.net> 02. doi:[10.7537/marscbj150125.02](https://doi.org/10.7537/marscbj150125.02)

Key words: cancer; immortality; AI; life; research; literature; cell

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

The following introduces recent reports as references in the related studies.

Abdolahi-Majd, M., et al. (2022). "Investigation of the Effect of *Prunus Amygdalus Amara* on the Expression of some Genes of Apoptosis and Immortality in Breast Cancer Cells (MCF- 7)." *Curr Drug Res Rev* **14**(1): 73-79.

BACKGROUND: Anti-cancer effects of almond nuts or oil have been approved, but there are a few pieces of research that have evaluated, in detail, almond and other seeds' effects on cancer. Therefore, in the present project, the aim was to explore the regulatory effect of the bitter almond extract (*Prunus amygdalus Batsch*) on the apoptotic and anti-cancer potency of MCF-7 cells. **OBJECTIVE:** In the current experimental research, the almond effect on MCF7 cells was evaluated by investigating the expression and the balance between Bcl-2, Bax genes to unmark the potential molecular mechanism. **METHODS:** For 24 and 48h, the MCF7 cells were treated with the bitter almond extract (187.5-3000 µg/mL). MTT assay was used to assess the viability, and Real-time-PCR was applied to determine the expression of Bax and Bcl-2, facing beta-actin. **RESULTS:** Our results

revealed a significant difference between different extract concentrations on the viability of MCF7 cell lines in 24 and 48 h; cell viability decreased time-dependently ($P < 0.05$). After 24 and 48h of extract facing MCF7 cells, the evaluated IC50 value was 3000 and 1500 µg/mL, respectively. Based on Real-Time-PCR analysis, after 24 and 48 h, the mRNA levels of BCL-2 decreased by the extract, whereas Bax was in the MCF-7 cell line. **CONCLUSION:** From the results, it can be concluded that bitter almond extract has anti-cancer properties that may influence the apoptotic pathways by regulating relative gene expression.

Adjiri, A. (2016). "Identifying and Targeting the Cause of Cancer is Needed to Cure Cancer." *Oncol Ther* **4**(1): 17-33.

Cancer cells continue to challenge scientists and oncologists due to the phenomenon of resistance. Moreover, recurrence, as seen in many treated patients, shows that currently-used anti-cancer drugs are unable to prevent the development of new cancer cells harboring new mutations. The purpose of this paper is to try to answer some of the questions regarding why cancer arises and why evolution would naturally lead to the development of cancer. Providing answers to these questions may shed new light on cancer development and potential causes of cancer. This work demonstrates that (1) cancer hallmarks are a series of events that can be organized in three consecutive stages; (2) cancer may develop when cells seek immortality; (3) heterogeneity in tumors may be explained by cancer cells not following universal laws for division; (4) evolution may not have selected for cancer; (5) currently-used anti-cancer drugs, with telomerase and poly adenosine diphosphate ribose polymerase inhibition given as examples, show that we may not be on the

right track, as these drugs are probably targeting molecular symptoms of tumors but not their cause; and (6) after an attempt to define the cause of cancer, the potentials of immunotherapy are discussed. Future anti-cancer drugs should be able to shrink the original tumor(s) and most importantly prevent the rise of new cancer cells in treated patients. In order to achieve this goal, new drugs must target the cause of cancer. Therefore, future research must focus on identifying potential causes of cancer common to all types of cancers. Finally, while immunotherapy holds great prospects for future cancer cure and prevention, global action is needed to reduce harmful substances known to contribute to the development of cancer in the environment.

Agbarya, A., et al. (2014). "Natural products as potential cancer therapy enhancers: A preclinical update." *SAGE Open Med* 2: 2050312114546924.

Cancer is a multifactorial disease that arises as a consequence of alterations in many physiological processes. Recently, hallmarks of cancer were suggested that include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis, along with two emerging hallmarks including reprogramming energy metabolism and escaping immune destruction. Treating multifactorial diseases, such as cancer with agents targeting a single target, might provide partial treatment and, in many cases, disappointing cure rates. Epidemiological studies have consistently shown that the regular consumption of fruits and vegetables is strongly associated with a reduced risk of developing chronic diseases, such as cardiovascular diseases and cancer. Since ancient times, plants, herbs, and other natural products have been used as healing agents. Moreover, the majority of the medicinal substances available today have their origin in natural compounds. Traditionally, pharmaceuticals are used to cure diseases, and nutrition and herbs are used to prevent disease and to provide an optimal balance of macro- and micro-nutrients needed for good health. We explored the combination of natural products, dietary nutrition, and cancer chemotherapeutics for improving the efficacy of cancer chemotherapeutics and negating side effects.

Ahmad, M. F. (2020). "Ganoderma lucidum: A rational pharmacological approach to surmount cancer." *J Ethnopharmacol* 260: 113047.

ETHNOPHARMACOLOGICAL

RELEVANCE: Ganoderma lucidum (*G. lucidum*) has been broadly used for health endorsement as well as longevity for over 2000 years in Asian countries. It

is an example of an ancient remedy and known as immortality mushroom. It has been employed as a health promoting agent owing to its broad pharmacological and therapeutical approaches. It has been confirmed that *G. lucidum* exhibits significant potency to prevent and treat different types of cancers such as breast, prostate, colon, lung and cervical. AIM OF THE STUDY: To explore anticancer effects of various pharmacologically active compounds obtained from *G. lucidum* and their possible mechanism of action. MATERIALS AND METHODS: A literature search was conducted using PubMed, Goggle Scholar, Saudi Digital Library and Cochrane Library until October 11, 2019. Search was made by using keywords such as anticancer evidence, mechanism of action, pharmacology, antioxidant, toxicity, chemotherapy, triterpenoids and polysaccharides of *G. lucidum*. RESULTS: Various chemical compounds from *G. lucidum* exhibit anticancer properties mainly through diverse mechanism such as cytotoxic properties, host immunomodulators, metabolizing enzymes induction, prohibit the expression of urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR) in cancer cells. Among the various compounds of *G. lucidum* triterpenoids and polysaccharides are under the major consideration of studies due to their several evidence of preclinical and clinical studies against cancer. CONCLUSION: Natural alternatives associated with mild side effects are the basic human need of present therapy to eradicate the new emerging disorders. This review is an attempt to compile pharmacologically active compounds of *G. lucidum* those exhibit anti cancer effects either alone or along with chemotherapy and anticancer mechanisms against various cancer cells, clinical trials, chemotherapy induced toxicity challenges with limitations. It acts as a possible substitute to combat cancer growth with advance and conventional combination therapies as natural alternatives.

Aird, J., et al. (2018). "Carcinogenesis in prostate cancer: The role of long non-coding RNAs." *Noncoding RNA Res* 3(1): 29-38.

LncRNAs appear to play a considerable role in tumorigenesis through regulating key processes in cancer cells such as proliferative signalling, replicative immortality, invasion and metastasis, evasion of growth suppressors, induction of angiogenesis and resistance to apoptosis. LncRNAs have been reported to play a role in prostate cancer, particularly in regulating the androgen receptor signalling pathway. In this review article, we summarise the role of 34 lncRNAs in prostate cancer with a particular focus on their role in the androgen

receptor signalling pathway and the epithelial to mesenchymal transition pathway.

Albakova, Z. (2024). "HSP90 multi-functionality in cancer." *Front Immunol* 15: 1436973.

The 90-kDa heat shock proteins (HSP90s) are molecular chaperones essential for folding, unfolding, degradation and activity of a wide range of client proteins. HSP90s and their cognate co-chaperones are subject to various post-translational modifications, functional consequences of which are not fully understood in cancer. Intracellular and extracellular HSP90 family members (HSP90alpha, HSP90beta, GRP94 and TRAP1) promote cancer by sustaining various hallmarks of cancer, including cell death resistance, replicative immortality, tumor immunity, angiogenesis, invasion and metastasis. Given the importance of HSP90 in tumor progression, various inhibitors and HSP90-based vaccines were developed for the treatment of cancer. Further understanding of HSP90 functions in cancer may provide new opportunities and novel therapeutic strategies for the treatment of cancer.

Ali, J. H. and M. Walter (2023). "Combining old and new concepts in targeting telomerase for cancer therapy: transient, immediate, complete and combinatory attack (TICCA)." *Cancer Cell Int* 23(1): 197.

Telomerase can overcome replicative senescence by elongation of telomeres but is also a specific element in most cancer cells. It is expressed more vastly than any other tumor marker. Telomerase as a tumor target inducing replicative immortality can be overcome by only one other mechanism: alternative lengthening of telomeres (ALT). This limits the probability to develop resistance to treatments. Moreover, telomerase inhibition offers some degree of specificity with a low risk of toxicity in normal cells. Nevertheless, only one telomerase antagonist reached late preclinical studies. The underlying causes, the pitfalls of telomerase-based therapies, and future chances based on recent technical advancements are summarized in this review. Based on new findings and approaches, we propose a concept how long-term survival in telomerase-based cancer therapies can be significantly improved: the TICCA (Transient Immediate Complete and Combinatory Attack) strategy.

Almeida-Lousada, H., et al. (2021). "Screening for Colorectal Cancer Leading into a New Decade: The "Roaring '20s" for Epigenetic Biomarkers?" *Curr Oncol* 28(6): 4874-4893.

Colorectal cancer (CRC) has an important

bearing (top five) on cancer incidence and mortality in the world. The etiology of sporadic CRC is related to the accumulation of genetic and epigenetic alterations that result in the appearance of cancer hallmarks such as abnormal proliferation, evasion of immune destruction, resistance to apoptosis, replicative immortality, and others, contributing to cancer promotion, invasion, and metastasis. It is estimated that, each year, at least four million people are diagnosed with CRC in the world. Depending on CRC staging at diagnosis, many of these patients die, as CRC is in the top four causes of cancer death in the world. New and improved screening tests for CRC are needed to detect the disease at an early stage and adopt patient management strategies to decrease the death toll. The three pillars of CRC screening are endoscopy, radiological imaging, and molecular assays. Endoscopic procedures comprise traditional colonoscopy, and more recently, capsule-based endoscopy. The main imaging modality remains Computed Tomography (CT) of the colon. Molecular approaches continue to grow in the diversity of biomarkers and the sophistication of the technologies deployed to detect them. What started with simple fecal occult blood tests has expanded to an armamentarium, including mutation detection and identification of aberrant epigenetic signatures known to be oncogenic. Biomarker-based screening methods have critical advantages and are likely to eclipse the classical modalities of imaging and endoscopy in the future. For example, imaging methods are costly and require highly specialized medical personnel. In the case of endoscopy, their invasiveness limits compliance from large swaths of the population, especially those with average CRC risk. Beyond mere discomfort and fear, there are legitimate iatrogenic concerns associated with endoscopy. The risks of perforation and infection make endoscopy best suited for a confirmatory role in cases where there are positive results from other diagnostic tests. Biomarker-based screening methods are largely non-invasive and are growing in scope. Epigenetic biomarkers, in particular, can be detected in feces and blood, are less invasive to the average-risk patient, detect early-stage CRC, and have a demonstrably superior patient follow-up. Given the heterogeneity of CRC as it evolves, optimal screening may require a battery of blood and stool tests, where each can leverage different pathways perturbed during carcinogenesis. What follows is a comprehensive, systematic review of the literature pertaining to the screening and diagnostic protocols used in CRC. Relevant articles were retrieved from the PubMed database using keywords including: "Screening", "Diagnosis", and "Biomarkers for CRC". American and European clinical trials in progress were included

as well.

Al-Sowayan, B. S., et al. (2019). "Exosomes, cancer's little army." *Stem Cell Investig* **6**: 9.

In an attempt to conceptualize the process of cancer formation, Hanahan and Weinberg [2000] have outlined six universal characteristics of tumorigenesis, and labelled them as the "hallmarks of cancer". These hallmarks include; unlimited proliferation, evading growth suppressors, resisting cell death, replicative immortality, inducing angiogenesis, initiating invasion and metastasis. Cancer cell signalling is crucial for initiating and controlling cellular pathways that are involved in these hallmarks. The intricate network of communication between cancer cells and other cancer or non-cancer cells is still being investigated, and is yet to be fully understood. Initially it was proposed that the main form of communication between cells within the tumour microenvironment are soluble growth factors, and gap junctions. Then, researchers reported another form of cell-to-cell communication, through the release of spherical particles called exosomes. It is believed that these exosomes enable communication through the transfer of active components from the releasing cell, and off-loading it into the recipient cell. As researchers continue to examine the development of the cancer hallmarks and the pathways involved, it became evident that cancer cell-derived exosomes play a major role in almost all of them. This review will examine the role played by cancer cell-derived exosomes in development of cancer.

Amen, A. M., et al. (2021). "Cancer-specific loss of TERT activation sensitizes glioblastoma to DNA damage." *Proc Natl Acad Sci U S A* **118**(13).

Most glioblastomas (GBMs) achieve cellular immortality by acquiring a mutation in the telomerase reverse transcriptase (TERT) promoter. TERT promoter mutations create a binding site for a GAB binding protein (GABP) transcription factor complex, whose assembly at the promoter is associated with TERT reactivation and telomere maintenance. Here, we demonstrate increased binding of a specific GABPB1L-isoform-containing complex to the mutant TERT promoter. Furthermore, we find that TERT promoter mutant GBM cells, unlike wild-type cells, exhibit a critical near-term dependence on GABPB1L for proliferation, notably also posttumor establishment in vivo. Up-regulation of the protein paralogue GABPB2, which is normally expressed at very low levels, can rescue this dependence. More importantly, when combined with frontline temozolomide (TMZ) chemotherapy, inducible GABPB1L knockdown and the associated TERT

reduction led to an impaired DNA damage response that resulted in profoundly reduced growth of intracranial GBM tumors. Together, these findings provide insights into the mechanism of cancer-specific TERT regulation, uncover rapid effects of GABPB1L-mediated TERT suppression in GBM maintenance, and establish GABPB1L inhibition in combination with chemotherapy as a therapeutic strategy for TERT promoter mutant GBM.

Antoniou, A., et al. (2013). "Cancer stem cells, a fuzzy evolving concept: a cell population or a cell property?" *Cell Cycle* **12**(24): 3743-3748.

The cancer stem cells (CSC) hypothesis represents a pathological extrapolation of the physiological concept of embryonic and somatic stem cells. In its initial definition, it encompassed the hypothesis of a qualitatively distinct population of immortal cancer cells originating from somatic stem cells, which generate in xenotransplants by a deterministic irreversible process, the hierarchy of more differentiated finite lifespan derived cells, which constitute, themselves, the bulk of the cancer. These CSC would express specific biomarkers and gene expressions related to chemo- and radioresistance, stemness, epithelial-mesenchymal transition, etc. No convincing congruence of several of these properties in one cell population has been demonstrated. The concept has greatly evolved with time and with different authors ("the plasticity of cancer stem cells"), leading to a minimal definition of cells generating a hierarchy of derived cells. In this article these concepts are analyzed. It is proposed that stemness is a property, more or less reversible, a hallmark of some cells at some time in a cancer cell population, as immortality, dormancy, chemo- or radioresistance, epithelial-mesenchymal transition etc. These phenotypic properties represent the result of independent, linked, or more or less congruent, genetic, epigenetic, or signaling programs.

Arizmendi-Izazaga, A., et al. (2023). "The NRSF/REST transcription factor in hallmarks of cancer: From molecular mechanisms to clinical relevance." *Biochimie* **206**: 116-134.

The RE-1 silencing transcription factor (REST), or neuron restrictive silencing factor (NRSF), was first identified as a repressor of neuronal genes in non-neuronal tissue. Interestingly, this transcription factor may act as a tumor suppressor or an oncogenic role in developing neuroendocrine and other tumors in patients. The hallmarks of cancer include six biological processes, including proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, inducing angiogenesis, and activating invasion and metastasis. In addition to

two emerging hallmarks, the reprogramming of energy metabolism and evasion of the immune response are all implicated in the development of human tumors. It is essential to know the role of these processes as they will affect the outcome of alternatives for cancer treatment. Various studies in this review demonstrate that NRSF/REST affects the different hallmarks of cancer that could position NRSF/REST as an essential target in the therapy and diagnosis of certain types of cancer.

Armando, R. G., et al. (2018). "Homology Model and Docking-Based Virtual Screening for Ligands of Human Dyskerin as New Inhibitors of Telomerase for Cancer Treatment." *Int J Mol Sci* **19**(10).

Immortality is one of the main features of cancer cells. Tumor cells have an unlimited replicative potential, principally due to the holoenzyme telomerase. Telomerase is composed mainly by dyskerin (DKC1), a catalytic retrotranscriptase (hTERT) and an RNA template (hTR). The aim of this work is to develop new inhibitors of telomerase, selecting the interaction between hTR(-)DKC1 as a target. We designed two models of the human protein DKC1: homology and ab initio. These models were evaluated by different procedures, revealing that the homology model parameters were the most accurate. We selected two hydrophobic pockets contained in the PUA (pseudouridine synthase and archaeosine transglycosylase) domain, using structural and stability analysis. We carried out a docking-based virtual screen on these pockets, using the reported mutation K314 as the center of the docking. The hDKC1 model was tested against a library of 450,000 drug-like molecules. We selected the first 10 molecules that showed the highest affinity values to test their inhibitory activity on the cell line MDA MB 231 (Monroe Dunaway Anderson Metastasis Breast cancer 231), obtaining three compounds that showed inhibitory effect. These results allowed us to validate our design and set the basis to continue with the study of telomerase inhibitors for cancer treatment.

Armas-Lopez, L., et al. (2017). "The Hedgehog-GLI pathway in embryonic development and cancer: implications for pulmonary oncology therapy." *Oncotarget* **8**(36): 60684-60703.

Transcriptional regulation and epigenetic mechanisms closely control gene expression through diverse physiological and pathophysiological processes. These include the development of germ layers and post-natal epithelial cell-tissue differentiation, as well as, involved with the induction, promotion and/or progression of human malignancies. Diverse studies have shed light on the

molecular similarities and differences involved in the stages of embryological epithelial development and dedifferentiation processes in malignant tumors of epithelial origin, of which many focus on lung carcinomas. In lung cancer, several transcriptional, epigenetic and genetic aberrations have been described to partly arise from environmental risk factors, but ethnic genetic predisposition factors may also play a role. The classification of the molecular hallmarks of cancer has been essential to study and achieve a comprehensive view of the interaction networks between cell signaling pathways and functional roles of the transcriptional and epigenetic regulatory mechanisms. This has in turn increased understanding on how these molecular networks are involved in embryo-layers and malignant diseases development. Ultimately, a major biomedicine goal is to achieve a thorough understanding of their roles as diagnostic, prognostic and treatment response indicators in lung oncological patients. Recently, several notable cell-signaling pathways have been studied based on their contribution to promoting and/or regulating the engagement of different cancer hallmarks, among them genome instability, exacerbated proliferative signaling, replicative immortality, tumor invasion-metastasis, inflammation, and immune-surveillance evasion mechanisms. Of these, the Hedgehog-GLI (Hh) cell-signaling pathway has been identified as a main molecular contribution into several of the abovementioned functional embryo-malignancy processes. Nonetheless, the systematic study of the regulatory epigenetic and transcriptional mechanisms has remained mostly unexplored, which could identify the interaction networks between specific biomarkers and/or new therapeutic targets in malignant tumor progression and resistance to lung oncologic therapy. In the present work, we aimed to revise the most important up-to-date experimental and clinical findings in biology, embryology and cancer research regarding the Hh pathway. We explore the potential control of the transcriptional-epigenetic programming versus reprogramming mechanisms associated with its Hh-GLI cell signaling pathway members. Last, we present a summary of this information to systematically integrate the Hh signaling pathway to identify and propose novel compound strategies or better oncological therapeutic schemes for lung cancer patients.

Bailey, S. M. (2023). "Editorial: Hallmark of cancer: replicative immortality." *Front Oncol* **13**: 1204094.

Balasubramanian, S. and N. Singh (1996). "The role of telomeres and telomerase in human cancer." *Indian J Physiol Pharmacol* **40**(3): 199-204.

Human cancers/malignant transformation of normal cells occur from multiple independent genetic changes/mutations that can subvert the normal growth controls of cells, leading to distinct phenotypic changes and immortalization. Normal human somatic cells have limited proliferative capacity both in vitro and in vivo and undergo senescence. Recent studies have implicated telomeres and telomerase in the regulation of lifespan of cells. Telomeres are the stretches of DNA consisting of tandem repeats of nucleotide sequences that cap chromosomes and prevent its degradation and play a role, both in normal control of cell proliferation and abnormal growth of cancers. They are highly conserved during evolution. Telomerase, the novel reverse transcriptase enzyme that synthesizes telomeric DNA is repressed in most human somatic cells, it results in telomere shortening with each cell division, leading to a process thought to contribute to senescence. Recent research proposes that activation of telomerase is important for cells to proliferate indefinitely and that all human cancer cells require activation of this enzyme to maintain telomeric DNA, to overcome cellular senescence and to attain immortality. Thus telomeres and telomerase offer potential for diagnostics, cancer therapy as well as for understanding the process of aging.

Barger, C. J., et al. (2022). "Conserved features of TERT promoter duplications reveal an activation mechanism that mimics hotspot mutations in cancer." *Nat Commun* 13(1): 5430.

Mutations in the TERT promoter represent the genetic underpinnings of tumor cell immortality. Beyond the two most common point mutations, which selectively recruit the ETS factor GABP to activate TERT, the significance of other variants is unknown. In seven cancer types, we identify duplications of wildtype sequence within the core promoter region of TERT that have strikingly similar features including an ETS motif, the duplication length and insertion site. The duplications recruit a GABP tetramer by virtue of the native ETS motif and its precisely spaced duplicated counterpart, activate the promoter and are clonal in a TERT expressing multifocal glioblastoma. We conclude that recurrent TERT promoter duplications are functionally and mechanistically equivalent to the hotspot mutations that confer tumor cell immortality. The shared mechanism of these divergent somatic genetic alterations suggests a strong selective pressure for recruitment of the GABP tetramer to activate TERT.

Barodawala, S. M., et al. (2019). "Cervical cancer screening by molecular Pap-transformation of gynecologic cytology." *Diagn Cytopathol* 47(5): 374-

381.

BACKGROUND: Cervical cancer is one of the common cancers in women accounting for 7.9% of all cancers. In India it is the second commonest cancer in women. The immortality of the cancer cell and the relatively long time frame from acquisition of infection to development of cervical cancer was established. As major advancements like LBC, HPV testing were introduced in the recent years, screening has taken a new avatar, the Molecular pap!! The objectives of this study were: To compare gynecologic cytology and abnormal results with respect to conventional and LBC. To study the role of HPV cotesting and ancillary tests performed, that is, HPV CISH, and p16ink4a by IHC. **METHODS:** About 71 924 Conventional and LBC samples were included from August 2009 to December 2017. Cases for HPV testing along the conventional smears were 1539. HPV can be tested from the same LBC vial as the sample remains stable at room temperature for 6 weeks. HPV DNA PCR was carried out in our laboratory for High and Low risk genotypes. Cytology findings were also correlated with histology. **RESULTS:** Detection rate of SILs in LBC samples were higher (2.20%). The commonest abnormality was LSIL in LBC and ASCUS in conventional smears. Commonest HR HPV and LR HPV detected was 1 61 856 and 61 142. **CONCLUSION:** LBC with HPV cotesting improves sensitivity and specificity and reduces ambiguous results; allows better compliance, as a negative result of both tests allows patients to get screening every 5 years, thereby increasing screening intervals, important in a resource limited situation.

Bartholoma, P., et al. (2005). "A more aggressive breast cancer spheroid model coupled to an electronic capillary sensor system for a high-content screening of cytotoxic agents in cancer therapy: 3-dimensional in vitro tumor spheroids as a screening model." *J Biomol Screen* 10(7): 705-714.

One major problem in cancer therapy is the immortality of tumor cells showing an active telomerase, which is responsible for the elongation of the telomeres after each cellular division and the knocking down of apoptotic suppressors. A further phenomenon occurring during cancer therapies is the problem of multicellular resistance. To develop therapeutic anticancer approaches inducing cellular apoptosis, gene-modified biological in vitro systems were established and evaluated for drug screening in a capillary system for a real-time, impedimetric monitoring. Multicellular spheroids of the human breast cancer cell line T-47D clone 11 were transfected with 1) antisense caspase-3 cDNA expression vectors for knocking down the main cell

death molecule and 2) sense Bcl-xl cDNA expression vectors for overexpressing the apoptotic suppressor, resulting in more aggressive tumor models. These gene-modified tumor spheroids less sensitive for apoptosis were developed for screening drugs such as methotrexate in tumor spheroid-based biosensor systems via impedance spectroscopy. In this report, it is demonstrated that this could successfully exhibit that this real-time monitoring system with tumor spheroids positioned in a capillary system with a 4-electrode configuration is the most efficient high-content screening module for impedimetric measurements of physiological alterations during gene modification and drug application.

Basu, S. C., et al. (2018). "Induction of Apoptosis in Metastatic Breast Cancer Cells: XV. Downregulation of DNA Polymerase-alpha - Helicase Complex (Replisomes) and Glyco-Genes." *Adv Exp Med Biol* **1112**: 199-221.

In normal and cancer cells, successful cell division requires accurate duplication of chromosomal DNA. All cells require a multiprotein DNA duplication system (replisomes) for their existence. However, death of normal cells in our body occurs through the apoptotic process. During apoptotic process several crucial genes are downregulated with the upregulation of caspase pathways, leading to ultimate degradation of genomic DNA. In metastatic cancer cells (SKBR-3, MCF -7, and MDA-462), this process is inhibited to achieve immortality as well as overexpression of the enzymes for the synthesis of marker molecules. It is believed that the GSL of the lacto family such as Le(X), SA-Le(X), Le(Y), Le(a), and Le(b) are markers on the human colon and breast cancer cells. Recently, we have characterized that a few apoptotic chemicals (cis-platin, L-PPMP, D-PDMP, GD3 ganglioside, GD1b ganglioside, betulinic acid, tamoxifen, and melphalan) in low doses kill metastatic breast cancer cells. The apoptosis-inducing agent (e.g., cis-platin) showed inhibition of DNA polymerase/helicase (part of the replisomes) and also modulated (positively) a few glycolipid-glycosyltransferase (GSL-GLTs) transcriptions in the early stages (within 2 h after treatment) of apoptosis. These Lc-family GSLs are also present on the surfaces of human breast and colon carcinoma cells. It is advantageous to deliver these apoptotic chemicals through the metastatic cell surfaces containing high concentration of marker glycolipids (Lc-GSLs). Targeted application of apoptotic chemicals (in micro scale) to kill the cancer cells would be an ideal way to inhibit the metastatic growth of both breast and colon cancer cells. It was observed in three different breast cancer lines (SKBR-3, MDA-468, and MCF-7) that in 2 h very

little apoptotic process had started, but predominant biochemical changes (including inactivation of replisomes) started between 6 and 24 h of the drug treatments. The contents of replisomes (replisomal complexes) during induction of apoptosis are not known. It is known that DNA helicase activities (major proteins catalyze the melting of dsDNA strands) change during apoptotic induction process. Previously DNA Helicase-III was characterized as a component of the replication complexes isolated from carcinoma cells and normal rapid growing embryonic chicken brain cells. Helicase activities were assayed by a novel method (combined immunoprecipitation-ROME assay), and DNA polymerase-alpha activities were determined by regular chain extension of nicked "ACT-DNA," by determining values obtained from +/- aphidicolin added to the incubation mixtures. Very little is known about the stability of the "replication complexes" (or replisomes) during the apoptotic process. DNA helicases are motor proteins that catalyze the melting of genomic DNA during replication, repair, and recombination processes. In all three breast carcinoma cell lines (SKBR-3, MCF-7, and MDA-468), a common trend, decrease of activities of DNA polymerase-alpha and Helicase-III (estimated and detected with a polyclonal antibody), was observed, after cis-platin- and L-PPMP-induced apoptosis. Previously our laboratory has documented downregulation (within 24-48 h) of several GSL-GLTs with these apoptotic reagents in breast and colon cancer cells also. Perhaps induced apoptosis would improve the prognosis in metastatic breast and colon cancer patients.

Beeraka, N. M., et al. (2020). "The Role of Exosomes in Stemness and Neurodegenerative Diseases-Chemoresistant-Cancer Therapeutics and Phytochemicals." *Int J Mol Sci* **21**(18).

Exosomes exhibit a wide range of biological properties and functions in the living organisms. They are nanometric vehicles and used for delivering drugs, as they are biocompatible and minimally immunogenic. Exosomal secretions derived from cancer cells contribute to metastasis, immortality, angiogenesis, tissue invasion, stemness and chemo/radio-resistance. Exosome-derived microRNAs (miRNAs) and long non-coding RNAs (lnc RNAs) are involved in the pathophysiology of cancers and neurodegenerative diseases. For instance, exosomes derived from mesenchymal stromal cells, astrocytes, macrophages, and acute myeloid leukemia (AML) cells are involved in the cancer progression and stemness as they induce chemotherapeutic drug resistance in several cancer cells. This review covered the recent research advances in

understanding the role of exosomes in cancer progression, metastasis, angiogenesis, stemness and drug resistance by illustrating the modulatory effects of exosomal cargo (ex. miRNA, lncRNAs, etc.) on cell signaling pathways involved in cancer progression and cancer stem cell growth and development. Recent reports have implicated exosomes even in the treatment of several cancers. For instance, exosomes-loaded with novel anti-cancer drugs such as phytochemicals, tumor-targeting proteins, anticancer peptides, nucleic acids are known to interfere with drug resistance pathways in several cancer cell lines. In addition, this review depicted the need to develop exosome-based novel diagnostic biomarkers for early detection of cancers and neurodegenerative disease. Furthermore, the role of exosomes in stroke and oxidative stress-mediated neurodegenerative diseases including Alzheimer's disease (AD), and Parkinson's disease (PD) is also discussed in this article.

Bhartiya, D., et al. (2023). "Very Small Embryonic-Like Stem Cells Transform Into Cancer Stem Cells and Are Novel Candidates for Detecting/Monitoring Cancer by a Simple Blood Test." *Stem Cells* **41**(4): 310-318.

Cancer continues to remain a "Black Box," as there is no consensus on how it initiates, progresses, metastasizes, or recurs. Many imponderables exist about whether somatic mutations initiate cancer, do cancer stem cells (CSCs) exist, and if yes, are they a result of de-differentiation or originate from tissue-resident stem cells; why do cancer cells express embryonic markers, and what leads to metastasis and recurrence. Currently, the detection of multiple solid cancers through liquid biopsy is based on circulating tumor cells (CTCs) or clusters, or circulating tumor DNA (ctDNA). However, quantity of starting material is usually adequate only when the tumor has grown beyond a certain size. We posit that pluripotent, endogenous, tissue-resident, very small embryonic-like stem cells (VSELs) that exist in small numbers in all adult tissues, exit from their quiescent state due to epigenetic changes in response to various insults and transform into CSCs to initiate cancer. VSELs and CSCs share properties like quiescence, pluripotency, self-renewal, immortality, plasticity, enrichment in side-population, mobilization, and resistance to oncotherapy. HrC test, developed by Epigeneres, offers the potential for early detection of cancer using a common set of VSEL/CSC specific bio-markers in peripheral blood. In addition, NGS studies on VSELs/CSCs/tissue-specific progenitors using the All Organ Biopsy (AOB) test provide exomic and transcriptomic information regarding impacted

organ(s), cancer type/subtype, germline/somatic mutations, altered gene expressions, and dysregulated pathways. To conclude, HrC and AOB tests can confirm the absence of cancer and categorize the rest of subjects into low/moderate/high risk of cancer, and also monitor response to therapy, remission, and recurrence.

Bilsland, A. E., et al. (2011). "Targeting the telomere and shelterin complex for cancer therapy: current views and future perspectives." *J Cell Mol Med* **15**(2): 179-186.

Aberrant telomere homeostasis is essential for cell immortality, enabling cells to evade telomere dependent senescence. Disruption of telomere structure and function in cancer cells is highly toxic as shown by detailed pre-clinical evaluation of telomerase inhibitors. Under telomerase inhibition, cells must divide sufficiently frequently to allow one or more telomeres to shorten to an unprotected length. Functioning telomeres are disguised from the DNA damage machinery by DNA remodelling and other activities of the telomere binding complex shelterin. Direct interference with shelterin has been shown to result in cell killing and small molecules directly targeting telomere DNA also have anti-tumour effects partially dependent on shelterin disruption. However, shelterin components have not generally been regarded as therapeutic targets in their own right. In this review, we explore the possibilities for therapeutic targeting of the shelterin complex.

Bisht, J., et al. (2023). "Caregivers with Cancer Patients: Focus on Hispanics." *Cancers (Basel)* **15**(3).

Cancer is a public health concern and causes more than 8 million deaths annually. Cancer triggers include population growth, aging, and variations in the prevalence and distribution of the critical risk factors for cancer. Multiple hallmarks are involved in cancer, including cell proliferation, evading growth suppressors, activating invasion and metastasis, resisting cell death, enabling replicative immortality, reprogramming energy metabolism, and evading immune destruction. Both cancer and dementia are age-related and potentially lethal, impacting survival. With increasing aging populations, cancer and dementia cause a burden on patients, family members, the health care system, and informal/formal caregivers. In the current article, we highlight cancer prevalence with a focus on different ethnic groups, ages, and genders. Our article covers risk factors and genetic causes associated with cancer and types of cancers and comorbidities. We extensively cover the impact of cancer in Hispanics in comparison to that in other ethnic groups. We also discuss the status of caregivers with cancer patients and urgent needs from

the state and federal support for caregivers.

Blagosklonny, M. V. (2003). "Cell immortality and hallmarks of cancer." *Cell Cycle* 2(4): 296-299.

In growth-limiting conditions, cells that express telomerase and inactivate tumor suppressors have a selective advantage due to resistance to growth arrest. Accidentally such cells become immortal.

Blagosklonny, M. V. (2005). "Molecular theory of cancer." *Cancer Biol Ther* 4(6): 621-627.

The mutation theory of cancer was always confronted by alternative (vitalistic) theories, which insist that cancer (like life itself) cannot be reduced to molecular interactions. In fact, the most fundamental feature of the somatic mutation theory of cancer is that it is a molecular theory, meaning that all the complexity of cancer on any level (e.g., tissue) can be explained on the molecular level. To emphasize the essence of mutation theory, cancer-causing mutation can be defined as any (a) molecular event that is (b) somatically inheritable and (c) selectable (e.g., provides selective advantage in restrictive/carcinogenic conditions). Here I review molecular (somatic mutation) theory and its alternatives and discuss that molecular interactions can completely explain complex tissue phenomena such as benign tumors and stroma initiated tumorigenesis. In addition, molecular theory predicts extragenetic somatic hereditary in cancer (e.g., posttranslational protein modifications that initiate and are supported by positive feedback loops) and also explains the relationship between selection for resistance, hallmarks of cancer and genetic instability. From molecules to cells to the organism, this review discusses how somatically heritable molecular alterations (genetic, epigenetic and extragenetic) alter translation of cellular signals, resulting in resistance to growth inhibition and apoptosis, that is manifested as secondary hallmarks of cancer (metastasis, angiogenesis and immortality) and, finally, as the amazing ability of some cancer cells such as canine transmissible sarcoma to 'live in a wild' like unicellular mammalian species.

Blagosklonny, M. V. (2007). "Cancer stem cell and cancer stemoids: from biology to therapy." *Cancer Biol Ther* 6(11): 1684-1690.

It has become a cliché that cancer therapy fails because it does not target rare cancer stem cells (CSCs). Here we are discuss that this is not how therapy fails and not any cancer cell with stem-like properties is CSC. Paradoxically, CSCs must be resting to explain their resistance to therapy yet must be cycling to explain their persistence in cell culture.

To solve contradictions, this article introduces the term cancer stemoids (or stem cell-like cells) to describe proliferating self-renewing cells. The stem cell hierarchy (stem--proliferating--terminal cells) exists exactly to separate self-renewal (immortality) from proliferation. Cancer stemoids break the stem cell hierarchy and eventually may replace other cells. While CSC is shielded from any selective pressure and therefore unable to drive tumor progression, cancer stemoids undergo clonal selection, accumulate mutations, thus determining tumor progression and therapeutic failures. Unlike CSC, cancer stemoids are a crucial target for cancer therapy, exactly because they proliferate. Furthermore, two normally mutually-exclusive properties (proliferation and stemness) provide a means to design therapy to kill cancer stemoids selectively without killing normal stem and non-stem cells. In contrast, true CSCs are not only a difficult, but also an insufficient and perhaps even an unnecessary therapeutic target, especially in advanced malignancies.

Blagosklonny, M. V. (2022). "Hallmarks of cancer and hallmarks of aging." *Aging (Albany NY)* 14(9): 4176-4187.

A thought-provoking article by Gems and de Magalhaes suggests that canonic hallmarks of aging are superficial imitations of hallmarks of cancer. I took their work a step further and proposed hallmarks of aging based on a hierarchical principle and the hyperfunction theory. To do this, I first reexamine the hallmarks of cancer proposed by Hanahan and Weinberg in 2000. Although six hallmarks of cancer are genuine, they are not hierarchically arranged, i.e., molecular, intra-cellular, cellular, tissue, organismal and extra-organismal. (For example, invasion and angiogenesis are manifestations of molecular alterations on the tissue level; metastasis on the organismal level, whereas cell immortality is observed outside the host). The same hierarchical approach is applicable to aging. Unlike cancer, however, aging is not a molecular disease. The lowest level of its origin is normal intracellular signaling pathways such as mTOR that drive developmental growth and, later in life, become hyperfunctional, causing age-related diseases, whose sum is aging. The key hallmark of organismal aging, from worms to humans, are age-related diseases. In addition, hallmarks of aging can be arranged as a timeline, wherein initial hyperfunction is followed by dysfunction, organ damage and functional decline.

Bonuccelli, G., et al. (2017). "Targeting cancer stem cell propagation with palbociclib, a CDK4/6 inhibitor: Telomerase drives tumor cell heterogeneity."

Oncotarget **8**(6): 9868-9884.

In this report, we systematically examined the role of telomerase activity in lung and ovarian cancer stem cell (CSC) propagation. For this purpose, we indirectly gauged telomerase activity, by linking the hTERT-promoter to eGFP. Using lung (A549) and ovarian (SKOV3) cancer cells, transduced with the hTERT-GFP reporter, we then employed GFP-expression levels to fractionate these cell lines into GFP-high and GFP-low populations. We functionally compared the phenotype of these GFP-high and GFP-low populations. More specifically, we now show that the cancer cells with higher telomerase activity (GFP-high) are more energetically activated, with increased mitochondrial mass and function, as well as increased glycolytic activity. This was further validated and confirmed by unbiased proteomics analysis. Cells with high telomerase activity also showed an increased capacity for stem cell activity (as measured using the 3D-spheroid assay) and cell migration (as measured using a Boyden chamber approach). These enhanced biological phenotypes were effectively inhibited by classical modulators of energy metabolism, which target either i) mitochondrial metabolism (i.e., oligomycin) or ii) glycolysis (i.e., 2-deoxy-glucose), or iii) by using the FDA-approved antibiotic doxycycline, which inhibits mitochondrial biogenesis. Finally, the level of telomerase activity also determined the ability of hTERT-high cells to proliferate, as assessed by measuring DNA synthesis via EdU incorporation. Consistent with these observations, treatment with an FDA-approved CDK4/6 inhibitor (PD-0332991/palbociclib) specifically blocked the propagation of both lung and ovarian CSCs. Virtually identical results were obtained with breast CSCs, which were also highly sensitive to palbociclib at concentrations in the nanomolar range. In summary, CSCs with high telomerase activity are among the most energetically activated, migratory and proliferative cell sub-populations. These observations may provide a mechanistic explanation for tumor metabolic heterogeneity, based on telomerase activity. FDA-approved drugs, such as doxycycline and palbociclib, were both effective at curtailing CSC propagation. Thus, these FDA-approved drugs could be used to target telomerase-high proliferative CSCs, in multiple cancer types. Finally, our experiments also allowed us to distinguish two different cellular populations of hTERT-high cells, one that was proliferative (i.e., replicative immortality) and the other that was non-proliferative (i.e., quiescent). We speculate that the non-proliferative population of hTERT-high cells that we identified could be mechanistically involved in tumor dormancy.

Borah, S., et al. (2015). "Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer." Science **347**(6225): 1006-1010.

Reactivation of telomerase, the chromosome end-replicating enzyme, drives human cell immortality and cancer. Point mutations in the telomerase reverse transcriptase (TERT) gene promoter occur at high frequency in multiple cancers, including urothelial cancer (UC), but their effect on telomerase function has been unclear. In a study of 23 human UC cell lines, we show that these promoter mutations correlate with higher levels of TERT messenger RNA (mRNA), TERT protein, telomerase enzymatic activity, and telomere length. Although previous studies found no relation between TERT promoter mutations and UC patient outcome, we find that elevated TERT mRNA expression strongly correlates with reduced disease-specific survival in two independent UC patient cohorts (n = 35; n = 87). These results suggest that high telomerase activity may be a better marker of aggressive UC tumors than TERT promoter mutations alone.

Brondum-Jacobsen, P., et al. (2014). "Authors' Response to: Skin cancer as a marker of sun exposure--a case of serious immortality bias." Int J Epidemiol **43**(3): 972-973.

Bruggeman, J. W., et al. (2023). "How germline genes promote malignancy in cancer cells." Bioessays **45**(1): e2200112.

Cancers often express hundreds of genes otherwise specific to germ cells, the germline/cancer (GC) genes. Here, we present and discuss the hypothesis that activation of a "germline program" promotes cancer cell malignancy. We do so by proposing four hallmark processes of the germline: meiosis, epigenetic plasticity, migration, and metabolic plasticity. Together, these hallmarks enable replicative immortality of germ cells as well as cancer cells. Especially meiotic genes are frequently expressed in cancer, implying that genes unique to meiosis may play a role in oncogenesis. Because GC genes are not expressed in healthy somatic tissues, they form an appealing source of specific treatment targets with limited side effects besides infertility. Although it is still unclear why germ cell specific genes are so abundantly expressed in cancer, from our hypothesis it follows that the germline's reproductive program is intrinsic to cancer development.

Burger, A. M. (1999). "Telomerase in cancer diagnosis and therapy: a clinical perspective." BioDrugs **12**(6): 413-422.

Curing cancers is one of the most

challenging tasks of modern medicine. The major problem is the heterogeneity of human tumours and thus finding a 'universal' target for cancer treatment. The discovery that the expression of the enzyme telomerase is a hallmark of immortality and cancer, and that it is found in the majority (>85%) of human tumours but is repressed in most normal cells, has therefore caused considerable excitement. These observations led to the design of potential telomerase inhibitors and ideas about targeting telomerase in the clinic. To date, several classes of telomerase inhibitory agents have been identified and are in preclinical development. However, the approach has not yet been tested clinically. Because of the proposed function of telomerase, and the understanding that replicative cell senescence or cell death result from progressive telomere shortening during successive cell divisions, even complete enzyme inhibition will not produce immediate cell death. Designing clinical trials for promising telomerase inhibitors requires consideration of the novel mechanism of action of these drugs. A lag period between initiation of treatment and occurrence of effects is likely, and thus anti-telomerase therapy might best be given in adjuvant treatment protocols after initial tumour debulking therapy and in combination with other cytostatic agents. The available knowledge of telomerase biology and its association with human tumours suggests that telomerase inhibition might prove a valuable addition to current cancer treatment regimens.

Button, L., et al. (2022). "Telomere and Telomerase-Associated Proteins in Endometrial Carcinogenesis and Cancer-Associated Survival." *Int J Mol Sci* **23**(2).

Risk of relapse of endometrial cancer (EC) after surgical treatment is 13% and recurrent disease carries a poor prognosis. Research into prognostic indicators is essential to improve EC management and outcome. "Immortality" of most cancer cells is dependent on telomerase, but the role of associated proteins in the endometrium is poorly understood. The Cancer Genome Atlas data highlighted telomere/telomerase associated genes (TTAGs) with prognostic relevance in the endometrium, and a recent in silico study identified a group of TTAGs and proteins as key regulators within a network of dysregulated genes in EC. We characterise relevant telomere/telomerase associated proteins (TTAPs) NOP10, NHP2, NOP56, TERF1, TERF2 and TERF2IP in the endometrium using quantitative polymerase chain reaction (qPCR) and immunohistochemistry (IHC). qPCR data demonstrated altered expression of multiple TTAPs; specifically, increased NOP10 ($p = 0.03$) and reduced NHP2 ($p = 0.01$), TERF2 ($p = 0.01$) and TERF2IP (p

< 0.003) in EC relative to post-menopausal endometrium. Notably, we report reduced NHP2 in EC compared to post-menopausal endometrium in qPCR and IHC ($p = 0.0001$) data; with survival analysis indicating high immunoscore is favourable in EC ($p = 0.0006$). Our findings indicate a potential prognostic role for TTAPs in EC, particularly NHP2. Further evaluation of the prognostic and functional role of the examined TTAPs is warranted to develop novel treatment strategies.

Carruba, G. and J. E. Trosko (2017). "The Long Evolutionary Journey of Cancer from Ancestor to Modern Humans." *Crit Rev Oncog* **22**(3-4): 323-352.

In this article, we review various key issues in cancer development and progression that have important implications for both cancer prevention and treatment: (1) evolutionary aspects of cancer appearance; (2) evidence of organ-specific adult stem cells as cancer-initiating cells; (3) the immortality of cancer-initiating cells; (4) cancer cell loss of growth control, contact inhibition, terminal differentiation, and apoptosis; (5) stem-cell versus de-differentiation theory of carcinogenesis; (6) mutations in cancer; (7) oncogenes and tumor suppressor genes; (8) epigenetics as the rate-limiting step in carcinogenesis; (9) the potential role of cultural, lifestyle, and nutritional behaviors in oncology; and (10) changes of commensal microbial community and its metagenome in carcinogenesis and tumor progression. Relevant, combined evidence is discussed from a standpoint whereby cancer is considered a multifaceted disease requiring integrated biomolecular and clinico-pathological information to design and implement strategies for either primary prevention or therapy.

Casacuberta-Serra, S., et al. (2024). "MYC and KRAS cooperation: from historical challenges to therapeutic opportunities in cancer." *Signal Transduct Target Ther* **9**(1): 205.

RAS and MYC rank amongst the most commonly altered oncogenes in cancer, with RAS being the most frequently mutated and MYC the most amplified. The cooperative interplay between RAS and MYC constitutes a complex and multifaceted phenomenon, profoundly influencing tumor development. Together and individually, these two oncogenes regulate most, if not all, hallmarks of cancer, including cell death escape, replicative immortality, tumor-associated angiogenesis, cell invasion and metastasis, metabolic adaptation, and immune evasion. Due to their frequent alteration and role in tumorigenesis, MYC and RAS emerge as highly appealing targets in cancer therapy. However, due to their complex nature, both oncogenes have

been long considered "undruggable" and, until recently, no drugs directly targeting them had reached the clinic. This review aims to shed light on their complex partnership, with special attention to their active collaboration in fostering an immunosuppressive milieu and driving immunotherapeutic resistance in cancer. Within this review, we also present an update on the different inhibitors targeting RAS and MYC currently undergoing clinical trials, along with their clinical outcomes and the different combination strategies being explored to overcome drug resistance. This recent clinical development suggests a paradigm shift in the long-standing belief of RAS and MYC "undruggability", hinting at a new era in their therapeutic targeting.

Causin, R. L., et al. (2021). "A Systematic Review of MicroRNAs Involved in Cervical Cancer Progression." *Cells* **10**(3).

To obtain a better understanding on the role of microRNAs in the progression of cervical cancer, a systematic review was performed to analyze cervical cancer microRNA studies. We provide an overview of the studies investigating microRNA expression in relation to cervical cancer (CC) progression, highlighting their common outcomes and target gene interactions according to the regulatory pathways. To achieve this, we systematically searched through PubMed MEDLINE, EMBASE, and Google Scholar for all articles between April 2010 and April 2020, in accordance with the PICO acronym (participants, interventions, comparisons, outcomes). From 27 published reports, totaling 1721 cases and 1361 noncancerous control tissue samples, 26 differentially expressed microRNAs (DEmiRNAs) were identified in different International Federation of Gynecology and Obstetrics (FIGO) stages of cervical cancer development. It was identified that some of the dysregulated microRNAs were associated with specific stages of cervical cancer development. The results indicated that DEmiRNAs in different stages of cervical cancer were functionally involved in several key hallmarks of cancer, such as evading growth suppressors, enabling replicative immortality, activation of invasion and metastasis, resisting cell death, and sustained proliferative signaling. These dysregulated microRNAs could play an important role in cervical cancer's development. Some of the stage-specific microRNAs can also be used as biomarkers for cancer classification and monitoring the progression of cervical cancer.

Cernaj, I. E. (2016). "Simultaneous dual targeting of Par-4 and G6PD: a promising new approach in

cancer therapy? Quintessence of a literature review on survival requirements of tumor cells." *Cancer Cell Int* **16**: 87.

The aim of this hypothesis is to propose a new approach in targeted therapy of cancer: The simultaneous, dual targeting of two single molecules, Par-4 and G6PD, rather than inhibition of full-length signaling pathways. RATIONALE: Targeted inhibition of especially two survival signaling pathways (PI3K/AKT/mTOR and MAPK/ERK) is frequently tried, however, a major breakthrough has not yet been reported. Inhibition of complete pathways naturally goes along with a variety of dose-limiting side effects thus contributing to poor efficacy of the administered drugs. This essay offers a synopsis of relevant studies to support the above mentioned idea-targeting of two single molecules which either are crucial for tumor growth and cancer-cell-survival: on one side, Par-4-activation selectively triggers apoptosis of tumor cells thus reversing their characteristic feature-immortality. On the other side inhibition of G6PD breaks the energy supply of tumor cells, weakens their defence against oxidative stress and thereby enhances the sensitivity of tumor cells to oxidative agents (e.g. chemotherapy). Advantage of the proposed dual Par-4/G6PD-therapy is good tolerability and-especially when administered along with conventional therapy-less frequent emergence of resistance.

Chang, H. L. and J. C. Lin (2019). "SRSF1 and RBM4 differentially modulate the oncogenic effect of HIF-1alpha in lung cancer cells through alternative splicing mechanism." *Biochim Biophys Acta Mol Cell Res* **1866**(12): 118550.

Alternative splicing (AS) constitutes a pivotal mechanism for expanding the transcriptome and proteome diversity in higher eukaryotes. In contrast, misregulated AS events are relevant to carcinogenic signatures, including migration, angiogenesis, immortality, and drug resistance of cancer cells. Using a transcriptome analysis, discriminative splicing profiles of hypoxia-inducible factor (HIF)-1alpha transcripts were identified in tumorous tissues compared to adjacent normal tissues of lung cancer (LC) patients. In cancerous tissues or LC-derived cells, relatively high levels of HIF-1alpha(-ex14) transcripts encoding the HIF-1alpha(S) isoform were noted compared to adjacent normal tissues and non-cancerous cells. The HIF-1alpha(S) isoform exhibited a more-prominent effect than that of the HIF-1alpha(L) isoform translated from HIF-1alpha(+ex14) transcripts on enhancing promoter activities of the vascular endothelial growth factor receptor 2 (VEGFR2), serine/arginine splicing factor 1 (SRSF1), and c13orf25 genes. An increase in the

SRSF1 protein facilitated the generation of HIF-1alpha(-ex14) transcripts, whereas overexpression of RNA-binding motif protein 4 (RBM4) enhanced the expression of HIF-1alpha(+ex14) transcripts in the A549 cells. Results of splicing reporter assays demonstrated the differential impacts of RBM4 and SRSF1 on the utilization of HIF-1alpha exon 14 in a CU element-dependent manner. In addition to transcriptional regulation, overexpression of the HIF-1alpha(S) and HIF-1alpha(L) isoforms differentially enhanced the metastatic signatures of A549 cells. Taken together, SRSF1 and RBM4 constitute an antagonistic mechanism on regulating the splicing profiles of HIF-1alpha gene, which is relevant to the oncogenic signatures of LC cells.

Chen, C. H. and R. J. Chen (2011). "Prevalence of telomerase activity in human cancer." *J Formos Med Assoc* **110**(5): 275-289.

Telomerase activity has been measured in a wide variety of cancerous and non-cancerous tissue types, and the vast majority of clinical studies have shown a direct correlation between it and the presence of cancerous cells. Telomerase plays a key role in cellular immortality and tumorigenesis. Telomerase is activated in 80-90% of human carcinomas, but not in normal somatic cells, therefore, its detection holds promise as a diagnostic marker for cancer. Measurable levels of telomerase have been detected in malignant cells from various samples: tissue from gestational trophoblastic neoplasms; squamous carcinoma cells from oral rinses; lung carcinoma cells from bronchial washings; colorectal carcinoma cells from colonic luminal washings; bladder carcinoma cells from urine or bladder washings; and breast carcinoma or thyroid cancer cells from fine needle aspirations. Such clinical tests for telomerase can be useful as non-invasive and cost-effective methods for early detection and monitoring of cancer. In addition, telomerase activity has been shown to correlate with poor clinical outcome in late-stage diseases such as non-small cell lung cancer, colorectal cancer, and soft tissue sarcomas. In such cases, testing for telomerase activity can be used to identify patients with a poor prognosis and to select those who might benefit from adjuvant treatment. Our review of the latest medical advances in this field reveals that telomerase holds great promise as a biomarker for early cancer detection and monitoring, and has considerable potential as the basis for developing new anticancer therapies.

