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Cancer and Immortal 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.
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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.

Aasen, T., et al. (2003). "The relationship between connexins, gap junctions, tissue architecture and tumour invasion, as studied in a novel in vitro model of HPV-16-associated cervical cancer progression." Oncogene **22**(39): 7969-7980.

Disruption of gap junctional intercellular communication (GJIC) and/or connexins (gap junction proteins) is frequently reported in malignant cell lines and tumours. Certain human papillomaviruses (HPV) associated with the development of cancers, especially of the cervix, have previously been reported to downregulate GJIC in vitro. There is also evidence for reduced gap junctions in cervical dysplasia. However, many squamous hyperproliferative conditions, including HPV-induced warts, often show extensive upregulation of certain connexins. The association between HPV and GJIC, and the mechanism and consequence of deregulated GJIC in cervical tumour progression, remains unclear. Therefore, using a variety of nonmalignant and malignant cell lines and an organotypic raft-culture system, we investigated the relationship between HPV, gap junctions and tumour progression. Established cervical tumour cell lines carrying HPV were unable to communicate via gap junctions (when assayed by dye-transfer techniques). This correlated with lack of connexin protein expression, while transfection with connexins 26 or 43 led to functional gap junction membrane plaques. On the other hand, immortal but nonmalignant cell lines that contained episomal or integrated HPV-16, but required feeder-layer and growth-factor support, were consistently well coupled, and expressed multiple connexins at membrane junctions. In vitro selection of feeder-layer and growth-factor-independent variants eventually lead to loss of GJIC, which correlated with loss of membrane and increased cytoplasmic connexin 43 localization. However, this was preceded by loss of differentiation and stromal invasion, as assayed on the organotypic raft-culture model. Using this model, a comparison between noncoupled, well-coupled and connexin-transfected cell lines revealed no firm correlation between GJIC and dysplasia, but GJIC appeared to favour increased stratification. These findings demonstrate that loss of GJIC is frequent, but appears to occur more as a consequence of, rather than being the cause of, epithelial dysplasia, and may be influenced by, but is not directly attributable to, HPV.

Afzal, A., et al. (2020). "Splanchnic vein thrombosis predicts worse survival in patients with advanced pancreatic cancer." Thromb Res **185**: 125-131.

INTRODUCTION: Pancreatic cancer is a thrombogenic malignancy with nearly half of venous thrombotic events occurring in the splanchnic

circulation. The effect of splanchnic vein thrombosis on mortality in pancreatic cancer is unknown. We studied the effect of splanchnic vein thrombosis on mortality in veterans with advanced pancreatic adenocarcinoma, and explored the association of anticoagulant therapy on mortality and hemorrhage. METHODS: Using International Classification of Diseases (ICD) codes, we identified eligible patients and outcomes in the Veterans Health Administration database. Using Cox proportional hazards regression, we analyzed the association between splanchnic vein thrombosis and mortality among patients with advanced pancreatic cancer. We used propensity score inverse probability-of-treatment weighting to balance the groups who did and did not receive anticoagulation. To understand the role of anticoagulant therapy, we used Cox proportional hazards regression to analyze mortality and competing risk analysis to assess the risk of hemorrhage. RESULTS: Of the patients with advanced pancreatic cancer (N = 6164), 122 developed splanchnic vein thrombosis. Splanchnic vein thrombosis was associated with a two-fold increase in mortality, aHR 2.02, 95% CI 1.65-2.47. The finding held true after restricting the analysis to patients undergoing treatment for pancreatic cancer, and after adjusting for immortal time bias by a 30-day landmark analysis. Anticoagulant therapy did not affect mortality (aHR 0.99, 95% CI 0.65-1.51), and increased the risk of hemorrhage (aHR 2.7, 95% CI 1.02-7.07). CONCLUSION: Splanchnic vein thrombosis predicts worse survival in patients with advanced pancreatic adenocarcinoma. Anticoagulant therapy may not mitigate this increased mortality, and increases the risk of hemorrhage.

Agarwal, P., et al. (2018). "Immortal Time Bias in Observational Studies of Time-to-Event Outcomes: Assessing Effects of Postmastectomy Radiation Therapy Using the National Cancer Database." Cancer Control **25**(1): 1073274818789355.

The objectives of this study are to illustrate the effects of immortal time bias (ITB) using an oncology outcomes database and quantify through simulations the magnitude and direction of ITB when different analytical techniques are used. A cohort of 11 626 women who received neoadjuvant chemotherapy and underwent mastectomy with pathologically positive lymph nodes were accrued from the National Cancer Database (2004-2008). Standard Cox regression, time-dependent (TD), and landmark models were used to compare overall survival in patients who did or did not receive postmastectomy radiation therapy (PMRT). Simulation studies showing ways to reduce the effect of ITB indicate that TD exposures should be included as variables in hazard-based analyses. Standard Cox regression models comparing overall survival in patients who did and did not receive PMRT showed a significant treatment effect (hazard ratio [HR]: 0.93, 95% confidence interval [CI]: 0.88-0.99). Time-dependent and landmark methods estimated no treatment effect with HR: 0.97, 95% CI: 0.92 to 1.03 and HR: 0.98, 95% CI, 0.92 to 1.04, respectively. In our simulation studies, the standard Cox regression model significantly overestimated treatment effects when no effect was present. Estimates of TD models were closest to the true treatment effect. Landmark model results were highly dependent on landmark timing. Appropriate statistical approaches that account for ITB are critical to minimize bias when examining relationships between receipt of PMRT and survival.

Ahrendt, S. A., et al. (1997). "Comparison of oncogene mutation detection and telomerase activity for the molecular staging of non-small cell lung cancer." Clin Cancer Res **3**(7): 1207-1214.

Novel oncogene mutation detection techniques have demonstrated that standard histopathological examination may fail to detect clinically significant metastatic cancer cells. Recently, telomerase activity has been detected in most immortal cell lines and human tumors, potentially providing a novel diagnostic marker. We compared standard histopathological examination with the telomeric repeat amplification protocol assay and either a p53 plaque hybridization or a K-ras mutation ligation assay in the lymph nodes of 12 patents with surgically resectable non-small cell lung cancer. Telomerase activity was detected in 10 of 10 (100%) evaluable tumors. Eight of 9 (89%) histopathologically positive lymph nodes were telomerase positive, and 26 of 48 (54%) histopathologically negative lymph nodes were telomerase positive. In comparison, oligonucleotide plaque hybridization detected metastases in all 3 histopathologically positive nodes and in 3 of 27 histopathologically negative nodes. Similarly, the K-ras mutation ligation assay detected metastases in all 6 histopathologically positive lymph nodes examined and in 1 of 21 histopathologically negative lymph nodes. Thus, most of the "positive" nodes by telomerase assay did not harbor occult neoplastic cells that shared the same genetic alteration as the primary tumor. The high rate of false positives associated with the telomeric repeat amplification protocol assay limits its role in staging lymph nodes in patients with non-small cell lung cancer.

An, J., et al. (2021). "Identification of spliceosome components pivotal to breast cancer survival." RNA Biol **18**(6): 833-842.

Cancer cells employ alternative splicing (AS) to acquire splicing isoforms favouring their survival.

However, the causes of aberrant AS in breast cancer are poorly understood. In this study, the METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) data were analysed with univariate feature selection. Of 122 analysed spliceosome components, U2SURP, PUF60, DDX41, HNRNPAB, EIF4A3, and PPIL3 were significantly associated with breast cancer survival. The top 4 four genes, U2SURP, PUF60, DDX41, and HNRNPAB, were chosen for further analyses. Their expression was significantly associated with cancer molecular subtype, tumour stage, tumour grade, overall survival (OS), and cancer-specific survival in the METABRIC data. These results were verifiable using other cohorts. The Cancer Genome Atlas data unveiled the elevated expression of PUF60, DDX41, and HNRNPAB in tumours compared with the normal tissue and confirmed the differential expression of the four genes among cancer molecular subtypes, as well as the associations of U2SURP, PUF60, and DDX41 expression with tumour stage. A meta-analysis data verified the associations of U2SURP, PUF60, and HNRNPAB expression with tumour grade, the associations of PUF60, DDX41, and HNRNPAB expression with OS and distant metastasis-free survival, and the associations of U2SURP and HNRNPAB expression with relapse-free survival. Experimentally, we demonstrated that inhibiting the expression of the four genes separately suppressed cell colony formation and slowed down cell growth considerably in breast cancer cells, but not in immortal breast epithelial cells. In conclusion, we have identified U2SURP, PUF60, DDX41, and HNRNPAB are spliceosome-related genes pivotal for breast cancer survival.

Andersen, T. I. (1998). "[Telomeres, telomerase and development of cancer]." Tidsskr Nor Laegeforen **118**(13): 2043-2046.

The chromosome ends, telomeres, shorten during each cell division due to the inability of DNA polymerase to replicate the ends of linear chromosomes. The telomere length serves as a clock determining the remaining replicative capacity of the cell. After 50-100 doublings, the cell becomes senescent. Rarely, a cell overcomes the senescence blockade, and eventually becomes immortal. Cellular immortalisation is almost always accompanied by the expression of the enzyme telomerase, which synthesises telomeric DNA. Telomerase is present in approximately 85% of malignancies. The detection of telomerase activity in cancer cells represents a possible cancer diagnostic and prognostic tool, and telomerase inhibition may become a novel therapeutic strategy in cancer patients.

Anderson, S., et al. (1997). "Telomerase activation in cervical cancer." Am J Pathol **151**(1): 25-31.

It has been hypothesized that infection with high-risk human papillomaviruses (HPVs), in conjunction with other cellular events, plays a critical role in the development of cervical cancer. Activation of telomerase, a ribonucleoprotein enzyme complex that synthesizes telomere repeats, has been associated with acquisition of the immortal phenotype in vitro and is commonly observed in human cancers. In this study, we have examined 10 high-grade cervical cancers for telomerase activity and for the presence of HPV. Telomerase activity was detected in all of the cancers but in none of the paired histopathologically normal uterine tissues or in normal cervical epithelium. Analysis of these same tissues for HPV nucleic acids by polymerase chain reaction (PCR) using primers from the HPV L1 and E6 open reading frames demonstrated that 7 of 10 cancers were positive for HPV, 3 for HPV type 16 (HPV-16), and 4 for HPV-18. In one case, HPV-16 was detected in histopathologically normal uterine tissue, the same type as that detected in the cancer from the same patient. HPV DNA was not detected in 3 of 10 cancers. These results indicate that telomerase activation is common in high-grade cervical cancers and suggests that telomerase activity may be a useful diagnostic marker for the disease.

Arbiser, J. L., et al. (2017). "Targeting the duality of cancer." NPJ Precis Oncol **1**.

Cancer is the second leading cause of death in the United States, and is an increasing cause of death in the developing world. While there is great heterogeneity in the anatomic site and mutations involved in human cancer, there are common features, including immortal growth, angiogenesis, apoptosis evasion, and other features, that are common to most if not all cancers. However, new features of human cancers have been found as a result of clinical use of novel "targeted therapies," angiogenesis inhibitors, and immunotherapies, including checkpoint inhibitors. These findings indicate that cancer is a moving target, which can change signaling and metabolic features based upon the therapies offered. It is well-known that there is significant heterogeneity within a tumor and it is possible that treatment might reduce the heterogeneity as a tumor adapts to therapy and, thus, a tumor might be synchronized, even if there is no major clinical response. Understanding this concept is important, as concurrent and sequential therapies might lead to improved tumor responses and cures. We posit that the repertoire of tumor responses is both predictable and limited, thus giving hope that eventually we can be more effective against solid tumors. Currently, among solid tumors, we observe a response of 1/3 of tumors to immunotherapy, perhaps

less to angiogenesis inhibition, a varied response to targeted therapies, with relapse and resistance being the rule, and a large fraction being insensitive to all of these therapies, thus requiring the older therapies of chemotherapy, surgery, and radiation. Tumor phenotypes can be seen as a continuum between binary extremes, which will be discussed further. The biology of cancer is undoubtedly more complex than duality, but thinking of cancer as a duality may help scientists and oncologists discover optimal treatments that can be given either simultaneously or sequentially.

Argyle, D. J., et al. (2004). "Evaluation of telomerase-targeted therapies in canine cancer cell lines." Vet Comp Oncol **2**(4): 214-221.

Despite advances in conventional therapeutics, cancer remains an invariably fatal disease, the major challenge being to develop tumour-specific cancer treatment strategies. Current treatments such as chemotherapy and radiotherapy rely on a crude distinction between cancer cells and normal cells. However, with an increased understanding of the molecular events in the development of cancer, it is possible that far more innovative and targeted approaches can be developed. From studies on humans and dogs, the enzyme telomerase has emerged as a central unifying mechanism underlying the immortal phenotype of cancer and has thus become a candidate for differentiating between normal and cancer cells. The level and frequency of telomerase activity and component gene expression in cancers reinforces this as a potential target for cancer therapies. This article describes two approaches to target cancer by capitalizing on the expression of this enzyme. In the first approach, we target the enzyme itself, the goal being to cause cancer cell death. In the second approach, we utilize the respective gene promoters for telomerase component enzymes to drive expression of a reporter gene in cancer cell lines. The results demonstrated that targeted gene expression using promoter elements can be achieved specifically in telomerase-positive cell lines. However, targeting the enzyme itself proved less successful and warrants investigations into alternative approaches.

Armstrong, C. A. and K. Tomita (2017). "Fundamental mechanisms of telomerase action in yeasts and mammals: understanding telomeres and telomerase in cancer cells." Open Biol **7**(3).

Aberrant activation of telomerase occurs in 85-90% of all cancers and underpins the ability of cancer cells to bypass their proliferative limit, rendering them immortal. The activity of telomerase is tightly controlled at multiple levels, from transcriptional regulation of the telomerase components to holoenzyme biogenesis and recruitment to the telomere, and finally activation and processivity. However, studies using cancer cell lines and other model systems have begun to reveal features of telomeres and telomerase that are unique to cancer. This review summarizes our current knowledge on the mechanisms of telomerase recruitment and activation using insights from studies in mammals and budding and fission yeasts. Finally, we discuss the differences in telomere homeostasis between normal cells and cancer cells, which may provide a foundation for telomere/telomerase targeted cancer treatments.

Au Yeung, S. L. and C. M. Schooling (2019). "Impact of glycemic traits, type 2 diabetes and metformin use on breast and prostate cancer risk: a Mendelian randomization study." BMJ Open Diabetes Res Care **7**(1): e000872.

Objectives: Observational studies suggest glycemic traits and type 2 diabetes are positively associated, and metformin inversely associated with breast and prostate cancer risk. However, observational studies are susceptible to unmeasured confounding while studies of metformin use are also vulnerable to immortal time bias. The use of Mendelian randomization may reduce confounding due to random allocation of relevant genetic markers at birth, and may reduce immortal time bias (for metformin-related variants analysis) since the start of exposure is at birth. Research design and methods: We identified strong genetic predictors of fasting glucose, glycated hemoglobin, and type 2 diabetes from the Meta-Analyses of Glucose and Insulin-related traits Consortium and Diabetes Genetics Replication And Meta-analysis Consortium (n=140 595 for glucose; n=123 665 for HbA1c; n=898 130 for type 2 diabetes) and of AMPK-instrumented HbA1c reduction as a proxy of metformin and applied them to large genome-wide association studies of breast cancer (Breast Cancer Association Consortium; BCAC) and prostate cancer (Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome; PRACTICAL). We used inverse variance weighting to obtain estimates. Sensitivity analyses included use of MR-Egger, weighted median, exclusion of pleiotropic instruments, and validation using UK Biobank (breast cancer only). Results: There was no association of fasting glucose (OR 1.03 per mmol/L, 95% CI 0.85 to 1.25), HbA1c (OR 1.02 per %, 95% CI 0.73 to 1.45), or type 2 diabetes (OR 0.98 per log odds, 95% CI 0.95 to 1.01) with breast cancer in BCAC, with similar findings from UK Biobank. There was no association of fasting glucose (OR 0.93 per mmol/L, 95% CI 0.73 to 1.17), HbA1c (OR 0.90 per %, 95% CI 0.58 to 1.40) or type 2 diabetes (OR 1.02 per log odds, 95% CI 0.97 to 1.07) with prostate cancer in PRACTICAL. No strong

evidence was observed for AMPK-instrumented HbA1c reduction on cancer risk. Conclusion: Glycemic traits and type 2 diabetes unlikely cause breast and prostate cancer. Whether metformin can be repurposed for cancer prevention remains unclear.

Ayyagari, S., et al. (2020). "Use of Acid-Suppressant Medications After Diagnosis Increases Mortality in a Subset of Gastrointestinal Cancer Patients." Dig Dis Sci **65**(9): 2691-2699.

BACKGROUND: Acid-suppressant medications, including proton pump inhibitors (PPIs) and H2 receptor antagonists (H2RAs), are frequently prescribed and have been linked to increased risk of some gastrointestinal cancers. AIMS: We examined whether post-diagnosis use of PPIs/H2RAs is associated with the risk of mortality in gastrointestinal cancer patients. METHODS: We used data from patients with esophageal cancer, gastric cancer, or hepatocellular carcinomas (HCCs) in the national VA Central Cancer Registry diagnosed between 2002 and 2016. We identified PPI/H2RA prescriptions that were filled before and after cancer diagnosis and used time-dependent Cox regression models to calculate adjusted hazard ratios (HRs) and 95% CIs for mortality risk. We used a time-varying exposure to avoid immortal-time bias and a 3-month lag to reduce reverse causation. A sensitivity analysis was conducted varying the lag duration between the date of cancer diagnosis and the start of follow-up. RESULTS: PPIs were used by the majority (54% post-diagnosis use) of patients. We found no association between post-diagnosis PPI use and cancer-specific mortality in esophageal adenocarcinoma (HR 0.93; 95% CI 0.84-1.02), esophageal squamous cell carcinoma (HR 0.99; 95% CI 0.87-1.12), or gastric cardia cancer (HR 1.04; 95% CI 0.89-1.22) patients. Post-diagnosis PPI use was, however, associated with the increased risk of cancer-specific mortality for patients with gastric non-cardia cancer (HR 1.50; 95% CI 1.29-1.74) and HCC (HR 1.31; 95% CI 1.23-1.40). The results were similar for associations with post-diagnosis use of H2RAs. There was no association with pre-diagnosis PPI/H2RA use. CONCLUSION: Post-diagnosis PPI/H2RA use was associated with the increased mortality in gastric non-cardia cancer and HCC patients.

Badrick, E. and A. G. Renehan (2014). "Diabetes and cancer: 5 years into the recent controversy." Eur J Cancer **50**(12): 2119-2125.

Diabetes and cancer are common chronic disorders. The literature has long recognised that type 2 diabetes (T2D) is associated with an increased incident risk of several cancer types, independent of the mutual risk factor, obesity. However, in June 2009, four papers were published simultaneously in Diabetologia, the official journal of the European Association for the Study of Diabetes, raising questions of a link between diabetes therapies, notably the long-acting insulin analogue, glargine, and increased cancer risk. These papers awakened an unprecedented debate in the diabetes community, drawing in cancer experts and bringing together representatives from these two large, traditionally non-intersecting, biomedical communities. This Current Perspective summarises the events that followed the 'breaking news' from summer 2009: the pitfalls encountered; the increased mutual understanding between diabetes and cancer researchers; and the direction of current research. Much of the debate on the clinical impact of this controversy has been played out in the diabetes literature: here, we update the oncology readership.

Bander, N. H. (1984). "Study of the normal human kidney and kidney cancer with monoclonal antibodies." Uremia Invest **8**(3-4): 263-273.

The ability to establish immortal tissue culture lines of human renal cancer as well as short-term lines of autologous normal kidney epithelium offers a unique system to probe the differences between a neoplastic cell and its normal counterpart. Monoclonal antibodies have been prepared against cell surface differentiation antigens of normal and neoplastic human kidney. The detected antigens have been biochemically characterized, and the molecular phenotypes of these cells is being unraveled. Differences in gene expression are becoming apparent between the normal and neoplastic kidney cell. Preliminary results indicate that these differences appear to have clinical significance.

Baykal, A., et al. (2004). "Telomerase in breast cancer: a critical evaluation." Adv Anat Pathol **11**(5): 262-268.

Human chromosomes have highly specialized structures at their ends termed telomeres, repetitive, non-coding DNA sequences (5'-TTAGGG-3'), ranging in size from 5 to 20 kb in human cells. These highly specialized structures prevent chromosome ends from being recognized as double-strand DNA breaks, and they also provide protection from destabilizing agents. The mechanism for maintaining telomere integrity is controlled by telomerase, a ribonucleoprotein enzyme that specifically restores telomere sequences lost during replication by using an intrinsic RNA component as a template for polymerization. Telomerase has two core functional components required for its activity: the catalytic subunit of human telomerase reverse transcriptase (hTERT) and a telomerase RNA template (hTR). Telomerase is activated in the majority of immortal cell lines in

culture and in most malignant tumors. This review outlines our current understanding of telomerase in breast cancer development and critically evaluates potential utilities in diagnosis, prognosis, and therapy.

Berger, Y., et al. (2021). "Cytoreductive Surgery for Selected Patients Whose Metastatic Gastric Cancer was Treated with Systemic Chemotherapy." Ann Surg Oncol.

BACKGROUND: The authors hypothesized that cytoreductive surgery (CRS, comprising gastrectomy combined with metastasectomy) in addition to systemic chemotherapy (SC) is associated with a better survival than chemotherapy alone for patients with metastatic gastric adenocarcinoma (MGA). METHODS: Patients with MGA who received SC between 2004 and 2016 were identified using the National Cancer Database (NCDB). Nearest-neighbor 1:1 propensity score-matching was used to create comparable groups. Overall survival (OS) was compared between subgroups using Kaplan-Meier analyses. Immortal bias analysis was performed among those who survived longer than 90 days. RESULTS: The study identified 29,728 chemotherapy-treated patients, who were divided into the following four subgroups: no surgery (NS, n = 25,690), metastasectomy alone (n = 1170), gastrectomy alone (n = 2248), and CRS (n = 620) with median OS periods of 8.6, 10.9, 14.8, and 16.3 months, respectively ($p < 0.001$). Compared with the patients who underwent NS, the patients who had CRS were younger (58.9 +/- 13.4 vs 62.0 +/- 13.1 years), had a lower proportion of disease involving multiple sites (4.6% vs 19.1%), and were more likely to be clinically occult (cM0 stage: 59.2% vs 8.3%) ($p < 0.001$ for all). The median OS for the propensity-matched patients who underwent CRS (n = 615) was longer than for those with NS (16.4 vs 9.3 months; $p < 0.001$), including in those with clinical M1 stage (n = 210). In the Cox regression model using the matched data, the hazard ratio for CRS versus NS was 0.56 (95% confidence interval [CI], 0.49-0.63). In the immortal-matched cohort, the corresponding median OS was 17.0 versus 9.5 months ($p < 0.001$). CONCLUSIONS: In addition to SC, CRS may be associated with an OS benefit for a selected group of MGA patients meriting further prospective investigation.

Bertram, J. S. (2000). "The molecular biology of cancer." Mol Aspects Med **21**(6): 167-223.

The process by which normal cells become progressively transformed to malignancy is now known to require the sequential acquisition of mutations which arise as a consequence of damage to the genome. This damage can be the result of endogenous processes such as errors in replication of DNA, the intrinsic chemical instability of certain DNA bases or from attack by free radicals generated during metabolism. DNA damage can also result from interactions with exogenous agents such as ionizing radiation, UV radiation and chemical carcinogens. Cells have evolved means to repair such damage, but for various reasons errors occur and permanent changes in the genome, mutations, are introduced. Some inactivating mutations occur in genes responsible for maintaining genomic integrity facilitating the acquisition of additional mutations. This review seeks first to identify sources of mutational damage so as to identify the basic causes of human cancer. Through an understanding of cause, prevention may be possible. The evolution of the normal cell to a malignant one involves processes by which genes involved in normal homeostatic mechanisms that control proliferation and cell death suffer mutational damage which results in the activation of genes stimulating proliferation or protection against cell death, the oncogenes, and the inactivation of genes which would normally inhibit proliferation, the tumor suppressor genes. Finally, having overcome normal controls on cell birth and cell death, an aspiring cancer cell faces two new challenges: it must overcome replicative senescence and become immortal and it must obtain adequate supplies of nutrients and oxygen to maintain this high rate of proliferation. This review examines the process of the sequential acquisition of mutations from the prospective of Darwinian evolution. Here, the fittest cell is one that survives to form a new population of genetically distinct cells, the tumor. This review does not attempt to be comprehensive but identifies key genes directly involved in carcinogenesis and demonstrates how mutations in these genes allow cells to circumvent cellular controls. This detailed understanding of the process of carcinogenesis at the molecular level has only been possible because of the advent of modern molecular biology. This new discipline, by precisely identifying the molecular basis of the differences between normal and malignant cells, has created novel opportunities and provided the means to specifically target these modified genes. Whenever possible this review highlights these opportunities and the attempts being made to generate novel, molecular based therapies against cancer. Successful use of these new therapies will rely upon a detailed knowledge of the genetic defects in individual tumors. The review concludes with a discussion of how the use of high throughput molecular arrays will allow the molecular pathologist/therapist to identify these defects and direct specific therapies to specific mutations.

Bhat, U. G., et al. (2011). "Nucleophosmin interacts

with FOXM1 and modulates the level and localization of FOXM1 in human cancer cells." J Biol Chem **286**(48): 41425-41433.

Using mass spectrometric analysis we found that oncogenic transcription factor FOXM1 that is overexpressed in a majority of human cancers interacts with multifunctional protein NPM, which is also overexpressed in a variety of human tumors. Coimmunoprecipitation and glutathione S-transferase pull-down experiments demonstrated that NPM forms a complex with FOXM1 and also identified the regions responsible for their interaction. Immunofluorescence microscopy confirmed the interaction between FOXM1 and NPM in cancer and immortal cells. Furthermore, knockdown of NPM in immortal and cancer cells led to significant down-regulation of FOXM1 similar to its levels in normal cells, suggesting that NPM might modulate FOXM1 level. In addition, in OCI/AML3 leukemia cells where mutant NPM is localized in the cytoplasm we found that typically nuclear FOXM1 was predominantly co-localized with NPM in the cytoplasm, while NPM knockdown led to the disappearance of FOXM1 from the cytoplasm, suggesting that NPM may also determine intracellular localization of FOXM1. Knockdown of FOXM1 or NPM in MIA PaCa-2 pancreatic cancer cells inhibited anchorage-dependent and independent growth in cell culture, and tumor growth in nude mice. In addition, over-expression of FOXM1 reversed the effect of NPM knockdown in vitro. Our data suggest that in cancer cells NPM interacts with FOXM1 and their interaction is required for sustaining the level and localization of FOXM1. Targeting the interaction between FOXM1 and NPM by peptides or small molecules may represent a novel therapeutic strategy against cancer.

Bischoff, F. Z., et al. (1991). "Tumorigenic transformation of spontaneously immortalized fibroblasts from patients with a familial cancer syndrome." Oncogene **6**(2): 183-186.

Immortal cell lines arose spontaneously during in vitro culture of initially normal fibroblasts, MDAH041 and MDAH087, from patients with Li-Fraumeni familial cancer syndrome. Fibroblasts from a control donor, MDAH170, maintained a normal morphology and senesced at 31 population doublings. The immortal fibroblasts have several properties of transformed cells. In addition to having acquired an altered morphology and chromosomal anomalies, MDAH041 and MDAH087 have escaped from senescence, growing beyond 300 and 100 population doublings (pd), respectively. As early as 50 pd, these cells can be transformed by an activated H-ras oncogene to form tumors in nude mice. However, MDAH041 immortal cells were resistant to tumorigenic transformation by transfection with the v-abl oncogene.

Bloomfield, M. and P. Duesberg (2018). "Is cancer progression caused by gradual or simultaneous acquisitions of new chromosomes?" Mol Cytogenet **11**: 4.

Background: Foulds defined, "Tumor progression (as a) permanent, irreversible qualitative change in one or more of its characters" (Cancer Res. 1954). Accordingly progressions, such as metastases and acquired drug-resistance, were since found to be subspecies of cancers with conserved and numerous new chromosomes. Here we ask whether cancers acquire numerous new chromosomes gradually or simultaneously in progressions. The currently prevailing theory of Nowell (Science, 1976) holds that unexplained "genetic instability" generates "variant sublines (with) changes in chromosome number" and that "clonal" progressions arise by "stepwise selection of more aggressive sublines". The literature, however, contains many examples of "immediate" selections of progressions with numerous new chromosomes - notably experimentally initiated fusions between cancers and heterologous cells. Furthermore, the stepwise progression theory predicts intermediate sublines of cancers with multiple non-clonal additions of new chromosomes. However, the literature does not describe such intermediates. Results: In view of these inconsistencies with stepwise progression we test here a saltational theory, in which the inherent variability of cancer-specific aneuploidy generates "immediate" progressions with individual clonal karyotypes, transcriptomes and phenotypes in single steps. Using cell fusion as an established controllable model of "immediate" progression, we generated seven immortal murine hybridomas by fusing immortal murine myeloma cells and normal antibody-producing B-cells with polyethylene glycol within a few minutes. These immortal hybridomas contained individual sets of 71 to 105 clonal chromosomes, compared to the 52 chromosomes of the parental myeloma. Thus the myeloma had gained 19 to 53 new clonal chromosomes in seven individual hybridomas in a single step. Furthermore, no stable intermediates were found, as would be predicted by a saltational process. Conclusions: We conclude that random fusions between myelomas and normal B-cells generate clonal hybridomas with multiple, individual chromosomes in single steps. Similar single-step mechanisms may also generate the "late" clonal progressions of cancers with gains of numerous new chromosomes and thus explain the absence of intermediates. Latency would reflect the low probability of rare stochastic progressions. In conclusion, the karyotypic clonality of hybridomas and spontaneous progressions suggests karyotypic

alterations as proximate causes of neoplastic progressions. Since cancer-specific aneuploidy catalyzes karyotypic variation, the degree of aneuploidy predicts the clinical risk of neoplastic progression, confirming classical predictions based on DNA content.

Boldrini, L., et al. (2003). "Evaluation of telomerase in non-melanoma skin cancer." Int J Mol Med **11**(5): 607-611.

Telomerase, a ribonucleoprotein, is capable of adding telomeric sequences (TTAGGG hexameric repeats) to the ends of chromosomes and, thereby, halting the erosion of chromosome at each cell division. Whereas most normal somatic cells contain minimal or no detectable telomerase activity, most immortal and tumour cells exhibit significant levels of telomerase activity and show no net loss of telomere length during proliferation. The evaluation of telomerase has been proposed for diagnostic and therapeutic purposes in human cancer. Skin cancer is the most common cancer in humans; the precise molecular events in skin carcinogenesis are numerous and complicated and not yet completely clarified. In this study, we evaluated telomerase in 35 basal cell carcinomas and in 14 squamous cell carcinomas in order to determine if activation of the telomerase enzyme was a pivotal step in the development of skin cancer and whether telomerase activity levels were different between the two histotypes. A higher enzymatic level was shown to be associated with squamous cell carcinomas, while low levels were mainly detected in the basal cell histotype (chi2 test; p=0.02). Telomerase complex activity is dependent on its catalytic subunit, telomerase reverse transcriptase hTERT. By reverse transcription-PCR, using primers within the reverse transcriptase domain of hTERT, we observed a significant correlation between hTERT expression and telomerase activity in our skin tumour samples (p=0.0003). We detected the presence of multiple, alternately spliced transcripts, corresponding to full-length messages as well as spliced messages with critical reverse transcriptase motifs deleted. A higher telomerase messenger level was shown to be associated with squamous cell carcinomas (chi2 test; p<0.0001), as for telomerase activity. Our results provide arguments supporting the role of telomerase in skin cancer and suggest RT-PCR of telomerase RNA as a tool easier and faster than TRAP assay to identify more aggressive malignancies among non-melanoma skin specimens.

Bradley, M. C., et al. (2018). "A Cohort Study of Metformin and Colorectal Cancer Risk among Patients with Diabetes Mellitus." Cancer Epidemiol Biomarkers Prev **27**(5): 525-530.

Background: Several epidemiologic studies have reported strong inverse associations between metformin use and risk of colorectal cancer, although time-related biases, such as immortal time bias, may in part explain these findings. We reexamined this association using methods to minimize these biases.Methods: A cohort study was conducted among 47,351 members of Kaiser Permanente Northern California with diabetes and no history of cancer or metformin use. Follow-up for incident colorectal cancer occurred from January 1, 1997, until June 30, 2012. Cox regression was used to calculate HRs and 95% confidence intervals (CIs) for colorectal cancer risk associated with metformin use (ever use, total duration, recency of use, and cumulative dose).Results: No association was observed between ever use of metformin and colorectal cancer risk (HR, 0.90; 95% CI, 0.76-1.07) and there was no consistent pattern of decreasing risk with increasing total duration, dose, or recency of use. However, long-term use (>/=5.0 years) appeared to be associated with reduced risk of colorectal cancer in the full population (HR, 0.78; 95% CI, 0.60-1.02), among current users (HR, 0.78; 95% CI, 0.59-1.04), and in men (HR, 0.65; 95% CI, 0.45-0.94) but not in women. Higher cumulative doses of metformin were associated with reduced risk. In initial users of sulfonylureas, switching to or adding metformin was also associated with decreased colorectal cancer risk.Conclusions: Our findings showed an inverse association between long-term use of metformin and colorectal cancer risk. Findings, especially the risk reduction among men, need to be confirmed in large, well-conducted studies.Impact: If our findings are confirmed, metformin may have a role in the chemoprevention of colorectal cancer. Cancer Epidemiol Biomarkers Prev; 27(5); 525-30. (c)2018 AACRSee related commentary by Jackson and Garcia-Albeniz, p. 520.

Brenner, A. J. and C. M. Aldaz (1995). "Chromosome 9p allelic loss and p16/CDKN2 in breast cancer and evidence of p16 inactivation in immortal breast epithelial cells." Cancer Res **55**(13): 2892-2895.

To define the extent of involvement of chromosome 9p in breast carcinogenesis, we performed microsatellite length polymorphism analysis of markers spanning this region. Of 24 primary breast carcinomas analyzed, we observed a high frequency (58%) of loss of heterozygosity or allelic imbalance affecting subregion 9p21-22. Mutational analysis of CDKN2 (p16) was performed to determine whether this gene was the target of such alterations. Of 21 tumors analyzed, only 1 showed a mutation of probable consequence, suggesting that CDKN2 appears not to be the target of loss of heterozygosity and indicating the possible existence of

another tumor suppressor gene within this region. Additionally, since it has been suggested that some CDKN2 deletions and mutations could be due to an in vitro phenomenon, four immortal breast cell lines derived from normal epithelium, MCF10F, MCF12F, 184A1, and 184B5, were examined for loss or mutation of CDKN2. Two lines (MCF10F and MCF12F) showed homozygous deletions of CDKN2, and one (184A1) revealed a hemizygous deletion and a nonsense mutation in the remaining allele. This could imply an important role of CDKN2 in the control of immortalization or in vitro adaptation and is the first evidence of such in nontumor-derived cell lines. Additionally, this is the first report of frequent loss of heterozygosity in the 9p21-22 chromosome subregion of uncultured primary breast tumors.

Bright, R. K., et al. (1997). "Generation and genetic characterization of immortal human prostate epithelial cell lines derived from primary cancer specimens." Cancer Res **57**(5): 995-1002.

Difficulty in establishing long-term human prostate epithelial cell lines has impeded efforts to understand prostate tumorigenesis and to develop alternative therapies for prostate cancer. In the current study, we describe a method that was successful in generating 14 immortal benign or malignant prostate epithelial cell cultures from primary adenocarcinomas of the prostate resected from six successive patients. Immortalization with the E6 and E7 transforming proteins of human papilloma virus serotype 16 was necessary to establish long-term cultures. Microscopic examination of fresh tumor specimens exhibited a variable mixture of benign and malignant epithelium. Thus, single-cell cloning of tumor-derived cell cultures was essential for defining tumor cell lines. Efforts to characterize these cultures using traditional criteria such as karyotype, growth in nude mice, and prostate-specific antigen expression were noninformative. However, allelic loss of heterozygosity (LOH) represents a powerful alternative method for characterizing tumor cell lines originating from primary adenocarcinomas of the prostate. Microdissected fresh tumors from four of six patients revealed LOH at multiple loci on chromosome 8p, as assessed by PCR. LOH on chromosome 8p matching the patterns found in microdissected tumors was also observed in a tumor-derived cell line and its clones, as well as in one clone from a tumor-derived cell line from a second patient. LOH was not observed in immortal lines generated from autologous benign prostatic epithelium, seminal vesicle epithelium, or fibroblasts. The multifocal nature of prostate cancer, as well as the presence of an entire spectrum of malignant transformation within individual prostate glands, necessitates this type of careful analysis of derivative cell cultures for their validation as in vitro models that accurately reflect the primary cancers from which they are derived.

Buchanan, N. S., et al. (2005). "Mass mapping of cancer cell lysates using two-dimensional liquid separations, electrospray-time of flight-mass spectrometry, and automated data processing." Electrophoresis **26**(1): 248-256.

Intact protein masses from immortal, nontransformed MCF10A, a human breast epithelial cell line, and its malignant derivative MCF10CA1a.cl1 have been mapped using a combination of all-liquid separations and automated data interpretation. Preparative liquid isoelectric focusing combined with nonporous silica reverse-phase high-performance liquid chromatography allows efficient separation of a large number of proteins in complex mixtures such as whole-cell lysates. Molecular weight determination of these proteins is achieved using electrospray-time of flight-mass spectrometry, however, manual data analysis for these separations is both complex and time-consuming. Protein mass mapping can be significantly enhanced by automating deconvolution functions typically performed manually, with resulting reductions in hands-on analysis time from 20-30 h per chromatogram to approximately 15 min. This reduction in analysis time allows for rapid screening of cancer cell lines for potential biomarkers over a wider pI range than would otherwise be possible.

Bykov, K., et al. (2019). "Glucose-lowering medications and the risk of cancer: A methodological review of studies based on real-world data." Diabetes Obes Metab **21**(9): 2029-2038.

AIM: To review the methodology of observational studies examining the association between glucose-lowering medications and cancer to identify the most common methodological challenges and sources of bias. METHODS: We searched PubMed systematically to identify observational studies on glucose-lowering medications and cancer published between January 2000 and January 2016. We assessed the design and analytical methods used in each study, with a focus on their ability to achieve study validity, and further evaluated the prevalence of major methodological choices over time. RESULTS: Of 155 studies evaluated, only 26% implemented a new-user design, 41% used an active comparator, 33% implemented a lag or latency period, and 51% adjusted for diabetes duration. Potential for immortal person-time bias was identified in 63% of the studies; 55% of the studies adjusted for variables measured during the follow-up without appropriate statistical methods. Aside from a decreasing trend in adjusting for variables measured during the follow-up, we observed

no trends in methodological choices over time. CONCLUSIONS: The prevalence of well-known design and analysis flaws that may lead to biased results remains high among observational studies on glucose-lowering medications and cancer, limiting the conclusions that can be drawn from these studies. Avoiding known pitfalls could substantially improve the quality and validity of real-world evidence in this field.

Cai, L., et al. (2005). "[Study on the relation between anticancer drug-sensitivity and differentional expression of anticancer drug sensitivity related genes in non-small cell lung cancer cell lines]." Zhongguo Fei Ai Za Zhi **8**(3): 163-169.