Chen, G. T. and M. L. Waterman (2015). "Cancer: leaping the E-cadherin hurdle." *EMBO J* **34**(18): 2307-2309.

Aberrant activation of the Wnt signaling pathway is a common cause of colon cancer and other tumor types, accomplishing many of the hallmarks of cancer including sustained proliferative signaling, replicative immortality, reprogrammed metabolism, angiogenesis, and invasion. Yet, the dominant mutation that leads to chronic Wnt signaling in colon cancer is quite different from the spectrum of mutations that activate Wnt signaling in other tumor types. In this issue of *The EMBO Journal*, Huels et al (2015) focus on the influential role E-cadherin plays in shaping these differences.

Chen, M. and S. W. McLeskey (2010). "Telomere-based cancer treatment: emerging targeted therapies." *Clin J Oncol Nurs* **14**(6): 720-726.

Chemotherapy and radiation therapy are standard care in cancer treatment; however, both have numerous adverse side effects because they affect healthy as well as cancerous cells. The side effects, including decreased white blood cell count, nausea, hair loss, and fatigue, can be severe enough that patients may decide to forgo treatment. Targeted therapies are treatments that focus on specific molecules in cancerous cells and avoid disruption of healthy cells. Telomeres, the ends of chromosomes, are possible targets. In healthy cells, telomeres become shorter with each cell division, limiting the number of divisions that a normal cell can undergo. Many cancer cells have telomerase activity, which rebuilds telomeres after each cell division and confers immortality to cancer cells. Telomerase is an enzyme normally present to a significant degree only in the cells of developing fetuses. Treatments that target the telomerase enzyme itself or the chromosomal telomeres are being developed and tested in early clinical trials. This article focuses on several approaches to telomere-targeted therapy.

Chen, W., et al. (2021). "Potential impact and mechanism of Long Non-coding RNAs on cancer and associated T cells." *J Cancer* **12**(16): 4873-4882.

The discovery of many aberrant expressions of long non-coding RNAs (lncRNAs) in various cancers has focused attention on the effects of lncRNA on cancer cells themselves, including cell proliferation, growth inhibition, cell migration, cell immortality, vascular regeneration and cell viability. But with the increasing role of immunotherapy in cancer therapy, a large number of studies have revealed that the regulatory role of lncRNAs in immunity such as differentiation of immune cells can also influence the development and progression of cancer. In particular, recent publications have suggested that lncRNAs play critical roles in T-lymphocyte activation, proliferation, differentiation,

function, apoptosis and metabolism. To elucidate the actual functions of lncRNAs at the molecular level of cancer pathogenesis, we summarize some of the current lncRNA regulatory mechanisms associated with T cell to discuss their effects in cancer in the hope of providing potential cancer therapeutic targets or cancer biomarkers. However, we all know that the differentiation and function of T cells is an extremely complex process that involves the expression and regulation of multiple lncRNAs. As a result, more regulatory mechanisms of lncRNAs need to be further studied.

Cheng, C. W., et al. (2000). "Diagnosis of bladder cancer using telomerase activity in voided urine." *J Formos Med Assoc* **99**(12): 920-925.

BACKGROUND AND PURPOSE: Telomerase is an essential enzyme for cellular immortality and tumorigenesis. Reactivation of telomerase is associated with many primary cancers. We evaluated the accuracy of a modified immunodiagnostic technique based on the telomeric repeat amplification protocol (TRAP) assay, by semi-quantitative measurement of telomerase activity in exfoliated urothelial cells in voided urine from patients with bladder cancer. **METHODS:** Telomerase activity was assayed in centrifuged urine cell pellets from 17 bladder cancer patients and from 32 patients with benign bladder diseases. Each specimen was collected from a 50-mL sample of single voided urine obtained before surgery, and telomerase activity was detected using a telomerase polymerase chain reaction and enzyme-linked immunosorbent assay (PCR-ELISA) protocol. Results of pathologic study, urine cytologic examination, and urine telomerase activity were determined independently. **RESULTS:** The cut-off value for relative telomerase activity was set at 0.059, which provided an optimal diagnostic accuracy of 88% (n = 49). At this cut-off value, the sensitivity and specificity for urine telomerase in bladder cancer were 82% (n = 17) and 91% (n = 32), respectively. Telomerase activity was found in 11 low-grade tumors and six high-grade tumors, whereas negative results for telomerase activity were found in urothelial cells of patients with inguinal hernia, urinary stones, acute urinary tract infection, or chronic cystitis. Only five cytology samples from the same patients were positive for bladder cancer. The difference in these two detection rates was significant (p = 0.002). **CONCLUSION:** The results of this study indicate that the measurement of telomerase activity from voided urine using our modified semi-quantitative PCR-ELISA technique may help provide earlier diagnosis of bladder cancer and earlier postoperative indication of recurrence.

Claude, E. and A. Decottignies (2020). "Telomere maintenance mechanisms in cancer: telomerase, ALT or lack thereof." *Curr Opin Genet Dev* **60**: 1-8.

Cancer cells acquire replicative immortality by activating a telomere maintenance mechanism (TMM), either the telomerase or the Alternative Lengthening of Telomeres (ALT) mechanism. ALT is frequently activated in tumors derived from mesenchymal cells, which are more frequent in childhood cancers. Recent studies showed that, occasionally, cancer cells can arise without any TMM activation. Here, we discuss the challenge in assessing which TMM is activated in tumors. We also evaluate the prevalence of ALT mechanism in pediatric cancers and review the associated survival prognosis in different tumor types. Finally, we discuss about possible anti-TMM therapies for new emerging cancer treatments.

Cleal, K., et al. (2018). "Telomere Length Dynamics and the Evolution of Cancer Genome Architecture." *Int J Mol Sci* **19**(2).

Telomeres are progressively eroded during repeated rounds of cell division due to the end replication problem but also undergo additional more substantial stochastic shortening events. In most cases, shortened telomeres induce a cell-cycle arrest or trigger apoptosis, although for those cells that bypass such signals during tumour progression, a critical length threshold is reached at which telomere dysfunction may ensue. Dysfunction of the telomere nucleoprotein complex can expose free chromosome ends to the DNA double-strand break (DSB) repair machinery, leading to telomere fusion with both telomeric and non-telomeric loci. The consequences of telomere fusions in promoting genome instability have long been appreciated through the breakage-fusion-bridge (BFB) cycle mechanism, although recent studies using high-throughput sequencing technologies have uncovered evidence of involvement in a wider spectrum of genomic rearrangements including chromothripsis. A critical step in cancer progression is the transition of a clone to immortality, through the stabilisation of the telomere repeat array. This can be achieved via the reactivation of telomerase, or the induction of the alternative lengthening of telomeres (ALT) pathway. Whilst telomere dysfunction may promote genome instability and tumour progression, by limiting the replicative potential of a cell and enforcing senescence, telomere shortening can act as a tumour suppressor mechanism. However, the burden of senescent cells has also been implicated as a driver of ageing and age-related pathology, and in the promotion of cancer through inflammatory signalling.

Considering the critical role of telomere length in governing cancer biology, we review questions related to the prognostic value of studying the dynamics of telomere shortening and fusion, and discuss mechanisms and consequences of telomere-induced genome rearrangements.

Costa, A. C., et al. (2021). "Impact of immune cells on the hallmarks of cancer: A literature review." *Crit Rev Oncol Hematol* **168**: 103541.

Tumor-infiltrating immune cells (TIICs) are critical players in the tumor microenvironment, modulating cancer cell functions. TIICs are highly heterogenic and plastic and may either suppress cancers or provide support for tumor growth. A wide range of studies have shed light on how tumor-associated macrophages, dendritic cells, neutrophils, mast cells, natural killer cells and lymphocytes contribute for the establishment of several hallmarks of cancer and became the basis for successful immunotherapies. Many of those TIICs play pivotal roles in several hallmarks of cancer. This review contributes to elucidate the multifaceted roles of immune cells in cancer development, highlighting molecular components that constitute promising therapeutic targets. Additional studies are needed to clarify the relation between TIICs and hallmarks such as enabling replicative immortality, evading growth suppressors, sustaining proliferative signaling, resisting cell death and genome instability and mutation, to further explore their therapeutic potential and improve the outcomes of cancer patients.

Cowell, J. K. (1999). "Telomeres and telomerase in ageing and cancer." *Age (Omaha)* **22**(2): 59-64.

Telomeres lie at the ends of human chromosomes and contain long tandem repeats of a simple nucleotide sequence. Because DNA replication cannot proceed to the very end of chromosomes, copies of these repeats are lost at each cell division. If the telomeres shorten below a critical length, the cells will eventually die as a result of genomic instability. Aging cells usually avoid death by entering senescence before the critical telomere length is reached. Malignantly transformed, immortal cells overcome senescence but they must still avoid the final, critical shortening of telomeres to survive. In the vast majority of cases, tumor cells achieve this by activating the telomerase enzyme, a ribonucleoprotein complex which repairs the end of chromosomes and prevents telomere shortening. Normal mortal cells do not normally express telomerase, although some stem cell populations which must regenerate through the life span of the organism, retain enzyme activity. Cellular senescence can be overcome by inducing telomerase expression

in mortal cells, firmly establishing the role of telomere length in the senescence signaling pathway. In tumor cells, the evidence of a role for telomerase in immortality is still largely correlative, with 80-90% of tumors expressing telomerase activity. To establish whether telomerase activity is important in maintaining the malignant phenotype, attempts have been made to inactivate it in tumor cells, using a variety of approaches, where there is evidence that disrupting telomerase function can result in the induction of apoptosis. The background and implications of these observations is discussed.

Creese, Z., et al. (2014). "Oligonucleotides and G-quadruplex stabilizers: targeting telomeres and telomerase in cancer therapy." *Curr Pharm Des* **20**(41): 6422-6437.

Cancer is a leading cause of death worldwide and an estimated 1 in 4 deaths in the United States is due to cancer. Despite recent advances in cancer treatment, adverse effects related to cancer therapy remain a limiting factor for many patients. The ideal cancer treatment would selectively target cancerous cells while sparing normal, healthy cells to offer maximal therapeutic benefit while minimizing toxicity. Telomeres are structurally unique DNA sequences at the end of human chromosomes, which play an integral role in the cellular mortality of normal cells. As telomeres shorten with successive cellular divisions, cells develop chromosomal instability and undergo either apoptosis or senescence. In many cancers, this apoptosis or senescence is avoided as normal telomere length is maintained by a ribonucleoprotein reverse transcriptase called telomerase. Telomerase is expressed in more than 85% of all cancers and confers cancerous cells with a replicative immortality, which is a hallmark of malignant tumors. In contrast, telomerase activity is not detectable in the majority of normal somatic cell populations. Therefore, the targeting of telomerase and telomere maintenance mechanisms represent a potentially promising therapeutic approach for various types of cancer. This review evaluates the roles of GRN163L, T-oligo and small molecule G-quadruplex stabilizers as potential anticancer therapies by targeting telomerase and other telomere maintenance mechanisms.

Criscitiello, C., et al. (2014). "Tumor-stroma crosstalk: targeting stroma in breast cancer." *Curr Opin Oncol* **26**(6): 551-555.

PURPOSE OF REVIEW: Combinatorial strategies in cancer medicine will not only target cancer cell-intrinsic pathways, but also cancer cell-extrinsic cells, pathways, and mediators of the tumor microenvironment. The aim of the present review is

to define the roles of the tumor microenvironment in primary and metastatic breast cancer progression. **RECENT FINDINGS:** The cancer microenvironment is composed of nontransformed host stromal cells, such as endothelial cells, fibroblasts, various immune cells, and a complex extracellular matrix secreted by both the normal and neoplastic cells embedded in it. The stromal constituents contribute to the core and emergent hallmarks of cancer. In particular, they can boost sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming energy metabolism, and evading immune destruction. **SUMMARY:** The stromal cells play a role in enabling or enhancing multiple hallmark capabilities in tumor microenvironment. This is a background for therapeutic-targeting strategies aimed to abrogate the stroma's contribution. Targeting tumor-associated fibroblasts, macrophages, angiogenesis and enhancing immune response may represent a paradigm-shifting approach to treating human cancer in the near future.

Cui, Z., et al. (2024). "PANoptosis: A new era for anti-cancer strategies." *Life Sci* **359**: 123241.

Cancer cells possess an extraordinary ability to dodge cell death through various pathways, granting them a form of immortality—a key obstacle in oncotherapy. Thus, it's vital to unravel the intricate mechanisms behind newly discovered types of cell death that drive tumor suppression, going beyond apoptosis alone. The emergence of PANoptosis, a form of cell death intertwining necroptosis, pyroptosis, and apoptosis, offers a fresh perspective, integrating these pathways into one cohesive process. When cells detect damage signals, they assemble PANoptosome complexes that disrupt their balance, trigger immune responses, and lead to their eventual collapse. PANoptosis has been associated with multiple cellular pathways, including ferroptosis. Mitochondrial dysfunction also plays a critical role in sparking and advancing PANoptosis. In this review, we map out the molecular machinery and regulatory web controlling PANoptosis. We explore cutting-edge research and future trends in PANoptosis-centered tumor therapies, spotlighting promising innovations that could amplify cancer treatment effectiveness through harnessing this multifaceted cell death pathway. The development of nanomedicines and nanomaterials provides solutions to the therapeutic challenges of clinical drugs. Developing novel tumor nano-PANoptosis inducers by leveraging the advantages of nanomedicine is of research value. Traditional Chinese medicine (TCM) treatment is characterized by multiple targets, and it

has distinct advantages in triggering PANoptosis through multiple pathways. Additionally, photodynamic Therapy (PDT) may offer new insights into promoting PANoptosis in tumor cells by increasing oxidative stress and reactive oxygen species levels. These will establish a solid theoretical groundwork for the development of integrated treatment methodologies.

Cuthbert, A. P., et al. (1999). "Telomerase repressor sequences on chromosome 3 and induction of permanent growth arrest in human breast cancer cells." *J Natl Cancer Inst* **91**(1): 37-45.

BACKGROUND: Activation of the enzyme telomerase, which has been associated with cellular immortality, may constitute a key step in the development of human cancer. Telomerase is repressed in most normal human somatic cells. This study was conducted, using a genetic complementation approach, with the aim of identifying and mapping the genes responsible for repressing telomerase and, simultaneously, to establish the effect of experimentally induced telomerase repression on human tumor cell growth. **METHODS:** Individual human chromosomes isolated from normal diploid cells and tagged with bacterial antibiotic resistance genes (for later selection) were introduced into cells of the human breast carcinoma cell line 21NT by means of microcell transfer. Selected hybrid clones were screened for telomerase activity by use of the polymerase chain reaction-based telomere repeat amplification protocol (TRAP) assay, and the proliferative fate of the hybrid clones was determined. Regions of the introduced chromosomes associated with telomerase repression were mapped using segregant hybrids and a deletion analysis that employed microsatellite DNA markers. **RESULTS:** Strong repression of telomerase was observed following transfer of human chromosome 3 into 21NT cells but not after transfer of chromosomes 8, 12, or 20. The vast majority of hybrid clones with repressed telomerase entered permanent growth arrest after 10-18 population doublings. Deletion analysis of nonrepressed segregant monochromosome 3 hybrids indicated two regions on the short arm of chromosome 3 (3p21.3-p22 and 3p12-21.1) where telomerase regulator genes may be located. **CONCLUSIONS:** Telomerase in human breast cancer cells is efficiently repressed by a gene or genes on normal human chromosome 3p, and this repression is associated with permanent growth arrest of the tumor cells.

Dagg, R. A., et al. (2017). "Extensive Proliferation of Human Cancer Cells with Ever-Shorter Telomeres."

Cell Rep **19**(12): 2544-2556.

Acquisition of replicative immortality is currently regarded as essential for malignant transformation. This is achieved by activating a telomere lengthening mechanism (TLM), either telomerase or alternative lengthening of telomeres, to counter normal telomere attrition. However, a substantial proportion of some cancer types, including glioblastomas, liposarcomas, retinoblastomas, and osteosarcomas, are reportedly TLM-negative. As serial samples of human tumors cannot usually be obtained to monitor telomere length changes, it has previously been impossible to determine whether tumors are truly TLM-deficient, there is a previously unrecognized TLM, or the assay results are false-negative. Here, we show that a subset of high-risk neuroblastomas (with approximately 50% 5-year mortality) lacked significant TLM activity. Cancer cells derived from these highly aggressive tumors initially had long telomeres and proliferated for >200 population doublings with ever-shorter telomeres. This indicates that prevention of telomere shortening is not always required for oncogenesis, which has implications for inhibiting TLMs for cancer therapy.

Dai, X., et al. (2016). "Cancer Hallmarks, Biomarkers and Breast Cancer Molecular Subtypes." J Cancer **7**(10): 1281-1294.

Breast cancer is a complex disease encompassing multiple tumor entities, each characterized by distinct morphology, behavior and clinical implications. Besides estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2, novel biomarkers have shown their prognostic and predictive values, complicating our understanding towards the heterogeneity of such cancers. Ten cancer hallmarks have been proposed by Weinberg to characterize cancer and its carcinogenesis. By reviewing biomarkers and breast cancer molecular subtypes, we propose that the divergent outcome observed from patients stratified by hormone status are driven by different cancer hallmarks. 'Sustaining proliferative signaling' further differentiates cancers with positive hormone receptors. 'Activating invasion and metastasis' and 'evading immune destruction' drive the differentiation of triple negative breast cancers. 'Resisting cell death', 'genome instability and mutation' and 'deregulating cellular energetics' refine breast cancer classification with their predictive values. 'Evading growth suppressors', 'enabling replicative immortality', 'inducing angiogenesis' and 'tumor-promoting inflammation' have not been involved in breast cancer classification which need more focus in the future biomarker-related research. This review novels

in its global view on breast cancer heterogeneity, which clarifies many confusions in this field and contributes to precision medicine.

de Oliveira, J. C., et al. (2019). "Long non-coding RNAs in cancer: Another layer of complexity." J Gene Med **21**(1): e3065.

We review the most well characterized long non-coding RNAs (lncRNAs) with important roles in hallmarks of cancer, additionally including lncRNAs with a higher potential for clinical application. lncRNAs are transcripts larger than 200 nucleotides in length that do not appear to have protein-coding potential, although some of those may produce small functional peptides. These transcripts have attracted significant attention from researchers as a result of their role in genetic regulation, including epigenetic, transcriptional and post-transcriptional regulation, being involved in numerous biological processes, as well as being associated with multifactorial diseases, including tumorigenesis. The hallmarks of cancer include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion/metastasis. Additionally, genome instability, inflammation, reprogramming of energy metabolism and evading immune destruction and lncRNAs are implicated in all hallmarks of cancer. Based on the great number of studies describing lncRNAs associated with diverse aspects of most tumor types, lncRNAs have essential roles in potentially all biological features of cancer cells and show great utility as diagnostic and prognostic markers, as exemplified by PCA3 lncRNA detection in prostate cancer diagnosis.

Deeg, K. I., et al. (2016). "Cancer Cells with Alternative Lengthening of Telomeres Do Not Display a General Hypersensitivity to ATR Inhibition." Front Oncol **6**: 186.

Telomere maintenance is a hallmark of cancer as it provides cancer cells with cellular immortality. A significant fraction of tumors uses the alternative lengthening of telomeres (ALT) pathway to elongate their telomeres and to gain an unlimited proliferation potential. Since the ALT pathway is unique to cancer cells, it represents a potentially valuable, currently unexploited target for anti-cancer therapies. Recently, it was proposed that ALT renders cells hypersensitive to ataxia telangiectasia- and RAD3-related (ATR) protein inhibitors (Flynn et al., *Science* 347, 273). Here, we measured the response of various ALT- or telomerase-positive cell lines to the ATR inhibitor VE-821. In addition, we compared the effect of the inhibitor on cell viability in isogenic cell lines, in which ALT was active or suppressed. In

these experiments, a general ATR inhibitor sensitivity of cells with ALT could not be confirmed. We rather propose that the observed variations in sensitivity reflect differences between cell lines that are unrelated to ALT.

Demeny, M. A. and L. Virag (2021). "The PARP Enzyme Family and the Hallmarks of Cancer Part 1. Cell Intrinsic Hallmarks." *Cancers (Basel)* **13**(9).

The 17-member poly (ADP-ribose) polymerase enzyme family, also known as the ADP-ribosyl transferase diphtheria toxin-like (ARTD) enzyme family, contains DNA damage-responsive and nonresponsive members. Only PARP1, 2, 5a, and 5b are capable of modifying their targets with poly ADP-ribose (PAR) polymers; the other PARP family members function as mono-ADP-ribosyl transferases. In the last decade, PARP1 has taken center stage in oncology treatments. New PARP inhibitors (PARPi) have been introduced for the targeted treatment of breast cancer 1 or 2 (BRCA1/2)-deficient ovarian and breast cancers, and this novel therapy represents the prototype of the synthetic lethality paradigm. Much less attention has been paid to other PARPs and their potential roles in cancer biology. In this review, we summarize the roles played by all PARP enzyme family members in six intrinsic hallmarks of cancer: uncontrolled proliferation, evasion of growth suppressors, cell death resistance, genome instability, reprogrammed energy metabolism, and escape from replicative senescence. In a companion paper, we will discuss the roles of PARP enzymes in cancer hallmarks related to cancer-host interactions, including angiogenesis, invasion and metastasis, evasion of the anticancer immune response, and tumor-promoting inflammation. While PARP1 is clearly involved in all ten cancer hallmarks, an increasing body of evidence supports the role of other PARPs in modifying these cancer hallmarks (e.g., PARP5a and 5b in replicative immortality and PARP2 in cancer metabolism). We also highlight controversies, open questions, and discuss prospects of recent developments related to the wide range of roles played by PARPs in cancer biology. Some of the summarized findings may explain resistance to PARPi therapy or highlight novel biological roles of PARPs that can be therapeutically exploited in novel anticancer treatment paradigms.

Demeny, M. A. and L. Virag (2021). "The PARP Enzyme Family and the Hallmarks of Cancer Part 2: Hallmarks Related to Cancer Host Interactions." *Cancers (Basel)* **13**(9).

Poly (ADP-ribose) polymerases (PARPs) modify target proteins with a single ADP-ribose unit or with a poly (ADP-ribose) (PAR) polymer. PARP

inhibitors (PARPis) recently became clinically available for the treatment of BRCA1/2 deficient tumors via the synthetic lethality paradigm. This personalized treatment primarily targets DNA damage-responsive PARPs (PARP1-3). However, the biological roles of PARP family member enzymes are broad; therefore, the effects of PARPis should be viewed in a much wider context, which includes complex effects on all known hallmarks of cancer. In the companion paper (part 1) to this review, we presented the fundamental roles of PARPs in intrinsic cancer cell hallmarks, such as uncontrolled proliferation, evasion of growth suppressors, cell death resistance, genome instability, replicative immortality, and reprogrammed metabolism. In the second part of this review, we present evidence linking PARPs to cancer-associated inflammation, anti-cancer immune response, invasion, and metastasis. A comprehensive overview of the roles of PARPs can facilitate the identification of novel cancer treatment opportunities and barriers limiting the efficacy of PARPi compounds.

Demetriou, C. A., et al. (2018). "Filling the gap between chemical carcinogenesis and the hallmarks of cancer: A temporal perspective." *Eur J Clin Invest* **48**(6): e12933.

BACKGROUND: Cancer is believed to arise through the perturbation of pathways and the order of pathway perturbation events can enhance understanding and evaluation of carcinogenicity. This order has not been examined so far, and this study aimed to fill this gap by attempting to gather evidence on the potential temporal sequence of events in carcinogenesis. **DESIGN:** The methodology followed was to discuss first the temporal sequence of hallmarks of cancer from the point of view of pathological specimens of cancer (essentially branched mutations) and then to consider the hallmarks of cancer that one well-known carcinogen, benzo(a)pyrene, can modify. **RESULTS:** Even though the sequential order of driving genetic alterations can vary between and within tumours, the main cancer pathways affected are almost ubiquitous and follow a generally common sequence: resisting cell death, insensitivity to antigrowth signals, sustained proliferation, deregulated energetics, replicative immortality and activation of invasion and metastasis. The first 3 hallmarks can be regarded as almost simultaneous while angiogenesis and avoiding immune destruction are perhaps the only hallmarks with a varying position in the above sequence. **CONCLUSIONS:** Our review of hallmarks of cancer and their temporal sequence, based on mutational spectra in biopsies from different cancer sites, allowed us to propose a hypothetical temporal

sequence of the hallmarks. This sequence can add molecular support to the evaluation of an agent as a carcinogen as it can be used as a conceptual framework for organising and evaluating the strength of existing evidence.

Denhardt, D. T. (1996). "Oncogene-initiated aberrant signaling engenders the metastatic phenotype: synergistic transcription factor interactions are targets for cancer therapy." *Crit Rev Oncog* 7(3-4): 261-291.

Certain p21GTPases (notably Ras) and some of their guanine nucleotide exchange factors (e.g., Ost, Dbl, Tiam) and downstream mediators (e.g., Raf, Myc) have the potential to promote the development of malignancies because they can enhance the transcription of genes that foster the tumorigenic and metastatic phenotype. Among these are genes that stimulate cell proliferation, confer immortality, and facilitate the invasion of normal tissues. Oncogenes upstream of Ras-cell surface receptors such as ErbB2/Neu, Met, or Trk (and their ligands), and nonreceptor cytoplasmic protein tyrosine kinases such as Src and Abl-not only can act through Ras but also contribute additional signals. This review presents a synopsis of our understanding of signaling pathways controlled by the p21GTPases, with a focus on transcription factors regulated by the pathways. Mutations in one or more of the elements in these signaling pathways are invariably found in cancer cells. Crosstalk among the pathways may explain how some forms of stress can contribute to the development of a malignancy. Abnormal signaling leads to modified cytoskeletal structures and permanently altered (i.e., self-sustaining or epigenetic) transcription of target genes. A common theme is that genes whose transcription is elevated to the greatest extent by Ras often have in their promoters juxtaposed binding sites for two different transcription factors (particularly those in the Fos/Jun, CREB/ATF, NFkB, and Ets families) each of which is activated and such that together they synergize to augment transcription substantially. Some of these transcription factors can also act as oncogenes in certain cell types when appropriately modified and expressed. This unifying theme among many different cancers suggests that strategies to restore the balance among the signaling pathways or to suppress synergistic interactions between transcription factors may prove broadly useful in reversing the malignant phenotype.

Dilley, R. L. and R. A. Greenberg (2015). "ALternative Telomere Maintenance and Cancer." *Trends Cancer* 1(2): 145-156.

Activation of a telomere maintenance mechanism (TMM) is permissive for replicative

immortality and a hallmark of human cancer. While most cancers rely on reactivation of telomerase, a significant fraction utilizes the recombination dependent alternative lengthening of telomeres (ALT) pathway. ALT is enriched in tumors of mesenchymal origin, including those arising from bone, soft tissue, and the nervous system, and usually portends a poor prognosis. Recent insights into the mechanisms of ALT are uncovering novel avenues to exploit vulnerabilities and may facilitate clinical development of ALT detection assays and personalized treatment decisions based on TMM status. Treatments targeting ALT may hold promise for a broadly applicable therapeutic modality specific to mesenchymal lineage tumors, something that has thus far remained elusive.

Dlugosz, A. and A. Ciechanowicz (1998). "[Telomerase and gastrointestinal cancer]." *Pol Merkuri Lekarski* 5(26): 57-59.

Activation of telomerase and stabilisation of telomeres are considered to be essential for the immortality of cancer cells. Telomerase activity is present in almost all carcinomas. It indicates that the detection of telomerase activity in tissues using a telomeric repeat amplification protocol (TRAP) is useful for cancer diagnosis. This review will describe the current state of knowledge of telomerase as it relates to gastrointestinal malignancies focussing primarily on published measurements of this enzymes activity in benign and malignant neoplasms of stomach, colon, pancreas and liver. Telomerase seems to be promising diagnostic and prognostic marker of gastrointestinal tumors which can be useful especially in early detection and even screening. Continuing research will determine its potential value in the control of cancer.

Dogan, F. and C. Biray Avci (2018). "Correlation between telomerase and mTOR pathway in cancer stem cells." *Gene* 641: 235-239.

Cancer stem cells (CSCs), which are defined as a subset of tumor cells, are able to self-renew, proliferate, differentiate similar to normal stem cells. Therefore, targeting CSCs has been considered as a new approach in cancer therapy. The mammalian target of rapamycin (mTOR) is a receptor tyrosine kinase which plays an important role in regulating cell proliferation, differentiation, cell growth, self-renewal in CSCs. On the other hand, hTERT overactivation provides replicative feature and immortality to CSCs, so the stemness and replicative properties of CSCs depend on telomerase activity. Therefore hTERT/telomerase activity may become a universal biomarker for anticancer therapy and it is an attractive therapeutic target for CSCs. It is known

that mTOR regulates telomerase activity at the translational and post-translational level. Researchers show that mTOR inhibitor rapamycin reduces telomerase activity without changing hTERT mRNA activity. Correlation between mTOR and hTERT is important for survival and immortality of cancer cells. In addition, the PI3K/AKT/mTOR signaling pathway and hTERT up-regulation are related with cancer stemness features and drug resistance. mTOR inhibitor and TERT inhibitor combination may construct a novel strategy in cancer stem cells and it can make a double effect on telomerase enzyme. Consequently, inhibition of PI3K/AKT/mTOR signaling pathway components and hTERT activation may prohibit CSC self-renewal and surpass CSC-mediated resistance in order to develop new cancer therapeutics.

Dou, X., et al. (2020). "Evidence for immortality and autonomy in animal cancer models is often not provided, which causes confusion on key issues of cancer biology." *J Cancer* **11**(10): 2887-2920.

Modern research into carcinogenesis has undergone three phases. Surgeons and pathologists started the first phase roughly 250 years ago, establishing morphological traits of tumors for pathologic diagnosis, and setting immortality and autonomy as indispensable criteria for neoplasms. A century ago, medical doctors, biologists and chemists started to enhance "experimental cancer research" by establishing many animal models of chemical-induced carcinogenesis for studies of cellular mechanisms. In this second phase, the two-hit theory and stepwise carcinogenesis of "initiation-promotion" or "initiation-promotion-progression" were established, with an illustrious finding that outgrowths induced in animals depend on the inducers, and thus are not authentically neoplastic, until late stages. The last 40 years are the third incarnation, molecular biologists have gradually dominated the carcinogenesis research fraternity and have established numerous genetically-modified animal models of carcinogenesis. However, evidence has not been provided for immortality and autonomy of the lesions from most of these models. Probably, many lesions had already been collected from animals for analyses of molecular mechanisms of "cancer" before the lesions became autonomous. We herein review the monumental work of many predecessors to reinforce that evidence for immortality and autonomy is essential for confirming a neoplastic nature. We extrapolate that immortality and autonomy are established early during sporadic human carcinogenesis, unlike the late establishment in most animal models. It is imperative to resume many forerunners' work by determining the genetic

bases for initiation, promotion and progression, the genetic bases for immortality and autonomy, and which animal models are, in fact, good for identifying such genetic bases.

Duesberg, P., et al. (2005). "The chromosomal basis of cancer." *Cell Oncol* **27**(5-6): 293-318.

Conventional genetic theories have failed to explain why cancer (1) is not heritable and thus extremely rare in newborns, (2) is caused by non-mutagenic carcinogens, (3) develops only years to decades after initiation by carcinogens, (4) follows pre-neoplastic aneuploidy, (5) is aneuploid, (6) is chromosomally and phenotypically "unstable", (7) carries specific aneusomies, (8) generates much more complex phenotypes than conventional mutation such as multidrug resistance, (9) generates nonselective phenotypes such as metastasis (no benefit at native site) and "immortality" (not necessary for tumorigenesis), and (10) does not contain carcinogenic mutations. We propose, instead, that cancer is a chromosomal disease. Accordingly carcinogenesis is initiated by random aneuploidies, which are induced by carcinogens or spontaneously. Since aneuploidy unbalances 1000s of genes, it corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is therefore a steady source of chromosomal variations from which, in classical Darwinian terms, selection encourages the evolution and malignant progression of cancer cells. The rates of specific chromosomal variations can exceed conventional mutations by 4-11 orders of magnitude, depending on the degrees of aneuploidy. Based on their chromosomal constitution cancer cells are new cell "species" with specific aneusomies, but unstable karyotypes. The cancer-specific aneusomies generate complex, malignant phenotypes through the abnormal dosages of 1000s of genes, just as trisomy 21 generates Down syndrome. In sum, cancer is caused by chromosomal disorganization, which increases karyotypic entropy. Thus, cancer is a chromosomal rather than a genetic disease. The chromosomal theory explains (1) non-heritable cancer because aneuploidy is not heritable, (2) non-mutagenic carcinogens as aneuploidogens, (3) long neoplastic latencies by the low probability of evolving new species, (4) nonselective phenotypes via genes hitchhiking with selective chromosomes, and (5) immortality because, through their cellular heterogeneity, cancers survive negative mutations and cytotoxic drugs via resistant subspecies.

Duesberg, P., et al. (2006). "Aneuploidy and cancer: from correlation to causation." *Contrib Microbiol* **13**: 16-44.

Conventional genetic theories have failed to

explain why cancer (1) is not found in newborns and thus not heritable; (2) develops only years to decades after 'initiation' by carcinogens; (3) is caused by non-mutagenic carcinogens; (4) is chromosomally and phenotypically 'unstable'; (5) carries cancer-specific aneuploidies; (6) evolves polygenic phenotypes; (7) nonselective phenotypes such as multidrug resistance, metastasis or affinity for non-native sites and 'immortality' that is not necessary for tumorigenesis; (8) contains no carcinogenic mutations. We propose instead that cancer is a chromosomal disease: Accordingly, carcinogens initiate chromosomal evolutions via unspecific aneuploidies. By unbalancing thousands of genes aneuploidy corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is thus a steady source of karyotypic-phenotypic variations from which, in classical Darwinian terms, selection of cancer-specific aneuploidies encourages the evolution and subsequent malignant 'progressions' of cancer cells. The rates of these variations are proportional to the degrees of aneuploidy, and can exceed conventional mutation by 4-7 orders of magnitude. This makes cancer cells new cell 'species' with distinct, but unstable karyotypes, rather than mutant cells. The cancer-specific aneuploidies generate complex, malignant phenotypes, through the abnormal dosages of the thousands of genes, just as trisomy 21 generates Down syndrome. Thus cancer is a chromosomal rather than a genetic disease. The chromosomal theory explains (1) nonheritability of cancer, because aneuploidy is not heritable; (2) long 'neoplastic latencies' by the low probability of evolving competitive new species; (3) nonselective phenotypes via genes hitchhiking on selective chromosomes, and (4) 'immortality', because chromosomal variations neutralize negative mutations and adapt to inhibitory conditions much faster than conventional mutation. Based on this article a similar one, entitled 'The chromosomal basis of cancer', has since been published by us in *Cellular Oncology* 2005;27:293-318.

Duesberg, P. and D. Rasnick (2000). "Aneuploidy, the somatic mutation that makes cancer a species of its own." *Cell Motil Cytoskeleton* 47(2): 81-107.

The many complex phenotypes of cancer have all been attributed to "somatic mutation." These phenotypes include anaplasia, autonomous growth, metastasis, abnormal cell morphology, DNA indices ranging from 0.5 to over 2, clonal origin but unstable and non-clonal karyotypes and phenotypes, abnormal centrosome numbers, immortality in vitro and in transplantation, spontaneous progression of malignancy, as well as the exceedingly slow kinetics from carcinogen to carcinogenesis of many months to

decades. However, it has yet to be determined whether this mutation is aneuploidy, an abnormal number of chromosomes, or gene mutation. A century ago, Boveri proposed cancer is caused by aneuploidy, because it correlates with cancer and because it generates "pathological" phenotypes in sea urchins. But half a century later, when cancers were found to be non-clonal for aneuploidy, but clonal for somatic gene mutations, this hypothesis was abandoned. As a result aneuploidy is now generally viewed as a consequence, and mutated genes as a cause of cancer although, (1) many carcinogens do not mutate genes, (2) there is no functional proof that mutant genes cause cancer, and (3) mutation is fast but carcinogenesis is exceedingly slow. Intrigued by the enormous mutagenic potential of aneuploidy, we undertook biochemical and biological analyses of aneuploidy and gene mutation, which show that aneuploidy is probably the only mutation that can explain all aspects of carcinogenesis. On this basis we can now offer a coherent two-stage mechanism of carcinogenesis. In stage one, carcinogens cause aneuploidy, either by fragmenting chromosomes or by damaging the spindle apparatus. In stage two, ever new and eventually tumorigenic karyotypes evolve autocatalytically because aneuploidy destabilizes the karyotype, ie. causes genetic instability. Thus, cancer cells derive their unique and complex phenotypes from random chromosome number mutation, a process that is similar to regrouping assembly lines of a car factory and is analogous to speciation. The slow kinetics of carcinogenesis reflects the low probability of generating by random chromosome reassortments a karyotype that surpasses the viability of a normal cell, similar again to natural speciation. There is correlative and functional proof of principle: (1) solid cancers are aneuploid; (2) genotoxic and non-genotoxic carcinogens cause aneuploidy; (3) the biochemical phenotypes of cells are severely altered by aneuploidy affecting the dosage of thousands of genes, but are virtually un-altered by mutations of known hypothetical oncogenes and tumor suppressor genes; (4) aneuploidy immortalizes cells; (5) non-cancerous aneuploidy generates abnormal phenotypes in all species tested, e.g., Down syndrome; (6) the degrees of aneuploidies are proportional to the degrees of abnormalities in non-cancerous and cancerous cells; (7) polyploidy also varies biological phenotypes; (8) variation of the numbers of chromosomes is the basis of speciation. Thus, aneuploidy falls within the definition of speciation, and cancer is a species of its own. The aneuploidy hypothesis offers new prospects of cancer prevention and therapy.

Durant, S. T. (2012). "Telomerase-independent paths

to immortality in predictable cancer subtypes." *J Cancer* **3**: 67-82.

The vast majority of cancers commandeer the activity of telomerase - the remarkable enzyme responsible for prolonging cellular lifespan by maintaining the length of telomeres at the ends of chromosomes. Telomerase is only normally active in embryonic and highly proliferative somatic cells. Thus, targeting telomerase is an attractive anti-cancer therapeutic rationale currently under investigation in various phases of clinical development. However, previous reports suggest that an average of 10-15% of all cancers lose the functional activity of telomerase and most of these turn to an Alternative Lengthening of Telomeres pathway (ALT). ALT-positive tumours will therefore not respond to anti-telomerase therapies and there is a real possibility that such drugs would be toxic to normal telomerase-utilising cells and ultimately select for resistant cells that activate an ALT mechanism. ALT exploits certain DNA damage response (DDR) components to counteract telomere shortening and rapid trimming. ALT has been reported in many cancer subtypes including sarcoma, gastric carcinoma, central nervous system malignancies, subtypes of kidney (Wilm's Tumour) and bladder carcinoma, mesothelioma, malignant melanoma and germ cell testicular cancers to name but a few. A recent heroic study that analysed ALT in over six thousand tumour samples supports this historical spread, although only reporting an approximate 4% prevalence. This review highlights the various methods of ALT detection, unravels several molecular ALT models thought to promote telomere maintenance and elongation, spotlights the DDR components known to facilitate these and explores why certain tissues are more likely to subvert DDR away from its usually protective functions, resulting in a predictive pattern of prevalence in specific cancer subsets.

Eckburg, A., et al. (2020). "Oligonucleotides and microRNAs Targeting Telomerase Subunits in Cancer Therapy." *Cancers (Basel)* **12**(9).

Telomerase provides cancer cells with replicative immortality, and its overexpression serves as a near-universal marker of cancer. Anti-cancer therapeutics targeting telomerase have garnered interest as possible alternatives to chemotherapy and radiotherapy. Oligonucleotide-based therapies that inhibit telomerase through direct or indirect modulation of its subunits, human telomerase reverse transcriptase (hTERT) and human telomerase RNA gene (hTERC), are a unique and diverse subclass of telomerase inhibitors which hold clinical promise. MicroRNAs that play a role in the upregulation or downregulation of hTERT and respective progression

or attenuation of cancer development have been effectively targeted to reduce telomerase activity in various cancer types. Tumor suppressor miRNAs, such as miRNA-512-5p, miRNA-138, and miRNA-128, and oncogenic miRNAs, such as miRNA-19b, miRNA-346, and miRNA-21, have displayed preclinical promise as potential hTERT-based therapeutic targets. Antisense oligonucleotides like GRN163L and T-oligos have also been shown to uniquely target the telomerase subunits and have become popular in the design of novel cancer therapies. Finally, studies suggest that G-quadruplex stabilizers, such as Telomestatin, preserve telomeric oligonucleotide architecture, thus inhibiting hTERC binding to the telomere. This review aims to provide an adept understanding of the conceptual foundation and current state of therapeutics utilizing oligonucleotides to target the telomerase subunits, including the advantages and drawbacks of each of these approaches.

Edwardson, D. W., et al. (2019). "Chemotherapy and Inflammatory Cytokine Signalling in Cancer Cells and the Tumour Microenvironment." *Adv Exp Med Biol* **1152**: 173-215.

Cancer is the result of a cell's acquisition of a variety of biological capabilities or 'hallmarks' as outlined by Hanahan and Weinberg. These include sustained proliferative signalling, the ability to evade growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and the ability to invade other tissue and metastasize. More recently, the ability to escape immune destruction has been recognized as another important hallmark of tumours. It is suggested that genome instability and inflammation accelerates the acquisition of a variety of the above hallmarks. Inflammation, is a product of the body's response to tissue damage or pathogen invasion. It is required for tissue repair and host defense, but prolonged inflammation can often be the cause for disease. In a cancer patient, it is often unclear whether inflammation plays a protective or deleterious role in disease progression. Chemotherapy drugs can suppress tumour growth but also induce pathways in tumour cells that have been shown experimentally to support tumour progression or, in other cases, encourage an anti-tumour immune response. Thus, with the goal of better understanding the context under which each of these possible outcomes occurs, recent progress exploring chemotherapy-induced inflammatory cytokine production and the effects of cytokines on drug efficacy in the tumour microenvironment will be reviewed. The implications of chemotherapy on host and tumour cytokine pathways and their effect on the treatment of cancer

patients will also be discussed.

Efficace, F. and R. Marrone (2002). "Spiritual issues and quality of life assessment in cancer care." *Death Stud* **26**(9): 743-756.

Being diagnosed with cancer forces most human beings to face their own death. The comfortable sense of both invulnerability and immortality is shattered, making the patient thoroughly aware that life is finite and limited. Approaching death, cancer patients commonly embark on an inner journey involving a search for meaning as well as a reordering of priorities involving physical, psychological, social, and spiritual needs. Although interest in the role of spirituality, relating to both adjustment to cancer and the overall quality of life of cancer patients, has increased in recent years, most of the commonly used quality of life (QOL) instruments in oncology typically do not include spiritual issues. In this article, it is argued that assessing QOL effectively should involve all aspects of the personality, including mind, body, and spirit as well. This article also reviews recent studies, which have shown that spiritual well-being, although a many-sided and difficult construct to define, is closely related to the QOL of cancer patients. It is also suggested that further research is needed to understand how the new focus on spirituality can contribute to a more comprehensive assessment of patient's QOL in cancer care.

Efimova, E. V., et al. (2016). "Linking Cancer Metabolism to DNA Repair and Accelerated Senescence." *Mol Cancer Res* **14**(2): 173-184.

Conventional wisdom ascribes metabolic reprogramming in cancer to meeting increased demands for intermediates to support rapid proliferation. Prior models have proposed benefits toward cell survival, immortality, and stress resistance, although the recent discovery of oncometabolites has shifted attention to chromatin targets affecting gene expression. To explore further effects of cancer metabolism and epigenetic deregulation, DNA repair kinetics were examined in cells treated with metabolic intermediates, oncometabolites, and/or metabolic inhibitors by tracking resolution of double-strand breaks (DSB) in irradiated MCF7 breast cancer cells. Disrupting cancer metabolism revealed roles for both glycolysis and glutaminolysis in promoting DSB repair and preventing accelerated senescence after irradiation. Targeting pathways common to glycolysis and glutaminolysis uncovered opposing effects of the hexosamine biosynthetic pathway (HBP) and tricarboxylic acid (TCA) cycle. Treating cells with the HBP metabolite N-acetylglucosamine (GlcNAc)

or augmenting protein O-GlcNAcylation with small molecules or RNAi targeting O-GlcNAcase each enhanced DSB repair, while targeting O-GlcNAc transferase reversed GlcNAc's effects. Opposing the HBP, TCA metabolites including alpha-ketoglutarate blocked DSB resolution. Strikingly, DNA repair could be restored by the oncometabolite 2-hydroxyglutarate (2-HG). Targeting downstream effectors of histone methylation and demethylation implicated the PRC1/2 polycomb complexes as the ultimate targets for metabolic regulation, reflecting known roles for Polycomb group proteins in nonhomologous end-joining DSB repair. Our findings that epigenetic effects of cancer metabolic reprogramming may promote DNA repair provide a molecular mechanism by which deregulation of metabolism may not only support cell growth but also maintain cell immortality, drive therapeutic resistance, and promote genomic instability. **IMPLICATIONS:** By defining a pathway from deregulated metabolism to enhanced DNA damage response in cancer, these data provide a rationale for targeting downstream epigenetic effects of metabolic reprogramming to block cancer cell immortality and overcome resistance to genotoxic stress.

Eissenberg, J. C. (2013). "Telomeres, cancer & aging: live long & prosper?" *Mo Med* **110**(1): 11-16.

Like our clothes, our chromosomes fray at the edges with age. Some believe that if we could discover a molecular tailor to patch our age-abraded chromosome ends, we could become modern Methuselahs. Notably, cancer cells achieve immortality by protecting their chromosome ends. Drugs that selectively fray the ends of cancer cell chromosomes would be potent and general anti-cancer therapies. Here, I summarize data on the role of chromosome ends in cellular and organismal aging.

Elbadawi, M. and T. Efferth (2024). "In Vivo and Clinical Studies of Natural Products Targeting the Hallmarks of Cancer." *Handb Exp Pharmacol*.

Despite more than 200 approved anticancer agents, cancer remains a leading cause of death worldwide due to disease complexity, tumour heterogeneity, drug toxicity, and the emergence of drug resistance. Accordingly, the development of chemotherapeutic agents with higher efficacy, a better safety profile, and the capability of bypassing drug resistance would be a cornerstone in cancer therapy. Natural products have played a pivotal role in the field of drug discovery, especially for the pharmacotherapy of cancer, infectious, and chronic diseases. Owing to their distinctive structures and multiple mechanistic activities, natural products and their derivatives have been utilized for decades in

cancer treatment protocols. In this review, we delve into the potential of natural products as anticancer agents by targeting cancer's hallmarks, including sustained proliferative signalling, evading growth suppression, resisting apoptosis and cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. We highlight the molecular mechanisms of some natural products, in vivo studies, and promising clinical trials. This review emphasizes the significance of natural products in fighting cancer and the need for further studies to uncover their fully therapeutic potential.

Erenpreisa, J. and M. S. Cragg (2010). "MOS, aneuploidy and the ploidy cycle of cancer cells." *Oncogene* **29**(40): 5447-5451.

After DNA or spindle damage, p53-defective tumor cells undergo a complex cycle of reversible polyploidy. How this process occurs and more importantly, why, has recently become the focus of several research groups, prompting this review in which we discuss two related phenomena that accompany the reversible polyploidy of tumor cells: the induction of meiosis genes such as MOS and the decrease in genomic instability observed during the reversion from polyploidy to para-diploidy. The reversible polyploidy likely provides the means through which the balance between increased chromosome instability (CIN), driving genetic variation and decreased CIN, necessary for perpetuating these malignant clones, is maintained. These concepts are integrated with recent findings that many meiotic and self-renewal genes become activated during reversible polyploidy and lead us to the hypothesis that tumor cell immortality may be achieved through germline-like transmission.

Erenpreisa, J. and M. S. Cragg (2013). "Three steps to the immortality of cancer cells: senescence, polyploidy and self-renewal." *Cancer Cell Int* **13**(1): 92.

Metastatic cancer is rarely cured by current DNA damaging treatments, apparently due to the development of resistance. However, recent data indicates that tumour cells can elicit the opposing processes of senescence and stemness in response to these treatments, the biological significance and molecular regulation of which is currently poorly understood. Although cellular senescence is typically considered a terminal cell fate, it was recently shown to be reversible in a small population of polyploid cancer cells induced after DNA damage. Overcoming genotoxic insults is associated with reversible polyploidy, which itself is associated with the induction of a stemness phenotype, thereby providing

a framework linking these separate phenomena. In keeping with this suggestion, senescence and autophagy are clearly intimately involved in the emergence of self-renewal potential in the surviving cells that result from de-polyploidisation. Moreover, subsequent analysis indicates that senescence may paradoxically be actually required to rejuvenate cancer cells after genotoxic treatments. We propose that genotoxic resistance is thereby afforded through a programmed life-cycle-like process which intimately unites senescence, polyploidy and stemness.

Erenpreisa, J., et al. (2015). "The "virgin birth", polyploidy, and the origin of cancer." *Oncoscience* **2**(1): 3-14.

Recently, it has become clear that the complexity of cancer biology cannot fully be explained by somatic mutation and clonal selection. Meanwhile, data have accumulated on how cancer stem cells or stemoids bestow immortality on tumour cells and how reversible polyploidy is involved. Most recently, single polyploid tumour cells were shown capable of forming spheroids, releasing EMT-like descendents and inducing tumours in vivo. These data refocus attention on the centuries-old embryological theory of cancer. This review attempts to reconcile seemingly conflicting data by viewing cancer as a pre-programmed phylogenetic life-cycle-like process. This cycle is apparently initiated by a meiosis-like process and driven as an alternative to accelerated senescence at the DNA damage checkpoint, followed by an asexual syngamy event and endopolyploid-type embryonal cleavage to provide germ-cell-like (EMT) cells. This cycle is augmented by genotoxic treatments, explaining why chemotherapy is rarely curative and drives resistance. The logical outcome of this viewpoint is that alternative treatments may be more efficacious - either those that suppress the endopolyploidy-associated 'life cycle' or, those that cause reversion of embryonal malignant cells into benign counterparts. Targets for these opposing strategies are components of the same molecular pathways and interact with regulators of accelerated senescence.

Ertel, A., et al. (2012). "Is cancer a metabolic rebellion against host aging? In the quest for immortality, tumor cells try to save themselves by boosting mitochondrial metabolism." *Cell Cycle* **11**(2): 253-263.

Aging drives large systemic reductions in oxidative mitochondrial function, shifting the entire body metabolically towards aerobic glycolysis, a.k.a, the Warburg effect. Aging is also one of the most significant risk factors for the development of human

cancers, including breast tumors. How are these two findings connected? One simplistic idea is that cancer cells rebel against the aging process by increasing their capacity for oxidative mitochondrial metabolism (OXPHOS). Then, local and systemic aerobic glycolysis in the aging host would provide energy-rich mitochondrial fuels (such as L-lactate and ketones) to directly "fuel" tumor cell growth and metastasis. This would establish a type of parasite-host relationship or "two-compartment tumor metabolism", with glycolytic/oxidative metabolic-coupling. The cancer cells ("the seeds") would flourish in this nutrient-rich microenvironment ("the soil"), which has been fertilized by host aging. In this scenario, cancer cells are only trying to save themselves from the consequences of aging, by engineering a metabolic mutiny, through the amplification of mitochondrial metabolism. We discuss the recent findings of Drs. Ron DePinho (MD Anderson) and Craig Thompson (Sloan-Kettering) that are also consistent with this new hypothesis, linking cancer progression with metabolic aging. Using data mining and bioinformatics approaches, we also provide key evidence of a role for PGC1 α /NRF1 signaling in the pathogenesis of (1) two-compartment tumor metabolism, and (2) mitochondrial biogenesis in human breast cancer cells.

Fadri-Moskwick, M., et al. (2013). "Beyond Telomerase: Telomere Instability as a Novel Target for Cancer Therapy." *J Mol Genet Med* 7(4).

Telomeres are areas of heterochromatin composed of TTAGGG repeats located at the ends of linear chromosomes. They play a critical role in keeping genome stable and preventing premature aging diseases and the development of cancer. Characterizing mechanisms of telomere maintenance and understanding how their deregulation contributes to human diseases are therefore important for developing novel therapies. A key mechanism driving telomere maintenance and replicative immortality in cancer cells is telomere elongation by telomerase, and many emerging potential telomerase-based therapies have focused on targeting telomerase components. By contrast, recent studies on telomere maintenance mechanism suggest that disrupting telomere stability by interfering with alternative mechanisms of telomere synthesis or protection may also yield new strategies for the treatment of cancer. This review will focus on emerging regulators of telomere synthesis or maintenance, such as G4 telomeric DNA, the CST complex, the t-loop, and shelterins, and discuss their potential as targets for anti-cancer chemotherapeutic intervention in the future.

Fan, H. C., et al. (2021). "Telomeres and Cancer." *Life (Basel)* 11(12).

Telomeres cap the ends of eukaryotic chromosomes and are indispensable chromatin structures for genome protection and replication. Telomere length maintenance has been attributed to several functional modulators, including telomerase, the shelterin complex, and the CST complex, synergizing with DNA replication, repair, and the RNA metabolism pathway components. As dysfunctional telomere maintenance and telomerase activation are associated with several human diseases, including cancer, the molecular mechanisms behind telomere length regulation and protection need particular emphasis. Cancer cells exhibit telomerase activation, enabling replicative immortality. Telomerase reverse transcriptase (TERT) activation is involved in cancer development through diverse activities other than mediating telomere elongation. This review describes the telomere functions, the role of functional modulators, the implications in cancer development, and the future therapeutic opportunities.

Fang, D. C., et al. (1999). "Detection of telomerase activity in biopsy samples of colorectal cancer." *J Gastroenterol Hepatol* 14(4): 328-332.

BACKGROUND: Telomerase is a ribonucleoprotein that synthesizes telomeric DNA onto chromosomal ends. The expression of telomerase is thought to be required for cellular immortality and oncogenesis. **METHODS:** To investigate the role of telomerase in the pathogenesis of colorectal cancer, we analysed telomerase activity in biopsy samples of colorectal cancer and colonic adenomas. Using a polymerase chain reaction-based assay, we examined telomerase activity in 52 samples of colorectal cancer, 12 colonic adenomas and 30 normal colonic mucosa samples obtained by endoscopic biopsy. **RESULTS:** Telomerase activity was detectable in 88.5% (46/52) of colorectal carcinomas, in 50% (6/12) of colonic adenomas but not in normal colorectal mucosa. There was no correlation between telomerase activity and tumour location, type, size and differentiation ($P > 0.05$). **CONCLUSIONS:** It was concluded that telomerase activation plays a role in the evolution of colorectal cancer, and that measurement of telomerase activity in biopsied colorectal mucosa samples may provide information both as a diagnostic marker to detect small numbers of cancer cells, and as a screening method for patients at high risk for colorectal carcinoma.

Farias, T. G., et al. (2024). "Low-power red laser and blue LED modulate telomere maintenance and length in human breast cancer cells." *Lasers Med Sci* 39(1):

248.

Cancer cells have the ability to undergo an unlimited number of cell divisions, which gives them immortality. Thus, the cancer cell can extend the length of its telomeres, allowing these cells to divide unlimitedly and avoid entering the state of senescence or cellular apoptosis. One of the main effects of photobiomodulation (PBM) is the increase in the production of adenosine triphosphate (ATP) and free radicals, mainly reactive oxygen species (ROS). Existing data indicates that high levels of ROS can cause shortening and dysfunctional telomeres. Therefore, a better understanding of the effects induced by PBM on cancer cell telomere maintenance is needed. This work aimed to evaluate the effects of low-power red laser (658 nm) and blue LED (470 nm) on the TRF1 and TRF2 mRNA levels and telomere length in human breast cancer cells. MCF-7 and MDA-MB-231 cells were irradiated with a low-power red laser (69 J cm⁻², 0.77 W/cm⁻²) and blue LED (482 J cm⁻², 5.35 W/cm⁻²), alone or in combination, and the relative mRNA levels of the genes and telomere length were assessed by quantitative reverse transcription polymerase chain reaction. The results suggested that exposure to certain red laser and blue LED fluences decreased the TRF1 and TRF2 mRNA levels in both human breast cancer cells. Telomere length was increased in MCF-7 cells after exposure to red laser and blue LED. However, telomere length in MDA-MB-231 was shortened after exposure to red laser and blue LED at fluences evaluated. Our research suggests that photobiomodulation induced by red laser and low-power blue LED could alter telomere maintenance and length.

Fernandez-Garcia, I., et al. (2008). "Telomeres and telomerase in lung cancer." *J Thorac Oncol* **3**(10): 1085-1088.

Protected telomeres ensure normal chromosomal segregation during mitosis but at the same time can endow genetically abnormal cancer cells with immortality. Telomerase has a pivotal role in telomere protection, both in normal and cancer cells. Understanding the functional interplay between telomere shortening and telomerase expression in cancer cells is of critical importance to elucidating the precise mechanisms by which these cells are able to bypass telomere crisis and become immortal.

Feuerbach, L., et al. (2019). "TelomereHunter - in silico estimation of telomere content and composition from cancer genomes." *BMC Bioinformatics* **20**(1): 272.

BACKGROUND: Establishment of telomere maintenance mechanisms is a universal step

in tumor development to achieve replicative immortality. These processes leave molecular footprints in cancer genomes in the form of altered telomere content and aberrations in telomere composition. To retrieve these telomere characteristics from high-throughput sequencing data the available computational approaches need to be extended and optimized to fully exploit the information provided by large scale cancer genome data sets. **RESULTS:** We here present TelomereHunter, a software for the detailed characterization of telomere maintenance mechanism footprints in the genome. The tool is implemented for the analysis of large cancer genome cohorts and provides a variety of diagnostic diagrams as well as machine-readable output for subsequent analysis. A novel key feature is the extraction of singleton telomere variant repeats, which improves the identification and subclassification of the alternative lengthening of telomeres phenotype. We find that whole genome sequencing-derived telomere content estimates strongly correlate with telomere qPCR measurements ($r = 0.94$). For the first time, we determine the correlation of in silico telomere content quantification from whole genome sequencing and whole genome bisulfite sequencing data derived from the same tumor sample ($r = 0.78$). An analogous comparison of whole exome sequencing data and whole genome sequencing data measured slightly lower correlation ($r = 0.79$). However, this is considerably improved by normalization with matched controls ($r = 0.91$). **CONCLUSIONS:** TelomereHunter provides new functionality for the analysis of the footprints of telomere maintenance mechanisms in cancer genomes. Besides whole genome sequencing, whole exome sequencing and whole genome bisulfite sequencing are suited for in silico telomere content quantification, especially if matched control samples are available. The software runs under a GPL license and is available at <https://www.dkfz.de/en/applied-bioinformatics/telomerehunter/telomerehunter.html>.

Firna, M. B. and A. Brieger (2022). "CK2 and the Hallmarks of Cancer." *Biomedicine* **10**(8).

Cancer is a leading cause of death worldwide. Casein kinase 2 (CK2) is commonly dysregulated in cancer, impacting diverse molecular pathways. CK2 is a highly conserved serine/threonine kinase, constitutively active and ubiquitously expressed in eukaryotes. With over 500 known substrates and being estimated to be responsible for up to 10% of the human phosphoproteome, it is of significant importance. A broad spectrum of diverse types of cancer cells has been already shown to rely on disturbed CK2 levels for their survival. The

hallmarks of cancer provide a rationale for understanding cancer's common traits. They constitute the maintenance of proliferative signaling, evasion of growth suppressors, resisting cell death, enabling of replicative immortality, induction of angiogenesis, the activation of invasion and metastasis, as well as avoidance of immune destruction and dysregulation of cellular energetics. In this work, we have compiled evidence from the literature suggesting that CK2 modulates all hallmarks of cancer, thereby promoting oncogenesis and operating as a cancer driver by creating a cellular environment favorable to neoplasia.

Floor, S., et al. (2011). "Cancer cells in epithelial-to-mesenchymal transition and tumor-propagating-cancer stem cells: distinct, overlapping or same populations." *Oncogene* 30(46): 4609-4621.

Cell populations of solid cancers and their distant models, the cancer cell lines, have been categorized in sub-populations: cancer stem-tumor-propagating cells (CSC-TPC) versus derived cells, epithelial- versus mesenchymal-type cells, dormant versus actively proliferating cells and so on. CSC-TPC are minimally defined by their operational properties: immortality and the ability to regenerate in vivo or in vitro the whole panel of cancer cells. The epithelial-to-mesenchymal transition (EMT), mostly observed in vitro, generates mesenchymal-type from epithelial-type cells. The converse transition is mesenchymal-to-epithelial transition. In vitro work suggests that CSC-TPC and EMT cell phenotypes overlap. An analysis of the properties of these sub-populations, as studied in vitro, shows that indeed these two phenotypes may be linked to some extent. However, the in vivo counterpart of this relation in human tumors has barely been investigated. A model in which among the EMT cells released from the tumor only the most competent CSC-TPC will succeed to metastasize is proposed. It is suggested that in the Darwinian evolution of cancer cells, many phenotypes reflecting the expression of various programs, reversible to irreversible, exclusive, overlapping or linked coexist and compete with each other.

Franco, P. I. R., et al. (2023). "Revisiting the hallmarks of cancer: A new look at long noncoding RNAs in breast cancer." *Pathol Res Pract* 243: 154381.

Breast cancer is one of the leading causes of death in women worldwide. The increasing understanding of the molecular mechanisms underlying its heterogeneity favors a better understanding of tumor biology and consequently the development of better diagnostic and treatment

techniques. The advent of tumor genome sequencing techniques has highlighted more participants in the process, in addition to protein-coding genes. Thus, it is now known that long noncoding RNAs, previously described as transcriptional noise with no biological function, are intimately associated with tumor development. In breast cancer, they are abnormally expressed and closely associated with tumor progression, which makes them attractive diagnostic biomarkers and prognostic and specific therapeutic targets. Therefore, a thorough understanding of the regulatory mechanisms of long noncoding RNAs in breast cancer is essential for the search for new treatment strategies. In this review, we summarize the major long noncoding RNAs and their association with the cancer characteristics of the ability to sustain proliferative signaling, evasion of growth suppressors, replicative immortality, activation of invasion and metastasis, induction of angiogenesis, resistance to cell death, reprogramming of energy metabolism, genomic instability and sustained mutations, promotion of tumor inflammation, and evasion of the immune system. In addition, we report and suggest how they can be used as prognostic biomarkers and possible therapeutic targets.

Freitag, L., et al. (2000). "[Telomerase in lung cancer. Testing the activity of the "immortality enzyme" bronchial biopsies increases the diagnostic yield in cases of suspected peripheral bronchogenic carcinomas]." *Pneumologie* 54(11): 480-485.

The proliferative capability is time-limited in normal somatic cells by the shortening of their chromosomal ends, the telomeres (Hayflick limit). An important feature of malignant cells is their immortality. The probably most common mechanism of tumour cells to achieve unlimited replicability is the activation of the enzyme telomerase. The reverse transcriptase can compensate the loss of telomeres. Using a PCR-based TRAP assay we found telomerase activity in tumour biopsies, exsudates and bronchial washings in various thoracic malignancies. In 38 of 47 patients with suspected peripheral lung cancer eventually surgery or invasive procedures proved a malignancy. In fluoroscopically guided bronchial brushings from 25 of these 38 patients (66%) the TRAP assay revealed telomerase activity. There was a single false positive case (tuberculosis) and with a single exception, the simultaneously taken brushes of the contralateral lobes were all telomerase negative. In 23 patients (61%) tumour cells were found in the cytological examination. In 33 patients at least one marker was positive. Thus the combination of cytology and telomerase test in bronchial brush biopsies attained a diagnostic yield of 87%.

Frias, C., et al. (2009). "Telomere function in colorectal cancer." *World J Gastrointest Oncol* **1**(1): 3-11.

Colorectal cancer is the third most common form of cancer and the second leading cause of cancer-related death in the western world. Tumour cells acquire the hallmarks of cancer during the carcinogenic selection process. Cell immortality is one of the principal features acquired during this process which involves the stabilization of telomere length. It is achieved mainly, by telomerase activation. Thus, the discovery of telomeres and telomerase allowed an understanding of the mechanisms by which cells can become immortalized. Different studies have shown that tumour cells have shorter telomeres than nontumour cells and have detected telomerase activity in the majority of tumours. Survival studies have determined that telomere maintenance and telomerase activity are associated with poor prognosis. Taking into account all the results achieved by different groups, quantification and evaluation of telomerase activity and measurement of telomere length may be useful methods for additional biologic and prognostic staging of colorectal carcinoma.

Frost, F. G., et al. (2020). "Pan-cancer RNA-seq data stratifies tumours by some hallmarks of cancer." *J Cell Mol Med* **24**(1): 418-430.

Numerous genetic and epigenetic alterations cause functional changes in cell biology underlying cancer. These hallmark functional changes constitute potentially tissue-independent anticancer therapeutic targets. We hypothesized that RNA-Seq identifies gene expression changes that underly those hallmarks, and thereby defines relevant therapeutic targets. To test this hypothesis, we analysed the publicly available TCGA-TARGET-GTEX gene expression data set from the University of California Santa CruzToil recompute project using WGCNA to delineate co-correlated 'modules' from tumour gene expression profiles and functional enrichment of these modules to hierarchically cluster tumours. This stratified tumours according to T cell activation, NK-cell activation, complement cascade, ATM, Rb, angiogenic, MAPK, ECM receptor and histone modification signalling. These correspond to the cancer hallmarks of avoiding immune destruction, tumour-promoting inflammation, evading growth suppressors, inducing angiogenesis, sustained proliferative signalling, activating invasion and metastasis, and genome instability and mutation. This approach did not detect pathways corresponding to the cancer enabling replicative immortality, resisting cell death or deregulating cellular energetics

hallmarks. We conclude that RNA-Seq stratifies tumours along some, but not all, hallmarks of cancer and, therefore, could be used in conjunction with other analyses collectively to inform precision therapy.

Gao, J. and H. A. Pickett (2022). "Targeting telomeres: advances in telomere maintenance mechanism-specific cancer therapies." *Nat Rev Cancer* **22**(9): 515-532.

Cancer cells establish replicative immortality by activating a telomere-maintenance mechanism (TMM), be it telomerase or the alternative lengthening of telomeres (ALT) pathway. Targeting telomere maintenance represents an intriguing opportunity to treat the vast majority of all cancer types. Whilst telomerase inhibitors have historically been heralded as promising anticancer agents, the reality has been more challenging, and there are currently no therapeutic options for cancer types that use ALT despite their aggressive nature and poor prognosis. In this Review, we discuss the mechanistic differences between telomere maintenance by telomerase and ALT, the current methods used to detect each mechanism, the utility of these tests for clinical diagnosis, and recent developments in the therapeutic strategies being employed to target both telomerase and ALT. We present notable developments in repurposing established therapeutic agents and new avenues that are emerging to target cancer types according to which TMM they employ. These opportunities extend beyond inhibition of telomere maintenance, by finding and exploiting inherent weaknesses in the telomeres themselves to trigger rapid cellular effects that lead to cell death.

Garcia-Chico, C., et al. (2023). "Physical Exercise and the Hallmarks of Breast Cancer: A Narrative Review." *Cancers (Basel)* **15**(1).

Growing evidence suggests that, among the different molecular/cellular pathophysiological mechanisms associated with cancer, there are 14 hallmarks that play a major role, including: (i) sustaining proliferative signaling, (ii) evading growth suppressors, (iii) activating invasion and metastasis, (iv) enabling replicative immortality, (v) inducing angiogenesis, (vi) resisting cell death, (vii) reprogramming energy metabolism, (viii) evading immune destruction, (ix) genome instability and mutations, (x) tumor-promoting inflammation, (xi) unlocking phenotypic plasticity, (xii) nonmutational epigenetic reprogramming, (xiii) polymorphic microbiomes, and (xiv) senescent cells. These hallmarks are also associated with the development of breast cancer, which represents the most prevalent

tumor type in the world. The present narrative review aims to describe, for the first time, the effects of physical activity/exercise on these hallmarks. In summary, an active lifestyle, and particularly regular physical exercise, provides beneficial effects on all major hallmarks associated with breast cancer, and might therefore help to counteract the progression of the disease or its associated burden.

Garlan, R. W., et al. (2010). "Perceived benefits and psychosocial outcomes of a brief existential family intervention for cancer patients/survivors." Omega (Westport) **62**(3): 243-268.

This study assessed a range of benefits from participation in a brief existential intervention consisting of a semi-structured videotaped interview with cancer patients and their families designed to illuminate a life legacy for the family (the Life Tape Project [LTP]). Results indicated the majority reported intervention-specific benefits, especially in the areas of symbolic immortality (passing on personal values and philosophy), self-reflection and growth, and improved family cohesion and communication. Participants, particularly those who had perceived their cancer as a threat of death, serious injury, or threat to their physical integrity, and responded with intense fear or helplessness, also reported more general reductions in mood disturbance, improvements in aspects of well-being (including overall quality of life), satisfaction with the understanding they received, and enhanced cancer-related posttraumatic growth. In short, the LTP is a brief, inexpensive, existential intervention that can yield broad positive psychosocial changes for a majority of participants.

Gaspar, T. B., et al. (2018). "Telomere Maintenance Mechanisms in Cancer." Genes (Basel) **9**(5).

Tumour cells can adopt telomere maintenance mechanisms (TMMs) to avoid telomere shortening, an inevitable process due to successive cell divisions. In most tumour cells, telomere length (TL) is maintained by reactivation of telomerase, while a small part acquires immortality through the telomerase-independent alternative lengthening of telomeres (ALT) mechanism. In the last years, a great amount of data was generated, and different TMMs were reported and explained in detail, benefiting from genome-scale studies of major importance. In this review, we address seven different TMMs in tumour cells: mutations of the TERT promoter (TERTp), amplification of the genes TERT and TERC, polymorphic variants of the TERT gene and of its promoter, rearrangements of the TERT gene, epigenetic changes, ALT, and non-defined TMM (NDTMM). We gathered information from over fifty

thousand patients reported in 288 papers in the last years. This wide data collection enabled us to portray, by organ/system and histotypes, the prevalence of TERTp mutations, TERT and TERC amplifications, and ALT in human tumours. Based on this information, we discuss the putative future clinical impact of the aforementioned mechanisms on the malignant transformation process in different setups, and provide insights for screening, prognosis, and patient management stratification.

Gazzaniga, P., et al. (2007). "Gemcitabine-induced apoptosis in 5637 cell line: an in-vitro model for high-risk superficial bladder cancer." Anticancer Drugs **18**(2): 179-185.

Recent data suggest that new treatment options for superficial bladder cancer are necessary, owing to the high recurrence rate after conventional treatment, especially in T1G3 and Bacillus Calmette-Guerin-refractory patients. Phase I and II studies have demonstrated that gemcitabine may represent a candidate for intravesical therapy in superficial bladder cancer. Despite clinical trials, the in-vitro cytotoxic and proapoptotic effects of gemcitabine have been poorly investigated. In the present study, we investigated how gemcitabine affects apoptosis in bladder cancer cell line 5637, which has the same molecular features of high-risk superficial bladder cancer. Apoptosis was evaluated by DNA fragmentation, flow cytometry and caspase activation. bcl-2, bcl-X, bax, survivin and fas gene expression were also evaluated by reverse-transcriptase polymerase chain reaction. Nuclear factor-kappa B activation was assessed by immunofluorescence. Gemcitabine induced apoptosis in 5637 cells in a time-dependent manner, with activation of caspase-3, -8 and -9. Expression of bcl-2, bax, survivin and bcl-X was not affected by treatment, whereas fas strongly increased after 24 h of treatment. After treatment, we failed to find any nuclear localization of nuclear factor-kappa B. As gemcitabine-induced apoptosis involves fas upregulation, these results may encourage the investigation of intravesical gemcitabine in fas-negative bladder tumors. Furthermore, as nuclear factor-kappa B activation by cisplatin, doxorubicin and adriamycin may result in enhanced proliferation, migration, immortality and inhibition of apoptosis, the observation that gemcitabine does not activate nuclear factor-kappa B may have implications in intravesical therapy of high-risk superficial bladder cancer.

Gentile, S. (2016). "hERG1 potassium channel in cancer cells: a tool to reprogram immortality." Eur Biophys J **45**(7): 649-655.

It has been well established that changes in

ion fluxes across cellular membranes as a function of time is fundamental in maintaining cellular homeostasis of every living cell. Consequently, dysregulation of ion channels activity is a critical event in pathological conditions of several tissues, including cancer. Nevertheless, the role of ion channels in cancer biology is still not well understood and very little is known about the possible therapeutic opportunities offered by the use of the vast collection of drugs that target ion channels. In this review, we focus on the recent advances in understanding the role of the voltage-gated hERG1 potassium channel in cancer and on the effects of pharmacologic manipulation of the hERG1 in cancer cells aiming to provide insights into the biochemical signaling and cellular processes that are altered by using these drugs.

Ghosh, P. K., et al. (2024). "Telomerase: a nexus between cancer nanotherapy and circadian rhythm." *Biomater Sci* **12**(9): 2259-2281.

Cancer represents a complex disease category defined by the unregulated proliferation and dissemination of anomalous cells within the human body. According to the GLOBOCAN 2020 report, the year 2020 witnessed the diagnosis of approximately 19.3 million new cases of cancer and 10.0 million individuals succumbed to the disease. A typical cell eventually becomes cancerous because of a long-term buildup of genetic instability and replicative immortality. Telomerase is a crucial regulator of cancer progression as it induces replicative immortality. In cancer cells, telomerase inhibits apoptosis by elongating the length of the telomeric region, which usually protects the genome from shortening. Many nanoparticles are documented as being available for detecting the presence of telomerase, and many were used as delivery systems to transport drugs. Furthermore, telomere homeostasis is regulated by the circadian time-keeping machinery, leading to 24-hour rhythms in telomerase activity and TERT mRNA expression in mammals. This review provides a comprehensive discussion of various kinds of nanoparticles used in telomerase detection, inhibition, and multiple drug-related pathways, as well as enlightens an imperative association between circadian rhythm and telomerase activity from the perspective of nanoparticle-based anticancer therapeutics.

Gimla, M. and A. Herman-Antosiewicz (2024). "Multifaceted Properties of Usnic Acid in Disrupting Cancer Hallmarks." *Biomedicines* **12**(10).

Cancer, a complex group of diseases marked by uncontrolled cell growth and invasive behavior, is characterized by distinct hallmarks acquired during

tumor development. These hallmarks, first proposed by Douglas Hanahan and Robert Weinberg in 2000, provide a framework for understanding cancer's complexity. Targeting them is a key strategy in cancer therapy. It includes inhibiting abnormal signaling, reactivating growth suppressors, preventing invasion and metastasis, inhibiting angiogenesis, limiting replicative immortality, modulating the immune system, inducing apoptosis, addressing genome instability and regulating cellular energetics. Usnic acid (UA) is a natural compound found in lichens that has been explored as a cytotoxic agent against cancer cells of different origins. Although the exact mechanisms remain incompletely understood, UA presents a promising compound for therapeutic intervention. Understanding its impact on cancer hallmarks provides valuable insights into the potential of UA in developing targeted and multifaceted cancer therapies. This article explores UA activity in the context of disrupting hallmarks in cancer cells of different origins based on recent articles that emphasize the molecular mechanisms of this activity.

Girotti, M. R., et al. (2020). "Sweetening the hallmarks of cancer: Galectins as multifunctional mediators of tumor progression." *J Exp Med* **217**(2).

Hanahan and Weinberg have proposed 10 organizing principles that enable growth and metastatic dissemination of cancer cells. These distinctive and complementary capabilities, defined as the "hallmarks of cancer," include the ability of tumor cells and their microenvironment to sustain proliferative signaling, evade growth suppressors, resist cell death, promote replicative immortality, induce angiogenesis, support invasion and metastasis, reprogram energy metabolism, induce genomic instability and inflammation, and trigger evasion of immune responses. These common features are hierarchically regulated through different mechanisms, including those involving glycosylation-dependent programs that influence the biological and clinical impact of each hallmark. Galectins, an evolutionarily conserved family of glycan-binding proteins, have broad influence in tumor progression by rewiring intracellular and extracellular circuits either in cancer or stromal cells, including immune cells, endothelial cells, and fibroblasts. In this review, we dissect the role of galectins in shaping cellular circuitries governing each hallmark of tumors, illustrating relevant examples and highlighting novel opportunities for treating human cancer.

Goding, C. R., et al. (2014). "Cancer: pathological nuclear reprogramming?" *Nat Rev Cancer* **14**(8):

568-573.

The ability of stem cells to self-renew and generate different lineages during development and organogenesis is a fundamental, tightly controlled, and generally unidirectional process, whereas the 'immortality' of cancer cells could be regarded as pathological self-renewal. The molecular mechanisms that underpin the generation of induced pluripotent stem cells are remarkably similar to those that are deregulated in cancer - so much so that aberrant reprogramming is tumorigenic. The similarities also suggest that mutations in genes implicated in DNA methylation dynamics might represent a hallmark of cancers with a stem cell origin, and they highlight an alternative view of cancer that may be of clinical benefit.

Gomez, D. L., et al. (2016). "Telomerase as a Cancer Target. Development of New Molecules." *Curr Top Med Chem* **16**(22): 2432-2440.

Telomeres are the terminal part of the chromosome containing a long repetitive and noncoding sequence that has as function protecting the chromosomes. In normal cells, telomeres lost part of such repetitive sequence in each mitosis, until telomeres reach a critical point, triggering at that time senescence and cell death. However, in most of tumor cells in each cell division a part of the telomere is lost, however the appearance of an enzyme called telomerase synthetize the segment that just has been lost, therefore conferring to tumor cells the immortality hallmark. Telomerase is significantly overexpressed in 80-95% of all malignant tumors, being present at low levels in few normal cells, mostly stem cells. Due to these characteristics, telomerase has become an attractive target for new and more effective anticancer agents. The capability of inhibiting telomerase in tumor cells should lead to telomere shortening, senescence and apoptosis. In this work, we analyze the different strategies for telomerase inhibition, either in development, preclinical or clinical stages taking into account their strong points and their caveats. We covered strategies such as nucleosides analogs, oligonucleotides, small molecule inhibitors, G-quadruplex stabilizers, immunotherapy, gene therapy, molecules that affect the telomere/ telomerase associated proteins, agents from microbial sources, among others, providing a balanced evaluation of the status of the inhibitors of this powerful target together with an analysis of the challenges ahead.

Gonzalez-Moles, M. A., et al. (2022). "Prognostic and Clinicopathological Significance of Telomerase Reverse Transcriptase Upregulation in Oral Cancer: A Systematic Review and Meta-Analysis." *Cancers*

(Basel) **14**(15).

The aim of this systematic review and meta-analysis was to evaluate the current evidence on the prognostic and clinicopathological significance value of telomerase reverse transcriptase (TERT) upregulation in patients with oral squamous cell carcinoma (OSCC). PubMed, Embase, Web of Science, and Scopus were searched for studies published before April 2022, not restricted by date or publication language. The methodological quality of primary-level studies was critically assessed using the Quality in Prognosis Studies (QUIPS) tool. We carried out meta-analyses, explored heterogeneity and its sources, and performed subgroup, meta-regression, sensitivity, and small-study effects analyses. Twenty-one studies (1698 patients) met inclusion criteria. TERT protein overexpression was significantly associated with worse overall survival (hazard ratio [HR] = 3.01, 95% CI = 1.70-5.35, $p < 0.001$), disease-free survival (HR = 4.03, 95% CI = 1.80-9.05, $p = 0.001$), and higher histological grade OSCC (odds ratio [OR] = 3.20, 95% CI = 1.83-5.62, $p < 0.001$). These large effect sizes were consistently obtained by homogeneous subgroups ($p > 0.10$, $I^2 = 0.0$, respectively), which reflects a high quality of evidence. On the other hand, TERT gene mutations obtained constantly nonsignificant null effect sizes for all outcomes investigated, evidencing no prognostic or clinicopathological value. In conclusion, our findings indicate that TERT upregulation is a prognostic indicator of poor survival in oral cancer. Our findings support the immunohistochemical assessment of TERT overexpression, which could probably be incorporated into the prognostic evaluation of OSCC.

Groza, M., et al. (2020). "Recent advancements in the study of breast cancer exosomes as mediators of intratumoral communication." *J Cell Physiol* **235**(2): 691-705.

Breast cancer is a heterogeneous disease, with a morbidity rate of 27.8% and a mortality rate of 15% among women population worldwide. Understanding how this cancer develops and the mechanisms behind tumor progression and chemoresistance is of utmost importance. Exosomes mediate communication in a population of heterogeneous tumoral cells. They have a cargo composed of oncogenes and oncomiRs which change the transcriptomic scenario of their targeted cells and activate numerous tumor-promoting signaling pathways. Exosomes secreted by breast cancer cells lead to enhanced cell proliferation, replicative immortality, angiogenesis, invasion, migration, and chemoresistance. Studying exosomes from this perspective offers more in depth understanding of

breast malignancy and may aid in the future development of early diagnostic, prognostic and therapeutic options. We present the latest findings in this area and offer practical solutions which may further stimulate the much-needed research of exosome in breast cancer.

Gruenbacher, G. and M. Thurnher (2015). "Mevalonate metabolism in cancer." *Cancer Lett* **356**(2 Pt A): 192-196.

Cancer cells are characterized by sustained proliferative signaling, insensitivity to growth suppressors and resistance to apoptosis as well as by replicative immortality, the capacity to induce angiogenesis and to perform invasive growth. Additional hallmarks of cancer cells include the reprogramming of energy metabolism as well as the ability to evade immune surveillance. The current review focuses on the metabolic reprogramming of cancer cells and on the immune system's capacity to detect such changes in cancer cell metabolism. Specifically, we focus on mevalonate metabolism, which is a target for drug and immune based cancer treatment.

Guerra, R. M., et al. (2018). "Precision Targeting of BFL-1/A1 and an ATM Co-dependency in Human Cancer." *Cell Rep* **24**(13): 3393-3403 e3395.

Cancer cells overexpress a diversity of anti-apoptotic BCL-2 family proteins, such as BCL-2, MCL-1, and BFL-1/A1, to enforce cellular immortality. Thus, intensive drug development efforts have focused on targeting this class of oncogenic proteins to overcome treatment resistance. Whereas a selective BCL-2 inhibitor has been FDA approved and several small molecule inhibitors of MCL-1 have recently entered phase I clinical testing, BFL-1/A1 remains undrugged. Here, we developed a series of stapled peptide design principles to engineer a functionally selective and cell-permeable BFL-1/A1 inhibitor that is specifically cytotoxic to BFL-1/A1-dependent human cancer cells. Because cancers harbor a diversity of resistance mechanisms and typically require multi-agent treatment, we further investigated BFL-1/A1 co-dependencies by mining a genome-scale CRISPR-Cas9 screen. We identified ataxia-telangiectasia-mutated (ATM) kinase as a BFL-1/A1 co-dependency in acute myeloid leukemia (AML), which informed the validation of BFL-1/A1 and ATM inhibitor co-treatment as a synergistic approach to subverting apoptotic resistance in cancer.

Guo, Y., et al. (2020). "Bisphenol A and polychlorinated biphenyls enhance the cancer stem cell properties of human ovarian cancer cells by activating the WNT signaling pathway."

Chemosphere **246**: 125775.

Cancer stem cells (CSCs) are a very small subpopulation that have stem-cell qualities, such as exhibiting self-renewal, immortality, and pluripotency, and the capability to differentiate into different tumor cell subtypes. CSCs contribute to tumor onset, expansion, metastasis, resistance and recurrence. Meanwhile, organic pollutants, including nonpersistent pollutants, such as bisphenol A (BPA), and persistent pollutants, such as polychlorinated biphenyls (PCBs), are toxic chemicals that can be readily ingested via dietary exposure and other exposure routes and can accumulate through the food chain. Many organic pollutants increase the risk of ovarian cancer depending on their estrogenic effects. Nonetheless, most previous studies have focused on the toxic effects of these pollutants on the proliferation, metastasis and development of ovarian cancer cells. However, little research has investigated the adverse effect of these pollutants on ovarian cancer stem cells. The current study found that BPA, PCB126 and PCB153 greatly enhanced the formation of cancer stem-like cell spheres of OVCAR-3 cells (human ovarian cancer cells) under low-dose exposure. In parallel, the CD44(high)CD24(low) cell subpopulation was increased in treated cells relative to untreated cells. Elevated expression of cancer stem cell markers, including ALDH1A1, CD133, SOX2, NANOG and OCT4, was demonstrated in treated cells compared to untreated cells. In summary, these data demonstrate that the oncogenic effects of pollutants can be evaluated according to changes in cancer stem cell properties.

Guterres, A. N. and J. Villanueva (2020). "Targeting telomerase for cancer therapy." *Oncogene* **39**(36): 5811-5824.

Telomere maintenance via telomerase reactivation is a nearly universal hallmark of cancer cells which enables replicative immortality. In contrast, telomerase activity is silenced in most adult somatic cells. Thus, telomerase represents an attractive target for highly selective cancer therapeutics. However, development of telomerase inhibitors has been challenging and thus far there are no clinically approved strategies exploiting this cancer target. The discovery of prevalent mutations in the TERT promoter region in many cancers and recent advances in telomerase biology has led to a renewed interest in targeting this enzyme. Here we discuss recent efforts targeting telomerase, including immunotherapies and direct telomerase inhibitors, as well as emerging approaches such as targeting TERT gene expression driven by TERT promoter mutations. We also address some of the challenges to telomerase-directed therapies including potential

therapeutic resistance and considerations for future therapeutic applications and translation into the clinical setting. Although much work remains to be done, effective strategies targeting telomerase will have a transformative impact for cancer therapy and the prospect of clinically effective drugs is boosted by recent advances in structural models of human telomerase.

Hanahan, D. and R. A. Weinberg (2011). "Hallmarks of cancer: the next generation." *Cell* **144**(5): 646-674.

The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumors. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Underlying these hallmarks are genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions. Conceptual progress in the last decade has added two emerging hallmarks of potential generality to this list-reprogramming of energy metabolism and evading immune destruction. In addition to cancer cells, tumors exhibit another dimension of complexity: they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the "tumor microenvironment." Recognition of the widespread applicability of these concepts will increasingly affect the development of new means to treat human cancer.

Hannen, R. and J. W. Bartsch (2018). "Essential roles of telomerase reverse transcriptase hTERT in cancer stemness and metastasis." *FEBS Lett* **592**(12): 2023-2031.

Maintenance of chromosomal telomere length is a hallmark of cancer cells and a prerequisite for stemness. In 85-90% of all human cancers, telomere length maintenance is achieved by reactivation of telomerase, whereas in the remaining 10-15% cancers, alternative lengthening of telomeres (ALT) is observed. Reactivation of telomerase occurs by various mechanisms, one of which is accumulation of point mutations in the promoter region of the gene encoding the protein subunit hTERT. There are numerous studies linking either hTERT overexpression or the presence of hTERT mutations to an aggressive phenotype of several human cancers. Recent findings demonstrate that hTERT expression is not only associated with replicative immortality, but also with cancer cell

motility and stem cell phenotype. However, the mechanisms by which hTERT affects cancer cell migration, invasion, and distant metastasis on the one hand, and stemness and resistance on the other hand, are still poorly understood. Within this review, we aim to provide an overview on the functional involvement of hTERT in these cellular processes, focusing on metastasis formation and maintenance of stemness in different human cancers.

Hansen, J. P., et al. (2021). "Bacteria-Cancer Interface: Awaiting the Perfect Storm." *Pathogens* **10**(10).

Epidemiological evidence reveal a very close association of malignancies with chronic inflammation as a result of persistent bacterial infection. Recently, more studies have provided experimental evidence for an etiological role of bacterial factors disposing infected tissue towards carcinoma. When healthy cells accumulate genomic insults resulting in DNA damage, they may sustain proliferative signalling, resist apoptotic signals, evade growth suppressors, enable replicative immortality, and induce angiogenesis, thus boosting active invasion and metastasis. Moreover, these cells must be able to deregulate cellular energetics and have the ability to evade immune destruction. How bacterial infection leads to mutations and enriches a tumour-promoting inflammatory response or micro-environment is still not clear. In this review we showcase well-studied bacteria and their virulence factors that are tightly associated with carcinoma and the various mechanisms and pathways that could have carcinogenic properties.

Harley, C. B. and N. W. Kim (1996). "Telomerase and cancer." *Important Adv Oncol*: 57-67.

The data reviewed here suggest that telomere dynamics and telomerase expression are fundamentally involved in cellular aging and cancer. Of particular importance is the stabilization of telomeres by activation of telomerase and the association of this process with cell immortality and human malignancies. Thus, we believe that cell immortalization is required for long-term growth of the vast majority of malignant or metastatic tumors and that advances in telomere biology and telomerase inhibition will improve the way cancers are diagnosed and treated. We look forward to the clinical evaluation of these bold predictions.

Hasina, R. and M. W. Lingen (2001). "Angiogenesis in oral cancer." *J Dent Educ* **65**(11): 1282-1290.

Head and neck squamous cell carcinoma (HNSCC) is an aggressive malignancy that develops after years of chronic exposure to alcohol and

tobacco products. Exposure to these agents results in alterations of genes that are important in the regulation of various cellular functions. This loss of regulation allows the tumor cells to survive and grow in an unchecked manner by allowing the cells to perform functions that contribute to the growth of the tumor. Some of these important changes include the acquisition of immortality and the ability to invade tissue and/or metastasize to other sites, as well as acquiring the ability to induce angiogenesis. Angiogenesis, the growth of new blood vessels from pre-existing ones, is a complex phenomenon that is absolutely required for the continued growth and survival of solid neoplasms. Without new blood vessels to provide nutrients and remove waste, tumors would be unable to grow larger than 2-3 mm in diameter. Therefore, one could envision its potential role in both the treatment and prevention of malignancies such as HNSCC. The concept of chemoprevention is extremely important in HNSCC since patients often develop multiple independent lesions throughout the mucosa of the upper aerodigestive tract. Therefore, the comprehensive treatment of this disease must address not only the initial primary neoplasm, but also prevent the progression of the premalignant lesions lurking throughout the rest of the mucosal surfaces. This review will outline the basic changes that occur in tumor cells that result in the switch to angiogenic phenotype. In addition, it will discuss the present status of using antiangiogenic agents in the treatment of cancer. Finally, this paper will present a rationale for the use of multiple antiangiogenic agents as a means of developing new chemotherapeutic and chemopreventive protocols that may result in reduced patient toxicity while maintaining similar clinical efficacies.

Hayflick, L. (1999). "[A brief overview of the discovery of cell mortality and immortality and of its influence on concepts about aging and cancer]." *Pathol Biol (Paris)* **47**(10): 1094-1104.

After having accomplished the miraculous performance that led us from conception to birth, then to sexual maturity and adulthood, natural selection failed to develop a more elementary mechanism capable of simply maintaining the results of this process forever. This failure is aging. Because few animals age in the wild, evolution could not give an advantage to animals with modifications due to aging. Natural selection benefits those animals that have the highest likelihood of effectively perpetuating their species because their vital systems have the larger reserve capacity they need to resist and survive predators, disease, injury, and extreme environmental conditions. Natural selection decreases

after sexual maturity has been reached because at that stage the species would not derive additional advantages from individuals with larger physiological reserves. A species increases its likelihood of survival by investing its resources and energy into increasing its opportunities for fruitful reproduction rather than into prolonging its postreproductive life span. Most animals are mortal and undergo aging because investment of resources into keeping the body eternally youthful does not promote species survival as much as their investment into strategies that make reproduction more successful.

He, S., et al. (2023). "The unfolding story of dying tumor cells during cancer treatment." *Front Immunol* **14**: 1073561.

Generally, the demise of cancer cells in different ways enables the body to clear these harmful cells. However, cancer cells obtain unlimited replication and immortality from successful circumvention of cell death via various mechanisms. Some evidence suggests that treatment-induced dying tumor cells even promote cancer progression. Notably, therapeutic interventions to harness the immune system against tumor cells have shown complicated influences in clinics. Herein, there is an urgent need to clarify the underlying mechanisms that influence the outcome and regulation of the immune system during cancer treatment. In this review, we provide an account on the cell death modes and the relationship between dying tumor cells with tumor immune microenvironment during cancer treatment, focusing on immunotherapy, from mechanistic standpoint to emerging limitations and future directions.

Hegde, M. V., et al. (2013). "What is a cancer cell? Why does it metastasize?" *Asian Pac J Cancer Prev* **14**(6): 3987-3989.

This is a commentary on what a cancer cell is and why cancer cells metastasize. Normal cell get transformed to a cancer cell, with excessive production of free radicals that mutate the DNA of a normal cell. The immortality and malignant stage of transformed cell is maintained by higher GSH levels. With the faster rate of proliferation, when the cancer cell finds the place of origin is not conducive to its further growth, cancer cell chooses to take the metastatic course. We argue that if we can stop the exit of cancer cell from place of origin, cancer spread can be stopped or even cured.

Heidenreich, B., et al. (2014). "TERT promoter mutations in cancer development." *Curr Opin Genet Dev* **24**: 30-37.

Human telomerase reverse transcriptase

(TERT) encodes a rate-limiting catalytic subunit of telomerase that maintains genomic integrity. TERT expression is mostly repressed in somatic cells with exception of proliferative cells in self-renewing tissues and cancer. Immortality associated with cancer cells has been attributed to telomerase over-expression. The precise mechanism behind the TERT activation in cancers has mostly remained unknown. The newly described germline and recurrent somatic mutations in melanoma and other cancers in the TERT promoter that create de novo E-twenty six/ternary complex factors (Ets/TCF) binding sites, provide an insight into the possible cause of tumor-specific increased TERT expression. In this review we discuss the discovery and possible implications of the TERT promoter mutations in melanoma and other cancers.

Helder, M. N., et al. (1999). "Telomerase targeting in cancer treatment: new developments." *Drug Resist Updat* 2(2): 104-115.

Telomerase, a ribonucleoprotein expressed in 85% of advanced cancers but not in most somatic cells, compensates for telomeric DNA erosion and as such stabilizes cell immortality. Telomerase inhibition might restore mortality in tumor cells. Recent progress is illustrated in studies on telomerase and telomere targeting with differentiation induction, reverse transcriptase inhibitors, promoter down regulation, antisense inhibition, and blockage of telomere/telomerase interactions. Also, new developments are described indicating that anti-telomerase treatment can induce apoptosis in tumor cells and can chemosensitize drug-resistant cell lines. Implications of these findings for anti-telomerase-based therapeutic applications, in particular in combination therapies, are discussed. Copyright 1999 Harcourt Publishers Ltd.

Helder, M. N., et al. (2002). "Telomerase and telomeres: from basic biology to cancer treatment." *Cancer Invest* 20(1): 82-101.

The limited capacity to divide is one of the major differences between normal somatic cells and cancerous cells. This 'finite life span' of somatic cells is closely linked to loss of telomeric DNA at telomeres, the 'chromosome caps' consisting of repeated (7TAGGG) sequences. In more than 85% of advanced cancers, this telomeric attrition is compensated by telomerase, 'the immortality enzyme', implying that telomerase inhibition may restore mortality in tumor cells. This review discusses the progress in research on the structure and function of telomeres and the telomerase holoenzyme. In addition, new developments in telomere/telomerase targeting compounds such as antisense

oligonucleotides and G-quadruplex stabilizing substances, but also new telomerase expression-related strategies such as telomerase promoter-driven suicide gene therapy and telomerase immunotherapy will be presented. It will be discussed how these data can be implemented in telomerase-directed therapies.

Hiyama, E. and K. Hiyama (2004). "Telomerase detection in the diagnosis and prognosis of cancer." *Cytotechnology* 45(1-2): 61-74.

Telomerase, a critical enzyme responsible for cellular immortality, is usually repressed in somatic cells except for lymphocytes and self-renewal cells, but is activated in approximately 85% of human cancer tissues. The human telomerase reverse transcriptase (hTERT) is the catalytic component of human telomerase. In cancers in which telomerase activation occurs at the early stages of the disease, telomerase activity and hTERT expression are useful markers for the detection of cancer cells. In other cancers in which telomerase becomes upregulated upon tumor progression, they are useful as prognostic indicators. However, careful attention should be paid to false-negative results caused by the instability of telomerase and of the hTERT mRNA and the presence of PCR inhibitors, as well as to false-positive results caused by the presence of alternatively spliced hTERT mRNA and normal cells with telomerase activity. Recently, methods for the in situ detection of the hTERT mRNA and protein have been developed. These methods should facilitate the unequivocal detection of cancer cells, even in tissues containing a background of normal telomerase-positive cells.

Hiyama, E., et al. (1995). "Telomerase activity in gastric cancer." *Cancer Res* 55(15): 3258-3262.

Although many genetic alterations have been reported in gastric cancer, it is not known whether all gastric tumors are capable of indefinite proliferative potential, e.g., immortality. The expression of telomerase and stabilization of telomeres are concomitant with the attainment of immortality in tumor cells; thus, the measurement of telomerase activity in clinically obtained tumor samples may provide important information useful both as a diagnostic marker to detect immortal cancer cells in clinical materials and as a prognostic indicator of patient outcome. Telomerase activity was analyzed in 66 primary gastric cancers with the use of a PCR-based assay. The majority of tumors (85%) displayed telomerase activity, but telomerase was undetectable in 10 tumors (15%), 8 of which were early stage tumors. Most of the tumors with telomerase activity were large and of advanced stages, including metastases. Survival rate of patients of

tumors with detectable telomerase activity was significantly shorter than that of those without telomerase activity. Alterations of telomere length (reduced/elongated terminal restriction fragments) were detected in 14 of 66 (21%) gastric cancers, and all 14 had telomerase activity. Cellular DNA contents revealed that all 22 aneuploid tumors had detectable telomerase activity. The present results indicate that telomerase activation may be required as a critical step in the multigenetic process of tumorigenesis, and that telomerase is frequently but not always activated as a late event in gastric cancer progression.

Hiyama, K., et al. (1997). "[Telomere and telomerase in human cancer]." *Gan To Kagaku Ryoho* **24**(2): 196-201.

Human somatic cells gradually lose their telomeric repeats each cell division, and when they become critically shortened, stop dividing. On the other hand, in immortal cancer cells and germline cells, telomerase, a ribonucleoprotein which can compensate for the loss of telomeric repeats synthesizing telomeric DNA onto chromosomal ends, is activated and the telomere lengths are stabilized. Thus, telomere length and telomerase activity are believed to be characteristic indicators of cell proliferation and cell immortality, and inhibition of telomerase activity is expected to be a new strategy of anti-cancer therapy.

Hiyama, K., et al. (1998). "[Telomerase activity as a novel marker of lung cancer]." *Nihon Rinsho* **56**(5): 1253-1257.

Telomerase activity is found in more than 80% of human malignant neoplasms, including lung cancer. Markers with high incidence in malignant samples and very low incidence in benign samples are useful in clinical diagnosis of cancer. Thus, telomerase activity in clinical materials may become a novel tumor marker of existing lung cancer cells. Moreover, since activation of telomerase is associated with cellular immortality and its activity level is quite different between lung cancer tissues, the activity level may become an indicator of some biological features in lung cancer.

Hiyama, K., et al. (1995). "Alterations in telomeric repeat length in lung cancer are associated with loss of heterozygosity in p53 and Rb." *Oncogene* **10**(5): 937-944.

In the two-stage model of controlling cellular senescence in cultured human fibroblasts, retinoblastoma (Rb) and p53 proteins may be key factors regulating the mortality stage 1 mechanism. In addition, the critical loss of telomeric DNA due to the end-replication problem may result in the

mortality stage 2 mechanism. Cells which acquire telomerase activity can overcome the M2 mechanism by stabilizing telomere length and thus become immortal (telomere hypothesis). At present it is known whether cellular immortality is a prerequisite for all human cancers. To investigate this question and the applicability of the two-stage model to human cancers, we analysed the relationship between alterations of telomere length and other genetic changes in lung cancer. Among 60 primary lung cancer tissues, telomere length alterations were observed in 16 tumors (26.7%) including 14 with short and two with elongated telomeres. Ten of them revealed allelic loss of both p53 and Rb genes, and remaining six showed no abnormalities in both genes. We propose that inactivation of both p53 and Rb genes may promote cell divisions causing telomere shortening in lung cancer as in the two-stage model, while there may be another pathway to overcome both M1 and M2 mechanisms, especially for adenocarcinoma.

Hjelmeland, A. and J. Zhang (2016). "Metabolic, autophagic, and mitophagic activities in cancer initiation and progression." *Biomed J* **39**(2): 98-106.

Cancer is a complex disease marked by uncontrolled cell growth and invasion. These processes are driven by the accumulation of genetic and epigenetic alterations that promote cancer initiation and progression. Contributing to genome changes are the regulation of oxidative stress and reactive species-induced damage to molecules and organelles. Redox regulation, metabolic plasticity, autophagy, and mitophagy play important and interactive roles in cancer hallmarks including sustained proliferation, activated invasion, and replicative immortality. However, the impact of these processes can differ depending on the signaling pathways altered in cancer, tumor type, tumor stage, and/or the differentiation state. Here, we highlight some of the representative studies on the impact of oxidative and nitrosative activities, mitochondrial bioenergetics, metabolism, and autophagy and mitophagy in the context of tumorigenesis. We discuss the implications of these processes for cellular activities in cancer for anti-cancer-based therapeutics.

Hortobagyi, G. N., et al. (1999). "Recent developments in breast cancer therapy." *Semin Oncol* **26**(4 Suppl 12): 11-20.

Over the past three decades conceptual approaches to breast cancer have led to improvements in locoregional therapy and early diagnosis. Systematic screening programs with mammography reduce disease-specific mortality by

25% to 30%, while many patients with early breast cancer receive optimal breast-conserving treatments. Our increased understanding of the biology of breast cancer helped develop successful adjuvant systemic therapies (cytotoxic and hormonal) that, in turn, reduce mortality by 15% to 25%. Newer therapeutic interventions are under intensive investigation. While continued progress in cytotoxic therapy is evident (taxanes, vinorelbine, gemcitabine, new antifolates, liposomal anthracyclines, etc), there is increasing interest in targeting growth factors and their receptors. Thus, a monoclonal antibody directed to the extracellular domain of the HER-2/neu oncoprotein was recently approved by the Food Drug Administration based on evidence of antitumor activity as a single agent and in combination with cytotoxic therapy. A similar approach against the epidermal growth factor receptor is under evaluation in clinical trials. Various methods of inhibiting intracellular signal transduction also are in clinical development. These include tyrosine kinase inhibition, dominant negative mutant inhibitors of GRB-2, farnesyl transferase inhibition and vaccines directed against various epitopes expressed by mammary cancer cells. Angiogenesis and the enzyme telomerase are other targets under intense scrutiny since they are integrally involved with metastases and cellular immortality, both common characteristics of the malignant cell. These lines of investigation are likely to provide innovative therapeutic interventions, which may improve the specificity and therapeutic index of anticancer treatments.

Hsu, Y. H. and J. J. Lin (2005). "Telomere and telomerase as targets for anti-cancer and regeneration therapies." *Acta Pharmacol Sin* **26**(5): 513-518.

Telomerase is a ribonucleoprotein that directs the synthesis of telomeric sequence. It is detected in majority of malignant tumors, but not in most normal somatic cells. Because telomerase plays a critical role in cell immortality and tumor formation, it has been one of the targets for anti-cancer and regeneration drug development. In this review, we will discuss therapeutic approaches based mainly on small molecules that have been developed to inhibit telomerase activity, modulate telomerase expression, and telomerase directed gene therapy.

Hu, K., et al. (2021). "Integrated evaluation of telomerase activation and telomere maintenance across cancer cell lines." *Elife* **10**.

In cancer, telomere maintenance is critical for the development of replicative immortality. Using genome sequences from the Cancer Cell Line Encyclopedia and Genomics of Drug Sensitivity in Cancer Project, we calculated telomere content across

1299 cancer cell lines. We find that telomerase reverse transcriptase (TERT) expression correlates with telomere content in lung, central nervous system, and leukemia cell lines. Using CRISPR/Cas9 screening data, we show that lower telomeric content is associated with dependency of CST telomere maintenance genes. Increased dependencies of shelterin members are associated with wild-type TP53 status. Investigating the epigenetic regulation of TERT, we find widespread allele-specific expression in promoter-wildtype contexts. TERT promoter-mutant cell lines exhibit hypomethylation at PRC2-repressed regions, suggesting a cooperative global epigenetic state in the reactivation of telomerase. By incorporating telomere content with genomic features across comprehensively characterized cell lines, we provide further insights into the role of telomere regulation in cancer immortality.

Iacobas, S. and D. A. Iacobas (2021). "A Personalized Genomics Approach of the Prostate Cancer." *Cells* **10**(7).

Decades of research identified genomic similarities among prostate cancer patients and proposed general solutions for diagnostic and treatments. However, each human is a dynamic unique with never repeatable transcriptomic topology and no gene therapy is good for everybody. Therefore, we propose the Genomic Fabric Paradigm (GFP) as a personalized alternative to the biomarkers approach. Here, GFP is applied to three (one primary-"A", and two secondary-"B" & "C") cancer nodules and the surrounding normal tissue ("N") from a surgically removed prostate tumor. GFP proved for the first time that, in addition to the expression levels, cancer alters also the cellular control of the gene expression fluctuations and remodels their networking. Substantial differences among the profiled regions were found in the pathways of P53-signaling, apoptosis, prostate cancer, block of differentiation, evading apoptosis, immortality, insensitivity to anti-growth signals, proliferation, resistance to chemotherapy, and sustained angiogenesis. ENTPD2, AP5M1 BAIAP2L1, and TOR1A were identified as the master regulators of the "A", "B", "C", and "N" regions, and potential consequences of ENTPD2 manipulation were analyzed. The study shows that GFP can fully characterize the transcriptomic complexity of a heterogeneous prostate tumor and identify the most influential genes in each cancer nodule.

Iida, A., et al. (2000). "Telomerase activity in colorectal cancer and its relationship to bcl-2 expression." *J Surg Oncol* **73**(4): 219-223.

BACKGROUND AND OBJECTIVES: Telomerase is thought to be responsible for cell immortality, and bcl-2 has been demonstrated to regulate apoptosis. Recent studies have shown a wide occurrence of telomerase activation and bcl-2 deregulation in human carcinoma cells. **METHODS:** We examined telomerase activity in tissues from 50 patients with colorectal carcinoma with a telomeric repeat amplification protocol assay. We also investigated the relationship between telomerase activity and expression of bcl-2 in 37 colorectal carcinoma specimens. **RESULTS:** We detected telomerase activity in 33 (66%) of 50 colorectal carcinomas, whereas no activity was detected in the adjacent noncancerous mucosa of 13 tumor specimens. There was no correlation between pathological stage and telomerase activity. Telomerase activity in the bcl-2-expressing cases was higher than that in the bcl-2-non-expressing cases. **CONCLUSIONS:** Expression of bcl-2 may be related to telomerase activity in colorectal carcinomas.