BACKGROUND: In order to enhance the chemotherapeutic efficacy of advanced lung cancer and to practise the individualized treatment, it is necessary to find out the difference of anticancer drug-sensitivity in lung cancer cells. Comparing the array profile of related gene of anticancer drug-sensitivity between non-small cell lung cancer (NSCLC) cell lines and immortal human bronchial epithelial cell line BET2A, the difference of expression of related genes of anticancer drug sensitivity was detected. METHODS: With the technique of cDNA macroarray, the different related gene proceeding of anticancer drug sensitivity expression was analysed in 6 NSCLC cell lines and BET2A cell line. RT-PCR was used to reconfirm the results. RESULTS: Seventy-three genes which were differentially expressed were found from 1291 candidate genes, and there were 45 genes upregulated, and 28 genes downregulated. The results of RT-PCR were consistent with those of cDNA macroarray. CONCLUSIONS: The main reason of different sensitivity might be the difference of related gene of anticancer drug sensitivity expression. The results of this study provide new targets for reversing multiple drug-resistance, and they also provide experimental evidence to develop some new drugs and to realize the individualized treatment clinically.

Cairns, D., et al. (2002). "Design of telomerase inhibitors for the treatment of cancer." Curr Pharm Des **8**(27): 2491-2504.

Telomerase is a cellular ribonucleoprotein reverse transcriptase responsible for the maintenance of telomeres, the tandemly repeating guanine-rich nucleic acid sequences at the 3'-ends of eukaryotic chromosomes that serve to protect chromosomal stability and maintain integrity. Telomerase enzyme activity is essential for the sustained proliferation of most immortal cells, including cancer cells, and is currently an important recognised target for the development of novel and potentially tumour-specific anticancer chemotherapeutics. Herein, we review recent advances in the design and development of telomerase inhibitors for the treatment of cancer. To date, these have included antisense strategies, reverse transcriptase inhibitors, and agents capable of interacting with high-order telomeric DNA tetraplex (or "G-quadruplex") structures in such a way as to prevent enzyme access to its required linear telomeric DNA substrate. Critical appraisal of each distinct approach is provided together with highlighted areas for continued development necessary to further refine the present disparate classes of telomerase inhibitors for use in clinically viable therapies.

Carmona-Bayonas, A., et al. (2018). "Top ten errors of statistical analysis in observational studies for cancer research." Clin Transl Oncol **20**(8): 954-965.

Observational studies using registry data make it possible to compile quality information and can surpass clinical trials in some contexts. However, data heterogeneity, analytical complexity, and the diversity of aspects to be taken into account when interpreting results makes it easy for mistakes to be made and calls for mastery of statistical methodology. Some questionable research practices that include poor analytical data management are responsible for the low reproducibility of some results; yet, there is a paucity of information in the literature regarding specific statistical pitfalls of cancer studies. In addition to proposing how to avoid or solve them, this article seeks to expose ten common problematic situations in the analysis of cancer registries: convenience, dichotomization, stratification, regression to the mean, impact of sample size, competing risks, immortal time and survivor bias, management of missing values, and data dredging.

Cassoni, P., et al. (1995). "Mitogenic effect of the 15-kDa gross cystic disease fluid protein (GCDFP-15) on breast-cancer cell lines and on immortal mammary cells." Int J Cancer **60**(2): 216-220.

The biological significance of a major protein component in the fluid of gross cystic breast disease and a recognized marker of apocrine metaplasia, i.e. the 15-kDa glycoprotein (GCDFP-15), is presently unknown. We have added GCDFP-15 to cell culture medium and tested its effect on proliferation of 4 human breast-cancer cell lines (MCF7, BT474, MDA-MB231 and T47D) and a "normal" human immortal breast-cell line (MCF10A). These breast-cell lines showed a mitogenic response to GCDFP-15 (10 micrograms/ml). GCDFP-15 enhanced cell growth of the MCF10A, MCF7, BT474 and MDA-MB231 cell lines at both 48 and 96 hr of exposure. The glycoprotein exerted a mitogenic effect on the T47D cell line at 48 hr but not at 96 hr. This may be due to an auto-regulatory effect of endogenous GCDFP-15

synthesized by the T47D cells. GCDFP-15 was ineffective on 2 colon-cancer cell lines (HT29 and NIC-H716), on the IMR32 neuroblastoma cell line and on the NIC-H209 small-cell lung carcinoma cells. A separate major breast cystic disease fluid protein of 24 kDa (GCDFP-24) was tested, following the same experimental design, on the 5 breast-cell lines, and showed no mitogenic activity. The mitogenic effect of GCDFP-15 observed in this study in both "normal" and malignant breast epithelial cells suggests a possible relationship between apocrine metaplasia in breast cystic disease and the development of breast epithelial hyperplasia. In addition, a possible role of GCDFP-15 in breast-cancer progression should be considered.

Castiglioni, S. and J. A. Maier (2011). "Magnesium and cancer: a dangerous liason." Magnes Res **24**(3): S92-100.

A complex relationship links magnesium and cancer. The aim of this review is to revisit current knowledge concerning the contribution of magnesium to tumorigenesis, from transformed cells to animal models, and ending with data from human studies. Cultured neoplastic cells tend to accumulate magnesium. High intracellular levels of the cation seem to confer a metabolic advantage to the cells, contribute to alterations of the genome, and promote the acquisition of an immortal phenotype. In magnesium-deficient mice, low magnesium both limits and fosters tumorigenesis, since inhibition of tumor growth at its primary site is observed in the face of increased metastatic colonization. Epidemiological studies identify magnesium deficiency as a risk factor for some types of human cancers. In addition, impaired magnesium homeostasis is reported in cancer patients, and frequently complicates therapy with some anti-cancer drugs. More studies should be undertaken in order to disclose whether a simple and inexpensive intervention to optimize magnesium intake might be helpful in the prevention and treatment of cancer.

Cesano, A., et al. (1995). "Effects of lethal irradiation and cyclosporin A treatment on the growth and tumoricidal activity of a T cell clone potentially useful in cancer therapy." Cancer Immunol Immunother **40**(3): 139-151.

The TALL-104 cell line, originally derived from a patient with T cell leukemia, can be maintained indefinitely in culture in the presence of interleukin-2 (IL-2) and is endowed with a highly potent major-histocompatibility-complex (MHC)-non-restricted tumoricidal activity both in vitro and in animal models. The present study analyzes in detail the short- and long-term effects of irradiation and cyclosporin A (CsA) treatment on the growth and tumoricidal function of this T cell clone as compared to polyclonal lymphokine-activated killer (LAK) cell preparations from healthy donors. DNA and RNA syntheses by both TALL-104 and LAK cells were irreversibly arrested a few hours after irradiation with 40 Gy. However, 4-h 51Cr-release assays, performed on different days (day 1 to day 7) after irradiation, showed that the cytotoxic efficiency of TALL-104 cells against hematopoietic and solid tumor targets was only modestly reduced, whereas that of LAK cells was severely inhibited. Moreover, the cytotoxic responses to recombinant human IL-2 and IL-12, measured 18 h after irradiation and cytokine addition, were normal in the case of TALL-104 cells but were abolished in the case of LAK cells. Co-culture of IL-2- or IL-12-preactivated TALL-104 cells with a tumor target for 5 days in the absence of cytokines resulted in a lower efficiency of lysis, as compared to the non-irradiated effectors, especially if the initial stimulus was IL-12. These findings suggest the requirement of multiple cytokine stimulation for optimal expression of tumoricidal activity by lethally irradiated TALL-104 cells. CsA, while abrogating TALL-104 cell proliferation at the low dose of 0.5 microgram/ml, inhibited their cytotoxic function marginally only at high doses (100 micrograms/ml). By contrast, CsA reduced dose-dependently the cytotoxicity of LAK cells starting at very low doses (0.5 microgram/ml). CsA did not impair the ability of TALL-104 and LAK cells to produce interferon (IFN) gamma, tumor necrosis factor (TNF) alpha, and granulocyte/macrophage-colony-stimulatory factor (GM-CSF) in response to IL-2, IL-12, or tumor targets. Irradiation reduced drastically IFN gamma production by LAK, but not TALL-104 cells; release of TNF alpha and GM-CSF by either type of effector was inhibited by 10%-50%, depending on the stimulus. The high resistance and immunosuppressive drugs renders tis immortal T cell clone a potentially safe and effective reagent for new adoptive-transfer approaches to cancer in MHC-incompatible recipients.

Chang, J. T., et al. (2005). "Upstream stimulatory factor (USF) as a transcriptional suppressor of human telomerase reverse transcriptase (hTERT) in oral cancer cells." Mol Carcinog **44**(3): 183-192.

Telomerase activity is suppressed in normal human somatic tissues but is activated in cancer cells and immortal cell lines. The reverse transcriptase (RT) subunit human telomerase reverse transcriptase (hTERT) is the key regulator of telomerase activity. The hTERT promoter contains E-box elements and may allow upstream stimulatory factor (USF), a basic helix-loop-helix (bHLH) leucine zipper family proteins, to bind and regulate the expression. In this study, we investigated whether and how USF effect on hTERT. Through luciferase reporter assays, we found

that both USF1 and USF2 possess a comparable effect on the inhibition of hTERT expression. Immunoprecipitation (IP) and immunoblotting (IB) analysis reveal that the suppression of hTERT by USF was not through the interaction of USF with c-myc or mad, nor disturbed the cellular protein levels of those. In gel mobility shift and chromatin immunoprecipitation (CHIP) assays, we found that the USF suppression is through direct binding at the E-box site of hTERT promoter and rendering the effect actively. Analysis on clinical normal and tumor tissues reveal that the expression of USF1 and USF2 was lower in the tumor tissues, correlated with hTERT expression and telomerase activity. Taking together, our results demonstrate that USF is a negative transcriptional repressor for hTERT in oral cancer cells. It is possible that USF lose the inhibitory effect on hTERT expression leading to telomerase reactivation and oral carcinogenesis.

Chen, P., et al. (2020). "Silencing of keratin 17 by lentivirus-mediated short hairpin RNA inhibits the proliferation of PANC-1 human pancreatic cancer cells." Oncol Lett **19**(5): 3531-3541.

Keratin 17 (KRT17) has been demonstrated to be a potential biological marker for the prediction of prognosis in particular types of cancer. The aim of the present study was to investigate the molecular mechanisms underlying the function of KRT17 in the pancreatic cancer (PAC) cell line PANC-1 and the potential of KRT17 as a therapeutic target for PAC. KRT17 expression levels were analyzed using quantitative PCR and compared with histological data using bioinformatics tools in PAC samples and three human PAC cell lines. Cell proliferation was determined using an MTT assay, in addition to cell cycle distribution and apoptosis analysis using flow cytometry, colony formation assay using Giemsa staining and cell motility analysis using a Transwell migration assay. Tumor growth was evaluated in vivo in nude mice. The expression levels of a number of signaling molecules were measured to establish the potential mechanism by which silencing KRT17 expression affected PAC PANC-1 cells. Increased levels of KRT17 expression were observed in human PAC compared with normal tissues, as well as in three human PAC cell lines (MIA PaCa-2, PANC-1 and KP-3 cells) compared with the H6c7 human immortal pancreatic duct epithelial cell line. High expression levels of KRT17 in PAC samples were associated with poor overall survival (P=0.036) and disease-free survival (P=0.017). Lentivirus-mediated KRT17 silencing inhibited cell proliferation, colony formation and migration, but promoted apoptosis and resulted in cell cycle arrest in the G0/G1 phase in PANC-1 cells. In addition, KRT17 knockdown inhibited in vivo tumor growth. KRT17 knockdown induced dysregulation of ERK1/2 and upregulation of the pro-apoptotic Bcl-2 protein Bad. In conclusion, the present study demonstrated that elevated KRT17 levels are positively associated with pancreatic cancer progression; KRT17 knockdown suppressed cell growth, colony formation, migration and tumor growth, and induced apoptosis and cell cycle arrest, affecting ERK1/2/Bad signaling. Therefore, the results of the present study suggested that KRT17 may be a potential target for the treatment of pancreatic cancer.

Chen, Q., et al. (2019). "Long Noncoding RNA IGF2AS is Acting as an Epigenetic Tumor Suppressor in Human Prostate Cancer." Urology **124**: 310 e311-310 e318.

OBJECTIVE: To assess the expression profile and functional mechanism of long noncoding RNA (lncRNA) insulin growth factor 2 antisense (IGF2AS) in human prostate cancer (PCa). METHODS: Quantitative reverse transcriptase-polymerase chain reaction was applied to assess IGF2AS expression in immortal PCa cell lines and in situ human PCa tumors. IGF2AS was overexpressed in VCaP and PC3 cells to assess its effect on PCa cell proliferation and invasion in vitro, and xenograft in vivo. The effect of IGF2AS overexpression on IGF2 was also assessed in PCa cells. Then, IGF2 was upregulated in IGF2AS-overexpressed PCa cells to assess the functional involvement of IGF2 in IGF2AS-mediated PCa cell development. RESULTS: IGF2AS was downregulated in both PCa cell lines and human PCa tumors. In VCaP and PC3 cells, lentivirus-induced IGF2AS overexpression suppressed cancer cell proliferation and invasion in vitro, and xenograft development in vivo. IGF2 was downregulated by IGF2AS overexpression. Conversely, IGF2 upregulation revered the suppressing function of IGF2AS on PCa proliferation and invasion. CONCLUSION: LncRNA IGF2AS is acting as an epigenetic tumor suppressor in human PCa, likely through inverse regulation on IGF2. IGF2AS/IGF2 axis may be a future therapeutic target for PCa treatment.

Chiou, W. Y., et al. (2019). "Effectiveness of 23-valent pneumococcal polysaccharide vaccine on elderly patients with colorectal cancer: A population-based propensity score-matched cohort study." Medicine (Baltimore) **98**(50): e18380.

The commonly used vaccine for adults with a high risk of pneumonia is 23-valent pneumococcal polysaccharide vaccine (PPSV23). However, its effectiveness in patients with colorectal cancer has not been investigated. This study aimed to investigate the effectiveness of PPSV23 in reducing the risk of

pneumonia among elderly patients with colorectal cancer.A total of 120,605 newly diagnosed patients with colorectal cancer were identified from the Taiwan National Health Insurance Research Database between 1996 and 2010. Of these patients, 18,468 were 75 years or older in 2007 to 2010, and 3515 received PPSV23. People aged 75 years or older have been considered eligible for receiving PPSV23 vaccination in Taiwan since 2007. The specific "vaccination period" of October 2008 to December 2008 was used to minimize the potential immortal time bias. Therefore, 893 patients who received PPSV23 outside this vaccination period or died before 2009 and 2960 unvaccinated patients who died before 2009 were excluded. After the propensity score was matched with a 1:3 ratio, 2622 vaccinated patients and 7866 unvaccinated patients were recruited. A multivariate log-linear Poisson regression model was performed and adjusted for potential confounders, including influenza vaccination, vaccination period, cancer treatment modalities, comorbidities, and sociodemographic variables.After 2 years of follow-up, the incidence rate of the pneumonia hospitalization of the vaccinated patients was significantly lower than that of the unvaccinated patients at 85.53 per 1000 person-years (PYs) of the former and 92.38 per 1000 PYs of the latter. The proportions of patients who had 2, 3, and >3 pneumonia hospitalizations per year were consistently lower in the vaccinated group than in the unvaccinated group (1.9% vs 2.0%, 0.5% vs 0.9%, and 0.7% vs 1.1%, respectively). After adjustment for covariates was made, PPSV23 vaccine was significantly associated with a reduced risk of pneumonia hospitalization, with an adjusted incidence rate ratio of 0.88 (P = .040). The overall pneumonia-free survival rate was also significantly higher in the vaccinated patients than in the unvaccinated patients (P = .001).PPSV23 vaccination was associated with a significantly reduced rate of pneumonia hospitalization in elderly patients with colorectal cancer.

Cho, M. H., et al. (2021). "Association of Aspirin, Metformin, and Statin Use with Gastric Cancer Incidence and Mortality: A Nationwide Cohort Study." Cancer Prev Res (Phila) **14**(1): 95-104.

Anticancer effects of aspirin, metformin, and statins against gastric cancer, one of the most common cancers in the world, have been reported. This retrospective cohort study aimed to investigate independent associations of aspirin, metformin, and statin use with gastric cancer incidence and mortality after adjustment for concomitant use of other drugs, using pooled cohort data extracted from the Korean National Health Insurance claim database. Follow-up started on January 1, 2004 and ended at the date of gastric cancer diagnosis, death, or December 31, 2013. Exposures to drugs were defined as cumulative duration of use for aspirin and cumulative defined daily dose for metformin and statin, and were entered as time-dependent variables in Cox analysis models to avoid immortal time bias. Use of aspirin for longer than 182.5 and 547.5 days during 2-year interval was associated with reduced risks of gastric cancer incidence and mortality, respectively. Patients with diabetes were at higher risk of gastric cancer incidence and mortality than nondiabetic people, regardless of metformin treatment. However, metformin use among patients with diabetes was associated with a reduction in gastric cancer mortality in a dose-response manner. Statin use was also associated with a reduction of gastric cancer mortality in the general population, but not with gastric cancer incidence. In conclusion, long-term use of aspirin was independently associated with reduced incidence and mortality of gastric cancer in the general population, but metformin or statin use was only associated with a reduction of gastric cancer mortality in patients with diabetes and in the general population, respectively. PREVENTION RELEVANCE: Long-term use of aspirin was independently associated with reduced incidence and mortality of gastric cancer in the general population. Metformin or statin use, however, was only associated with a reduction of gastric cancer mortality in diabetic patients and in the general population in a dose-response manner, respectively.

Chung, H., et al. (2020). "Statins and metachronous recurrence after endoscopic resection of early gastric cancer: a nationwide Korean cohort study." Gastric Cancer **23**(4): 659-666.

BACKGROUND: Statins have shown to reduce the risk of various cancers. However, their effects on metachronous recurrence (MR) after endoscopic resection (ER) for early gastric cancer (EGC) are unknown. We evaluate their effects on MR development after ER for EGC. METHODS: We selected 11,568 patients who received ER for EGC from 2002 to 2011 from the Korean National Health Insurance database and classified into 2 groups: control and statins using propensity score matching. Metachronous recurrence was defined as the second ER or gastrectomy performed 6 months after the first ER. RESULTS: Mean follow-up period was 8.8 +/- 3.1 years. Statins showed a significantly lower incidence of MR than the control group (12.5% vs 2.2%, respectively, P < 0.01). After conducting competing risk analyses and time-dependent cox regression analysis considering immortal time bias, statins still showed a lower incidence rate of MR compared to that observed in the control group. For the multivariate analysis, statins remained significant (HR 0.17; 95% CI 0.13-0.24, P < 0.01). In the dose-response analysis,

an inverse dose-response relationship was identified between MR and statins ($P < 0.01$). CONCLUSION: Statins was significantly associated with a reduced risk of MR after ER for EGC with an inverse dose-response relationship.

Cole, A. L., et al. (2018). "Review of methodological challenges in comparing the effectiveness of neoadjuvant chemotherapy versus primary debulking surgery for advanced ovarian cancer in the United States." Cancer Epidemiol **55**: 8-16.

Randomized trials outside the U.S. have found non-inferior survival for neoadjuvant chemotherapy (NACT) versus primary debulking surgery (PDS) for advanced ovarian cancer (AOC). However, these trials reported lower overall survival and lower rates of optimal debulking than U.S. studies, leading to questions about generalizability to U.S. practice, where aggressive debulking is more common. Consequently, comparative effectiveness in the U.S. remains controversial. We reviewed U.S. comparative effectiveness studies of NACT versus PDS for AOC. Here we describe methodological challenges, compare results to trials outside the U.S., and make suggestions for future research. We identified U.S. studies published in 2010 or later that evaluated the comparative effectiveness of NACT versus PDS on survival in AOC through a PubMed search. Two independent reviewers abstracted data from eligible articles. Nine of 230 articles were eligible for review. Methodological challenges included unmeasured confounders, heterogeneous treatment effects, treatment variations over time, and inconsistent measurement of treatment and survival. Whereas some limitations were unavoidable, several limitations noted across studies were avoidable, including conditioning on mediating factors and immortal time introduced by measuring survival beginning from diagnosis. Without trials in the U.S., non-randomized studies are an important source of evidence for the ideal treatment for AOC. However, several methodological challenges exist when assessing the comparative effectiveness of NACT versus PDS in a non-randomized setting. Future observational studies must ensure that treatment is consistent throughout the study period and that treatment groups are comparable. Rapidly-evolving oncology data networks may allow for identification of treatment intent and other important confounders.

Crowe, D. L. and D. C. Nguyen (2001). "Rb and E2F-1 regulate telomerase activity in human cancer cells." Biochim Biophys Acta **1518**(1-2): 1-6.

The ends of human chromosomes (telomeres) lose up to 200 bp of DNA per cell division. Chromosomal shortening ultimately leads to senescence and death in normal cells. Many human carcinoma lines are immortal in vitro, suggesting that these cells have a mechanism for maintaining the ends of their chromosomes. Telomerase is a ribonucleoprotein complex that synthesizes telomeric DNA onto chromosomes using its RNA component as a template. Recent studies have shown that inactivation of the retinoblastoma gene product pRb and the cyclin dependent kinase inhibitor p16(INK4A) is required for telomerase activity in epithelial cells. We have demonstrated previously that restoration of functional retinoblastoma (Rb) expression is sufficient to downregulate telomerase activity in carcinoma cells. To determine mechanisms by which Rb regulates telomerase expression, we examined the effects of cyclin dependent kinase (cdk) mediated Rb inactivation and the release of E2F-1 on telomerase activity in human carcinoma cells. Overexpression of cdk2 and cdk4 but not a dominant negative cdk2 rescued Rb mediated downregulation of telomerase activity. Overexpression of the cdk regulatory subunit cyclin D1 also rescued telomerase downregulation and p16 expression alone was sufficient to ablate activity. E2F-1 overexpression was sufficient to rescue Rb mediated reduction of telomerase activity, but an E2F-1 mutant defective in DNA and Rb binding activities failed to produce this effect. Tumor tissue from E2F-1 -/- mice was negative for telomerase activity, indicating a key regulatory role for this transcription factor.

Cui, Y., et al. (2019). "Use of Antihypertensive Medications and Survival Rates for Breast, Colorectal, Lung, or Stomach Cancer." Am J Epidemiol **188**(8): 1512-1528.

Using time-dependent Cox regression models, we examined associations of common antihypertensive medications with overall cancer survival (OS) and disease-specific survival (DSS), with comprehensive adjustment for potential confounding factors. Participants were from the Shanghai Women's Health Study (1996-2000) and Shanghai Men's Health Study (2002-2006) in Shanghai, China. Included were 2,891 incident breast, colorectal, lung, and stomach cancer cases. Medication use was extracted from electronic medical records. With a median 3.4-year follow-up after diagnosis (interquartile range, 1.0-6.3), we found better outcomes among users of angiotensin II receptor blockers with colorectal cancer (OS: adjusted hazard ratio (HR) = 0.62, 95% confidence interval (CI): 0.44, 0.86; DSS: adjusted HR = 0.61, 95% CI: 0.43, 0.87) and stomach cancer (OS: adjusted HR = 0.62, 95% CI: 0.41, 0.94; DSS: adjusted HR = 0.63, 95% CI: 0.41, 0.98) and among users of beta-adrenergic receptor blockers with colorectal cancer (OS: adjusted HR = 0.50, 95% CI: 0.35, 0.72; DSS: adjusted HR = 0.50, 95%

CI: 0.34, 0.73). Better survival was also found for calcium channel blockers (DSS: adjusted HR = 0.67, 95% CI: 0.47, 0.97) and diuretics (OS: adjusted HR = 0.66, 95% CI: 0.45, 0.96; DSS: adjusted HR = 0.57, 95% CI: 0.38, 0.85) with stomach cancer. Our findings suggest angiotensin II receptor blockers, beta-adrenergic receptor blockers, and calcium channel blockers might be associated with improved survival outcomes of gastrointestinal cancers.

Dairkee, S. H., et al. (2004). "A molecular 'signature' of primary breast cancer cultures; patterns resembling tumor tissue." BMC Genomics **5**(1): 47.

BACKGROUND: To identify the spectrum of malignant attributes maintained outside the host environment, we have compared global gene expression in primary breast tumors and matched short-term epithelial cultures. RESULTS: In contrast to immortal cell lines, a characteristic 'limited proliferation' phenotype was observed, which included over expressed genes associated with the TGFbeta signal transduction pathway, such as SPARC, LOXL1, RUNX1, and DAPK1. Underlying this profile was the conspicuous absence of hTERT expression and telomerase activity, a significant increase in TbetaRII, its cognate ligand, and the CDK inhibitor, p21CIP1/WAF1. Concurrently, tumor tissue and primary cultures displayed low transcript levels of proliferation-related genes, such as, TOP2A, ANKT, RAD51, UBE2C, CENPA, RRM2, and PLK. CONCLUSIONS: Our data demonstrate that commonly used immortal cell lines do not reflect some aspects of tumor biology as closely as primary tumor cell cultures. The gene expression profile of malignant tissue, which is uniquely retained by cells cultured on solid substrates, could facilitate the development and testing of novel molecular targets for breast cancer.

Dent, P. (2013). "The multi-hit hypothesis in basal-like breast cancer." Cancer Biol Ther **14**(9): 778-779.

It has been known for many years that for a "normal" un-transformed cell to become immortal and subsequently tumorigenic requires multiple pro-oncogenic changes in the levels of protein expression and function. Genes most commonly associated with the process of oncogenesis include: p53 inactivating mutation; hDM2 overexpression; p16 reduced expression; K-/H-RAS activating mutation; PTEN inactivating mutation/deletion; EGFR activating mutation and overexpression; retinoblastoma inactivating mutation and deletion; Cyclin proteins overexpression; CD95 reduced expression; protective BCL-2 proteins overexpression; to name but just a few of such molecules.(1-5) That the minimally required specific proteins for oncogenesis are not known for many specific tumor types remains a challenge for the rational design of molecular targeted therapies.

Diaconu, C. C., et al. (2004). "The development of larger cells that spontaneously escape senescence--a step during the immortalization of a human cancer cell line." J Cell Mol Med **8**(1): 93-101.

There are few information concerning the changes associated with the transition interval when slow growing, primary explanted human cancer cells are displaced by new selected faster growing cells and became an immortal cell line. In a previous paper (J. Cell. Mol. Med., 5: 49-59, 2001) we described the TV cell line derived from a laryngeal tumor which harbors human papillomavirus (HPV) gene sequences throughout more than sixty in vitro passages. In this paper we analyze the modifications observed during the crisis interval when significant amount of cells senesce but occasional cells acquire some mutations that make them immortal. Confocal microscopy analysis revealed the heterogeneity of the cells in terms of their size and nucleus/cell ratio. Proliferation capacity was assessed by flow cytometry analyzing DNA content and expression of transferrin receptor (CD71). We discussed the possibility that HPV genome sequences alleviate a proliferation block during the crisis growth arrest of human larynx carcinoma cell line and the possibility that the cells monitor their size and growth by measuring the levels of some protein whose synthesis is coupled to cell development.

Dimri, M., et al. (2007). "Modeling breast cancer-associated c-Src and EGFR overexpression in human MECs: c-Src and EGFR cooperatively promote aberrant three-dimensional acinar structure and invasive behavior." Cancer Res **67**(9): 4164-4172.

Epidermal growth factor receptor (EGFR), a member of the ErbB family of receptor tyrosine kinases, is overexpressed in as many as 60% cases of breast and other cancers. EGFR overexpression is a characteristic of highly aggressive molecular subtypes of breast cancer with basal-like and BRCA1 mutant phenotypes distinct from ErbB2-overexpressing breast cancers. Yet, EGFR is substantially weaker compared with ErbB2 in promoting the oncogenic transformation of nontumorigenic human mammary epithelial cells (human MEC), suggesting a role for cooperating oncogenes. Here, we have modeled the co-overexpression of EGFR and a biologically and clinically relevant potential modifier c-Src in two distinct immortal but nontumorigenic human MECs. Using a combination of morphologic analysis and confocal imaging of polarity markers in three-dimensional Matrigel culture together with functional analyses of early oncogenic traits, we show for the first time that EGFR and c-Src co-overexpression but

not EGFR or c-Src overexpression alone unleashes an oncogenic signaling program that leads to hyperproliferation and loss of polarity in three-dimensional acinar cultures, marked enhancement of migratory and invasive behavior, and anchorage-independent growth. Our results establish that EGFR overexpression in an appropriate context (modeled here using c-Src overexpression) can initiate oncogenic transformation of nontumorigenic human MECs and provide a suitable in vitro model to interrogate human breast cancer-relevant oncogenic signaling pathways initiated by overexpressed EGFR and to identify modifiers of EGFR-mediated breast oncogenesis.

Dokukin, M. E., et al. (2015). "Emerging of fractal geometry on surface of human cervical epithelial cells during progression towards cancer." New J Phys **17**(3).

Despite considerable advances in understanding the molecular nature of cancer, many biophysical aspects of malignant development are still unclear. Here we study physical alterations of the surface of human cervical epithelial cells during stepwise in vitro development of cancer (from normal to immortal (premalignant), to malignant). We use atomic force microscopy to demonstrate that development of cancer is associated with emergence of simple fractal geometry on the cell surface. Contrary to the previously expected correlation between cancer and fractals, we find that fractal geometry occurs only at a limited period of development when immortal cells become cancerous; further cancer progression demonstrates deviation from fractal. Because of the connection between fractal behaviour and chaos (or far from equilibrium behaviour), these results suggest that chaotic behaviour coincides with the cancer transformation of the immortalization stage of cancer development, whereas further cancer progression recovers determinism of processes responsible for cell surface formation.

Du, J., et al. (2011). "Role of Rac1-dependent NADPH oxidase in the growth of pancreatic cancer." Cancer Gene Ther **18**(2): 135-143.

K-ras mutations occur in as high as 95% of patients with pancreatic cancer. K-ras activates Rac1-dependent NADPH oxidase, a key source of superoxide. Superoxide has an important function in pancreatic cancer cell proliferation, and scavenging or decreasing the levels of superoxide inhibits pancreatic cancer cell growth both in vitro and in vivo. DNA microarray analysis and RT-PCR has demonstrated that Rac1 is also upregulated in pancreatic cancer. The aim of this study was to determine whether inhibiting Rac1 would alter pancreatic tumor cell behavior. Human pancreatic cancer cells with mutant K-ras (MIA PaCa-2), wild-type K-ras (BxPC-3) and the immortal H6c7 cell line (pancreatic ductal epithelium) expressing K-ras oncogene (H6c7eR-KrasT) that is tumorigenic, were infected with a dominant/negative Rac1 construct (AdN17Rac1). In cells with mutant K-ras, AdN17Rac1 decreased rac activity, decreased superoxide levels and inhibited in vitro growth. However, in the BxPC-3 cell line, AdN17Rac1 did not change rac activity, superoxide levels or in vitro cell growth. Additionally, AdN17Rac1 decreased superoxide levels and inhibited in vitro growth in the KrasT tumorigenic cell line, but had no effect in the immortalized H6c7 cell line. In human pancreatic tumor xenografts, intratumoral injections of AdN17Rac1 inhibited tumor growth. These results suggest that activation of Rac1-dependent superoxide generation leads to pancreatic cancer cell proliferation. In pancreatic cancer, inhibition of Rac1 may be a potential therapeutic target.

Elbanna, M., et al. (2021). "Impact of Lung Parenchymal-Only Failure on Overall Survival in Early-Stage Lung Cancer Patients Treated With Stereotactic Ablative Radiotherapy." Clin Lung Cancer **22**(3): e342-e359.

INTRODUCTION: The impact of lung parenchymal-only failure on patient survival after stereotactic ablative body radiotherapy (SABR) for early-stage non-small-cell lung cancer (NSCLC) remains unclear. PATIENTS AND METHODS: The study population included 481 patients with early-stage NSCLC who were treated with 3- to 5-fraction SABR between 2000 and 2016. The primary study objective was to assess the impact of out-of-field lung parenchymal-only failure (OLPF) on overall survival (OS). RESULTS: At a median follow-up of 5.9 years, the median OS was 2.7 years for all patients. Patients with OLPF did not have a significantly different OS compared to patients without failure (P = .0952, median OS 4.1 years with failure vs. 2.6 years never failure). Analysis in a 1:1 propensity score-matched cohort for Karnofsky performance status, comorbidity score, and smoking status showed no differences in OS between patients without failure and those with OLPF (P = .8). In subgroup analyses exploring the impact of time of failure on OS, patients with OLPF 6 months or more after diagnosis did not have significantly different OS compared to those without failure, when accounting for immortal time bias (P = .3, median OS 4.3 years vs. 3.5 years never failure). Only 7 patients in our data set experienced failure within 6 months of treatment, of which only 4 were confirmed to be true failures; therefore, limited data are available in our cohort on the impact of OLPF for </= 6 months on OS. CONCLUSION: OLPF after SABR for early-stage NSCLC does not appear to adversely affect OS,

especially if occurring at least 6 months after SABR. More studies are needed to understand if OLPF within 6 months of SABR is associated with adverse OS. These data are useful when discussing prognosis of lung parenchymal failures after initial SABR.

Eldholm, V., et al. (2014). "CTCF mediates the TERT enhancer-promoter interactions in lung cancer cells: identification of a novel enhancer region involved in the regulation of TERT gene." Int J Cancer **134**(10): 2305-2313.

Telomerase activation is a hallmark of cancer. Although the regulation of the telomerase reverse transcriptase catalytic subunit (TERT), the rate-limiting factor for telomerase activity, has been studied intensively it remains incompletely understood. In cells devoid of telomerase activity, TERT is embedded in a region of condensed chromatin and the chromatin remodeling protein CCCTC-binding factor (CTCF) has been implicated in the inhibition of TERT expression. The importance of TERT activation for cellular immortalization and carcinogenesis is attested by the fact that the gene is expressed in more than 90% of immortal cell lines and tumors and that gain of TERT is the most frequent amplification event in early stage lung cancer. This study was designed to study the mechanisms of regulation of the TERT gene expression by the CTCF transcription factor in three human lung cancer cell lines, A427, A549 and H838. Depletion of CTCF by siRNA resulted in reduced TERT mRNA levels in two (A427 and A549) of the three cell lines. A novel enhancer element was identified approximately 4.5 kb upstream of the TERT transcription start site. Chromatin immunoprecipitation experiments revealed recruitment of CTCF to this enhancer element. Chromosome conformation capture experiments demonstrated the presence of CTCF-dependent chromatin loops between this enhancer element and the TERT proximal promoter in A427 and A549 cell lines. In summary, the results show that CTCF plays an important role in maintaining TERT expression in a subset of human lung cancer cell lines. This role may be due to CTCF-dependent enhancer-promoter interactions.

Elmore, L. W., et al. (2002). "Telomerase protects cancer-prone human cells from chromosomal instability and spontaneous immortalization." Cancer Biol Ther **1**(4): 391-397.

Studies were conducted to directly test whether the introduction of telomerase protects cancer-prone human mammary epithelial cells from chromosomal instability and spontaneous immortalization. Using a model for Li Fraumeni Syndrome (LFS), infection of human telomerase resulted in maintenance of telomere lengths, extension of in vitro lifespan, and prevention of spontaneous immortalization. In stark contrast to the spontaneously immortalized LFS cells, cells expressing ectopic telomerase displayed a remarkably stable karyotype and even after >150 population doublings, did not express endogenous telomerase. Since the hTERT-infected and spontaneously immortal LFS cells, like the parental cells, exhibit loss of p53 function, our data suggests that telomere shortening is the primary driving force for the genomic instability characteristic of LFS cells, while p53 inactivation is necessary for triggering the spontaneous immortalization event. Collectively, our data indicate that exogenous telomerase prevents chromosomal instability and spontaneous immortalization of LFS cells, suggesting a unique protective role for telomerase in the progression to immortalization.

Emilsson, L., et al. (2018). "Examining Bias in Studies of Statin Treatment and Survival in Patients With Cancer." JAMA Oncol **4**(1): 63-70.

Importance: Patients with cancer who use statins appear to have a substantially better survival than nonusers in observational studies. However, this inverse association between statin use and mortality may be due to selection bias and immortal-time bias. Objective: To emulate a randomized trial of statin therapy initiation that is free of selection bias and immortal-time bias. Design, Setting, and Participants: We used observational data on 17372 patients with cancer from the Surveillance, Epidemiology, and End Results (SEER)-Medicare database (2007-2009) with complete follow-up until 2011. The SEER-Medicare database links 17 US cancer registries and claims files from Medicare and Medicaid in 12 US states. We included individuals with a new diagnosis of colorectal, breast, prostate, or bladder cancer who had not been prescribed statins for at least 6 months before the cancer diagnosis. Individuals were duplicated, and each replicate was assigned to either the strategy "statin therapy initiation within 6 months after diagnosis" or "no statin therapy initiation." Replicates were censored when they stopped following their assigned strategy, and the potential selection bias was adjusted for via inverse-probability weighting. Hazard ratios (HRs), cumulative incidences, and risk differences were calculated for all-cause mortality and cancer-specific mortality. We then compared our estimates with those obtained using the same analytic approaches used in previous observational studies. Exposures: Statin therapy initiation within 6 months after cancer diagnosis. Main Outcomes and Measures: Cancer-specific and all-cause mortality using SEER-Medicare data and data from previous studies. Results: Of the 17372 patients whose data were analyzed, 8440 (49%) were men, and 8932 (51%) were women (mean

[SD] age, 76.4 [7.4] years; range, 66-115 years). The adjusted HR (95% CI) comparing statin therapy initiation vs no initiation was 1.00 (0.88-1.15) for cancer-specific mortality and 1.07 (0.93-1.21) for overall mortality. Cumulative incidence curves for both groups were almost overlapping (the risk difference never exceeded 0.8%). In contrast, the methods used by prior studies resulted in an inverse association between statin use and mortality (pooled hazard ratio 0.69). Conclusion and Relevance: After using methods that are not susceptible to selection bias from prevalent users and to immortal time bias, we found that initiation of therapy with statins within 6 months after cancer diagnosis did not appear to improve 3-year cancer-specific or overall survival.

Erdem, E., et al. (2003). "Telomerase activity in diagnosis of bladder cancer." Scand J Urol Nephrol **37**(3): 205-209.

OBJECTIVE: Telomerase is an enzyme that can reconstitute the ends of chromosomes after cell division and thus circumvent the damage that occurs in normal adult somatic cells during successive mitotic cycles. Immortal cells have short but stable chromosomes and increased telomerase activity. Transitional cell carcinoma (TCC) has only a few useful markers of diagnostic or prognostic importance. The objectives of this study were to determine whether there was a correlation between telomerase activities and the grade or stage of TCC and whether the activity of the enzyme could serve as a biochemical marker of this tumor. MATERIAL AND METHODS: Telomerase activity was determined by examining, using a polymerase chain reaction-based assay designed using the telomeric repeat amplification protocol (TRAP), urine cell pellets obtained from 42 bladder cancer patients, 18 patients with primary hematuria, 19 patients with benign urologic disease, 14 patients with urologic malignancies other than TCC and 20 healthy volunteers. RESULTS: Telomerase activity was found in 24/31 patients with bladder tumors (77.4% sensitivity) and in 5/77 patients without tumors (93.5% specificity). No correlation was found between telomerase activity and the grade or stage of the tumor. Although none of the urine cell pellets obtained from the 20 healthy volunteers demonstrated telomerase activity, positive telomerase activity was found in two subjects in the benign urologic disease group and in three subjects in the other urologic malignancy group. It was demonstrated that gross hematuria was the cause of false-negative results in six of the nine patients (66.7%). but washing the pellets four times and diluting them before the TRAP assay solved this problem. CONCLUSION: These results indicate that telomerase activity may be a promising marker for TCC but the technical aspects of the technique must be improved before it is used in routine clinical practice as a standard method. False-negative results obtained using gross hematuric urine should be carefully reevaluated and cell pellets should be washed again and diluted before analysis.