Ikeda, S., et al. (2003). "Correlation between the expression of telomerase reverse transcriptase and proliferative activity in breast cancer cells using an immunocytochemical restaining method." *Pathol Int* 53(11): 762-768.

Telomerase activity is thought to contribute to the immortality of cancers. Recently, some investigators described a correlation between the activity of telomerase and the proliferative activity of cancer cells. The aim of this study was to evaluate the correlation between the expression of telomerase-associated protein and proliferative activity. Telomerase reverse transcriptase (TERT) is one of the proteins that correlates with telomerase activity. We investigated TERT protein and its mRNA, and examined the correlation between the TERT protein and Ki-67, which reflects proliferative activity with immunostaining, and its mRNA, which correlates with telomerase activity, using in situ hybridization. Imprint smears from 17 invasive ductal adenocarcinomas were investigated. In most cases positive for TERT mRNA, the percentage of TERT protein-positive cells was also high and was closely related to mRNA ($P = 0.024$). The positive rates of TERT for the cases with lymph node metastasis were significantly higher than those for the cases without metastasis ($P = 0.046$). The positivity of TERT protein also correlated significantly with the Ki-67-positive rate ($r = 0.82$). As the proliferation activity increased, the number of cells positive for both proteins also increased ($r = 0.89$). In conclusion, it was suggested that the expression of TERT protein is associated with the expression of Ki-67, and is concerned with maintenance of the high proliferative

activity in cancer cells with aggressive proliferation.

Iskender, B., et al. (2015). "Myrtucommulone-A treatment decreases pluripotency- and multipotency-associated marker expression in bladder cancer cell line HTB-9." *J Nat Med* 69(4): 543-554.

Cancer and stem cells exhibit similar features, including self-renewal, differentiation and immortality. The expression of stem-cell-related genes in cancer cells is demonstrated to be potentially correlated with cancer cell behaviour, affecting both drug response and tumor recurrence. There is an emerging body of evidence that subpopulations of tumors carry a distinct molecular sign and are selectively resistant to chemotherapy. Therefore, it is important to find novel therapeutic agents that could suppress the stem-like features of cancer cells while inhibiting their proliferation. Myrtucommulone-A (MC-A) is an active compound of a nonprenylated acylphloroglucinol isolated from the leaves of myrtle. Here we have investigated the potential of MC-A in inhibiting the expression of self-renewal regulatory factors and cancer stem cell markers in a bladder cancer cell line HTB-9. We used RT-PCR, immunocytochemistry, flow cytometry and western blotting to examine the expression of pluripotency- and multipotency-associated markers with or without treatment with MC-A. Treatment with MC-A not only decreased cancer cell viability and proliferation but also resulted in a decrease in the expression of pluripotency- and multipotency-associated markers such as NANOG, OCT-4, SOX-2, SSEA-4, TRA-1-60, CD90, CD73 and CD44. MC-A treatment was also observed to decrease the sphere-forming ability of HTB-9 cells. In summary, this study provides valuable information on the presence of stem-cell marker expression in HTB-9 cells and our results imply that MC-A could be utilized to target cancer cells with stem-like characteristics.

Ito, H., et al. (1998). "Expression of human telomerase subunits and correlation with telomerase activity in urothelial cancer." *Clin Cancer Res* 4(7): 1603-1608.

The activation of telomerase and stabilization of telomeres are thought to be required for cellular immortality and oncogenesis. Three major components of human telomerase--human telomerase RNA (hTERC), telomerase-associated protein (TEP1), and human telomerase catalytic subunit (hTERT)--have recently been identified. However, the roles played by these subunits in the regulation of telomerase activity are still unclear. In the present study, a total of 37 urothelial cancers, including one metastatic lesion, and adjacent normal tissues as well as cell lines derived from bladder

cancers were examined for the expression of each telomerase subunit. Reverse transcription-PCR analysis revealed that more than 90% of urothelial cancers expressed hTERT mRNA, whereas less than 20% of normal adjacent tissues did. In contrast, hTERC and TEP1 mRNA were commonly expressed in both cancers and normal tissues. All of the three cell lines derived from bladder cancer expressed each of the telomerase subunits, whereas the two normal primary fibroblast cell lines expressed hTERC and TEP1 mRNA but not hTERT mRNA. Telomerase activity was examined using telomeric repeat amplification protocol assay. All of the cancers examined exhibited telomerase activity, whereas only 2 of 12 normal tissues exhibited weak activity. There was a significant association of telomerase activity with hTERT mRNA expression but not with hTERC or TEP1 mRNA expression. These findings provide strong evidence that the expression of hTERT is a rate-limiting determinant of the enzymatic activity of human telomerase and that the up-regulation of hTERT expression may play a critical role in human carcinogenesis.

Ito, H., et al. (1998). "Detection of human telomerase reverse transcriptase messenger RNA in voided urine samples as a useful diagnostic tool for bladder cancer." *Clin Cancer Res* 4(11): 2807-2810.

Activation of telomerase and stabilization of telomeres are thought to be required for cellular immortality and oncogenesis. Telomerase activity is detected in >90% of various cancers, including urothelial cancers. Of the three subunits comprising telomerase complex, human telomerase reverse transcriptase (hTERT) is a rate-limiting determinant of the enzymatic activity of telomerase. In the present study, spontaneously voided urine specimens from 33 patients with bladder cancer and 26 without bladder lesions were examined for the expression of hTERT mRNA, and the usefulness of detecting hTERT mRNA in urine samples for screening of bladder cancer was evaluated. RT-PCR analysis revealed that approximately 80% of urinary sediments from patients with bladder cancer expressed hTERT mRNA, regardless of clinical stage or pathological grade, whereas only 4% of sediments from patients without urothelial lesions did. Interestingly, hTERT mRNA expression was observed, even in some urine samples from bladder cancer patients with negative urinary cytology. These findings suggest that the expression of hTERT in urine sample may be a useful diagnostic marker for bladder cancer.

Ivancich, M., et al. (2017). "Treating Cancer by Targeting Telomeres and Telomerase." *Antioxidants (Basel)* 6(1).

Telomerase is expressed in more than 85% of cancer cells. Tumor cells with metastatic potential may have a high telomerase activity, allowing cells to escape from the inhibition of cell proliferation due to shortened telomeres. Human telomerase primarily consists of two main components: hTERT, a catalytic subunit, and hTR, an RNA template whose sequence is complementary to the telomeric 5'-dTAGGG-3' repeat. In humans, telomerase activity is typically restricted to renewing tissues, such as germ cells and stem cells, and is generally absent in normal cells. While hTR is constitutively expressed in most tissue types, hTERT expression levels are low enough that telomere length cannot be maintained, which sets a proliferative lifespan on normal cells. However, in the majority of cancers, telomerase maintains stable telomere length, thereby conferring cell immortality. Levels of hTERT mRNA are directly related to telomerase activity, thereby making it a more suitable therapeutic target than hTR. Recent data suggests that stabilization of telomeric G-quadruplexes may act to indirectly inhibit telomerase action by blocking hTR binding. Telomeric DNA has the propensity to spontaneously form intramolecular G-quadruplexes, four-stranded DNA secondary structures that are stabilized by the stacking of guanine residues in a planar arrangement. The functional roles of telomeric G-quadruplexes are not completely understood, but recent evidence suggests that they can stall the replication fork during DNA synthesis and inhibit telomere replication by preventing telomerase and related proteins from binding to the telomere. Long-term treatment with G-quadruplex stabilizers induces a gradual reduction in the length of the G-rich 3' end of the telomere without a reduction of the total telomere length, suggesting that telomerase activity is inhibited. However, inhibition of telomerase, either directly or indirectly, has shown only moderate success in cancer patients. Another promising approach of targeting the telomere is the use of guanine-rich oligonucleotides (GROs) homologous to the 3' telomere overhang sequence (T-oligos). T-oligos, particularly a specific 11-base oligonucleotide (5'-dGTTAGGGTTAG-3') called T11, have been shown to induce DNA damage responses (DDRs) such as senescence, apoptosis, and cell cycle arrest in numerous cancer cell types with minimal or no cytostatic effects in normal, non-transformed cells. As a result, T-oligos and other GROs are being investigated as prospective anticancer therapeutics. Interestingly, the DDRs induced by T-oligos in cancer cells are similar to the effects seen after progressive telomere degradation in normal cells. The loss of telomeres is an important tumor suppressor mechanism that is commonly absent in transformed malignant cells, and hence, T-oligos have garnered

significant interest as a novel strategy to combat cancer. However, little is known about their mechanism of action. In this review, we discuss the current understanding of how T-oligos exert their antiproliferative effects in cancer cells and their role in inhibition of telomerase. We also discuss the current understanding of telomerase in cancer and various therapeutic targets related to the telomeres and telomerase.

Jagadeesh, S. and P. P. Banerjee (2006). "Inositol hexaphosphate represses telomerase activity and translocates TERT from the nucleus in mouse and human prostate cancer cells via the deactivation of Akt and PKC α ." *Biochem Biophys Res Commun* **349**(4): 1361-1367.

Inositol hexaphosphate (IP6) has anti-proliferative effects on a variety of cancer cells, including prostate cancer. However, the molecular mechanism of anti-proliferative effects of IP6 is not entirely understood. Since the activation of telomerase is crucial for cells to gain immortality and proliferation ability, we examined the role of IP6 in the regulation of telomerase activity in prostate cancer cells. Here, we show that IP6 represses telomerase activity in mouse and human prostate cancer cells dose-dependently. In addition, IP6 prevents the translocation of TERT to the nucleus. Since phosphorylation of TERT by Akt and/or PKC α is necessary for nuclear translocation, we examined phosphorylation of Akt and PKC α after IP6 treatments. Our results show that IP6 inhibits phosphorylation of Akt and PKC α . These results show for the first time that IP6 represses telomerase activity in prostate cancer cells by posttranslational modification of TERT via the deactivation of Akt and PKC α .

Jagadeesh, S., et al. (2006). "Genistein represses telomerase activity via both transcriptional and posttranslational mechanisms in human prostate cancer cells." *Cancer Res* **66**(4): 2107-2115.

Genistein, the most abundant isoflavone present in soybean has antiproliferative effects on a variety of cancer cells, including prostate cancer. However, the molecular mechanism of antiproliferative effects of genistein is not entirely understood. Because the activation of telomerase is crucial for cells to gain immortality and proliferation ability, we examined the role of genistein in the regulation of telomerase activity in prostate cancer cells. Here, we show that genistein-induced inhibition in cell proliferation is associated with a reduction in telomerase activity. Using reverse transcriptase-PCR and hTERT promoter activity assays, we showed that genistein decreased hTERT expression and

transcriptional activity dose-dependently. Using various deleted hTERT promoter constructs, we defined that the hTERT core promoter is enough to observe the genistein-induced repression of hTERT transcriptional activity. Because c-Myc is involved in transcriptional regulation of hTERT, c-Myc expression was examined. A dose-dependent decrease in c-Myc message and proteins was observed with genistein treatment. These results indicate that genistein represses hTERT transcriptional activity via the down-regulation of c-Myc expression. However, genistein-induced repression of hTERT transcriptional activity was not blocked by the mutation of c-Myc at the hTERT promoter, suggesting that additional factors are involved in genistein-dependent repression of telomerase activity. Interestingly, we observed that genistein down-regulates the activation of Akt thereby phosphorylation of hTERT and inhibits its translocation to the nucleus. These results show for the first time that genistein represses telomerase activity in prostate cancer cells not only by repressing hTERT transcriptional activity via c-Myc but also by posttranslational modification of hTERT via Akt.

Janknecht, R. (2004). "On the road to immortality: hTERT upregulation in cancer cells." *FEBS Lett* **564**(1-2): 9-13.

Telomere attrition limits the replicative potential of most somatic cells. In contrast, tumor cells acquire immortality by continuous telomere maintenance which is predominantly due to the transcriptional upregulation of the limiting component of telomerase, hTERT (human telomerase reverse transcriptase). Recent findings have provided mechanistic insight into how oncogenic activation as well as derepression, often due to the inactivation of tumor suppressors, stimulate the hTERT promoter. Knowledge gained from the study of hTERT transcriptional regulation may prove instrumental in the development of cancer therapies directed at the suppression of telomerase activity in tumor cells.

Jiang, J., et al. (2004). "Ganoderma lucidum suppresses growth of breast cancer cells through the inhibition of Akt/NF-kappaB signaling." *Nutr Cancer* **49**(2): 209-216.

Ganoderma lucidum (Reishi, Lingzhi) is a popular Asian mushroom that has been used for more than 2 millennia for the general promotion of health and was therefore called the "Mushroom of Immortality." Ganoderma lucidum was also used in traditional Chinese medicine to prevent or treat a variety of diseases, including cancer. We previously demonstrated that Ganoderma lucidum suppresses the invasive behavior of breast cancer cells by inhibiting

the transcription factor NF-kappaB. However, the molecular mechanisms responsible for the inhibitory effects of *Ganoderma lucidum* on the growth of highly invasive and metastatic breast cancer cells has not been fully elucidated. Here, we show that *Ganoderma lucidum* inhibits proliferation of breast cancer MDA-MB-231 cells by downregulating Akt/NF-kappaB signaling. *Ganoderma lucidum* suppresses phosphorylation of Akt on Ser473 and downregulates the expression of Akt, which results in the inhibition of NF-kappaB activity in MDA-MB-231 cells. The biological effect of *Ganoderma lucidum* was demonstrated by cell cycle arrest at G0/G1, which was the result of the downregulation of expression of NF-kappaB-regulated cyclin D1, followed by the inhibition of cdk4. Our results suggest that *Ganoderma lucidum* inhibits the growth of MDA-MB-231 breast cancer cells by modulating Akt/NF-kappaB signaling and could have potential therapeutic use for the treatment of breast cancer.

Jiang, S., et al. (2016). "Molecular Mechanisms of Anti-cancer Activities of β -elemene: Targeting Hallmarks of Cancer." *Anticancer Agents Med Chem* **16**(11): 1426-1434.

Increasing knowledge on the hallmark characteristics of cancer and tumor pharmacology has promoted the introduction of phytochemicals, such as traditional Chinese medicine (TCM) in cancer therapy, which modulate numerous molecular targets and exert anticancer activities. β -elemene, an active and non-toxic compound isolated from the Chinese medicinal herb *Rhizoma Zedoariae*, has been explored as a potent anti-cancer agent against multiple cancers in extensive clinical trials and experimental research in vivo and in vitro. β -elemene exerts therapeutic potential via modulation of core hallmark capabilities of cancer by suppressing proliferative signaling, such as MAPK and PI3K/Akt/mTOR pathway, inducing cell death, up-regulating growth suppressors, deactivating invasion and metastasis and interacting replicative immortality and attenuating angiogenesis. Recent studies have significantly improved our understanding of anti-cancer activities and underlying molecular mechanisms of this Chinese medicine. This review presents these novel findings regarding the unique properties of β -elemene as an agent for cancer treatment, with an emphasis on multi-targeting biological and molecular regulation.

Jung, I., et al. (2023). "Epigenetic Regulators of DNA Cytosine Modification: Promising Targets for Cancer Therapy." *Biomedicines* **11**(3).

Epigenetic modifications are crucial regulators of gene expression that critically impact

cell lineage differentiation, survival, and proliferation, and dysregulations are commonly observed in various cancers. The aberrantly modified epigenome confers unique features on tumor cells, including sustained proliferative potential, resistance to growth-suppressive or cell death signals, augmented replicative immortality, invasion, and metastasis. As a result, epigenetic abnormalities exhibit significant impacts on all stages of oncogenesis from its onset to progression to metastasis. Among various epigenetic mechanisms in mammals, DNA cytosine methylation-demethylation is recurrently disrupted in cancers. Due to its inherent reversibility, targeting DNA methylation dynamics has gained tremendous attention as a promising therapeutic option that can ameliorate the effects of cancer-specific epigenetic abnormalities by restoring normal conditions. Various small molecules targeting DNA (de)methylation regulators have been developed as potential cancer therapeutics, some of which are approved for usage in clinics. Clinical trials of many other molecules are underway for both hematological malignancies and solid tumors. In this review, we discuss the DNA methylation/demethylation pathway as a promising target for therapeutic intervention in cancer and highlight the development of various epigenetic drugs targeting DNA-modifying enzymes such as DNA methyltransferases (DNMTs) and ten-eleven translocation (TET) enzymes.

Katayama, H., et al. (2008). "Activation of focal adhesion kinase in detached human epidermal cancer cells and their long-term survival might be associated with cell surface expression of laminin-5." *Acta Derm Venereol* **88**(2): 100-107.

While normal epithelial cells are anchorage-dependent, cancer cells are anchorage-independent. To elucidate the mechanism underlying the anchorage-independence of cancer cells, we cultured detached cells in medium containing elastase. Detached human epidermal cancer cells (DJM-1) survived for at least 3 weeks and focal adhesion kinase was still phosphorylated. In contrast, most detached keratinocytes underwent rapid apoptosis and focal adhesion kinase was not phosphorylated while the cells were alive. Thus, discontinuation of the phosphorylation of focal adhesion kinase preceded cell death. Immunostaining showed laminin-5 expression on the surface of detached DJM-1 cells, but not on detached keratinocytes. Receptors for laminin-5 (i.e. integrins) were detected on both detached DJM-1 cells and keratinocytes. Laminins are secreted proteins, so we speculated that laminin-5 adhered to the surface of secreting DJM-1 cells via integrins and evoked activation of focal adhesion kinase, with the resultant signalling cascade

promoting cellular survival. If this hypothesis is correct, cell surface expression of laminin-5 may be used to explain the characteristics of cancer, immortality, tumour formation, metastasis and angiogenesis.

Kato, H., et al. (2006). "Induction of human endometrial cancer cell senescence through modulation of HIF-1 α activity by EGLN1." *Int J Cancer* **118**(5): 1144-1153.

Previous observations indicate that transfer of human chromosome (chr.) 1 induces senescence of endometrial cancer cells. To identify the gene(s) responsible for the senescence, we first analyzed the structural integrity of the introduced chr. 1 in immortal revertant from chr.1-transferred HHUA cells. The data demonstrated a correlation between nonrandom deletions within the 1q31-qter region and reversion to immortality. Next, by using a panel of 12 microsatellite markers, we found high frequencies of loss of heterozygosity in the particular 1q region (1q41-42), in surgically removed samples. Then, we screened the genetic mutation of the genes involved in this region, with endometrial cancer panel. Among them, EGLN1, that is a member of prolyl hydroxylase and can facilitate HIF-1 degradation by ubiquitination through the hydroxylation of HIF-1, was mutated at significantly higher frequencies (12/20, 60%). Introduction of wild-type EGLN1 into endometrial cancer cell lines (HHUA, Ishikawa and HWCA), that carry EGLN1 gene mutations induced senescence. This was invoked through the negative regulation of HIF-1 expression. In addition, alternative way of negative regulation of HIF-1 by Factor inhibiting HIF-1(FIH), SiRNA against HIF-1, and HIF-1 inhibitor, YC-1, could also induce senescence. Thus, EGLN1 can be considered as a candidate tumor suppressor on chr. 1q, and our observation could open the new aspect in exploring the machinery of senescence induction associated with HIF-1 signal transduction. These results also suggested the availability of negative regulation of HIF-1 signals for uterine cancer treatment, especially for uterine sarcomas that have worse prognosis and show a high frequency of EGLN1 gene abnormality.

Kato, T., et al. (2007). "Activation of Holliday junction recognizing protein involved in the chromosomal stability and immortality of cancer cells." *Cancer Res* **67**(18): 8544-8553.

We identified a novel gene HJURP (Holliday junction-recognizing protein) whose activation seemed to play a pivotal role in the immortality of cancer cells. HJURP was considered a possible downstream target for ataxia telangiectasia mutated signaling, and its expression was increased

by DNA double-strand breaks (DSB). HJURP was involved in the homologous recombination pathway in the DSB repair process through interaction with hMSH5 and NBS1, which is a part of the MRN protein complex. HJURP formed nuclear foci in cells at S phase and those subjected to DNA damage. In vitro assays implied that HJURP bound directly to the Holliday junction and rDNA arrays. Treatment of cancer cells with small interfering RNA (siRNA) against HJURP caused abnormal chromosomal fusions and led to genomic instability and senescence. In addition, HJURP overexpression was observed in a majority of lung cancers and was associated with poor prognosis as well. We suggest that HJURP is an indispensable factor for chromosomal stability in immortalized cancer cells and is a potential novel therapeutic target for the development of anticancer drugs.

Kaul, Z., et al. (2021). "Functional characterization of miR-708 microRNA in telomerase positive and negative human cancer cells." *Sci Rep* **11**(1): 17052.

Activation of a telomere length maintenance mechanism (TMM), including telomerase and alternative lengthening of telomeres (ALT), is essential for replicative immortality of tumor cells, although its regulatory mechanisms are incompletely understood. We conducted a microRNA (miRNA) microarray analysis on isogenic telomerase positive (TEP) and ALT cancer cell lines. Amongst nine miRNAs that showed difference in their expression in TEP and ALT cancer cells in array analysis, miR-708 was selected for further analysis since it was consistently highly expressed in a large panel of ALT cells. miR-708 in TEP and ALT cancer cells was not correlated with C-circle levels, an established feature of ALT cells. Its overexpression induced suppression of cell migration, invasion, and angiogenesis in both TEP and ALT cells, although cell proliferation was inhibited only in TEP cells suggesting that ALT cells may have acquired the ability to escape inhibition of cell proliferation by sustained miR-708 overexpression. Further, cell proliferation regulation in TEP cells by miR708 appears to be through the CARF-p53 pathway. We demonstrate here that miR-708 (i) is the first miRNA shown to be differentially regulated in TEP and ALT cancer cells, (ii) possesses tumor suppressor function, and (iii) deregulates CARF and p21(WAF1)-mediated signaling to limit proliferation in TEP cells.

Kaur, D., et al. (2024). "Identification of novel inhibitors of cancer target telomerase using a dual structure-based pharmacophore approach to virtually screen libraries, molecular docking and validation by molecular dynamics simulations." *J Biomol Struct*

Dyn: 1-24.

In about 85% of cancer malignancies, replicative immortality caused by increased telomerase activity makes it an attractive target for developing anticancer therapeutics. However, the lack of approved small-molecule inhibitors rooted in the structural ambiguity of telomerase has impeded drug development for decades. In this study, we have exploited the FVYL pocket in the thumb domain, which plays a key role in the enzyme's processivity. Due to the unavailability of a co-crystallized structure of BIBR1532 with the catalytic hTERT thumb domain, we utilized the molecular dynamics method to identify the precise binding site of the inhibitor. Two pharmacophore models were generated and validated for the putative (Site-I) and newly identified (Site-II) binding pockets which were screened virtually through the ChemDiv anticancer library, Otava drug-like green collection to identify novel lead compounds, and Binding database to screen out thumb domain-specific telomerase inhibitors. The top hits obtained were filtered using drug-likeness parameters followed by redocking using a three-level screening strategy into their binding site. The structural investigation, molecular docking studies, and confirmatory molecular dynamics revealed that the exact binding site of BIBR1532 is away from the reported FVYL pocket with characteristic interactions conserved. Subsequently, the lead compounds with the highest docking scores and significant interactions in the newly discovered extended FVYL pocket were validated using 100 ns MD simulations. Additionally, cross-validated binding free energy calculations were performed using MM-PB(GB)SA methods followed by PCA and FEL characterization. The identified top lead compounds can be validated in vitro and taken forward for anticancer drug development.

Kawahara, R., et al. (2007). "Analysis of hTERT mRNA expression in biliary tract and pancreatic cancer." *J Hepatobiliary Pancreat Surg* **14**(2): 189-193.

BACKGROUND/PURPOSE: Telomerase, an enzyme that prevents the loss of telomere regions consisting of TTAGGG repeats, which maintain the stability of cells, is considered to be involved in cell immortality and cancer growth. Recent genetic analysis has shown that the mRNA for the catalytic subunit of human telomerase reverse transcriptase (hTERT) is expressed in many cancer tissues. **METHODS:** In this study, we measured hTERT mRNA levels in bile samples from patients with pancreatobiliary disease, and we combined the hTERT mRNA analysis with conventional cytology to achieve an accurate preoperative diagnosis. Bile

samples were obtained from 19 patients with biliary tract cancer, 6 with gallbladder cancer, 10 with pancreatic cancer, 1 with gastric cancer, and 10 with benign disease. These samples were examined cytologically, and analyzed for hTERT mRNA levels. **RESULTS:** The Combination of cytological examination and hTERT mRNA analysis achieved a positive rate of 78.9% in diagnosing biliary tract cancer, significantly improving the diagnostic accuracy over that for either method alone ($P = 0.01$). The diagnostic sensitivity for malignant disease was 66.6%, also significantly improving the diagnostic accuracy compared with either method alone ($P = 0.001$). **CONCLUSIONS:** The combination of cytological examination and hTERT mRNA analysis appeared useful for the preoperative diagnosis of malignant biliary tract diseases, but was not superior to diagnostic imaging studies, and therefore remains an adjunct to cytological examination. Further studies should lead to improvements in the combination's diagnostic capabilities.

Kaye, J., et al. (1981). "Attitudes of medical students and residents toward cancer." *J Psychol* **107**(1st Half): 87-96.

Attitudes toward cancer and heart disease were evaluated in 99 freshmen medical students, 76 seniors, and 66 residents using the Cancer Attitude Survey and a Semantic Differential test. The Survey revealed a rise in positive attitudes towards patients' inner resources to cope with serious illness and toward personal immortality and a rise in negative attitudes toward early diagnosis of cancer as students progressed in their training. The Semantic Differential test demonstrated more negative attitudes toward cancer than heart disease in all groups (freshman, seniors, and residents in medicine, psychiatry, or surgery). The seniors had the most positive attitudes toward cancer and freshman the least positive attitudes. The residents had more positive attitudes than the freshmen but less positive attitudes than the seniors. The residents in psychiatry had more positive attitudes than the residents in medicine, who had more positive attitudes than the residents in surgery.

Ke, S., et al. (2015). "Downregulation of high mobility group box 1 modulates telomere homeostasis and increases the radiosensitivity of human breast cancer cells." *Int J Oncol* **46**(3): 1051-1058.

The functions of the high mobility group box 1 (HMGB1) in tumor cells include replenishing telomeric DNA and maintaining cell immortality. There is a negative correlation between human telomerase reverse transcriptase (hTERT) and

radiosensitivity in tumor cells. Our aim was to elucidate the relationship among HMGB1, telomere homeostasis and radiosensitivity in MCF-7 cells. In this study, we established stably transfected control (MCF-7-NC) and HMGB1 knockdown (MCF-7-shHMGB1) cell lines. The expression of HMGB1 mRNA and the relative telomere length were examined by real-time PCR. Radiosensitivity was detected by clonogenic assay. The protein expressions were determined by western blot analysis. The telomerase activity was detected by PCR-ELISA. Proliferation ability was examined by CCK-8 assay. Cell cycle and apoptosis were examined by flow cytometry. DNA damage foci were detected by immunofluorescence.

ShRNA-mediated downregulation of HMGB1 expression increased the radiosensitivity of MCF-7 cells, and reduced the accumulation of hTERT and cyclin D1. Moreover, knockdown of HMGB1 in MCF-7 cells inhibited telomerase activity and cell proliferation, while increasing the extent of apoptosis. Downregulation of HMGB1 modulated telomere homeostasis by changing the level of telomere-binding proteins, such as TPP1 (PTOP), TRF1 and TRF2. This downregulation also inhibited the ATM and ATR signaling pathways. The current data demonstrate that knockdown of HMGB1 breaks telomere homeostasis, enhances radiosensitivity, and suppresses the repair of DNA damage in human breast cancer cells. These results suggested that HMGB1 might be a potential radiotherapy target in human breast cancer.

Keith, W. N. (2004). "From stem cells to cancer: balancing immortality and neoplasia." *Oncogene* 23(29): 5092-5094.

In this issue of *Oncogene*, Serakinci et al show that adult stem cells can be targets for neoplastic transformation. After transducing human adult mesenchymal stem cells (hMSC) with the telomerase hTERT gene, and growing them for many population doublings in culture, Serakinci et al observed that the transduced cells developed characteristics consistent with transformation including loss of contact inhibition, anchorage independence and tumour formation in mice. Underlying these changes were alterations to genes involved in cell cycle regulation and senescence as well as oncogene activation. The importance of these observations is twofold. Firstly, showing that stem cells can become tumours raises a note of caution for stem cell therapeutics. Secondly, the findings lend support to the stem cell hypothesis of cancer development, and provide an experimental system in which the tantalizing hint of new diagnostic, prognostic, and therapeutic opportunities offered by

this concept can be explored further.

Keith, W. N., et al. (2004). "Drug insight: Cancer cell immortality-telomerase as a target for novel cancer gene therapies." *Nat Clin Pract Oncol* 1(2): 88-96.

Rapid advances in our understanding of the molecular basis of cancer development and progression over the past three decades have led to the design of new potential cancer therapies. High throughput target validation and expression studies are expected to yield a powerful arsenal of new cancer treatments, but untangling the complex pathways underlying the major cancer phenotypes remains a significant challenge. A considerable body of evidence in recent years implicates deregulated expression of a single multi-component enzyme, telomerase, as a causative factor at the heart of immortalization in the vast majority of human tumors. This review highlights the potential of telomerase as a target for novel cancer therapies. The potential of exploiting the selectivity of the telomerase family of genes within cancer cells to develop gene therapy strategies is discussed, and the progress towards translating these novel therapeutics from the laboratory to the clinic is reviewed.

Kelland, L. R. (2005). "Overcoming the immortality of tumour cells by telomere and telomerase based cancer therapeutics--current status and future prospects." *Eur J Cancer* 41(7): 971-979.

A key property of malignant tumours is their immortality or limitless replicative potential. Cell replication is associated with the maintenance of telomeres and in the great majority of cases, through the reactivation of the reverse transcriptase telomerase. Targeting the telomere/telomerase machinery offers a novel and potentially broad-spectrum anticancer therapeutic strategy since telomerase is constitutively overexpressed in the vast majority of human cancers. Telomeres are also critically short in most tumours compared to normal tissues. Strategies that exploit these differences include the direct targeting of components of telomerase: the protein component hTERT or RNA component hTR. Examples of such agents include the small molecule hTERT inhibitor BIBR1532 and GRN163L, a thio-phosphoramidate oligonucleotide targeting the template region of hTR as a "template antagonist". Anti-tumour effects have been observed in both cell lines and, especially for GRN163L, in xenografted human tumours in mice. Effects, however, are largely dependent upon initial telomere length, which can result in a substantial lag before antitumour activity is observed in tumours possessing relatively long telomeres. An alternative approach is to target the telomere itself (Telomere Targeting

Agents, TTAs). Several classes of small molecules have been described that induce the G-rich single-stranded overhang of telomeric DNA to fold into 4-stranded G-quadruplex structures. Such folding is incompatible with telomerase function and may induce rapid telomere uncapping. These molecules have shown potent telomerase inhibition in nanomolar concentrations in vitro and the rapid induction of senescence in cancer cells. The trisubstituted acridine based TTA, BRACO19, has demonstrated single agent activity against human tumour xenografts with anti-tumour effects apparent from only 7 days of treatment. In the near future, it is expected that lead examples from both the direct telomerase targeted agents (e.g., GRN163L) and from the distinct class of those targeting telomeres (e.g., AS1410 based on BRACO19) will enter Phase I clinical trial where clinical benefit from this class of novel drugs will be determined.

Kent, T., et al. (2019). "Alternative Lengthening of Telomeres in Pediatric Cancer: Mechanisms to Therapies." *Front Oncol* **9**: 1518.

Achieving replicative immortality is a crucial step in tumorigenesis and requires both bypassing cell cycle checkpoints and the extension of telomeres, sequences that protect the distal ends of chromosomes during replication. In the majority of cancers this is achieved through the enzyme telomerase, however a subset of cancers instead utilize a telomerase-independent mechanism of telomere elongation-the Alternative Lengthening of Telomeres (ALT) pathway. Recent work has aimed to decipher the exact mechanism that underlies this pathway. To this end, this pathway has now been shown to extend telomeres through exploitation of DNA repair machinery in a unique process that may present a number of druggable targets. The identification of such targets, and the subsequent development or repurposing of therapies to these targets may be crucial to improving the prognosis for many ALT-positive cancers, wherein mean survival is lower than non-ALT counterparts and the cancers themselves are particularly unresponsive to standard of care therapies. In this review we summarize the recent identification of many aspects of the ALT pathway, and the therapies that may be employed to exploit these new targets.

Khanam, R., et al. (2017). "Inhibitory growth evaluation and apoptosis induction in MCF-7 cancer cells by new 5-aryl-2-butylthio-1,3,4-oxadiazole derivatives." *Cancer Chemother Pharmacol* **80**(5): 1027-1042.

BACKGROUND: Cancer has become one of the global health issues and it is the life-

threatening disease characterized by unrestrained growth of cells. Despite various advances being adopted by chemotherapeutic management, the use of the current anticancer drugs such as Doxorubicin, Asparaginase, Methotrexate, Vincristine remains limited due to high toxicity, side effects and developing drug resistance. Apoptosis is a crucial cellular process and improper regulation of apoptotic signaling pathways may lead to cancer formation. Subsequently, the synthesis of effective chemotherapeutic agents that can induce apoptosis in tumor cell has emerged as a significant approach in cancer drug discovery. **METHODS:** The goal of this work is to develop a potential antitumor agent exerting significant inhibitory effects on cancer cell and low cytotoxicity, for which we focused on the structural features of 1,3,4-oxadiazoles as it a privileged scaffold in modern medicinal chemistry and have the ability to inhibit growth factors, enzymes and kinases potentially involved in the attainment of cellular immortality and carcinogenesis. **RESULT:** In vitro MTT screening assay showed the compound 5-aminophenyl-2-butylthio-1,3,4-oxadiazole (5e) showing the highest inhibitory effect against MCF-7 cancer cell with IC(50) value 10.05 +/- 1.08 microM while it is much safer and less toxic on normal cell line (HEK-293). The dose-dependent treatment of MCF-7 cells with 5e resulted in inhibition of cell migration in the wound healing assay. The flow-cytometry analysis showed the cells arrested in G0/G1 phase of the cell cycle. Compound 5e induced apoptosis of MCF-7 cells was characterized using DAPI staining and Annexin V-PE/7-AAD dual binding assay. Reduction of NBT by compound 5e showed a reduced generation of ROS. Western blotting studies showed high activation of apoptotic protein Caspase3 and decrease in expression of anti-apoptotic protein BCL-2. **CONCLUSION:** Based on the results of in vitro studies, it could be concluded that compound 5e showed a significant inhibitory growth effect on MCF-7 cells and have the potential to be developed as lead molecule and further structural modifications may result in promising new anticancer agents.

Khorramizadeh, M. R., et al. (2007). "Suppression of telomerase activity by pyrimethamine: implication to cancer." *Iran Biomed J* **11**(4): 223-228.

BACKGROUND: Although pyrimethamine (Tindurin) appears to be effective in the prevention and treatment of some infectious diseases, very little information exists on its unpredictable properties. We design this study to evaluate its anti-tumoral effect on a model of cell line. **METHODS:** The cytotoxic influence of Pyrimethamine on prostate cell line was investigated using an in vitro colometric assay. The

potential modulatory effects on metastasis, apoptosis, and immortality characteristics of cells were assessed with gelatin zymography, terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay and telomeric repeat amplification protocol, respectively. RESULTS: Cytotoxicity analysis of pyrimethamine revealed a dose-dependent fashion. An apoptotic influence of pyrimethamine was also confirmed by data obtained from TUNEL assay. Dose-dependent inhibitory effect on matrix metalloproteinases (MMP) was seen in pyrimethamine. A potent inhibitory effect of pyrimethamine was also established by data achieved from TRAPeze telomerase detection kit. CONCLUSIONS: Collectively, as induction of apoptosis together with MMP and telomerase inhibition could be indicative of cancer treatment, pyrimethamine might be considered as a chemopreventative agent in cancer.

Kienzl, P., et al. (2024). "Telomere transcripts act as tumor suppressor and are associated with favorable prognosis in colorectal cancer with low proliferating cell nuclear antigen expression." *Cell Oncol (Dordr)*.

Telomeric repeat-containing RNAs (TERRA) and telomerase RNA component (TERC) regulate telomerase activity (TA) and thereby contribute to telomere homeostasis by influencing telomere length (TL) and the cell immortality hallmark of cancer cells. Additionally, the non-canonical functions of telomerase reverse transcriptase (TERT) and TERRA appear to be involved in the epithelial-mesenchymal transition (EMT), which is important for cancer progression. However, the relationship between TERRA and patient prognosis has not been fully characterized. In this small-scale study, 68 patients with colorectal cancer (CRC) were evaluated for correlations between telomere biology, proliferation, and EMT gene transcripts and disease outcome. The proliferating cell nuclear antigen (PCNA) and the epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2) showed a positive correlation with TERRA, while TA and TERRA exhibited an inverse correlation. Consistent with previous findings, the present study revealed higher expression levels of TERT and TERC, and increased TA and TL in CRC tumor tissue compared to adjacent non-tumor tissue. In contrast, lower expression levels of TERRA were observed in tumor tissue. Patients with high TERRA expression and low PCNA levels exhibited favorable overall survival rates compared to individuals with the inverse pattern. Furthermore, TERRA suppressed CRC tumor growth in severe combined immunodeficiency disease (SCID) mice. In conclusion, our study extends previously published research on TERRA suggesting its potential

therapeutic role in telomerase-positive CRC.

Kim, H. R., et al. (2000). "Change of telomerase activity in rectal cancer with chemoradiation therapy." *J Korean Med Sci* 15(2): 167-172.

Telomerase, an enzyme associated with cellular immortality, is expressed by most malignant cells and is inactive in most normal somatic cells, with the exception of proliferative stem cells, germ cells and activated lymphocytes. Measuring telomerase activity clinically may provide useful diagnostic and prognostic information of cancer. The purpose of this study was to investigate the change in telomerase activity following chemoradiation in rectal cancer, which almost always produces positive enzymatic activity. A total of 24 tumor tissue samples were used in this study, consisting of 12 paired specimens before and 4 weeks after chemoradiation. Telomerase activity was determined by PCR-based telomeric repeat amplification protocol (TRAP) assay. The telomerase activity was positive in 10 out of 12 patients (83%) in pre-irradiated and post-irradiated states. The levels of telomerase activity was decreased in 8 out of 10 patients after chemoradiation (80%) and two cases showed no change in enzymatic activity. One case showed no activity in either sample. The other case showed no enzymatic activity in the pre-irradiated sample, but showed weak activity in the post-irradiated sample. These data indicate that telomerase activity in rectal cancer is reduced after neoadjuvant chemoradiation therapy, possibly suggesting a mechanism of downstaging following chemoradiation therapy in cancer.

Kim, N. W. (1997). "Clinical implications of telomerase in cancer." *Eur J Cancer* 33(5): 781-786.

Cellular immortality is believed to be a critical step in tumorigenesis. As an important component of the telomere maintenance mechanism, the activation of the enzyme telomerase is tightly associated with cellular immortality and cancer. Telomerase expression is detected in a majority of tumours, but is absent in most somatic tissues and correlates to clinical outcome in a number of cancer types. Telomerase expression is associated with the stage of differentiation but not necessarily with the rate of cell proliferation. Data also indicate that inhibition or absence of telomerase may result in cell crisis in cancer cells and tumour regression in cancer patients. These results suggest that cancer therapy based on telomerase inhibition could be a more effective and safer treatment for cancer, as well as provide a more accurate means for diagnosing and predicting clinical outcome in cancer. Complete understanding of the role of telomerase in tumorigenesis through well-designed clinical studies

will have a significant clinical impact on the treatment and diagnosis of cancer.

Kitagawa, Y., et al. (2000). "Demethylating reagent 5-azacytidine inhibits telomerase activity in human prostate cancer cells through transcriptional repression of hTERT." *Clin Cancer Res* 6(7): 2868-2875.

Telomerase activation is thought to be a critical step in cellular immortality and oncogenesis. Several reagents including differentiation-inducing and antineoplastic agents are known to inhibit telomerase activity, although the molecular mechanisms through which they inhibit telomerase activity remain unclear. Demethylating reagents have recently been used as potential antineoplastic drugs for some types of cancers including those of the prostate. In the present study, we examined the effect of the demethylating reagent 5-azacytidine (5-aza-CR) on telomerase activity using cells of two prostate cancer cell lines, DU-145 and TSU-PR1. 5-aza-CR treatment significantly reduced telomerase activity in TSU-PR1 cells, but not in DU-145 cells, although growth inhibition was observed to a similar extent in both cell lines. Reverse transcription-PCR analyses revealed that inhibition of telomerase activity was accompanied by down-regulation of telomerase catalytic subunit (hTERT) mRNA expression. Transient expression assays showed that 5-aza-CR repressed the transcriptional activity of the hTERT promoter and that the E-box within the core promoter was responsible for this down-regulation. Western blot analyses revealed that 5-aza-CR reactivated p16 expression and repressed c-Myc expression in TSU-PR1 cells but not in DU-145 cells. Overexpression of p16 in TSU-PR1 cells led to significant repression of c-Myc transcription. These findings suggest that 5-aza-CR inhibits telomerase activity via transcriptional repression of hTERT, in which p16 and c-Myc may play a key role.

Kolat, D., et al. (2022). "AP-2delta Is the Most Relevant Target of AP-2 Family-Focused Cancer Therapy and Affects Genome Organization." *Cells* 11(24).

Formerly hailed as "undruggable" proteins, transcription factors (TFs) are now under investigation for targeted therapy. In cancer, this may alter, inter alia, immune evasion or replicative immortality, which are implicated in genome organization, a process that accompanies multi-step tumorigenesis and which frequently develops in a non-random manner. Still, targeting-related research on some TFs is scarce, e.g., among AP-2 proteins, which are known for their altered functionality in cancer and prognostic importance. Using public

repositories, bioinformatics tools, and RNA-seq data, the present study examined the ligandability of all AP-2 members, selecting the best one, which was investigated in terms of mutations, targets, co-activators, correlated genes, and impact on genome organization. AP-2 proteins were found to have the conserved "TF_AP-2" domain, but manifested different binding characteristics and evolution. Among them, AP-2delta has not only the highest number of post-translational modifications and extended strands but also contains a specific histidine-rich region and cleft that can receive a ligand. Uterine, colon, lung, and stomach tumors are most susceptible to AP-2delta mutations, which also co-depend with cancer hallmark genes and drug targets. Considering AP-2delta targets, some of them were located proximally in the spatial genome or served as co-factors of the genes regulated by AP-2delta. Correlation and functional analyses suggested that AP-2delta affects various processes, including genome organization, via its targets; this has been eventually verified in lung adenocarcinoma using expression and immunohistochemistry data of chromosomal conformation-related genes. In conclusion, AP-2delta affects chromosomal conformation and is the most appropriate target for cancer therapy focused on the AP-2 family.

Kovalenko, O. A., et al. (2010). "Expression of (NES-)hTERT in cancer cells delays cell cycle progression and increases sensitivity to genotoxic stress." *PLoS One* 5(5): e10812.

Telomerase is a reverse transcriptase associated with cellular immortality through telomere maintenance. This enzyme is activated in 90% of human cancers, and inhibitors of telomerase are currently in clinical trials to counteract tumor growth. Many aspects of telomerase biology have been investigated for therapy, particularly inhibition of the enzyme, but little was done regarding its subcellular shuttling. We have recently shown that mutations in the nuclear export signal of hTERT, the catalytic component of telomerase, led to a mutant ((NES-)hTERT) that failed to immortalize cells despite nuclear localization and catalytic activity. Expression of (NES-)hTERT in primary fibroblast resulted in telomere-based premature senescence and mitochondrial dysfunction. Here we show that expression of (NES-)hTERT in LNCaP, SQ20B and HeLa cells rapidly and significantly decreases their proliferation rate and ability to form colonies in soft agar while not interfering with endogenous telomerase activity. The cancer cells showed increased DNA damage at telomeric and extra-telomeric sites, and became sensitive to ionizing radiation and hydrogen peroxide exposures. Our data

show that expression of (NES-)hTERT efficiently counteracts cancer cell growth in vitro in at least two different ways, and suggest manipulation with the NES of hTERT or its subcellular shuttling as a new strategy for cancer treatment.

Kraemer, K., et al. (2004). "Chemosensitization of bladder cancer cell lines by human telomerase reverse transcriptase antisense treatment." *J Urol* **172**(5 Pt 1): 2023-2028.

PURPOSE: Responses of transitional cell carcinoma of the bladder (TCC) to commonly used chemotherapy agents such as mitomycin C (MMC), cisplatin and gemcitabine are often disappointing. Since human telomerase reverse transcriptase (hTERT) is tumor specifically expressed and contributes to the immortality and malignancy of the majority of tumors, it is regarded as a suitable antitumor target. We investigated whether combinations of hTERT antisense (AS)-oligonucleotides (ODNs) with common chemotherapy (CT) schedules may improve drug mediated antitumor effects. **MATERIALS AND METHODS:** Initial screening for enhancement of the inhibitory effects of MMC, cisplatin and gemcitabine on viability by treatment with the 2 hTERT AS-ODNs ASt2206 and ASt2331 was performed in 4 TCC cell lines prior to CT. Apoptosis was assessed by annexin V staining and detection of activated caspase-3 using Western blot analysis. Nonsense (NS)-ODN served as a control in all experiments. **RESULTS:** All cell lines responded to the anticancer agents tested. Treatment with AS plus CT resulted in a significantly stronger inhibition of viability than the NS plus CT control in the majority of combinations, indicating an AS specific enhancement effect. For example, ASt2331 plus MMC decreased viability to 17% in contrast to NS plus MMC (58%) in EJ28 cells. All ASt2331 plus CT combinations specifically increased the rate of apoptosis 1.3 to 3.0-fold compared with NS plus CT. Apoptosis induction was associated with caspase 3 activation. **CONCLUSIONS:** Enhancement of cytotoxic drug effects on the growth of TCC cells by hTERT AS-ODNs presented herein allows a dose decrease in chemotherapy and confirms the suitability of hTERT as a target in a specific therapy approach.

Kroll, J. (2007). "Molecular chaperones and the epigenetics of longevity and cancer resistance." *Ann N Y Acad Sci* **1100**: 75-83.

The inherent immortality of embryonic stem cells demonstrates that replicative senescence as possibly the aging of species are epigenetic phenomena. The cellular level of expression of the housekeeping molecular chaperones correlates with

longevity and cancer resistance of species. The chaperones are cancer antagonists by acting as genetic buffers, stabilizing the normal phenotype. Probably the progressive age-related silencing of the housekeeping genes contributes to the phenotype of aging, with the associated increase in cancer incidence. The present review concerns epigenetic chemical, immunological, and hormonal mechanisms, activating chaperone- and immune-response genes, which have proved effective in increasing longevity and cancer resistance. The relation of steroid hormone levels to species longevity, the anticarcinogenic activity of pregnancy hormones, and the influence of hormones on the longevity of social insects, illustrates the importance of hormonal mechanisms for the activation of longevity genes.

Krupp, G., et al. (2000). "Telomerase, immortality and cancer." *Biotechnol Annu Rev* **6**: 103-140.

Replication of eukaryotic linear chromosomes is incomplete and leaves terminal gaps. The evolutionary widely distributed solution to this "end replication" is twofold: chromosome ends are capped with telomeres, bearing multiple copies of redundant telomeric sequences, and the telomerase enzyme can add (lost) telomeric repeats. Telomerase in humans, as in all mammals, is ubiquitous in all embryonic tissues. In adults, telomerase remains active in germs cells, and, although down-regulated in most somatic tissues, telomerase is active in regenerative tissues and notably, in tumor cells. Telomerase activity is linked to cellular proliferation, and its activation seems to be a mandatory step in carcinogenesis. In contrast to mammals, indeterminately growing multicellular organisms, like fish and crustaceae, maintain unlimited growth potential or 'immortality' in all somatic tissues throughout their entire life. Also this cell immortalization is brought about by maintaining telomerase expression. Disease prognosis for human tumors includes evaluation of cell proliferation, based on the detection of proliferation markers with monoclonal antibodies. The significance of the classical marker Ki-67, and of a novel marker repp-86 are compared with semiquantitative telomerase assays. For tumor therapy, telomerase inhibitors are attractive tools. Results with telomerase knock-out mice have revealed promise, but also risk of this approach. On the other side, telomerase stimulation is attractive for expanding the potential of cellular proliferation in vitro, with possible applications for transplantation of in vitro expanded human cells, for immortalizing primary human cells as improved tissue models, and for the isolation of otherwise intractable products, like genuine human monoclonal antibodies.

Kunej, T., et al. (2014). "The decalog of long non-coding RNA involvement in cancer diagnosis and monitoring." *Crit Rev Clin Lab Sci* **51**(6): 344-357.

Long non-coding RNAs (lncRNAs) are transcripts without protein-coding capacity; initially regarded as "transcriptional noise", lately they have emerged as essential factors in both cell biology and mechanisms of disease. In this article, we present basic knowledge of lncRNA molecular mechanisms, associated physiological processes and cancer association, as well as their diagnostic and therapeutic value in the form of a decalog: (1) Non-coding RNAs (ncRNAs) are transcripts without protein-coding capacity divided by size (short and long ncRNAs), function (housekeeping RNA and regulatory RNA) and direction of transcription (sense/antisense, bidirectional, intronic and intergenic), containing a broad range of molecules with diverse properties and functions, such as messenger RNA, transfer RNA, microRNA and long non-coding RNAs. (2) Long non-coding RNAs are implicated in many molecular mechanisms, such as transcriptional regulation, post-transcriptional regulation and processing of other short ncRNAs. (3) Long non-coding RNAs play an important role in many physiological processes such as X-chromosome inactivation, cell differentiation, immune response and apoptosis. (4) Long non-coding RNAs have been linked to hallmarks of cancer: (a) sustaining proliferative signaling; (b) evading growth suppressors; (c) enabling replicative immortality; (d) activating invasion and metastasis; (e) inducing angiogenesis; (f) resisting cell death; and (g) reprogramming energy metabolism. (5) Regarding their impact on cancer cells, lncRNAs are divided into two groups: oncogenic and tumor-suppressor lncRNAs. (6) Studies of lncRNA involvement in cancer usually analyze deregulated expression patterns at the RNA level as well as the effects of single nucleotide polymorphisms and copy number variations at the DNA level. (7) Long non-coding RNAs have potential as novel biomarkers due to tissue-specific expression patterns, efficient detection in body fluids and high stability. (8) lncRNAs serve as novel biomarkers for diagnostic, prognostic and monitoring purposes. (9) Tissue specificity of lncRNAs enables the development of selective therapeutic options. (10) Long non-coding RNAs are emerging as commercial biomarkers and therapeutic agents.

Kushlinskii, N. E. and M. V. Nemtsova (2014). "[Molecular biological characteristics of cancer]." *Vestn Ross Akad Med Nauk*(1-2): 5-15.

The review presents the main and additional

features that distinguish tumor cells from normal tissue cells. They include sustained proliferative signaling, evasion from growth suppressors, resisting cell death, enabling replicative immortality, angiogenesis induction, and invasion and metastasis activation. Basis for the formation of these features is provided by tumor genome instability. Tumors are complex tissues that consist of different cell types interacting with each other as well as with normal cells. An important characteristic of tumor cells is the ability to interact with the tumor microenvironment and the formation of tumor stroma.

Lambert, C. A., et al. (2017). "Chemotherapy induces alternative transcription and splicing: Facts and hopes for cancer treatment." *Int J Biochem Cell Biol* **91**(Pt B): 84-97.

Alternative promoter usage, alternative splicing and alternative cleavage/polyadenylation (referred here as to alternative transcription and splicing) are main instruments to diversify the transcriptome from a limited set of genes. There is a good deal of evidence that chemotherapeutic drugs affect these processes, but the therapeutic incidence of these effects is poorly documented. The scope of this study is to review the impact of chemotherapy on alternative transcription and splicing and to discuss potential implications in cancer therapy. A literature survey identified >2200 events induced by chemotherapeutic drugs. The molecular pathways involved in these regulations are briefly discussed. The GO terms associated with the alternative transcripts are mainly related to cell cycle/division, mRNA processing, DNA repair, macromolecules catabolism and chromatin. A large fraction (43%) of transcripts are also related to the new hallmarks of cancer, mostly genetic instability and replicative immortality. Finally, we ask the question of the impact of alternative transcription and splicing on drug efficacy and of the possible curative benefit of combining chemotherapy and pharmaceutical regulation of this process.

Lange, T. and N. Keiding (2014). "Skin cancer as a marker of sun exposure: a case of serious immortality bias." *Int J Epidemiol* **43**(3): 971.

Lapinska, K., et al. (2018). "Cancer Progenitor Cells: The Result of an Epigenetic Event?" *Anticancer Res* **38**(1): 1-6.