Esparza-Lopez, J., et al. (2019). "Deriving Primary Cancer Cell Cultures for Personalized Therapy." Rev Invest Clin **71**(6): 369-380.

Cancer is the second-leading cause of death in the world, accounting for one out of six deaths. Consequently, there is an urgent need for new and more effective therapeutic options as well as drug screening methods. Immortal, "stable" cancer cell lines have been employed since the past century to assess drug response but face several disadvantages. They often accumulate new genetic aberrations due to long-term culture and lack the indisputable heterogeneity of solid tumors, therefore, compromising the recapitulation of molecular features from parental tumors. Primary cancer cells have emerged as an attractive alternative to commercial cell lines since they can preserve such properties more closely. Here, we provide an overview of the basic concepts underlying generation and characterization of primary cell cultures from tumor samples. We emphasize the advantages and disadvantages of using these types of cancer cell cultures, and we make a comparison with other types of cultures used for personalized therapy. Finally, we consider the use of primary cancer cell cultures in personalized therapy as a means to improve drug response prediction and therapeutic outcomes.

Ewald, J. A., et al. (2010). "Therapy-induced senescence in cancer." J Natl Cancer Inst **102**(20): 1536-1546.

Cellular senescence is a response to nonlethal stress that results in persistent cytostasis with a distinct morphological and biochemical phenotype. The senescence phenotype, detected in tumors through the expression of mRNA and protein markers, can be generated in cancer cells lacking functional p53 and retinoblastoma protein. Current research suggests that therapy-induced senescence (TIS) represents a novel functional target that may improve cancer therapy. TIS can be induced in immortal and transformed cancer cells by selected anticancer compounds or radiation, and accumulating data indicate that TIS may produce reduced toxicity-related side effects and increased tumor-specific immune activity. This review examines the current status of TIS-regulated mechanisms, agents, and senescence biomarkers with the goal of encouraging further development of this approach to cancer therapy. Remaining hurdles include the lack of efficient senescence-inducing agents and incomplete biological data on tumor response. The identification

of additional compounds and other targeted approaches to senescence induction will further the development of TIS in the clinical treatment of cancer.

Fairhurst, C., et al. (2016). "Sodium channel-inhibiting drugs and cancer survival: protocol for a cohort study using the CPRD primary care database." BMJ Open **6**(9): e011661.

INTRODUCTION: Voltage-gated sodium channel (VGSC)-inhibiting drugs are commonly used to treat epilepsy and cardiac arrhythmia. VGSCs are also widely expressed in various cancers, including those of the breast, bowel and prostate. A number of VGSC-inhibiting drugs have been shown to inhibit cancer cell proliferation, invasion, tumour growth and metastasis in preclinical models, suggesting that VGSCs may be novel molecular targets for cancer treatment. Surprisingly, we previously found that prior exposure to VGSC-inhibiting drugs may be associated with reduced overall survival in patients with cancer, but we were unable to control for the cause of death or indication for prescription. The purpose of the present study is to interrogate a different database to further investigate the relationship between VGSC-inhibiting drugs and cancer-specific survival. METHODS AND ANALYSIS: A cohort study using primary care data from the Clinical Practice Research Datalink database will include patients with diagnosis of breast, bowel and prostate cancer (13 000). The primary outcome will be cancer-specific survival from the date of cancer diagnosis. Cox proportional hazards regression will be used to compare survival of patients taking VGSC-inhibiting drugs (including antiepileptic drugs and class I antiarrhythmic agents) with patients with cancer not taking these drugs, adjusting for cancer type, age and sex. Drug exposure will be treated as a time-varying covariate to account for potential immortal time bias. Various sensitivity and secondary analyses will be performed. ETHICS AND DISSEMINATION: The project has been reviewed and approved by the University of York Ethical Review Process. Results will be presented at an international conference and published in open access peer-reviewed journals according to the STROBE and RECORD guidelines.

Farmer, R. E., et al. (2017). "Metformin and cancer in type 2 diabetes: a systematic review and comprehensive bias evaluation." Int J Epidemiol **46**(2): 728-744.

Background: Existing observational studies provide conflicting evidence for the causal effect of metformin use on cancer risk in patients with type-2 diabetes, and there are concerns about bias affecting a number of studies. Methods: MEDLINE was used to identify observational studies investigating the association between metformin and overall or site-specific cancer in people with type-2 diabetes. A systematic data extraction and bias assessment was conducted, in which risk of eight bias domains (outcome, exposure, control selection, baseline confounding, time-dependent confounding, immortal time, missing data, censoring methods) were assessed against pre-defined criteria, and rated as unlikely, low, medium or high. Results: Of 46 studies identified, 21 assessed the effect of metformin on all cancer. Reported relative risks ranged from 0.23 to 1.22, with 12/21 reporting a statistically significant protective effect and none a harmful effect. The range of estimates was similar for site-specific cancers; 3/46 studies were rated as low or unlikely risk of bias in all domains. Two of these had results consistent with no effect of metformin; one observed a moderate protective effect overall, but presented further analyses that the authors concluded were inconsistent with causality. However, 28/46 studies were at risk from bias through exposure definition, 22 through insufficient baseline adjustment and 35 from possible time-dependent confounding. Conclusions: Observational studies on metformin and cancer varied in design, and the majority were at risk of a range of biases. The studies least likely to be affected by bias did not support a causal effect of metformin on cancer risk.

Fenton, J. I. and N. G. Hord (2006). "Stage matters: choosing relevant model systems to address hypotheses in diet and cancer chemoprevention research." Carcinogenesis **27**(5): 893-902.

Clinical evidence reveals that the efficacy of dietary factors to prevent cancer is probably stage-dependent. The ability to demonstrate stage-specific effects of dietary compounds on normal, preneoplastic and malignant cell models may provide insights into puzzling clinical results from cancer chemoprevention trials. The relevance of these models to the field of cancer prevention is immense and will undoubtedly facilitate the ability to discover which dietary factors are most effective at preventing cancer and which, if any, specific steps in neoplastic transformation render cells refractory to the effects of dietary compounds. There are illustrative examples where exposure of high-risk individuals to dietary chemopreventive agents increases rather than decreases cancer risk. While geneticists and clinical oncologists acknowledge the morphological continuum along which tumors develop in specific tissues, tumor cells, rather than normal and preneoplastic cells, continue to be the primary in vitro reductionist tool employed to elucidate mechanisms underlying disease progression and to investigate the potential utility of dietary as well as other chemopreventive agents. Currently, there are few relevant model systems to study the progression of

neoplastic transformation, especially in epithelial cells. We highlight examples of model systems isolated from prostate, breast, endometrial and intestinal tissue, with special emphasis on a specific set of non-tumorigenic, conditionally immortal cell lines derived from C57/BL6 mice [YAMC (Young Adult Mouse Colon cells; Apc+/+) cells and IMCE (Immorto-Min Colonic Epithelium cells; ApcMin/+) cells] that have yielded important information on early events in colorectal neoplasia development. These cell lines are an illustrative example of how researchers can examine stage-dependent effects of specific dietary components on carcinogenesis. The utilization of cell culture systems modeling early, middle and late stages of tumorigenesis will yield important insights into mechanisms by which dietary components impact cancer progression.

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.

Ferris, M. J., et al. (2018). "Radiation Therapy Is Associated With an Increased Incidence of Cardiac Events in Patients with Small Cell Lung Cancer." Int J Radiat Oncol Biol Phys **102**(2): 383-390.

PURPOSE: Cardiac radiation dose was a predictor of inferior overall survival in the Radiation Therapy Oncology Group 0617 non-small cell lung cancer trial. We examined the association between radiation therapy (RT) and cardiac events (CE) for patients with small cell lung cancer (SCLC). METHODS AND MATERIALS: The US population-based Surveillance, Epidemiology, and End Results Program and Medicare claims databases were queried for rates of CE among patients with SCLC treated with chemotherapy (CTX) +/- RT. Propensity score matching (PSM) and multivariate analysis were conducted. Patients were matched for actual/theoretical RT start date (to prevent immortal time bias) and then full PSM balanced clinical characteristics. Cumulative incidence function curves were generated. RESULTS: From 2000 to 2011, 7060 patients were included: 2892 limited-stage SCLC (LS-SCLC) and 4168 extensive-stage SCLC. Grouping LS-SCLC and extensive-stage SCLC together, the incidence of CE for the CTX + RT and CTX-only groups was 44.1% versus 39% at 60 months (P = .008). After PSM (5286 patients), the incidence of CE for the CTX + RT and CTX-only groups was 43% versus 38.6% at 60 months (P = .033). Analysis of only LS-SCLC (2016 patients) demonstrated that the incidence of CE for CTX + RT versus CTX-only groups was 50.3% versus 42% at 60 months (P = .0231). Multivariate analysis again demonstrated an association between CE and RT (hazard ratio 1.20; 95% confidence interval 1.06-1.37; P = .005). After PSM (1614 patients), the incidence of CE for CTX + RT versus CTX-only groups was 51.7% versus 41.6% at 60 months (P = .0042). CONCLUSIONS: Patients with SCLC are at significant risk of developing CE posttreatment; RT is associated with an absolute increase in the rate of CE at 5 years of approximately 5% for all patients with SCLC and up to 10% for patients with LS-SCLC. Cardiac risk management and cardiac-sparing RT techniques should be further evaluated for patients with SCLC.

Fournier, M. V., et al. (2006). "Gene expression signature in organized and growth-arrested mammary acini predicts good outcome in breast cancer." Cancer Res **66**(14): 7095-7102.

Nonmalignant human mammary epithelial cells (HMEC) seeded in laminin-rich extracellular matrix (lrECM) form polarized acini and, in doing so, transit from a disorganized proliferating state to an organized growth-arrested state. We hypothesized that the gene expression pattern of organized and growth-arrested HMECs would share similarities with breast tumors with good prognoses. Using Affymetrix HG-U133A microarrays, we analyzed the expression of 22,283 gene transcripts in 184 (finite life span) and HMT3522 S1 (immortal nonmalignant) HMECs on successive days after seeding in a lrECM assay. Both HMECs underwent growth arrest in G0-G1 and differentiated into polarized acini between days 5 and 7. We identified gene expression changes with the same temporal pattern in both lines and examined the expression of these genes in a previously published panel of microarray data for 295 breast cancer samples. We show that genes that are significantly lower in the organized, growth-arrested HMEC than in their proliferating counterparts can be used to classify breast cancer patients into poor and good prognosis groups with high accuracy. This study represents a novel unsupervised approach to identifying breast cancer markers that may be of use clinically.

Gagos, S., et al. (1996). "Cell senescence and a mechanism of clonal evolution leading to continuous cell proliferation, loss of heterozygosity, and tumor heterogeneity: studies on two immortal colon cancer

cell lines." Cancer Genet Cytogenet **90**(2): 157-165.

Extensive karyotypic analysis was performed on early and late passages of two continuous human cell lines, SW480 and SW620, that were derived from the same colon cancer patient. We cultivated these two cell lines in vitro for a period of 24 months and periodically examined their chromosome constitution. SW480 cells, from passage 138, were injected subcutaneously into 20 nude mice. The tumors that grew in nude mice were then cultivated in vitro for several passages to compare histopathologic findings and tumor growth patterns with clonal chromosomal profiles. Despite some karyotypic diversity, the two cell lines exhibited common marker chromosomes and followed similar patterns of evolution. During subsequent passages, acquisition of new chromosomal abnormalities gave rise to sidelines with a near-diploid genome that frequently underwent endoreduplication. Genomic instability seemed to play an important role in the emergence, growth, and subsequent elimination of the heterogenous sidelines by selection, clonal expansion, and cell death by senescence. Despite continuous growth, both the cell lines occasionally showed telomeric associations and random dicentric and multicentric formations. These lesions were considered evidence of cell senescence and were related to the disappearance of particular sidelines through evolution. Successful evolutionary steps were characterized by elimination of pre-existing marker chromosomes that were subsequently replaced in the karyotype by their cytologically intact homologous chromosomes possibly after selective endoreduplication. Frequent loss of heterozygosity for the chromosomes taking part in this process is postulated. We suggest that one of the mechanisms by which cancer cells bypass senescence may be related to their potential for continuous clonal diversification.

Gao, G., et al. (2019). "miR-129-5p inhibits prostate cancer proliferation via targeting ETV1." Onco Targets Ther **12**: 3531-3544.

Background: Prostate cancer is one of the most commonly diagnosed diseases in males. Methods: RT-qPCR was used to detect miR-129-5p expression in tumor tissues and adjacent normal tissues from patients with prostate cancer. The cell proliferation assay and colony forming assay were used to study the role of miR-129-5p in mediating prostate cancer cell growth. Bioinformatic analysis and dual luciferase assay were performed to predict and confirm ETV1 as a target gene of miR-129-5p. Results: We found that miR-129-5p levels were decreased significantly in human prostate cancer tissues compared with matched normal tissues from patients with prostate cancer. Overexpression of miR-129-5p suppressed prostate cancer cell growth while antagonist of miR-129-5p promoted cell proliferation in immortal prostate cell line RWPE-1. In addition, elevation of miR-129-5p decreased ETV1 expression in prostate cancer cells while downregulation of miR-129-5p increased ETV1 expression in RWPE-1. Mechanistically, ETV1 is confirmed a direct target of miR-129-5p in prostate cancer cells. Through repression of ETV1 expression, miR-129-5p could inactivate YAP signaling in prostate cancer cells. In addition, overexpression of ETV1 attenuated miR-129-5p induced cell proliferation in prostate cancer cells. Correlation analysis further revealed that there was a negative correlation between miR-129-5p levels and ETV1 mRNA levels in tumor tissues from patients with prostate cancer. Conclusion: Our results identified miR-129-5p as a tumor suppressor in prostate cancer via repression of ETV1.

Gao, R., et al. (2017). "Enemies or weapons in hands: investigational anti-diabetic drug glibenclamide and cancer risk." Expert Opin Investig Drugs **26**(7): 853-864.

INTRODUCTION: Epidemiological evidence suggests that diabetes is associated with elevated cancer risk through the actions of hyperglycemia, hyperinsulinemia and chronic inflammation. Metformin, a first-line medication for type 2 diabetes mellitus, arouses growing concerns on its anti-cancer effect. However, data regarding the effect of glibenclamide on tumor growth and cancer risk are less consistent, which may be a potential anti-cancer drug. Areas covered: In this review, we clarified probable underlying mechanisms in preclinical studies and reviewed epidemiological evidence on glibenclamide's cancer risk in clinical studies. Glibenclamide inhibited carcinogenesis through ATP-binding cassette protein super-family and ATP-sensitive potassium channels, while majority of clinical researches reported an increased or non-significant elevated cancer risk of glibenclamide users compared with metformin users. Other sulfonylureas and diarylsulfonylureas were also briefly introduced. Expert opinion: The inconsistency between the results of studies was probably ascribed to undiscovered mechanisms, confounding factors, inconsistent comparators and publication bias. Existing clinical trials were prone to be afflicted by time-related bias including immortal time bias, time-window bias, and time-lag bias. Glibenclimiade could be a promising and well-tolerated anti-neoplastic drug targeting ATP-binding cassette protein super-family and KATP channels, but its efficacy still needs to be proven in well-designed long-term randomized controlled clinical trials.

Garkavtsev, I., et al. (1998). "Molecular aspects of the relationship between cancer and aging: tumor

suppressor activity during cellular senescence." Exp Gerontol **33**(1-2): 81-94.

Normal cells cultured in vitro lose their proliferative potential after a finite number of doublings in a process termed replicative cellular senescence (Hayflick, 1965). The roles that growth inhibitory tumor suppressors play in the establishment and maintainence of cellular senescence have been reported in many different systems. The Rb and p53 tumor suppressors are examples of growth inhibitors that lose the ability to be regulated and are constantly activated during senescence. Other proteins that inhibit the initiation of DNA synthesis in early passage fibroblasts and that link the action of tumor suppressors with the cell cycle machinery, are also expressed at higher levels in senescent cells. For example, the increased expression of the cyclin-dependent kinase inhibitor p16 may contribute to arresting the growth of senescent cells. Identification and characterization of additional genes encoding growth inhibitors that are upregulated in senescent cells, such as the recently isolated p33ING1 protein, should provide a better understanding of the "aging program" that ceases to operate in the generation of immortal cancer cells.

Glaser, S. M., et al. (2016). "Anaplastic thyroid cancer: Prognostic factors, patterns of care, and overall survival." Head Neck **38 Suppl 1**: E2083-2090.

BACKGROUND: Anaplastic thyroid cancer (ATC) represents a rare, aggressive malignancy. We analyzed factors predictive for overall survival (OS) and treatment modality utilization. METHODS: Using the National Cancer Data Base, we identified 3552 patients with ATC. Factors associated with surgery, high-dose radiotherapy (RT; >/=59.4 Gy), and chemotherapy utilization were evaluated using multivariable logistic regression. From this, an inverse probability-weighted propensity score was incorporated into multivariable Cox regression analyses for OS. RESULTS: Numerous factors predictive for high-dose RT, total thyroidectomy, and chemotherapy utilization are described. Factors associated with improved survival were absence of clinical or pathologic lymph node involvement, absence of metastasis, tumor size </=6 cm, negative surgical margins, surgery, RT, and chemotherapy. On conditional landmark analysis, improved survival seen with chemotherapy and surgery other than total thyroidectomy was lost, but persisted for total thyroidectomy and high-dose RT. CONCLUSION: Even after correction for selection and immortal time bias, high-dose RT resulted in improved survival. (c) 2016 Wiley Periodicals, Inc. Head Neck 38: E2083-E2090, 2016.

Gleiss, A., et al. (2016). "Re-analysis of survival data of cancer patients utilizing additive homeopathy." Complement Ther Med **27**: 65-67.

In this short communication we present a re-analysis of homeopathic patient data in comparison to control patient data from the same Outpatient s Unit "Homeopathy in malignant diseases" of the Medical University of Vienna. In this analysis we took account of a probable immortal time bias. For patients suffering from advanced stages of cancer and surviving the first 6 or 12 months after diagnosis, respectively, the results show that utilizing homeopathy gives a statistically significant ($p<0.001$) advantage over control patients regarding survival time. In conclusion, bearing in mind all limitations, the results of this retrospective study suggest that patients with advanced stages of cancer might benefit from additional homeopathic treatment until a survival time of up to 12 months after diagnosis.

Goodison, S., et al. (2003). "Prolonged dormancy and site-specific growth potential of cancer cells spontaneously disseminated from nonmetastatic breast tumors as revealed by labeling with green fluorescent protein." Clin Cancer Res **9**(10 Pt 1): 3808-3814.

This study used an isogenic pair of metastatic (M4A4) and nonmetastatic (NM2C5), green fluorescent protein-labeled human breast cancer cell lines derived from the same patient and inoculated into the mammary glands of nude mice to investigate the dissemination patterns and fate of cells that escaped spontaneously from the resulting tumors. After tumors appeared, fluorescing single tumor cells were regularly seen in the lungs, even in animals inoculated with NM2C5, which fails to form secondary tumors in other organs. The sensitivity of the technique confirmed the continuing presence of scattered NM2C5 cells after primary tumor resection, although they formed no metastases by 6 months. These self-disseminated human tumor cells were retrievable from the tissues and were still viable and malignant, manifested by indefinite proliferation in vitro and green fluorescence and local tumorigenicity in vivo. Therefore, these scattered tumor cells were still immortal but rendered indefinitely quiescent by the microenvironmental conditions in the lung tissue. This is the first unequivocal demonstration of spontaneous distant dissemination of human cancer cells by undisturbed nonmetastatic tumors and comprises a valuable system for the analysis of tumor dormancy. In contrast, although many of the cells disseminating from M4A4 tumors grew into fluorescing metastases in the lungs, others remained solitary and quiescent. Therefore, even in a clonally derived cell population with metastatic properties, many cells do not, or cannot, mobilize the organ-specific growth properties needed

to generate metastases. This experimental approach, by using self-disseminating, green fluorescent protein-labeled, sister cell lines of opposing metastatic phenotypes, opens new avenues for investigating topics of clinical relevance, including tumor cell dormancy, anatomical distribution of metastases, and host factors influencing the metastatic process.

Granados Lopez, A. J. and J. A. Lopez (2014). "Multistep model of cervical cancer: participation of miRNAs and coding genes." Int J Mol Sci **15**(9): 15700-15733.

Aberrant miRNA expression is well recognized as an important step in the development of cancer. Close to 70 microRNAs (miRNAs) have been implicated in cervical cancer up to now, nevertheless it is unknown if aberrant miRNA expression causes the onset of cervical cancer. One of the best ways to address this issue is through a multistep model of carcinogenesis. In the progression of cervical cancer there are three well-established steps to reach cancer that we used in the model proposed here. The first step of the model comprises the gene changes that occur in normal cells to be transformed into immortal cells (CIN 1), the second comprises immortal cell changes to tumorigenic cells (CIN 2), the third step includes cell changes to increase tumorigenic capacity (CIN 3), and the final step covers tumorigenic changes to carcinogenic cells. Altered miRNAs and their target genes are located in each one of the four steps of the multistep model of carcinogenesis. miRNA expression has shown discrepancies in different works; therefore, in this model we include miRNAs recording similar results in at least two studies. The present model is a useful insight into studying potential prognostic, diagnostic, and therapeutic miRNAs.

Greider, C. W. (1999). "Telomerase activation. One step on the road to cancer?" Trends Genet **15**(3): 109-112.

Ever since the discovery that telomeres are short in cancer cells and telomerase is activated in immortal cells, telomerase has been an oncogene wannabe. Oncogenes have been the glamour genes of molecular biology for 20 years, garnering flashy headlines and name recognition. More recently, tumor-suppressor genes have joined oncogenes on center stage. Recent evidence has shown that MYC upregulates the catalytic subunit of telomerase, TERT, and that TERT cooperates with HPV E7 in cell immortalization. This evidence now supports the placement of telomerase among the cancer gene elite.

Guerreiro Da Silva, I. D., et al. (2000). "S100P calcium-binding protein overexpression is associated with immortalization of human breast epithelial cells in vitro and early stages of breast cancer development in vivo." Int J Oncol **16**(2): 231-240.

The mechanism of cell immortalization of human breast epithelial cells leading to neoplastic transformation is not clear. The isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10F, have provided a valuable tool to identify genes involved in this process. Using the technique of differential display, we have identified seven cDNA bands differentially displayed in the MCF-10F cells when compared with the mortal S130 cells from which MCF-10F was originated. One of these bands was isolated and cloned. Sequence analysis revealed 99% homology to the EF-hand calcium-binding protein S100P (Placental). The clone was overexpressed in the immortal cell line MCF-10F when compared to the mortal counterpart S130 or other primary cultures of human breast epithelial cells. In addition, it was highly expressed in chemically transformed breast epithelial cell lines (BP1E and D3. 1), breast cancer cell line T47D, as well as in three invasive ductal carcinomas when compared to their normal adjacent tissue. The S100P protein was localized by immunohistochemistry, using a monoclonal antibody against the same amino acid sequence of the gene cloned, in ductal hyperplasias, in situ and invasive ductal carcinoma, but not in the normal tissues. We concluded that S100P overexpression is an early event that might play an important role in the immortalization of human breast epithelial cells in vitro and tumor progression in vivo.

Guijarro, M. V., et al. (2012). "p38alpha limits the contribution of MAP17 to cancer progression in breast tumors." Oncogene **31**(41): 4447-4459.

MAP17 is a small, 17-kDa, non-glycosylated membrane protein that is overexpressed in a percentage of carcinomas. In the present work, we have analyzed the role of MAP17 expression during mammary cancer progression. We have found that MAP17 is expressed in 60% human mammary tumors while it is not expressed in normal or benign neoplasias. MAP17 levels increased with breast tumor stage and were strongly correlated with mammary tumoral progression. A significant increase in the levels of reactive oxygen species (ROS) was observed in MAP17-expressing cells, as compared with parental cells. This increase was further paralleled by an increase in the tumorigenic capacity of carcinoma cells but not in immortal non-tumoral breast epithelial cells, which provides a selective advantage once tumorigenesis has begun. Expression of specific MAP17 shRNA in protein-expressing tumor cells reduced their tumorigenic capabilities, which suggests that this effect is dependent upon MAP17 protein expression. Our data show that ROS functions as a

second messenger that enhances tumoral properties, which are inhibited in non-tumoral cells. We have found that p38alpha activation mediates this response. MAP17 triggers a ROS-dependent, senescence-like response that is abolished in the absence of p38a activation. Furthermore, in human breast tumors, MAP17 activation is correlated with a lack of phosphorylation of p38alpha. Therefore, MAP17 is overexpressed in late-stage breast tumors, in which oncogenic activity relies on p38 insensitivity to induce intracellular ROS.

Gunes, C., et al. (2018). "Telomeres in cancer." Differentiation **99**: 41-50.

Telomere shortening as a consequence of cell divisions during aging and chronic diseases associates with an increased cancer risk. Experimental data revealed that telomere shortening results in telomere dysfunction, which in turn affects tumorigenesis in two ways. First, telomere dysfunction suppresses tumor progression by the activation of DNA damage checkpoints, which induce cell cycle arrest (senescence) or apoptosis, as well as by inducing metabolic compromise and activation of immune responses directed against senescent cells. Second, telomere dysfunction promotes tumorigenesis by inducing chromosomal instability in tumor initiating cells, by inhibiting proliferative competition of non-transformed cells, and possibly, also by influencing tumor cell plasticity. The tumor promoting effects of telomere dysfunction are context dependent and require the loss of p53-dependent DNA damage checkpoints or other genetic modifiers that attenuate DNA damage responses possibly involving complex interactions of different genes. The activation of telomere stabilizing mechanisms appears as a subsequent step, which is required to enable immortal grotwh of emerging cancer cells. Here, we conceptually discuss our current knowledge and new, unpublished experimental data on telomere dependent influences on tumor initiation and progression.

Guo, S., et al. (2012). "Transcriptional regulation of hTREX84 in human cancer cells." PLoS One **7**(8): e43610.

TREX (transcription/export) is a multiprotein complex that plays a key role in the transcriptional elongation and transport of mRNA from the nucleus to the cytoplasm. We previously reported the purification of the human TREX protein and found that expression of a member of this complex, p84N5 (referred to as hTREX84 or hHPR1), a RB binding protein, correlated with breast tumor size and metastasis. Here we examine the mechanisms of aberrant expression of hTREX84 in breast and ovarian cancer cells and evaluate its role in tumorigenesis. We show that ovarian tumor cells over-express hTREX84 4-fold and 10-fold compared to immortal, non-tumorigenic and primary ovarian surface epithelial cells, respectively. Reduction of hTREX84 levels by small interfering RNA result in inhibition of cellular proliferation and G(2/M) arrest. Even though we observed that hTREX84 expression was induced by treatment with a demethylation agent, 5-aza-2'-deoxycytidine (5-aza-dC), sodium bisulfite DNA sequencing and methylation specific PCR found no evidence of changes in DNA methylation in the CpG islands in the regulator region of hTREX84. We subsequently identify several transcriptional factors, including NF-kappaB binding sites in the hTREX84 gene promoter and demonstrate by chromatin immunoprecipation (ChIP) and site directed mutagenesis that RelA/p65 binds the NF-kB binding sites and induces hTREX84 expression. Finally, we show by immunohistochemistry (IHC) that RelA/p65 is abundantly expressed in malignant cells that aberrantly express hTREX84 indicating that RelA/p65 might play a pivotal role in regulating hTREX84 expression in cancer. Our results indicate that overexpression of hTREX84 is associated with cancer cell transformation, proliferation and may be regulated by RelA/p65.

Guz, N. V., et al. (2015). "Towards early detection of cervical cancer: Fractal dimension of AFM images of human cervical epithelial cells at different stages of progression to cancer." Nanomedicine **11**(7): 1667-1675.

UNLABELLED: We used AFM HarmoniX modality to analyse the surface of individual human cervical epithelial cells at three stages of progression to cancer, normal, immortal (pre-malignant) and carcinoma cells. Primary cells from 6 normal strains, 6 cancer, and 6 immortalized lines (derived by plasmid DNA-HPV-16 transfection of cells from 6 healthy individuals) were tested. This cell model allowed for good control of the cell phenotype down to the single cell level, which is impractical to attain in clinical screening tests (ex-vivo). AFM maps of physical (nonspecific) adhesion are collected on fixed dried cells. We show that a surface parameter called fractal dimension can be used to segregate normal from both immortal pre-malignant and malignant cells with sensitivity and specificity of more than 99%. The reported method of analysis can be directly applied to cells collected in liquid cytology screening tests and identified as abnormal with regular optical methods to increase sensitivity. FROM THE CLINICAL EDITOR: Despite cervical smear screening, sometimes it is very difficult to differentiate cancers cells from pre-malignant cells. By using AFM to analyze the surface properties of human cervical epithelial cells, the

authors were able to accurately identify normal from abnormal cells. This method could augment existing protocols to increase diagnostic accuracy.

Hamid, S., et al. (2007). "Establishment and characterization of Asian oral cancer cell lines as in vitro models to study a disease prevalent in Asia." Int J Mol Med **19**(3): 453-460.

We have established 3 cell lines ORL-48, -115 and -136 from surgically resected specimens obtained from untreated primary human oral squamous cell carcinomas of the oral cavity. The in vitro growth characteristics, epithelial origin, in vitro anchorage independency, human papilloma-virus (HPV) infection, microsatellite instability status, karyotype and the status of various cell cycle regulators and gatekeepers of these cell lines were investigated. All 3 cell lines grew as monolayers with doubling times ranging between 26.4 and 40.8 h and were immortal. Karyotyping confirmed that these cell lines were of human origin with multiple random losses and gains of entire chromosomes and regions of chromosomes. Immunohistochemistry staining of cytokeratins confirmed the epithelial origin of these cell lines, and the low degree of anchorage independency expressed by these cell lines suggests non-transformed phenotypes. Genetic analysis identified mutations in the p53 gene in all cell lines and hypermethylation of p16INK4a in ORL-48 and -136. Analysis of MDM2 and EGFR expression indicated MDM2 overexpression in ORL-48 and EGFR overexpression in ORL-136 in comparison to the protein levels in normal oral keratinocytes. Analysis of the BAT-26 polyadenine repeat sequence and MLH-1 and MSH-2 repair enzymes demonstrated that all 3 cell lines were microsatellite stable. The role of HPV in driving carcinogenesis in these tumours was negated by the absence of HPV. Finally, analysis of the tissues from which these cell lines were derived indicated that the cell lines were genetically representative of the tumours, and, therefore, are useful tools in the understanding of the molecular changes associated with oral cancers.

Hanna, N., et al. (2018). "Effectiveness of Neoadjuvant Chemotherapy for Muscle-invasive Bladder Cancer in the Current Real World Setting in the USA." Eur Urol Oncol **1**(1): 83-90.

BACKGROUND: The use of neoadjuvant chemotherapy (NAC) before radical cystectomy (RC) is supported by results from several randomized control trials, including SWOG-8710. OBJECTIVE: To look at the effectiveness of NAC before RC in current real world practice in the USA. DESIGN, SETTING, AND PARTICIPANTS: We used the National Cancer Data Base (NCDB) to identify patients with nonmetastatic muscle-invasive urothelial carcinoma of the bladder who underwent RC between 2004 and 2012. INTERVENTION: Receipt of NAC before RC. OUTCOME MEASUREMENTS AND STATISTICAL ANALYSIS: The primary endpoint was overall survival (OS). Secondary endpoints were rates of complete pathologic response (pT0), positive pathologic lymph nodes (pN+), and margin status. Using a landmark analysis to adjust for an immortal-time bias, OS comparison was performed using Cox regression analysis. Furthermore, logistic regression models examining secondary outcomes were fitted. To adjust for potential selection bias, propensity score-weighted analyses were performed. RESULTS AND LIMITATIONS: Of 8732 patients who underwent RC, 1619 (19%) received NAC. Following propensity score adjustment, receipt of NAC was not associated with an OS benefit (hazard ratio 0.97; p=0.591). On secondary outcome analysis, higher pT0 rates (odds ratio 5.03; p<0.001) were recorded among patients who received NAC, although rates of pT0 were lower than for patients treated with NAC within the SWOG-8710 trial (13% vs 38%). Limitations include the retrospective design and limited details available regarding type of chemotherapy. CONCLUSIONS: Important baseline differences between patients from the SWOG-8710 trial and those in general urologic practice exist. After adjusting for immortal-time bias, we did not find a clear survival advantage of NAC before RC when compared to RC alone in current general urology practice in the USA. PATIENT SUMMARY: The benefit of chemotherapy before radical cystectomy is supported by few randomized control trials. In this study, using a large national data set from the USA we found that preoperative chemotherapy is not associated with a survival benefit in all patients in general urology practice. Hence, better selection criteria are needed to determine who will benefit the most from chemotherapy before radical cystectomy.

Hawkins, O. E., et al. (2008). "Identification of breast cancer peptide epitopes presented by HLA-A*0201." J Proteome Res **7**(4): 1445-1457.

Cellular immune mechanisms detect and destroy cancerous and infected cells via the human leukocyte antigen (HLA) class I molecules that present peptides of intracellular origin on the surface of all nucleated cells. The identification of novel, tumor-specific epitopes is a critical step in the development of immunotherapeutics for breast cancer. To directly identify peptide epitopes unique to cancerous cells, secreted human class I HLA molecules (sHLA) were constructed by deletion of the transmembrane and cytoplasmic domain of HLA A*0201. The resulting sHLA-A*0201 was transferred and expressed in breast

cancer cell lines MCF-7, MDA-MB-231, and BT-20 as well as in the immortal, nontumorigenic cell line MCF10A. Stable transfectants were seeded into bioreactors for production of > 25 mg of sHLA-A*0201. Peptides eluted from affinity purified sHLA were analyzed by mass spectroscopy. Comparative analysis of HLA-A*0201 peptides revealed 5 previously uncharacterized epitopes uniquely presented on breast cancer cells. These peptides were derived from intracellular proteins with either well-defined or putative roles in breast cancer development and progression: Cyclin Dependent Kinase 2 (Cdk2), Ornithine Decarboxylase (ODC1), Kinetochore Associated 2 (KNTC2 or HEC1), Macrophage Migration Inhibitory Factor (MIF), and Exosome Component 6 (EXOSC6). Cellular recognition of the MIF, KNTC2, EXOSC6, and Cdk2 peptides by circulating CD8+ cells was demonstrated by tetramer staining and IFN-gamma ELISPOT. The identification and characterization of peptides unique to the class I of breast cancer cells provide putative targets for the development of immune diagnostic tools and therapeutics.

He, C., et al. (2018). "Ratiometric Fluorescent Biosensor for Visual Discrimination of Cancer Cells with Different Telomerase Expression Levels." ACS Sens **3**(4): 757-762.

Telomerase is inactive in normal somatic cells but highly activated in tumor cells to maintain their indefinite proliferation and immortal phenotype. As a specific marker for the generation and progress of almost all tumors, the detection of telomerase activity by classical PCR techniques has served in the biological research of tumors. However, the detection of in situ telomerase activity in cell extracts to evaluate the malignancy, progress, and metastasis of tumors remains a daunting challenge. Here, a precisely designed FRET-based ratiometric fluorescent oligonucleotide probe has achieved high-fidelity detection of telomerase activity for accurate discrimination of different cancer cells toward advanced diagnosis of tumors. Our method is superior to other methods in its capabilities to quantify telomerase activity in cell extracts and visualize various tumor cell extracts with different telomerase expression levels by the naked eye for clinical diagnosis. In particular, the ratiometric fluorescent probe used in the assay could exclude other experimental factors influence, and further avoid false positive signal generation. The method reported here could provide a reliable, accurate, and convenient way in medical diagnostics and therapeutic response assessment.

Hershey, J. W. (2015). "The role of eIF3 and its individual subunits in cancer." Biochim Biophys Acta **1849**(7): 792-800.

Specific individual subunits of eIF3 are elevated or reduced in numerous human tumors, and their ectopic overexpression in immortal cells can result in malignant transformation. The structure and assembly of eIF3 and its role in promoting mRNA and methionyl-tRNAi binding to the ribosome during the initiation phase of protein synthesis are described. Methods employed to detect altered levels of eIF3 subunits in cancers are critically evaluated in order to conclude rigorously that such subunits may cause malignant transformation. Strong evidence is presented that the individual overexpression of eIF3 subunits 3a, 3b, 3c, 3h, 3i and 3m may cause malignant transformation, whereas underexpression of subunits 3e and 3f may cause a similar outcome. Possible mechanisms to explain the malignant phenotypes are examined. The involvement of eIF3 in cancer reinforces the view that translational control plays an important role in the regulation of cell proliferation, and provides new targets for the development of therapeutic agents. This article is part of a Special Issue entitled: Translation and Cancer.

Herskovic, A., et al. (2017). "Role of Postoperative Radiotherapy in Pathologic Stage IIIA (N2) Non-Small Cell Lung Cancer in a Prospective Nationwide Oncology Outcomes Database." J Thorac Oncol **12**(2): 302-313.

INTRODUCTION: The role of postoperative radiotherapy (PORT) in the treatment of pathologic stage IIIA (N2) NSCLC remains controversial. We investigated practice patterns and outcomes for these patients in a prospectively maintained nationwide oncology outcomes database. METHODS: Patients with known histologic features of pathologic stage IIIA (N2) NSCLC who underwent an operation with negative margins and received adjuvant multiagent chemotherapy from 2004 to 2013 were identified from the National Cancer Data Base and stratified by the use of PORT. Multivariable logistic regression modeling was used to examine factors associated with receiving PORT, and multivariable proportional hazards regression was used to examine the association of treatment and mortality, adjusting for demographic, socioeconomic and clinicopathologic factors. Landmark analysis and covariate balancing propensity score (CBPS) weighting were also explored to account for immortal time bias and nonrandomization. RESULTS: A total of 2691 patients were identified, with a median follow-up of 32.32 months. In multivariable analysis, improved overall survival was associated with multiple factors, including younger age, female sex, lower Charlson-Deyo comorbidity index, histologic type (with

squamous cell being better than adenocarcinoma), smaller tumor size, lower pathologic T stage, surgical procedure (with pneumonectomy or lobectomy being better than sublobar resection), and receipt of PORT (all $p < 0.05$). Before landmark analysis, the hazard ratio (HR) showed an overall survival benefit for patients receiving PORT (adjusted HR = 0.83, 95% CI [confidence interval]: 0.72-0.95; p = 0.008). This benefit remained significant after CBPS weighting (HR = 0.81, 95% CI: 0.70-0.94, p = 0.005), almost significant after landmark analysis (adjusted HR = 0.84, 95% CI: 0.69-1.007, p = 0.059), and significant after landmark analysis with CBPS weighting (HR = 0.77, 95% CI: 0.63-0.94, p = 0.009). Median survival past landmark time was 27.43 months in the PORT group and 25.86 months in the non-PORT group. Factors significantly associated with receiving PORT were facility location, facility type, Charlson-Deyo comorbidity index, and grade (all $p < 0.05$). CONCLUSIONS: Improved survival is associated with receipt of PORT for patients with pathologic stage IIIA (N2) NSCLC treated with complete resection and multiagent chemotherapy.