The concept of cancer stem cells was proposed in the late 1990s. Although initially the idea seemed controversial, the existence of cancer stem cells is now well established. However, the process leading to the formation of cancer stem cells is still not clear and thus requires further research. This

article discusses epigenetic events that possibly produce cancer progenitor cells from predisposed cells by the influence of their environment. Every somatic cell possesses an epigenetic signature in terms of histone modifications and DNA methylation, which are obtained during lineage-specific differentiation of pluripotent stem cells, which is specific to that particular tissue. We call this signature an epigenetic switch. The epigenetic switch is not fixed. Our epigenome alters with aging. However, depending on the predisposition of the cells of a particular tissue and their microenvironment, the balance of the switch (histone modifications and the DNA methylation) may be tilted to immortality in a few cells, which generates cancer progenitor cells.

Laursen, M. B., et al. (2014). "Human B-cell cancer cell lines as a preclinical model for studies of drug effect in diffuse large B-cell lymphoma and multiple myeloma." *Exp Hematol* **42**(11): 927-938.

Drug resistance in cancer refers to recurrent or primary refractory disease following drug therapy. At the cellular level, it is a consequence of molecular functions that ultimately enable the cell to resist cell death—one of the classical hallmarks of cancer. Thus, drug resistance is a fundamental aspect of the cancer cell phenotype, in parallel with sustained proliferation, immortality, angiogenesis, invasion, and metastasis. Here we present a preclinical model of human B-cell cancer cell lines used to identify genes involved in specific drug resistance. This process includes a standardized technical setup for specific drug screening, analysis of global gene expression, and the statistical considerations required to develop resistance gene signatures. The state of the art is illustrated by the first-step classical drug screen (including the CD20 antibody rituximab, the DNA intercalating topoisomerase II inhibitor doxorubicin, the mitotic inhibitor vincristine, and the alkylating agents cyclophosphamide and melphalan) along with the generation of gene lists predicting the chemotherapeutic outcome as validated retrospectively in clinical trial datasets. This B-cell lineage-specific preclinical model will allow us to initiate a range of laboratory studies, with focus on specific gene functions involved in molecular resistance mechanisms.

Lee, D. D., et al. (2020). "DNA methylation of the TERT promoter and its impact on human cancer." *Curr Opin Genet Dev* **60**: 17-24.

Telomere maintenance is a hallmark of human cancer that enables replicative immortality. Most cancer cells acquire telomere maintenance by telomerase activation through expression of

telomerase reverse transcriptase (TERT), a rate-limiting component of the telomerase holoenzyme. Although multiple cancer-specific genetic alterations such as gain of TERT copy number and recurrent TERT promoter mutations (TPM) have been identified, the majority of cancers still express TERT via unknown mechanisms. In the last decade, DNA methylation of the TERT promoter emerged as a putative epigenetic regulatory mechanism of telomerase activation in cancer. Here, we comparatively discuss studies that investigated the DNA methylation landscape of the TERT promoter. We further review the biological and clinical impacts of TERT promoter hypermethylation in cancer and provide insight into future applications of this phenomenon.

Lee, D. D., et al. (2019). "DNA hypermethylation within TERT promoter upregulates TERT expression in cancer." *J Clin Invest* **129**(1): 223-229.

Replicative immortality is a hallmark of cancer cells governed by telomere maintenance. Approximately 90% of human cancers maintain their telomeres by activating telomerase, driven by the transcriptional upregulation of telomerase reverse transcriptase (TERT). Although TERT promoter mutations (TPMs) are a major cancer-associated genetic mechanism of TERT upregulation, many cancers exhibit TERT upregulation without TPMs. In this study, we describe the TERT hypermethylated oncological region (THOR), a 433-bp genomic region encompassing 52 CpG sites located immediately upstream of the TERT core promoter, as a cancer-associated epigenetic mechanism of TERT upregulation. Unmethylated THOR repressed TERT promoter activity regardless of TPM status, and hypermethylation of THOR counteracted this repressive function. THOR methylation analysis in 1,352 human tumors revealed frequent (>45%) cancer-associated DNA hypermethylation in 9 of 11 (82%) tumor types screened. Additionally, THOR hypermethylation, either independently or along with TPMs, accounted for how approximately 90% of human cancers can aberrantly activate telomerase. Thus, we propose that THOR hypermethylation is a prevalent telomerase-activating mechanism in cancer that can act independently of or in conjunction with TPMs, further supporting the utility of THOR hypermethylation as a prognostic biomarker.

Lee, J. C., et al. (1998). "Telomerase activity in lung cancer cell lines and tissues." *Lung Cancer* **21**(2): 99-103.

Using TRAP assay, we studied the activity of the telomerase in the lung cancer cell lines, and lung cancer and normal tissues in which expression

appears to be related to the immortality of cancer cells. All the human lung cancer cell lines and the majority of human lung cancer tissues (78%) expressed telomerase activity, but this was undetectable in normal human lung tissues. Positivity for telomerase activity in lung cancer cell lines was higher than in lung cancer tissues; this result implies the expression of telomerase activity may play a crucial role in the development or progression of lung cancer, and also suggests that improved method of detection may lead to the higher positivity for telomerase activity in primary lung cancer tissues. To determine whether there is a definite causal relationship between telomerase and cancer, and to develop new anti-cancer agents which inhibit telomerase, further study is needed.

Li, C., et al. (2013). "Genetic variations in TERT-CLPTM1L genes and risk of lung cancer in Chinese women nonsmokers." *PLoS One* 8(5): e64988.

BACKGROUND: The TERT gene is the reverse transcriptase component of telomerase and is essential for the maintenance of telomere DNA length, chromosomal stability and cellular immortality. CLPTM1L gene encodes a protein linked to cisplatin resistance, and it is well conserved and express in various normal or malignant tissues, including lung. **METHODS:** To test this hypothesis, we genotyped for two significant SNPs TERT-rs2736098 and CLPTM1L-rs4016981 in a case-control study with 501 cancer cases and 576 cancer-free controls in Chinese nonsmoking population. Information concerning demographic and risk factors was obtained for each case and control by a trained interviewer. Gene polymorphisms were determined by TaqMan methodology. **RESULTS:** We found that the homozygous variant genetic model of TERT gene was associated with a significantly increased risk of lung cancer with adjusted OR of 1.72(95%CI = 1.19-2.51, P = 0.004 for heterogeneity). The joint effect of TERT and CLPTM1L increased risk for lung cancer with adjusted OR is 1.31(95%CI = 1.00-1.74, P = 0.052 for heterogeneity). **CONCLUSION:** Genetic variants in TERT and CLPTM1L may affect the susceptibility of lung cancer, especially adenocarcinoma in Chinese women nonsmokers.

Li, C. H., et al. (2021). "Stationed or Relocating: The Seesawing EMT/MET Determinants from Embryonic Development to Cancer Metastasis." *Biomedicines* 9(9).

Epithelial and mesenchymal transition mechanisms continue to occur during the cell cycle and throughout human development from the embryo stage to death. In embryo development, epithelial-mesenchymal transition (EMT) can be divided into

three essential steps. First, endoderm, mesoderm, and neural crest cells form, then the cells are subdivided, and finally, cardiac valve formation occurs. After the embryonic period, the human body will be subjected to ongoing mechanical stress or injury. The formation of a wound requires EMT to recruit fibroblasts to generate granulation tissues, repair the wound and re-create an intact skin barrier. However, once cells transform into a malignant tumor, the tumor cells acquire the characteristic of immortality. Local cell growth with no growth inhibition creates a solid tumor. If the tumor cannot obtain enough nutrition in situ, the tumor cells will undergo EMT and invade the basal membrane of nearby blood vessels. The tumor cells are transported through the bloodstream to secondary sites and then begin to form colonies and undergo reverse EMT, the so-called "mesenchymal-epithelial transition (MET)." This dynamic change involves cell morphology, environmental conditions, and external stimuli. Therefore, in this manuscript, the similarities and differences between EMT and MET will be dissected from embryonic development to the stage of cancer metastasis.

Li, K., et al. (2023). "AKAP12 promotes cancer stem cell-like phenotypes and activates STAT3 in colorectal cancer." *Clin Transl Oncol* 25(11): 3263-3276.

BACKGROUND: Cancer stem cells (CSCs) have unique biological characteristics, including tumorigenicity, immortality, and chemoresistance. Colorectal CSCs have been identified and isolated from colorectal cancers by various methods. AKAP12, a scaffolding protein, is considered to act as a potential suppressor in colorectal cancer, but its role in CSCs remains unknown. In this study, we investigated the function of AKAP12 in Colorectal CSCs. **METHODS:** Herein, Colorectal CSCs were enriched by cell culture with a serum-free medium. CSC-associated characteristics were evaluated by Flow cytometry assay and qPCR. AKAP12 gene expression was regulated by lentiviral transfection assay. The tumorigenicity of AKAP12 in vivo by constructing a tumor xenograft model. The related pathways were explored by qPCR and Western blot. **RESULTS:** The depletion of AKAP12 reduced colony formation, sphere formation, and expression of stem cell markers in colorectal cancer cells, while its knockdown decreased the volume and weight of tumor xenografts in vivo. AKAP12 expression levels also affected the expression of stemness markers associated with STAT3, potentially via regulating the expression of protein kinase C. **CONCLUSION:** This study suggests Colorectal CSCs overexpress AKAP12 and maintain stem cell characteristics

through the AKAP12/PKC/STAT3 pathway. AKAP12 may be an important therapeutic target for blocking the development of colorectal cancer in the field of cancer stem cells.

Liggett, L. A. and J. DeGregori (2017). "Changing mutational and adaptive landscapes and the genesis of cancer." *Biochim Biophys Acta Rev Cancer* **1867**(2): 84-94.

By the time the process of oncogenesis has produced an advanced cancer, tumor cells have undergone extensive evolution. The cellular phenotypes resulting from this evolution have been well studied, and include accelerated growth rates, apoptosis resistance, immortality, invasiveness, and immune evasion. Yet with all of our current knowledge of tumor biology, the details of early oncogenesis have been difficult to observe and understand. Where different oncogenic mutations may work together to enhance the survival of a tumor cell, in isolation they are often pro-apoptotic, pro-differentiative or pro-senescent, and therefore often, somewhat paradoxically, disadvantageous to a cell. It is also becoming clear that somatic mutations, including those in known oncogenic drivers, are common in tissues starting at a young age. These observations raise the question: how do we largely avoid cancer for most of our lives? Here we propose that evolutionary forces can help explain this paradox. As humans and other organisms age or experience external insults such as radiation or smoking, the structure and function of tissues progressively degrade, resulting in altered stem cell niche microenvironments. As tissue integrity declines, it becomes less capable of supporting and maintaining resident stem cells. These stem cells then find themselves in a microenvironment to which they are poorly adapted, providing a competitive advantage to those cells that can restore their functionality and fitness through mutations or epigenetic changes. The resulting oncogenic clonal expansions then increase the odds of further cancer progression. Understanding how the causes of cancer, such as aging or smoking, affect tissue microenvironments to control the impact of mutations on somatic cell fitness can help reconcile the discrepancy between marked mutation accumulation starting early in life and the somatic evolution that leads to cancer at advanced ages or following carcinogenic insults. This article is part of a Special Issue entitled: Evolutionary principles - heterogeneity in cancer?, edited by Dr. Robert A. Gatenby.

Lin, Y., et al. (1996). "Telomerase activity in human bladder cancer." *Clin Cancer Res* **2**(6): 929-932.

Telomerase can synthesize telomeric DNA

repeats onto chromosome ends. Telomere length and telomerase activity have recently been implicated in the control of the proliferative capacity of normal and malignant cells. The expression of telomerase activity is concomitant with the attainment of immortality in tumor tissues and cells. Thus, enzyme activity may indicate a prevalent or even ubiquitous tumor producer. In this report, telomerase activity was analyzed in 40 human bladder cancers, 7 normal tissues, and 2 bladder epithelia with dysplasia using a PCR-based telomeric repeat amplification protocol assay. Telomerase activity was detected in almost all bladder tumors (97.5%); only one sample, which was in an early stage, did not express telomerase activity. None of the normal tissues displayed telomerase activity. One of the two bladder epithelia with dysplasia expressed low telomerase activity. The expression of telomerase activity has a clear association with the pathological grade and clinical stage. Most of the tumors with high telomerase activity were in an advanced grade and had deep invasion. Thus, telomerase activity might be suggested to represent an additional required event in the multigenetic process of tumorigenesis in human bladder cancer.

Liu, H., et al. (2023). "Cancer-associated SMARCAL1 loss-of-function mutations promote alternative lengthening of telomeres and tumorigenesis in telomerase-negative glioblastoma cells." *Neuro Oncol* **25**(9): 1563-1575.

BACKGROUND: Telomere maintenance mechanisms are required to enable the replicative immortality of malignant cells. While most cancers activate the enzyme telomerase, a subset of cancers uses telomerase-independent mechanisms termed alternative lengthening of telomeres (ALT). ALT occurs via homology-directed-repair mechanisms and is frequently associated with ATRX mutations. We previously showed that a subset of adult glioblastoma (GBM) patients with ATRX-expressing ALT-positive tumors harbored loss-of-function mutations in the SMARCAL1 gene, which encodes an annealing helicase involved in replication fork remodeling and the resolution of replication stress. However, the causative relationship between SMARCAL1 deficiency, tumorigenesis, and de novo telomere synthesis is not understood. **METHODS:** We used a patient-derived ALT-positive GBM cell line with native SMARCAL1 deficiency to investigate the role of SMARCAL1 in ALT-mediated de novo telomere synthesis, replication stress, and gliomagenesis in vivo. **RESULTS:** Inducible rescue of SMARCAL1 expression suppresses ALT indicators and inhibits de novo telomere synthesis in GBM and osteosarcoma cells, suggesting that

SMARCAL1 deficiency plays a functional role in ALT induction in cancers that natively lack SMARCAL1 function. SMARCAL1-deficient ALT-positive cells can be serially propagated in vivo in the absence of detectable telomerase activity, demonstrating that the SMARCAL1-deficient ALT phenotype maintains telomeres in a manner that promotes tumorigenesis. **CONCLUSIONS:** SMARCAL1 deficiency is permissive to ALT and promotes gliomagenesis. Inducible rescue of SMARCAL1 in ALT-positive cell lines permits the dynamic modulation of ALT activity, which will be valuable for future studies aimed at understanding the mechanisms of ALT and identifying novel anticancer therapeutics that target the ALT phenotype.

Livingstone, J., et al. (2021). "The telomere length landscape of prostate cancer." *Nat Commun* **12**(1): 6893.

Replicative immortality is a hallmark of cancer, and can be achieved through telomere lengthening and maintenance. Although the role of telomere length in cancer has been well studied, its association to genomic features is less well known. Here, we report the telomere lengths of 392 localized prostate cancer tumours and characterize their relationship to genomic, transcriptomic and proteomic features. Shorter tumour telomere lengths are associated with elevated genomic instability, including single-nucleotide variants, indels and structural variants. Genes involved in cell proliferation and signaling are correlated with tumour telomere length at all levels of the central dogma. Telomere length is also associated with multiple clinical features of a tumour. Longer telomere lengths in non-tumour samples are associated with a lower rate of biochemical relapse. In summary, we describe the multi-level integration of telomere length, genomics, transcriptomics and proteomics in localized prostate cancer.

Lleonart, M. E., et al. (2009). "Senescence induction; a possible cancer therapy." *Mol Cancer* **8**: 3.

Cellular immortalization is a crucial step during the development of human cancer. Primary mammalian cells reach replicative exhaustion after several passages in vitro, a process called replicative senescence. During such a state of permanent growth arrest, senescent cells are refractory to physiological proliferation stimuli: they have altered cell morphology and gene expression patterns, although they remain viable with preserved metabolic activity. Interestingly, senescent cells have also been detected in vivo in human tumors, particularly in benign lesions. Senescence is a mechanism that limits cellular lifespan and constitutes a barrier against

cellular immortalization. During immortalization, cells acquire genetic alterations that override senescence. Tumor suppressor genes and oncogenes are closely involved in senescence, as their knockdown and ectopic expression confer immortality and senescence induction, respectively. By using high throughput genetic screening to search for genes involved in senescence, several candidate oncogenes and putative tumor suppressor genes have been recently isolated, including subtypes of micro-RNAs. These findings offer new perspectives in the modulation of senescence and open new approaches for cancer therapy.

Loftus, L. V., et al. (2022). "Interplay between Cell Death and Cell Proliferation Reveals New Strategies for Cancer Therapy." *Int J Mol Sci* **23**(9).

Cell division and cell death are fundamental processes governing growth and development across the tree of life. This relationship represents an evolutionary link between cell cycle and cell death programs that is present in all cells. Cancer is characterized by aberrant regulation of both, leading to unchecked proliferation and replicative immortality. Conventional anti-cancer therapeutic strategies take advantage of the proliferative dependency of cancer yet, in doing so, are triggering apoptosis, a death pathway to which cancer is inherently resistant. A thorough understanding of how therapeutics kill cancer cells is needed to develop novel, more durable treatment strategies. While cancer evolves cell-intrinsic resistance to physiological cell death pathways, there are opportunities for cell cycle agnostic forms of cell death, for example, necroptosis or ferroptosis. Furthermore, cell cycle independent death programs are immunogenic, potentially licensing host immunity for additional antitumor activity. Identifying cell cycle independent vulnerabilities of cancer is critical for developing alternative strategies that can overcome therapeutic resistance.

Losson, H., et al. (2016). "Natural Compound Histone Deacetylase Inhibitors (HDACi): Synergy with Inflammatory Signaling Pathway Modulators and Clinical Applications in Cancer." *Molecules* **21**(11).

The remarkable complexity of cancer involving multiple mechanisms of action and specific organs led researchers Hanahan and Weinberg to distinguish biological capabilities acquired by cancer cells during the multistep development of human tumors to simplify its understanding. These characteristic hallmarks include the abilities to sustain proliferative signaling, evade growth suppressors, resist cell death, enable replicative

immortality, induce angiogenesis, activate invasion and metastasis, avoid immune destruction, and deregulate cellular energetics. Furthermore, two important characteristics of tumor cells that facilitate the acquisition of emerging hallmarks are tumor-promoting inflammation and genome instability. To treat a multifactorial disease such as cancer, a combination treatment strategy seems to be the best approach. Here we focus on natural histone deacetylase inhibitors (HDACi), their clinical uses as well as synergies with modulators of the pro-inflammatory transcription factor signaling pathways.

LS, D. E. H., et al. (2019). "Characterization of Telomerase (hTERT) in Solid and Hematopoietic Cancer Cell Lines Reveals Different Expression Patterns." *Anticancer Res* 39(9): 4743-4748.

BACKGROUND/AIM: Overexpression of human telomerase reverse transcriptase (hTERT) allows disordered proliferation and immortality of malignant cells, which has been of interest for the development of targeted therapies. The present study aimed to characterize hTERT gene expression in a series of cancer cell lines. **MATERIALS AND METHODS:** Leukemia cell lines K-562, its vincristine-resistant derivative K-562-Lucena1 and daunorubicin-resistant derivative FEPS; gastric adenocarcinoma lines AGP01, ACP02 and ACP03; melanoma SK-Mel-103 cells; and MN01 and MRC5, two non-neoplastic cell lines were analyzed by real-time polymerase chain reaction in order to evaluate hTERT gene expression. **RESULTS:** In leukemia cells, hTERT gene expression was significantly increased only in K-562 ($p < 0.05$) and K-562-Lucena1 ($p < 0.001$) when compared to the calibrator MRC5. For solid tumor types, only ACP03 presented a significant hTERT gene expression when compared to ACP02 ($p < 0.05$). hTERT gene expression in K-562 and K-562-Lucena1 was significantly increased ($p < 0.05$ to $p < 0.001$) compared to all other cell lines except ACP03. **CONCLUSION:** In leukemia cell lines, hTERT gene overexpression was shown to be a potential target for pharmacological assays for drugs aiming to inhibit telomerase activity and control cell proliferation in oncohematological diseases.

Luo, M., et al. (2023). "Targeting lipid metabolism for ferroptotic cancer therapy." *Apoptosis* 28(1-2): 81-107.

It has been 10 years since the concept of ferroptosis was put forward and research focusing on ferroptosis has been increasing continuously. Ferroptosis is driven by iron-dependent lipid peroxidation, which can be antagonized by glutathione peroxidase 4 (GPX4), ferroptosis inhibitory protein 1 (FSP1), dihydroorotate

dehydrogenase (DHODH) and Fas-associated factor 1 (FAF1). Various cellular metabolic events, including lipid metabolism, can modulate ferroptosis sensitivity. It is worth noting that the reprogramming of lipid metabolism in cancer cells can promote the occurrence and development of tumors. The metabolic flexibility of cancer cells opens the possibility for the coordinated targeting of multiple lipid metabolic pathways to trigger cancer cells ferroptosis. In addition, cancer cells must obtain immortality, escape from programmed cell death including ferroptosis, to promote cancer progression, which provides new perspectives for improving cancer therapy. Targeting the vulnerability of ferroptosis has received attention as one of the significant possible strategies to treat cancer given its role in regulating tumor cell survival. We review the impact of iron and lipid metabolism on ferroptosis and the potential role of the crosstalk of lipid metabolism reprogramming and ferroptosis in antitumor immunity and sum up agents targeting lipid metabolism and ferroptosis for cancer therapy.

Madden, R. E. and R. L. Dornbush (1986). "Attitudes of medical students and faculty toward cancer." *J Cancer Educ* 1(3): 177-181.

The Cancer Attitude Survey (CAS) was administered to second year students before, during, and after a year-long "Introduction to Clinical Medicine" course. The course contained a 14-hour oncology segment. Faculty of the Clinical Medicine Course and nonclinical faculty teaching oncology related topics also responded to the CAS. The CAS contains four scales: Attitudes toward: I, the patient's inner resources to cope with cancer; IIA, the value of early diagnosis; IIB, aggressive treatment; and III, personal immortality and preparation for and acceptance of death. At the beginning of the course, student attitudes were positive; there was no change in attitudes across the three testing periods. Among faculty, dedicated oncologists had the most positive attitudes; students and nononcology clinical faculty exhibited similar trends in attitudes; nonclinical faculty teaching oncology related material had least positive attitudes.

Mahfouz, N., et al. (2017). "Gastrointestinal cancer cells treatment with bevacizumab activates a VEGF autoregulatory mechanism involving telomerase catalytic subunit hTERT via PI3K-AKT, HIF-1 α and VEGF receptors." *PLoS One* 12(6): e0179202.

BACKGROUND: Targeting angiogenesis has been considered a promising treatment of choice for a large number of malignancies, including gastrointestinal cancers. Bevacizumab is an anti-vascular endothelial growth factor (anti-VEGF) being

used for this purpose. However, treatment efficacy is largely questioned. Telomerase activity, responsible for cancer cell immortality, is detected in 85-95% of human cancers and is considered a potential regulator of VEGF. The aim of our study was to investigate the interrelationship between VEGF and hTERT in gastrointestinal cancers and to explore cell response to a combined inhibition of telomerase and VEGF. **METHODS:** AGS (gastric cancer), Caco-2 (colorectal cancer) and HepG2/C3A (hepatocellular carcinoma), were treated with telomerase inhibitors BIBR-1232 (10μM) and costunolide (10μM), with bevacizumab (Avastin(R) at 5 ng/ml or 100μg/ml) or with a combination of both types of inhibitors. VEGF and hTERT mRNA levels, and telomerase activity were detected by RT-PCR. VEGF levels were quantified by ELISA. Telomerase was knocked down using hTERT siRNA and hTERT was overexpressed in the telomerase negative cell line, Saos-2 (osteosarcoma), using constructs expressing either wild type hTERT (hTERT-WT) or dominant negative hTERT (hTERT-DN). Tube formation by HUVECs was assessed using ECMatrix (EMD Millipore). **RESULTS:** Our results showed that telomerase regulates VEGF expression and secretion through its catalytic subunit hTERT in AGS, Caco2, and HepG2/C3A, independent of its catalytic activity. Interestingly, VEGF inhibition with bevacizumab (100μg/ml) increased hTERT expression 42.3% in AGS, 94.1% in Caco2, and 52.5% in HepG2/C3A, and increased telomerase activity 30-fold in AGS, 10.3-fold in Caco2 and 8-fold in HepG2/C3A. A further investigation showed that VEGF upregulates hTERT expression in a mechanism that implicates the PI3K/AKT/mTOR pathway and HIF-1α. Moreover, bevacizumab treatment increased VEGFR1 and VEGFR2 expression in cancer cells and human umbilical vein endothelial cells (HUVECs) through hTERT. Thus, the combination of bevacizumab with telomerase inhibitors decreased VEGF expression and secretion by cancer cells, inhibited VEGFR1 and VEGFR2 upregulation, and reduced tube formation by HUVECs. **CONCLUSIONS:** Taken together, our results suggest that bevacizumab treatment activates a VEGF autoregulatory mechanism involving hTERT and VEGF receptors and that an inhibition of this pathway could improve tumor cell response to anti-VEGF treatment.

Mangosh, T. L., et al. (2021). "SLX4IP Promotes Telomere Maintenance in Androgen Receptor-Independent Castration-Resistant Prostate Cancer through ALT-like Telomeric PML Localization." *Mol Cancer Res* **19**(2): 301-316.

In advanced prostate cancer, resistance to

androgen deprivation therapy is achieved through numerous mechanisms, including loss of the androgen receptor (AR) allowing for AR-independent growth. Therapeutic options are limited for AR-independent castration-resistant prostate cancer (CRPC), and defining mechanisms critical for survival is of utmost importance for targeting this lethal disease. Our studies focus on identifying telomere maintenance mechanism (TMM) hallmarks adopted by CRPC to promote survival. TMMs are responsible for telomere elongation to instill replicative immortality and prevent senescence, with the two TMM pathways available being telomerase and alternative lengthening of telomeres (ALT). Here, we show that AR-independent CRPC demonstrates an atypical ALT-like phenotype with variable telomerase expression and activity, whereas AR-dependent models lack discernible ALT hallmarks. In addition, AR-independent CRPC cells exhibited elevated levels of SLX4IP, a protein implicated in promoting ALT. SLX4IP overexpression in AR-dependent C4-2B cells promoted an ALT-like phenotype and telomere maintenance. SLX4IP knockdown in AR-independent DU145 and PC-3 cells led to ALT-like hallmark reduction, telomere shortening, and induction of senescence. In PC-3 xenografts, this effect translated to reduced tumor volume. Using an in vitro model of AR-independent progression, loss of AR in AR-dependent C4-2B cells promoted an atypical ALT-like phenotype in an SLX4IP-dependent manner. Insufficient SLX4IP expression diminished ALT-like hallmarks and resulted in accelerated telomere loss and senescence. **IMPLICATIONS:** This study demonstrates a unique reliance of AR-independent CRPC on SLX4IP-mediated ALT-like hallmarks and loss of these hallmarks induces telomere shortening and senescence, thereby impairing replicative immortality.

Mangosh, T. L., et al. (2021). "SLX4IP N-terminus dictates telomeric localization in ALT-like castration-resistant prostate cancer cell lines." *Prostate* **81**(15): 1235-1251.

BACKGROUND: To ensure replicative immortality in cancer, telomeres must be maintained through activation of telomere maintenance mechanisms (TMMs) that are dependent on telomerase or the alternative lengthening of telomeres (ALT) pathway. Although TMM pathways have traditionally been considered to be mutually exclusive, ALT hallmarks have been identified in cancers defined as being telomerase-positive, supporting TMM coexistence. In castration-resistant prostate cancer (CRPC), in vitro models were thought to be universally dependent on telomerase as the primary TMM; however, CRPC models with

androgen receptor (AR) loss demonstrate ALT hallmarks with limited telomerase activity and require ALT-associated PML bodies (APBs) for sustained telomere maintenance. The TMM coexistence in AR-negative CRPC is reliant on the ALT regulator protein, SLX4IP. METHODS: To identify the regions of SLX4IP responsible for the induction of APBs and telomere preservation in CRPC models, five 3xFLAG-tagged SLX4IP constructs were designed and stably introduced into parental C4-2B, DU145, and PC-3 cells. Once generated, these cell lines were interrogated for APB abundance and SLX4IP construct localization via immunofluorescence-fluorescence in situ hybridization (IF-FISH) and coimmunoprecipitation experiments for telomeric localization. Similarly, PC-3 cells with endogenous SLX4IP knockdown and SLX4IP construct introduction were interrogated for APB abundance, telomere length preservation, and senescent rescue. RESULTS: Here, we define the N-terminus of SLX4IP as being responsible for the promotion of the ALT-like phenotype of AR-negative CRPC models. Specifically, the N-terminus of SLX4IP was sufficient for promoting APB formation to a similar degree as full-length SLX4IP across CRPC cell lines. Additionally, APB promotion by the N-terminus of SLX4IP rescued telomere shortening and senescent induction triggered by SLX4IP knockdown in AR-negative CRPC cells. Moreover, APB formation and telomere maintenance were dependent on the ability of the N-terminus to direct SLX4IP localization at telomeres and APBs. CONCLUSIONS: These findings identify the role of the uncharacterized ALT regulator SLX4IP in the promotion of TMM coexistence to perpetuate replicative immortality in CRPC in vitro.

Manirakiza, A., et al. (2021). "Aloe and its Effects on Cancer: A Narrative Literature Review." *East Afr Health Res J* 5(1): 1-16.

Many years ago, Aloe Vera was cited to have a lot of therapeutic properties including; anti-microbial, anti-viral, anti-cancer, anti-oxidant, anti-inflammatory, skin protection, wound healing, and regulation of blood glucose and cholesterol. However, Aloe could present some side effects. This review focused on the latest discoveries regarding the therapeutic role of Aloe plant or its compounds on the acquired biological capabilities for tumour growth and progression namely; evading growth suppressor, avoiding immune destruction, enabling replicative immortality, tumour promoting inflammation, activating invasion and metastasis, inducing angiogenesis, genome instability and mutation, resisting cell death, deregulating cellular energetics and sustaining proliferating signalling. It

clarified the anti-cancer activities it exerts on different types of cancer and also highlighted some pro-oncogenic pathways that can be disrupted by different compounds of Aloe.

Marahatta, S. B., et al. (2005). "Cancer: determinants and progression." *Nepal Med Coll J* 7(1): 65-71.

Cancer is the combination of uncontrolled cellular proliferation and immortality. It is a multi-step disease with a multi-factorial etiology. The determinants of cancer are many and varied including genetic predisposition, environmental influences, infectious agents, nutritional factors, hormonal and reproductive factors, radiation etc. However, the extent of the genetic involvement and their interaction with environment in tumorigenesis is still elusive. The six essential alterations in cell are proposed which determines the transition from normal cell to malignant. It includes--self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Nevertheless, the last two decades have seen rapid improvements in understanding the complex molecular mechanisms underlying tumorigenesis, yet the quest for unraveling the mystery is not over. Further study in this area is indispensable that could hold the promise of increasing our understanding of cancer etiology and possible preventive strategy.

Martineau, C. A., et al. (2024). "From viruses to cancer: exploring the role of the hepatitis C virus NS3 protein in carcinogenesis." *Infect Agent Cancer* 19(1): 40.

Hepatitis C virus (HCV) chronically infects approximately 170 million people worldwide and is a known etiological agent of hepatocellular carcinoma (HCC). The molecular mechanisms of HCV-mediated carcinogenesis are not fully understood. This review article focuses on the oncogenic potential of NS3, a viral protein with transformative effects on cells, although the precise mechanisms remain elusive. Unlike the more extensively studied Core and NS5A proteins, NS3's roles in cancer development are less defined but critical. Research indicates that NS3 is implicated in several carcinogenic processes such as proliferative signaling, cell death resistance, genomic instability and mutations, invasion and metastasis, tumor-related inflammation, immune evasion, and replicative immortality. Understanding the direct impact of viral proteins such as NS3 on cellular transformation is crucial for elucidating HCV's role in HCC development. Overall, this review sheds light on the

molecular mechanisms used by NS3 to contribute to hepatocarcinogenesis, and highlights its significance in the context of HCV-associated HCC, underscoring the need for further investigation into its specific molecular and cellular actions.

McKenzie, K. E., et al. (1999). "Applications of telomerase research in the fight against cancer." *Mol Med Today* 5(3): 114-122.

Telomerase, an enzyme that confers immortality upon cells and that is active in the majority of human tumors, has emerged as a powerful new marker and potential prognostic indicator and therapeutic target for cancer. Furthermore, investigations into the biology of telomerase have revealed important clues into the causes of cell death and have made progress toward answering one of the most important questions of cancer research - what gives a tumor cell an advantage over normal cells? In this article, we present the current state of telomerase research and critically assess both its potential and the pitfalls of its application in cancer diagnosis and treatment.

McKinney, A. M., et al. (2022). "GABP couples oncogene signaling to telomere regulation in TERT promoter mutant cancer." *Cell Rep* 40(12): 111344.

Telomerase activation counteracts senescence and telomere erosion caused by uncontrolled proliferation. Epidermal growth factor receptor (EGFR) amplification drives proliferation while telomerase reverse transcriptase promoter (TERTp) mutations underlie telomerase reactivation through recruitment of GA-binding protein (GABP). EGFR amplification and TERTp mutations typically co-occur in glioblastoma, the most common and aggressive primary brain tumor. To determine if these two frequent alterations driving proliferation and immortality are functionally connected, we combine analyses of copy number, mRNA, and protein data from tumor tissue with pharmacologic and genetic perturbations. We demonstrate that proliferation arrest decreases TERT expression in a GABP-dependent manner and elucidate a critical proliferation-to-immortality pathway from EGFR to TERT expression selectively from the mutant TERTp through activation of AMP-mediated kinase (AMPK) and GABP upregulation. EGFR-AMPK signaling promotes telomerase activity and maintains telomere length. These results define how the tumor cell immortality mechanism keeps pace with persistent oncogene signaling and cell cycling.

McNally, E. J., et al. (2019). "Long telomeres and cancer risk: the price of cellular immortality." *J Clin Invest* 129(9): 3474-3481.

The distribution of telomere length in humans is broad, but it has finite upper and lower boundaries. Growing evidence shows that there are disease processes that are caused by both short and long telomere length extremes. The genetic basis of these short and long telomere syndromes may be linked to mutations in the same genes, such as the telomerase reverse transcriptase (TERT), but through differential effects on telomere length. Short telomere syndromes have a predominant degenerative phenotype marked by organ failure that most commonly manifests as pulmonary fibrosis and are associated with a relatively low cancer incidence. In contrast, insights from studies of cancer-prone families as well as genome-wide association studies (GWAS) have identified both rare and common variants that lengthen telomeres as being strongly associated with cancer risk. We have hypothesized that these cancers represent a long telomere syndrome that is associated with a high penetrance of cutaneous melanoma and chronic lymphocytic leukemia. In this Review, we will synthesize the clinical and human genetic observations with data from mouse models to define the role of telomeres in cancer etiology and biology.

Meehan, K. and L. J. Vella (2016). "The contribution of tumour-derived exosomes to the hallmarks of cancer." *Crit Rev Clin Lab Sci* 53(2): 121-131.

Exosomes are small, biologically active extracellular vesicles and over the last decade, both stromal and tumour-derived exosomes (TDE) have been implicated in cancer onset, progression and metastases. Cancer is a complex disease that is underpinned by several "cancer hallmarks", originally described by Hanahan and Weinberg in 2000 and then revised in 2011. The hallmarks of cancer comprise six biological capabilities, along with two emerging hallmarks and two enabling characteristics that facilitate tumour growth and metastatic dissemination. Ample evidence supports a clear role for TDE in four of the original biological hallmarks (sustaining proliferative signalling, resisting cell death, inducing angiogenesis and activating invasion and metastases). A less-defined role exists for TDE in evading growth suppressors, and currently, there is no evidence to suggest a role for TDE in enabling replicative immortality. TDE are intimately involved in the newly defined hallmarks of cancer and enabling characteristics, most evidently in immune inhibition and tumour-promoting inflammation, which ultimately enable escape from immune destruction and tumour progression. Herein, we discuss the role of TDE in the context of the hallmarks and enabling characteristics of cancer as defined by Hanahan and Weinberg.

Meeker, A. K. (2018). "Cancer telomeres and white crows." Am J Clin Exp Urol **6**(2): 93-100.

This mini-review article discusses past and present prostate-focused research on telomere and telomerase biology conducted at Johns Hopkins, through the eyes of a Donald S Coffey trainee. Included are past discoveries of abnormalities in telomere biology in the context of prostate cancer and its pre-malignant precursor prostatic intraepithelial neoplasia (PIN); the finding that telomerase activity is androgen-regulated in the prostate, and the potential role of telomerase in prostate epithelial stem cells. Also reviewed are more recent results showing that in situ telomere length measurements in patient tissue specimens may have utility in risk assessment and as a prognostic biomarker. Highlighted throughout the article are some of the training and mentorship approaches employed by the late Dr. Coffey, former Director of Urologic Research at the Brady Urological Research Institute, which inspired new research ideas, team science, and discovery.

Meeker, A. K. and D. S. Coffey (1997). "Telomerase: a promising marker of biological immortality of germ, stem, and cancer cells. A review." Biochemistry (Mosc) **62**(11): 1323-1331.

This review will describe the current state of knowledge of telomerase as it relates to human malignancies, focusing primarily on published measurements of this enzymes activity in benign and malignant neoplasms and their normal tissue counterparts. Key questions concerning the potential clinical utility of assaying for telomerase activity will be addressed and the implications of recent findings discussed.

Meerwein, F., et al. (1976). "[Remarks on the physician-patient relationship with cancer patients. Prerequisites, function, and goal of so-called Balint groups in an internal-oncological department]." Z Psychosom Med Psychoanal **22**(3): 278-300.

Drawing on their experience with their own cancer patients and with the Balint Group in the Oncological Department of Zurich's University Hospital, the authors describe the special problems arising in the doctor-patient relationship in this field. They show how the diagnosis of cancer can give rise to a feeling of sudden and complete object loss in the patient, thereby confronting the doctor with his own fear of death. The mobilization of archaic defence mechanisms in both the doctor and the patients can lead to an insoluble double-blind situation unless the doctor is able to give up his defence position and thus make it possible for the patient to give up his own fear of death and to accept the nature of his illness.

The authors show how the doctor can break through the isolation of the patient in whose body-ego an archaic bad inner-object has been activated by the cancer, and build up good inner objects for him again. In the last chapter Winnicott's idea the "intermediate area" is shown to shed a new light on the phenomenon of redential or belief in immortality.

Meiyanto, E. and Y. A. Larasati (2019). "The Chemopreventive Activity of Indonesia Medicinal Plants Targeting on Hallmarks of Cancer." Adv Pharm Bull **9**(2): 219-230.

Cancer remains a complex disease with increasing global mortality and morbidity. Numerous theories have been established to understand the biological mechanism underlying cancer. One of the most renowned frameworks is the hallmark of cancer proposed by Hanahan and Weinberg that covers ten eminent characteristics of cancer: (i) genome instability and mutation, (ii) sustaining proliferative signaling, (iii) evading growth suppressor, (iv) enabling replicative immortality, (v) resisting cell death, (vi) inducing angiogenesis, (vii) activating invasion and metastasis, (viii) avoiding immune destruction, (ix) tumor-promoting inflammation, and (x) deregulating cellular energetics. These hallmarks provide a rational approach to design an anticancer therapy. In the current review, we summarized specific target molecules on each hallmark of cancer. Further, we evaluated the biological activity of several Indonesia medicinal plants against those specific targets. We explicated the anticancer and chemopreventive activities of some medicinal plants that have been used for centuries by local communities in Indonesia, including *Curcuma* genus, *Brucea javanica*, *Boesenbergia pandurata*, *Caesalpinia sappan*, and *Nigella sativa*. Interestingly, these medicinal plants target several hallmarks of cancer, and even *Curcuma* genus exhibited biological activities that target all hallmarks of cancer. Further, we also discuss several strategies to develop those medicinal plants and/or their active compounds as anticancer and chemopreventive agents.

Menendez, J. A., et al. (2012). "Metformin is synthetically lethal with glucose withdrawal in cancer cells." Cell Cycle **11**(15): 2782-2792.

Glucose deprivation is a distinctive feature of the tumor microecosystem caused by the imbalance between poor supply and an extraordinarily high consumption rate. The metabolic reprogramming from mitochondrial respiration to aerobic glycolysis in cancer cells (the "Warburg effect") is linked to oncogenic transformation in a manner that frequently implies the inactivation of metabolic checkpoints such as the energy rheostat

AMP-activated protein kinase (AMPK). Because the concept of synthetic lethality in oncology can be applied not only to genetic and epigenetic intrinsic differences between normal and cancer cells but also to extrinsic ones such as altered microenvironment, we recently hypothesized that stress-energy mimickers such as the AMPK agonist metformin should produce metabolic synthetic lethality in a glucose-starved cell culture milieu imitating the adverse tumor growth conditions in vivo. Under standard high-glucose conditions, metformin supplementation mostly caused cell cycle arrest without signs of apoptotic cell death. Under glucose withdrawal stress, metformin supplementation circumvented the ability of oncogenes (e.g., HER2) to protect breast cancer cells from glucose-deprivation apoptosis. Significantly, representative cell models of breast cancer heterogeneity underwent massive apoptosis (by >90% in some cases) when glucose-starved cell cultures were supplemented with metformin. Our current findings may uncover crucial issues regarding the cell-autonomous metformin's anti-cancer actions: (1) The offently claimed clinically irrelevant, non-physiological concentrations needed to observe the metformin's anti-cancer effects in vitro merely underlie the artifactual interference of erroneous glucose-rich experimental conditions that poorly reflect glucose-starved in vivo conditions; (2) the preferential killing of cancer stem cells (CSC) by metformin may simply expose the best-case scenario for its synthetically lethal activity because an increased dependency on Warburg-like aerobic glycolysis (hyperglycolytic phenotype) is critical to sustain CSC stemness and immortality; (3) the microenvironment-mediated contextual synthetic lethality of metformin should be expected to enormously potentiate the anti-cancer effect of anti-angiogenesis agents that promote severe oxygen and glucose deprivation in certain areas of the tumor tissues.

Menyhart, O., et al. (2016). "Guidelines for the selection of functional assays to evaluate the hallmarks of cancer." *Biochim Biophys Acta* **1866**(2): 300-319.

The hallmarks of cancer capture the most essential phenotypic characteristics of malignant transformation and progression. Although numerous factors involved in this multi-step process are still unknown to date, an ever-increasing number of mutated/alterd candidate genes are being identified within large-scale cancer genomic projects. Therefore, investigators need to be aware of available and appropriate techniques capable of determining characteristic features of each hallmark. We review the methods tailored to experimental cancer

researchers to evaluate cell proliferation, programmed cell death, replicative immortality, induction of angiogenesis, invasion and metastasis, genome instability, and reprogramming of energy metabolism. Selecting the ideal method is based on the investigator's goals, available equipment and also on financial constraints. Multiplexing strategies enable a more in-depth data collection from a single experiment - obtaining several results from a single procedure reduces variability and saves time and relative cost, leading to more robust conclusions compared to a single end point measurement. Each hallmark possesses characteristics that can be analyzed by immunoblot, RT-PCR, immunocytochemistry, immunoprecipitation, RNA microarray or RNA-seq. In general, flow cytometry, fluorescence microscopy, and multiwell readers are extremely versatile tools and, with proper sample preparation, allow the detection of a vast number of hallmark features. Finally, we also provide a list of hallmark-specific genes to be measured in transcriptome-level studies. Although our list is not exhaustive, we provide a snapshot of the most widely used methods, with an emphasis on methods enabling the simultaneous evaluation of multiple hallmark features.

Mihich, E. and L. Hartwell (1997). "Eighth Annual Pezcoller Symposium: genomic instability and immortality in cancer." *Cancer Res* **57**(19): 4437-4441.

Minakshi, R., et al. (2017). "Implications of aging and the endoplasmic reticulum unfolded protein response on the molecular modality of breast cancer." *Exp Mol Med* **49**(11): e389.

The endoplasmic reticulum (ER) is an important subcellular organelle that is involved in numerous activities required to achieve and maintain functional proteins in addition to its role in the biosynthesis of lipids and as a repository of intracellular Ca(2+). The inability of the ER to cope with protein folding beyond its capacity causes disturbances that evoke ER stress. Cells possess molecular mechanisms aimed at clearing unwanted cargo from the ER lumen as an adaptive response, but failing to do so navigates the system towards cell death. This systemic approach is called the unfolded protein response. Aging insults cells through various perturbations in homeostasis that involve curtailing ER function by mitigating the expression of its resident chaperones and enzymes. Here the unfolded protein response (UPR) cannot protect the cell due to the weakening of its protective arm, which exacerbates imbalanced homeostasis. Aging predisposed breast malignancy activates the UPR, but

tumor cells maneuver the mechanistic details of the UPR, favoring tumorigenesis and thereby eliciting a treacherous condition. Tumor cells exploit UPR pathways via crosstalk involving various signaling cascades that usher tumor cells to immortality. This review aims to present a collection of data that can delineate the missing links of molecular signatures between aging and breast cancer.

Misawa, M., et al. (2002). "Inhibition of human telomerase enhances the effect of chemotherapeutic agents in lung cancer cells." *Int J Oncol* **21**(5): 1087-1092.

Telomerase is a ribonucleoprotein enzyme that maintains protective structures at the ends of eukaryotic chromosomes. Earlier studies have reported that the presence of telomerase activity in tumors of patients with non-small cell lung cancer patients correlates with a high proliferation rate and advanced pathological stage. Thus, the modification of telomerase activity may be a potential therapeutic modality for the treatment of lung and other cancers. We introduced vectors encoding dominant negative (DN)-hTERT, or wild-type (WT)-hTERT, or a control vector expressing only a drug-resistance marker, into the A549 lung cancer cell line, and assessed the biological effect of telomerase inhibition on cellular immortality. Ectopic expression of DN-hTERT resulted in complete inhibition of telomerase activity and reduction of telomere length. The entire population of telomerase-inhibited A549 cells exhibited cytoplasmic blebbing and chromatin condensation, which are features of apoptosis. In contrast, A549 cells expressing wild-type hTERT, which differs from the mutants by only two amino acids, exhibited normal morphology. Evidence for apoptosis in the telomerase-inhibited cells was provided by flow cytometric analysis with APO2.7 monoclonal antibody. We also observed enhanced induction of apoptosis by chemotherapeutic reagents, including cisplatin, docetaxel and etoposide, in DN-hTERT-expressing A549 cells, as compared with WT-hTERT-expressing cells. These results demonstrate that disruption of telomere maintenance limits the cellular lifespan of lung cancer cells, and show that the combined use of chemotherapeutic agents and telomere maintenance inhibition may be effective in the treatment of patients with non-small cell lung cancer.

Miyachi, K., et al. (2002). "Correlation between telomerase activity and telomeric-repeat binding factors in gastric cancer." *J Exp Clin Cancer Res* **21**(2): 269-275.

Telomeres of a specific length are essential for continuous cell proliferation. The length of

telomeres must be maintained by telomerase action and the telomeric DNA-repeat binding protein must be protected. Therefore, there seems to be a relationship between cell immortality due to telomerase activity and telomeric DNA-repeat binding protein. We examined telomerase activity and the expression of telomeric-repeat binding factor 1 and 2 (TRF1 and TRF2) in gastric cancer. Telomerase activity was semi-quantified using the f-TRAP technique in 53 cancerous and non-cancerous gastric tissue specimens. TRF1 and TRF2 were also studied using an immunohistochemical method to determine the frequency of these factors in cell nuclei. Telomerase activity was observed in 79.2% of the cancerous tissue and in 39.6% of the non-cancerous tissue. The average semi-quantitative values for telomerase activity were 67.3 total product generated (TPG) unit/microg protein in cancerous tissue and 6.0 TPG unit/microg protein in non-cancerous tissue. Moreover, T0/1 tumor had the same incidence of telomerase activity as T2 or deeper tumors. These results indicated that the activation of telomerase begins at an early stage of carcinogenesis. TRF1 and TRF2 were detected in 45.1% and 42.9% of the cancerous tissue and in 70.6% and 65.6% of the non-cancerous tissue, respectively. In addition, low positive staining ratios were found for TRF1 and TRF2 when cancer had more deeply invaded. However, telomerase activity did not correlate with either TRF1 or TRF2. These findings suggest that optimal conditions for efficient telomerase are produced as cancer progresses, via suppression of TRFs.

Mizumoto, K. and M. Tanaka (2005). "Detection of telomerase activity in patients with pancreatic cancer." *Methods Mol Med* **103**: 199-205.

Telomerase, which ensures the unlimited proliferation by adding TTAGGG repeat at the end of the chromosome, is strongly activated at a very high incidence in a variety of malignant neoplasms including pancreatic cancer. In addition to the acquisition of the immortality, telomerase plays an important role in the aggressive behavior of pancreatic cancer. Invasiveness of human pancreatic cancer cells correlates well with telomerase activity. Exposure of pancreatic cancer to anticancer drugs up-regulates telomerase activity, and the increase in telomerase activity correlates with resistance to the drug-induced apoptosis. More important, diagnostic values of telomerase activity are highly focused because of the lack of other specific genetic markers for pancreatic cancer. Samples of pancreatic juice are obtained at endoscopic retrograde pancreatography using a balloon catheter after intravenous injection of secretin. Because the pancreatic juice has strong

protease and RNase activity, addition of protease inhibitors and RNase inhibitors in the telomerase extraction buffer is necessary for the detection of telomerase activity in the pancreatic juice. A telomeric ladder was detected in 80% patients with carcinoma, whereas only 4.3% patients with adenoma and none with chronic pancreatitis showed positive telomerase activity.

Mokbel, K. (2003). "The evolving role of telomerase inhibitors in the treatment of cancer." *Curr Med Res Opin* **19**(6): 470-472.

Telomerase is a ribonucleoprotein that maintains telomeres and is essential for cellular immortality and tumour growth. The differential expression of telomerase in cancer cells makes it an attractive therapeutic target. Anti-sense oligonucleotides directed against the RNA template of hTR and small molecules that can interact and stabilise the G-quadruplex represent promising therapeutic strategies. Human trials investigating the potential role of the catalytic subunit hTERT as a universal cancer vaccine have already commenced. Alternative lengthening of telomeres (ALT) and efficacy delay remain important limitations to anti-telomerase therapy.

Mokbel, K., et al. (2000). "The association between telomerase activity and hormone receptor status and p53 expression in breast cancer." *Int J Surg Investig* **1**(6): 509-516.

BACKGROUND: Telomerase is a ribonucleoprotein enzyme that seems to play an important role in cellular immortality and carcinogenesis. p53 mutations account for approximately 50% of human cancers and represent the most frequent genetic lesion in breast cancer. **AIMS:** This study aims to examine the association between telomerase reactivation and hormonal receptor status and p53 expression in invasive breast cancer. **METHODS:** Using a polymerase chain reaction-based assay, telomerase activity was determined in 47 invasive breast carcinomas and 21 adjacent non-cancerous breast tissue specimens (stored at -80 degrees C) prospectively collected from 47 women undergoing elective surgical treatment in our centre. The histopathological features of the tumour were determined by experienced breast pathologists using light microscopy and haematoxylin and eosin staining. Oestrogen receptor (ER), progesterone receptor (PR) and p53 expressions were determined using immunohistochemistry techniques. **RESULTS:** Telomerase activity was detected in 34 (72%) of 47 breast carcinomas and in none of the adjacent non-cancerous breast specimens. There was a significant

association between telomerase reactivation, tumour size and nodal status. Telomerase positive tumours were more likely to be poorly differentiated (65% versus 46%), but this association failed to reach statistical significance. There was no significant difference in ER expression (68% versus 85%). PR expression (62% versus 62%) and p53 expression (19% versus 27%) between telomerase positive and telomerase negative cancers. **CONCLUSION:** Telomerase reactivation is associated with important prognostic factors such as tumour size and nodal status in invasive breast cancer and seems to be independent of hormonal receptor status and p53 expression.

Moloudizargari, M., et al. (2022). "Targeting Hippo signaling pathway by phytochemicals in cancer therapy." *Semin Cancer Biol* **80**: 183-194.

The current era of cancer research has been continuously advancing upon identifying novel aspects of tumorigenesis and the principal mechanisms behind the unleashed proliferation, invasion, drug resistance and immortality of cancer cells in hopes of exploiting these findings to achieve a more effective treatment for cancer. In pursuit of this goal, the identification of the first components of an extremely important regulatory pathway in *Drosophila melanogaster* that largely determines cell fate during the developmental stages, ended up in the discovery of the highly sophisticated Hippo signaling cascade. Soon after, it was revealed that deregulation of the components of this pathway either via mutations or through epigenetic alterations can be observed in a vast variety of tumors and these alterations greatly contribute to the neoplastic transformation of cells, their survival, growth and resistance to therapy. As more hidden aspects of this pathway such as its widespread entanglement with other major cellular signaling pathways are continuously being uncovered, many researchers have sought over the past decade to find ways of therapeutic interventions targeting the major components of the Hippo cascade. To date, various approaches such as the use of exogenous targeting miRNAs and different molecular inhibitors have been recruited herein, among which naturally occurring compounds have shown a great promise. On such a basis, in the present work we review the current understanding of Hippo pathway and the most recent evidence on targeting its components using natural plant-derived phytochemicals.

Mori, J. O., et al. (2024). "Alternative lengthening of telomeres: mechanism and the pathogenesis of cancer." *J Clin Pathol* **77**(2): 82-86.