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 and immune-associated lung diseases." Int J Mol Med **1**(3): 545-549.

Telomerase maintains telomere length and is considered to be necessary for the indefinite proliferation of human cells. Telomerase activity is detected not only in germline and immortal cancer cells, but also in stem/progenitor cells of renewal tissues and activated lymphocytes. While it is generally agreed that telomerase is a useful tumor marker, the utility of telomerase activity in non-cancerous cells should also be considered. In the present study, we quantitatively examined telomerase activity in 56 cytology samples and 106 bronchoalveolar lavage samples obtained from patients with various respiratory diseases. Fourteen of 34 samples obtained from lung cancer patients showed detectable telomerase activity, while only 7 of 128 samples obtained from patients without lung cancer did ($p<0.001$). Moreover, 12 of 14 telomerase-positive samples with lung cancer showed strong signals, while none without lung cancer did. Among 106 non-cancerous bronchoalveolar lavage samples, 4 telomerase positive samples had increased number of lymphocytes and increased disease progression. These findings indicate that evaluation of telomerase activity may not only be a useful diagnostic test for lung cancer, but may also be a marker of disease aggressiveness for immune-associated lung diseases.

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.

Hoffmann, F. and F. Andersohn (2010). "Is guideline adherence the key to increased survival in patients with breast cancer?" Oncology **79**(3-4): 301-302; author reply 303-305.

Recently, Varga et al. reported in an observational study that guideline adherence could prevent 56.3-78.9% of all deaths in patients with early onset breast cancer [Oncology 2010;78:189-195]. This would mean that nearly all deaths due to breast cancer can be avoided by guideline-adherent treatment. We argue that some methodological issues like immortal time bias or healthy adherer bias may have contributed to these implausible findings. However, the non-transparent reporting of the methods, especially regarding the operationalization of guideline adherence, hampers critical assessment of this study.

Holt, J. A. (1993). "The glutathione cycle is the creative reaction of life and cancer. Cancer causes oncogenes and not vice versa." Med Hypotheses **40**(5): 262-266.

Life is definable as a chemical reaction which obeys exponential growth and dies if reversed. Such a reaction must be the commencement of all life so that every evolved form of it inherits these characteristics. As no single reaction known has these two features, life must be a combination of two or more reactions which whilst obeying all the classical laws of physics and chemistry assume an exponential form and effectively act as being irreversible. The reactions of glutathione--oxidation and reduction--when combined in sequence as a cyclical process fulfill these criteria. The cyclic changes of glutathione from reduced to oxidised to reduced forms must therefore be the reaction which creates life and is responsible for cancer's growth. 434 mHz electromagnetic radiation stimulates cancer growth rate by forcing this cycle into activity. Proof of this hypothesis is the long-term control of cancer in 11 patients treated with oxidised glutathione and 434 mHz radiation. Genetic material does not contain any energy system with exponential form, neither is it self-replicating. Genetic material will only reproduce if placed within an immortal cell in which all controls of the glutathione system have been lost, as in a cancer cell. Oncogenes must be the product of cancer and not the reverse.

Holt, S. E. and J. W. Shay (1999). "Role of telomerase in cellular proliferation and cancer." J Cell Physiol **180**(1): 10-18.

Telomerase is a cellular reverse transcriptase that helps to provide genomic stability in highly proliferative normal, immortal, and tumor cells by maintaining the integrity of the chromosome ends, the telomeres. The activity of telomerase is associated with the majority of malignant human cancers. Telomerase or another mechanism for telomere maintenance is required for continuous tumor cell proliferation. Telomerase-positive cells that exit the cell cycle via quiescence downregulate telomerase through a transcriptional repression pathway. In the case of cell cycle exit via terminal differentiation, proteolysis of telomerase may also be involved. In response to mitogenic or growth factor signaling, telomerase-competent quiescent cells reenter the cell cycle and express telomerase activity independent of DNA synthesis. Under normal growth conditions, inhibition of telomerase activity in tumor-derived cells results in continued cell division coupled with telomere shortening, eventually followed by cellular senescence or death. Thus, repression of telomerase activity may be a novel adjuvant therapy for the treatment of human cancer and detection of telomerase activity may be important for cancer diagnostics.

Hong, J. L., et al. (2017). "Comparative Effect of Initiating Metformin Versus Sulfonylureas on Breast Cancer Risk in Older Women." Epidemiology **28**(3): 446-454.

BACKGROUND: Several observational studies have reported that metformin may be associated with reduced risk of breast cancer; however, many of these studies were affected by time-related biases such as immortal time bias and time-window bias. This study aimed to examine the relative risk of breast cancer for older women initiating metformin versus sulfonylureas while avoiding such biases. METHODS: The study cohort consisted of women aged 65+ who initiated monotherapy with metformin

(n = 45,900) or sulfonylureas (n = 13,904) and were free of cancer and renal disease within 6 months before treatment initiation using 2007-2012 US Medicare claims data. We followed treatment initiators for incident breast cancer, and estimated hazard ratios using weighted Cox models. Unmeasured confounding by body mass index and smoking was further adjusted by propensity score calibration using external information from Medicare Current Beneficiary Survey 2006-2009 panels. RESULTS: During 58,835 and 16,366 person-years of follow-up, 385 initiators of metformin treatment and 95 of sulfonylurea were diagnosed with breast cancer. Metformin initiators did not have a reduced risk of breast cancer compared with sulfonylurea initiators (hazard ratio: 1.2; 95% confidence interval: 0.94, 1.6). Externally controlling for body mass index and smoking did not affect the estimates. CONCLUSION: The findings of this study provide no support for a reduced risk of breast cancer after initiation of metformin compared with a clinical alternative in older women. This study is limited by the relatively short follow-up time and we cannot exclude the possible benefits of long-time metformin use on breast cancer risk.

Hopkinson, B. M., et al. (2017). "Establishment of a normal-derived estrogen receptor-positive cell line comparable to the prevailing human breast cancer subtype." Oncotarget **8**(6): 10580-10593.

Understanding human cancer increasingly relies on insight gained from subtype specific comparisons between malignant and non-malignant cells. The most frequent subtype in breast cancer is the luminal. By far the most frequently used model for luminal breast cancer is the iconic estrogen receptor-positive (ERpos) MCF7 cell line. However, luminal specific comparisons have suffered from the lack of a relevant non-malignant counterpart. Our previous work has shown that transforming growth factor-beta receptor (TGFbetaR) inhibition suffices to propagate prospectively isolated ERpos human breast luminal cells from reduction mammoplasties (HBEC). Here we demonstrate that transduction of these cells with hTERT/shp16 renders them immortal while remaining true to the luminal lineage including expression of functional ER (iHBECERpos). Under identical culture conditions a major difference between MCF7 and normal-derived cells is the dependence of the latter on TGFbetaR inhibition for ER expression. In a breast fibroblast co-culture model we further show that whereas MCF7 proliferate concurrently with ER expression, iHBECERpos form correctly polarized acini, and segregate into proliferating and ER expressing cells. We propose that iHBECERpos may serve to shed light on hitherto unappreciated differences in ER regulation and function between normal breast and breast cancer.

Huard, S. and C. Autexier (2002). "Targeting human telomerase in cancer therapy." Curr Med Chem Anticancer Agents **2**(5): 577-587.

Telomerase is a specialized RNA template-containing reverse transcriptase that mediates telomere repeat synthesis at chromosome ends. The maintenance of telomere length and integrity is essential for cell survival. Telomerase is active in most immortal and tumor cells, whereas the majority of normal human cells demonstrate no detectable activity and undergo telomere shortening. The identification of a possible role for telomerase in cellular aging and cancer has led to numerous studies designed to characterize this ribonucleoprotein enzyme. Inhibiting telomerase activity in immortal human cells reduces cellular proliferative capacity and can lead to cell death. Identifying mechanisms to specifically inhibit telomerase activity in malignant cells could thus be of great therapeutic value in the treatment of cancer. In this review, we summarize the current understanding of the mechanism of action of human telomerase. The biochemical characterization of telomerase is necessary for the design and evaluation of antitelomerase therapies. Different strategies are currently under investigation to design inhibitors that target the reverse transcriptase and RNA components of the telomerase complex. Recent advances in the design of these inhibitors and their properties are discussed.

Hung, Y. P., et al. (2015). "Secondary Primary Malignancy Risk in Patients With Ovarian Cancer in Taiwan: A Nationwide Population-Based Study." Medicine (Baltimore) **94**(38): e1626.

To evaluate the incidence of secondary primary malignancy (SPM) in patients with ovarian cancer using a nationwide retrospective population-based dataset. Patients newly diagnosed with ovarian cancer between 1997 and 2010 were identified using Taiwan's National Health Insurance database. Patients with antecedent malignancies were excluded. Standardized incidence ratios (SIRs) for SPM were calculated and compared with the cancer incidence in the general population. Risk factors for cancer development were analyzed using Cox proportional hazard models. Effects of surgery, chemotherapy, and radiotherapy after ovarian cancer diagnosis were regarded as time-dependent variables to prevent immortal time bias. During the 14-year study period (follow-up of 56,214 person-years), 707 cancers developed in 12,127 patients with ovarian cancer. The SIR for all cancers was 2.78 (95% confidence interval 2.58-3.00). SIRs for follow-up periods of >5, 1-5, and <1 year were 1.87, 2.04, and 6.40, respectively. After

the exclusion of SPM occurring within 1 year of ovarian cancer diagnosis, SIRs were significantly higher for cancers of the colon, rectum, and anus (2.14); lung and mediastinum (1.58); breast (1.68); cervix (1.65); uterus (7.96); bladder (3.17), and thyroid (2.23); as well as for leukemia (3.98) and others (3.83). Multivariate analysis showed that age >/= 50 years was a significant SPM risk factor (hazard ratio [HR] 1.60). Different treatments for ovarian cancer, including radiotherapy (HR 2.07) and chemotherapy (HR 1.27), had different impacts on SPM risk. Patients with ovarian cancer are at increased risk of SPM development. Age >/= 50 years, radiotherapy, and chemotherapy are independent risk factors. Close surveillance of patients at high risk should be considered for the early detection of SPM.

Hunter, K. D., et al. (2006). "Divergent routes to oral cancer." Cancer Res **66**(15): 7405-7413.

Most head and neck squamous cell carcinoma (HNSCC) patients present with late-stage cancers, which are difficult to treat. Therefore, early diagnosis of high-risk premalignant lesions and incipient cancers is important. HNSCC is currently perceived as a single progression mechanism, resulting in immortal invasive cancers. However, we have found that approximately 40% of primary oral SCCs are mortal in culture, and these have a better prognosis. About 60% of oral premalignancies (dysplasias) are also mortal. The mortal and immortal tumors are generated in vivo as judged by p53 mutations and loss of p16(INK4A) expression being found only in the original tumors from which the immortal cultures were derived. To investigate the relationships of dysplasias to SCCs, we did microarray analysis of primary cultures of 4 normal oral mucosa biopsies, 19 dysplasias, and 16 SCCs. Spectral clustering using the singular value decomposition and other bioinformatic techniques showed that development of mortal and immortal SCCs involves distinct transcriptional changes. Both SCC classes share most of the transcriptional changes found in their respective dysplasias but have additional changes. Moreover, high-risk dysplasias that subsequently progress to SCCs more closely resemble SCCs than nonprogressing dysplasias. This indicates for the first time that there are divergent mortal and immortal pathways for oral SCC development via intermediate dysplasias. We believe that this new information may lead to new ways of classifying HNSCC in relation to prognosis.

Iarrobino, N. A., et al. (2018). "Targeting Tumor Metabolism With Statins During Treatment for Advanced-stage Pancreatic Cancer." Am J Clin Oncol **41**(11): 1125-1131.

INTRODUCTION: A growing body of preclinical data suggests that statins may exert potent antitumor effects, yet the interactions of these medications with standard therapies and clinical outcomes in this population is less clear. We assessed the impact of statin use on outcomes in patients with advanced-stage pancreatic adenocarcinoma undergoing various treatments. MATERIALS AND METHODS: After institutional review board approval, we conducted a retrospective-cohort study consisting of 303 newly diagnosed advanced-stage pancreatic adenocarcinoma patients to determine the impact of statin use on outcomes. Univariate and multivariable Cox proportional hazard regression models were utilized to estimate hazard ratios (HRs). Time-to-event was estimated using Kaplan-Meier survival analysis for overall survival, distant metastasis, and locoregional failure. Baseline and active statin usage were assessed and to mitigate risk of immortal time bias, subanalysis excluding patients with under 6 months of follow-up was conducted. RESULTS: Both prior (P=0.021) and active (P=0.030) statin usage correlated with improved survival in this cohort. Surgery, chemoradiation, and statin use improved 2-year survival rates (84.1% vs. 55.0%; P<0.001). On multivariable analysis, statin exposure was associated with overall survival (HR, 0.662; P=0.027) and trended to significance for freedom from distant metastasis (HR, 0.577; P=0.060). Comorbid conditions were not significantly associated with outcomes. CONCLUSIONS: Statin use was associated with improved overall survival in advanced-stage pancreatic adenocarcinoma patients. This data supports previous findings in early-stage pancreatic adenocarcinoma and other cancer sites. To our knowledge this is the first report to examine the efficacy of statin use as a supplementary treatment option in advanced-stage pancreatic adenocarcinoma patients.

Ikeda, N., et al. (2003). "Combination treatment with 1alpha,25-dihydroxyvitamin D3 and 9-cis-retinoic acid directly inhibits human telomerase reverse transcriptase transcription in prostate cancer cells." Mol Cancer Ther **2**(8): 739-746.

The vitamin D(3) receptor, which is the nuclear receptor for 1alpha,25-dihydroxyvitamin D(3) (VD(3)), forms a heterodimer with the retinoid X receptor (RXR), which is the nuclear receptor for 9-cis-retinoic acid (9-cis-RA). The heterodimer binds to a specific response element consisting of two directly repeated pairs of motifs, AGGTGA, spaced by three nucleotides [direct repeat (DR) 3] and modulates the expression of VD(3)-responsive genes. Telomerase activity, which is seen in most immortal cells and germ cells, is a complex of enzymes that maintain the length of telomeres. One of the major components of human

telomerase, human telomerase reverse transcriptase (hTERT), is the catalytic subunit, and the expression of hTERT might correlate most strongly with telomerase activity. We found that the sequence of 5'-AGTTCATGGAGTTCA-3' (DR3') is similar to that of DR3 in the promoter region of hTERT. Our results showed that the combination of VD(3) and 9-cis-RA inhibited telomerase activity through direct interaction of the heterodimer of vitamin D(3) receptor and RXR with the DR3' sequence in the hTERT promoter as well as the combination of VD(3) and selective RXR ligand did. Also, in vivo data showed that the growth of xenografts in nude mice was inhibited by VD(3) and 9-cis-RA. The results of the present study provide evidence on the molecular mechanism of the inhibition of cell growth by these agents, and they could be novel therapeutic agents for prostate cancer.

Imam, S. A., et al. (1997). "Systematic determination of telomerase activity and telomerase length during the progression of human breast cancer in cell culture models." Anticancer Res **17**(6D): 4435-4441.

The purpose of the study was to determine systematically the expression of telomerase activity and the length of telomere repeat arrays by utilizing two different cell culture models that derive from normal individual donors, and probably represent various stages of human breast oncogenesis in cell culture. The models consist of mortal, non-tumorigenic immortal and tumorigenic immortal human mammary epithelial cell (MEC) lines. Using a recently developed polymerase chain reaction (PCR)-based telomeric repeat amplification protocol (TRAP) assay, telomerase activity was undetectable in mortal MEC cells. In contrast, the immortal MEC that were nontumorigenic or tumorigenic in immunosuppressed athymic mice, showed telomerase activity. The absence of telomerase activity in mortal and its presence in both non-tumorigenic and tumorigenic immortal cell lines did not reflect their proliferative rate, as demonstrated by the similar pattern and intensity of reactivity of these cell lines with anti-Ki 67 antibody which recognizes a human nuclear cell proliferation--associated antigen. Southern blot analyses of Hinf I-digested genomic DNA hybridized with a (TTAGGG)4 probe revealed arrays of telomeric repeat lengths ranging from 3 to 5, 3.5 to 9, 3.2 to 9 or 3 to 15 kilobase pair (kbp) for mortal, nontumorigenic immortal, and tumorigenic immortal or established MEC lines respectively. These results suggest that telomerase activity and stable telomeric repeat lengths may be a molecular phenotype of the early stages in the progression of breast cancer.

Jackson, J. W. and X. Garcia-Albeniz (2018). "Studying the Effects of Nonindicated Medications on Cancer: Etiologic versus Action-Focused Analysis of Epidemiologic Data." Cancer Epidemiol Biomarkers Prev **27**(5): 520-524.

The study of nonindicated medications on cancer outcomes is challenged by potential time-related biases. The literature has strongly advocated for treating the exposure as time-varying and summarizing the outcomes through a dose-response model (an etiologic-focused analysis). An alternative is to refashion the data to resemble a hypothetical randomized trial of drug use (an action-focused analysis). To our knowledge, their relative treatment of time-related bias and aspects of interpretation have not been compared. In this commentary, using the study of metformin use on colorectal cancer risk by Bradley and colleagues (2018) as motivation, we compare the etiologic versus action-focused analysis of epidemiologic data. We examine their treatment of immortal person-time, time-varying confounding, selection bias, and the biological and clinical relevance of their results. In doing so, we aim to establish areas of common ground and points of departure that can guide future observational studies of medications on cancer risk, recurrence, and survival. Cancer Epidemiol Biomarkers Prev; 27(5); 520-4. (c)2018 AACRSee related article by Bradley et al., p. 525.

Jia, M., et al. (2010). "Proteome profiling of immortalization-to-senescence transition of human breast epithelial cells identified MAP2K3 as a senescence-promoting protein which is downregulated in human breast cancer." Proteomics Clin Appl **4**(10-11): 816-828.

PURPOSE: immortalization is one of the first changes in cells undergoing carcinogenic transformation. Proteome profiling of the immortalization-senescence transition is expected to provide insights into the molecular mechanisms of early tumorigenesis. EXPERIMENTAL DESIGN: 2-DE and MALDI-MS were used to identify proteins in primary human breast epithelial cells, relevant to the immortalization-senescence transition. Cell and molecular biology and immunohistochemistry were used to validate involvement of mitogen-activated protein kinase kinase 3 (MAP2K3) in the immortalization-senescence transition. RESULTS: we identified 71 proteins whose expression changed upon induction of senescence. The identified proteins include regulators of cell growth, death, cell assembly and organization. Analysis of the network formed by the identified proteins suggested that the immortalization-to-senescence transition could affect regulators of the cell cycle, protein synthesis, transport, post-translational modifications, DNA recombination and repair, and lipid and amino acid metabolism. We observed that MAP2K3 was downregulated in

immortal human breast epithelial cells and that upregulation of MAP2K3 expression promoted cell senescence. Decreased expression of MAP2K3 was observed in human breast infiltrating ductal carcinomas, as compared to non-cancerous human breast tissues. CONCLUSION AND CLINICAL RELEVANCE: we described a proteome profile of the immortalization-to-senescence transition for human breast epithelial cells, and identified MAP2K3 as a protein that promotes senescence in these cells.

Jiang, M., et al. (2016). "Elevated O-GlcNAcylation promotes gastric cancer cells proliferation by modulating cell cycle related proteins and ERK 1/2 signaling." Oncotarget **7**(38): 61390-61402.

O-GlcNAc transferase (OGT) is the only enzyme in mammals that catalyzes the attachment of beta-D-N-acetylglucosamine (GlcNAc) to serine or threonine residues of target proteins. Hyper-O-GlcNAcylation is becoming increasingly realized as a general feature of cancer and contributes to rapid proliferation of cancer cells. In this study, we demonstrated that O-GlcNAc and OGT levels were increased in all six gastric cancer (GC) cell lines as compared with immortal gastric epithelial cells. Downregulation of the O-GlcNAcylation level by silencing OGT inhibited cell viability and growth rate via the cdk-2, cyclin D1 and ERK 1/2 pathways. In vivo xenograft assays also demonstrated that the hyper-O-GlcNAc level markedly promoted the proliferation of tumors. Moreover, compared with noncancerous tissues, the O-GlcNAcylation level was increased in cancerous tissues. GC patients with higher levels of O-GlcNAcylation exhibited large tumor sizes (>/=5 cm), deep tumor invasion (T3 and T4), high AJCC stages (stage III and IV), more lymph node metastases and lower overall survival. Notably, the phosphorylation level of ERK 1/2 was increased progressively with the increase of O-GlcNAcylation in both SGC 7901 and AGS cells. Consistently, human GC tissue arrays also revealed that ERK 1/2 signaling was positively correlated to O-GlcNAcylation (r = 0.348; P = 0.015). Taken together, here we reported that hyper-O-GlcNAcylation significantly promotes GC cells proliferation by modulating cell cycle related proteins and ERK 1/2 signaling, suggesting that inhibition of OGT may be a potential novel therapeutic target of GC.

Jin, L. P., et al. (2020). "Design, synthesis, and biological activity of a novel series of benzofuran derivatives against oestrogen receptor-dependent breast cancer cell lines." Bioorg Chem **95**: 103566.

A docking study of a novel series of benzofuran derivatives with ERalpha was conducted. In this study, we report the synthesis of a novel series of benzofuran derivatives and evaluation of their anticancer activity in vitro against MCF-7 human breast cancer cells, as well as their potential toxicity to ER-independent MDA-MB-231 breast cancer cells, human renal epithelial HEK-293 cells, and human immortal keratinocytes (HaCaT cells) by using the MTT colorimetric assay. The screening results indicated that the target compounds exhibited anti-breast cancer activity. The target compound 2-benzoyl-3-methyl-6-[2-(morpholin-4-yl)ethoxy]benzofuran hydrochloride (4e) exhibited excellent activity against anti-oestrogen receptor-dependent breast cancer cells and low toxicity. The preliminary structure-activity relationships of the target benzofuran derivatives have been summarised. In conclusion, the novel benzofuran scaffold may be a promising lead for the development of potential oestrogen receptor inhibitors.

Jones, C. J., et al. (1998). "Dissociation of telomere dynamics from telomerase activity in human thyroid cancer cells." Exp Cell Res **240**(2): 333-339.

Prevention of telomere erosion through acquisition of telomerase activity is thought to be an essential mechanism in most human cancer cells for avoidance of cellular senescence and crisis. It has been generally assumed that once telomerase has been activated, no further telomere shortening should ensue. We show here, however, that a much more complex pattern of telomere dynamics can exist in telomerase-positive immortal cancer cells. Using a panel of subclones derived from a human thyroid cancer cell line, K1E7, we found that some clones show persistent decline in mean telomere restriction fragment (TRF) length by up to 2 kb over 450 population doublings (pd), despite sustained high telomerase activity (as assessed by the in vitro "TRAP" assay). TRF length subsequently stabilized at around 5 kb, but with no corresponding increase in telomerase activity. One clone showed an even more unexpected biphasic time course, with the mean TRF length initially increasing by 1.5 kb over 90 pd, before "plateauing" and then returning over a similar period to its original value, again without any correlation to TRAP activity. Such dissociations between telomere dynamics and telomerase activity support the existence of additional controls on telomere length in the intact cell. Our observations are consistent with current negative-feedback models of telomere length regulation by telomere binding proteins and these cell lines should prove useful experimental tools for their further evaluation.

Jung, S. Y., et al. (2011). "The effect of delays in treatment for breast cancer metastasis on survival." Breast Cancer Res Treat **130**(3): 953-964.

It is generally accepted that delay in receiving

treatment for breast cancer results in adverse outcomes. The purpose of this study was to evaluate the impact of delay in treatment after the diagnosis of metastatic disease on survival measured from metastatic breast cancer diagnosis and from first treatment while controlling for immortal time effect among patients with metastatic breast cancer. A total of 553 patients with breast cancer metastasis diagnosis from one large urban practice have been followed between January 1, 1999 and June 30, 2008. Prognostic factors and outcomes of these patients were analyzed using log-rank test and Cox regression model. Backward stepwise selection of covariates was conducted to assess the association of treatment delay with survival. The median survival was 40 months (range 1-114 months), with 265 (47.9%) women alive and 288 (52.1%) having died at the end of the follow-up period. Treatment delays of more than 12 weeks had impact on poor survival from first treatment than the delays of 4-12 weeks with borderline significance level (HR 1.76, 95% CI 0.99-3.13, P = 0.056) in multivariate analysis, adjusted by BMI, history of hypertension, ER/PR status, HER2 status, number of metastatic sites, and liver metastasis. Moreover, the interval of 12-24 weeks, compared to the interval of 4-12 weeks was associated with greater risk of death from first treatment (HR 2.39, 95% CI 1.19-4.77, P = 0.014). The treatment delay interval of >12 weeks was not related with survival since metastatic breast cancer diagnosis, compared to the 4-12 weeks of treatment delays. This study demonstrated that delays of over 12 weeks in receiving treatment for metastatic breast cancer were related to adverse survival outcomes measured from initiation of first treatment. The findings of this study support targeted efforts to ensure prompt treatment initiation in patients diagnosed with metastatic breast cancer.

Kageyama, Y., et al. (1997). "Telomere length and telomerase activity in bladder and prostate cancer cell lines." Int J Urol **4**(4): 407-410.

BACKGROUND: Specific repeats of oligonucleotides at the ends of chromosomes (telomeres) are shortened by cell division in somatic cells and are thought to be related to aging. Immortal cells such as germ-line cells or cancer cells have demonstrated increased activity of the telomere-elongating enzyme (telomerase). The length of the telomeres of these cells is stable regardless of cell division. We examined the telomere length and telomerase activity in 3 bladder (JTC30, JTC32, and T24) and 2 prostate cancer (LNCaP and DU145) cell lines. METHODS: Telomere lengths were evaluated by Southern blot analysis with a oligonucleotide probe, (TTAGGG)5, and telomerase activities were detected with a polymerase chain reaction-based assay method. RESULTS: Telomerase activity was detected in all of the cell lines. Each cell line had a specific telomere length. In 2 bladder cancer cell lines (JTC30 and JTC32), the telomere length decreased with increased passage of the cells. CONCLUSION: The presence of telomerase may be a biological character of bladder and prostate cancers as well as other malignancies, although it does not always compensate telomere shortening.

Kakeji, Y., et al. (2001). "Gastric cancer with high telomerase activity shows rapid development and invasiveness." Oncol Rep **8**(1): 107-110.

Telomerase activity was reported to be activated in most immortal cells and cancers. As the clinical significance of telomerase activity in human gastric cancer is controversial, we investigated this activity using a telomeric repeat amplification protocol. The telomerase activity was tentatively defined by strength of activity as follows: 3+, observed with 0.06 microg of protein; 2+, observed with 0.6 microg of protein; 1+, observed with 6 microg of protein; 0, not observed under these three conditions. Telomerase activity was detected in 35 of 39 (89.7%) gastric cancer specimens. Tumors with high telomerase activities (2+/3+) tended to have a deeper invasion, lymphatic and vascular invasion, lymph node metastasis, liver metastasis, and peritoneal dissemination, as compared to findings in case of low telomerase activities (-/1+). Thus, telomerase activity of gastric cancer tissue may reflect the malignant potential of the tumor and intensive postoperative care might be required for such patients.

Kang, J., et al. (2021). "The Associations of Aspirin, Statins, and Metformin With Lung Cancer Risk and Related Mortality: A Time-Dependent Analysis of Population-Based Nationally Representative Data." J Thorac Oncol **16**(1): 76-88.

INTRODUCTION: The aim of this study was to investigate the associations of aspirin, metformin, and statins with lung cancer risk and mortality using population-based nationwide cohort data. METHODS: This study included a total of 732,199 participants who underwent a national health check-up from 2002 to 2003. Lung cancer incidence and mortality were identified using a registered lung cancer diagnosis code (International Classification of Diseases, 10th revision, code C34) and the Korean National Death Registry. The study participants were followed up from January 1, 2004 to December 31, 2013. Medication exposure was defined by the cumulative duration of use and cumulative defined daily dose per 2-year interval. To avoid immortal-time bias, drug exposure was inserted as a time-dependent variable in Cox analysis, which evaluated the associations of

these medications with lung cancer. RESULTS: Metformin use had a protective association with lung cancer incidence (p's for trend 0.008) and mortality (p's for trend < 0.001) in a dose-response fashion, and these associations were prominent among participants with a metformin cumulative defined daily dose of 547.5 and above compared with patients without diabetes. Lung cancer mortality was dose-dependently reduced with the use of aspirin (p's for trends 0.046) and statin (p's for trends < 0.001). The combined use of aspirin, statins, and metformin exhibited more prominent protective associations with lung cancer risk and mortality. CONCLUSIONS: The use of aspirin, metformin, and statins had independent protective associations with lung cancer mortality, and metformin had an inverse association with lung cancer risk. Further studies are necessary to develop clinically applicable anticancer strategies using these drugs for the reduction of lung cancer and related mortality.

Kato, H., et al. (2006). "Induction of human endometrial cancer cell senescence through modulation of HIF-1alpha 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.

Kaur, G. P., et al. (2005). "Functional identification of a BAC clone from 16q24 carrying a senescence gene SEN16 for breast cancer cells." Oncogene **24**(1): 47-54.

We have identified an 85 kb BAC clone, 346J21, that carries a cell senescence gene (SEN16), previously mapped to 16q24.3. Transfer and retention of 346J21 in breast cancer cell lines leads to growth arrest after 8-10 cell doublings, accompanied by the appearance of characteristic senescent cell morphology and senescence-associated acid beta-galactosidase activity. Loss of transferred BAC results in reversion to the immortal growth phenotype of the parental cancer cell lines. BAC 346J21 restores senescence in the human breast cancer cell lines, MCF.7 and MDA-MB468, and the rat mammary tumor cell line LA7, but not in the human glioblastoma cell line T98G. We postulate that inactivation of both copies of SEN16 is required for the immortalization of breast epithelial cells at an early stage of tumorigenesis. Positional mapping of 346J21 shows that SEN16 is distinct from other candidate tumor suppressor genes reported at 16q24.

Kellen, J. A. (2002). "Gene therapy in cancer." J Exp Ther Oncol **2**(6): 312-316.

Genetics have and will continue to have a strong and often controversial impact on our lives. Since the first human gene therapy in 1989, data from some 400 officially approved trials have been reported; only a handful appears promising. Of course, one must discern between gene replacements that correct inherited genetic disorders for life and the specific replacement of mutant genes that initiate or maintain the malignant phenotype with a correct gene copy. A truly reliable, safe, and efficient gene delivery system is not yet available, and many techniques have serious limitations or may be outright dangerous. Nevertheless, strong scientific and economic forces keep driving genetic research in cancer, with the promise of immortal fame (and even greater monetary rewards).

Khanuja, P. S., et al. (1993). "Nuclear matrix proteins in normal and breast cancer cells." Cancer Res **53**(14): 3394-3398.

The progression from normal breast epithelium to a malignant phenotype may depend on changes in genetic events as well as failure of host mechanisms. Intermediate biomarkers are needed to more effectively identify malignant progression as well as to develop the potential for more specific treatments and prevention strategies. The nuclear

matrix is the RNA-protein network which forms the skeleton of the nucleus and participates in DNA organization as well as multiple cellular functions. Nuclear matrix proteins have been demonstrated to be tissue and cell type specific as well as to reflect the state of cell differentiation and/or transformation. We prepared nuclear matrices from normal and cancer breast tissue from 10 patients with infiltrating ductal carcinoma of the breast as well as the MCF-10 mortal, immortal, and transfected breast cell lines. Nuclear matrices derived from normal human breast tissue and tumor tissue share common nuclear matrix proteins as well as demonstrate specific changes which appear to occur with the acquisition of the cancer phenotype. The MCF-10 cell lines demonstrate a phenotype that is intermediate between the normal and cancer tissue. These data suggest that the nuclear matrix may be an important biomarker in the pathogenesis of breast cancer.

Kim, B., et al. (2015). "Chemotherapy induces Notch1-dependent MRP1 up-regulation, inhibition of which sensitizes breast cancer cells to chemotherapy." BMC Cancer **15**: 634.

BACKGROUND: Multi-drug Resistance associated Protein-1 (MRP1) can export chemotherapeutics from cancer cells and is implicated in chemoresistance, particularly as is it known to be up-regulated by chemotherapeutics. Our aims in this study were to determine whether activation of Notch signalling is responsible for chemotherapy-induced MRP1 expression Notch in breast cancers, and whether this pathway can be manipulated with an inhibitor of Notch activity. METHODS: MRP1 and Notch1 were investigated in 29 patients treated with neoadjuvant chemotherapy (NAC) for breast cancer, using immunohistochemistry on matched biopsy (pre-NAC) and surgical samples (post-NAC). Breast epithelial cell cultures (T47D, HB2) were treated with doxorubicin in the presence and absence of functional Notch1, and qPCR, siRNA, Western blots, ELISAs and flow-cytometry were used to establish interactions. RESULTS: In clinical samples, Notch1 was activated by neoadjuvant chemotherapy (Wilcoxon signed-rank $p < 0.0001$) and this correlated with induction of MRP1 expression (rho = 0.6 p = 0.0008). In breast cell lines, doxorubicin induced MRP1 expression and function (non-linear regression $p < 0.004$). In the breast cancer line T47D, doxorubicin activated Notch1 and, critically, inhibition of Notch1 activation with the gamma-secretase inhibitor DAPT abolished the doxorubicin-induced increase in MRP1 expression and function (t-test $p < 0.05$), resulting in enhanced cellular retention of doxorubicin and increased doxorubicin-induced apoptosis (t-test p = 0.0002). In HB2 cells, an immortal but non-cancer derived breast cell line, Notch1-independent MRP1 induction was noted and DAPT did not enhance doxorubicin-induced apoptosis. CONCLUSIONS: Notch inhibitors may have potential in sensitizing breast cancer cells to chemotherapeutics and therefore in tackling chemoresistance.

Kim, E., et al. (2003). "Ad-mTERT-delta19, a conditional replication-competent adenovirus driven by the human telomerase promoter, selectively replicates in and elicits cytopathic effect in a cancer cell-specific manner." Hum Gene Ther **14**(15): 1415-1428.

Human telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase, functions to stabilize telomere length during chromosomal replication. Previous studies have shown that hTERT promoter is highly active in most tumor and immortal cell lines but inactive in normal somatic cell types. The use of wild-type hTERT promoter, however, may be limited by its inability to direct high level and cancer cell-specific expression necessary for effective targeted gene therapy. To improve cancer cell specificity and the strength of the hTERT promoter, a modified hTERT, m-hTERT promoter was generated in which additional copies of c-Myc and Sp1 binding sites were incorporated adjacent to the promoter. As assessed using relative lacZ expression, hTERT and m-hTERT promoter activity was significantly upregulated in cancer cells but not in normal cells, and within these upregulated cancer cells, m-hTERT promoter strength was substantially higher than that of the wild-type hTERT. Next, to restrict viral replication to tumor cells, a conditional replication-competent adenoviruses, Ad-TERT-Delta19 and Ad-mTERT-delta19 were generated in which the E1A gene, which is essential for viral replication, was placed under the control of the hTERT and m-hTERT promoter, respectively. While the wild-type Ad-TERT-delta19 replicated in and induced cytopathic effect in cancer and in some normal cell lines, Ad-mTERT-delta19 enhanced viral replication and cytopathic effect only in cancer cells. Furthermore, the growth of established human cervical carcinoma in nude mice was significantly suppressed by intratumoral injection of Ad-mTERT-delta19. Taken together, present results strongly suggest that the use of the m-hTERT promoter is not only useful in the regulation of therapeutic gene expression but also that replication-competent oncolytic adenovirus under the control of the m-hTERT promoter may be a new promising tool for the treatment of human malignancies.

Kim, G. A., et al. (2019). "Effect of Statin Use on Liver Cancer Mortality Considering Hypercholesterolemia and Obesity in Patients with

Non-Cirrhotic Chronic Hepatitis B." Yonsei Med J **60**(12): 1203-1208.

Little is known about the benefits of statin use on liver cancer mortality among patients with chronic hepatitis B (CHB) considering hypercholesterolemia and obesity. A nationwide retrospective cohort study was conducted using data from a Health Examination Cohort of the National Health Insurance Service of Korea. Data on CHB patients with no other concurrent liver disease were acquired, and statin use was defined as a cumulative daily dose >/=28. A 3-year landmark analysis was performed to avoid immortal time bias. Patients who started statin therapy within the landmark date were considered statin users. A Cox regression analysis was applied to assess associations between statin use and liver cancer mortality considering hypercholesterolemia and obesity. Among 13063 patients, 193 (1.5%) died of liver cancer during the mean follow-up period of 10.6 years. After adjusting for demographic and metabolic factors, statin use [hazard ratio (HR), 0.17; 95% confidence interval (CI), 0.04-0.70] and hypercholesterolemia (HR, 0.46; 95% CI, 0.24-0.88 for total cholesterol >/=240 mg/dL) were associated with a decreased risk of liver cancer mortality, whereas body mass index (BMI) >/=30 kg/m(2) was associated with an increased risk of liver cancer mortality (HR, 2.46; 95% CI, 1.20-5.06). This study showed that statin use was associated with decreased liver cancer mortality when adjusting for cholesterol levels and BMI. This study found that hypercholesterolemia was independently associated with decreased liver cancer mortality regardless of statin use.

Kim, J. H., et al. (2002). "A novel telomere elongation in an adriamycin-resistant stomach cancer cell line with decreased telomerase activity." Mol Cells **13**(2): 228-236.

Actively dividing cells show progressive loss of telomeric DNA during successive rounds of replication due to end-replication problem. Telomere shortening has been proposed as a regulatory mechanism that controls the replicative capacity of primary cells before undergoing cellular senescence. In immortal cells including cancer, cellular senescence can be overcome by reactivation of telomerase or by a telomerase-independent mechanism for lengthening telomeres. In this work, we present a novel example of telomere elongation mechanism in a human stomach adenocarcinoma cell line which was selected for resistance to adriamycin. The resistant cell line (MKN/ADR) had long terminal restriction fragments (TRFs) of up to approximately 50 kb, while its parent cell line (MKN-45) had the TRFs, consisting of a smear extending from approximately 4 to approximately 25 kb. The very large TRFs in MKN/ADR cell line were proven to be telomeric by digestion with the exonuclease Bal31. When telomerase activity was examined using the PCR-based telomeric repeat amplification protocol (TRAP) assay, MKN/ADR cell line showed reduced activity to about 10% of that in MKN-45 cell line. The correlation between reduced telomerase activity and mRNA expression of telomerase subunits in MKN/ADR cell line was assessed by the reverse transcriptase-PCR analysis. The level of human telomerase reverse transcriptase (hTERT) mRNA was lower in MKN/ADR cell line than in MKN-45 cell line. This observation correlates with the finding that telomerase activity is reduced about 10-fold in MKN/ADR cell line. Reverse transcriptase-PCR analysis also revealed a close correlation between telomerase-associated protein (TP1) mRNA expression and telomerase activity in MKN/ADR cell line. In contrast, expression levels of human telomerase RNA (hTR) were identical in both MKN/ADR and MKN-45 cell lines. Taken together, these data suggest that telomeres in MKN/ADR cell line may be regulated through a novel mechanism other than telomerase. Although the basis for telomere elongation mechanism in MKN/ADR cell line is not yet understood, the occurrence of alternative mechanism for telomere elongation in drug-resistant cancer cells may have an important implication for use of telomerase inhibitors in human cancer treatment.

Kim, M. H., et al. (2019). "Aspirin Does Not Prevent Pancreatic Cancer in a Large Asian Cohort." Cancer Epidemiol Biomarkers Prev **28**(4): 826-828.

BACKGROUND: Evidence has suggested that aspirin reduces the incidence of several cancers, but these benefits may not occur with pancreatic cancer. METHODS: A 12-year nationwide longitudinal cohort merged with the health checkup data was divided into "exposure ascertainment period" and "outcome ascertainment period" to avoid immortal time bias. The daily defined dose system was used to indicate the drug exposure. RESULTS: We found no significant association between aspirin use and incident pancreatic cancer based on HR. CONCLUSIONS: Aspirin does not prevent pancreatic cancer. IMPACT: A large Asian cohort study with reliable medication information affirms no impact of aspirin on pancreatic cancer development.

Kim, N. W., et al. (1994). "Specific association of human telomerase activity with immortal cells and cancer." Science **266**(5193): 2011-2015.

Synthesis of DNA at chromosome ends by telomerase may be necessary for indefinite proliferation of human cells. A highly sensitive assay

for measuring telomerase activity was developed. In cultured cells representing 18 different human tissues, 98 of 100 immortal and none of 22 mortal populations were positive for telomerase. Similarly, 90 of 101 biopsies representing 12 human tumor types and none of 50 normal somatic tissues were positive. Normal ovaries and testes were positive, but benign tumors such as fibroids were negative. Thus, telomerase appears to be stringently repressed in normal human somatic tissues but reactivated in cancer, where immortal cells are likely required to maintain tumor growth.

Kinnunen, P. T., et al. (2020). "Anticoagulants and Breast Cancer Survival: A Nationwide Cohort Study." Cancer Epidemiol Biomarkers Prev **29**(1): 208-215.

BACKGROUND: Various components of the coagulation cascade have been linked to breast cancer progression. In vivo results suggest that anticoagulants possess anticancer properties, but there are virtually no studies in human populations. Our nationwide study explored the association between anticoagulant use and breast cancer survival. METHODS: All anticoagulants used from 1995 to 2015 in women (n = 73,170) diagnosed with invasive breast cancer in Finland between 1995 and 2013 were identified from the national prescription database; women were identified from the Finnish Cancer Registry. Cox regressions were performed to analyze breast cancer survival as a function of pre- and postdiagnostic anticoagulant use; analyses were conducted for different anticoagulant subtypes and overall. Models were adjusted for age, mammography screening, tumor clinical characteristics, comorbidities, statin use, antidiabetic use, and antihypertensive use. To control for immortal time bias, postdiagnostic anticoagulant use was analyzed as a time-dependent variable. RESULTS: At a median of 5.8 years after breast cancer diagnosis, 10,900 (15%) women had died from breast cancer. In total, 25,622 (35%) women had used anticoagulants during the study period. Postdiagnostic anticoagulant use increased the risk of breast cancer death (HR = 1.41; 95% confidence interval, 1.33-1.49). The risk was especially high for low-molecular weight heparin, although the effect disappeared in long-term users. CONCLUSIONS: Anticoagulant use provides no clinical benefit for breast cancer survival; however, the association between thrombosis and cancer might mask potential survival benefits. IMPACT: Future pharmacoepidemiologic studies should adjust for anticoagulant use. Research should focus on the use of new oral anticoagulants because these are rarely studied and might be associated with improved breast cancer survival.

Kinnunen, P. T. T., et al. (2017). "Prostate cancer-specific survival among warfarin users in the Finnish Randomized Study of Screening for Prostate Cancer." BMC Cancer **17**(1): 585.

BACKGROUND: Venous thromboembolic events (VTE) are common in cancer patients and associated with higher mortality. In vivo thrombosis and anticoagulation might be involved in tumor growth and progression. We studied the association of warfarin and other anticoagulant use as antithrombotic medication and prostate cancer (PCa) death in men with the disease. METHODS: The study included 6,537 men diagnosed with PCa during 1995-2009. Information on anticoagulant use was obtained from a national reimbursement registry. Cox regression with adjustment for age, PCa risk group, primary therapy and use of other medication was performed to compare risk of PCa death between warfarin users with 1) men using other types of anticoagulants and 2) non-users of anticoagulants. Medication use was analyzed as a time-dependent variable to minimize immortal time bias. RESULTS: In total, 728 men died from PCa during a median follow-up of 9 years. Compared to anticoagulant non-users, post-diagnostic use of warfarin was associated with an increased risk of PCa death (overall HR 1.47, 95% CI 1.13-1.93). However, this was limited to low-dose, low-intensity use. Otherwise, the risk was similar to anticoagulant non-users. Additionally, we found no risk difference between warfarin and other types of anticoagulants. Pre-diagnostic use of warfarin was not associated with the risk of PCa death. CONCLUSIONS: We found no reduction in risk of PCa death associated with warfarin use. Conversely, the risk was increased in short-term use, which is probably explained by a higher risk of thrombotic events prompting warfarin use in patients with terminal PCa.

Kinnunen, P. T. T., et al. (2019). "Anticoagulants and cancer mortality in the Finnish randomized study of screening for prostate cancer." Cancer Causes Control **30**(8): 877-888.

PURPOSE: Anticoagulants may reduce mortality of cancer patients, though the evidence remains controversial. We studied the association between different anticoagulants and cancer death. METHODS: All anticoagulant use during 1995-2015 was analyzed among 75,336 men in the Finnish Randomized Study of Screening for Prostate Cancer. Men with prevalent cancer were excluded. Multivariable Cox regression was performed to compare risk of death from any cancer and disease-specific death from 9 specific cancer types between (1) anticoagulant users overall and (2) warfarin users compared to anticoagulant non-users and (3) warfarin or (4) low-molecular-weight heparins (LMWH) compared to users of other anticoagulants. Medication

use was analyzed as time-dependent variable to minimize immortal time bias. 1-, 2- and 3-year lag-time analyses were performed. RESULTS: During a median follow-up of 17.2 years, a total of 27,233 men died of whom 8033 with cancer as the primary cause of death. In total, 32,628 men (43%) used anticoagulants. Any anticoagulant use was associated with an increased risk of cancer death (HR = 2.50, 95% CI 2.37-2.64) compared to non-users. Risk was similar independent of the amount, duration, or intensity of use. The risk increase was observed both among warfarin and LMWH users, although not as strong in warfarin users. Additionally, cancer-specific risks of death were similar to overall cancer mortality in all anticoagulant categories. CONCLUSION: Our study does not support reduced cancer mortality among anticoagulant users. Future studies on drug use and cancer mortality should be adjusted for anticoagulants as they are associated with significantly higher risk of cancer death.

Klingelhutz, A. J. (1999). "The roles of telomeres and telomerase in cellular immortalization and the development of cancer." Anticancer Res **19**(6A): 4823-4830.

Normal human cells have a limited lifespan in culture called the Hayflick limit. Recent studies have indicated that telomere shortening is one of the important meters utilized by cells to determine the Hayflick limit, and that activation of a mechanism to maintain telomere length is essential for cells to become immortal. It is generally believed that cells must have a means to maintain telomeres in order to progress to malignancy. Most cancers do this by activating an enzyme called telomerase which adds telomeric repeats to the telomere ends. Recently, expression of this enzyme has been shown to extend the lifespan of cells. This review discusses the research that led to the discovery of telomerase, the characteristics of telomerase complex, and how recent and future advances in the telomerase field may lead to better diagnostic and treatment protocols for many different cancer types.

Knowles, M. A. and M. Williamson (1993). "Mutation of H-ras is infrequent in bladder cancer: confirmation by single-strand conformation polymorphism analysis, designed restriction fragment length polymorphisms, and direct sequencing." Cancer Res **53**(1): 133-139.

A series of 152 human bladder tumors, 14 bladder tumor cell lines, and 1 immortal urothelial cell line were examined by single-strand conformation polymorphism (SSCP) and designed restriction fragment.length polymorphism analyses for mutations in exons 1 and 2 of the H-ras gene. Nine tumors (6%) contained mutations. There was complete concordance between SSCP and restriction fragment length polymorphism analyses. Six mutations in exon 1 and three in exon 2 were identified by SSCP analysis. Subsequent restriction fragment length polymorphism analysis showed that of the exon 1 mutations, four were in codon 12 and two in codon 13, and all three exon 2 mutations were in codon 61. Eight mutations were confirmed by direct sequencing. One codon 13 mutation could not be identified by direct sequencing. Distinct strand mobility shifts detected by SSCP analysis identified specific point mutations, and in all cases, strands containing different mutations migrated differently. The base substitutions identified in these bladder tumors were diverse and included four transversions (three G-->T and one A-->T) and four transitions (two G-->A and two A-->G). This pattern of base substitutions is compatible with interactions of the urothelium with more than one class of environmental agent during bladder tumor development. No correlation was found between tumor grade and/or stage and the presence of H-ras mutation. We conclude that H-ras mutation does not play a role in the development of the majority of transitional cell tumors of the bladder.

Kok, V. C., et al. (2015). "Regular inhaled corticosteroids in adult-onset asthma and the risk for future cancer: a population-based cohort study with proper person-time analysis." Ther Clin Risk Manag **11**: 489-499.

BACKGROUND: Recent studies have shown that inhaled corticosteroids (ICS) can exert anti-inflammatory effects for chronic airway diseases, and several observational studies suggest that they play a role as cancer chemopreventive agents, particularly against lung cancer. We aimed to examine whether regular ICS use was associated with a reduced risk for future malignancy in patients with newly diagnosed adult-onset asthma. METHODS: We used a population-based cohort study between 2001 and 2008 with appropriate person-time analysis. Participants were followed up until the first incident of cancer, death, or to the end of 2008. The Cox model was used to derive an adjusted hazard ratio (aHR) for cancer development. Kaplan-Meier cancer-free survival curves of two groups were compared. RESULTS: The exposed group of 2,117 regular ICS users and the nonexposed group of 17,732 non-ICS users were assembled. After 7,365 (mean, 3.5 years; standard deviation 2.1) and 73,789 (mean, 4.1 years; standard deviation 2.4) person-years of follow-up for the ICS users and the comparator group of non-ICS users, respectively, the aHR for overall cancer was nonsignificantly elevated at 1.33 with 95% confidence interval (CI), 1.00-1.76, P=0.0501. The Kaplan-Meier curves for overall cancer-free proportions of both

groups were not significant (log-rank, P=0.065). Synergistic interaction of concurrent presence of regular ICS use was conducted using "ICS-negative and chronic obstructive pulmonary disease (COPD)-negative" as the reference. The aHR for the group of "ICS-positive, COPD-negative" did not reach statistically significant levels with aHR at 1.38 (95% CI, 0.53-3.56). There was a statistically significant synergistic interaction of concurrent presence of regular ICS use and COPD with aHR at 3.78 (95% CI, 2.10-6.81). CONCLUSION: The protective effect of regular ICS use in the studied East Asian patients with adult-onset asthma was not detectable, contrary to reports of previous studies that ICS might prevent the occurrence of future cancer.

Korenchuk, S., et al. (2001). "VCaP, a cell-based model system of human prostate cancer." In Vivo **15**(2): 163-168.

OBJECTIVES: We report the isolation and characterization of a novel prostate cancer cell line derived from a vertebral metastatic lesion, Vertebral-Cancer of the Prostate (VCaP). METHODS: Prostate cancer tissue was harvested at autopsy from a metastatic lesion to a lumbar vertebral body of a patient with hormone refractory prostate cancer. This tissue was aseptically xenografted into SCID mice and later harvested and plated on tissue culture dishes. For characterization, soft agar clonegenic assay, in vivo xenograft growth, in vitro doubling time, karyotype analysis, immunocytochemistry for cytokeratin-18 expression immunochemistry for PSA (prostate specific antigen), RT PCR for PAP (prostatic acid phosphatase) and northern blot and western blot analysis to determine expression of Rb and p53, were performed. Androgen receptor expression was measured by transient transfection with a luciferase reporter construct. RESULTS: VCaP cells are immortal in vitro and can be passaged serially in vivo. They express large quantities of prostate specific antigen (PSA). This cell line also expresses prostatic acid phosphatase (PAP), cytokeratin-18 and the androgen receptor, and is androgen sensitive in vitro and in vivo. CONCLUSIONS: This cell line was derived from a metastatic tumor to the vertebrae of a prostate cancer patient. It exhibits many of the characteristics of clinical prostate carcinoma, including expression of PSA, PAP, and AR. We believe that VCaP will be a useful addition to the existing models of prostate cancer, and enable more advanced study of the mechanisms of prostate cancer progression and metastasis.

Koyanagi, K., et al. (1998). "[Telomerase assay for diagnosis of esophageal cancer]." Nihon Rinsho **56**(5): 1171-1175.

Esophageal squamous cell carcinoma is one of the aggressive diseases that has poor outcome. Therefore it is appeared that early diagnosis is very important for improving its outcome. Iodine staining method is useful for detecting the abnormal squamous epithelium and unstaining lesions by iodine contain the early esophageal cancers. Recently, telomerase activity that provides an immortal capacity for the cells has been measured in many tissues. We measured the telomerase activity in the samples of unstaining lesion by iodine using a polymerase chain reaction-based assay and described the relation between telomerase activity and histopathological findings.

Kraemer, K., et al. (2006). "Microarray analyses in bladder cancer cells: inhibition of hTERT expression down-regulates EGFR." Int J Cancer **119**(6): 1276-1284.

The human telomerase reverse transcriptase (hTERT) contributes to the immortal phenotype of the majority of cancers. Targeting hTERT by transfection with antisense oligonucleotides (AS-ODNs) induced immediate growth inhibition in human bladder cancer (BCa) cells. The molecular basis of the antiproliferative capacity of hTERT AS-ODNs was investigated by oligonucleotide microarray analyses and was compared to effects caused by siRNA-mediated knock-down of hTERT in EJ28 BCa cells. Two different AS-ODNs -- both down-regulated the expression of hTERT -- changed the expression of different genes mainly involved in stress response (including EGR1, ATF3 and GDF15), but without an association to telomerase function. This indicates that the immediate growth inhibition was caused, at least in part, by off-target effects. In comparison to that the blockade of the expression of hTERT using 2 different siRNAs was accompanied by the down-regulation of the oncogenes FOS-like antigen 1 (FOSL1) and epidermal growth factor receptor (EGFR), known to be overexpressed in BCa. We show here for the first time that repression of the hTERT transcript number decreased the expression of EGFR both at the mRNA and protein levels, suggesting a potential new function of hTERT in the regulation of EGFR-stimulated proliferation. Furthermore, the suppression of hTERT by siRNAs caused an enhancement of the antiproliferative capacity of the chemotherapeutics mitomycin C and cisplatin. The results presented herein may support the hypothesis that hTERT promotes the growth of tumor cells by mechanisms independent from telomere lengthening. The detailed clarification of these processes will shed light on the question, whether telomerase inhibitors might constitute suitable anticancer tools.

Kroemer, G. (2007). "Cancer: defeating the immortal."

Cancer Biol Ther **6**(8): 1324-1328.

My first reaction when I was invited to contribute an article for the section "Profiles and Legacies" of Cancer Biology & Therapy was a shock. Am I that old, at the age of 46 years, that I should write my own obituary? But then I realized that I would have the opportunity to share some of my intimate convictions, dilemmas and doubts. So instead of an auto-apotheosis, this piece will simply translate what I could tell a friend during an after-dinner conversation, knowing that the plans for the future (optimistically) outnumber the experiences in the past.

Kuczkowski, J., et al. (2006). "[Prognostic and diagnostic value of telomerase in the head and neck cancer]." Otolaryngol Pol **60**(5): 723-728.

UNLABELLED: Telomerase is an enzyme responsible for maintaining the constant length of chromosomal telomers, which are necessary for normal function of eucaryotic cells. Presence of active telomerase in neoplastic cells prevents shortening of telomers what makes this cells immortal. Telomerase plays an important role in carcinogenesis. Aim of this study was to investigate the activity of telomerase in head and neck tumors and assess its diagnostic and prognostic value. MATERIAL AND METHODS: Material consisted of 30 head and neck tumors treated surgically and 9 samples from healthy skin and mucous membranes. Telomerase activity was investigated using TeloTAGGG Telomerase PCR ELISA method. RESULTS: Telomerase activity was found in 24 (80%) malignant tumors. Relative activity of telomerase in neoplasms was from 16.9 to 766,2 RTA. Telomerase expression was much lower in samples of healthy skin and mucous membrane (RTA <5). Statistically significant difference was proven for T1 and T2 tumors, comparing to T4 tumors ($p < 0.05$). No statistically significant difference in telomerase activity for G1 and G2 differentiation tumors comparing to G3 and for tumors without lymph nodes metastases comparing to tumors with metastases. CONCLUSION: Malignant head and neck tumors show high activity of telomerase comparing to healthy tissue. Detection of telomerase activity in head and neck malignant neoplasms can be a useful marker for cancer assessment. The quantification of telomerase activity has clinical diagnostic value for head and neck malignant neoplasms. To make a convenience of telomerase as a marker for diagnostic and prognosis in patients with malignant head and neck tumor needs further investigations.

Kukko, V., et al. (2019). "Allopurinol and the risk of prostate cancer in a Finnish population-based cohort." Prostate Cancer Prostatic Dis **22**(3): 483-490.

BACKGROUND: Allopurinol reduces oxidative stress and may thus have an anti-inflammatory effect. Previous studies suggest that allopurinol use might decrease the risk of prostate cancer (PCa) among gout patients. We studied the association between allopurinol use and PCa incidence. METHODS: The cohort consists of 76,874 men without prevalent PCa, originally identified for the Finnish Randomized Study of Screening for Prostate Cancer (FinRSPC). The follow-up started at entry to the trial. We excluded men using allopurinol in the year before entry (wash-out). PCa cases detected during 1996-2015 were identified from the Finnish Cancer Registry. Information on tumor Gleason score and TNM stage were obtained from medical files. Information on PSA level was obtained from screening samples for men in the FinRSPC screening arm and from laboratory databases for men in the control arm. Information on BMI was based on a questionnaire sent to men in the FinRSPC screening arm in 2004-2008. Drug purchase information were obtained from the national prescription database. We used Cox regression (adjusted for age, FinRSPC trial arm, PCa family history and use of other medication) to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) of PCa risk by allopurinol use. We analyzed medication as a time-dependent variable to minimize immortal time bias. RESULTS: There were 9062 new PCa diagnoses in the cohort. Follow-up time did not differ by allopurinol use (median 17 yr; IQR 11-19 vs median 17 yr; IQR 12.33-19). The risk of PCa did not differ by allopurinol use (multivariable adjusted HR 1.03; 95% CI 0.92-1.16). Allopurinol use did not associate with the risk of high-grade or metastatic cancer. Cumulative duration or average yearly dose of allopurinol use showed no association with PCa risk. No delayed risk associations were observed in the lag-time analyses. CONCLUSIONS: We observed no difference in the PCa risk by allopurinol use.

Lamy, E., et al. (2013). "hTERT: another brick in the wall of cancer cells." Mutat Res **752**(2): 119-128.

In human cancer, expression of telomerase is positively correlated with tumour aggressiveness and metastatic potential. There is accumulating evidence that hTERT (the catalytic subunit of telomerase) favours an immortal phenotype by blocking programmed cell death (apoptosis) independently of its protective function at the telomere ends. This review summarized existing evidence for the anti-apoptotic role of hTERT in the context of tumour-cell resistance against DNA damage and aims to put hTERT in the context of cell-signal-transduction pathways leading either to survival or cell death. We found evidence that telomerase is cross-linked with many different signalling pathways that regulate cell

proliferation, DNA damage repair, and also cell death. Thereby, hTERT survival function seems to occur at early stages of DNA damage recognition. We found some discrepancies in the published data though. Based on our findings, we suggest further exploration is needed of the interplay of the DNA damage response signalling network, including MAPK and p53 family activation, on telomerase regulation. This interaction is probably an important factor for fine tuning of the sensitivity of the cell to genotoxic stress. Using anti-neoplastic agents, further dose relationships on timing and extent of DNA damage, cellular repair and death should be established and correlated with hTERT expression/telomerase activation. Closing the data gaps identified here could profoundly improve our understanding of the relevance of telomerase for protecting the cell against anti-cancer agents and would contribute to developing new strategies for cancer therapy.

Lane, G., et al. (2019). "Persistent muscle-invasive bladder cancer after neoadjuvant chemotherapy: an analysis of Surveillance, Epidemiology and End Results-Medicare data." BJU Int **123**(5): 818-825.

OBJECTIVES: To evaluate whether patients with persistent muscle-invasive bladder cancer (MIBC) after undergoing neoadjuvant chemotherapy (NAC) and radical cystectomy (RC) have worse overall survival (OS) and cancer-specific survival (CSS) than patients with similar pathology who undergo RC alone. MATERIALS AND METHODS: Using the Surveillance, Epidemiology and End Results (SEER)-Medicare database, we identified the records of patients with pT2-4N0M0 disease who underwent RC, with and without NAC, for MIBC between 2004 and 2011. To evaluate survival outcomes in those with MIBC after NAC vs patients with MIBC who underwent RC alone, we used Kaplan-Meier time-to-event analysis and Cox proportional hazard regression modelling. Landmark analysis was conducted to mitigate immortal time bias. Propensity scoring was used to decrease the risk of selection bias. RESULTS: Of the 1 886 patients with persistent pT2-4 disease at the time of RC, 1505 underwent RC alone and 381 received NAC + RC. After adjusting for confounders, the propensity-weighted risk of death from bladder cancer after diagnosis did not differ between the groups (hazard ratio [HR] 0.72, 95% confidence interval [CI] 0.72-1.08; P = 0.23); however, the risk of death from all causes was worse in the RC-alone group (HR 0.79, 95% CI0.67-0.94; P = 0.006). CONCLUSIONS: Patients who had persistent MIBC after platinum-based NAC + RC vs RC alone derived an OS benefit but not a CSS benefit from NAC. This may represent a selection bias favouring patients who were selected for NAC; however, the OS benefit was not evident in patients with persistent pT3-T4N0M0 disease. This study underscores the importance of future research investigating methods to identify patients who will respond to NAC for bladder cancer. It also highlights the need to consider adjuvant therapy in patients who have persistent MIBC after NAC.

Lashinger, L. M., et al. (2016). "Starving cancer from the outside and inside: separate and combined effects of calorie restriction and autophagy inhibition on Ras-driven tumors." Cancer Metab **4**: 18.

BACKGROUND: Calorie restriction (CR) prevents obesity and exerts anticancer effects in many preclinical models. CR is also increasingly being used in cancer patients as a sensitizing strategy prior to chemotherapy regimens. While the beneficial effects of CR are widely accepted, the mechanisms through which CR affects tumor growth are incompletely understood. In many cell types, CR and other nutrient stressors can induce autophagy, which provides energy and metabolic substrates critical for cancer cell survival. We hypothesized that limiting extracellular and intracellular substrate availability by combining CR with autophagy inhibition would reduce tumor growth more effectively than either treatment alone. RESULTS: A 30 % CR diet, relative to control diet, in nude mice resulted in significant decreases in body fat, blood glucose, and serum insulin, insulin-like growth factor-1, and leptin levels concurrent with increased adiponectin levels. In a xenograft model in nude mice involving H-Ras(G12V)-transformed immortal baby mouse kidney epithelial cells with (Atg5 (+/+)) and without (Atg5 (-/-)) autophagic capacity, the CR diet (relative to control diet) genetically induced autophagy inhibition and their combination, each reduced tumor development and growth. Final tumor volume was greatest for Atg5 (+/+) tumors in control-fed mice, intermediate for Atg5 (+/+) tumors in CR-fed mice and Atg5 (-/-) tumors in control-fed mice, and lowest for Atg5 (-/-) tumors in CR mice. In Atg5 (+/+) tumors, autophagic flux was increased in CR-fed relative to control-fed mice, suggesting that the prosurvival effects of autophagy induction may mitigate the tumor suppressive effects of CR. Metabolomic analyses of CR-fed, relative to control-fed, nude mice showed significant decreases in circulating glucose and amino acids and significant increases in ketones, indicating CR induced negative energy balance. Combining glucose deprivation with autophagy deficiency in Atg5 (-/-) cells resulted in significantly reduced in vitro colony formation relative to glucose deprivation or autophagy deficiency alone. CONCLUSIONS: Combined restriction of extracellular (via CR in vivo or glucose deprivation in vitro) and intracellular (via autophagy inhibition) sources of energy and nutrients suppresses Ras-driven tumor growth more effectively

than either CR or autophagy deficiency alone. Interventions targeting both systemic energy balance and tumor-cell intrinsic autophagy may represent a novel and effective anticancer strategy.

Lasorella, A., et al. (2001). "Id proteins at the cross-road of development and cancer." Oncogene **20**(58): 8326-8333.

A large body of evidence has been accumulated that demonstrates dominant effects of Id proteins on different aspects of cellular growth. Generally, constitutive expression of Id not only blocks cell differentiation but also drives proliferation. In some settings, it is sufficient to render cells immortal or induce oncogenic transformation. The participation of Id proteins in advanced human malignancy, where they are frequently deregulated, has been dramatically bolstered by the recent discovery that Id exert pivotal contributions to many of the essential alterations that collectively dictate malignant growth. Relentless proliferation associated with self-sufficiency in growth signals and insensitivity to growth inhibitory signals, sustained neoangiogenesis, tissue invasiveness and migration capabilities of tumor cells all share dependency on the unlimited availability of Id proteins. It is remarkable that many of these features recapitulate those physiologically propelled by Id proteins to support normal development. We propose that the participation of Id in multiple fundamental traits of cancer may be the basis for unprecedented therapeutic opportunities.

Lebedeva, I. V., et al. (2002). "The cancer growth suppressing gene mda-7 induces apoptosis selectively in human melanoma cells." Oncogene **21**(5): 708-718.

Human melanoma cells growth arrest irreversibly, lose tumorigenic potential and terminally differentiate after treatment with a combination of fibroblast interferon (IFN-beta) and the protein kinase C activator mezerein (MEZ). Applying subtraction hybridization to this model differentiation system permitted cloning of melanoma differentiation associated gene-7, mda-7. Expression of mda-7 inversely correlates with melanoma development and progression, with elevated expression in normal melanocytes and nevi and increasingly reduced expression in radial growth phase, vertical growth phase and metastatic melanoma. When expressed by means of a replication incompetent adenovirus (Ad.mda-7) growth of melanoma, but not normal early passage or immortal human melanocytes, is dramatically suppressed and cells undergo programmed cell death (apoptosis). Infection of metastatic melanoma cells with Ad.mda-7 results in an increase in cells in the G(2)/M phase of the cell cycle and changes in the ratio of pro-apoptotic (BAX, BAK) to anti-apoptotic (BCL-2, BCL-XL) proteins. Ad.mda-7 infection results in a temporal increase in mda-7 mRNA and intracellular MDA-7 protein in most of the melanocyte/melanoma cell lines and secretion of MDA-7 protein is readily detected following Ad.mda-7 infection of both melanocytes and melanoma cells. The present studies document a differential response of melanocytes versus melanoma cells to ectopic expression of mda-7 and support future applications of mda-7 for the gene-based therapy of metastatic melanoma.

Lee, M. B., et al. (2019). "Defining the impact of mutation accumulation on replicative lifespan in yeast using cancer-associated mutator phenotypes." Proc Natl Acad Sci U S A **116**(8): 3062-3071.

Mutations accumulate within somatic cells and have been proposed to contribute to aging. It is unclear what level of mutation burden may be required to consistently reduce cellular lifespan. Human cancers driven by a mutator phenotype represent an intriguing model to test this hypothesis, since they carry the highest mutation burdens of any human cell. However, it remains technically challenging to measure the replicative lifespan of individual mammalian cells. Here, we modeled the consequences of cancer-related mutator phenotypes on lifespan using yeast defective for mismatch repair (MMR) and/or leading strand (Polepsilon) or lagging strand (Poldelta) DNA polymerase proofreading. Only haploid mutator cells with significant lifetime mutation accumulation (MA) exhibited shorter lifespans. Diploid strains, derived by mating haploids of various genotypes, carried variable numbers of fixed mutations and a range of mutator phenotypes. Some diploid strains with fewer than two mutations per megabase displayed a 25% decrease in lifespan, suggesting that moderate numbers of random heterozygous mutations can increase mortality rate. As mutation rates and burdens climbed, lifespan steadily eroded. Strong diploid mutator phenotypes produced a form of genetic anticipation with regard to aging, where the longer a lineage persisted, the shorter lived cells became. Using MA lines, we established a relationship between mutation burden and lifespan, as well as population doubling time. Our observations define a threshold of random mutation burden that consistently decreases cellular longevity in diploid yeast cells. Many human cancers carry comparable mutation burdens, suggesting that while cancers appear immortal, individual cancer cells may suffer diminished lifespan due to accrued mutation burden.

Lehr, J. E., et al. (1998). "A model to study c-myc and v-H-ras induced prostate cancer progression in the Copenhagen rat." Cell Mol Biol (Noisy-le-grand) **44**(6): 949-959.

Normal rat prostate epithelial cells (EPYP-1) were isolated and immortalized with the Simian Virus-40 (SV40) large T-antigen, and transfected with the v-H-ras (EPYP-1-ras) and the c-myc oncogenes (EPYP-1-myc; EPYP-1-ras-myc) to serially create a step-wise model of tumor development in the rat prostate. Pronounced morphological differences were observed between EPYP-1 and the transfected sublines. The immortal epithelial cells (EPYP-1) maintained a cuboidal shape with orderly, contact mediated inhibition of growth. Oncogene transfected clones displayed a spindle shaped structure with multiple overlapping pseudopodia. Transfected cells also exhibited a greater degree of dysplasia, loss of contact inhibition growth and the upregulation of an epithelial tumor marker, cytokeratin-18. All cells exhibited anchorage independent and androgen independent growth. In vivo, EPYP-1 cells and EPYP-1-myc and formed slowly growing non-metastatic, benign tumors in immune compromised mice, while EPYP-1-ras and EPYP-1-ras-myc transfected cells produced rapidly growing, malignant tumors in similar animals. This model augments the hypothesis that tumor initiation and progression can be caused by as few as two discrete genetic events. In addition, the "normal" rat prostate epithelium and transfected daughter cell lines represent a tumor model system with distinct, well understood genetic alterations: activation of ras and myc. This model will be a valuable addition to the current cell lines used in the investigation of prostate cancer carcinogenesis.

Li, B., et al. (2021). "Calcium channel blockers are associated with lower gastric cancer risk: A territory-wide study with propensity score analysis." Int J Cancer **148**(9): 2148-2157.

Prior studies showed that calcium channel blockers (CCBs) could modify cancer risk, but data on gastric cancer (GC) are limited. We aimed to investigate whether CCBs could modify GC risk in Helicobacter pylori-eradicated patients. H pylori-infected patients with hypertension who are aged >/=50 and had received clarithromycin-based triple therapy between 2003 and 2016 were identified from a territory-wide healthcare database. Patients with eradication failure, GC diagnosed within 6 months after HP eradication, and gastric ulcer were excluded. Time-fixed Cox model with one-to-one propensity score matching was used to calculate hazard ratio (HR) of GC with CCBs. Sensitivity analysis using time-dependent multivariable Cox model in which CCB use was treated as time-varying covariate was also performed to address immortal time bias. 17 622 (29.6%) H pylori-eradicated patients with hypertension were included. During a median follow-up of 8.6 years, 105 (0.6%) developed GC. After PS matching, CCBs were associated with a lower GC risk (HR: 0.56; 95% CI: 0.32-0.97). Time-dependent analysis showed consistent result (aHR: 0.50; 95% CI: 0.33-0.75). A longer duration of CCB use was associated with even lower GC risk (adjusted HR [aHR]: 0.69; 95% CI: 0.61-0.79 for every 1-year increase in use). Long-acting CCBs (aHR: 0.47; 95% CI: 0.29-0.76) and dihydropyridines (aHR: 0.49; 95% CI: 0.32-0.73) conferred greater benefit than short-acting ones (aHR: 0.60; 95% CI: 0.36-1.03) and nondihydropyridines (aHR: 0.76; 95% CI: 0.24-2.48). The aHR was 0.57 (95% CI: 0.34-0.97) for noncardia and 0.59 (95% CI: 0.27-1.31) for cardia cancer. Use of CCBs was associated with lower risk of GC development in H pylori-eradicated patients, in a duration- and dose-response manner.

Li, B., et al. (2021). "Nonaspirin nonsteroidal anti-inflammatory drugs and gastric cancer risk after Helicobacter pylori eradication: A territory-wide study." Cancer **127**(11): 1805-1815.

BACKGROUND: Despite Helicobacter pylori (HP) eradication, individuals can still develop gastric cancer (GC). Prior studies have demonstrated that nonaspirin nonsteroidal anti-inflammatory drugs (NA-NSAIDs) reduce the risk of GC, but this may be caused by immortal time bias and failure to adjust for HP status. The objective of this study was to investigate whether NA-NSAIDs reduced the risk of GC in patients who undergo H. pylori eradication. METHODS: Adult patients who had received clarithromycin-based triple therapy between 2003 and 2016 were identified from a territory-wide health care database. Exclusion criteria included prior GC or GC diagnosed <6 months after HP eradication, prior gastrectomy, gastric ulcer after HP eradication, and failure of triple therapy. Covariates included age, sex, prior peptic ulcer disease, other comorbidities, and concurrent medications (aspirin, proton pump inhibitors, statins, and metformin). To avoid immortal time bias, NA-NSAID use (>/=90 days) was treated as a time-dependent variable in a multivariable Cox model (time-dependent analysis). Time-independent analysis was also performed. RESULTS: During a median follow-up of 8.9 years (interquartile range, 5.4-12.6 years), 364 of 92,017 patients (0.4%) who underwent HP eradication developed GC. NA-NSAID use was associated with a significant reduction in the risk of GC in time-fixed analysis (adjusted hazard ratio [aHR], 0.65; 95% CI, 0.47-0.90), but not in time-dependent multivariable analysis (aHR, 1.35; 95% CI, 0.97-1.87). Time-dependent subgroup analyses also did not indicate any significant association between NA-NSAID use and either cardia GC (aHR, 0.75; 95% CI, 0.27-2.06) or noncardia GC (aHR, 1.28; 95% CI, 0.83-1.98). CONCLUSIONS: NA-NSAID use was not

associated with a reduced risk of GC among patients who underwent HP eradication. The chemopreventive effect of NA-NSAIDs observed in prior studies may have been confounded by immortal time bias.

Li, B., et al. (2019). "Isobavachalcone exerts antiproliferative and proapoptotic effects on human liver cancer cells by targeting the ERKs/RSK2 signaling pathway." Oncol Rep **41**(6): 3355-3366.

Aberrant activation of the extracellular signalregulated kinases (ERKs)/ribosomal S6 kinase 2 (RSK2) signaling pathway is frequently determined in various human tumor types, including liver cancer, and has been considered as a promising target for cancer chemoprevention and therapy. In the present study, using computeraided virtual screening and molecular docking, isobavachalcone (IBC), a natural chalcone compound, was identified to be an ATPcompetitive inhibitor targeting ERK1/2 and RSK2. Cell Counting Kit8, EdU incorporation and colony formation assays were used to detect the effects of IBC on cell viability and proliferation, and the results demonstrated that IBC effectively inhibited the proliferation of liver cancer HepG2 and Hep3B cells, whereas it had no notable cytotoxic effect on immortal liver L02 cells. Flow cytometric analysis and western blotting further revealed that IBC caused significant levels of apoptosis on liver cancer cells via the caspasedependent mitochondria pathway. The computer prediction was confirmed with pulldown and in vitro kinase assays, in which IBC directly bound with ERK1/2 and RSK2, and dosedependently blocked RSK2 kinase activity in liver cancer cells. Treatment of HepG2 or Hep3B cells with IBC significantly attenuated epidermal growth factorinduced phosphorylation of RSK2 and resulted in the reduced activation of its downstream substrates including cAMP response elementbinding protein, activating transcription factor 1, histone H3 and activating protein1. Enforced RSK2 expression in L02 cells could increase the effect of IBC on suppressing cell growth. Conversely, knockdown of RSK2 reduced the inhibitory effect of IBC on HepG2 cell proliferation. Overall, the present data indicated that ERKs/RSK2 signaling serves a pivotal role in IBCinduced suppression of liver cancer cells and that IBC may be a potential therapeutic candidate for human cancer with elevated ERKs/RSK2 activity.

Li, D., et al. (2018). "Long noncoding RNA UCA1 promotes papillary thyroid cancer cell proliferation via miR204mediated BRD4 activation." Mol Med Rep **18**(3): 3059-3067.

Long noncoding RNA (lncRNA) urothelial carcinomaassociated 1 (UCA1) has been used in tumor development and progression in many types of cancer. However, the function and mechanism underlying the action of UCA1 in papillary thyroid cancer (PTC) remains unclear. Therefore, these topics were investigated in the present study by in vitro and in vivo experiments. It was demonstrated that the expression level of UCA1 was more significantly upregulated in PTC cell lines and tissues when compared with the immortal human thyroid follicular cell line and adjacent normal tissues, respectively. UCA1 knockdown significantly inhibited PTC cell viability, colony formation and the bromodomain containing 4 (BRD4) expression level in vitro, and retarded PTC tumor growth in vivo. In the previous study, microRNA (miR)204 inhibited thyroid cancer progression and was regulated by UCA1 in other types of cancer. In addition, by conducting dual luciferase reporter assays, it was confirmed that miR204 directly binds to UCA1 and the 3'untranslated region of BRD4. Furthermore, UCA1 competed with BRD4 for miR204 binding. miR204 knockdown enhanced BRD4 expression, which can be partially restored by short hairpinUCA1. The results of the present study illustrated that UCA1 promotes PTC progression by acting as a competing endogenous RNA by sponging miR204. In conclusion, UCA1 may be regarded as an oncogenic lncRNA, promoting PTC cell proliferation, and be a potential target for human PTC treatment.

Li, H., et al. (2006). "TGF-beta and cancer: is Smad3 a repressor of hTERT gene?" Cell Res **16**(2): 169-173.

Transforming growth factor beta (TGF-beta) carries out tumor suppressor activity in epithelial and lymphoid cells, whereas telomerase is required for most cancers. Although the molecular mechanisms by which TGF-beta acts as a tumor suppressor are yet to be fully established, a link between TGFb and its tumor suppressor activity by telomerase has been suggested. Recently, we have noted a novel mode of action for TGF-beta through which human telomerase reverse transcriptase (hTERT) gene is repressed in immortal and neoplastic cells, confirming that one of the mechanisms underlying TGF-beta suppression of tumor growth may be through inhibiting hTERT gene transcription. Moreover, the inhibition of hTERT gene by TGF-beta suggests a cis action of the TGF-beta signaling molecule Smad3 on hTERT promoter directly. This article examines our current understanding and investigation of TGF-beta regulation of telomerase activity, and presents a model in which Smad3 participates in regulating hTERT gene transcription by acting as a repressor directly. Engineering the interface between Smad3 and hTERT gene may lead to a new strategy to inhibit telomerase activity in cancer.