Telomere maintenance and elongation

allows cells to gain replicative immortality and evade cellular senescence during cancer development. While most cancers use telomerase to maintain telomere lengths, a subset of cancers engage the alternative lengthening of telomeres (ALT) pathway for telomere maintenance. ALT is present in 5%-10% of all cancers, although the prevalence is dramatically higher in certain cancer types, including complex karyotype sarcomas, isocitrate dehydrogenase-mutant astrocytoma (WHO grade II-IV), pancreatic neuroendocrine tumours, neuroblastoma and chromophobe hepatocellular carcinomas. ALT is maintained through a homology-directed DNA repair mechanism. Resembling break-induced replication, this aberrant process results in dramatic cell-to-cell telomere length heterogeneity, widespread chromosomal instability and chronic replication stress. Additionally, ALT-positive cancers frequently harbour inactivating mutations in either chromatin remodelling proteins (ATRX, DAXX and H3F3A) or DNA damage repair factors (SMARCA1 and SLX4IP). ALT can readily be detected in tissue by assessing the presence of unique molecular characteristics, such as large ultrabright nuclear telomeric foci or partially single-stranded telomeric DNA circles (C-circles). Importantly, ALT has been validated as a robust diagnostic and prognostic biomarker for certain cancer types and may even be exploited as a therapeutic target via small molecular inhibitors and/or synthetic lethality approaches.

Mungan, I., et al. (2020). "Correction to: Does the preoperative platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio predict morbidity after gastrectomy for gastric cancer?" *Mil Med Res* 7(1): 12.

In the original publication of this article [1] there are two garbled codes in the second sentence, the fourth paragraph of the Background section. The correct sentence should be: Tumor growth leads to the increased production of inflammatory cytokines and growth factors (mainly IL-1, IL-3, IL-6, IL-11, IL-23, and TNF-), and this perpetual process ensures immortality. These promoting factors are also important for angiogenesis and hematopoiesis, which explains the increase in blood cell types in cancerous diseases. The original publication has been corrected.

Nagy, A., et al. (2021). "Pancancer survival analysis of cancer hallmark genes." *Sci Rep* 11(1): 6047.

Cancer hallmark genes are responsible for the most essential phenotypic characteristics of malignant transformation and progression. In this study, our aim was to estimate the prognostic effect of the established cancer hallmark genes in multiple distinct cancer types. RNA-seq HTSeq counts and

survival data from 26 different tumor types were acquired from the TCGA repository. DESeq was used for normalization. Correlations between gene expression and survival were computed using the Cox proportional hazards regression and by plotting Kaplan-Meier survival plots. The false discovery rate was calculated to correct for multiple hypothesis testing. Signatures based on genes involved in genome instability and invasion reached significance in most individual cancer types. Thyroid and glioblastoma were independent of hallmark genes (61 and 54 genes significant, respectively), while renal clear cell cancer and low grade gliomas harbored the most prognostic changes (403 and 419 genes significant, respectively). The eight genes with the highest significance included BRCA1 (genome instability, HR 4.26, $p < 1E-16$), RUNX1 (sustaining proliferative signaling, HR 2.96, $p = 3.1E-10$) and SERPINE1 (inducing angiogenesis, HR 3.36, $p = 1.5E-12$) in low grade glioma, CDK1 (cell death resistance, HR = 5.67, $p = 2.1E-10$) in kidney papillary carcinoma, E2F1 (tumor suppressor, HR 0.38, $p = 2.4E-05$) and EREG (enabling replicative immortality, HR 3.23, $p = 2.1E-07$) in cervical cancer, FBP1 (deregulation of cellular energetics, HR 0.45, $p = 2.8E-07$) in kidney renal clear cell carcinoma and MYC (invasion and metastasis, HR 1.81, $p = 5.8E-05$) in bladder cancer. We observed unexpected heterogeneity and tissue specificity when correlating cancer hallmark genes and survival. These results will help to prioritize future targeted therapy development in different types of solid tumors.

Nash, J. M. (1997). "The immortality enzyme. A newly discovered gene may help scientists combat cancer and ailments linked to aging." *Time* 150(9): 65.

Negrini, S., et al. (2020). "Anti-cancer Immunotherapies Targeting Telomerase." *Cancers (Basel)* 12(8).

Telomerase is a reverse transcriptase that maintains telomeres length, compensating for the attrition of chromosomal ends that occurs during each replication cycle. Telomerase is expressed in germ cells and stem cells, whereas it is virtually undetectable in adult somatic cells. On the other hand, telomerase is broadly expressed in the majority of human tumors playing a crucial role in the replicative behavior and immortality of cancer cells. Several studies have demonstrated that telomerase-derived peptides are able to bind to HLA (human leukocyte antigen) class I and class II molecules and effectively activate both CD8(+) and CD4(+) T cells subsets. Due to its broad and selective expression in cancer cells and its significant immunogenicity, telomerase

is considered an ideal universal tumor-associated antigen, and consequently, a very attractive target for anti-cancer immunotherapy. To date, different telomerase targeting immunotherapies have been studied in pre-clinical and clinical settings, these approaches include peptide vaccination and cell-based vaccination. The objective of this review paper is to discuss the role of human telomerase in cancer immunotherapy analyzing recent developments and future perspectives in this field.

Newbold, R. F. (1999). "Telomerase as an anti-cancer drug target: will it fulfil its early promise?" *Anticancer Drug Des* **14**(4): 349-354.

The discovery that the ribonucleoprotein telomerase is responsible for the immortality of human cancer cells represents a major advance in our quest to identify a distinguishing biochemical feature of the malignant phenotype that could be useful as a target for novel anti-cancer drug development. However, recent observations on telomere dynamics and cell lifespan using telomerase 'knockout' mouse models together with improved techniques to assay telomerase in normal human tissues have raised certain questions regarding potential side effects of anti-telomerase treatments. More importantly, such work has also demonstrated the propensity of mouse cell populations, in which telomerase has been experimentally inactivated, to generate immortal variants capable of maintaining their telomeres by alternative mechanisms. These recent findings and their implications for the potential success of anti-telomerase therapies are subjected to critical review. The wide differences between telomerase and telomere biology in mouse and human cells are highlighted, and the urgent need to obtain direct experimental evidence concerning the behaviour of a wide variety of human cancer cells under conditions of telomerase inhibition is stressed. It is concluded that, despite the caveats, the development of small molecule drugs that powerfully inhibit telomerase should remain a top priority area for those engaged in the rational design of novel cancer therapeutics.

Niitsu, Y. and K. H. Plate (1999). "Regulation of machinery for cancer cell growth, immortality, apoptosis and invasion--the Eighteenth International Symposium of Sapporo Cancer Seminar." *Jpn J Cancer Res* **90**(3): 365-366.

Nyalali, A. M. K., et al. (2023). "CD147: an integral and potential molecule to abrogate hallmarks of cancer." *Front Oncol* **13**: 1238051.

CD147 also known as EMMPRIN, basigin, and HAb18G, is a single-chain type I transmembrane protein shown to be overexpressed in aggressive

human cancers of CNS, head and neck, breasts, lungs, gastrointestinal, genitourinary, skin, hematological, and musculoskeletal. In these malignancies, the molecule is integral to the diverse but complimentary hallmarks of cancer: it is pivotal in cancerous proliferative signaling, growth propagation, cellular survival, replicative immortality, angiogenesis, metabolic reprogramming, immune evasion, invasion, and metastasis. CD147 also has regulatory functions in cancer-enabling characteristics such as DNA damage response (DDR) and immune evasion. These neoplastic functions of CD147 are executed through numerous and sometimes overlapping molecular pathways: it transduces signals from upstream molecules or ligands such as cyclophilin A (CyPA), CD98, and S100A9; activates a repertoire of downstream molecules and pathways including matrix metalloproteinases (MMPs)-2,3,9, hypoxia-inducible factors (HIF)-1/2alpha, PI3K/Akt/mTOR/HIF-1alpha, and ATM/ATR/p53; and also functions as an indispensable chaperone or regulator to monocarboxylate, fatty acid, and amino acid transporters. Interestingly, induced loss of functions to CD147 prevents and reverses the acquired hallmarks of cancer in neoplastic diseases. Silencing of Cd147 also alleviates known resistance to chemoradiotherapy exhibited by malignant tumors like carcinomas of the breast, lung, pancreas, liver, gastric, colon, ovary, cervix, prostate, urinary bladder, glioblastoma, and melanoma. Targeting CD147 antigen in chimeric and induced-chimeric antigen T cell or antibody therapies is also shown to be safer and more effective. Moreover, incorporating anti-CD147 monoclonal antibodies in chemoradiotherapy, oncolytic viral therapy, and oncolytic virus-based-gene therapies increases effectiveness and reduces on and off-target toxicity. This study advocates the expedition and expansion by further exploiting the evidence acquired from the experimental studies that modulate CD147 functions in hallmarks of cancer and cancer-enabling features and strive to translate them into clinical practice to alleviate the emergency and propagation of cancer, as well as the associated clinical and social consequences.

Ohki, S., et al. (1998). "[Telomerase activity in colorectal cancer--a semi-quantitative procedure]." *Gan To Kagaku Ryoho* **25 Suppl 3**: 469-474.

Telomerase maintains telomere at the end of chromosome and stabilizes chromosome. It is thought to have important roles in cancer progression and cell immortality. We evaluated the role of telomerase expression in colorectal carcinogenesis. Materials included 13 colonic adenomas, 9 early colorectal cancers, 32 advanced colorectal cancers, 5 metastatic tumors, and 30 non-cancerous colon mucosas. The

telomerase activity was analyzed using TRAP-eze (Oncor Inc.) for a semi-quantitative method. The positive rate of telomerase activity was 13.3% in non-cancerous colonic mucosa, 15.4% in colonic adenomas, 77.8% in early colorectal cancers, 93.8% in advanced colorectal cancers, and 100% in metastatic tumors; the mean value was 18.0, 29.9, 65.8, 97.0 and 161.3. The correlation between telomerase activity and tumor size, histologic type, or depth of invasion was noted. Sensitivity, specificity and accuracy were on the order of 89%, 98% and 93% at the cut-off level as two times the mean value of non-cancerous mucosa. Telomerase had an important role in carcinogenesis, and progression of colorectal cancer, and it was suggested to be useful for a tumor marker.

Oshi, M., et al. (2023). "Accelerated glycolysis in tumor microenvironment is associated with worse survival in triple-negative but not consistently with ER+/HER2- breast cancer." *Am J Cancer Res* **13**(7): 3041-3054.

Metabolic reprogramming to sustain immortality is a hallmark of cancer and glycolysis is an important way to attain this. Thus, we investigate the association of glycolysis and associated pathways in the survival of breast cancer. A total of 5,176 breast cancer patients from multiple independent cohorts were analyzed. We determined the glycolytic signaling score by the degree of enrichment by Gene Set Variant Analysis and the median was used to divide each cohort into high vs low score groups. Glycolysis high breast cancer significantly enriched the hallmark cell proliferation-related gene sets (E2F targets, G2M checkpoint, and MYC targets v1 and v2) and was associated with high MKI67 expression. In all cohorts, triple-negative breast cancer (TNBC) was associated with the highest glycolysis score. It was found that in TNBC, glycolysis high breast cancer was associated with worse survival but in ER-positive/HER2-negative breast cancer this was not observed consistently. The glycolysis high TNBC enriched multiple pro-cancerous gene sets and was infiltrated with a low level of B-cells and anti-cancerous immune cells, and significantly associated with a decreased level of cytolytic activity. It was also observed that the glycolysis was higher in the metastatic sites than in the primary breast cancer and the survival was not affected by the metastatic sites. In conclusion, accelerated glycolysis is associated with cancer cell proliferation and worse survival in TNBC.

Park, W. C., et al. (2005). "Deregulation of estrogen induced telomerase activity in tamoxifen-resistant breast cancer cells." *Int J Oncol* **27**(5): 1459-1466.

Telomerase, a ribonucleoprotein enzyme that functions as a reverse transcriptase, is detected exclusively in immortal cells such as germ cells, stem cells and cancer cells. Telomerase activity is present in almost all human cancers. Telomerase activation is considered to be essential to maintain the integrity of the replicating tumor cell and to establish immortality. Based on this concept antiestrogen should initially regulate estrogen-stimulated telomerase but the enzyme would be expected to be constitutive in tamoxifen-resistant tumor cells. We have studied the estrogen regulation of telomerase in T47D:A18 breast cancer cells with a TRAPEZE Telomerase detection kit. Estradiol significantly increased telomerase activity after a 2-day treatment. Telomerase activity induced by estradiol was up to 10-fold higher within 4 days. Antiestrogens 4-hydroxytamoxifen (4-OHT) and ICI 182,780 were inactive alone and significantly blocked estradiol-stimulated increase in telomerase. These effects were correlated with changes in cell replications and changes in the cell cycle. In contrast, 4-OHT resistant T47D:A18 cells (T47D:A18/4-OHT, cultured in 1 microM 4-OHT for 6 months) grew spontaneously and had no changes in the cell cycle with estrogen treatment. The estrogen receptor (ERalpha) was present and still regulated at an estrogen responsive luciferase reporter gene with estrogen despite the fact that progesterone receptor was not increased in response to estradiol in T47D:A18/4-OHT cells. However, telomerase activity was increased about 40-fold in T47D:A18/4-OHT cells and this was not regulated by ICI 182,780. We conclude that the differential regulation of telomerase gene might be an important transition for tamoxifen resistance in T47D:A18 breast cancer cells.

Pedersen, P. L. (2012). "Mitochondria in relation to cancer metastasis: introduction to a mini-review series." *J Bioenerg Biomembr* **44**(6): 615-617.

This introductory article and those that follow focus on the roles that mitochondria may have in cancer metastasis (spreading) that all too frequently leads to death of cancer patients. The history of cancer dates back in time to several thousand years BC and continues to this day. Although billions of dollars have been invested, numerous cancer researchers/scientists and oncologist located at universities, hospitals, cancer centers, commercial entities (companies), and government agencies have been unable to discover "magic bullets" to quickly silence most cancers. That is, agents that are effective not only in eradicating the primary tumor at its site of origin, but eradicating also distant tumors that have arisen therefrom via metastatic cells. Fortunately, in recent years some

researchers have obtained evidence that the mitochondria of cancer cells are involved not only in providing in part the necessary energy (ATP) to fuel their growth, but hold the secrets to their immortality, and propensity to metastasize (spread) from their original site of origin to other body locations. This introductory article, as well as those that follow, focus on the possible roles of mitochondria in cancer metastasis as well as strategies to arrest cancer metastasis based on this knowledge. Ideally, for a patient to become "cancer free" the anticancer agent/agents used must 1) eradicate the primary tumor at its site of origin, 2) eradicate any tumors at other body locations that have arisen via metastasis, and 3) eradicate any tumor cells that remain in the blood, i.e., circulating tumor cells. One such agent that holds promise for doing all three is the small molecule 3-bromopyruvate (3BP) discovered in the author's laboratory by Dr. Young H. Ko near the turn of the century to be a potent anti-cancer agent [Ko et al.(2001) *Can Lett* 173:83-91].

Perillo, M., et al. (2024). "Peto's paradox: Nature has used multiple strategies to keep cancer at bay while evolving long lifespans and large body masses. A systematic review." *Biomed J* 47(2): 100654.

Comparative oncology is an understudied field of science. We are far from understanding the key mechanisms behind Peto's paradox, i.e., understanding how long-lived and large animals are not subject to a higher cancer burden despite the longer exposure time to mutations and the larger number of cells exposed. In this work, we investigated the scientific evidence on such mechanisms through a systematic mini-review of the literature about the relation of longevity and/or large body mass with physiological, genetic, or environmental traits among mammalian species. More than forty thousand articles were retrieved from three repositories, and 383 of them were screened using an active-learning-based tool. Of those, 36 articles on longevity and 37 on body mass were selected for the review. Such articles were examined focusing on: number and type of species considered, statistical methods used, traits investigated, and observed relationship with longevity and/or body mass. Where applicable, the traits investigated were matched with one or more hallmarks of cancer. We obtained a list of potential candidate traits to explain Peto's paradox related to replicative immortality, cell senescence, genome instability and mutations, proliferative signaling, growth suppression evasion, and cell resistance to death. Our investigation suggests that different strategies have been followed to prevent cancer in large and long-lived species. The large number of papers retrieved emphasizes that

more studies can be launched in the future, using more efficient analytical approaches to comprehensively evaluate the convergent biological mechanisms essential for acquiring longevity and large body mass without increasing cancer risk.

Perri, F., et al. (2022). "Cancer Cell Metabolism Reprogramming and Its Potential Implications on Therapy in Squamous Cell Carcinoma of the Head and Neck: A Review." *Cancers (Basel)* 14(15).

Carcinogenesis is a multistep process that consists of the transformation of healthy cells into cancer cells. Such an alteration goes through various stages and is closely linked to random mutations of genes that have a key role in the neoplastic phenotype. During carcinogenesis, cancer cells acquire and exhibit several characteristics including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, and expressing an immune phenotype, which allow them to evade recognition and destruction through cognate immune cells. In addition, cancer cells may acquire the ability to reprogram their metabolism in order to further promote growth, survival, and energy production. This phenomenon, termed metabolic reprogramming, is typical of all solid tumors, including squamous carcinomas of the head and neck (SCCHN). In this review, we analyze the genetic and biological mechanisms underlying metabolic reprogramming of SCCHN, focusing on potential therapeutic strategies that are able to counteract it.

Philippi, C., et al. (2010). "Telomerase as an emerging target to fight cancer--opportunities and challenges for nanomedicine." *J Control Release* 146(2): 228-240.

Telomerase as an enzyme is responsible for the renewal of the chromosomal ends, the so-called telomeres. By preventing them from shortening with each cell cycle, telomerase is able to inhibit cellular senescence and apoptosis. Telomerase activity, which is detectable in the majority of cancer cells, allows them to maintain their proliferative capacity. The thus obtained immortality of those cells again is a key to their malignancy. Based on these discoveries, it is obvious that telomerase inhibitors would represent an innovative approach to fight cancer, and a variety of such candidate molecules are currently in the pipeline. Telomerase inhibitors largely fall in two classes of compounds: small synthetic molecules and nucleotide-based biologicals. For several candidates, some proof of concept studies have been demonstrated, either on cell cultures or in animal models. But the same studies also revealed that

inefficient delivery is largely limiting the translational step into the clinic. The most appealing feature of telomerase inhibitors, which distinguishes them from conventional anticancer drugs, is probably seen in their intrinsic non-toxicity to normal cells. Nevertheless, efficient delivery to the target cells, i.e. to the tumor, is still required. Here, some well-known biopharmaceutical problems such as insufficient solubility, permeability or even metabolic stability are frequently encountered. To address these challenges, there is a clear need for adequate delivery technologies, for example by using nanomedicines, that would allow to overcome their biopharmaceutical shortcomings and to warrant a sufficient bioavailability at the target side. This review first briefly explains the concept of telomerase and telomerase inhibition in cancer therapy. It secondly aims to provide an overview of the different currently known telomerase inhibitors. Finally, the biopharmaceutical limitations of these molecules are discussed as well as the possibilities to overcome those limits by novel drug carrier systems and formulation approaches.

Pickup, M. W., et al. (2014). "The extracellular matrix modulates the hallmarks of cancer." *EMBO Rep* **15**(12): 1243-1253.

The extracellular matrix regulates tissue development and homeostasis, and its dysregulation contributes to neoplastic progression. The extracellular matrix serves not only as the scaffold upon which tissues are organized but provides critical biochemical and biomechanical cues that direct cell growth, survival, migration and differentiation and modulate vascular development and immune function. Thus, while genetic modifications in tumor cells undoubtedly initiate and drive malignancy, cancer progresses within a dynamically evolving extracellular matrix that modulates virtually every behavioral facet of the tumor cells and cancer-associated stromal cells. Hanahan and Weinberg defined the hallmarks of cancer to encompass key biological capabilities that are acquired and essential for the development, growth and dissemination of all human cancers. These capabilities include sustained proliferation, evasion of growth suppression, death resistance, replicative immortality, induced angiogenesis, initiation of invasion, dysregulation of cellular energetics, avoidance of immune destruction and chronic inflammation. Here, we argue that biophysical and biochemical cues from the tumor-associated extracellular matrix influence each of these cancer hallmarks and are therefore critical for malignancy. We suggest that the success of cancer prevention and therapy programs requires an intimate understanding of the reciprocal feedback between the

evolving extracellular matrix, the tumor cells and its cancer-associated cellular stroma.

Polese, C. and D. Mottet (2014). "[HDAC5 inhibition: a tool to stop cancer cell immortality]." *Med Sci (Paris)* **30**(8-9): 730-732.

Pyne, N. J. and S. Pyne (2020). "Recent advances in the role of sphingosine 1-phosphate in cancer." *FEBS Lett* **594**(22): 3583-3601.

Sphingosine 1-phosphate (S1P) is a bioactive lipid that binds to a family of G protein-coupled receptors (S1P(1-5)) and intracellular targets, such as HDAC1/2, that are functional in normal and pathophysiologic cell biology. There is a significant role for sphingosine 1-phosphate in cancer underpinning the so-called hallmarks, such as transformation and replicative immortality. In this review, we survey the most recent developments concerning the role of sphingosine 1-phosphate receptors, sphingosine kinase and S1P lyase in cancer and the prognostic indications of these receptors and enzymes in terms of disease-specific survival and recurrence. We also provide evidence for identification of new therapeutic approaches targeting sphingosine 1-phosphate to prevent neovascularisation, to revert aggressive and drug-resistant cancers to more amenable forms sensitive to chemotherapy, and to induce cytotoxicity in cancer cells. Finally, we briefly describe current advances in the development of isoform-specific inhibitors of sphingosine kinases for potential use in the treatment of various cancers, where these enzymes have a predominant role. This review will therefore highlight sphingosine 1-phosphate signalling as a promising translational target for precision medicine in stratified cancer patients.

Rahman, R., et al. (2009). "Cellular immortality in brain tumours: an integration of the cancer stem cell paradigm." *Biochim Biophys Acta* **1792**(4): 280-288.

Brain tumours are a diverse group of neoplasms that continue to present a formidable challenge in our attempt to achieve curable intervention. Our conceptual framework of human brain cancer has been redrawn in the current decade. There is a gathering acceptance that brain tumour formation is a phenotypic outcome of dysregulated neurogenesis, with tumours viewed as abnormally differentiated neural tissue. In relation, there is accumulating evidence that brain tumours, similar to leukaemia and many solid tumours, are organized as a developmental hierarchy which is maintained by a small fraction of cells endowed with many shared properties of tissue stem cells. Proof that neurogenesis persists throughout adult life,

compliments this concept. Although the cancer cell of origin is unclear, the proliferative zones that harbour stem cells in the embryonic, post-natal and adult brain are attractive candidates within which tumour-initiation may ensue. Dysregulated, unlimited proliferation and an ability to bypass senescence are acquired capabilities of cancerous cells. These abilities in part require the establishment of a telomere maintenance mechanism for counteracting the shortening of chromosomal termini. A strategy based upon the synthesis of telomeric repeat sequences by the ribonucleoprotein telomerase, is prevalent in approximately 90% of human tumours studied, including the majority of brain tumours. This review will provide a developmental perspective with respect to normal (neurogenesis) and aberrant (tumourigenesis) cellular turnover, differentiation and function. Within this context our current knowledge of brain tumour telomere/telomerase biology will be discussed with respect to both its developmental and therapeutic relevance to the hierarchical model of brain tumourigenesis presented by the cancer stem cell paradigm.

Raj, A., et al. (2024). "Multi-Omics Integration for Liver Cancer Using Regression Analysis." *Curr Issues Mol Biol* **46**(4): 3551-3562.

Genetic biomarkers have played a pivotal role in the classification, prognostication, and guidance of clinical cancer therapies. Large-scale and multi-dimensional analyses of entire cancer genomes, as exemplified by projects like The Cancer Genome Atlas (TCGA), have yielded an extensive repository of data that holds the potential to unveil the underlying biology of these malignancies. Mutations stand out as the principal catalysts of cellular transformation. Nonetheless, other global genomic processes, such as alterations in gene expression and chromosomal re-arrangements, also play crucial roles in conferring cellular immortality. The incorporation of multi-omics data specific to cancer has demonstrated the capacity to enhance our comprehension of the molecular mechanisms underpinning carcinogenesis. This report elucidates how the integration of comprehensive data on methylation, gene expression, and copy number variations can effectively facilitate the unsupervised clustering of cancer samples. We have identified regressors that can effectively classify tumor and normal samples with an optimal integration of RNA sequencing, DNA methylation, and copy number variation while also achieving significant p-values. Further, these regressors were trained using linear and logistic regression with k-means clustering. For comparison, we employed autoencoder- and stacking-based omics integration and computed

silhouette scores to evaluate the clusters. The proof of concept is illustrated using liver cancer data. Our analysis serves to underscore the feasibility of unsupervised cancer classification by considering genetic markers beyond mutations, thereby emphasizing the clinical relevance of additional global cellular parameters that contribute to the transformative process in cells. This work is clinically relevant because changes in gene expression and genomic re-arrangements have been shown to be signatures of cellular transformation across cancers, as well as in liver cancers.

Raseley, K., et al. (2023). "Single-Molecule Telomere Assay via Optical Mapping (SMTA-OM) Can Potentially Define the ALT Positivity of Cancer." *Genes (Basel)* **14**(6).

Telomeres play an essential role in protecting the ends of linear chromosomes and maintaining the integrity of the human genome. One of the key hallmarks of cancers is their replicative immortality. As many as 85-90% of cancers activate the expression of telomerase (TEL+) as the telomere maintenance mechanism (TMM), and 10-15% of cancers utilize the homology-dependent repair (HDR)-based Alternative Lengthening of Telomere (ALT+) pathway. Here, we performed statistical analysis of our previously reported telomere profiling results from Single Molecule Telomere Assay via Optical Mapping (SMTA-OM), which is capable of quantifying individual telomeres from single molecules across all chromosomes. By comparing the telomeric features from SMTA-OM in TEL+ and ALT+ cancer cells, we demonstrated that ALT+ cancer cells display certain unique telomeric profiles, including increased fusions/internal telomere-like sequence (ITS+), fusions/internal telomere-like sequence loss (ITS-), telomere-free ends (TFE), super-long telomeres, and telomere length heterogeneity, compared to TEL+ cancer cells. Therefore, we propose that ALT+ cancer cells can be differentiated from TEL+ cancer cells using the SMTA-OM readouts as biomarkers. In addition, we observed variations in SMTA-OM readouts between different ALT+ cell lines that may potentially be used as biomarkers for discerning subtypes of ALT+ cancer and monitoring the response to cancer therapy.

Rath, S., et al. (2017). "Inhibition of histone/lysine acetyltransferase activity kills CoCl₂-treated and hypoxia-exposed gastric cancer cells and reduces their invasiveness." *Int J Biochem Cell Biol* **82**: 28-40.

Hypoxia enhances immortality and metastatic properties of solid tumors. Deregulation of histone acetylation has been associated with several

metastatic cancers but its effect on hypoxic responses of cancer cells is not known. This study aimed at understanding the effectiveness of the hydrazinocurcumin, CTK7A, an inhibitor of p300 lysine/histone acetyltransferase (KAT/HAT) activity, in inducing apoptosis of gastric cancer cells (GCCs) exposed to cobalt chloride (CoCl₂), a hypoxia-mimetic chemical, or 1% O₂. Here, we show that CTK7A-induced hydrogen peroxide (H₂O₂) generation in CoCl₂-exposed and invasive gastric cancer cells (GCCs) leads to p38 MAPK-mediated Noxa expression and thereafter, mitochondrial apoptotic events. Noxa induction in normal immortalized gastric epithelial cells after CTK7A and hypoxia-exposure is remarkably less in comparison to similarly-treated GCCs. Moreover, hypoxia-exposed GCCs, which have acquired invasive properties, become apoptotic after CTK7A treatment to a significantly higher extent than normoxic cells. Thus, we show the potential of CTK7A in sensitizing hypoxic and metastatic GCCs to apoptosis induction.

Rathi, A., et al. (1999). "Telomerase RNA expression during progression of gastric cancer." *Hum Pathol* **30**(11): 1302-1308.

Telomerase, an enzyme associated with cellular immortality and malignancy, is stringently repressed in most normal somatic cells but is reactivated in malignant tumor cells and immortal cell lines, indicating that activation of telomerase may play an important role in tumorigenesis and immortalization. The pattern of human telomerase RNA (hTR) expression during progression of gastric cancer was investigated by a radioactive in situ hybridization (ISH) assay. Paraffin-embedded sections of 85 archival samples from Korean patients with benign and various malignant stages of gastric carcinomas as well as normal and regenerative tissues were studied. In normal gastric mucosae and regenerative lesions such as chronic peptic ulcer and hyperplastic polyps, only a weak degree of hTR expression was noted, and the expression was limited to basal cells of the gastric glands. Also, a moderate degree of hTR expression was present in the germinal centers of lymphoid follicles present in the submucosa. In tubular adenomas, the degree of hTR expression was also generally weak, but, unlike normal gastric mucosa, the expression was rather diffuse and occasionally focal in distribution. However, moderate to intense and usually diffuse hTR expression was present in all cancerous tissues at different stages. Although some heterogeneity of hTR expression was noted, there was a tendency for intensity of hTR expression to increase gradually as the cancer progressed to a more advanced stage. Our results indicate that upregulation of telomerase

expression is associated with gastric cancer development or plays some role in gastric carcinogenesis. Upregulation of hTR expression detected by ISH assay may be a useful marker or tool for the early detection of gastric cancer.

Ravi, D., et al. (2000). "Apoptosis: future directions in cancer therapy." *Natl Med J India* **13**(2): 71-78.

Cancer as a multifactorial disease results in gain of immortality due to defective apoptosis. The primary mode of cell death by apoptosis induced by various modes of treatment often fail in vivo. The in vitro environment is less complex while the in vivo environment is influenced by various external regulatory signals besides the existence of multiple, parallel and independent apoptotic pathways. Further, specific preference for an apoptotic pathway in a certain cell type would significantly alter the apoptotic responses. Identification of defects in preferred pathways and choosing alternative and potentially inducible pathways would help in deciding on apoptosis-based treatment protocols. Mechanisms involved in the execution of apoptosis may also not be unique to apoptotic pathways since similar events, possibly with strict control, do occur during mitosis. Further evaluation may yield new dimensions to apoptosis and apoptosis-based therapy.

Reda, A., et al. (2019). "Next-generation nanotheranostics targeting cancer stem cells." *Nanomedicine (Lond)* **14**(18): 2487-2514.

Cancer is depicted as the most aggressive malignancy and is one the major causes of death worldwide. It originates from immortal tumor-initiating cells called 'cancer stem cells' (CSCs). This devastating subpopulation exhibit potent self-renewal, proliferation and differentiation characteristics. Dynamic DNA repair mechanisms can sustain the immortality phenotype of cancer to evade all treatment strategies. To date, current conventional chemo- and radio-therapeutic strategies adopted against cancer fail in tackling CSCs. However, new advances in nanotechnology have paved the way for creating next-generation nanotheranostics as multifunctional smart 'all-in-one' nanoparticles. These particles integrate diagnostic, therapeutic and targeting agents into one single biocompatible and biodegradable carrier, opening up new avenues for breakthroughs in early detection, diagnosis and treatment of cancer through efficient targeting of CSCs.

Roake, C. M. and S. E. Artandi (2017). "Control of Cellular Aging, Tissue Function, and Cancer by p53 Downstream of Telomeres." *Cold Spring Harb Perspect Med* **7**(5).

Telomeres, the nucleoprotein complex at the ends of eukaryotic chromosomes, perform an essential cellular role in part by preventing the chromosomal end from initiating a DNA-damage response. This function of telomeres can be compromised as telomeres erode either as a consequence of cell division in culture or as a normal part of cellular ageing in proliferative tissues. Telomere dysfunction in this context leads to DNA-damage signaling and activation of the tumor-suppressor protein p53, which then can prompt either cellular senescence or apoptosis. By culling cells with dysfunctional telomeres, p53 plays a critical role in protecting tissues against the effects of critically short telomeres. However, as telomere dysfunction worsens, p53 likely exacerbates short telomere-driven tissue failure diseases, including pulmonary fibrosis, aplastic anemia, and liver cirrhosis. In cells lacking p53, unchecked telomere shortening drives chromosomal end-to-end fusions and cycles of chromosome fusion-bridge-breakage. Incipient cancer cells confronting these telomere barriers must disable p53 signaling to avoid senescence and eventually up-regulate telomerase to achieve cellular immortality. The recent findings of highly recurrent activating mutations in the promoter for the telomerase reverse transcriptase (TERT) gene in diverse human cancers, together with the widespread mutations in p53 in cancer, provide support for the idea that circumvention of a telomere-p53 checkpoint is essential for malignant progression in human cancer.

Robinson, N. J. and W. P. Schieman (2022). "Telomerase in Cancer: Function, Regulation, and Clinical Translation." *Cancers (Basel)* **14**(3).

During the process of malignant transformation, cells undergo a series of genetic, epigenetic, and phenotypic alterations, including the acquisition and propagation of genomic aberrations that impart survival and proliferative advantages. These changes are mediated in part by the induction of replicative immortality that is accompanied by active telomere elongation. Indeed, telomeres undergo dynamic changes to their lengths and higher-order structures throughout tumor formation and progression, processes overseen in most cancers by telomerase. Telomerase is a multimeric enzyme whose function is exquisitely regulated through diverse transcriptional, post-transcriptional, and post-translational mechanisms to facilitate telomere extension. In turn, telomerase function depends not only on its core components, but also on a suite of binding partners, transcription factors, and intra- and extracellular signaling effectors. Additionally, telomerase exhibits telomere-independent regulation

of cancer cell growth by participating directly in cellular metabolism, signal transduction, and the regulation of gene expression in ways that are critical for tumorigenesis. In this review, we summarize the complex mechanisms underlying telomere maintenance, with a particular focus on both the telomeric and extratelomeric functions of telomerase. We also explore the clinical utility of telomeres and telomerase in the diagnosis, prognosis, and development of targeted therapies for primary, metastatic, and recurrent cancers.

Robinson, N. J., et al. (2019). "Stem cells, immortality, and the evolution of metastatic properties in breast cancer: telomere maintenance mechanisms and metastatic evolution." *J Cancer Metastasis Treat* **5**.

Breast cancer is the most significant cause of cancer-related death in women around the world. The vast majority of breast cancer-associated mortality stems from metastasis, which remains an incurable disease state. Metastasis results from evolution of clones that possess the insidious properties required for dissemination and colonization of distant organs. These clonal populations are descended from breast cancer stem cells (CSCs), which are also responsible for their prolonged maintenance and continued evolution. Telomeres impose a lifespan on cells that can be extended when they are actively elongated, as occurs in CSCs. Thus, changes in telomere structure serve to promote the survival of CSCs and subsequent metastatic evolution. The selection of telomere maintenance mechanism (TMM) has important consequences not only for CSC survival and evolution, but also for their coordination of various signaling pathways that choreograph the metastatic cascade. Targeting the telomere maintenance machinery may therefore provide a boon to the treatment of metastatic breast cancer. Here we review the two major TMMs and the roles they play in the development of stem and metastatic breast cancer cells. We also highlight current and future approaches to targeting these mechanisms in clinical settings to alleviate metastatic breast cancers.

Romaniuk-Drapala, A., et al. (2021). "hTERT Downregulation Attenuates Resistance to DOX, Impairs FAK-Mediated Adhesion, and Leads to Autophagy Induction in Breast Cancer Cells." *Cells* **10**(4).

Telomerase is known to contribute to telomere maintenance and to provide cancer cell immortality. However, numerous reports are showing that the function of the enzyme goes far beyond chromosome ends. The study aimed to explore how

telomerase downregulation in MCF7 and MDA-MB-231 breast cancer cells affects their ability to survive. Consequently, sensitivity to drug resistance, proliferation, and adhesion were assessed. The lentiviral-mediated human telomerase reverse transcriptase (hTERT) downregulation efficiency was performed at gene expression and protein level using qPCR and Western blot, respectively. Telomerase activity was evaluated using the Telomeric Repeat Amplification Protocol (TRAP) assay. The study revealed that hTERT downregulation led to an increased sensitivity of breast cancer cells to doxorubicin which was demonstrated in MTT and clonogenic assays. During a long-term doubling time assessment, a decreased population doubling level was observed. Interestingly, it did not dramatically affect cell cycle distribution. hTERT downregulation was accompanied by an alteration in beta1-integrin and by focal adhesion kinase (FAK)-driven pathways together with the reduction of target proteins phosphorylation, i.e., paxillin and c-Src. Additionally, autophagy activation was observed in MDA-MB-231 cells manifested by alternations in Atg5, Beclin 1, LC3II/I ratio, and p62. These results provide new evidence supporting the possible therapeutic potential of telomerase downregulation leading to induction of autophagy and cancer cells elimination.

Rooiintan, A., et al. (2016). "A comparison between PLGA-PEG and NIPAAm-MAA nanocarriers in curcumin delivery for hTERT silencing in lung cancer cell line." *Cell Mol Biol (Noisy-le-grand)* **62**(9): 51-56.

Lung cancer is the most common cancer among men. Since the main reason of cancer cells immortality is telomerase activity, targeting of such enzyme can be a promising approach in cancer therapy. Curcumin is a safe and efficient anticancer agent in this context, but its applications in cancer therapy are limited because of its hydrophobic structure and low solubility in water. Today, using nanocarriers for delivery of such anticancer agents is a well performed method. Here, we developed and compared the efficiency of two nanocarriers (PLGA-PEG and NIPAAm-MAA) in delivery of curcumin and also in levels of hTERT silencing in lung cancer cell line (calu-6). Scanning electron microscopy, MTT assays and real-time PCR were used for imaging, cytotoxicity testing and measuring the expression levels of hTERT after treatment of cells with different concentrations of free curcumin and curcumin loaded nanocarriers. The MTT results demonstrated that the IC₅₀ values of curcumin loaded nanocarriers were in lower concentrations than free curcumin. The hTERT expression levels were decreased by curcumin loaded PLGA-PEG

more than curcumin loaded NIPAAm-MAA and free curcumin. Our results showed that the curcumin loaded PLGA-PEG can be a useful nano based carrier for delivery of anti-cancer agents such as curcumin to fight lung cancer.

Ross, S. A. and C. D. Davis (2011). "MicroRNA, nutrition, and cancer prevention." *Adv Nutr* **2**(6): 472-485.

MicroRNA (miRNA) are small noncoding RNA molecules that are involved in post-transcriptional gene silencing. Alterations in miRNA expression are observed in and may underlie many different human diseases, including cancer. In fact, miRNA have been shown to affect the hallmarks of cancer, including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Genetic and epigenetic alterations may explain aberrant miRNA expression in cancer cells and may also contribute to cancer risk. It is now thought that by circulating through the bloodstream, miRNA can exert their effects at distant sites as well as within the cells of origin. Recent evidence suggests that nutrients and other bioactive food components protect against cancer through modulation of miRNA expression. Moreover, dietary factors have been shown to modify miRNA expression and their mRNA targets in various cancer processes, including apoptosis, cell cycle regulation, differentiation, inflammation, angiogenesis, and metastasis as well as pathways in stress response. Herein, we provide a brief overview of dietary modulation of miRNA expression and its potential role in cancer prevention. Understanding the affect of dietary factors on miRNA expression and function may provide insight on prevention strategies to reduce the burden of cancer.

Sadr, Z., et al. (2024). "Beginning at the ends: telomere and telomere-based cancer therapeutics." *Mol Genet Genomics* **300**(1): 1.

Telomeres, which are situated at the terminal ends of chromosomes, undergo a reduction in length with each cellular division, ultimately reaching a critical threshold that triggers cellular senescence. Cancer cells circumvent this senescence by utilizing telomere maintenance mechanisms (TMMs) that grant them a form of immortality. These mechanisms can be categorized into two primary processes: the reactivation of telomerase reverse transcriptase and the alternative lengthening of telomeres (ALT) pathway, which is dependent on homologous recombination (HR). Various strategies have been developed to inhibit telomerase activation

in 85-95% of cancers, including the use of antisense oligonucleotides such as small interfering RNAs and endogenous microRNAs, agents that simulate telomere uncapping, expression modulators, immunotherapeutic vaccines targeting telomerase, reverse transcriptase inhibitors, stabilization of G-quadruplex structures, and gene therapy approaches. Conversely, in the remaining 5-15% of human cancers that rely on ALT, mechanisms involve modifications in the chromatin environment surrounding telomeres, upregulation of TERRA long non-coding RNA, enhanced activation of the ataxia telangiectasia and Rad-3-related protein kinase signaling pathway, increased interactions with nuclear receptors, telomere repositioning driven by HR, and recombination events between non-sister chromatids, all of which present potential targets for therapeutic intervention. Additionally, combinatorial therapy has emerged as a strategy that employs selective agents to simultaneously target both telomerase and ALT, aiming for optimal clinical outcomes. Given the critical role of anti-TMM strategies in cancer treatment, this review provides an overview of the latest insights into the structure and function of telomeres, their involvement in tumorigenesis, and the advancements in TMM-based cancer therapies.

Salhab, M., et al. (2008). "The expression of gene transcripts of telomere-associated genes in human breast cancer: correlation with clinico-pathological parameters and clinical outcome." *Breast Cancer Res Treat* **109**(1): 35-46.

BACKGROUND: Telomerase is a ribonucleoprotein enzyme that synthesises telomeres in human germ cells, embryogenesis and in cancer, maintaining chromosomal length, stability and cellular immortality. The hTERT gene is the rate-limiting determinant of telomerase reactivation during immortalization and malignant transformation. Telomeric DNA-binding proteins have been attracting increasing interest due to their essential role in the regulation of telomeric DNA length and in protecting against chromosomal end-to-end fusion. These proteins include hTR, TRF1, TRF2, TANK1, TANK2, POT1, TIN2, EST1, and TEP. This study represents the first comprehensive investigation of the mRNA expression of key telomere-related genes in human breast cancer. **METHODS:** One hundred and twenty seven tumour tissues and 33 normal tissues were analyzed. Levels of transcription of hTERT, hTR, TRF1, TRF2, TANK1, TANK2, POT1, TIN2, EST1, and TEP1 were determined using real-time quantitative PCR. The mRNA expression of these genes was normalized against CK19 and was then analyzed against the pathological parameters

and clinical outcome over a 10 year follow up period. **RESULTS:** The mRNA expressions of hTERT, hTR, TANK1, EST1, and TEP1 were higher in tumour samples compared with normal breast tissue. This reached statistical significance for EST1 when comparing good prognosis tumours with normal breast tissue (means=11013 vs 1160, $P=0.05$). Both hTERT and TEP1 levels significantly predicted overall survival ($P=0.012$ and 0.005 respectively) and disease-free survival ($P=0.0011$ and 0.01 respectively). The mRNA levels of TANK2 and POT1 were lower in malignant tissues compared with non-malignant breast tissues and this difference reached statistical significance when comparing the levels in normal tissues with those in advanced tumours ($P=0.0008$ and $P=0.038$ respectively). Their levels fell further with increasing tumour's stage and were higher in tumours from patients who remained disease free compared with those who developed local recurrence or distant metastasis or died from breast cancer. TRF2 showed a trend similar to that of TANK2 and POT1. Furthermore, there was a highly significant correlation between TANK1 expression and that of hTERT, hTR, TRF1, TRF2 and EST1, ($r=0.533, 0.586, 0.608, 0.644$ and 0.551 respectively, $P<0.001$). **CONCLUSIONS:** Genes encoding telomere-associated proteins display different patterns of mRNA expression in human breast cancer, and in normal breast tissue, suggesting different and sometimes opposing roles in mammary carcinogenesis. hTERT, hTR, TANK1, EST1 and TEP1 seem to be up-regulated, with hTERT and TEP1 correlating with clinical outcome. Conversely, TANK2 and POT1 transcription levels demonstrate a compelling trend to be lower in malignant tissues and lower still in those patients who develop recurrent disease suggesting that TANK2 and POT1 may act as tumour suppressor genes possibly by negatively regulating telomerase activity.

Saluja, T. S., et al. (2016). "Equating salivary lactate dehydrogenase (LDH) with LDH-5 expression in patients with oral squamous cell carcinoma: An insight into metabolic reprogramming of cancer cell as a predictor of aggressive phenotype." *Tumour Biol* **37**(4): 5609-5620.

Oral squamous cell carcinoma (OSCC) is the sixth most common human malignancy. According to World Health Organization, oral cancer has been reported to have the highest morbidity and mortality and a survival rate of approximately 50 % at 5 years from diagnosis. This is attributed to the subjectivity in TNM staging and histological grading which may result in less than optimum treatment outcomes including tumour recurrence. One of the hallmarks of cancer is aerobic glycolysis also known

as the Warburg effect. This glycolytic phenotype (hypoxic state) not only confers immortality to cancer cells, but also correlates with the belligerent behaviour of various malignancies and is reflected as an increase in the expression of lactate dehydrogenase 5 (LDH-5), the main isoform of LDH catalysing the conversion of pyruvate to lactate during glycolysis. The diagnostic role of salivary LDH in assessing the metabolic phenotype of oral cancer has not been studied. Since salivary LDH is mainly sourced from oral epithelial cells, any pathological changes in the epithelium should reflect diagnostically in saliva. Thus in our current research, we made an attempt to ascertain the biological behaviour and aggressiveness of OSCC by appraising its metabolic phenotype as indirectly reflected in salivary LDH activity. We found that salivary LDH can be used to assess the aggressiveness of different histological grades of OSCC. For the first time, an evidence of differing metabolic behaviour in similar histologic tumour grade is presented. Taken together, our study examines the inclusion of salivary LDH as potential diagnostic parameter and therapeutic index in OSCC.

Samare-Najaf, M., et al. (2024). "Non-apoptotic cell death programs in cervical cancer with an emphasis on ferroptosis." *Crit Rev Oncol Hematol* **194**: 104249.

BACKGROUND: Cervical cancer, a pernicious gynecological malignancy, causes the mortality of hundreds of thousands of females worldwide. Despite a considerable decline in mortality, the surging incidence rate among younger women has raised serious concerns. Immortality is the most important characteristic of tumor cells, hence the carcinogenesis of cervical cancer cells pivotally requires compromising with cell death mechanisms. **METHODS:** The current study comprehensively reviewed the mechanisms of non-apoptotic cell death programs to provide possible disease management strategies. **RESULTS:** Comprehensive evidence has stated that focusing on necroptosis, pyroptosis, and autophagy for disease management is associated with significant limitations such as insufficient understanding, contradictory functions, dependence on disease stage, and complexity of intracellular pathways. However, ferroptosis represents a predictable role in cervix carcinogenesis, and ferroptosis-related genes demonstrate a remarkable correlation with patient survival and clinical outcomes. **CONCLUSION:** Ferroptosis may be an appropriate option for disease management strategies from predicting prognosis to treatment.

Sankaranarayanan, P., et al. (2015). "Tensor GSVD

of patient- and platform-matched tumor and normal DNA copy-number profiles uncovers chromosome arm-wide patterns of tumor-exclusive platform-consistent alterations encoding for cell transformation and predicting ovarian cancer survival." *PLoS One* **10**(4): e0121396.

The number of large-scale high-dimensional datasets recording different aspects of a single disease is growing, accompanied by a need for frameworks that can create one coherent model from multiple tensors of matched columns, e.g., patients and platforms, but independent rows, e.g., probes. We define and prove the mathematical properties of a novel tensor generalized singular value decomposition (GSVD), which can simultaneously find the similarities and dissimilarities, i.e., patterns of varying relative significance, between any two such tensors. We demonstrate the tensor GSVD in comparative modeling of patient- and platform-matched but probe-independent ovarian serous cystadenocarcinoma (OV) tumor, mostly high-grade, and normal DNA copy-number profiles, across each chromosome arm, and combination of two arms, separately. The modeling uncovers previously unrecognized patterns of tumor-exclusive platform-consistent co-occurring copy-number alterations (CNAs). We find, first, and validate that each of the patterns across only 7p and Xq, and the combination of 6p+12p, is correlated with a patient's prognosis, is independent of the tumor's stage, the best predictor of OV survival to date, and together with stage makes a better predictor than stage alone. Second, these patterns include most known OV-associated CNAs that map to these chromosome arms, as well as several previously unreported, yet frequent focal CNAs. Third, differential mRNA, microRNA, and protein expression consistently map to the DNA CNAs. A coherent picture emerges for each pattern, suggesting roles for the CNAs in OV pathogenesis and personalized therapy. In 6p+12p, deletion of the p21-encoding CDKN1A and p38-encoding MAPK14 and amplification of RAD51AP1 and KRAS encode for human cell transformation, and are correlated with a cell's immortality, and a patient's shorter survival time. In 7p, RPA3 deletion and POLD2 amplification are correlated with DNA stability, and a longer survival. In Xq, PABPC5 deletion and BCAP31 amplification are correlated with a cellular immune response, and a longer survival.

Saretzki, G. (2014). "Extra-telomeric functions of human telomerase: cancer, mitochondria and oxidative stress." *Curr Pharm Des* **20**(41): 6386-6403.

Telomerase activity is essential for human cancer cells in order to maintain telomeres and provide unlimited proliferation potential and cellular

immortality. However, additional non-telomeric roles emerge for the telomerase protein TERT that can impact tumorigenesis and cancer cell properties. This review summarises our current knowledge of non-telomeric functions of telomerase in human cells, with a special emphasis on cancer cells. Non-canonical functions of telomerase can be performed within the nucleus as well as in other cellular compartments. These telomere-independent activities of TERT influence various essential cellular processes, such as gene expression, signalling pathways, mitochondrial function as well as cell survival and stress resistance. Emerging data show the interaction of telomerase with intracellular signalling pathways such as NF-kappaB and WNT/beta-catenin; thereby contributing to inflammation, epithelial to mesenchymal transition (EMT) and cancer invasiveness. All these different functions might contribute to tumorigenesis, and have serious consequences for cancer therapies due to increased resistance against damaging agents and prevention of cell death. In addition, TERT has been detected in non-nuclear locations such as the cytoplasm and mitochondria. Within mitochondria TERT has been shown to decrease ROS generation, improve respiration, bind to mitochondrial DNA, increase mitochondrial membrane potential and interact with mitochondrial tRNAs. All these different non-telomere-related mechanisms might contribute towards the higher resistance of cancer cells against DNA damaging treatments and promote cellular survival. Understanding these different mechanisms and their complexity in cancer cells might help to design more effective cancer therapies in the future.

Saretzki, G., et al. (2002). "hTERT gene dosage correlates with telomerase activity in human lung cancer cell lines." *Cancer Lett* **176**(1): 81-91.

Maintenance of telomeres, most often by telomerase, is a necessary prerequisite for immortality of eukaryotic cells. To better understand the mechanisms of telomerase up-regulation during tumorigenesis, we analysed the gene dosage of hTERT on chromosome 5p15, a region known to be overrepresented in a variety of malignancies, in 20 lung cancer cell lines by Southern blotting, fluorescence in-situ hybridization, and comparative genomic hybridization. We found a significant correlation between hTERT gene dosage, hTERT mRNA expression and telomerase activity. Imbalances of chromosome 5p may exert functionally relevant hTERT gene dosage effects in human lung cancer.

Sasahira, T. and T. Kirita (2018). "Hallmarks of

Cancer-Related Newly Prognostic Factors of Oral Squamous Cell Carcinoma." *Int J Mol Sci* **19**(8).

Head and neck cancer, including oral squamous cell carcinoma (OSCC), is the sixth leading malignancy worldwide. OSCC is an aggressive tumor and its prognosis has exhibited little improvement in the last three decades. Comprehensive elucidation of OSCC's molecular mechanism is imperative for early detection and treatment, improving patient survival. Based on broadly accepted notions, OSCC arises from multiple genetic alterations caused by chronic exposure to carcinogens. In 2011, research revealed 10 key alterations fundamental to cancer cell development: sustaining proliferative signaling, evading growth suppressors, avoiding immune destruction, activating invasion and metastasis, tumor-promoting inflammation, enabling replicative immortality, inducing angiogenesis, genome instability and mutation, resisting cell death, and deregulating energetics. This review describes molecular pathological findings on conventional and novel hallmarks of OSCC prognostic factors. In addition, the review summarizes the functions and roles of several molecules as novel OSCC prognosticators.

Sato, N., et al. (1998). "9-Hydroxyellipticine inhibits telomerase activity in human pancreatic cancer cells." *FEBS Lett* **441**(2): 318-321.

There is increasing interest in identifying potent inhibitors of telomerase because the enzyme plays a crucial role in the development of cellular immortality and carcinogenesis. We hypothesized that 9-hydroxyellipticine (9-HE), an antitumor alkaloid, would inhibit telomerase activity because the drug has a unique mechanism of inhibiting phosphorylation of mutant p53 protein via inhibition of protein kinases, thereby restoring wild-type p53 function. This study was conducted to examine the effect of 9-HE on telomerase activity in human pancreatic cancer cells with differing p53 gene status. 9-HE treatment at relatively high concentrations resulted in rapid, complete inhibition of telomerase activity, irrespective of the p53 status. We conclude that 9-HE may exert a strong inhibitory effect on telomerase activity possibly through inhibition of protein kinases rather than through restoration of functional wild-type p53.

Sato, N., et al. (2000). "Up-regulation of telomerase activity in human pancreatic cancer cells after exposure to etoposide." *Br J Cancer* **82**(11): 1819-1826.