Li, W., et al. (2018). "Long noncoding RNA BDNF-

AS is associated with clinical outcomes and has functional role in human prostate cancer." Biomed Pharmacother **102**: 1105-1110.

BACKGROUND: The underlying molecular mechanisms of prostate cancer (CaP) are largely unknown. We investigated the expression, prognostic value and functional role of long non-coding RNA (lncRNA) brain-derived neurotrophin factor antisense (BDNF-AS) in CaP. METHODS: Clinical tumor samples were excised from patients with CaP. Their endogenous BDNF-AS expression levels were evaluated by qRT-PCR. Correlations between CaP patients' endogenous BDNF-AS expression and their clinicopathological factors, overall survival were statistically analyzed. BDNF-AS expression levels were also probed in immortal CaP cell lines. In LNCaP and PC-3 cells, BDNF-AS was ectopically overexpressed through lentiviral transduction. The functions of BDNF-AS upregulation on CaP cell development were evaluated both in vitro and in vivo. RESULTS: BDNF-AS was downregulated in human CaP tumors. Low BDNF-AS expression was correlated with CaP patients' poor prognosis and shorter overall survival. BDNF-AS was also found to be lowly expressed in CaP cell lines. In LNCaP and PC-3 cells, lentivirus-driven BDNF-AS overexpression exerted significantly tumor-suppressing effects on hindering cancer cell proliferation and invasion in vitro, and explant growth in vivo. CONCLUSION: Downregulated BDNF-AS in CaP patients could be a potential prognostic biomarker for predicating poor prognosis and survival. Upregulating BDNF-AS may be a novel molecular intervening target for CaP treatment.

Li, W., et al. (2010). "Reptin is required for the transcription of telomerase reverse transcriptase and over-expressed in gastric cancer." Mol Cancer **9**: 132.

BACKGROUND: Telomerase is activated in oncogenesis, which confers an immortal phenotype to cancer cells. The AAA + ATPase Reptin is required for telomerase biogenesis by maintaining telomerase RNA (hTER) stability and is aberrantly expressed in certain cancers. Given its role in chromatin remodeling and transcription regulation, we determined the effect of Reptin on the transcription of the telomerase reverse transcriptase (hTERT) gene, a key component of the telomerase complex and its expression in gastric cancer. RESULTS: Knocking down Reptin or its partner Pontin using small interfering RNA in gastric and cervical cancer cells led to significant decreases in hTERT mRNA, but hTERT promoter activity was inhibited in only Reptin-depleted cells. Reptin interacted with the c-MYC oncoprotein and its stimulatory effect on the hTERTpromoter was significantly dependent on functional E-boxes in the promoter. Moreover, Reptin bound to the hTERT proximal promoter and the loss of the Reptin occupancy led to dissociation of c-MYC from the hTERT promoter in Reptin-depleted cells. Reptin inhibition dramatically impaired clonogenic potential of gastric cancer cells by inducing cell growtharrest and over-expression of Reptin was observed in primary gastric cancer specimens. CONCLUSIONS: The hTERT gene is a direct target of Reptin, and hTERT transcription requires constitutive expression of Reptin and its cooperation with c-MYC. Thus, Reptin regulates telomerase at two different levels. This finding, together with the requirementof Reptin for the clonogenic potential of cancer cells and its over-expression in gastriccancer and other solid tumors, suggests that Reptin may be a putative therapeutic target.

Li, X., et al. (2020). "Circular RNA circNRIP1 promotes migration and invasion in cervical cancer by sponging miR-629-3p and regulating the PTP4A1/ERK1/2 pathway." Cell Death Dis **11**(5): 399.

Emerging evidence indicates that circRNAs play essential roles in tumorigenesis and development. However, the role of circRNAs in cervical cancer (CC) remains unclear. CircRNA microarrays performed on the immortal cervical cell line H8 and the cervical cancer cell line SiHa were used to identify a circRNA, termed circNRIP1 (hsa_circ_0004771), which was upregulated in SiHa. QRT-PCR confirmed that circNRIP1 was upregulated in CC tissues, where its expression was correlated with lymphovascular space invasion. Besides, both in vitro and in vivo experiments demonstrated that circNRIP1 promotes cell proliferation, migration, and invasion. Additionally, we found that miR-629-3p induced tumor suppression by regulating PTP4A1 and the ERK1/2 pathway. Finally, we confirmed that circNRIP1 exerts its effect, at least partially, by sponging miR-629-3p and thereby regulating the PTP4A1/ERK1/2 pathway. Therefore, circNRIP1 may be useful as a potential prognostic biomarker and therapeutic target in CC patients.

Li, Z., et al. (2002). "Genome-wide allelotyping of a new in vitro model system reveals early events in breast cancer progression." Cancer Res **62**(20): 5980-5987.

Toward the goal of identifying early genetic losses, which mediate the release of human breast epithelium from replicative suppression leading to cellular immortalization, we have used a newly developed in vitro model system. This system consists of epithelial cultures derived from noncancerous breast tissue, treated with the chemical carcinogen N-ethyl-N-nitrosourea, and continuously passaged to yield cell

populations culminating in the immortal phenotype. Genome-wide allelotyping of early passage N-ethyl-N-nitrosourea-exposed cell populations revealed aberrations at >10% (18 of 169) loci examined. Allelic losses encompassing chromosomes 6q24-6q27, implicating immortalization-associated candidate genes, hZAC and SEN6, occurred in two independently derived cell lines before the Hayflick limit. Additional LOH sites were present in one cell line at 3p11-3p26, 11p15, and 20p12-13. Allelic losses reported in this cell line preceded detectable levels of telomerase activity and the occurrence of p53-related aberrations. Information gained from the search for early immortalization-associated genetic deletions in cultured cells was applied in a novel approach toward the analysis of morphologically normal terminal ductal lobular units microdissected from 20 cases of ductal carcinoma in situ. Notably, clonal allelic losses at chromosome 3p24 and 6q24 were an early occurrence in adjoining terminal ductal lobular units of a proportion of primary tumors, which displayed loss of heterozygosity (3 of 11 and 3 of 6, respectively). The biological insights provided by the new model system reported here strongly suggest that early allelic losses delineated in immortalized cultures and validated in vivo could serve as surrogate endpoints to assist in the identification and intervention of high-risk benign breast tissue, which sustains the potential for continuous proliferation.

Ligr, M., et al. (2012). "Mifepristone inhibits GRbeta coupled prostate cancer cell proliferation." J Urol **188**(3): 981-988.

PURPOSE: The GR gene produces GRalpha and GRbeta isoforms by alternative splicing of a C-terminal exon. GRalpha binds glucocorticoids, modulates transcription in a glucocorticoid dependent manner and has a growth inhibitory role in prostate cells. Due to this role glucocorticoids are often used to treat androgen independent prostate cancer. In contrast, GRbeta has intrinsic transcriptional activity and binds mifepristone (RU486) but not glucocorticoids to control gene expression. To our knowledge the role of GRbeta in prostate cell proliferation is unknown. MATERIALS AND METHODS: We determined GRbeta levels in various prostate cancer cell lines by reverse transcriptase-polymerase chain reaction and Western blot. The effect of GRbeta on the kinetics of prostate cancer cell growth was determined by cell counting and flow cytometry upon mifepristone and dexamethasone treatment. Cell proliferation was also examined after siRNA mediated knockdown and over expression of GRbeta. RESULTS: GRbeta mRNA and protein were up-regulated in LNCaP cells that over expressed the androgen receptor co-factor ARA70beta. Treatment of LNCaP-ARA70beta with mifepristone or siRNA targeting GRbeta inhibited proliferation compared to that of parental LNCaP cells. The immortal but nontumorigenic RC165 prostate cell line and the tumorigenic DU145 prostate cell line with endogenous GRbeta also showed partial growth reduction upon GRbeta depletion but to a lesser extent than LNCaP-ARA70beta cells. The growth stimulatory effect of ARA70beta on LNCaP cells was partly GRbeta dependent, as was the proliferation of RC165 cells and to a lesser extent of DU145 cells. CONCLUSIONS: Results suggest that patients with a primary tumor that expresses GRbeta and ARA70beta may benefit from mifepristone.

Lin, C. C., et al. (2015). "Can aspirin reduce the risk of colorectal cancer in people with diabetes? A population-based cohort study." Diabet Med **32**(3): 324-331.

AIM: To evaluate whether aspirin can reduce the risk of colorectal cancer in people with diabetes. METHODS: We studied >/= 30-year-old people with diabetes, included in the Longitudinal Health Insurance Database 2005 in Taiwan, who were treated with hypoglycaemic drugs. We used a time-varying Cox regression model to adjust for immortal time bias and to estimate the adjusted hazard ratio and 95% CI for the association between aspirin use and colorectal cancer occurrence. RESULTS: We studied a total of 60 828 people with diabetes (31 176 men and 29 652 women). Their mean (sd) age was 58.72 (13.33) years. A total of 26 494 people were taking aspirin. Aspirin use 3-5 times/week (moderate frequency) for > 5 years (long duration) was found to reduce the risk of colorectal cancer by 46% (hazard ratio 0.54, 95% CI 0.34-0.86). Aspirin use > 5 times/week (high frequency) for 4-5 years (moderate duration) and > 5 years reduced the risk of colorectal cancer by 56 and 68%, respectively (hazard ratio 0.44, 95% CI 0.24-0.80; hazard ratio 0.32, 95% CI 0.20-0.50). Low frequency (</= 2 times/week) and/or short duration (</= 3 years) of aspirin use did not reduce the risk of colorectal cancer. CONCLUSIONS: Aspirin use with high frequency and long duration reduced the risk of colorectal cancer in people with diabetes in a frequency- and duration-dependent manner, whereas low frequency and/or short duration of aspirin use did not. The dosage, frequency and duration of aspirin use that are sufficient to prevent the incidence of colorectal cancer in people with diabetes require further study.

Lin, P., et al. (2021). "Inhaled corticosteroids and risk of lung cancer: A systematic review and meta-analysis." Eur J Clin Invest **51**(2): e13434.

INTRODUCTION: Current studies investigating the association between inhaled corticosteroid (ICS) use and risk of lung cancer have

yielded inconsistent findings. The aim of this systematic review and meta-analysis was to pool all currently available data to estimate this association. METHODS: We systematically searched MEDLINE (1946 to July 2020), EMBASE (1974 to July 2020) and the Cochrane Library (June 2020) via Ovid to identify relevant articles investigating the association between the ICS use and the risk of lung cancer. Random-effects analysis was used to calculate pooled relative risks (RRs) with 95% confidence intervals (CIs). RESULTS: Ten articles including 234 920 patients were analysed. ICS use was identified to have a decreased risk of lung cancer in chronic obstructive pulmonary disease (8 studies, 1806 patients; RR = 0.73, 95% CI: 0.61-0.87, P < .01; I(2) = 60.0 %), asthma (1 study, 41 438 patients; RR = 0.44, 95% CI: 0.34-0.57, P < .01) and mixed (1 study, 46 225 patients; RR = 0.79, 95% CI: 0.69-0.90, P < .01) patients. The findings of reduced risk of lung cancer were consistent in all subgroup analyses except for the short-term follow-up (</=5 years) (RR = 0.94, 95% CI: 0.81-1.07, P = .34) and free of immortal time bias (RR = 0.94, 95% CI: 0.82-1.08, P = .38) subgroups. CONCLUSIONS: The present study suggested that ICS use was associated with decreased risk of lung cancer. However, our findings should be interpreted with caution because most original studies were judged to be at high risk of immortal time bias.

Lin, X., et al. (2019). "Transcriptomewide piRNA profiling in human gastric cancer." Oncol Rep **41**(5): 3089-3099.

Piwiinteracting RNAs (piRNAs) comprise the largest class of noncoding RNAs. They represent a molecular feature shared by all nonaging biological systems, including germline and somatic cancer stem cells, which display an indefinite capacity of renewal and proliferation and are potentially immortal. They have been identified in animal stomachs, but their relationship with human gastric cancers remains largely unclear. The present study aimed to identify the piRNAs associated with human gastric cancers across the whole transcriptome. Fresh tumor tissues and adjacent nontumorous tissues from stomachs were examined using a piRNA microarray (23,677 piRNAs) that was then validated by qPCR. The differential expression of piRNAs between cases and controls was analyzed. The transposable elements (TEs) that are potentially targeted by the risk piRNAs were searched. The expression of the nearest genes that are complementary to the sequences of the piRNAs was examined in the stomach tissue. The regulatory effects of genomewide significant and replicated cancerrisk DNA variants on the piRNA expression in stomach were tested. Based on the findings, we identified a total of 8,759 piRNAs in human stomachs. Of all, 50 were significantly (P<0.05) and differentially (>2fold change) expressed between the cases and controls, and 64.7% of the proteincoding genes potentially regulated by the gastric cancerassociated piRNAs were expressed in the human stomach. The expression of many cancerassociated piRNAs was correlated with the genomewide and replicated cancerrisk SNPs. In conclusion, we conclude that piRNAs are abundant in human stomachs and may play important roles in the etiological processes of gastric cancers.

Lin, Z., et al. (2018). "Overexpressing PRMT1 Inhibits Proliferation and Invasion in Pancreatic Cancer by Inverse Correlation of ZEB1." IUBMB Life **70**(10): 1032-1039.

Pancreatic cancer (PC) is one of the most malign human cancers, with its underlying molecular mechanisms largely unknown. In this work, we investigated the mechanistic role of protein arginine methyltransferase 1 (PRMT1) gene in PC. Expression of PRMT1 in immortal PC cell lines and clinical human PC tumors was evaluated by quantitative real-time polymerase chain reaction (qRT-PCR) and western blot. In PANC-1 and SW1990 cells, PRMT1 was either downregulated by lentiviral-mediated short hairpin RNA (shRNA) or upregulated by overexpression plasmid. The effects of PRMT1 downregulation or upregulation on PC proliferation and invasion in vitro, and xenograft in vivo, were evaluated. Gene expression of PRMT1 downstream target, zinc finger E-box binding homeobox 1 (ZEB1) was measured in PRMT1-downregulated PC cells. ZEB1 was also upregulated in PRMT1-downregulated PC cells to evaluate its functional role in PRMT1-mediated regulation in PC. PRMT1 was downregulated in both PC cell lines and human tumors. PRMT1 downregulation in PANC-1 and SW1990 cells significantly suppressed cancer proliferation and invasion in vitro and xenograft in vivo. However, PRMT1 overexpression did not have function impact in PC cells. ZEB1 gene expression was suppressed in PRMT1-downregulated PC cells. Subsequently, overexpressing ZEB1 reversed the antitumor effects of PRMT1 downregulation in PC cells. PRMT1 was aberrantly upregulated in PC. PRMT1 inhibition, possibly inversely acting through ZEB1, might be an effective molecular intervention to inhibit PC growth and invasion. (c) 2018 IUBMB Life, 70(10):1032-1039, 2018.

Liu, B. C. and K. R. Loughlin (2000). "Telomerase in human bladder cancer." Urol Clin North Am **27**(1): 115-123, x.

Biomarkers for human bladder cancer are currently available and more are being developed. However, the ultimate goal of diagnosing bladder

cancer consistently in a noninvasive fashion has not yet been achieved. Telomerase is an enzyme that may play a role in maintaining telomere sequences in the ends of chromosomes and its activity may reflect the presence of immortal or cancer cells. In this article, we reviewed the potential applications of telomerase in the diagnosis, monitoring, and treatment of human bladder cancer.

Liu, J., et al. (2016). "Inhibition of microRNA-383 has tumor suppressive effect in human epithelial ovarian cancer through the action on caspase-2 gene." Biomed Pharmacother **83**: 1286-1294.

BACKGROUND: MicroRNAs are important cancer regulators. In this work, we examined the expression pattern and mechanistic implications of microRNA-383 (miR-383) in human epithelial ovarian cancer (EOC). METHODS: Gene expression level of miR-383 was compared by qRT-PCR between EOC cell lines and normal ovarian epithelial cell line, and between clinical EOC tumors and adjacent non-tumor ovarian epithelial tissues. Endogenous miR-383 was downregulated through lentiviral infection. Its effects on regulating EOC proliferation, cell cycle, invasion and in vivo explant development were assessed. Possible downstream target of miR-383 in EOC, human caspase-2 gene (CASP2), was evaluated by luciferase assay and qRT-PCR. CASP2 was then genetically knocked down by siRNA to assess its functional relationship with miR-383 in regulating EOC development both in vitro and in vivo. RESULTS: MiR-383 was overexpressed in both immortal EOC cell lines and human EOC tumors. In stably miR-383-downregulated EOC cell lines, cancer proliferation, cell cycle progression, invasion and in vivo explant development were significantly suppressed. CASP2 was confirmed to be downstream of miR-383 in EOC. SiRNA-mediated CASP2 downregulation had reverse relationship with miR-383 downregulation in regulating EOC development both in vitro and in vivo. CONCLUSION: Inhibition of miR-383 has profound tumor suppressing effect on EOC development. And the functional regulation of miR-383 in EOC is very likely inversely associated with CASP2 gene.

Liu, J. H., et al. (2014). "Expression and prognostic significance of lncRNA MALAT1 in pancreatic cancer tissues." Asian Pac J Cancer Prev **15**(7): 2971-2977.

BACKGROUND: Long non-coding RNAs (lncRNAs) have been recently observed in various human cancers. However, the role of lncRNAs in pancreatic duct adenocarcinoma (PDAC) remains unclarified. The aim of this study was to detect the expression of lncRNA MALAT1 in PDAC formalin-fixed, paraffin embedded (FFPE) tissues and to investigate the clinical significance of the MALAT1 level. METHODS: The expression of MALAT1 was examined in 45 PDAC and 25 adjacent non-cancerous FFPE tissues, as well as in five PDAC cell lines and a normal pancreatic epithelium cell line HPDE6c-7, using qRT-PCR. The relationship between MALAT1 level and clinicopathological parameters of PDAC was analyzed with the Kaplan-Meier method and Cox proportional hazards model. RESULTS: The relative level of MALAT1 was significantly higher in PDAC compared to the adjacent normal pancreatic tissues ($p=0.009$). When comparing the MALAT1 level in the cultured cell lines, remarkably higher expression of MALAT1 was found in aspc-1 PDAC cells compared with the immortal pancreatic duct epithelial cell line HPDE6c-7 ($q=7.573$, $p<0.05$). Furthermore, MALAT1 expression level showed significant correlation with tumor size ($r=0.35$, $p=0.018$), tumor stage ($r=0.439$, $p=0.003$) and depth of invasion ($r=0.334$, $p=0.025$). Kaplan-Meier analysis revealed that patients with higher MALAT1 expression had a poorer disease free survival ($p=0.043$). Additionally, multivariate analysis indicated that overexpression of MALAT1, as well as the tumor location and nerve invasion, was an independent predictor of disease-specific survival of PDAC. CONCLUSION: MALAT1 might be considered as a potential prognostic indicator and may be a target for diagnosis and gene therapy for PDAC.

Liu, N., et al. (2007). "RhoC is essential for the metastasis of gastric cancer." J Mol Med (Berl) **85**(10): 1149-1156.

Rho family members are known to regulate malignant transformation and motility of cancer cells, but the clinicopathological significance of RhoC remains unclear yet in the case of gastric cancer. In this study, we evaluated the protein expression level of RhoC in gastric cancer tissues and cell lines. Results showed that only weak staining of RhoC was detected in 3 of 33 non-tumorous cases by immunohistochemistry. The expression of RhoC was significantly higher in gastric cancer tissues (23/42, 54.8%) than in non-tumorous tissues ($p < 0.01$). Further analysis demonstrated that RhoC had high specificity (80.0%) in detecting gastric carcinomas with metastatic potential. RhoC was positively expressed in 18 out of 20 metastases (90.0%), even higher than that in primary gastric cancer tissues. Western blot showed that RhoC was up-regulated in five different gastric cancer cell lines but not expressed in SV40-transformed immortal gastric epithelial cell GES-1. Overexpression of RhoC GTPase in GES-1 cells could produce the motile and invasive phenotype but did not alter the monolayer growth rate. To further study the functions of RhoC, we took the powerful siRNA technology to knock down the expression of

RhoC in SGC7901 cells. It was shown that down-regulation of RhoC did not affect the proliferation of SGC7901 cells. However, interference of RhoC expression could inhibit migration, invasion, and anchorage-independent growth of SGC7901 cells. In conclusion, RhoC may play a very important role in the metastasis of gastric carcinoma. Therapeutic strategies targeting RhoC and RhoC-mediated pathways may be a novel approach for treating metastasis of gastric cancer.

Liu, Y., et al. (2004). "Polyoma enhancer activator 3, an ets transcription factor, mediates the induction of cyclooxygenase-2 by nitric oxide in colorectal cancer cells." J Biol Chem **279**(18): 18694-18700.

Abundant evidence supports the role of cyclooxygenase-2 (COX-2) in colorectal cancer. Nitric oxide (NO), a pro-inflammatory signaling factor, may regulate COX-2 expression and activity thereby linking hyper-inflammatory states to cancer susceptibility. Previously we showed that NO induced COX-2 expression. Although NO also activated the beta-catenin.T-cell factor/lymphocyte enhancing factor transcriptional pathway, a direct causal link between this pathway and COX-2 expression was not demonstrated. In this current study, we focused on NO-induced transcriptional activity and elucidated its role in COX-2 expression. NO donors stimulated the expression of peroxisome proliferator-activated receptor-delta and c-myc, both downstream genes of beta-catenin. They also induced the expression of polyoma enhancer activator 3 (PEA3) and increased its DNA-binding activity. To establish a role for PEA3 to beta-catenin-induced COX-2, we transfected RKO cells with beta-catenin and found that beta-catenin increased PEA3 expression. Also, there was higher PEA3 in immortal mouse colon epithelium cells (Apc(Min/)(+)) compared with young adult mouse colon cells (Apc(+/+)). Luciferase reporter assays revealed that, although several transcription factors/coactivator, acting alone or in synergistic combination, induced COX-2 promoter activity, PEA3 was one of the most potent. Interestingly, NO from NO donors or generated endogenously from transfected inducible nitric-oxide synthase, increased PEA3/p300-induced COX-2 promoter activity. We also found that an ETS site (-75/-72) and the NF-IL6 site were responsible for COX-2 activity induced by PEA3, PEA3/p300, and NO. Taken together, our results demonstrated that NO through beta-catenin signaling stimulated PEA3 to increase COX-2 activity. In addition, NO augmented the synergistic interaction between PEA3 and CBP/p300.

Londono-Vallejo, J. A. (2008). "[Cancer as a microevolutionary process affecting telomere structure and dynamics: the contribution of telomeres to cancer]." Ai Zheng **27**(7): 775-783.

Telomeres play fundamental roles in genome stability, nuclear architecture and chromosome pairing during meiosis. They shorten at every cell division and may be re-elongated or not depending on the presence of the dedicated enzyme, telomerase. Since in most human somatic cells telomerase is not expressed, shortening of telomeres during development and aging is the rule. Short telomeres being, under physiological conditions, incompatible with extended cell proliferation, telomere length defines the proliferation potential of a cell and operates as a mechanism to prevent uncontrolled cell growth. Conversely, in cells in which proliferation checkpoints have been abolished, shortening of telomeres causes chromosomes to fuse and to initiate cycles of breakage-fusion-bridge thus becoming a strong driving force for genome instability. In vitro, transformed cells with highly unstable genomes because of severe telomere shortening accumulate deleterious genetic changes and die (crisis). At the same time, random genetic or epigenetic changes may allow cells to acquire a telomere maintenance mechanism (as well as other tumor phenotypes) and to become immortal. Although telomere shortening and other types of telomere dysfunction probably contribute to the genome instability detected in early tumors in vivo, the direct contributions of dysfunctional telomeres to the acquisition of tumor phenotypes in humans remain largely unspecified.

Londono-Vallejo, J. A. (2008). "Telomere instability and cancer." Biochimie **90**(1): 73-82.

Telomeres are required to preserve genome integrity, chromosome stability, nuclear architecture and chromosome pairing during meiosis. Given that telomerase activity is limiting or absent in most somatic tissues, shortening of telomeres during development and aging is the rule. In vitro, telomere length operates as a mechanism to prevent uncontrolled cell growth and therefore defines the proliferation potential of a cell. In vitro, in somatic cells that have lost proliferation control, shortening of telomeres becomes the main source of genome instability leading to genetic or epigenetic changes that may allow cells to become immortal and to acquire tumor phenotypes. In vivo, mice models have indisputably shown both the protective and the promoting role of very short telomeres in cancer development. In humans, although telomere shortening and other types of telomere dysfunction probably contribute to the genome instability often detected in tumors, the specific contributions of such instability to the development of cancer remain largely undetermined.

Lund, J. R., et al. (2008). "Inhibition of canine telomerase in vitro and in vivo using RNAi: further development of a natural canine model for telomerase-based cancer therapies." Vet J **177**(2): 192-197.

Despite advances in cancer therapy, cancer related morbidity and mortality among humans and companion animals remains high, and there is a clear need to develop novel targeted therapies. Expression of the enzyme telomerase has emerged as a central unifying mechanism underlying the immortal phenotype of canine cancer cells and has thus become a candidate for targeted molecular therapies. In this study, the value of telomerase inhibition to target telomerase expressing cancer cells was explored using the novel mechanism of RNA interference (RNAi). Using a Lentiviral expression construct, targeting the RNA component of canine telomerase was effective at inhibiting telomerase in vitro and tumour growth in vivo, but possible resistance mechanisms are highlighted. As canine telomerase biology is more closely related to human telomerase biology than the murine system, it is proposed that this study highlights the value of natural canine models to study anti-telomerase therapies for human patients.

Luo, S., et al. (2020). "Evaluating the impact of AMPK activation, a target of metformin, on risk of cardiovascular diseases and cancer in the UK Biobank: a Mendelian randomisation study." Diabetologia **63**(11): 2349-2358.

AIMS/HYPOTHESIS: Whether metformin reduces cardiovascular or cancer risk is unclear owing to concerns over immortal time bias and confounding in observational studies. This study evaluated the effect of AMP-activated protein kinase (AMPK), the target of metformin, on risk of cardiovascular disease and cancer. METHODS: This is a Mendelian randomisation design, using AMPK, the pharmacological target of metformin, to infer the AMPK pathway-dependent effects of metformin on risk of cardiovascular disease and cancer in participants of white British ancestry in the UK Biobank. RESULTS: A total of 391,199 participants were included (mean age 56.9 years; 54.1% women), including 26,690 cases of type 2 diabetes, 38,098 cases of coronary artery disease and 80,941 cases of overall cancer. Genetically predicted reduction in HbA1c (%) instrumented by AMPK variants was associated with a 61% reduction in risk of type 2 diabetes (OR 0.39; 95% CI 0.20, 0.78; p = 7.69 x 10(-3)), a 53% decrease in the risk of coronary artery disease (OR 0.47; 95% CI 0.26, 0.84; p = 0.01) and a 44% decrease in the risk of overall cancer (OR 0.56; 95% CI 0.36, 0.85; p = 7.23 x 10(-3)). Results were similar using median or quartiles of AMPK score, with dose-response effects (p for trend = 4.18 x 10(-3) for type 2 diabetes, 4.37 x 10(-3) for coronary artery disease and 4.04 x 10(-3) for overall cancer). CONCLUSIONS/INTERPRETATION: This study provides some genetic evidence that AMPK activation by metformin may protect against cardiovascular disease and cancer, which needs to be confirmed by randomised controlled trials.

Luyendijk, M., et al. (2020). "Assessment of Studies Evaluating Incremental Costs, Effectiveness, or Cost-Effectiveness of Systemic Therapies in Breast Cancer Based on Claims Data: A Systematic Review." Value Health **23**(11): 1497-1508.

OBJECTIVES: Large secondary databases, such as those containing insurance claims data, are increasingly being used to compare the effects and costs of treatments in routine clinical practice. Despite their appeal, however, caution must be exercised when using these data. In this study, we aimed to identify and assess the methodological quality of studies that used claims data to compare the effectiveness, costs, or cost-effectiveness of systemic therapies for breast cancer. METHODS: We searched Embase, the Cochrane Library, Medline, Web of Science, and Google Scholar for English-language publications and assessed the methodological quality using the Good Research for Comparative Effectiveness principles. This study was registered with the International Prospective Register of Systematic Reviews (PROSPERO) under number CRD42018103992. RESULTS: We identified 1251 articles, of which 106 met the inclusion criteria. Most studies were conducted in the United States (74%) and Taiwan (9%) and were based on claims data sets (35%) or claims data linked to cancer registries (58%). Furthermore, most included large samples (mean 17 130 patients) and elderly patients, and they covered various outcomes (eg, survival, adverse events, resource use, and costs). Key methodological shortcomings were the lack of information on relevant confounders, the risk of immortal time bias, and the lack of information on the validity of outcomes. Only a few studies performed sensitivity analyses. CONCLUSIONS: Many comparative studies of cost, effectiveness, and cost-effectiveness have been published in recent decades based on claims data, and the number of publications has increased over time. Despite the availability of guidelines to improve quality, methodological issues persist and are often inappropriately addressed or reported.

Majidi, A., et al. (2020). "Common medications and survival in women with ovarian cancer: A systematic review and meta-analysis." Gynecol Oncol **157**(3): 678-685.

OBJECTIVES: Ovarian cancer is usually

diagnosed at an advanced stage when five-year relative survival is <50%. Therefore, strategies to improve survival are required. Studies suggest associations between common chronic disease medications such as metformin, statins, beta-blockers, aspirin and non-aspirin non-steroidal anti-inflammatory drugs (NA-NSAIDs) and improved cancer survival. We aimed to review the evidence for a possible relation between these medications and survival among women with ovarian cancer. METHODS: We conducted four systematic reviews and evaluated the risk of bias in the included studies. Where possible, we calculated pooled hazard ratios (pHR) and 95% confidence intervals (CI), excluding studies considered to have the potential for immortal time bias (ITB) which, in practice, was the major source of bias. RESULTS: We identified 36 studies evaluating one or more of the medications (metformin n = 8, statins n = 12, beta-blockers n = 11, aspirin and/or NA-NSAIDs n = 9). We rated 21 studies as ITB-free. The meta-analysis of the ITB-free studies suggested improved survival in statin users compared to non-users (pHR: 0.76, 95%CI: 0.68-0.85), but no overall survival benefit associated with use of metformin, beta-blockers, aspirin or NA-NSAIDs. The pooled result of two studies did, however, suggest a possible association between perioperative beta-blocker use and improved survival. Studies considered to have potential ITB were more likely to report survival benefits associated with these medications. CONCLUSION: Statin use is associated with better ovarian cancer survival but further study, preferably a clinical trial, is required. There are insufficient data to draw conclusions regarding metformin, beta-blockers, aspirin and NA-NSAIDs.

Marchioni, M., et al. (2018). "Effect of African-American race on cancer specific mortality differs according to clear cell vs. non-clear cell histologic subtype in metastatic renal cell carcinoma." Cancer Epidemiol **54**: 112-118.

AIM: To test the effect of African-American race on cancer specific mortality (CSM) in clear cell metastatic renal cell carcinoma (ccmRCC) and non-ccmRCC. PATIENTS AND METHODS: Within Surveillance, Epidemiology and End Results registry (2001-2014), we identified patients with ccmRCC and non-ccmRCC. We relied on propensity score (PS) matching to reduce the effect of inherent differences between African-American vs. Caucasian patients. After PS matching that included access to cytoreductive nephrectomy (CNT), cumulative incidence, competing-risks regression (CRR) models and landmark analyses tested the effect of race on CSM. RESULTS: Before PS matching, African-American patients accounted for 7.0 and 24.5% of respectively ccmRCC (N=6742) and non-ccmRCC patients (N=766). After PS matching, African-American patients accounted for 22.3 and 33.5% of respectively ccmRCC (N=2050) and non-ccmRCC (N=391) matched cohorts. In multivariable CRR models focusing on ccmRCC, higher CSM was recorded in African-Americans (HR:1.27, p<0.001). Conversely, in non-ccmRCC, lower CSM was recorded in African-Americans (HR:0.54, p<0.001). Landmark analyses rejected the hypothesis of immortal time bias. CONCLUSION: African-Americans experienced higher CSM in ccmRCC. Conversely, African-Americans experienced lower CSM, when diagnosed with non-ccmRCC. These differences are independent of access to CNT and warrant further study since they may have an impact on efficacy or access to systemic therapies.

Marchioni, M., et al. (2019). "Comparison of Partial Versus Radical Nephrectomy Effect on Other-cause Mortality, Cancer-specific Mortality, and 30-day Mortality in Patients Older Than 75 Years." Eur Urol Focus **5**(3): 467-473.

BACKGROUND: Historically, partial nephrectomy (PN) showed no benefit on other-cause mortality (OCM) in elderly patients with small renal masses. OBJECTIVE: To test the effect of PN versus radical nephrectomy (RN) on OCM, cancer-specific mortality (CSM), as well as 30-d mortality in patients with nonmetastatic T1a renal cell carcinoma (RCC), aged >/=75 yr old. DESIGN, SETTING, AND PARTICIPANTS: Within the Surveillance, Epidemiology and End Results registry (2004-2014), we identified surgically treated patients with nonmetastatic pT1a RCC aged >/=75 yr. OUTCOME MEASUREMENTS AND STATISTICAL ANALYSIS: We relied on propensity score (PS) matching to reduce the effect of inherent differences between PN and RN. After PS matching, cumulative incidence, multivariable competing-risks regression (CRR) and logistic regression models were used. LOESS plots graphically depicted the relation between nephrectomy type and OCM after adjustment for all the covariates. Landmark analyses at 6 mo tested for immortal time bias. RESULTS AND LIMITATIONS: Of all 4541 patients, 41.6% underwent PN. After 1:1 PS matching, 2826 patients remained. In multivariable CRR models, lower OCM rates were recorded in PN patients (hazard ratio [HR]: 0.67, confidence interval [CI]: 0.54-0.84; p<0.001). LOESS plots showed lower OCM rates after PN across all examined ages. Lower CSM rates were also recorded in PN patients (HR: 0.64, CI=0.44-0.92; p=0.02). Landmark analyses rejected the hypothesis of immortal time bias. Finally, PN did not result in different 30-d mortality rates (odds ratio: 1.87; CI: 0.79-4.47; p=0.2) versus RN. Data are retrospective. CONCLUSIONS: PN results in lower OCM in elderly

patients with pT1a RCC. Moreover, PN does not contribute to higher CSM or 30-d mortality in patients aged >/=75 yr. In consequence, PN should be given strong consideration, even in elderly patients. PATIENT SUMMARY: Partial nephrectomy (PN) may protect from renal insufficiency, hypertension, and other unfavorable health outcomes, even in elderly patients. This protective effect results in lower other-cause mortality. Moreover, PN benefits are not undermined by higher cancer-specific mortality or 30-d mortality.

Maringe, C., et al. (2020). "Reflection on modern methods: trial emulation in the presence of immortal-time bias. Assessing the benefit of major surgery for elderly lung cancer patients using observational data." Int J Epidemiol **49**(5): 1719-1729.

Acquiring real-world evidence is crucial to support health policy, but observational studies are prone to serious biases. An approach was recently proposed to overcome confounding and immortal-time biases within the emulated trial framework. This tutorial provides a step-by-step description of the design and analysis of emulated trials, as well as R and Stata code, to facilitate its use in practice. The steps consist in: (i) specifying the target trial and inclusion criteria; (ii) cloning patients; (iii) defining censoring and survival times; (iv) estimating the weights to account for informative censoring introduced by design; and (v) analysing these data. These steps are illustrated with observational data to assess the benefit of surgery among 70-89-year-old patients diagnosed with early-stage lung cancer. Because of the severe unbalance of the patient characteristics between treatment arms (surgery yes/no), a naive Kaplan-Meier survival analysis of the initial cohort severely overestimated the benefit of surgery on 1-year survival (22% difference), as did a survival analysis of the cloned dataset when informative censoring was ignored (17% difference). By contrast, the estimated weights adequately removed the covariate imbalance. The weighted analysis still showed evidence of a benefit, though smaller (11% difference), of surgery among older lung cancer patients on 1-year survival. Complementing the CERBOT tool, this tutorial explains how to proceed to conduct emulated trials using observational data in the presence of immortal-time bias. The strength of this approach is its transparency and its principles that are easily understandable by non-specialists.

Mathew, R., et al. (2008). "Immortalized mouse epithelial cell models to study the role of apoptosis in cancer." Methods Enzymol **446**: 77-106.

Human cancer cell lines are widely used to model cancer but also have serious limitations. As an alternate approach, we have developed immortalized mouse epithelial cell model systems that are applicable to different tissue types and involve generation of immortalized cell lines that are genetically defined. By applying these model systems to mutant mice, we have extended the powerful approach of mouse genetics to in vitro analysis. By use of this model we have generated immortal epithelial cells that are either competent or deficient for apoptosis by different gain- and loss-of-function mutations that have revealed important mechanisms of tumor progression and treatment resistance. Furthermore, we have derived immortalized, isogenic mouse kidney, mammary, prostate, and ovarian epithelial cell lines to address the issues of tissue specificity. One of the major advantages of these immortalized mouse epithelial cell lines is the ability to perform biochemical analysis, screening, and further genetic manipulations. Moreover, the ability to generate tumor allografts in mice allows the integration of in vitro and in vivo approaches to delineate the mechanistic aspects of tumorigenesis. These model systems can be used effectively to determine the molecular requirements of epithelial tumorigenesis and tumor-promoting functions. This approach provides an efficient way to study the role of apoptosis in cancer and also enables the interrogation and identification of potential chemotherapeutic targets involving this pathway. Applying this technology to other mouse models can provide insight into additional aspects of oncogenesis.

Mazzone, E., et al. (2019). "Is neoadjuvant chemotherapy for pT2 bladder cancer associated with a survival benefit in a population-based analysis?" Cancer Epidemiol **58**: 83-88.

BACKGROUND: Patients with organ confined muscle-invasive bladder cancer (MIBC) who are candidates for radical cystectomy (RC) should receive neoadjuvant chemotherapy (CHT). However, the most contemporary CHT use rates indicate low adherence to these guidelines. We tested contemporary neoadjuvant CHT rates and associated cancer-specific mortality (CSM) and overall mortality (OM) in pT2N0 MIBC patients treated with RC. MATERIALS AND METHODS: Within the SEER database (2004-2015), we identified patients with pT2N0 MIBC patients who underwent RC. CHT administration rates were evaluated using estimated annual percentage changes (EAPCs) analyses. After inverse probability of treatment weighting (IPTW), Kaplan-Meier (KM) analyses and Cox regression models (CRMs) were used to test the effect of CHT vs no CHT on survival. Landmark analyses tested for immortal time bias. RESULTS: Of 3978 RC patients, 38.2% of patients received CHT. Between 2004 and 2015, CHT rates increased from 15.9% to 66.2% (EAPC: +14.2%; p <

0.001). IPTW-adjusted KM showed 10-year CSM-free survival rates of 78.9% for CHT vs 76.7% for no CHT patients (p = 0.6). Similarly, IPTW-adjusted KM showed 10-year OM-free survival rates of 54.6% for CHT vs 57.9% for no CHT patients (p = 0.8). In IPTW-adjusted MCRMs, CHT was not significantly associated with lower CSM (HR 0.97, CI 0.82-1.14; p = 0.7) or OM (HR 1.02, CI 0.90-1.16; p = 0.7). Virtually the same CSM and OM rates were recorded after landmark analyses. CONCLUSIONS: CHT use in pT2N0 MIBC RC patients sharply increased over the study span. However, neoadjuvant CHT was not associated with better survival in this patient group.

Meena, J., et al. (2015). "Telomere Dysfunction, Chromosomal Instability and Cancer." Recent Results Cancer Res **200**: 61-79.

Telomeres form protective caps at the ends of linear chromosomes to prevent nucleolytic degradation, end-to-end fusion, irregular recombination, and chromosomal instability. Telomeres are composed of repetitive DNA sequences (TTAGGG)n in humans, that are bound by specialized telomere binding proteins. Telomeres lose capping function in response to telomere shortening, which occurs during each division of cells that lack telomerase activity-the enzyme that can synthesize telomeres de novo. Telomeres have a dual role in cancer: telomere shortening can lead to induction of chromosomal instability and to the initiation of tumors, however, initiated tumors need to reactivate telomerase in order to stabilize chromosomes and to gain immortal growth capacity. In this review, we summarize current knowledge on the role of telomeres in the maintenance of chromosomal stability and carcinogenesis.

Mehta, K., et al. (2004). "Prognostic significance of tissue transglutaminase in drug resistant and metastatic breast cancer." Clin Cancer Res **10**(23): 8068-8076.

PURPOSE: Drug resistance and metastasis pose major impediments in the successful treatment of cancer. We previously reported that multidrug-resistant breast cancer cells exhibit high levels of tissue transglutaminase (TG2; EC 2.3.2.13). Because the drug-resistant and metastatic phenotypes are thought to share some common pathways, we sought to determine whether metastatic breast cancer cells express high levels of TG2. EXPERIMENTAL DESIGN: The metastatic breast cancer cell line MDA-MB-231 and the sublines derived from it were tested for TG2 expression. Similarly, several sublines derived from an immortal but normal breast epithelial cell line, MCF10A, representing various stages in breast cancer progression were studied for TG2 expression. The primary and nodal tumor samples from 30 patients with breast cancer were also studied for TG2 expression. RESULTS: The MDA-MB-231 cells expressed high basal levels of TG2. Two clones derived from this cell line, MDA231/cl.9 and MDA231/cl.16, showed a 10- to 15-fold difference in TG2 level. TG2-deficient MDA231/cl.9 cells exhibited higher sensitivity to doxorubicin and were less invasive than were the TG2-sufficient MDA231/cl.16 cells. The MCF10A-derived sublines had increased TG2 expression as they advanced from noninvasive to an invasive phenotype. Importantly, the metastatic lymph node tumors from patients with breast cancer showed significant higher levels of TG2 expression compared with the primary tumors from the same patients. CONCLUSIONS: TG2 expression is up-regulated in drug-resistant and metastatic breast cancer cells, and it can serve as a valuable prognostic marker for these phenotypes.

Mera, S. L. (1998). "The role of telomeres in ageing and cancer." Br J Biomed Sci **55**(3): 221-225.

Telomeres are regions of DNA that cap the ends of linear chromosomes. In somatic cells the telomeres shorten progressively with every cell division, reducing the number of tandem repeat sequences. Eventually the chromosomes become unstable and the cell is no longer able to replicate. This represents an inherent biological clock in which the somatic cell has only a finite capacity for division. In contrast, germ cells do not undergo telomeric shortening and have relatively unlimited capacities for cell division. The difference is that germ cells retain the enzyme telomerase which is able to restore the telomere ends that are lost during cell division. Although telomerase activity is absent in most somatic cells, cancer cells acquire the ability to activate the enzyme, ensuring their immortal growth characteristics and selective advantage over normal somatic cells.

Mielczarek-Puta, M., et al. (2020). "Telmisartan Influences the Antiproliferative Activity of Linoleic Acid in Human Colon Cancer Cells." Nutr Cancer **72**(1): 98-109.

Aim: Linoleic acid (LA) and telmisartan as PPARgamma agonists exhibit anticancer activity. The LA effect is observed for high non-achievable in vivo concentrations and in short treatment period, therefore we evaluate the effect of supplemental LA and pharmacological telmisartan plasma concentrations on human primary (SW480) and metastatic (SW620) colon cancer cells and immortal keratinocytes (HaCaT) cells in long-term treatment. Methods: Cell viability and proliferation were determined by TB and MTT and pro-apoptotic effect was measured by Annexin V binding assays, respectively.Results: LA decreased cancer cell viability and proliferation in a

concentration-dependent manner, whereas no significant effect was found for HaCaT cells. Telmisartan (0.2 microM) suppresses antiproliferative effect of 60 microM LA on cancer cells in short-term treatment. Long-term administration of 60 microM LA reduced cancer cells viability after one week, while telmisartan delayed this effect by two weeks. Growth of all cell lines with 20 microM LA was unchanged during all treatment time. Telmisartan decreased late apoptosis of cancer and normal cells with 60 and 120 microM LA. Conclusion: The cytotoxic LA action depends not only on its concentration but also duration of treatment. Telmisartan exhibits biphasic but not synergistic effect on LA cytotoxicity in cancer cells.

Minami, K., et al. (2001). "Dysregulation of telomerase activity and expression in lymphokine-activated killer cells from advanced cancer patients: possible involvement in cancer-associated immunosuppression mechanism." Oncol Rep **8**(3): 649-653.

There exists cancer-associated immunosuppression, and the generation of lymphokine-activated killer (LAK) cells is impaired in patients with advanced cancer. Telomerase has been reported to be upregulated in the activation of lymphocytes to proliferate against immune stimulation as well as in the malignant transformation of immortal cancer cells. We attempted to clarify the involvement of telomerase in the impairment of LAK cell generation in patients with advanced cancer. LAK cells were generated by stimulation with interleukin (IL)-2 and immobilized anti-CD3 antibody (IL-2/CD3 system) from peripheral blood mononuclear cells of healthy volunteers (he-LAK) or patients with advanced cancer (ca-LAK), and proliferative potential of LAK cells was evaluated on the basis of population doubling level (PDL). Telomere length and telomerase activity of LAK cells were measured by the hybridization with oligonucleotide (TTAGGG)4 and by the telomeric repeat amplification protocol (TRAP) assay, respectively. Effects on telomerase activity in LAK cells of serum from cancer patients, transforming growth factor (TGF)-beta, and IL-10 were also examined. The lifespan of ca-LAK (15.2 +/- 5.1 PDLs) was significantly shorter than that of he-LAK (22.6 +/- 8.3 PDLs) ($p = 0.0358$). There were no significant differences between he- and ca-LAK in telomere length before IL-2/CD3 stimulation and maximal telomerase activity induced. The telomerase activity induced in ca-LAK failed to elongate sufficiently the telomeric ends (-35.2 +/- 46.2 bp) compared with that in he-LAK (16.8 +/- 41.5 bp) ($p = 0.0448$). The telomerase activity was initially detectable on day 2 in all he-LAK, whereas 8 (61.5%) of 13 ca-LAK expressed telomerase activity on day 3 or later following the stimulation, showing a significant retardation of telomerase expression ($p = 0.0116$). The addition to the LAK cell generation system of serum from cancer patients, as well as IL-10, but not transforming growth factor (TGF)-beta, suppressed the telomerase activity. This serum-induced suppression of telomerase activity in LAK cells was abrogated with the addition of anti-IL-10 antibody but not with anti-TGF-beta antibody. It is suggested that the dysregulation of telomerase activity and expression exists in LAK cells of cancer patients, resulting in the impairment of LAK cell generation in patients with advanced cancer. Serum IL-10 may be involved in the impairment of LAK cell generation by the suppression of telomerase activity of lymphocytes in vivo. Thus, the dysregulation mechanism of telomerase activity and expression in lymphocytes of cancer patients may be attributable, in part, to cancer-associated immunosuppression.

Mira, Y. L. R., et al. (2000). "Retinol conversion to retinoic acid is impaired in breast cancer cell lines relative to normal cells." J Cell Physiol **185**(2): 302-309.

The bioactivity of retinol (vitamin A) is in part dependent on its metabolism to retinoic acid (RA). We investigated the ability of breast epithelial cells to synthesize RA when challenged with a physiological retinol dose (2 microM). Normal human mammary epithelial cells (HMEC) cultured from reduction mammoplasties were competent in RA synthesis and the ability to synthesize RA was retained by immortal, nontumorigenic breast epithelial cell lines (MTSV1.7, MCF-10F, and 184B5). In contrast, most (five of six) breast cancer cell lines could not synthesize RA or did so at low rates relative to normal cells. A notable exception was the MDA-MB-468 cell line, which was fully competent in RA synthesis. Most (>/=68%) of the RA synthesized by breast cells was recovered from the culture medium. Cellular retinol binding protein and cellular RA binding protein II, both expressed in HMEC, had various expression patterns in the cell lines that did not correlate with the observed differences in RA synthesizing ability. Strong RA induction of the RA hydroxylase P450RAI (CYP26) was confined to ERalpha-positive T47D and MCF-7 breast cancer cells and did not appear to explain the lack of detectable RA levels in these cells since RA remained undetectable when the cells were treated with 5-10 microM liarozole, a P450RAI inhibitor. We hypothesize that retinol bioactivity is impaired in breast cancer cells that cannot synthesize RA. In preliminary support of this hypothesis, we found that retinol (0.5-2 microM) inhibited MCF-10F but not T47D or MCF-7 cell growth.

Mitra, A., et al. (2013). "Technologies for deriving primary tumor cells for use in personalized cancer therapy." Trends Biotechnol **31**(6): 347-354.

For decades, immortal cancer cell lines have constituted an accessible, easily usable set of biological models to investigate cancer biology and explore the potential efficacy of anticancer drugs. However, numerous studies have suggested that these cell lines poorly represent the diversity, heterogeneity, and drug-resistant tumors occurring in patients. The derivation and short-term culture of primary cells from solid tumors have thus gained significant importance in personalized cancer therapy. This review focuses on our current understanding and the pros and cons of different methods for primary tumor cell culture. Furthermore, various culture matrices such as biomimetic scaffolds and chemically defined media supplemented with essential nutrients, have been prepared for different tissues. These well-characterized primary tumor cells redefine cancer therapies with high translational relevance.

Morris, E. and T. Treasure (2017). "If a picture is worth a thousand words, take a good look at the picture: Survival after liver metastasectomy for colorectal cancer." Cancer Epidemiol **49**: 152-155.

INTRODUCTION: An analysis of NHS data published in by Morris et al. in 2010 is widely used as evidence in support of liver metastasectomy for colorectal cancer and its wider application. Recent evidence concerning better overall survival for patients with metastatic colorectal cancer challenges the notional assumptions about what survival would be without metastasectomy. Earlier detection of metastases for local treatments has not resulted in a survival benefit. MATERIALS AND METHODS: The interpretation of its central graphical display is critically reviewed and the common the limitations of the analysis of registry data and resulting immortal time bias are explored. RESULTS AND DISCUSSION: Recent evidence, including the 2017 CLOCC trial report make the original interpretation of the analysis suspect. Randomised trials are essential to detect a treatment effect of specific interventions among variable disease progression, selection bias, and multiple and repeated treatments that are inherent in the management of advanced cancer.

Nagapoosanam, A. L., et al. (2019). "Knockdown of human telomerase reverse transcriptase induces apoptosis in cervical cancer cell line." Indian J Med Res **149**(3): 345-353.

Background & objectives: : Human telomerase reverse transcriptase (hTERT) is the catalytic subunit of telomerase enzyme that maintains telomere ends by the addition of telomeric repeats to the ends of chromosomal DNA, and that may generate immortal cancer cells. Hence, the activity of telomerase is raised in cancer cells including cervical cancer. The present study aimed to validate the unique siRNA loaded chitosan coated poly-lactic-co-glycolic acid (PLGA) nanoparticle targeting hTERT mRNA to knock down the expression of hTERT in HeLa cells. Methods: : The siRNA loaded chitosan coated polylactic-co-glycolic acid (PLGA) nanoparticles were synthesized by double emulsion solvent diffusion method. The characterization of nano-formulation was done to determine efficient siRNA delivery. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot were performed to evaluate silencing efficiency of nano-formulation. Results: :Size, zeta potential and encapsulation efficiency of nanoparticles were 249.2 nm, 12.4 mV and 80.5 per cent, respectively. Sustained release of siRNA from prepared nanoparticle was studied for 72 h by ultraviolet method. Staining assays were performed to confirm senescence and apoptosis. Silencing of hTERT mRNA and protein expression were analyzed in HeLa cells by RT-PCR and Western blot. Interpretation & conclusions: : The findings showed that biodegradable chitosan coated PLGA nanoparticles possessed an ability for efficient and successful siRNA delivery. The siRNA-loaded PLGA nanoparticles induced apoptosis in HeLa cells. Further studies need to be done with animal model.

Nakano, K., et al. (1998). "Telomerase activity and expression of telomerase RNA component and telomerase catalytic subunit gene in cervical cancer." Am J Pathol **153**(3): 857-864.

Telomerase, a ribonucleoprotein complex that includes the telomerase RNA component (hTR) and the telomerase catalytic subunit gene (hTERT) product, has been shown to be activated in the majority of cancer tissues and immortalized cells. To study telomerase activation during the progression of cervical cancer, the expression of hTR and hTERT RNAs in tissues of various stages of cervical cancer was analyzed using the in situ hybridization method and compared with proliferative activity as estimated by Ki-67 immunostaining. To test whether expression of these components is reflected in enzyme activity, we determined the levels of the RNAs in cervical cancer and normal tissues and in primary and immortal keratinocytes by reverse transcription-polymerase chain reaction and RNase protection assays and compared the results to telomerase activities as detected by telomeric repeat amplification protocol assay. In situ hybridization signals of hTR and hTERT were present not only in carcinoma tissues but also in normal epidermal layers. In many adenocarcinoma and

fewer squamous cell carcinoma tissues, both signals were focally increased where high proliferative activity was present at the stages of dysplasia/metaplasia, in situ carcinoma, and invasive carcinoma. The level of bTERT, as quantitated by RNase protection assay, was not different between cancer and control tissues or immortal and a subset of primary keratinocytes and did not correlate with telomerase activity. These results indicate that expression of hTR and bTERT is up-regulated in at least a subset of neoplastic cells at an early stage of carcinogenesis and that unidentified factors, such as the modulation or coordination of its protein level with other products, may contribute to the activation of telomerase in cervical cancer.

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.

Newman, N. B., et al. (2020). "Immortal Time Bias in National Cancer Database Studies." Int J Radiat Oncol Biol Phys **106**(1): 5-12.

PURPOSE: In studies evaluating the benefit of adjuvant therapies, immortal time bias (ITB) can affect the results by incorrectly reporting a survival advantage. It does so by including all deceased patients who may have been planned to receive adjuvant therapy within the observation cohort. Given the increase in National Cancer Database (NCDB) analyses evaluating postoperative radiation therapy (PORT) as an adjuvant therapy, we sought to examine how often such studies accounted and adjusted for ITB. METHODS AND MATERIALS: A systematic review was undertaken to search MEDLINE and EMBASE from January 2014 until May 2019 for NCDB studies evaluating PORT. After appropriate exclusion criteria were applied, 60 peer-reviewed manuscripts in which PORT was compared with postoperative observation or maintenance therapy were reviewed. The manuscripts were reviewed to evaluate whether ITB was accounted for, the method with which it was adjusted for, impact factor, year of publication, and whether PORT was beneficial. RESULTS: Of the 60 publications reviewed, 23 studies (38.3%) did not include an adjustment for ITB. Most studies that did adjust for ITB employed a single landmark (LM) time (n = 31), 4 used a sequential landmark analyses, and 2 used a time-dependent Cox model. In 23 of 31 studies (74.2%) that did adjust for ITB via a single LM time, the rationale behind why the specified LM time was chosen was not clearly explained. There was no relationship between adjusting for ITB and year of publication (P = .074) or whether the study was published in a high-impact journal (P = .55). CONCLUSIONS: Studies assessing adjuvant radiation therapy by analyzing the NCDB are susceptible to ITB, which overestimates the effect size of adjuvant therapies and can provide misleading results. Adjusting for this bias is essential for accurate data representation and to better quantify the impact of adjuvant therapies such as PORT.

Nguyen, T., et al. (2018). "The Association Between Statin Use After Diagnosis and Mortality Risk in Patients With Esophageal Cancer: A Retrospective Cohort Study of United States Veterans." Am J Gastroenterol **113**(9): 1310.

OBJECTIVE: Statins may reduce mortality from esophageal cancer by both reducing incidence but also improving prognosis. However, prior studies of statin use and mortality have reported conflicting results. METHODS: We identified 7882 patients with esophageal adenocarcinoma (EAC) and 3868 with esophageal squamous cell carcinoma (ESCC) from the VA Central Cancer Registry diagnosed between 2002 and 2016. We identified prescriptions for statins that were filled before and after cancer diagnosis. Time-dependent Cox regression models were used to calculate hazard ratios (HR) and 95% CIs for mortality risk. We used a time-varying exposure to avoid immortal-time bias and a 3 month lag (following patients from 3 months after cancer diagnosis) to

reduce reverse causation. A sensitivity analysis was conducted varying the lag duration between date of cancer diagnosis and start of follow-up. RESULTS: Statin use after diagnosis was recorded in 27.4% of EAC and 17.1% of ESCC patients. In EAC patients, statin use after diagnosis was associated with a decreased risk of cancer specific (HR, 0.79; 95% CI 0.70-0.88) and all-cause mortality (HR, 0.80; 95% CI 0.74-0.86). Similarly, statin use after diagnosis of ESCC was associated with a decreased risk of cancer specific (HR, 0.77; 95% CI 0.63-0.92) and all-cause mortality (HR, 0.83; 95% CI 0.74-0.95). The inverse associations were attenuated towards the null with a 6-month lag. CONCLUSIONS: Post-diagnosis statin use was associated with reduced mortality in esophageal cancer patients; however, the effect may be partially due to reverse causality as patients with poor prognosis are unlikely to initiate statin therapy.

Nortcliffe, A., et al. (2014). "Synthesis and biological evaluation of nitric oxide-donating analogues of sulindac for prostate cancer treatment." Bioorg Med Chem **22**(2): 756-761.

A series of analogues of the non-steroidal anti-inflammatory drug (NSAID) sulindac 1 were synthesised tethered to nitric oxide (NO) donating functional groups. Sulindac shows antiproliterative effects against immortal PC3 cell lines. It was previously demonstrated that the effect can be enhanced when tethered to NO releasing groups such as nitrate esters, furoxans and sydnonimines. To explore this approach further, a total of fifty-six sulindac-NO analogues were prepared and they were evaluated as NO-releasing cytotoxic agents against prostate cancer (PCa) cell lines. Compounds 1k and 1n exhibited significant cytotoxic with IC50 values of 6.1+/-4.1 and 12.1+/-3.2muM, respectively, coupled with observed nitric oxide release.

Okusa, Y., et al. (2000). "Clinical significance of telomerase activity in biopsy specimens of gastric cancer." J Clin Gastroenterol **30**(1): 61-63.

Telomerase has been reported to be activated in most immortal cells and human cancers. The purpose of this study was to assess the clinical significance of telomerase activity in biopsy specimens of gastric cancer. Telomerase activity in endoscopic biopsy specimens obtained preoperatively from 31 patients with gastric cancer was determined semiquantitatively using the telomeric repeat amplification protocol assay, a polymerase chain reaction-based assay. Cancer tissues had significantly higher telomerase activity than adjacent normal tissues (13.9 +/-2.0% vs. 7.0 +/- 0.8%; $p < 0.05$). The ratio of the telomerase activity in cancer tissues to that in normal tissues (telomerase index) was significantly higher in tumors invading the proper muscle layer or deeper or in tumors with moderate or marked lymphatic invasion than in tumors without these invasive factors (4.7 +/- 1.4 vs. 1.1 +/- 0.1 for depth of invasion and 4.4 +/- 1.3 vs. 1.2 +/- 0.2 for lymphatic invasion; $p < 0.05$ for both). These results suggest that the analysis of telomerase activity in biopsy specimens might contribute to preoperative assessment of the invasive activity or stage of gastric cancer.

Okusa, Y., et al. (1998). "Correlation between telomerase activity and DNA ploidy in gastric cancer." Oncology **55**(3): 258-264.

Telomerase has been reported to be activated in most immortal cells and human cancers. In the present study, we assessed the correlation between telomerase activity and cellular DNA ploidy level in gastric cancer. Telomerase activity was determined semiquantitatively using the telomeric repeat amplification protocol assay, a polymerase-chain-reaction-based assay, in surgical specimens of primary tumors obtained from 36 patients with gastric cancer. No correlation was observed between telomerase activity and the proliferating cell nuclear antigen labeling index. In contrast, a positive linear correlation was observed between telomerase activity and the DNA index ($r = 0.59$; $p < 0.01$). Tumor cells with aneuploid patterns showed higher telomerase activity than those with diploid patterns (27.6+/-5.8 vs. 5.8+/-1.1%; $p < 0.01$). Telomerase activity of tumors with liver metastases was significantly higher than activity of those without metastases (34.5+/-16.6 vs. 11.8+/-2.4; $p < 0.05$). There was a trend toward a lower survival rate in 9 patients with a telomerase activity of 20% or higher compared to 27 patients with telomerase activity lower than 20%. These results suggest that the telomerase activity of gastric cancer tissue may reflect the malignant potential of the tumor.

Osborne, D. L. and R. Hames (2014). "A life history perspective on skin cancer and the evolution of skin pigmentation." Am J Phys Anthropol **153**(1): 1-8.

The ancestral state of human skin pigmentation evolved in response to high ultraviolet radiation (UVR) stress. Some argue that pigmentation evolved to limit folate photolysis, therein limiting neural tube defects. Pigmentation also protects against sunburn which decreases the efficiency of sweating and potentiates skin infection. Pigmentation increases the efficacy of skin as a barrier to infection. Skin cancer has been rejected or minimized as a selective pressure because it is believed to have little or no effect on mortality during reproductive years. This argument ignores evidence of human longevity as a derived life history trait and the adaptive value of investment in offspring and kin, particularly during the

post-reproductive lifespan. Opponents argue that lifespan in prehistoric hunter-gatherers was too short to be relevant to the evolution of skin pigmentation. This argument is flawed in that it relies on estimates of longevity at birth rather than adolescence. When appropriate estimates are used, it is clear that human longevity has a deep evolutionary history. We use a life history perspective to demonstrate the value of skin pigmentation as an adaptation to skin cancer with the following points: UVR exposure increases dysregulation of gene expression in skin cells leading to immortal cell lines; cutaneous malignant melanoma (CMM) affects individuals throughout reproductive years; and lifespan was longer than has previously been acknowledged, providing the opportunity for kin selection. This hypothesis is not at odds with the folate or barrier hypotheses. We stress that the evolution of skin pigmentation is complex and is an ongoing process.

Ota, S., et al. (2009). "The role of senescence and prosurvival signaling in controlling the oncogenic activity of FGFR2 mutants associated with cancer and birth defects." Hum Mol Genet **18**(14): 2609-2621.

Mutations in fibroblast growth factor receptors (FGFRs) cause human birth defect syndromes and are associated with a variety of cancers. Although forced expression of mutant activated FGFRs has been shown to oncogenically transform some immortal cell types, their activity in primary cells remains unclear. Here, we show that birth defect and cancer-associated FGFR2 mutants promote DNA-damage signaling and p53-dependent senescence in primary mouse and human cells. Senescence promoted by FGFR mutants was associated with downregulation of c-Myc and forced expression of c-Myc facilitated senescence escape. Whereas c-Myc expression facilitated senescence bypass, mutant FGFR2 signaling suppressed c-Myc-dependent apoptosis and led to oncogenic transformation. Cells transformed by coexpression of a constitutively activated FGFR2 mutant plus c-Myc appeared to be become highly addicted to FGFR-dependent prosurvival activities, as small molecule inhibition of FGFR signaling resulted in robust p53-dependent apoptosis. Our data suggest that senescence-promoting activities of mutant FGFRs may normally limit their oncogenic potential and may be relevant to their ability to disrupt morphogenesis and cause birth defects. Our results also raise the possibility that cancers originating through a combination of constitutive FGFR activation and deregulated Myc expression may be particularly sensitive to small molecule inhibitors of FGF receptors.

Ozen, M., et al. (1998). "Telomeric DNA: marker for human prostate cancer development?" Prostate **36**(4): 264-271.

BACKGROUND: Telomeres that protect chromosomes at both ends are shortened with each somatic cell division through replication-dependent sequence loss at DNA termini. The chromosomes with shortened telomeres tend to become unstable, leading to cell death. Due largely to reactivation/upregulation of telomerase, a ribonucleoprotein that adds nucleotide sequences onto chromosome ends, cancer cells become immortal and neoplastically transformed. METHODS: The purpose of the present study was to study three newly established human prostate cancer cell lines and three prostate-derived fibroblastic cell cultures at different passages for telomeric DNA signal intensity, telomeric restriction fragment length (TRFL), telomerase activity, and spontaneous apoptotic index. RESULTS: Compared with the three fibroblastic cell cultures, the three new prostate cancer cell lines showed: 1) telomerase activity, 2) stronger telomeric signals, 3) relatively longer TRFLs, and 4) much lower apoptotic indices. On the other hand, three fibroblastic cell cultures showed: 1) no telomerase activity, 2) weaker telomeric signals, 3) shorter TRFLs (fibroblasts derived from surrounding tissue of prostate tumor showed intermediate TRFLs), and 4) comparatively higher apoptotic indices. CONCLUSIONS: Based on these results, we conclude that telomeric DNA signal intensity, TRFL, and telomerase activity can be used to distinguish prostate cancer cells from adjacent fibroblasts.

Palmqvist, R., et al. (2005). "hTERT gene copy number is not associated with hTERT RNA expression or telomerase activity in colorectal cancer." Int J Cancer **116**(3): 395-400.

In a majority of malignant human tumors telomerase activity can be detected, suggesting an immortal phenotype. Expression of the reverse transcriptase subunit, hTERT, in the human telomerase complex is required for telomerase activity. The regulation of hTERT, from gene level to a fully functional protein, is still a poorly understood process. Increased copy number of the hTERT gene has been demonstrated in a significant portion of established cell lines and tumors of different origin but its relevance for telomerase activity levels is unclear. In the present study, we examined the hTERT gene copy number using fluorescence in situ hybridization (FISH) in samples from 64 colorectal carcinomas and an increased copy number (> or = 3 hTERT gene copies/nucleus) was observed in 31 cases (48%). No statistical association existed between hTERT gene copy number and hTERT RNA expression or telomerase activity. However, a significant relationship was found between an increase in hTERT gene copy number and p53 protein accumulation (p = 0.002) and

aneuploidy (p = 0.036). Only 4 tumors showed microsatellite instability, 3 of which had a normal hTERT gene copy number. The data indicated that the increased copy number of the hTERT gene in colorectal carcinoma was a result of genomic instability with no obvious consequence for telomerase activity levels.

Pandita, T. K., et al. (1996). "Chromosome end-to-end associations and telomerase activity during cancer progression in human cells after treatment with alpha-particles simulating radon progeny." Oncogene **13**(7): 1423-1430.

Chromosome end-to-end associations seen at metaphase involve telomeres and are commonly observed in cells derived from individuals with ataxia telangiectasia and most types of human tumors. The associations may arise because of short telomeres and/or alterations of chromatin structure. There is a growing consensus that telomere length is stabilized by the activity of telomerase in immortal cells; however, it is not clear why some immortal cells display chromosome end-to-end associations. In the present study we evaluated chromosome end-to-end associations, telomere length and telomerase activity with the tumorigenic status of human bronchial epithelial cells immortalized with human papillomavirus. Oncogenic transformation was initiated using radon simulated alpha-particles and cells evaluated as primary, secondary and metastatic transformants. The fewest chromosome end associations and lowest telomerase activity were observed in the parental immortalized cells. However, increased levels of telomerase activity were detected in alpha-particle survivors while robust telomerase activity was seen in the tumorigenic cell lines. The tumorigenic cells that were telomerase positive and had the highest frequency of cells with chromosome end-to-end associations were also metastatic. No correlation was found between telomere length and the different stages of carcinogenicity.

Park, H. S., et al. (2015). "Prophylactic Cranial Irradiation for Patients With Locally Advanced Non-Small-Cell Lung Cancer at High Risk for Brain Metastases." Clin Lung Cancer **16**(4): 292-297.

BACKGROUND: Although there is no proven survival benefit of prophylactic cranial irradiation (PCI) for patients with locally advanced (LA) non-small-cell lung cancer (NSCLC), some speculate that PCI might be helpful for certain subpopulations at higher risk of brain metastases (< 60 years, adenocarcinoma, or stage IIIB). In this study we evaluated the effect of PCI on survival among these high-risk LA-NSCLC patients on a national scale. MATERIALS AND METHODS: Using the Surveillance, Epidemiology, and End Results database, we included all adult patients with primary stage III NSCLC, diagnosed from 1988 to 1997 (years during which PCI was recorded) with follow-up until 2008. The Kaplan-Meier estimator, log-rank test, and Cox proportional hazard regression were used to evaluate the survival effect of PCI. Sequential landmark analysis excluding patients from 1 to 6 months after diagnosis was used to account for immortal time bias. RESULTS: A total of 17,852 patients were included in the analysis, among whom 326 (1.8%) received PCI. Patients younger than 60 years and those with adenocarcinoma were significantly more likely to receive PCI. After adjustment for available covariates, there was no statistically significant survival difference between PCI and non-PCI patients (hazard ratio, 1.04; 95% confidence interval, 0.93-1.16). Similar results were found in all subgroup analyses of high-risk patients. Sequential landmark analysis suggested a potential survival detriment associated with PCI when analyzing only patients who survived at least 3 months after diagnosis. CONCLUSION: Our population-based analysis suggested no overall survival benefit of PCI for LA-NSCLC patients, even among a group of patients who were at higher risk for brain metastases.

Park, Y. J., et al. (2014). "Human telomerase reverse transcriptase is a promising target for cancer inhibition in squamous cell carcinomas." Anticancer Res **34**(11): 6389-6395.

BACKGROUND/AIM: The present study aimed to investigate whether the down-regulation of human telomerase reverse transcriptase (hTERT) may induce an anti-invasive effect in oral squamous cell cancer cell lines. MATERIALS AND METHODS: A genetically-engineered squamous carcinoma cell line overexpressing hTERT in immortalized oral keratinocytes transfected by human papilloma virus (HPV)-16 E6/E7 (IHOK) was used. In vivo tumorigenicity was examined using an orthotopic xenograft model of nude mice. For evaluating anti-invasive activity by knockdown of hTERT expression, transwell invasion assay and real-time polymerase chain reaction (PCR) for matrix metalloproteinases (MMP) were employed. RESULTS: The down-regulation of hTERT expression reduced the invasive activity and MMP expression. This result was re-confirmed in the HSC3 oral squamous carcinoma cell line. CONCLUSION: Targeting hTERT may lead to novel therapeutic approaches.

Parkinson, E. K., et al. (2000). "Replicative senescence as a barrier to human cancer." Biochem Soc Trans **28**(2): 226-233.

There is evidence that one critically short

telomere may be recognized as DNA damage and, as a consequence, induce a p53/p21WAF- and p16INK4A-dependent G1 cell cycle checkpoint to cause senescence. Additionally, senescence via a p53- and p16(INK4A)-dependent mechanism can be induced by the over- or under-stimulation of certain signalling pathways that are involved in cancer. Central to this alternative senescence mechanism is the p14ARF protein, which connects oncogene activation, but not DNA damage, to p53 activation and senescence. We find that immortal keratinocytes almost invariably have dysfunctional p53 and p16 and have high levels of telomerase, but very often express a wild-type p14(ARF). Furthermore, when normal keratinocytes senesce they show a striking elevation of p16 protein, but not of p14(ARF) or its downstream targets p53 and p21(WAF). These results suggest that p16, rather than p14(ARF), is the more important gene in human keratinocyte senescence, but do not exclude a co-operative role for p14(ARF), perhaps in the induction of senescence by activated oncogenes in neoplasia. Regardless of mechanism, these results suggest that replicative senescence acts as a barrier to human cancer development.

Parkinson, K. E. (2005). "Telomerase as a target for cancer therapy." Curr Opin Investig Drugs **6**(6): 605-610.

Normal human somatic cells undergo telomeric attrition and replicative senescence because of inadequate levels of telomerase; however, most immortal cancer cells cope with this by deregulating telomerase. Inhibiting telomerase causes renewed telomeric attrition and eventually highly specific death in cancer cells that express the enzyme. However, most cancer cells undergo many cell divisions before they die, opening the way for acquired drug resistance. Recent attempts to solve this problem include the development of drugs that are more potent catalytic inhibitors, that deny telomerase access to the telomere in situ, or affect telomere structure. Combinations of these approaches may ultimately produce the best clinical results.

Pathak, R., et al. (2020). "Association of Survival With Adjuvant Chemotherapy Among Patients With Early-Stage Non-Small Cell Lung Cancer With vs Without High-Risk Clinicopathologic Features." JAMA Oncol **6**(11): 1741-1750.

Importance: Tumor size larger than 4 cm is accepted as an indication for adjuvant chemotherapy in patients with node-negative non-small cell lung cancer (NSCLC). Treatment guidelines suggest that high-risk features are also associated with the efficacy of adjuvant chemotherapy among patients with early-stage NSCLC, yet this association is understudied. Objective: To assess the association between adjuvant chemotherapy and survival in the presence and absence of high-risk pathologic features in patients with node-negative early-stage NSCLC. Design, Setting, and Participants: This retrospective cohort study using data from the National Cancer Database included 50814 treatment-naive patients with a completely resected node-negative NSCLC diagnosed between January 1, 2010, and December 31, 2015. The study was limited to patients who survived at least 6 weeks after surgery (ie, estimated median time to initiate adjuvant chemotherapy after surgery) to mitigate immortal time bias. Statistical analysis was performed from December 1, 2018, to February 29, 2020. Exposures: Adjuvant chemotherapy use vs observation, stratified according to the presence or absence of high-risk pathologic features (visceral pleural invasion, lymphovascular invasion, and high-grade histologic findings), sublobar surgery, and tumor size. Main Outcomes and Measures: The association of high-risk pathologic features with survival after adjuvant chemotherapy vs observation was evaluated using Cox proportional hazards regression models. Results: Overall, 50814 eligible patients with NSCLC (27365 women [53.9%]; mean [SD] age, 67.4 [9.5] years]) were identified, including 4220 (8.3%) who received adjuvant chemotherapy and 46594 (91.7%) who did not receive adjuvant chemotherapy. Among patients with tumors 3 cm or smaller, chemotherapy was not associated with improved survival (hazard ratio [HR], 1.10; 95% CI, 0.96-1.26; P = .17). For patients with tumors larger than 3 cm to 4 cm, adjuvant chemotherapy was associated with a survival benefit among patients who underwent sublobar surgery (HR, 0.72; 95% CI, 0.56-0.93; P = .004). For tumors larger than 4 cm to 5 cm, a survival benefit was associated with adjuvant chemotherapy only in patients with at least 1 high-risk pathologic feature (HR, 0.67; 95% CI, 0.56-0.80; P = .02). For tumors larger than 5 cm, adjuvant chemotherapy was associated with a survival benefit irrespective of the presence of high-risk pathologic features (HR, 0.75; 95% CI, 0.61-0.91; P = .004). Conclusions and Relevance: In this cohort study, tumor size alone was not associated with improved efficacy of adjuvant chemotherapy in patients with early-stage (node-negative) NSCLC. High-risk clinicopathologic features and tumor size should be considered simultaneously when evaluating patients with early-stage NSCLC for adjuvant chemotherapy.

Paules, R. S., et al. (1995). "Defective G2 checkpoint function in cells from individuals with familial cancer syndromes." Cancer Res **55**(8): 1763-1773.

The early events in the G2 checkpoint response to ionizing radiation (IR) were analyzed in

diploid normal human fibroblasts (NHFs) and fibroblasts from patients with two heritable cancer syndromes. Exposure to gamma-radiation of asynchronously growing NHFs resulted in a rapid reduction in the number of cells in mitosis (G2 delay) and was accompanied by a quantitatively similar reduction in the p34CDC2/cyclin B in vitro histone H1 kinase activity as compared with sham-treated controls. This G2 delay was strong by 1 h following exposure to IR, maximal by 2 h, and was accompanied by an accumulation of tyrosine-phosphorylated p34CDC2 molecules. In contrast, fibroblasts from individuals with ataxia telangiectasia displayed significantly less reduction of the mitotic index or histone H1 kinase activity after IR. Low passage fibroblasts from individuals with Li-Fraumeni syndrome having one wild-type and one mutated p53 allele were similar to NHFs in their immediate G2 checkpoint response to IR, as were NHFs expressing the human papilloma virus type 16 E6 gene product (functionally inactivating p53) and low passage cells from p53-deficient mouse embryos. However, the p53-deficient fibroblasts were genomically unstable and became defective in their early G2 checkpoint response to IR. Furthermore, immortal Li-Fraumeni syndrome fibroblasts lacking wild-type p53 displayed an attenuated G2 checkpoint response. These results link the early events in G2 checkpoint response to IR in NHFs with a rapid inhibition of p34CDC2/cyclin B protein kinase activity and demonstrate that while not required for this immediate G2 delay, lack of p53 can lead to subsequent genetic alterations that result in defective G2 checkpoint function.

Perhavec, A., et al. (2008). "The hTERT mRNA in plasma samples of early breast cancer patients, non-cancer patients and healthy individuals." Neoplasma **55**(6): 549-554.

One of the most important changes, which make cancer cells immortal, is reactivation of the telomerase enzyme. Human telomerase is composed of at least two subunits, hTERT and hTR. Many investigators have already detected telomerase mRNA in bodily fluids. The first aim of our study was to find out if there is a difference in the appearance frequency of detectable hTERT mRNA in plasma of early breast cancer patients, non-cancer patients and healthy individuals. The second aim was to determine whether surgical removal of the tumor affects the presence of hTERT mRNA in plasma of early breast cancer patients. 87 patients with early breast cancer, 22 non-cancer patients and 21 healthy individuals were included in the study. From early breast cancer patients, two blood samples were collected, the first prior and the second 24 hours after the surgical removal of the tumor. From other individuals one blood sample was collected. The presence or absence of hTERT mRNA was determined from all blood samples. 47% of early breast cancer patients, 32% of non-cancer patients and 5% of healthy individuals tested positive for the presence of hTERT mRNA in plasma. The difference between early breast cancer patients and healthy individuals was statistically significant ($p<0,001$). Among early breast cancer patients, 26% were positive for the presence of plasma hTERT mRNA before and after the surgical removal of the tumor, 21% were positive before and negative after, 36% were negative before and after and 17% were negative before and positive after the surgical removal of the tumor. In conclusion, we found statistically significant difference of hTERT mRNA presence in plasma of early breast cancer patients when compared to healthy individuals. Second, we found that hTERT mRNA in plasma of early breast cancer patients is affected by the surgical removal of the tumor.