Telomerase plays a critical role in the development of cellular immortality and oncogenesis. Activation of telomerase occurs in a majority of

human malignant tumours, and the relation between telomerase and vulnerability to drug-mediated apoptosis remains unclear. In this study, we demonstrate, for the first time, up-regulation of telomerase activity in human pancreatic cancer cells treated with etoposide, a topoisomerase II inhibitor. Exposure of MIA PaCa-2 cells to etoposide at various concentrations (1-30 µM) resulted in two- to threefold increases in telomerase activity. Up-regulation was detectable 24 h after drug exposure and was accompanied by enhanced expression of mRNA of the human telomerase reverse transcriptase. Telomerase activation was also observed in AsPC-1 and PANC-1 cells but not in KP-3 and KP-1N cells. Furthermore, we found a negative correlation between increased telomerase activity and the percentage of dead cells after etoposide treatment. These findings suggest the existence of an anti-apoptotic pathway through which telomerase is up-regulated in response to DNA damage. This telomerase activation pathway may be one of the mechanisms responsible for the development of etoposide resistance in certain pancreatic cancer cells.

Schrank, Z., et al. (2018). "Oligonucleotides Targeting Telomeres and Telomerase in Cancer." *Molecules* **23**(9).

Telomeres and telomerase have become attractive targets for the development of anticancer therapeutics due to their involvement in cancer cell immortality. Currently, several therapeutics have been developed that directly target telomerase and telomeres, such as telomerase inhibitors and G-quadruplex stabilizing ligands. Telomere-specific oligonucleotides that reduce telomerase activity and disrupt telomere architecture are also in development as novel anticancer therapeutics. Specifically, GRN163L and T-oligos have demonstrated promising anticancer activity in multiple cancers types via induction of potent DNA damage responses. Currently, several miRNAs have been implicated in the regulation of telomerase activity and may prove to be valuable targets in the development of novel therapies by reducing expression of telomerase subunits. Targeting miRNAs that are known to increase expression of telomerase subunits may be another strategy to reduce carcinogenesis. This review aims to provide a comprehensive understanding of current oligonucleotide-based anticancer therapies that target telomeres and telomerase. These studies may help design novel therapeutic approaches to overcome the challenges of oligonucleotide therapy in a clinical setting.

Sebastian, S., et al. (2005). "Telomeres, telomerase and oral cancer (Review)." *Int J Oncol* **27**(6): 1583-

1596.

Oral squamous cell carcinoma (oral cancer) and many squamous cell carcinomas of the head and neck arise as a consequence of multiple molecular events induced by the effects of various carcinogens related to tobacco use, environmental factors, and viruses in some instances (e.g., mucosal oncogenic human papillomaviruses), against a background of inheritable resistance or susceptibility. Consequent genetic damage affects many chromosomes and genes, and it is the accumulation of these changes that appears to lead to carcinoma. Telomere maintenance by telomerase or, in its absence, alternative lengthening of telomeres protect this acquired altered genetic information ensuring immortality without losing eukaryotic linear DNA; when this does not occur DNA is lost and end-replication problems arise. Telomerase is reactivated in 80-90% of cancers thus attracting the attention of pathologists and clinicians who have explored its use as a target for anticancer therapy and to develop better diagnostic and prognostic markers. In the last few years, valuable research from various laboratories has provided major insights into telomerase and telomeres leading to their use as diagnostic and prognostic markers in several types of cancer. Moreover, many strategies have emerged which inhibit this complex enzyme for anticancer therapy and are one step ahead of clinical trials. This review explains the basic biology and the clinical implications of telomerase-based diagnosis and prognosis, the prospects for its use in anticancer therapy, and the limitations it presents in the context of oral cancer.

Sell, S. and H. L. Leffert (2008). "Liver cancer stem cells." *J Clin Oncol* **26**(17): 2800-2805.

In an effort to review the evidence that liver cancer stem cells exist, two fundamental questions must be addressed. First, do hepatocellular carcinomas (HCC) arise from liver stem cells? Second, do HCCs contain cells that possess properties of cancer stem cells? For many years the finding of preneoplastic nodules in the liver during experimental induction of HCCs by chemicals was interpreted to support the hypothesis that HCC arose by dedifferentiation of mature liver cells. More recently, recognition of the role of small oval cells in the carcinogenic process led to a new hypothesis that HCC arises by maturation arrest of liver stem cells. Analysis of the cells in HCC supports the presence of cells with stem-cell properties (ie, immortality, transplantability, and resistance to therapy). However, definitive markers for these putative cancer stem cells have not yet been found and a liver cancer stem cell has not been isolated.

Seo, D. H., et al. (2024). "Promoter mutation-independent TERT expression is related to the immune-enriched milieu in papillary thyroid cancer." *Endocr Relat Cancer* **31**(11).

Telomerase reverse transcriptase promoter mutation (pTERT MT) promotes human carcinogenesis via aberrant expression of telomerase reverse transcriptase (TERT). However, the tumorigenic impact of TERT expression independent of pTERT MT remains unclear despite numerous mechanisms of TERT being suggested. To tackle this issue, we employed comprehensive bioinformatics to assess biological variations noticed among different TERT expression mechanisms. Papillary thyroid cancer (PTC) with pTERT MT (pTERT MT PTC) presented aggressive clinical behavior and exhibited biological profiles associated with cellular immortality and genomic instability. PTC with TERT expression but without pTERT MT (TERT (+) PTC), also exhibited poor clinicopathological characteristics and was enriched with immune responses. In accordance, c-MYC/E2F and nuclear factor kappa B (NFkappaB) were dominant transcription factors in pTERT MT PTC and TERT (+) PTC, respectively. Notably, we revealed TERT hypermethylated oncological region (THOR) as a potential TERT expressing mechanism in TERT (+) PTC patients. Furthermore, three unique subtypes of papillary thyroid cancer were deciphered using a combination of machine learning-based scoring systems. Our proposed scoring system was clinically significant, especially in microcarcinoma, predicting survival outcomes and inferring therapeutic responses to radioactive iodine therapy. Finally, our analysis was expanded to endocrine-related cancers, unveiling various regulatory mechanisms of TERT with poor clinical outcomes and biological behaviors.

Shin, P. K., et al. (2019). "Walnut phenolic extracts reduce telomere length and telomerase activity in a colon cancer stem cell model." *Nutr Res Pract* **13**(1): 58-63.

BACKGROUND/OBJECTIVES: Telomeres are located at the chromosomal ends and progressively shortened during each cell cycle. Telomerase, which is regulated by hTERT and c-MYC, maintains telomeric DNA sequences. Especially, telomerase is active in cancer and stem cells to maintain telomere length for replicative immortality. Recently we reported that walnut phenolic extract (WPE) can reduce cell viability in a colon cancer stem cell (CSC) model. We, therefore, investigated the effect of WPE on telomere maintenance in the same model. **MATERIALS/METHODS:** CD133(+)/CD44(+) cells

from HCT116, a human colon cancer cell line, were sorted by Fluorescence-activated cell sorting (FACS) and treated with WPE at the concentrations of 0, 10, 20, and 40 microg/mL for 6 days. Telomere lengths were assessed by quantitative real-time PCR (qRT-PCR) using telomere specific primers and DNA extracted from the cells, which was further adjusted with single-copy gene and reference DNA (ddC(t)). Telomerase activity was also measured by qRT-PCR after incubating the PCR mixture with cell protein extracts, which was adjusted with reference DNA (dC(t)). Transcriptions of hTERT and c-MYC were determined using conventional RT-PCR. **RESULTS:** Telomere length of WPE-treated cells was significantly decreased in a dose-dependent manner (5.16 +/- 0.13 at 0 microg/mL, 4.79 +/- 0.12 at 10 microg/mL, 3.24 +/- 0.08 at 20 microg/mL and 3.99 +/- 0.09 at 40 microg/mL; P = 0.0276). Telomerase activities concurrently decreased with telomere length (1.47 +/- 0.04, 1.09 +/- 0.01, 0.76 +/- 0.08, and 0.88 +/- 0.06; P = 0.0067). There was a positive correlation between telomere length and telomerase activity (r = 0.9090; P < 0.0001). Transcriptions of both hTERT and c-MYC were also significantly decreased in the same manner. **CONCLUSION:** In the present cell culture model, WPE reduced telomere maintenance, which may provide a mechanistic link to the effect of walnuts on the viability of colon CSCs.

Shirotani, Y., et al. (1994). "Alteration in length of telomeric repeats in lung cancer." *Lung Cancer* **11**(1-2): 29-41.

We investigated the relationship between telomere length and various characteristics of tumor cells in 46 lung cancer specimens (40 primary lesions and six metastatic lesions). Three variant patterns of telomere length were observed in 16 cases (34.8%): reduction in 13 cases, elongation in two cases, and convergence in one case. These variant patterns were frequently observed in small cell carcinomas, in metastatic lesions, and in cases which possessed the S-type allele of the L-myc gene. All three cases with telomere elongation or convergence were associated with a poor prognosis. This is compatible with the previous report suggesting that telomerase activity may be an indicator of immortality in vitro. In adenocarcinoma, telomere reduction or elongation was also observed in the early stages with a low percentage of cells in the S-phase, while in cases with other histologic types, these changes were observed only in late stage, in metastatic lesions, or in cancerous tissues with a high percentage of cells in the S-phase. Although the reduction of telomere length in these tissues may be a result of many cell divisions, it may represent another stage of

carcinogenesis in early-stage adenocarcinoma.

Shukla, V., et al. (2023). "Bioinformatic Analysis of miR-200b/429 and Hub Gene Network in Cervical Cancer." *Biochem Genet* **61**(5): 1898-1916.

The miR-200b/429 located at 1p36 is a highly conserved miRNA cluster emerging as a critical regulator of cervical cancer. Using publicly available miRNA expression data from TCGA and GEO followed by independent validation, we aimed to identify the association between miR-200b/429 expression and cervical cancer. miR-200b/429 cluster was significantly overexpressed in cancer samples compared to normal samples. miR-200b/429 expression did not correlate with patient survival; however, its overexpression correlated with histological type. Protein-protein interaction analysis of 90 target genes of miR-200b/429 identified EZH2, FLT1, IGF2, IRS1, JUN, KDR, SOX2, MYB, ZEB1, and TIMP2 as the top ten hub genes. PI3K-AKT and MAPK signaling pathways emerged as major target pathways of miR-200b/429 and their hub genes. Kaplan-Meier survival analysis showed the expression of seven miR-200b/429 target genes (EZH2, FLT1, IGF2, IRS1, JUN, SOX2, and TIMP2) to influence the overall survival of patients. The miR-200a-3p and miR-200b-5p could help predict cervical cancer with metastatic potential. The cancer hallmark enrichment analysis showed hub genes to promote growth, sustained proliferation, resistance to apoptosis, induction of angiogenesis, activation of invasion, and metastasis, enabling replicative immortality, evading immune destruction, and tumor-promoting inflammation. The drug-gene interaction analysis identified 182 potential drugs to interact with 27 target genes of miR-200b/429 with paclitaxel, doxorubicin, dabrafenib, bortezomib, docetaxel, ABT-199, eribulin, vorinostat, etoposide, and mitoxantrone emerging as the top ten best candidate drugs. Taken together, miR-200b/429 and associated hub genes can be helpful for prognostic application and clinical management of cervical cancer.

Siddhartha, R. and M. Garg (2021). "Molecular and clinical insights of matrix metalloproteinases into cancer spread and potential therapeutic interventions." *Toxicol Appl Pharmacol* **426**: 115593.

Matrix metalloproteinases (MMPs) are the group of enzymes that belong to the family of zinc dependent endopeptidases. These proteases degrade collagen and other important proteins in extracellular matrix (ECM) and regulate cytoskeletal proteins, growth factors, chemokines and cytokines, thereby play significant role during organogenesis and normal tissue turnover. Recent studies highlight the tumorigenic functions of MMPs by modulating tumor

microenvironment. Dysregulated MMPs/TIMPs cause an imbalance in crucial cell signals, and lead to serious pathological conditions related to inflammation, uncontrolled cell growth, ECM degradation, increased cell migration, cell death resistance, replicative immortality and the establishment of metastatic niche at secondary sites. Recently established correlation between the higher expression of active MMPs and cancer aggressiveness makes them probable target candidate of cancer diagnosis, prognosis and therapy. The present review focuses on the tumourigenic functions of MMPs and recent advancements in the development of MMP inhibitors of therapeutic potential in cancer treatment.

Simpson, A. J., et al. (2005). "Cancer/testis antigens, gametogenesis and cancer." *Nat Rev Cancer* **5**(8): 615-625.

Cancer/testis (CT) antigens, of which more than 40 have now been identified, are encoded by genes that are normally expressed only in the human germ line, but are also expressed in various tumour types, including melanoma, and carcinomas of the bladder, lung and liver. These immunogenic proteins are being vigorously pursued as targets for therapeutic cancer vaccines. CT antigens are also being evaluated for their role in oncogenesis--recapitulation of portions of the germline gene-expression programme might contribute characteristic features to the neoplastic phenotype, including immortality, invasiveness, immune evasion, hypomethylation and metastatic capacity.

Skolnick, A. A. (1995). "Cancer cells' immortality may prove their undoing." *JAMA* **273**(16): 1247-1248.

Slusher, A. L., et al. (2020). "The Role of Alternative RNA Splicing in the Regulation of hTERT, Telomerase, and Telomeres: Implications for Cancer Therapeutics." *Cancers (Basel)* **12**(6).

Alternative RNA splicing impacts the majority (>90%) of eukaryotic multi-exon genes, expanding the coding capacity and regulating the abundance of gene isoforms. Telomerase (hTERT) is a key example of a gene that is alternatively spliced during human fetal development and becomes dysregulated in nearly all cancers. Approximately 90% of human tumors use telomerase to synthesize de novo telomere repeats and obtain telomere-dependent cellular immortality. Paradigm shifting data indicates that hTERT alternative splicing, in addition to transcription, plays an important role in the regulation of active telomerase in cells. Our group and others are pursuing the basic science studies to

progress this emerging area of telomerase biology. Recent evidence demonstrates that switching splicing of hTERT from the telomerase activity producing full-length hTERT isoform to alternatively spliced, non-coding isoforms may be a novel telomerase inhibition strategy to prevent cancer growth and survival. Thus, the goals of this review are to detail the general roles of telomerase in cancer development, explore the emerging regulatory mechanisms of alternative RNA splicing of the hTERT gene in various somatic and cancer cell types, define the known and potential roles of hTERT splice isoforms in cancer cell biology, and provide insight into new treatment strategies targeting hTERT in telomerase-positive cancers.

Slusher, A. L., et al. (2022). "Intronic Cis-Element DR8 in hTERT Is Bound by Splicing Factor SF3B4 and Regulates hTERT Splicing in Non-Small Cell Lung Cancer." *Mol Cancer Res* **20**(10): 1574-1588.

Splicing of the hTERT gene to produce the full-length (FL) transcript is necessary for telomerase enzyme activity and telomere-dependent cellular immortality in the majority of human tumors, including non-small cell lung cancer (NSCLC) cells. The molecular machinery to splice hTERT to the FL isoform remains mostly unknown. Previously, we reported that an intron 8 cis-element termed "direct repeat 8" (DR8) promotes FL hTERT splicing, telomerase, and telomere length maintenance when bound by NOVA1 and PTBP1 in NSCLC cells. However, some NSCLC cells and patient tumor samples lack NOVA1 expression. This leaves a gap in knowledge about the splicing factors and cis-elements that promote telomerase in the NOVA1-negative context. We report that DR8 regulates FL hTERT splicing in the NOVA1-negative and -positive lung cancer contexts. We identified splicing factor 3b subunit 4 (SF3B4) as an RNA trans-factor whose expression is increased in lung adenocarcinoma (LUAD) tumors compared with adjacent normal tissue and predicts poor LUAD patient survival. In contrast to normal lung epithelial cells, which continued to grow with partial reductions of SF3B4 protein, SF3B4 knockdown reduced hTERT splicing, telomerase activity, telomere length, and cell growth in lung cancer cells. SF3B4 was also demonstrated to bind the DR8 region of hTERT pre-mRNA in both NOVA1-negative and -positive NSCLC cells. These findings provide evidence that DR8 is a critical binding hub for trans-factors to regulate FL hTERT splicing in NSCLC cells. These studies help define mechanisms of gene regulation important to the generation of telomerase activity during carcinogenesis. **IMPLICATIONS:** Manipulation of a core spliceosome protein reduces

telomerase/hTERT splicing in lung cancer cells and results in slowed cancer cell growth and cell death, revealing a potential therapeutic strategy.

Smith, H. S., et al. (1987). "Immortalization in culture: occurrence at a late stage in the progression of breast cancer." *J Natl Cancer Inst* **78**(4): 611-615.

The properties in culture of 3 breast cancer effusion metastases, obtained over approximately 2 years from the same patient, were examined. Despite repeated attempts with cryopreserved cells, only the last specimen reproducibly exhibited immortality in culture; the first 2 specimens grew initially but failed to develop into cell lines. Each specimen was unique in morphology and growth properties, although karyotypic markers indicated a common origin. Aberrations of chromosomes 1 and 11 marked these near-diploid cells, and further structural alterations of chromosome 11 accompanied the transition of biological properties observed in the third specimen.

Smith, K. J., et al. (2000). "Perspectives in dermatopathology: telomeres and telomerase in ageing and cancer; with emphasis on cutaneous disease." *J Cutan Pathol* **27**(1): 2-18.

Shortening of telomeres occurs with cell proliferation and correlates well with ageing in humans. Telomerase is a ribonucleoprotein, and is the body's most widely studied mechanism for extension of telomeres to circumvent cellular ageing. Telomerase levels remain at low or unmeasurable levels in most somatic cell populations with only a few exceptions. However, in transformed cell populations, upregulation of telomerase or some other mechanism for telomere extension is required for immortality. The telomere-telomerase system has been proposed to be an adaptation of organisms with prolonged lifespan to avoid malignant tumors, at the cost of the cellular dysfunctions associated with the aged phenotype. Therapies to modulate telomere length and telomerase levels hold promise for therapy of cancer and ageing as well as for genetic conditions that predispose to an aged phenotype.

Soder, A. I., et al. (1997). "Amplification, increased dosage and in situ expression of the telomerase RNA gene in human cancer." *Oncogene* **14**(9): 1013-1021.

Telomere length is maintained by the enzyme, telomerase, which has been linked to cellular immortality and tumour progression. However, the reasons for the high levels of telomerase found in human tumours are unknown. We have mapped the human telomerase RNA gene, (hTR), to chromosome 3q26.3 and show the hTR gene to be amplified in four carcinomas, (2/33 cervix, 1/31 head and neck, 1/9 lung). In addition, increased

copy numbers of the hTR locus was also observed in 97% of tumours. By in situ hybridisation, the histological distribution of high levels of hTR expression could be demonstrated in a lung tumour and its metastasis with hTR amplification. These results are the first report of genetic alterations involving a known component of telomerase in human cancer. Indeed, it is also the first report of the amplification of a specific locus within the chromosome 3q region frequently subject to copy number gains in human tumours. In addition, we also show for the first time the histological distribution of the RNA component of telomerase in human tumours.

Soundararajan, V., et al. (2010). "Multifunctional nanoscale platforms for targeting of the cancer cell immortality spectrum." *Macromol Rapid Commun* **31**(2): 202-216.

In the post-genomic era, "omics" platforms and cancer systems biology are greatly advancing our knowledge of the molecular and cellular underpinnings of cancer. In this article, we begin by outlining the factors governing the development of cancer (tumorigenesis) and use this framework to motivate the need for systems-approaches to cancer diagnostics and therapeutics. We review recent efforts to tap into the remarkable potential of nanotechnology for (i) systems-surveillance (or "sensing") of the molecular signatures of tumorigenesis, and (ii) spatiotemporally-regulated delivery (or "targeting") of combination therapeutics to cancer cells. Specifically, we highlight the salient role of polymeric biomaterials and describe the physicochemical characteristics that render them attractive for the design of such nanoscale platforms. We conclude with discussions on the emerging role of macromolecular biophysics and computational nanotechnology in engineering spatiotemporally-regulated anti-cancer systems.

Suenaga, M., et al. (2002). "Histone deacetylase inhibitors suppress telomerase reverse transcriptase mRNA expression in prostate cancer cells." *Int J Cancer* **97**(5): 621-625.

Telomerase activity is involved in cellular immortality. We have recently demonstrated that telomerase activity is closely associated with cell proliferation in prostate cancers. Telomerase is composed primarily of the catalytic subunit (hTERT) and the RNA template (hTERC), and hTERT expression is regulated by several factors such as c-MYC and p21(Waf1). Histone deacetylase (HDAC) inhibitors are known to modulate transcription and exhibit antiproliferative effects on cancer cells. The present study was designed to evaluate the effects of HDAC inhibitors on hTERT mRNA expression in

prostate cancer cells. LNCaP and PC-3 cells were treated with HDAC inhibitors, trichostatin A (TSA) and sodium butyrate (NaB); mRNA expression and telomerase activity were evaluated by RT-PCR and the TRAP assay, respectively. In LNCaP cells, hTERT mRNA expression was suppressed at 1 and 3 hr after treatment with 1 microM TSA and 4 mM NaB, respectively, followed by inhibition of telomerase activity. The inhibition of hTERT mRNA expression preceded suppression of cell proliferation. In PC-3 cells, TSA and NaB also inhibited cell proliferation, hTERT mRNA expression and telomerase activity. In both cell lines, TSA and NaB had no effect on hTERC expression, or on expression of c-myc and p21(Waf1) mRNA. These effects of TSA and NaB were unlikely to be consequences of cell cycle arrest, apoptosis, or cell differentiation. Thus, HDAC inhibitors down-regulated telomerase activity via suppression of hTERT mRNA expression. Our study identified a novel mechanism for the antiproliferative effects of HDAC inhibitors on prostate cancer cells.

Tabori, U. and J. S. Dome (2007). "Telomere biology of pediatric cancer." *Cancer Invest* **25**(3): 197-208.

One of the hallmarks of cancer is limitless proliferative capacity, which is tightly associated with the ability to maintain telomeres. Over the last decade, the telomere biology of pediatric cancers has begun to be elucidated. Most pediatric leukemias and embryonal solid tumors activate the enzyme telomerase, a specialized reverse transcriptase that adds nucleotide repeats to telomeres. In general, high levels of tumor telomerase expression are associated with unfavorable outcome, although results vary according to tumor type. Some pediatric tumors, including osteosarcoma and glioblastoma multiforme, lack telomerase activity and maintain telomeres via a recombination-based mechanism called ALT (alternative lengthening of telomeres). Telomerase is a highly attractive therapeutic target for pediatric cancer because the enzyme plays a key role in conferring cellular immortality, is present in most tumors, and is relatively specific for cancer cells. Telomerase inhibitors have been evaluated in preclinical models of adult cancers, but few studies have been conducted on pediatric cancers. Further research is required to define how telomere biology can be used to clinical advantage in malignancies of childhood.

Taillade, L., et al. (2007). "Immunohistochemical expression of biomarkers: a comparative study between diagnostic bronchial biopsies and surgical specimens of non-small-cell lung cancer." *Ann Oncol* **18**(6): 1043-1050.

BACKGROUND: The increasing use of biomarkers as molecular determinants of responsiveness to conventional chemotherapy or molecular targeted therapy has raised the question of the reliability and reproducibility of their evaluation in bronchial biopsies as compared with corresponding resected surgical specimens. **PATIENTS AND METHODS:** Immunohistochemical expression of five markers related to signal transduction [epidermal growth factor receptor (EGFR), phospho-Akt], cell proliferation (Ki-67), DNA repair [excision repair cross-complementing (ERCC)1] and cellular 'immortality' [human telomerase catalytic component (hTERT)], was assessed in 41 patients with operable non-small-cell lung cancer in both bronchial biopsies and whole surgical specimens. **RESULTS:** High correlation coefficients were observed between the expression of ERCC1, hTERT and Ki-67 in the biopsies and the surgical specimens [0.83 ($P < 0.0001$); 0.55 ($P < 0.001$) and 0.64 ($P < 0.0001$), respectively]. On the other hand, biomarker expression in biopsy was less correlated with the expression in the whole tissue sample for the markers of signal response and transduction [0.24 ($P = 0.17$) and 0.29 ($P = 0.09$) for EGFR and phospho-Akt, respectively]. **CONCLUSIONS:** Our results indicate a lack of association in the expression of important biomarkers between lung biopsies and corresponding resected tumors, with discordance rates ranging between 9% and 41%. Although these results need to be further validated in larger cohorts, they indicate that the evaluation of the expression of biomarkers in bronchial biopsies can be misleading.

Takakura, M., et al. (1998). "Expression of human telomerase subunits and correlation with telomerase activity in cervical cancer." *Cancer Res* **58**(7): 1558-1561.

Activation of telomerase and stabilization of telomeres are thought to be required for both cellular immortality and oncogenesis. Three major components of human telomerase, human telomerase RNA (hTR), telomerase-associated protein (TP1/TLP1), and human telomerase catalytic subunit (hTERT/hEST2), have been identified recently. However, it remains unclear what roles these subunits play in the regulation of telomerase activity. In the present study, a total of 25 cervical cancers and 14 normal cervixes as well as various cell lines derived from cervical cancer were examined for the expression of hTR, TP1 mRNA, and hTERT mRNA, and the correlations between expression of these and telomerase activity were evaluated in 23 cancers and 14 normal cervixes. Reverse transcription-PCR analysis revealed that hTR and TP1 mRNA were

commonly expressed in cancers and noncancerous tissues. However, hTERT mRNA was observed only in cervical cancers and cell lines, and more than 80% of cervical cancers expressed it, whereas neither normal cervical tissues nor normal primary fibroblast cells did. There was a strong correlation of telomerase activity with hTERT mRNA expression but not with TP1 or hTR expression. Cervical exfoliated cells were subjected to reverse transcription-PCR analysis for detection of hTERT mRNA, and approximately 70% of cervical cancers were positive for such expression. These findings provide strong evidence that expression of hTERT is a rate-limiting determinant of the enzymatic activity of human telomerase and that up-regulation of hTERT expression may play a critical role in human carcinogenesis. Our findings also indicate that detection of hTERT mRNA is useful for cytological screening for cervical cancer.

Takakura, M., et al. (2020). "A Novel Liquid Biopsy Strategy to Detect Small Amounts of Cancer Cells Using Cancer-Specific Replication Adenoviruses." *J Clin Med* **9**(12).

Circulating tumor cells (CTCs) are a promising source of clinical and biological cancer information and can be a material for liquid biopsy. However, detecting and capturing these cells remains a challenge. Various biological factors (e.g., cell surface proteins, cell size, deformability, or dielectrophoresis) have been applied to detect CTCs. Cancer cells dramatically change their characteristics during tumorigenesis and metastasis. Hence, defining a cell as malignant using such a parameter is difficult. Moreover, immortality is an essential characteristic of cancer cells. Telomerase elongates telomeres and plays a critical role in cellular immortality and is specifically activated in cancer cells. Thus, the activation of telomerase can be a good fingerprint for cancer cells. Telomerase cannot be recognized by antibodies in living cells because it is a nuclear enzyme. Therefore, telomerase-specific replication adenovirus, which expresses the green fluorescent protein, has been applied to detect CTCs. This review explores the overview of this novel technology and its application in gynecological cancers.

Talib, W. H. (2018). "Melatonin and Cancer Hallmarks." *Molecules* **23**(3).

Melatonin is a natural indoleamine produced by the pineal gland that has many functions, including regulation of the circadian rhythm. Many studies have reported the anticancer effect of melatonin against a myriad of cancer types. Cancer hallmarks include sustained proliferation, evading growth suppressors, metastasis, replicative immortality, angiogenesis, resisting cell death,

altered cellular energetics, and immune evasion. Melatonin anticancer activity is mediated by interfering with various cancer hallmarks. This review summarizes the anticancer role of melatonin in each cancer hallmark. The studies discussed in this review should serve as a solid foundation for researchers and physicians to support basic and clinical studies on melatonin as a promising anticancer agent.

Talib, W. H., et al. (2024). "Allicin and Cancer Hallmarks." *Molecules* **29**(6).

Natural products, particularly medicinal plants, are crucial in combating cancer and aiding in the discovery and development of new therapeutic agents owing to their biologically active compounds. They offer a promising avenue for developing effective anticancer medications because of their low toxicity, diverse chemical structures, and ability to target various cancers. Allicin is one of the main ingredients in garlic (*Allium sativum* L.). It is a bioactive sulfur compound maintained in various plant sections in a precursor state. Numerous studies have documented the positive health benefits of this natural compound on many chronic conditions, including gastric, hepatic, breast, lung, cervical, prostate, and colon cancer. Moreover, allicin may target several cancer hallmarks or fundamental biological traits and functions that influence cancer development and spread. Cancer hallmarks include sustained proliferation, evasion of growth suppressors, metastasis, replicative immortality, angiogenesis, resistance to cell death, altered cellular energetics, and immune evasion. The findings of this review should provide researchers and medical professionals with a solid basis to support fundamental and clinical investigations of allicin as a prospective anticancer drug. This review outlines the anticancer role of allicin in each hallmark of cancer.

Tarabichi, M., et al. (2013). "Systems biology of cancer: entropy, disorder, and selection-driven evolution to independence, invasion and "swarm intelligence"." *Cancer Metastasis Rev* **32**(3-4): 403-421.

Our knowledge of the biology of solid cancer has greatly progressed during the last few years, and many excellent reviews dealing with the various aspects of this biology have appeared. In the present review, we attempt to bring together these subjects in a general systems biology narrative. It starts from the roles of what we term entropy of signaling and noise in the initial oncogenic events, to the first major transition of tumorigenesis: the independence of the tumor cell and the switch in its physiology, i.e., from subservience to the organism to

its own independent Darwinian evolution. The development after independence involves a constant dynamic reprogramming of the cells and the emergence of a sort of collective intelligence leading to invasion and metastasis and seldom to the ultimate acquisition of immortality through inter-individual infection. At each step, the probability of success is minimal to infinitesimal, but the number of cells possibly involved and the time scale account for the relatively high occurrence of tumorigenesis and metastasis in multicellular organisms.

Tarkanyi, I. and J. Aradi (2008). "Pharmacological intervention strategies for affecting telomerase activity: future prospects to treat cancer and degenerative disease." *Biochimie* **90**(1): 156-172.

Telomerase enzyme is a ribonucleoprotein maintaining the length of the telomeres by adding G-rich repeats to the end of the eukaryotic chromosomes. Normal human somatic cells, cultured in vitro, have a strictly limited proliferative potential undergoing senescence after about 50-70 population doublings. In contrast, most of the tumor cells have unlimited replicative potential. Although the mechanisms of immortalization are not understood completely at a genetic level, the key role of the telomere/telomerase system in the process is clear. The DNA replication machinery is not able to replicate fully the DNA at the very end of the chromosomes; therefore, about 50-200 nucleotides are lost during each of the replication cycles resulting in a gradual decrease of telomere length. Critically short telomere induces senescence, subsequent crisis and cell death. In tumor cells, however, the telomerase enzyme prevents the formation of critically short telomeres, adding GGTTAG repeats to the 3' end of the chromosomes immortalizing the cells. Immortality is one of the hallmarks of cancer. Besides the catalytic activity dependent telomere maintenance, catalytic activity-independent effects of telomerase may also be involved in the regulation of cell cycle. The telomere/telomerase system offers two possibilities to intervene the proliferative activity of the cell: (1) inhibition the telomere maintenance by inhibiting the telomerase activity; (2) activating the residual telomerase enzyme or inducing telomerase expression. Whilst the former approach could abolish the limitless replicative potential of malignant cells, the activation of telomerase might be utilized for treating degenerative diseases. Here, we review the current status of telomerase therapeutics, summarizing the activities of those pharmacological agents which either inhibit or activate the enzyme. We also discuss the future opportunities and challenges of research on pharmacological intervention of telomerase activity.

Tewari, A. B., et al. (2023). "Extirpating the cancer stem cell hydra: Differentiation therapy and Hyperthermia therapy for targeting the cancer stem cell hierarchy." *Clin Exp Med* **23**(7): 3125-3145.

Ever since the discovery of cancer stem cells (CSCs), they have progressively attracted more attention as a therapeutic target. Like the mythical hydra, this subpopulation of cells seems to contribute to cancer immortality, spawning more cells each time that some components of the cancer cell hierarchy are destroyed. Traditional modalities focusing on cancer treatment have emphasized apoptosis as a route to eliminate the tumor burden. A major problem is that cancer cells are often in varying degrees of dedifferentiation contributing to what is known as the CSCs hierarchy and cells which are known to be resistant to conventional therapy. Differentiation therapy is an experimental therapeutic modality aimed at the conversion of malignant phenotype to a more benign one. Hyperthermia therapy (HT) is a modality exploiting the changes induced in cells by the application of heat produced to aid in cancer therapy. While differentiation therapy has been successfully employed in the treatment of acute myeloid leukemia, it has not been hugely successful for other cancer types. Mounting evidence suggests that hyperthermia therapy may greatly augment the effects of differentiation therapy while simultaneously overcoming many of the hard-to-treat facets of recurrent tumors. This review summarizes the progress made so far in integrating hyperthermia therapy with existing modules of differentiation therapy. The focus is on studies related to the successful application of both hyperthermia and differentiation therapy when used alone or in conjunction for hard-to-treat cancer cell niche with emphasis on combined approaches to target the CSCs hierarchy.

Thelen, P., et al. (2004). "Inhibition of telomerase activity and secretion of prostate specific antigen by silibinin in prostate cancer cells." *J Urol* **171**(5): 1934-1938.

PURPOSE: The androgen sensitive prostate cancer cell line LNCaP is strongly positive for dihydrotestosterone (DHT) dependent telomerase activity, which is an important factor in cellular immortality and carcinogenesis. In this study we determined the potential of silibinin as an anticancer drug that down-regulates telomerase activity and prostate specific antigen (PSA) together with the co-activator of the androgen receptor prostate epithelium specific Ets transcription factor. **MATERIALS AND METHODS:** LNCaP cells were treated with various concentrations of silibinin in the presence or absence

of 5alpha-DHT. We used real-time reverse transcriptase-polymerase chain reaction to quantify mRNA expression of PSA, prostate epithelium specific Ets transcription factor and the catalytic subunit of telomerase vs the housekeeping gene porphobilinogen deaminase with gene specific, dual labeled fluorescence probes. PSA secretion from LNCaP cells in conditioned medium was measured with an Elecsys System 2010 (Roche Diagnostics, Mannheim, Germany) and telomerase activity in extracts from LNCaP cells was measured with a TRAP (telomeric repeat amplification protocol) assay. **RESULTS:** Silibinin down-regulated PSA mRNA expression and PSA secretion in conditioned medium. Simultaneous stimulation with silibinin and 10(-8) M DHT also resulted in PSA down-regulation, whereas DHT alone increased PSA secretion. Telomerase catalytic subunit mRNA decreased significantly after silibinin stimulation. Telomerase activity was down-regulated by silibinin and stimulated by DHT. The 2 agents in combination resulted in telomerase down-regulation. **CONCLUSIONS:** The down-regulation of PSA by silibinin and its counteraction on DHT effects indicate that this compound can interact with the expression of genes that are regulated through the androgen receptor. Silibinin can also inhibit the telomerase activity that mediates cell immortality and carcinogenesis. The 2 effects underline the possible therapeutic use of silibinin as an antiproliferative agent in intervention for prostate cancer.

Tilborghs, S., et al. (2017). "The role of Nuclear Factor-kappa B signaling in human cervical cancer." *Crit Rev Oncol Hematol* **120**: 141-150.

Background The Nuclear Factor kappaB (NF-kB) family consists of transcription factors that play a complex and essential role in the regulation of immune responses and inflammation. NF-kB has recently generated considerable interest as it has been implicated in human cancer initiation, progression and resistance to treatment. In the present comprehensive review the different aspects of NF-kB signaling in the carcinogenesis of cancer of the uterine cervix are discussed. NF-kB functions as part of a network, which determines the pattern of its effects on the expression of several other genes (such as crosstalks with reactive oxygen species, p53, STAT3 and miRNAs) and thus its function. Activation of NF-kB triggered by a HPV infection is playing an important role in the innate and adaptive immune response of the host. The virus induces down regulation of NF-kB to liquidate the inhibitory activity for its replication triggered by the immune system leading a status of persistent HPV infection. During the progression to high grade intraepithelial neoplasia and cervical cancer NF-KB becomes

constitutionally activated again. Mutations in NF- κ B genes are rare in solid tumors but mutations of upstream signaling molecules such as RAS, EGFR, PGF, HER2 have been implicated in elevated NF- κ B signaling. NF- κ B can stimulate transcription of proliferation regulating genes (eg. cyclin D1 and c-myc), genes involved in metastasis, VEGF dependent angiogenesis and cell immortality by telomerase. NF- κ B activation can also induce the expression of activation-induced cytosine deaminase (AID) and the APOBEC proteins, providing a mechanistic link between the NF- κ B pathway and mutagenic characteristic of cervical cancer. Inhibition of NF- κ B has the potential to be used to reverse resistance to radiotherapy and systemic anti-cancer medication, but currently no clinically active NF- κ B targeting strategies are available.

Tomasin, R. and A. Bruni-Cardoso (2022). "The role of cellular quiescence in cancer - beyond a quiet passenger." *J Cell Sci* **135**(15).

Quiescence, the ability to temporarily halt proliferation, is a conserved process that initially allowed survival of unicellular organisms during inhospitable times and later contributed to the rise of multicellular organisms, becoming key for cell differentiation, size control and tissue homeostasis. In this Review, we explore the concept of cancer as a disease that involves abnormal regulation of cellular quiescence at every step, from malignant transformation to metastatic outgrowth. Indeed, disrupted quiescence regulation can be linked to each of the so-called 'hallmarks of cancer'. As we argue here, quiescence induction contributes to immune evasion and resistance against cell death. In contrast, loss of quiescence underlies sustained proliferative signalling, evasion of growth suppressors, pro-tumorigenic inflammation, angiogenesis and genomic instability. Finally, both acquisition and loss of quiescence are involved in replicative immortality, metastasis and deregulated cellular energetics. We believe that a viewpoint that considers quiescence abnormalities that occur during oncogenesis might change the way we ask fundamental questions and the experimental approaches we take, potentially contributing to novel discoveries that might help to alter the course of cancer therapy.

Tsuburaya, A., et al. (2002). "An anti-apoptosis gene, survivin and telomerase expression in gastric cancer." *Hepatogastroenterology* **49**(46): 1150-1152.

BACKGROUND/AIMS: Survivin is frequently expressed in various malignancies as an inhibitor of programmed cell death, while telomerase is associated with cell immortality and consequent accumulation of a malignant genetic property. We

surmised that an activation of telomerase in combination with an inhibition of apoptosis might further accelerate growth of malignant tumors. To test this hypothesis, we investigated the expression of survivin and telomerase in stomach cancer. **METHODOLOGY:** Twenty-five consecutive patients who underwent gastrectomy for stomach cancer were studied including 13 patients at stage 4, 7 of whom had peritoneal metastasis. The expression of survivin and telomerase in the tumors were studied by a reverse transcriptase-polymerase chain reaction. **RESULTS:** Survivin mRNA was detected in 16 (64%) out of 25 tumors and telomerase mRNA in 24 (96%) of them. Survivin expression was inversely associated with the depth of tumor and peritoneal metastasis, while histology, lymph node metastasis, stage, and telomerase expression were not associated. DNA fragmentation was observed in 8 out of 15 tumors including 4 of 9 survivin positive tumors and 4 of 6 survivin negative tumors (n.s.). **CONCLUSIONS:** Survivin was not likely to be related with an inhibition of apoptosis or induction of telomerase but may have some role in gastric cancer extension.

Ucaryilmaz Metin, C. and G. Ozcan (2022). "The HIF-1alpha as a Potent Inducer of the Hallmarks in Gastric Cancer." *Cancers (Basel)* **14**(11).

Hypoxia is the principal architect of the topographic heterogeneity in tumors. Hypoxia-inducible factor-1alpha (HIF-1alpha) reinforces all hallmarks of cancer and donates cancer cells with more aggressive characteristics at hypoxic niches. HIF-1alpha potently induces sustained growth factor signaling, angiogenesis, epithelial-mesenchymal transition, and replicative immortality. Hypoxia leads to the selection of cancer cells that evade growth suppressors or apoptotic triggers and deregulates cellular energetics. HIF-1alpha is also associated with genetic instability, tumor-promoting inflammation, and escape from immunity. Therefore, HIF-1alpha may be an important therapeutic target in cancer. Despite that, the drug market lacks safe and efficacious anti-HIF-1alpha molecules, raising the quest for fully unveiling the complex interactome of HIF-1alpha in cancer to discover more effective strategies. The knowledge gap is even wider in gastric cancer, where the number of studies on hypoxia is relatively low compared to other well-dissected cancers. A comprehensive review of the molecular mechanisms by which HIF-1alpha induces gastric cancer hallmarks could provide a broad perspective to the investigators and reveal missing links to explore in future studies. Thus, here we review the impact of HIF-1alpha on the cancer hallmarks with a specific focus on gastric cancer.

Vahidi, S. and A. Zabeti Touchaei (2024). "Telomerase-based vaccines: a promising frontier in cancer immunotherapy." *Cancer Cell Int* **24**(1): 421.

Telomerase, an enzyme crucial for maintaining telomere length, plays a critical role in cellular immortality and is overexpressed in most cancers. This ubiquitous presence makes telomerase, and specifically its catalytic subunit, human telomerase reverse transcriptase (hTERT), an attractive target for cancer immunotherapy. This review explores the development and application of telomerase-based vaccines, focusing on DNA and peptide-based approaches. While DNA vaccines demonstrate promising immunogenicity, peptide vaccines, such as UV1, UCPVax, and Vx-001, have shown clinical efficacy in certain cancer types. Recent advancements in vaccine design, including multiple peptides and adjuvants, have enhanced immune responses. However, challenges remain in achieving consistent and durable anti-tumor immunity. Accordingly, we discuss the mechanisms of action, preclinical and clinical data, and the potential of these vaccines to elicit robust and durable anti-tumor immune responses. This review highlights the potential of telomerase-based vaccines as a promising strategy for cancer treatment and identifies areas for future research.

Van Roosbroeck, K. and G. A. Calin (2017). "Cancer Hallmarks and MicroRNAs: The Therapeutic Connection." *Adv Cancer Res* **135**: 119-149.

Human cancers are characterized by a number of hallmarks, including sustained proliferative signaling, evasion of growth suppressors, activated invasion and metastasis, replicative immortality, angiogenesis, resistance to cell death, and evasion of immune destruction. As microRNAs (miRNAs) are deregulated in virtually all human cancers, they show involvement in each of the cancer hallmarks as well. In this chapter, we describe the involvement of miRNAs in cancer from a cancer hallmarks and targeted therapeutics point of view. As no miRNA-based cancer therapeutics are available to date, and the only clinical trial on miRNA-based cancer therapeutics (MRX34) was terminated prematurely due to serious adverse events, we are focusing on protein-coding miRNA targets for which targeted therapeutics in oncology are already approved by the FDA. For each of the cancer hallmarks, we selected major protein-coding players and describe the miRNAs that target them.

Van Tongelen, A., et al. (2017). "Oncogenic roles of DNA hypomethylation through the activation of cancer-germline genes." *Cancer Lett* **396**: 130-137.

Global loss of DNA methylation is frequently observed in the genome of human tumors. Although this epigenetic alteration is clearly associated with cancer progression, the way it exerts its pro-tumoral effect remains incompletely understood. A remarkable consequence of DNA hypomethylation in tumors is the aberrant activation of "cancer-germline" genes (also known as "cancer-testis" genes), which comprise a diverse group of germline-specific genes that use DNA methylation as a primary mechanism for repression in normal somatic tissues. Here we review the evidence that such cancer-germline genes contribute to key processes of tumor development. Notably, several cancer-germline genes were found to stimulate oncogenic pathways involved in cell proliferation (SSX, DDX43, MAEL, PIWIL1), angiogenesis (DDX53), immortality (BORIS/CTCF), and metastasis (CT-GABRA3). Others appear to inhibit tumor suppressor pathways, including those controlling growth inhibition signals (MAGEA11, MAGEB2), apoptosis (MAGEA2, MAGEC2), and genome integrity (HORMAD1, NXF2). Cancer-germline genes were also implicated in the regulation of tumor metabolism (MAGEA3/MAGEA6). Together, our survey substantiates the concept that DNA hypomethylation promotes tumorigenesis via transcriptional activation of oncogenes. Importantly, considering their highly restricted pattern of expression, cancer-germline genes may represent valuable targets for the development of anti-cancer therapies with limited side effects.

VanDyke, D., et al. (2016). "Nanoparticle Based Combination Treatments for Targeting Multiple Hallmarks of Cancer." *Int J Nano Stud Technol Suppl* **4**: 1-18.

Treatment of cancer remains one of the most challenging tasks facing the healthcare system. Cancer affects the lives of millions of people and is often fatal. Current treatment methods include surgery, chemotherapy, radiation therapies or some combinations of these. However, recurrence is a major problem. These treatments can be invasive with severe side effects. Inefficacies in treatments are a result of the complex and variable biology of cancerous cells. Malignant tumor cells and normal functioning cells share many of the same biological characteristics but the main difference is that in cancer cells there is an overuse and over expression of these biological characteristics. These pertinent characteristics can be grouped into eight hallmarks, as illustrated by Hanahan and Weinberg. These characteristics include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing

angiogenesis, activating invasion and metastasis, reprogramming energy metabolism, and evading immune destruction. In order to provide a noninvasive, effective treatment, delivery methods must be explored in order to transport cytotoxic agents used for targeting the hallmarks of cancer in a safer and more effective fashion. The use of nanoparticles as drug delivery carriers provides an effective method in which multiple cytotoxic agents can be safely delivered to cancer tissue to simultaneously target multiple hallmarks. By targeting multiple hallmarks of cancer at once, the efficacy of cancer treatments could be improved drastically. This review explores the uses and efficacy of combination therapies using nanoparticles that can simultaneously target multiple hallmarks of cancer.

Varadi, V., et al. (2009). "A functional promoter polymorphism in the TERT gene does not affect inherited susceptibility to breast cancer." *Cancer Genet Cytogenet* **190**(2): 71-74.

Telomere dysfunction is a key mechanism in cancer development. The human telomerase reverse transcriptase (TERT) is the rate-limiting catalytic subunit of the telomerase enzyme, which is necessary for the maintenance of telomere DNA length, chromosomal stability, and cellular immortality. In our attempt to identify functional polymorphisms in the TERT gene and their effect on breast cancer risk, we sequenced the promoter of the gene and identified three single nucleotide polymorphisms (SNPs) with a frequency of at least 10%. One of these SNPs, rs2853669 (-244 T > C), has been shown to affect telomerase activity and telomere length. Recently, this SNP has been suggested to affect familial breast cancer risk. In our case-control study using two large breast cancer sample series, including one with 841 cases with inherited susceptibility to breast cancer, we did not find any association with familial or sporadic breast cancer risk. This well-powered study excludes an effect of the functional -244 T > C SNP and two other correlated SNPs on breast cancer risk.

Veisi, A., et al. (2020). "Role of crocin in several cancer cell lines: An updated review." *Iran J Basic Med Sci* **23**(1): 3-12.

Cancer is a major public health problem worldwide. The most important considerable features of cancer cells are uncontrolled proliferation, up-regulated differentiation, and immortality. Crocin, as a bioactive compound of saffron and as a water-soluble carotenoid has radical scavenging, anti-hyperlipidemia, memory improving, and inhibition of tumor growth effects. The present review was designed to evaluate molecular mechanisms

underlying crocin effects against cancer cell lines. Data of this review have been collected from the scientific articles published in databases such as Science Direct, Scopus, PubMed, and Scientific Information Database from 1982 to 2019. According to various literature, crocin inhibits tumor growth, and its spread in several types of cancer including colorectal, pancreatic, breast, and prostate, as well as chronic myelogenous and leukemia. It inhibits telomerase activity, microtubule polymerization, cyclin D1, nuclear factor kappa B (NF- κ B), multidrug resistance-associated protein (MRP1), and MRP2 overexpression. Crocin can induce apoptosis through activation of caspase 8, up-regulation of p53 expression, Bax/Bcl-2 ratio, and down-regulation expression of Bcl-2, survivin, and cyclin D1. It also down-regulates matrix metalloproteinase 2 and 9 (MMP2 and MMP9), N-cadherin, and beta-catenin expression, which are involved in tumor invasion and metastasis. Tumor invasion was also inhibited by crocin through increasing E-cadherin expression, cell cycle suppression at G1, G0/G1, S, and G2/M phases. Crocin has therapeutic and preventive effects on cancer cells line. Therefore, it has been suggested that this agent can be administered in patients that suffer from this problem.

Veltri, R. W., et al. (2012). "Nuclear morphometry, nucleomics and prostate cancer progression." *Asian J Androl* **14**(3): 375-384.

Prostate cancer (PCa) results from a multistep process. This process includes initiation, which occurs through various aging events and multiple insults (such as chronic infection, inflammation and genetic instability through reactive oxygen species causing DNA double-strand breaks), followed by a multistep process of progression. These steps include several genetic and epigenetic alterations, as well as alterations to the chromatin structure, which occur in response to the carcinogenic stress-related events that sustain proliferative signaling. Events such as evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis are readily observed. In addition, in conjunction with these critical drivers of carcinogenesis, other factors related to the etiopathogenesis of PCa, involving energy metabolism and evasion of the immune surveillance system, appear to be involved. In addition, when cancer spread and metastasis occur, the 'tumor microenvironment' in the bone of PCa patients may provide a way to sustain dormancy or senescence and eventually establish a 'seed and soil' site where PCa proliferation and growth may occur over time. When PCa is initiated and progression ensues, significant

alterations in nuclear size, shape and heterochromatin (DNA transcription) organization are found, and key nuclear transcriptional and structural proteins, as well as multiple nuclear bodies can lead to precancerous and malignant changes. These series of cellular and tissue-related malignancy-associated events can be quantified to assess disease progression and management.

Villarinho, N. J., et al. (2022). "Effects of long-term exposure to MST-312 on lung cancer cells tumorigenesis: Role of SHH/GLI-1 axis." *Cell Biol Int* **46**(9): 1468-1479.

Replicative immortality is a key feature of cancer cells and it is maintained by the expression of telomerase, a promising target of novel therapies. Long-term telomerase inhibition can induce resistance, but the mechanisms underlying this process remain unclear. The Sonic hedgehog pathway (SHH) is an embryogenic pathway involved in tumorigenesis and modulates the transcription of telomerase. We evaluated the effects of long-term treatment of the telomerase inhibitor MST-312 in morphology, proliferation, resistance, and in the SHH pathway molecules expression levels in lung cancer cells. Cells treated for 12 weeks with MST-312 showed changes in morphology, such as spindle-shaped cells, and a shift in the distribution of F-ACTIN from cortical to diffuse. Treatment also significantly reduced cells' efficiency to form spheroids and their clonogenic potential, independently of the cell cycle and telomeric DNA content. Moreover, GLI-1 expression levels were significantly reduced after 12 weeks of MST-312 treatment, indicating a possible inhibition of this signaling axis in the SHH pathway, without hindering NANOG and OCT4 expression. Here, we described a novel implication of long-term treatment with MST-312 functionally and molecularly, shedding new light on the molecular mechanisms of this drug in vitro.

Vinay, D. S., et al. (2015). "Immune evasion in cancer: Mechanistic basis and therapeutic strategies." *Semin Cancer Biol* **35 Suppl**: S185-S198.

Cancer immune evasion is a major stumbling block in designing effective anticancer therapeutic strategies. Although considerable progress has been made in understanding how cancers evade destructive immunity, measures to counteract tumor escape have not kept pace. There are a number of factors that contribute to tumor persistence despite having a normal host immune system. Immune editing is one of the key aspects why tumors evade surveillance causing the tumors to lie dormant in patients for years through "equilibrium" and "senescence" before re-emerging.

In addition, tumors exploit several immunological processes such as targeting the regulatory T cell function or their secretions, antigen presentation, modifying the production of immune suppressive mediators, tolerance and immune deviation. Besides these, tumor heterogeneity and metastasis also play a critical role in tumor growth. A number of potential targets like promoting Th1, NK cell, gammadelta T cell responses, inhibiting Treg functionality, induction of IL-12, use of drugs including phytochemicals have been designed to counter tumor progression with much success. Some natural agents and phytochemicals merit further study. For example, use of certain key polysaccharide components from mushrooms and plants have shown to possess therapeutic impact on tumor-imposed genetic instability, anti-growth signaling, replicative immortality, dysregulated metabolism etc. In this review, we will discuss the advances made toward understanding the basis of cancer immune evasion and summarize the efficacy of various therapeutic measures and targets that have been developed or are being investigated to enhance tumor rejection.

Vincent, M. D. (2011). "Cancer: beyond speciation." *Adv Cancer Res* **112**: 283-350.

A good account of the nature of cancer should provide not only a description of its consistent features, but also how they arise, how they are maintained, why conventional chemotherapy succeeds, and fails, and where to look for better targets. Cancer was once regarded as enigmatic and inexplicable; more recently, the "mutation theory," based on random alterations in a relatively small set of proto-oncogenes and tumor suppressor genes, has enjoyed widespread acceptance. The "mutation theory," however, is noticeable for its failure to explain the basis of differential chemosensitivity, for providing a paucity of targets, especially druggable ones, and for justifying the development of targeted therapies with, in general, disappointingly abbreviated clinical benefit. Furthermore, this theory has mistakenly predicted a widespread commonality of consistent genetic abnormalities across the range of cancers, whereas the opposite, that is, roiling macrogenomic instability, is generally the rule. In contrast, concerning what actually is consistent, that is, the suite of metabolic derangements common to virtually all, especially aggressive, cancers, the "Mutation Theory" has nothing to say. Other hypotheses merit serious consideration "aneuploidy theories" posit whole-genome instability and imbalance as causally responsible for the propagation of the tumor. Another approach, that is, "derepression atavism," suggests cancer results from the release of an ancient survival program, characterized by the

emergence of remarkably primitive features such as unicellularity, fermentation, and immortality; existential goals are served by heuristic genomic instability coupled with host-to-tumor biomass interconversion, mediated by the Warburg effect, a major component of the program. Carcinogenesis is here seen as a process of de-speciation; however, genomic nonrestabilization raises issues as to where on the tree of life cancers belong, as a genuinely alternative *modus vivendi*. Philosophical considerations aside, genomic instability offers the prospect of subtle new therapies based on loss of information rather than gain; and the consistent, specific, and broad-spectrum perfidy of the Warburg effect highlights a supplemental target of the highest priority.

Vinnitsky, V. (2014). "The development of a malignant tumor is due to a desperate asexual self-cloning process in which cancer stem cells develop the ability to mimic the genetic program of germline cells." *Intrinsically Disord Proteins* 2(1): e29997.

To date there is no explanation why the development of almost all types of solid tumors occurs sharing a similar scenario: (1) creation of a cancer stem cell (CSC), (2) CSC multiplication and formation of a multicellular tumor spheroid (TS), (3) vascularization of the TS and its transformation into a vascularized primary tumor, (4) metastatic spreading of CSCs, (5) formation of a metastatic TSs and its transformation into metastatic tumors, and (6) potentially endless repetition of this cycle of events. The above gaps in our knowledge are related to the biology of cancer and specifically to tumorigenesis, which covers the process from the creation of a CSC to the formation of a malignant tumor and the development of metastases. My Oncogerminative Theory of Tumorigenesis considers tumor formation as a dynamic self-organizing process that mimics a self-organizing process of early embryo development. In the initial step in that process, gene mutations combined with epigenetic dysregulation cause somatic cells to be reprogrammed into CSCs, which are immortal pseudo-germline cells. Mimicking the behavior of fertilized germline cells, the CSC achieves immortality by passing through the stages of its life-cycle and developing into a pseudo-blastula-stage embryo, which manifests in the body as a malignant tumor. In this view, the development of a malignant tumor from a CSC is a phenomenon of developmental biology, which we named a desperate asexual self-cloning event. The theory explains seven core characteristics of malignant tumors: (1) CSC immortality, (2) multistep development of a malignant tumor from a single CSC, (3) heterogeneity of malignant tumor cell populations, (4)

metastatic spread of CSCs, (5) invasive growth, (6) malignant progression, and (7) selective immune tolerance toward cancer cells. The Oncogerminative Theory of Tumorigenesis suggests new avenues for discovery of revolutionary therapies to treat, prevent, and eradicate cancer.

Vrba, L. and B. W. Futscher (2017). "Epigenetic Silencing of MORT Is an Early Event in Cancer and Is Associated with Luminal, Receptor Positive Breast Tumor Subtypes." *J Breast Cancer* 20(2): 198-202.

Immortality is an essential characteristic of cancer cells; a recent transcriptomic study of epithelial cell immortalization has linked epigenetic silencing of the long noncoding RNA Mortal Obligate RNA Transcript (MORT; alias ZNF667-AS1) to this process. This study evaluated the epigenetic and transcriptional state of MORT in two premalignant conditions-ductal carcinomas in situ and colon adenomas. Results show that MORT silencing is an early epigenetic event in human carcinogenesis, likely occurring near the point where premalignant cells gain immortality; this epigenetic silencing is maintained throughout malignant transformation and metastatic growth. Additional associations between MORT loss and clinical and molecular features of breast tumors showed that silencing of MORT occurs predominantly in luminal, receptor-positive breast cancer; is associated with overexpression of CCND1 and mutations of GATA3; and is negatively correlated with TP53 mutations. Taken in toto, MORT silencing occurs early in breast carcinogenesis, probably during cellular immortalization, and precedes the development of invasive luminal breast cancer.

Wagner, N. and K. D. Wagner (2020). "PPAR Beta/Delta and the Hallmarks of Cancer." *Cells* 9(5).

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear hormone receptor family. Three different isoforms, PPAR alpha, PPAR beta/delta and PPAR gamma have been identified. They all form heterodimers with retinoic X receptors to activate or repress downstream target genes dependent on the presence/absence of ligands and coactivators or corepressors. PPARs differ in their tissue expression profile, ligands and specific agonists and antagonists. PPARs attract attention as potential therapeutic targets for a variety of diseases. PPAR alpha and gamma agonists are in clinical use for the treatment of dyslipidemias and diabetes. For both receptors, several clinical trials as potential therapeutic targets for cancer are ongoing. In contrast, PPAR beta/delta has been suggested as a therapeutic target for metabolic syndrome. However, potential risks in the settings of cancer are less clear. A variety

of studies have investigated PPAR beta/delta expression or activation/inhibition in different cancer cell models in vitro, but the relevance for cancer growth in vivo is less well documented and controversial. In this review, we summarize critically the knowledge of PPAR beta/delta functions for the different hallmarks of cancer biological capabilities, which interplay to determine cancer growth.

Wahi, A., et al. (2024). "Progress in discovery and development of natural inhibitors of histone deacetylases (HDACs) as anti-cancer agents." *Naunyn Schmiedebergs Arch Pharmacol* **397**(2): 675-702.

The study of epigenetic translational modifications had drawn great interest for the last few decades. These processes play a vital role in many diseases and cancer is one of them. Histone acetyltransferase (HAT) and histone deacetylases (HDACs) are key enzymes involved in the acetylation and deacetylation of histones and ultimately in post-translational modifications. Cancer frequently exhibits epigenetic changes, particularly disruption in the expression and activity of HDACs. It includes the capacity to regulate proliferative signalling, circumvent growth inhibitors, escape cell death, enable replicative immortality, promote angiogenesis, stimulate invasion and metastasis, prevent immunological destruction, and genomic instability. The majority of tumours develop and spread as a result of HDAC dysregulation. As a result, HDAC inhibitors (HDACis) were developed, and they today stand as a very promising therapeutic approach. One of the most well-known and efficient therapies for practically all cancer types is chemotherapy. However, the efficiency and safety of treatment are constrained by higher toxicity. The same has been observed with the synthetic HDACi. Natural products, owing to many advantages over synthetic compounds for cancer treatment have always been a choice for therapy. Hence, naturally available molecules are of particular interest for HDAC inhibition and HDAC has drawn the attention of the research fraternity due to their potential to offer a diverse array of chemical structures and bioactive compounds. This diversity opens up new avenues for exploring less toxic HDAC inhibitors to reduce side effects associated with conventional synthetic inhibitors. The review presents comprehensive details on natural product HDACi, their mechanism of action and their biological effects. Moreover, this review provides a brief discussion on the structure activity relationship of selected natural HDAC inhibitors and their analogues which can guide future research to discover selective, more potent HDACi with minimal toxicity.

Wang, H. and J. J. Unternaehrer (2019). "Epithelial-mesenchymal Transition and Cancer Stem Cells: At the Crossroads of Differentiation and Dedifferentiation." *Dev Dyn* **248**(1): 10-20.

In this review, we explore the connections between epithelial-mesenchymal transition (EMT) and differentiation status. EMTs in development have been described as differentiation events, while in most cases EMTs in cancer have been depicted as dedifferentiation events. We will briefly summarize both embryo development and cancer progression with regard to the involvement of EMT and cell differentiation status. We further present the studies that provide evidence that EMT results in both differentiation and dedifferentiation. Finally, we present our resolution to this dilemma by suggesting that EMT brings about dedifferentiation that enables subsequent differentiation. In normal development, EMT events may cause a partial reversal of differentiation to overcome differentiation barriers. When EMT is aberrantly activated in cancer, cells gain attributes of stem cells that contribute to self-renewal capabilities and are able to differentiate to all cell types represented in the tumor. The resulting cancer stem cells attain hallmarks of cancer, including replicative immortality, resistance to cell death, and invasiveness. *Developmental Dynamics* 248:10-20, 2019. (c) 2018 Wiley Periodicals, Inc.

Wang, T., et al. (2015). "Aberrant regulation of the LIN28A/LIN28B and let-7 loop in human malignant tumors and its effects on the hallmarks of cancer." *Mol Cancer* **14**: 125.

RNA binding proteins (RBPs) and microRNAs (miRNAs) are two of the most important post-transcriptional regulators of gene expression, and their aberrant expression contributes to the development of human malignancies. Let-7, one of the most well-known tumor suppressors, is frequently down-regulated in a variety of human cancers. The RBP LIN28A/LIN28B, a direct target of the let-7 family of miRNAs, is an inhibitor of let-7 biogenesis and is frequently up-regulated in cancers. Aberrant regulation of the LIN28A/LIN28B and let-7 loop in human malignant tumors is reportedly involved in cancer development, contributing to cellular proliferation, cell death resistance, angiogenesis, metastasis, metabolism reprogramming, tumor-associated inflammation, genome instability, acquiring immortality and evading immune destruction. In this review, we summarized the mechanisms of LIN28A/LIN28B and let-7 loop aberrant regulation in human cancer and discussed the roles and potential mechanisms of the LIN28A/LIN28B and let-7 loop in regulating the

hallmarks of cancer. The crosstalk between LIN28A/LIN28B and let-7 loop and certain oncogenes (such as MYC, RAS, PI3K/AKT, NF-kappaB and beta-catenin) in regulating hallmarks of cancer has also been discussed.

Wang, W., et al. (2013). "Inhibition of UBE2D3 expression attenuates radiosensitivity of MCF-7 human breast cancer cells by increasing hTERT expression and activity." *PLoS One* **8**(5): e64660.

The known functions of telomerase in tumor cells include replenishing telomeric DNA and maintaining cell immortality. We have previously shown the existence of a negative correlation between human telomerase reverse transcriptase (hTERT) and radiosensitivity in tumor cells. Here we set out to elucidate the molecular mechanisms underlying regulation by telomerase of radiosensitivity in MCF-7 cells. Toward this aim, yeast two-hybrid (Y2H) screening of a human laryngeal squamous cell carcinoma radioresistant (Hep2R) cDNA library was first performed to search for potential hTERT interacting proteins. We identified ubiquitin-conjugating enzyme E2D3 (UBE2D3) as a principle hTERT-interacting protein and validated this association biochemically. ShRNA-mediated inhibition of UBE2D3 expression attenuated MCF-7 radiosensitivity, and induced the accumulation of hTERT and cyclin D1 in these cells. Moreover, down-regulation of UBE2D3 increased hTERT activity and cell proliferation, accelerating G1 to S phase transition in MCF-7 cells. Collectively these findings suggest that UBE2D3 participates in the process of hTERT-mediated radiosensitivity in human breast cancer MCF-7 cells by regulating hTERT and cyclin D1.

Wardi, L., et al. (2014). "Glucose restriction decreases telomerase activity and enhances its inhibitor response on breast cancer cells: possible extra-telomerase role of BIBR 1532." *Cancer Cell Int* **14**: 60.

BACKGROUND: Considerable progress has been made to understand the association between lifestyle and diet in cancer initiation and promotion. Because excessive glucose consumption is a key metabolic hallmark of cancer cells, glucose restriction (GR) decreases the proliferation, and promotes the differentiation and transformation of cancer cells to quiescent cells. The immortality of cancerous cells is largely assured by telomerase, which is an interesting target for inhibition by BIBR 1532. In this study, we investigated the effect of GR on telomerase activity and on the efficacy of its inhibition by BIBR 1532. **METHODS:** Breast cancer MDA-MB 231 and MCF-7 cells were cultured in

DMEM (Dulbecco's modified eagle's media) with 0, 1 or 4.5 g/l of glucose. The telomerase activity was measured via quantitative Real-Time PCR, and the two telomerase subunits were semi-quantified by RT-PCR. Proliferation test and mitochondrial metabolism were assessed via tetrazolium salt reduction and cell counts; apoptosis was assessed via caspase-3 quantification and flow cytometry. **RESULTS:** A decrease in the telomerase activity of more than 75% was associated with a significant reduction in the mRNA expression of its catalytic subunit hTERT (Reverse Transcriptase) and a decrease in the mitochondrial metabolism by more than 80% under restricted glucose conditions. In addition, GR increased the effect of BIBR 1532. Glucose deprivation induces apoptosis via BIBR 1532-mediated telomerase inhibition in triple negative breast cancer cells, as assessed by caspase-3 measurements and Annexin analysis. **CONCLUSIONS:** Taken together, our results suggest that the effect of BIBR 1532 is potentiated by GR to induce triple negative breast cancer cell death.

Watanabe, D., et al. (2023). "Prognostic impact of severe neutropenia in colorectal cancer patients treated with TAS-102 and bevacizumab, addressing immortal-time bias." *BMC Cancer* **23**(1): 1078.

BACKGROUND: Several studies have reported an association between severe neutropenia and long-term survival in patients treated with trifluridine-tipiracil (TAS-102). Because some of these studies failed to address immortality time bias, however, their findings should be interpreted with caution. Additionally, the association between severe neutropenia and survival in patients receiving TAS-102 in combination with bevacizumab (Bmab) remains unclear. **PATIENTS AND METHODS:** We conducted a single-center retrospective cohort study in patients with colorectal cancer who received Bmab + TAS-102. We compared overall survival (OS) between patients who developed grade ≥ 3 neutropenia during the treatment period and those who did not. To account for immortal time bias, we used two approaches, time-varying Cox regression and landmark analysis. **RESULTS:** Median OS was 15.3 months [95% CI: 14.1-NA] in patients with grade ≥ 3 neutropenia and 10.0 months [95% CI: 8.1-NA] in those without. In time-varying Cox regression, onset grade ≥ 3 neutropenia was significantly related to longer survival after adjustment for age and modified Glasgow Prognostic Score. Additionally, 30-, 60-, 90-, and 120-day landmark analysis showed that grade ≥ 3 neutropenia was associated with longer survival after adjustment for age and modified Glasgow Prognostic Score, with respective HRs of 0.30 [0.10-0.90], 0.65

[0.30-1.42], 0.39 [0.17-0.90], and 0.41 [0.18-0.95].
CONCLUSION: We identified an association between long-term survival and the development of severe neutropenia during the early cycle of Bmab + TAS-102 using an approach that addressed immortality time bias.

Watanabe, N. (2001). "[Telomerase, cell immortality and cancer]." *Hokkaido Igaku Zasshi* **76**(3): 127-132.

Telomerase is an enzyme that replaces repetitive (TTAGGG)_n sequences on the ends of chromosomes that would otherwise be lost during successive cell divisions. Telomerase activity is closely linked to attainment of cellular immortality, a step in carcinogenesis, while lack of such activity contributes to cellular senescence. Telomerase is activated in more than 85% of malignant tumors. However, with the exception of some self-renewing tissues with high regenerative potential, telomerase activity is usually repressed in normal somatic tissues. Based on these reports, we investigated telomerase activity in gastric mucosal tissues. Telomerase activity is highest in cancer, followed by intestinal metaplasia, chronic gastritis, and normal mucosa. In patients with intestinal-type gastric cancer, telomerase activity was higher in those with intestinal metaplasia and *H. pylori* infection than in patients without infection. Our results suggest that *H. pylori* infection may influence telomerase activity in cancer and noncancerous tissue. Genes encoding three major components of human telomerase have been recently cloned. They included those for human telomerase RNA component (hTR), human telomerase reverse transcriptase (hTERT), and telomerase-associated protein 1 (TEP1). More recently, two human telomeric repeat binding factors (TRFs) have also been cloned: TRF1, considered to inhibit the action of telomerase at the telomeric region, and TRF2, believed to prevent fusion of chromosome ends and, in vitro, to remodel linear telomeric DNA into large duplex loops. However, the details of mechanisms regulating telomerase activity are still poorly understood, and specific components or binding proteins that might represent suitable targets for cancer gene therapy have not yet been identified. Therefore, we established quantitative assays using a TaqMan RT-PCR for mRNAs encoding the telomerase components hTR, hTERT, and TEP1, as well as for those encoding TRF1 and TRF2. By using our quantitative assays, we found the following results: 1) Expression of TRF1 and TRF2 mRNA was greater in the normal cells than in human malignant hematopoietic cell lines or in patients with acute leukemia, 2) hTERT mRNA expression showed changes paralleling telomerase activity and became undetectable with HL60 cell differentiation, 3)

initially low expression of TRF1 and TRF2 mRNA increased during differentiation. Our results suggest that not only hTERT but also TRF1 and 2 are important regulators of telomerase activity.

Wazir, U., et al. (2019). "Correlation of TERT and Stem Cell Markers in the Context of Human Breast Cancer." *Cancer Genomics Proteomics* **16**(2): 121-127.

BACKGROUND: Telomerase reverse transcriptase (TERT) has a well-known role in carcinogenesis due to its functions in inducing cell immortality and preventing senescence. In this study, the relationships between TERT and a panel of known stem cell markers was examined in order to direct future enquiries into the role of 'stem-ness' in human breast cancer. **MATERIALS AND METHODS:** Breast cancer tissues (n=124) and adjacent normal tissues (n=30) underwent reverse transcription and quantitative polymerase chain reaction. Transcript levels were analyzed for the correlation with that of TERT. **RESULTS:** A significant direct correlation was found in cancerous tissue between TERT and BMI1 proto-oncogene polycomb ring finger 4 (BMI1; n=88, p<0.001), nestin (NES; n=88, p<0.001), POU domain, class 5, transcription factor 1 (POU5F1; n=88, p<0.001), aldehyde dehydrogenase 1 family member A2 (ALDH1A2; n=87, p=0.0298), cyclin-dependent kinase inhibitor 1A (CDKN1A; n=88, p<0.001), integrin subunit beta 1 (ITGNB1; n=88, p<0.001), integrin subunit alpha 6 (ITGA6; n=88, p<0.001), cluster of differentiation antigen 24 (CD24; n=88, p=0.0114), MET proto-oncogene (MET; n=78, p<0.001) and noggin (NOG; n=88, p<0.001). **CONCLUSION:** The evidence presented in this article of possible interactions between TERT and a discrete subset of known stem cell markers would significantly contribute to further enquiries regarding clonal dynamics in the context of human breast cancer.

Welfer, G. A. and B. D. Freudenthal (2023). "Recent advancements in the structural biology of human telomerase and their implications for improved design of cancer therapeutics." *NAR Cancer* **5**(1): zcad010.

Telomerase is a specialized reverse transcriptase that synthesizes telomeric repeats at the ends of linear chromosomes. Telomerase is transiently expressed in germ and stem cells, but nearly all somatic cells silence it after differentiating. However, the vast majority of cancer cells reactivate and constitutively express telomerase to maintain replicative immortality. Because of this, telomerase has remained a promising broad-spectrum

chemotherapeutic target for over 30 years. However, various challenges associated with obtaining high-resolution structural data for telomerase have limited the development of rationally designed structure-based therapeutics. Various techniques and model systems have been utilized to advance our understanding of the structural biology of telomerase. In particular, multiple high-resolution cryogenic electron microscopy (cryo-EM) structures published within the past few years have revealed new components of the telomerase complex with near atomic resolution structural models. Additionally, these structures have provided details for how telomerase is recruited to telomeres and its mechanism of telomere synthesis. With these new pieces of evidence, and the promising outlook for future refinements to our current models, the possibility of telomerase specific chemotherapeutics is becoming more tangible than ever. This review summarizes these recent advancements and outlines outstanding questions in the field.

Wen, L., et al. (2020). "CRISPR/Cas9-Mediated TERT Disruption in Cancer Cells." *Int J Mol Sci* **21**(2).

Mammalian telomere lengths are primarily regulated by telomerase, a ribonucleoprotein consisting of a reverse transcriptase (TERT) and an RNA subunit (TERC). TERC is constitutively expressed in all cells, whereas TERT expression is temporally and spatially regulated, such that in most adult somatic cells, TERT is inactivated and telomerase activity is undetectable. Most tumor cells activate TERT as a mechanism for preventing progressive telomere attrition to achieve proliferative immortality. Therefore, inactivating TERT has been considered to be a promising means of cancer therapy. Here we applied the CRISPR/Cas9 gene editing system to target the TERT gene in cancer cells. We report that disruption of TERT severely compromises cancer cell survival in vitro and in vivo. Haploinsufficiency of TERT in tumor cells is sufficient to result in telomere attrition and growth retardation in vitro. In vivo, TERT haploinsufficient tumor cells failed to form xenograft after transplantation to nude mice. Our work demonstrates that gene editing-mediated TERT knockout is a potential therapeutic option for treating cancer.

Williams, D. (2015). "Thyroid Growth and Cancer." *Eur Thyroid J* **4**(3): 164-173.

It is proposed that most papillary thyroid cancers originate in infancy and childhood, based on the early rise in sporadic thyroid carcinoma incidence, the pattern of radiation-induced risk (highest in those exposed as infants), and the high prevalence of

sporadic papillary thyroid cancers in children and adolescents (ultrasound screening after the Fukushima accident). The early origin can be linked to the growth pattern of follicular cells, with a high mitotic rate in infancy falling to very low replacement levels in adult life. The cell of origin of thyroid cancers, the differentiated follicular cell, has a limited growth potential. Unlike cancers originating in stem cells, loss of the usually tight link between differentiation and replicative senescence is required for immortalisation. It is suggested that this loss distinguishes larger clinically significant papillary thyroid cancers from micro-papillary thyroid cancers of little clinical significance. Papillary carcinogenesis can then be divided into 3 stages: (1) initiation, the first mutation in the carcinogenic cascade, for radiation-induced papillary thyroid cancers usually a RET rearrangement, (2) progression, acquisition of the additional mutations needed for low-grade malignancy, and (3) escape, further mutations giving immortality and a higher net growth rate. Most papillary thyroid cancers will not have achieved full immortality by adulthood, and remain as so-called micro-carcinomas with a very low growth rate. The use of the term 'cancer' to describe micro-papillary thyroid cancers in older patients encourages overtreatment and alarms patients. Invasive papillary thyroid tumours show a spectrum of malignancy, which at its lowest poses no threat to life. The treatment protocols and nomenclature for small papillary carcinomas need to be reconsidered in the light of the new evidence available, the continuing discovery of smaller lesions, and the model of thyroid carcinogenesis proposed.

Wong, S. C., et al. (2006). "Detection of telomerase activity in gastric lavage fluid: a novel method to detect gastric cancer." *J Surg Res* **131**(2): 252-255.

BACKGROUND: Telomerase is a ribonucleoprotein polymerase that is essential for cell immortality. Recent studies have demonstrated that a high percentage of gastric cancer tissue expressed telomerase. This study describes the presence of telomerase activity in gastric lavage fluid in patients with gastric cancer. **METHODS:** Gastric lavage fluid was collected during esophageogastroduodenoscopy in 70 patients: 25 with gastric cancer, 25 with peptic ulcer disease, and 20 with normal stomach. The fluid and biopsy samples were analyzed for telomerase activity by a polymerase chain reaction-based telomerase repeat amplification protocol. The findings were related to the histological results. **RESULTS:** Telomerase activity was present in 24 of the 25 (96%) gastric cancer tissue and in 7 of the 25 tissue specimens from peptic ulcer or gastritis. In the gastric lavage fluid, telomerase was detected in 20

patients (80%) with gastric cancer, 7 patients (28%) with peptic ulcer, and none in normal subjects ($P < 0.001$). The sensitivity, specificity, positive predictive value, and negative predictive value of gastric fluid telomerase expression in gastric cancer patients was 80%, 84%, 74%, and 88%, respectively. **CONCLUSIONS:** The presence of telomerase activity is present in gastric lavage fluid of patients with gastric cancer as compared to those without, may represent a novel method for diagnosis of gastric cancer.

Wu, D., et al. (2015). "Association of genetic polymorphisms in the telomerase reverse transcriptase gene with prostate cancer aggressiveness." *Mol Med Rep* **12**(1): 489-497.

Telomerase reverse transcriptase (TERT), encoded by the TERT gene, is an essential component of telomerase, essential for the maintenance of telomere DNA length, chromosomal stability and cellular immortality. The aim of the present study was to evaluate the association between common genetic variations across the TERT gene region and prostate cancer (PCa) aggressiveness in a Chinese population. A total of 12 TERT tagging single-nucleotide polymorphisms (SNPs) were genotyped on the Sequenom Mass-ARRAY iPLEX(R) platform in a case-case study with 1,210 Chinese patients with PCa. Unconditional logistic regression was used to investigate the association of genotypes with PCa aggressiveness, Gleason grade and risk of developing early-onset PCa. It was observed that the C allele of the TERT intron 2 SNP (rs2736100) was significantly associated with reduced risk of PCa aggressiveness [odds ratio (OR)=0.81; 95% confidence interval (CI): 0.66-0.99; $P=0.037$]. This allele was also significantly correlated with a reduced risk of developing a tumor with a high Gleason score (>7 ; OR=0.83; 95% CI: 0.70-0.99; $P=0.039$). The T allele of the intron 4 SNP (rs10069690) was found to be significantly associated with a decreased risk for an aggressive form of PCa (OR=0.76; 95% CI: 0.59-0.97; $P=0.030$). In addition, the A allele of rs10078761 located at the 3' end of the TERT gene exhibited a statistically significant association with the reduced risk of developing a higher grade disease (OR=0.48; 95% CI: 0.28-0.81; $P=0.006$). However, no association between TERT polymorphisms and age at diagnosis was observed in the present study. The present findings demonstrated for the first time, to the best of our knowledge, that genetic variations across the TERT gene are associated with PCa aggressiveness in a Chinese Han population.

Wu, L., et al. (2020). "Telomerase: Key regulator of inflammation and cancer." *Pharmacol Res* **155**:

104726.

The telomerase holoenzyme, which has a highly conserved role in maintaining telomere length, has long been regarded as a high-profile target in cancer therapy due to the high dependency of the majority of cancer cells on constitutive and elevated telomerase activity for sustained proliferation and immortality. In this review, we present the salient findings in the telomerase field with special focus on the association of telomerase with inflammation and cancer. The elucidation of extra-telomeric roles of telomerase in inflammation, reactive oxygen species (ROS) generation, and cancer development further complicated the design of anti-telomerase therapy. Of note, the discovery of the unique mechanism that underlies reactivation of the dormant telomerase reverse transcriptase TERT promoter in somatic cells not only enhanced our understanding of the critical role of TERT in carcinogenesis but also opens up new intervention ideas that enable the differential targeting of cancer cells only. Despite significant effort invested in developing telomerase-targeted therapeutics, devising efficacious cancer-specific telomerase/TERT inhibitors remains an uphill task. The latest discoveries of the telomere-independent functionalities of telomerase in inflammation and cancer can help illuminate the path of developing specific anti-telomerase/TERT therapeutics against cancer cells.

Wymenga, L. F., et al. (2000). "Telomerase activity in needle biopsies from prostate cancer and benign prostates." *Eur J Clin Invest* **30**(4): 330-335.

BACKGROUND: Telomerase activation is thought to be essential for the immortality of cancer cells. It may be a prognostic factor in small volume well differentiated prostate cancers and hence a guide for the aggressiveness of the approach. The length of the chromosome tips (telomeres) are maintained by a specific enzyme (telomerase) independently of the normal cell division cycle. Although telomerase is not expressed in most normal human tissues, it is expressed in most human tumours. For the detection of telomerase in small prostate needle biopsy samples a recently developed telomeric repeat amplification protocol (TRAP) assay was used. The aim of the present study was: to measure telomerase activity in human prostate samples, and to evaluate the applicability of this assay on specimens from a prostate biopsy. **MATERIALS AND METHODS:** From 36 patients referred for lower urinary tract symptoms (LUTS) or suspicion of having prostate cancer a total of 288 prostate biopsy samples were obtained (8 in each patient). When the digital rectal examination was abnormal and/or when the PSA level was elevated in L.U.T.S., or asymptomatic

patients' tissue samples were obtained by transrectal ultrasound (TRUS) guided biopsies. Samples were tested for telomerase activity by a modified TRAP and forwarded for histology. RESULTS: In 19 out of 36 patients prostate cancer was diagnosed on histology. In 11 of these 19 tumours substantial telomerase activity was detected, whereas only very low telomerase activity existed in 2 of 17 samples from benign prostatic hypertrophy (BPH) patients. In this small series the relative telomerase activity in prostate cancer correlated with histopathological grade. CONCLUSIONS: Our results show the applicability of a TRAP assay to measure telomerase activity in small needle biopsied prostate samples. In poorly differentiated and metastatic cancer we observed that levels of telomerase activity were high. To establish accuracy and to distinguish the 'relative good from the ugly' further study is needed.

Wynter, C. V. (2006). "The dialectics of cancer: A theory of the initiation and development of cancer through errors in RNAi." *Med Hypotheses* **66**(3): 612-635.

The recent discoveries of the RNA-mediated interference system in cells could explain all of the known features of human carcinogenesis. A key, novel idea, proposed here, is that the cell has the ability to recognise a mutated protein and/or mRNA. Secondly, the cell can generate its own short interfering RNA (siRNA) using an RNA polymerase to destroy mutated mRNA, even when only a single base pair in the gene has mutated. The anti-sense strand of the short RNA molecule (called sicRNA), targets the mutated mRNA of an oncogene or a tumour suppressor. The resulting double stranded RNA, using the RNA-induced silencing complex in the cytoplasm dices the mutated mRNA. In cancer-prone tissues, during cell mitosis, the sicRNA complex can move into the nucleus to target the mutated gene. The sicRNA, possibly edited by dsRNA-specific adenosine deaminase, converting adenosines to inosines, can be retained in the nucleus, with enhanced destructive capability. The sicRNA triggers the assembly of protein complexes leading to epigenetic modification of the promoter site of the mutant gene, specifically methylation of cytosines. In some instances, instead of methylation, the homologous DNA is degraded, leading to loss of heterozygosity. The factors controlling these two actions are unknown but the result is gene silencing or physical destruction of the mutant gene. The cell survives dependent on the functioning of the single, wild-type allele. An error in RNAi defence occurs when the sicRNA enters the nucleus and targets the sense strand of the wrong DNA. The sicRNA, because of the similarity of its short sequence and

relaxed stringency, can target other RNAs, which are being transcribed. This can result in the methylation of the wrong promoter site of a gene or LOH of that region. In the vast majority of these cases, the aberrant hybridisations will have no effect on cell function or apoptosis eliminates non-viable cells. On a rare occasion, a preneoplastic cell is initiated when aberrant hybridisations switches on/off a gene involved in apoptosis, as well as a gene involved in cell proliferation and DNA damage surveillance. Genetic instability results when the sicRNA competes for a repeat sequence in the centromere or telomere, leading to gross chromosomal rearrangements. A malignancy develops when the sicRNAs fortuitously targets a microRNA (miRNA) or activates a transcription factor, resulting in the translation of a large number of new genes, alien to that tissue. This leads to dedifferentiation of the tissue, a resculpting of the histone code, chromosomal rearrangements, along a number of specific pathways, the gain of immortality and the dissemination of a metastatic cancer.

Xiao, N., et al. (2012). "Interaction of Berberine derivative with protein POT1 affect telomere function in cancer cells." *Biochem Biophys Res Commun* **419**(3): 567-572.

The protein POT1 plays an important role in telomere protection, which is related with telomere elongation and cell immortality. The protein has been recognized as a promising drug target for cancer treatment. In the present study, we cloned, overexpressed in *Escherichia coli* for the first time, and purified recombinant human POT1. The protein was proved to be active through filter binding assay, FRET and CD experiments. In the initial screening for protein binding ligands using SPR, compound Sysu-00692 was found to bind well with the POT1, which was confirmed with EMSA. Its in vivo activity study showed that compound Sysu-00692 could interfere with the binding between human POT1 and the telomeric DNA through chromatin immunoprecipitation. Besides, the compound showed mild inhibition on telomerase and cell proliferation. As we know, compound Sysu-00692 is the first reported POT1-binding ligand, which could serve as a lead compound for further improvement. This work offered a potentially new approach for drug design for the treatment of cancers.

Yang, T. and K. Rycaj (2015). "Targeted therapy against cancer stem cells." *Oncol Lett* **10**(1): 27-33.

Research into cancer stem cells (CSCs), which have the ability to self-renew and give rise to more mature (differentiated) cancer cells, and which may be the cells responsible for the overall

organization of a tumor, has progressed rapidly and concomitantly with recent advances in studies of normal tissue stem cells. CSCs have been reported in a wide spectrum of human tumors. Like normal tissue stem cells, CSCs similarly exhibit significant phenotypic and functional heterogeneity. The ability of CSCs to self-renew results in the immortality of malignant cells at the population level, whereas the ability of CSCs to differentiate, either fully or partially, generates the cellular hierarchy and heterogeneity commonly observed in solid tumors. CSCs also appear to have maximized their pro-survival mechanisms leading to their relative resistance to anti-cancer therapies and subsequent relapse. Studies in animal models of human cancers have also provided insight into the heterogeneity and characteristics of CSCs, helping to establish a platform for the development of novel targeted therapies against specific CSCs. In the present study, we briefly review the most recent progress in dissecting CSC heterogeneity and targeting CSCs in various human tumor systems. We also highlight a few examples of CSC-targeted drug development and clinical trials, with the ultimate aim of developing more effective therapeutic regimens that are capable of preventing tumor recurrence and metastasis.

Yasui, W., et al. (1999). "Molecular-pathological diagnosis of gastrointestinal tissues and its contribution to cancer histopathology." *Pathol Int* 49(9): 763-774.

Multiple genetic and epigenetic alterations of cancer-related genes and molecules are involved in the course of the development and progression of gastrointestinal cancers. These include telomerase activation, genetic instability, and abnormalities of oncogenes, tumor suppressor genes, cell cycle regulators, cell adhesion molecules and DNA repair genes. By analyzing these alterations in pathology specimens, we can improve differential diagnosis of cancer, obtain information of grade of malignancy, and identify patients at high risk for developing multiple primary cancers. Since 1993, a system of molecular-pathological diagnosis was established, and has been performed as a routine service in collaboration with Hiroshima City Medical Association Clinical Laboratory. More than 10 000 cases of gastrointestinal biopsy and surgery have been analyzed, and additional information of differential diagnosis, biological malignancy and tumor multiplicity could be obtained. Molecular-pathological diagnosis may provide a new approach to cancer diagnosis and novel therapeutics for the 21st century. Furthermore, the analysis of the genetic and epigenetic abnormalities in clinical materials may clarify the molecular mechanism of

carcinogenesis and comparative morphological changes. From the analyses of p27KIP1 and telomerase in gastrointestinal adenomas, we have learned that morphological abnormality of the nucleus is an indicator for cells with immortality and malignant potential that must participate in super-early diagnosis (detection of true precancerous lesions) of gastrointestinal cancer. Molecular-pathological diagnosis thus contributes to detailed understanding of cancer histopathology and improves the histopathological diagnosis.

Yoshioka, K., et al. (2015). "Development of cancer-initiating cells and immortalized cells with genomic instability." *World J Stem Cells* 7(2): 483-489.

Cancers that develop after middle age usually exhibit genomic instability and multiple mutations. This is in direct contrast to pediatric tumors that usually develop as a result of specific chromosomal translocations and epigenetic aberrations. The development of genomic instability is associated with mutations that contribute to cellular immortalization and transformation. Cancer occurs when cancer-initiating cells (CICs), also called cancer stem cells, develop as a result of these mutations. In this paper, we explore how CICs develop as a result of genomic instability, including looking at which cancer suppression mechanisms are abrogated. A recent in vitro study revealed the existence of a CIC induction pathway in differentiating stem cells. Under aberrant differentiation conditions, cells become senescent and develop genomic instabilities that lead to the development of CICs. The resulting CICs contain a mutation in the alternative reading frame of CDKN2A (ARF)/p53 module, i.e., in either ARF or p53. We summarize recently established knowledge of CIC development and cellular immortality, explore the role of the ARF/p53 module in protecting cells from transformation, and describe a risk factor for genomic destabilization that increases during the process of normal cell growth and differentiation and is associated with the downregulation of histone H2AX to levels representative of growth arrest in normal cells.

Yousefi, T., et al. (2024). "Innovating Cancer Treatment Through Cell Cycle, Telomerase, Angiogenesis, and Metastasis." *DNA Cell Biol* 43(9): 438-451.

Cancer remains a formidable challenge in the field of medicine, necessitating innovative therapeutic strategies to combat its relentless progression. The cell cycle, a tightly regulated process governing cell growth and division, plays a pivotal role in cancer development. Dysregulation of

the cell cycle allows cancer cells to proliferate uncontrollably. Therapeutic interventions designed to disrupt the cell cycle offer promise in restraining tumor growth and progression. Telomerase, an enzyme responsible for maintaining telomere length, is often overactive in cancer cells, conferring them with immortality. Targeting telomerase presents an opportunity to limit the replicative potential of cancer cells and hinder tumor growth. Angiogenesis, the formation of new blood vessels, is essential for tumor growth and metastasis. Strategies aimed at inhibiting angiogenesis seek to deprive tumors of their vital blood supply, thereby impeding their progression. Metastasis, the spread of cancer cells from the primary tumor to distant sites, is a major challenge in cancer therapy. Research efforts are focused on understanding the underlying mechanisms of metastasis and developing interventions to disrupt this deadly process. This review provides a glimpse into the multifaceted approach to cancer therapy, addressing critical aspects of cancer biology-cell cycle regulation, telomerase activity, angiogenesis, and metastasis. Through ongoing research and innovative strategies, the field of oncology continues to advance, offering new hope for improved treatment outcomes and enhanced quality of life for cancer patients.

Yu, S., et al. (2020). "MCMs in Cancer: Prognostic Potential and Mechanisms." *Anal Cell Pathol (Amst)* **2020**: 3750294.

Enabling replicative immortality and uncontrolled cell cycle are hallmarks of cancer cells. Minichromosome maintenance proteins (MCMs) exhibit helicase activity in replication initiation and play vital roles in controlling replication times within a cell cycle. Overexpressed MCMs are detected in various cancerous tissues and cancer cell lines. Previous studies have proposed MCMs as promising proliferation markers in cancers, while the prognostic values remain controversial and the underlying mechanisms remain unascertained. This review provides an overview of the significant findings regarding the cellular and tumorigenic functions of the MCM family. Besides, current evidence of the prognostic roles of MCMs is retrospectively reviewed. This work also offers insight into the mechanisms of MCMs prompting carcinogenesis and adverse prognosis, providing information for future research. Finally, MCMs in liver cancer are specifically discussed, and future perspectives are provided.

Zaidi, N. E., et al. (2022). "Crosstalk between fatty acid metabolism and tumour-associated macrophages in cancer progression." *Biomedicine (Taipei)* **12**(4):

9-19.

Over the last few decades, cancer has been regarded as an independent and self sustaining progression. The earliest hallmarks of cancer comprise of sustaining proliferative signalling, avoiding growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Nonetheless, two emerging hallmarks are being described: aberrant metabolic pathways and evasion of immune destruction. Changes in tumour cell metabolism are not restricted to tumour cells alone; the products of the altered metabolism have a direct impact on the activity of immune cells inside the tumour microenvironment, particularly tumour-associated macrophages (TAMs). The complicated process of cancer growth is orchestrated by metabolic changes dictating the tight mutual connection between these cells. Here, we discuss approaches to exploit the interaction of cancer cells' abnormal metabolic activity and TAMs. We also describe ways to exploit it by reprogramming fatty acid metabolism via TAMs.

Zeng, T., et al. (2011). "Up-regulation of kin17 is essential for proliferation of breast cancer." *PLoS One* **6**(9): e25343.

BACKGROUND: Kin17 is ubiquitously expressed at low levels in human tissue and participates in DNA replication, DNA repair and cell cycle control. Breast cancer cells are characterized by enabling replicative immortality and accumulated DNA damage. However, whether kin17 contributes to breast carcinogenesis remains unknown. **METHODOLOGY/PRINCIPAL FINDINGS:** In this study, we show for the first time that kin17 is an important molecule related to breast cancer. Our results show that kin17 expression was markedly increased in clinical breast tumors and was associated with tumor grade, Ki-67 expression, p53 mutation status and progesterone receptor expression, which were assessed in a clinicopathologic characteristics review. Knockdown of kin17 inhibited DNA replication and repair, blocked cell cycle progression and inhibited anchorage-independent growth, while increasing sensitivity to chemotherapy in breast cancer cells. Moreover, kin17 silencing decreased EGF-stimulated cell growth. Furthermore, overexpression of kin17 promoted DNA replication and cell proliferation in MCF-10A. **CONCLUSIONS/SIGNIFICANCE:** Our findings indicate that up-regulation of kin17 is strongly associated with cellular proliferation, DNA replication, DNA damage response and breast cancer development. The increased level of kin17 was not only a consequence of immortalization but also

associated with tumorigenesis. Therefore, kin17 could be a novel therapeutic target for inhibiting cell growth in breast cancer.

Zeng, X., et al. (2018). "Administration of a Nucleoside Analog Promotes Cancer Cell Death in a Telomerase-Dependent Manner." *Cell Rep* **23**(10): 3031-3041.

Telomerase, the end-replication enzyme, is reactivated in malignant cancers to drive cellular immortality. While this distinction makes telomerase an attractive target for anti-cancer therapies, most approaches for inhibiting its activity have been clinically ineffective. As opposed to inhibiting telomerase, we use its activity to selectively promote cytotoxicity in cancer cells. We show that several nucleotide analogs, including 5-fluoro-2'-deoxyuridine (5-FdU) triphosphate, are effectively incorporated by telomerase into a telomere DNA product. Administration of 5-FdU results in an increased number of telomere-induced foci, impedes binding of telomere proteins, activates the ATR-related DNA-damage response, and promotes cell death in a telomerase-dependent manner. Collectively, our data indicate that telomerase activity can be exploited as a putative anti-cancer strategy.

Zhang, D. Y., et al. (2021). "Mechanisms of cancer stem cell senescence: Current understanding and future perspectives." *Clin Exp Pharmacol Physiol* **48**(9): 1185-1202.

Cancer stem cells (CSCs) are a small population of heterogeneous tumor cells with the capacity of self-renewal and aberrant differentiation for immortality and divergent lineages of cancer cells. In contrast to bulky tumor cells, CSCs remain less differentiated and resistant to therapy even when targeted with tissue-specific antigenic markers. This makes CSCs responsible for not only tumor initiation, development, but also tumor recurrence. Emerging evidence suggests that CSCs can undergo cell senescence, a non-proliferative state of cells in response to stress. While cell senescence attenuates tumor cell proliferation, it is commonly regarded as a tumor suppressive mechanism. However, mounting research indicates that CSC senescence also provides these cells with the capacity to evade cytotoxic effects from cancer therapy, exacerbating cancer relapse and metastasis. Recent studies demonstrate that senescence drives reprogramming of cancer cell toward stemness and promotes CSC generation. In this review, we highlight the origin, heterogeneity and senescence regulatory mechanisms of CSCs, the complex relationship between CSC senescence and tumor therapy, and the recent beneficial effects of senotherapy on eliminating senescent tumor cells.

Zhao, C., et al. (2010). "Spontaneously immortalised bovine mammary epithelial cells exhibit a distinct gene expression pattern from the breast cancer cells." *BMC Cell Biol* **11**: 82.

BACKGROUND: Spontaneous immortalisation of cultured mammary epithelial cells (MECs) is an extremely rare event, and the molecular mechanism behind spontaneous immortalisation of MECs is unclear. Here, we report the establishment of a spontaneously immortalised bovine mammary epithelial cell line (BME65Cs) and the changes in gene expression associated with BME65Cs cells. **RESULTS:** BME65Cs cells maintain the general characteristics of normal mammary epithelial cells in morphology, karyotype and immunohistochemistry, and are accompanied by the activation of endogenous bTERT (bovine Telomerase Reverse Transcriptase) and stabilisation of the telomere. Currently, BME65Cs cells have been passed for more than 220 generations, and these cells exhibit non-malignant transformation. The expression of multiple genes was investigated in BME65Cs cells, senescent BMECs (bovine MECs) cells, early passage BMECs cells and MCF-7 cells (a human breast cancer cell line). In comparison with early passage BMECs cells, the expression of senescence-relevant apoptosis-related gene were significantly changed in BME65Cs cells. P16INK4a was downregulated, p53 was low expressed and Bax/Bcl-2 ratio was reversed. Moreover, a slight upregulation of the oncogene c-Myc, along with an undetectable level of breast tumor-related gene Bag-1 and TRPS-1, was observed in BME65Cs cells while these genes are all highly expressed in MCF-7. In addition, DNMT1 is upregulated in BME65Cs. These results suggest that the inhibition of both senescence and mitochondrial apoptosis signalling pathways contribute to the immortality of BME65Cs cells. The expression of p53 and p16INK4a in BME65Cs was altered in the pattern of down-regulation but not "loss", suggesting that this spontaneous immortalization is possibly initiated by other mechanism rather than gene mutation of p53 or p16INK4a. **CONCLUSIONS:** Spontaneously immortalised BME65Cs cells maintain many characteristics of normal BMEC cells and exhibit non-malignant transformation. Although this cell line displays altered patterns of gene expression, it is clearly distinct from malignant breast cancer cell line. It showed that co-inhibition of cellular senescence and mitochondrial apoptosis pathways coordinates BME65Cs cells immortalisation. Additionally, mechanisms other than gene mutation are likely to be involved in regulation of cellular functions. This study provides an insight into the relationship between cell

senescence and immortalisation. BME65Cs cells will be useful in future studies of cellular senescence and tumorigenesis.

Zhao, L., et al. (2022). "Ferroptosis in cancer and cancer immunotherapy." *Cancer Commun (Lond)* 42(2): 88-116.

The hallmark of tumorigenesis is the successful circumvention of cell death regulation for achieving unlimited replication and immortality. Ferroptosis is a newly identified type of cell death dependent on lipid peroxidation which differs from classical programmed cell death in terms of morphology, physiology and biochemistry. The broad spectrum of injury and tumor tolerance are the main reasons for radiotherapy and chemotherapy failure. The effective rate of tumor immunotherapy as a new treatment method is less than 30%. Ferroptosis can be seen in radiotherapy, chemotherapy, and tumor immunotherapy; therefore, ferroptosis activation may be a potential strategy to overcome the drug resistance mechanism of traditional cancer treatments. In this review, the characteristics and causes of cell death by lipid peroxidation in ferroptosis are briefly described. In addition, the three metabolic regulations of ferroptosis and its crosstalk with classical signaling pathways are summarized. Collectively, these findings suggest the vital role of ferroptosis in immunotherapy based on the interaction of ferroptosis with tumor immunotherapy, chemotherapy and radiotherapy, thus, indicating the remarkable potential of ferroptosis in cancer treatment.

Zmitrovich, I. V., et al. (2019). "Cancer without Pharmacological Illusions and a Niche for Mycotherapy (Review)." *Int J Med Mushrooms* 21(2): 105-119.

In this review we outline a framework in which mycotherapy is effective in the field of oncology. We suppose that irreversible epigenomic changes in cancer cells and achieving their replicative immortality when cancer-specific targets are absent should take away any illusions about a fundamental possibility of pharmacological blockage of the cancer process once ontogenesis begins. At the same time, however, we believe that effects of both traditional and alternative medicines on cancer clonogenic units within a particular range can lead to prolonged remission; with this in mind, we carefully consider the various possibilities of mycotherapy in controlling cancer activity. The aforementioned range is limited to nondisseminated cancer processes and depends on the absence of large secondary tumor nodes and the inexhaustibility of immune depots after chemotherapeutic treatment. The main therapeutic

effect of fungal bioactive complexes is dectin-1-mediated immunity, including the reprogramming of dendritic cells, which significantly increases the period during which tumors generate immune tolerance. An inhibitory effect of fungal bioactive complexes on some molecular mediators of proliferative signaling and components of proinflammatory (synergistic with cancer) immunity can be considered less significant. The effect of fungal bioactive complexes on vital (including overexpressed) targets of cancer cells is even more limited. The results of this study stress that mycotherapy is only one of the tools that can be used to balance remission. Palliative mycotherapy is associated with polyphenols composites, which contribute to detoxification and to the suppression of inflammation and pain sensation.

Zohreh, B., et al. (2019). "Apigenin-mediated Alterations in Viability and Senescence of SW480 Colorectal Cancer Cells Persist in The Presence of L-thyroxine." *Anticancer Agents Med Chem* 19(12): 1535-1542.

INTRODUCTION: Deregulation of Thyroid Hormones (THs) system in Colorectal Cancer (CRC) suggests that these hormones may play roles in CRC pathogenesis. Flavonoids are polyphenolic compounds, which possess potent antitumor activities and interfere, albeit some of them, with all aspects of THs physiology. Whether the antitumor actions of flavonoids are affected by THs is unknown. Therefore, we investigated the effects of apigenin (Api), a well-known flavone, on some tumorigenic properties of SW480 CRC cells in the presence and absence of L-thyroxine (T4). **METHODS:** Cell viability was assessed by MTT assay. Flow cytometry and DNA electrophoresis were used to evaluate cell death. Cell senescence was examined by in situ detection of beta-galactosidase activity. Protein expression was assessed by antibody array technique. **RESULTS:** While T4 had minimal effects, Api reduced cell growth and senescence by induction of apoptosis. Expression of anti-apoptotic and pro-apoptotic proteins were differentially affected by Api and T4. Survivin, HSP60 and HTRA were the most expressed proteins by the cells. Almost all Api-induced effects persisted in the presence of T4. **CONCLUSION:** These data suggest that Api may inhibit CRC cell growth and progression through induction of apoptosis rather than cell necrosis or senescence. In addition, they suggest that T4 has minimal effects on CRC cell growth, and is not able to antagonize the anti-growth effects of Api. Regardless of the treatments, cells expressed high levels of survivin, HSP60 and HTRA, indicating that these proteins may play central roles in SW480 CRC

cell immortality.

Zuo, W. F., et al. (2024). "Heat shock proteins as hallmarks of cancer: insights from molecular mechanisms to therapeutic strategies." *J Hematol Oncol* 17(1): 81.

Heat shock proteins are essential molecular chaperones that play crucial roles in stabilizing protein structures, facilitating the repair or degradation of damaged proteins, and maintaining proteostasis and cellular functions. Extensive research has demonstrated that heat shock proteins are highly expressed in cancers and closely associated with tumorigenesis and progression. The "Hallmarks of Cancer" are the core features of cancer biology that collectively define a series of functional characteristics acquired by cells as they transition from a normal state to a state of tumor growth, including sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, enabled replicative immortality, the induction of angiogenesis, and the activation of invasion and metastasis. The pivotal roles of heat shock proteins in modulating the hallmarks of cancer through the activation or inhibition of various signaling pathways has been well documented. Therefore, this review provides an overview of the roles of heat shock proteins in vital biological processes from the perspective of the hallmarks of cancer and summarizes the small-molecule inhibitors that target heat shock proteins to regulate various cancer hallmarks. Moreover, we further discuss combination therapy strategies involving heat shock proteins and promising dual-target inhibitors to highlight the potential of targeting heat shock proteins for cancer treatment. In summary, this review highlights how targeting heat shock proteins could regulate the hallmarks of cancer, which will provide valuable information to better elucidate and understand the roles of heat shock proteins in oncology and the mechanisms of cancer occurrence and development and aid in the development of more efficacious and less toxic novel anticancer agents.

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References

1. Baidu. <http://www.baidu.com>. 2024.
2. Cancer Biology. <http://www.cancerbio.net>. 2024.
3. Google. <http://www.google.com>. 2024.
4. Journal of American Science. <http://www.jofamericanscience.org>. 2024.
5. Life Science Journal. <http://www.lifesciencesite.com>. 2024.
6. Ma H, Chen G. Stem cell. The Journal of American Science 2005;1(2):90-92. doi:[10.7537/marsjas010205.14](https://doi.org/10.7537/marsjas010205.14). <http://www.jofamericanscience.org/journals/am-sci/0102/14-mahongbao.pdf>.
7. Ma H, Cherng S. Eternal Life and Stem Cell. Nature and Science. 2007;5(1):81-96. doi:[10.7537/marsnsj050107.10](https://doi.org/10.7537/marsnsj050107.10). <http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf>.
8. Ma H, Cherng S. Nature of Life. Life Science Journal 2005;2(1):7-15. doi:[10.7537/marslsj020105.03](https://doi.org/10.7537/marslsj020105.03). <http://www.lifesciencesite.com/ljs/life0201/life-0201-03.pdf>.
9. Ma H, Yang Y. Turritopsis nutricula. Nature and Science 2010;8(2):15-20. doi:[10.7537/marsnsj080210.03](https://doi.org/10.7537/marsnsj080210.03). http://www.sciencepub.net/nature/ns0802/03_12_79_hongbao_turritopsis_ns0802_15_20.pdf.
10. Ma H. The Nature of Time and Space. Nature and science 2003;1(1):1-11. doi:[10.7537/marsnsj010103.01](https://doi.org/10.7537/marsnsj010103.01). <http://www.sciencepub.net/nature/0101/01-ma.pdf>.
11. Marsland Press. <http://www.sciencepub.net>. 2024; <http://www.sciencepub.org>. 2024.
12. National Center for Biotechnology Information, U.S. National Library of Medicine. <http://www.ncbi.nlm.nih.gov/pubmed>. 2024.
13. Nature and Science. <http://www.sciencepub.net/nature>. 2024.
14. Stem Cell. <http://www.sciencepub.net/stem>. 2024.
15. Wikipedia. The free encyclopedia. <http://en.wikipedia.org>. 2024.

12/20/2024