Perry, P. J., et al. (2001). "Telomerase inhibitors for the treatment of cancer: the current perspective." Expert Opin Investig Drugs **10**(12): 2141-2156.

Telomerase is a holoenzyme responsible for the maintenance of telomeres, the protein-nucleic acid complexes at the ends of eukaryotic chromosomes that serve to maintain chromosomal stability and integrity. Telomerase activity is essential for the sustained proliferation of most immortal cells, including cancer cells. Since the discovery that telomerase activity is detected in 85-90% of all human tumours and tumour-derived cell lines but not in most normal somatic cells, telomerase has become the focus of much attention as a novel and potentially highly-specific target for the development of new anticancer chemotherapeutics. Herein we review the current perspective for the development of telomerase inhibitors as cancer chemotherapeutics. These include antisense strategies, reverse transcriptase inhibitors and compounds capable of interacting with high-order telomeric DNA tetraplex ("G-quadruplex") structures, so as to prevent enzyme access to the necessary linear telomere substrate. Critical appraisal of each individual approach is provided together with highlighted areas of likely future development.

Perry, P. J. and T. C. Jenkins (1999). "Recent advances in the development of telomerase inhibitors for the treatment of cancer." Expert Opin Investig Drugs **8**(12): 1981-2008.

Telomerase is an holoenzyme responsible for the maintenance of telomeres, the protein-nucleic acid structures which exist at the ends of eukaryotic chromosomes that serve to protect chromosomal stability and integrity. Telomerase activity is essential for the sustained proliferation of most immortal cells,

including cancer cells. Since the discovery that telomerase activity is expressed in 85 - 90% of all human tumours and tumour-derived cell lines but not in most normal somatic cells, telomerase has become the focus of much attention as a novel and potentially highly-specific target for the development of new anticancer chemotherapeutics. Herein we review recent advances in the development of telomerase inhibitors for the treatment of cancer. To date, these have included antisense strategies, reverse transcriptase inhibitors and compounds capable of interacting with high-order telomeric DNA tetraplex ('G-quadruplex') structures to prevent enzyme access to the necessary linear telomere substrate. In addition, a number of telomerase-inhibitory therapies have been shown to synergistically enhance the effects of clinically-established anticancer drugs. Critical appraisal of each individual approach is provided, together with highlighted areas of likely future development. We also review recent developments in telomere and telomerase biology, of which a more detailed understanding would be essential in order to further develop the present classes of telomerase inhibitors into viable, clinically applicable therapies.

Petersen, O. W., et al. (2003). "Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma." Am J Pathol **162**(2): 391-402.

A breast carcinoma biopsy showed cytochemical evidence of epithelial mesenchymal transition and an alpha-smooth muscle actin-positive stromal reaction. To study the lineage, and the nature of the cells in the stromal reaction, we derived a novel cell line, HBFL-1, from the explanted biopsy. HBFL-1 cells are immortal and exhibit a shared non-random X-chromosome inactivation pattern with the epithelial tumor of origin. Yet they closely resemble normal, finite-life-span fibroblasts by morphology, lack of tumor formation in nude mice, marker expression profile, protein pattern using two-dimensional gel electrophoresis and the ability to undergo myofibroblast conversion. HBFL-1 interacts reciprocally with tumor cells in collagen gel to induce activation of MMP2, leading to tumor-like behavior of epithelial colonies. In vivo, HBFL-1 cells resembled normal-derived myofibroblasts and conferred a significant 3.5- to 7-fold increase in MCF-7 tumor size in nude mice. However, that they were indeed not normal fibroblasts was revealed by residual keratin expression and formation of epithelial microfoci in a reconstituted basement membrane and in nude mice. We conclude that breast cancer can generate its own nonmalignant stroma and that one function for this is that of a reciprocal interaction with epithelial tumor cells to facilitate tumor growth.

Pfitzenmaier, J., et al. (2006). "Telomerase activity in disseminated prostate cancer cells." BJU Int **97**(6): 1309-1313.

OBJECTIVE: To analyse telomerase activity in disseminated prostate cancer cells isolated from bone marrow aspirates taken from men with localized prostate cancer before radical prostatectomy (RP). PATIENTS AND METHODS: Disseminated epithelial prostate cancer cells were isolated from bone marrow aspirates from 69 men with localized prostate cancer before RP, by magnetic column-chromatography enrichment, followed by isolation of fluorescently labelled epithelial cells by micropipetting. We used pools of 10 non-epithelial bone marrow cells after tumour cell enrichment as control samples. These pure cell pools were tested for the presence of telomerase activity. RESULTS: In all, 49 of the patient samples contained disseminated prostate cancer cells. Homogeneous pools of 10 cells were obtained from 35 of these; 49% of the 35 specimens showed telomerase activity, whereas all five control samples did not. Telomerase activity in the 35 samples was not significantly associated with Gleason score, preoperative prostate-specific antigen level, tumour stage, or surgical margin status. Follow-up is continuing to assess an association with disease recurrence. CONCLUSION: This work shows the feasibility of isolating disseminated cancer cells for analysing individual or pooled cells. Compared to tissue staining, where telomerase is detected in 80-90% of samples, we found lower rates of telomerase activity in the disseminated tumour cells (49%). Telomerase-negative cells might provide information about cell dormancy, as telomerase is a marker of cell proliferation in immortal and cancer cells. Telomerase-positive cells might predict early disease recurrence, but a longer follow-up is needed to test this possibility.

Plumb, J. A., et al. (2001). "Telomerase-specific suicide gene therapy vectors expressing bacterial nitroreductase sensitize human cancer cells to the pro-drug CB1954." Oncogene **20**(53): 7797-7803.

Telomerase activation is considered to be a critical step in cancer progression due to its role in cellular immortalization. The prevalence of telomerase expression in human cancers makes it an attractive candidate for new mechanism-based targets for cancer therapy. The selective killing of cancer cells can be achieved by gene-directed enzyme pro-drug therapy (GDEPT). In this study we have tested the feasibility of using the transcriptional regulatory sequences from the hTERT and hTR genes to regulate expression of the bacterial nitroreductase enzyme in combination with the pro-drug CB1954 in a suicide gene therapy strategy. hTERT and hTR promoter activity was

compared in a panel of 10 cell lines and showed a wide distribution in activity; low activity was observed in normal cells and telomerase-negative immortal ALT cell lines, with up to 300-fold higher activity observed in telomerase positive cancer lines. Placing the nitroreductase gene under the control of the telomerase gene promoters sensitized cancer cells in tissue culture to the pro-drug CB1954 and promoter activity was predictive of sensitization to the pro-drug (2-20-fold sensitization), with cell death restricted to lines exhibiting high levels of promoter activity. The in vivo relevance of these data was tested using two xenograft models (C33a and GLC4 cells). Significant tumour reduction was seen with both telomerase promoters and the promoter-specific patterns of sensitization observed in tissue culture were retained in xenograft models. Thus, telomerase-specific suicide gene therapy vectors expressing bacterial nitroreductase sensitize human cancer cells to the pro-drug CB1954.

Prime, S. S., et al. (1997). "Early genetic and functional events in the pathogenesis of oral cancer." Radiat Oncol Investig **5**(3): 93-96.

Oral squamous cell carcinoma (SCC) is a major world health problem, but the changes leading to the development of malignancy remain essentially unknown. Early changes are thought to include the loss of tumour suppressor genes on chromosomes 3p, 9p, and 17p. Although what genes are involved on chromosome 3 remains speculative, p16 (9p21) and p53 (17p13) are inactivated in a high proportion of oral dysplastic lesions and carcinomas. SCC-derived cell lines are immortal, have decreased growth requirements in vitro, and show a variable capacity to form tumours in athymic mice. Normal oral keratinocytes and cells from potentially malignant lesions invariably senesce at early culture passage, have strict growth requirements in vitro, and are nontumorigenic in vivo. By contrast to normal oral keratinocytes, cells from potentially malignant lesions are defective in their capacity to terminally differentiate in suspension culture. Loss of cellular senescence and gain of the immortal phenotype is associated with inactivation of p16 and p53.

Qu, Y., et al. (2008). "Over-expression of FRZB in gastric cancer cell suppresses proliferation and induces differentiation." J Cancer Res Clin Oncol **134**(3): 353-364.

PURPOSE: Frizzled motif associated with bone development (FRZB) was a member of secreted frizzled related proteins (sFRPs) family. Previous evidences showed that FRZB played role in embryogenesis and diseases such as osteoarthritis and prostate cancer. The purpose of our study is to clarify the role of FRZB in gastric cancer cell proliferation and differentiation. METHODS: The expression of FRZB in gastric cancer tissues were detected by immunohistochemistry. The expression of FRZB in eight gastric cancer cell lines and one immortal gastric epithelial cell GES-1 were detected by western blotting and real-time quantitative PCR. To investigate the role of over-expressed FRZB in gastric cancer cells, FRZB/pcDNA3.1 plasmid was constructed and transfected into gastric cancer cell line SGC7901. The changes of biological features in these stable transfectants were examined. RESULTS: FRZB was highly expressed in gastric cancer (90%), intestinal metaplasia (100%) and gastric dysplasia (90%), but no or just weakly (3/40) expressed in normal gastric mucosa. FRZB staining was stronger in intestinal-type gastric cancer tissues than that in diffuse-type ones and was positive correlated with differentiation grade. The expression of FRZB in eight gastric cancer cell lines was higher than in GES-1. Over-expressed FRZB inhibited cell proliferation in vitro and in vivo which was first caused by prolonged cell division progression in G2/M phase, and second by higher sensitivity to apoptotic inducing factors and spontaneous apoptosis. Our findings gave evidences that FRZB suppressed gastric cancer cell proliferation and modulated the balance between proliferation and differentiation in gastric cancer.

Quinn, T. J., et al. (2020). "Patterns of care and outcomes for adjuvant treatment of pT3N0 rectal cancer using the National Cancer Database." J Gastrointest Oncol **11**(1): 1-12.

Background: The standard of care in locally advanced rectal cancer is preoperative chemoradiation followed by surgical resection. However, the optimal treatment paradigm is currently controversial for patients with pathological T3N0 (pT3N0) in the era of total mesorectal excision (TME). Given the paucity of data, we conducted an analysis using the National Cancer Database (NCDB) to identify patterns of care and outcomes. Methods: We utilized the NCDB to identify 7,836 non-metastatic, pT3N0 rectal cancer patients who did not receive neoadjuvant therapy from 2004-2014. Univariate and multivariable analysis for factors affecting treatment selection were completed using logistic regression. Overall survival (OS) analyses were completed using Cox regression modeling, incorporating propensity scores with inverse probability of treatment weighting (IPTW) and conditional landmark analysis. Results: There was a significant improvement in OS in patients receiving adjuvant chemotherapy ($P<0.01$) or radiotherapy (RT) with chemotherapy ($P<0.01$) vs. observation alone. There was no significant difference between RT vs. observation ($P=0.54$) and chemotherapy vs. chemotherapy with RT cohorts ($P=0.15$). Multivariable

analysis showed age, gender, race, insurance status, income, Charlson-Deyo Comorbidity Condition (CDCC) score, facility location, grade, surgical margin, RT, and chemotherapy to be statistically significant predictors of OS. After correcting for indication and immortal time biases, chemotherapy, with or without RT, improved OS compared with observation [hazard ratio (HR) 0.48, P<0.001]. This benefit was maintained in the margin negative cohort. Conclusions: Practice patterns vary in the management of pT3N0 rectal cancer patients. This analysis suggests that the use of adjuvant therapy, particularly adjuvant chemotherapy with or without RT, appears to improve OS.

Rahbari, N. N., et al. (2019). "Time of Metastasis and Outcome in Colorectal Cancer." Ann Surg **269**(3): 494-502.

OBJECTIVE: The aim of this study was to evaluate outcomes of metastases at various time intervals after colorectal cancer (CRC) diagnosis. BACKGROUND: Earlier studies have indicated a short time interval between CRC diagnosis and distant metastases to be associated with poor prognosis. The majority of studies assessed outcome from CRC diagnosis or metastasis resection rather than from metastasis diagnosis and might be subject to immortal time bias. METHODS: Patients in the population-based DACHS study were stratified: metastases at/within 1 month (immediate), 2 to 6 months (early), 7 to 12 months (intermediate), and >12 months (late) after CRC diagnosis. The primary endpoint was overall survival (OS) from metastasis diagnosis. Cox proportional hazards regression models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CI). HRs were adjusted for important confounders and immortal time. RESULTS: A total of 1027 patients were included. T4 (P < 0.0001) and node-positive tumors (P < 0.0001) were more frequent in the immediate group. Lung metastases (P < 0.0001) and single-site metastases (P < 0.0001) were more prevalent in the late group. In multivariable analysis, immediate metastases were not associated with poor OS compared to metastases at later time points (late vs immediate: HR 1.21; 95% CI, 0.98-1.48). Subgroup analyses revealed poor OS of late versus immediate metastases for females (1.45; 1.08-1.96), proximal colon cancer (1.54; 1.09-2.16), and N0 (1.46; 1.00-2.12) or N1 disease (1.88; 1.17-3.05). CONCLUSIONS: Immediate or early metastases are not associated with unfavorable outcome compared to late metastases. These findings challenge the current notion of poor outcome for CRC with immediate or early metastases.

Rane, N. S., et al. (2011). "Restoration of senescence in breast and ovarian cancer cells following the transfer of the YAC carrying SEN6A gene located at 6q16.3." Cancer Genomics Proteomics **8**(5): 227-233.

We previously located a senescence gene locus (SEN6A), at chromosome 6q14-21 by a functional strategy using chromosome transfer into immortal ovarian tumor cells. To further elucidate the SEN6A locus, intact chromosome 6 or 6q was transferred into rat ovarian tumor cells and a panel of immortal revertant clones of senescent cells was generated. The panel of independent colonies as well as mixed populations of revertant cells was analyzed for the presence or absence of chromosome 6 specific markers. These investigations led to the identification of a fine deletion of approximately 1cM at chromosomal interval 6q16.3. A contiguous stretch containing five yeast artificial chromosome (YAC) clones was constructed across the deleted region. The non-chimeric YAC clones were retrofitted and transferred into mouse A9 cells by spheroplast fusion to generate YAC/A9 hybrids. YAC DNA present in YAC/A9 hybrids was subsequently transferred by microcell fusion into immortal tumor cells, and the hybrid cells were characterized for their senescence phenotype. Using this functional strategy, the transfer of YAC clone 966b10 was shown to restore senescence in both rat and human ovarian and breast tumor cells. Our results demonstrate that the SEN6A gene is carried on a 1 Mb YAC, 966b10, which maps at 6q16.3.

Rao, Y. J., et al. (2017). "Patterns of care and treatment outcomes of patients with Craniopharyngioma in the national cancer database." J Neurooncol **132**(1): 109-117.

To investigate the patterns of care and outcomes in patients with craniopharyngioma in the National Cancer Data Base (NCDB). This study included 697 patients (166 pediatric and 531 adult cases) treated for craniopharyngioma between 2004 and 2012 in the NCDB. Adjuvant radiotherapy (RT) was defined if within 6 months of surgery. Limited surgery (LS) was defined as biopsy or subtotal resection. Proportional-hazards models were used to evaluate associations between covariates and overall survival (OS). A time-dependent analysis of RT was performed to account for early deaths after surgery. Median follow-up was 46 months. Overall, 21% of patients received adjuvant RT. Of patients with known surgical extent (n = 195), 71% had LS. Utilization of adjuvant RT increased from 18% in 2004-2007 to 24% in 2008-2012. Patterns of care regarding adjuvant RT or LS were not significantly different between adult and pediatric patients. Tumor size, low comorbidity, and LS were associated with increased utilization of adjuvant RT. The 5-year OS among patients treated

with LS, LS+RT, and gross total resection were 75, 85, and 82% (p = 0.02). On multivariate analysis of the 195 patients with known surgical extent, LS+RT was associated with improved OS compared to LS (HR 0.22, 95% CI 0.05-0.99, p = 0.04), but was not significant when early deaths (<2 months from surgery) were removed to adjust for immortal-time bias. Medical practice regarding surgical approach and adjuvant RT are similar for pediatric and adult craniopharyngiomas. Immortal-time bias may confound assessment of OS for adjuvant RT. Prospective studies comparing adjuvant RT versus observation after LS are warranted.

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.

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.

Reddel, R. R. (2014). "Telomere maintenance mechanisms in cancer: clinical implications." Curr Pharm Des **20**(41): 6361-6374.

The presence of immortal cell populations with an up-regulated telomere maintenance mechanism (TMM) is an almost universal characteristic of cancers, whereas normal somatic cells are unable to prevent proliferation-associated telomere shortening and have a limited proliferative potential. TMMs and related aspects of telomere structure and function therefore appear to be ideal targets for the development of anticancer therapeutics. Such treatments would be targeted to a specific cancer-related molecular abnormality, and also be broad-spectrum in that they would be expected to be potentially applicable to most cancers. However, the telomere biology of normal and malignant human cells is a relatively young research field with large numbers of unanswered questions, so the optimal design of TMM-targeted therapeutic approaches remains unclear. This review outlines the opportunities and challenges presented by telomeres and TMMs for clinical management of cancer.

Reddy, D. E., et al. (2000). "Identification of a YAC from 16q24 carrying a senescence gene for breast cancer cells." Oncogene **19**(2): 217-222.

We have identified a 360 kb YAC that carries a cell senescence gene, SEN16. In our earlier studies, we localized SEN16 within a genetic interval of 3 - 7 cM at 16q24.3. Six overlapping YACs spanning the chromosomal region of senescence activity, were assembled in a contig. Candidate YACs, identified by the markers located in the vicinity of SEN16, were retrofitted to introduce a neo selectable marker. Retrofitted YACs were first transferred into mouse A9

cells to generate A9/YAC hybrids. YAC DNA present in A9/YAC hybrids was further transferred by microcell fusion into immortal cell lines derived from human and rat mammary tumors. YAC d792t2 restored senescence in both human and rat mammary tumor cell lines, while an unrelated YAC from chromosome 6q had no senescence activity.

Rha, S. Y., et al. (1999). "Changes of telomerase and telomere lengths in paired normal and cancer tissues of breast." Int J Oncol **15**(4): 839-845.

To attain the immortal phenotype, cancer cells must overcome the mitotic clock. Telomerase activity has been identified to be activated in malignant tumors including breast cancer. Telomerase activity was evaluated in 71 breast cancer tissues and paired normal tissues with the TRAP (telomerase repeat amplification protocol) assay. Telomerase activity was calculated and translated into arbitrary units by computer-assisted densitometry with the control of telomerase activity in the 293 control cell line. In 59 paired breast tissues with telomerase activity, terminal restriction fragment (TRF) lengths were measured using Southern blotting. Relative inhibition (RI), the ratio of inhibited telomerase activity in each tumor tissue compared to that of the 293 control cell line after pre-treatment with 150 microg/ml of RNAse A, was measured. Sixty-three of 71 cancer tissues showed telomerase activity (88.7%) with 75.3+/-17.9 units in densitometry, while no telomerase activity was detected in their paired normal tissues. Telomerase activity was correlated to node metastasis (p=0.02) and stage (p=0.005), but not to tumor size or the hormonal receptor status. TRF lengths were 11. 0+/-4.7 kb in 59 tumor tissues and 11.7+/-2.2 kb in paired normal tissues. TRF lengths did not correlate to any of the clinical parameters. However changes of TRF lengths in tumor tissues compared to those of normal tissues correlated to telomerase activity. RI in the tumor tissues was proportional to telomerase activity without RNAse A pre-treatment. In breast cancer, telomerase activity was specific to tumor tissues and increased with tumor progression. Telomerase activity and changes in TRF lengths can be used as guidelines in detecting candidates for the telomerase inhibitor.

Riudavets, M., et al. (2020). "Immune-Related Adverse Events and Corticosteroid Use for Cancer-Related Symptoms Are Associated With Efficacy in Patients With Non-small Cell Lung Cancer Receiving Anti-PD-(L)1 Blockade Agents." Front Oncol **10**: 1677.

Background: Immune-related adverse events (irAEs) have been associated with improved efficacy in advanced non-small cell lung cancer (NSCLC) patients receiving anti-PD-(L)1 blockade agents, while the concurrent use of corticosteroids seems to worsen it. We evaluated outcomes in advanced NSCLC patients treated with anti-PD-(L)1 blockade agents in relation to the presence of irAEs and the reasons for using corticosteroids: whether for palliative cancer-related reasons or for the management of irAEs. Methods: Clinical outcomes in advanced NSCLC patients treated with anti-PD-(L)1 blockade agents were calculated with regard to the presence of irAEs and the use of corticosteroids. A landmark analysis was performed to avoid immortal time bias due to the time-dependent nature of irAEs. Results: Out of a total of 267 patients, the 56.9% of patients who experienced irAEs had significantly improved outcomes. In the landmark analysis, median progression-free survival (PFS) was 12.4 months for patients with irAEs vs. 4.1 months for patients without irAEs ($p < 0.001$), while median overall survival (OS) was 28.2 vs. 12.5 months, respectively ($p < 0.001$). Likewise, objective response and disease control rates were significantly higher in patients experiencing irAEs: 48.6 vs. 22.8% and 77.1 vs. 39.6% ($p < 0.001$), respectively. Median OS was significantly shorter for patients receiving >/=10 mg of prednisone equivalent daily for cancer-related symptoms than for the rest of patients (<10 mg prednisone equivalent daily or for management of irAEs): 6 vs. 15.9 months ($p < 0.001$). Conclusions: IrAEs were associated with improved efficacy in advanced NSCLC patients when a landmark analysis was applied. Patients receiving corticosteroids had significantly poorer outcomes when they were used for cancer-related symptoms.

Roos, G., et al. (1998). "Telomerase activity in relation to p53 status and clinico-pathological parameters in breast cancer." Int J Cancer **79**(4): 343-348.

Cell cycle deregulation can occur at different levels in cancer. In human breast cancer it includes overexpression of cyclins D1 and E, down-regulation of cyclin-dependent kinase inhibitors and inactivation of the retinoblastoma and p53 tumor suppressor proteins. Telomerase activity is strongly associated with an immortal phenotype and expression of telomerase is linked to the cell cycle. We have recently demonstrated a connection between specific cell cycle defects within the pRB pathway and levels of telomerase activity in breast cancer. In the present study, 106 tumors were investigated for p53 gene and protein status. By single strand conformation polymorphism (SSCP) analysis, 15% showed mutations within exons 5-8 and by immunohistochemistry (IHC), 29% were p53 positive. Tumors with a telomerase activity above median (i.e., telomerase(high)) were significantly associated with p53 protein accumulation ($p = 0.004$), but not with p53

gene mutations. The strongest telomerase expression was found in tumors with p53 protein accumulation. Morphologic grade, estrogen and progesterone receptor expression differed significantly between the telomerase(high) and telomerase(low) groups ($p < 0.0001$, $p = 0.016$ and $p = 0.046$, respectively), but no difference was observed for stage or nodal status. Telomerase(high) tumors were significantly associated with a poor prognosis for node-negative (N0) patients ($p = 0.008$), but not for node-positive (N+) patients, whereas the opposite was demonstrated for tumors with p53 accumulation. The survival data indicated that telomerase expression has biological importance particularly for N0 tumors, suggesting that telomerase(low) tumors constitute a group of "pre-immortalized" tumors with a good prognosis.

Sameni, M., et al. (1995). "Cathepsin B and D are Localized at the Surface of Human Breast Cancer Cells." Pathol Oncol Res **1**(1): 43-53.

Alterations in trafficking of cathepsins B and D have been reported in human and animal tumors. In MCF10 human breast epithelial cells, altered trafficking of cathepsin B occurs during their progression from a preneoplastic to neoplastic state. We now show that this is also the case for altered trafficking of cathepsin D. Nevertheless, the two cathepsins are not necessarily trafficked to the same vesicles. Perinuclear vesicles of immortal MCF10A cells label for both cathepsins B and D, yet the peripheral vesicles found in ras-transfected MCF10AneoT cells label for cathepsin B, cathepsin D or both enzymes. Studies at the electron microscopic level confirm these findings and show in addition surface labeling for both enzymes in the transfected cells. By immunofluorescence staining, cathepsin B can be localized on the outer surface of the cells. Similar patterns of peripheral intracellular and surface staining for cathepsin B are seen in the human breast carcinoma lines MCF7 and BT20. We suggest that the altered trafficking of cathepsins B and D may be of functional significance in malignant progression of human breast epithelial cells. Translocation of vesicles containing cathepsins B and D toward the cell periphery occurs in human breast epithelial cells that are at the point of transition between the pre-neoplastic and neoplastic state and remains part of the malignant phenotype of breast carcinoma cells.

Saretzki, G. (2003). "Telomerase inhibition as cancer therapy." Cancer Lett **194**(2): 209-219.

A number of different approaches have been developed to inhibit telomerase activity in human cancer cells. Different components and types of inhibitors targeting various regulatory levels have been regarded as useful for telomerase inhibition. Most methods, however, rely on successive telomere shortening. This process is very slow and causes a long time lag between the onset of inhibition and the occurrence of senescence or apoptosis as a reversal of the immortal phenotype. Many telomerase inhibitors seem to be most efficient when combined with conventional chemotherapeutics. There are some promising approaches that seem to circumvent the slow way of telomere shortening and induce fast apoptosis in treated tumor cells. It has been demonstrated that telomerase may be involved in triggering apoptosis, but the underlying molecular mechanism remains unclear.

Saretzki, G. and T. von Zglinicki (2003). "Telomerase as a promising target for human cancer gene therapy." Drugs Today (Barc) **39**(4): 265-276.

A number of different approaches have been developed to target human cancer on the basis of its specific expression of telomerase activity. The most common approach relies on inhibition of telomerase activity for reversion of the immortal phenotype of tumor cells. Different components and types of inhibitors targeting various regulatory levels have been regarded as useful for telomerase inhibition. Most methods, however, rely on successive telomere shortening. This process is very slow and causes a long time lag between the onset of inhibition and the occurrence of senescence or apoptosis as a reversal of the immortal phenotype. Many telomerase inhibitors seem to be most efficient when combined with conventional chemotherapeutics. There are some promising approaches to circumvent the slow track of telomere shortening and induce fast apoptosis in treated tumor cells. It has been demonstrated that telomerase may be involved in triggering apoptosis, but the underlying molecular mechanism remains unclear. Other important strategies are the use of telomerase promoters for the application of toxic or pro-apoptotic drugs into human cancer cells or the use of immunological properties of the telomerase enzyme for possible cancer therapies.

Sarkar, D., et al. (2008). "A cancer terminator virus eradicates both primary and distant human melanomas." Cancer Gene Ther **15**(5): 293-302.

The prognosis and response to conventional therapies of malignant melanoma inversely correlate with disease progression. With increasing thickness, melanomas acquire metastatic potential and become inherently resistant to radiotherapy and chemotherapy. These harsh realities mandate the design of improved therapeutic modalities, especially those targeting metastases. To develop an approach to effectively treat this aggressive disease, we constructed a conditionally replication-competent adenovirus in which expression

of the adenoviral E1A gene, necessary for replication, is driven by the cancer-specific promoter of progression-elevated gene-3 (PEG-3) and which simultaneously expresses mda-7/IL-24 in the E3 region of the adenovirus (Ad.PEG-E1A-mda-7), a cancer terminator virus (CTV). This CTV produces large quantities of MDA-7/IL-24 protein as a function of adenovirus replication uniquely in cancer cells. Infection of Ad.PEG-E1A-mda-7 (CTV) in normal human immortal melanocytes and human melanoma cells demonstrates cancer cell-selective adenoviral replication, mda-7/IL-24 expression, growth inhibition and apoptosis induction. Injecting Ad.PEG-E1A-mda-7 CTV into xenografts derived from MeWo human metastatic melanoma cells in athymic nude mice completely eliminated not only primary treated tumors but also distant non-treated tumors (established in the opposite flank), thereby implementing a cure. These provocative findings advocate potential therapeutic applications of this novel virus for treating patients with advanced melanomas with metastases.

Seo, Y. S., et al. (2016). "Association of Metformin Use With Cancer-Specific Mortality in Hepatocellular Carcinoma After Curative Resection: A Nationwide Population-Based Study." Medicine (Baltimore) **95**(17): e3527.

Many preclinical reports and retrospective population studies have shown an anticancer effect of metformin in patients with several types of cancer and comorbid type 2 diabetes mellitus (T2DM). In this work, the anticancer effect of metformin was assessed in hepatocellular carcinoma (HCC) patients with T2DM who underwent curative resection.A population-based retrospective cohort design was used. Data were obtained from the National Health Insurance Service and Korea Center Cancer Registry in the Republic of Korea, identifying 5494 patients with newly diagnosed HCC who underwent curative resection between 2005 and 2011. Crude and adjusted hazard ratios (HRs) were calculated using Cox proportional hazard models to estimate effects. In the sensitivity analysis, we excluded patients who started metformin or other oral hypoglycemic agents (OHAs) after HCC diagnosis to control for immortal time bias.From the patient cohort, 751 diabetic patients who were prescribed an OHA were analyzed for HCC-specific mortality and retreatment upon recurrence, comparing 533 patients treated with metformin to 218 patients treated without metformin. In the fully adjusted analyses, metformin users showed a significantly lower risk of HCC-specific mortality (HR 0.38, 95% confidence interval [CI] 0.30-0.49) and retreatment events (HR 0.41, 95% CI 0.33-0.52) compared with metformin nonusers. Risks for HCC-specific mortality were consistently lower among metformin-using groups, excluding patients who started metformin or OHAs after diagnosis.In this large population-based cohort of patients with comorbid HCC and T2DM, treated with curative hepatic resection, metformin use was associated with improvement of HCC-specific mortality and reduced occurrence of retreatment events.

Shammas, M. A. and R. J. Shmookler Reis (1999). "Recombination and its roles in DNA repair, cellular immortalization and cancer." Age (Omaha) **22**(2): 71-88.

Genetic recombination is the creation of new gene combinations in a cell or gamete, which differ from those of progenitor cells or parental gametes. In eukaryotes, recombination may occur at mitosis or meiosis. Mitotic recombination plays an indispensable role in DNA repair, which presumably directed its early evolution; the multiplicity of recombination genes and pathways may be best understood in this context, although they have acquired important additional functions in generating diversity, both somatically (increasing the immune repertoire) and in germ line (facilitating evolution). Chromosomal homologous recombination and HsRad51 recombinase expression are increased in both immortal and preimmortal transformed cells, and may favor the occurrence of multiple oncogenic mutations. Tumorigenesis in vivo is frequently associated with karyotypic instability, locus-specific gene rearrangements, and loss of heterozygosity at tumor suppressor loci - all of which can be recombinationally mediated. Genetic defects which increase the rate of somatic mutation (several of which feature elevated recombination) are associated with early incidence and high risk for a variety of cancers. Moreover, carcinogenic agents appear to quite consistently stimulate homologous recombination. If cells with high recombination arise, either spontaneously or in response to "recombinogens," and predispose to the development of cancer, what selective advantage could favor these cells prior to the occurrence of growth-promoting mutations? We propose that the augmentation of telomere-telomere recombination may provide just such an advantage, to hyper-recombinant cells within a population of telomerase-negative cells nearing their replicative (Hayflick) limit, by extending telomeres in some progeny cells and thus allowing their continued proliferation.

Sharma, S., et al. (2005). "DNA helicases as targets for anti-cancer drugs." Curr Med Chem Anticancer Agents **5**(3): 183-199.

DNA helicases have essential roles in nucleic acid metabolism by facilitating cellular processes including replication, recombination, DNA repair, and

transcription. The vital roles of helicases in these pathways are reflected by their emerging importance in the maintenance of genomic stability. Recently, a number of human diseases with cancer predisposition have been shown to be genetically linked to a specific helicase defect. This has led researchers to further investigate the roles of helicases in cancer biology, and to study the efficacy of targeting human DNA helicases for anti-cancer drug treatment. Helicase-specific inhibition in malignant cells may compromise the high proliferation rates of cancerous tissues. The role of RecQ helicases in response to replicational stress suggests a molecular target for selectively eliminating malignant tumor cells by a cancer chemotherapeutic agent. Alternate DNA secondary structures such as G-quadruplexes that may form in regulatory regions of oncogenes or G-rich telomere sequences are potential targets for cancer therapy since these sequence-specific structures are proposed to affect gene expression and telomerase activation, respectively. Small molecule inhibitors of G-quadruplex helicases may be used to regulate cell cycle progression by modulating promotor activation or disrupting telomere maintenance, important processes of cellular transformation. The design of small molecules which deter helicase function at telomeres may provide a molecular target since telomerase activity is necessary for the proliferation of numerous immortal cells. Although evidence suggests that helicases are specifically inhibited by certain DNA binding compounds, another area of promise in anti-cancer therapy is siRNA technology. Specific knockdown of helicase expression can be utilized as a means to sensitize oncogenic proliferating cell lines. This review will address these topics in detail and summarize the current avenues of research in anti-cancer therapy targeting helicases through small molecule inhibitors of DNA-protein complexes, DNA binding drugs, or down-regulation of helicase gene expression.

Shay, J., et al. (1993). "Loss of telomeric DNA during aging may predispose cells to cancer (review)." Int J Oncol **3**(4): 559-563.

In normal human somatic cells, gradual shortening of telomeres may activate the complex cascade of molecular events known as cellular senescence. Experimental evidence from our laboratory suggests that cellular mortality is regulated by two separate mechanisms that we have termed mortality stage 1 (M1) and mortality stage 2 (M2). In mammary epithelial cells, the M1 mechanism involves de-regulation of p53 whereas in fibroblasts both the retinoblastoma (Rb) and p53 gene products are implicated. Cells that overcome the function of these antiproliferative proteins (M1 controls) continue to divide until a second entirely independent mechanism, M2 is induced. As somatic cells age they gradually lose telomeric sequences at the termini of their chromosomes, a process that continues during the extended lifespan period between M1 and M2. Immortal and cancer cells, as well as cells that maintain telomere length (e.g. germ cells), express telomerase, a ribonucleoprotein which maintains (stabilizes) telomere length by synthesizing TTAGGG repeats. Because normal human somatic cells and cells prior to M2 do not express telomerase, we propose that the M2 mechanism involves either the direct or indirect induction of telomerase activity. In order for cells to overcome senescence and become immortal, they must first escape the checkpoints that limit the proliferative capacity of normal cells, the MI and M2 controls (a very rare event). However, the probability of immortalization and that of tumorigenesis increases with age and we propose telomere shortening and reactivation of telomerase are important components in these processes. Once immortal, cells can then follow many pathways that result in the acquisition and progression of cancer.

Shay, J. W. (1995). "Aging and cancer: are telomeres and telomerase the connection?" Mol Med Today **1**(8): 378-384.

There is substantial evidence for the progressive loss of the telomeric ends of chromosomes during aging, both in cell culture and in vivo. The loss of telomeres may eventually induce antiproliferative signals that result in cellular senescence. A hypothesis gaining prominence is that the activation of telomerase, a ribonucleoprotein enzyme that is important in maintaining telomere length stability, is necessary for the sustained growth of most tumors. The interrelationships between telomere shortening and aging, and how activation of telomerase may be necessary for cells to become immortal and malignant, are reviewed here.

Shibui, T., et al. (2008). "Changes in expression of imprinted genes following treatment of human cancer cell lines with non-mutagenic or mutagenic carcinogens." Int J Oncol **33**(2): 351-360.

It remains possible that chemicals that act by mutagenic mechanisms as well as chemicals that do not induce gene mutations may affect epigenetic gene expression. To test the possibility, we investigated the ability of both types of chemicals to alter the expression of five imprinted genes, PEG3, SNRPN, NDN, ZAC and H19, using two human colon cancer cell lines and a human breast cancer cell line. The expression of imprinted genes was changed by some non-mutagenic and mutagenic carcinogens independent of their mutagenic activity. The genes

most commonly exhibiting the changes in expression were SNRPN and PEG3. Alterations of the expression of NDN and ZAC were also observed in some conditions. Methylation-specific PCR and chromatin immunoprecipitation assays suggest the possibility that changes in the expression of SNRPN may be associated with DNA hypomethylation and histone acetylation of the promoters and euchromatinization of the heterochromatic domains of the promoters. Changes in expression of the imprinted genes, PEG3 and NDN, were also observed in cells immortalized by treatment of normal human fibroblasts with 4-nitroquinoline 1-oxide or aflatoxin B1. We previously demonstrated that expression of the cancer-related gene, INK4a, in these immortal cells was lost via epigenetic mechanisms. The results prove that, in cancer cells, some mutagenic or non-mutagenic carcinogens can epigenetically influence the transcription levels of imprinted genes and also suggest the possibility that some chemical carcinogens may have epigenetic carcinogenic effects in human cells.

Sigley, J., et al. (2017). "Diffusion and Binding of Mismatch Repair Protein, MSH2, in Breast Cancer Cells at Different Stages of Neoplastic Transformation." PLoS One **12**(1): e0170414.

The interior of cells is a highly complex medium, containing numerous organelles, a matrix of different fibers and a viscous, aqueous fluid of proteins and small molecules. The interior of cells is also a highly dynamic medium, in which many components move, either by active transport or passive diffusion. The mobility and localization of proteins inside cells can provide important insights into protein function and also general cellular properties, such as viscosity. Neoplastic transformation affects numerous cellular properties, and our goal was to investigate the diffusional and binding behavior of the important mismatch repair (MMR) protein MSH2 in live human cells at various stages of neoplastic transformation. Toward this end, noncancerous, immortal, tumorigenic, and metastatic mammary epithelial cells were transfected with EGFP and EGFP-tagged MSH2. MSH2 forms two MMR proteins (MutSalpha and MutSbeta) and we assume MSH2 is in the complex MutSalpha, though our results are similar in either case. Unlike the MutS complexes that bind to nuclear DNA, EGFP diffuses freely. EGFP and MutSalpha-EGFP diffusion coefficients were determined in the cytoplasm and nucleus of each cell type using fluorescence recovery after photobleaching. Diffusion coefficients were 14-24 mum2/s for EGFP and 3-7 mum2/s for MutSalpha-EGFP. EGFP diffusion increased in going from noncancerous to immortal cells, indicating a decrease in viscosity, with smaller changes in subsequent stages. MutSalpha produces an effective diffusion coefficient that, coupled with the free EGFP diffusion measurements, can be used to extract a pure diffusion coefficient and a pseudo-equilibrium constant K*. The MutSalpha nuclear K* increased sixfold in the first stage of cancer and then decreased in the more advanced stages. The ratio of nuclear to cytoplasmic K*for MutSalpha increased almost two orders of magnitude in going from noncancerous to immortal cells, suggesting that this quantity may be a sensitive metric for recognizing the onset of cancer.

Smith, L. M., et al. (1997). "Breast cancer cells have lower activating protein 1 transcription factor activity than normal mammary epithelial cells." Cancer Res **57**(14): 3046-3054.

To determine whether normal breast cells have different levels of activating protein 1 (AP-1) expression and activation relative to breast cancer cells, we have compared the level of c-Jun and c-Fos expression and AP-1 activity in human mammary epithelial cells (HMECs) at different stages of transformation (normal proliferating HMECs, immortal HMECs, oncogene-transformed HMECs, and breast cancer cell lines). These studies demonstrated that normal and immortal HMECs have a high basal level of expression of cJun and cFos and higher AP-1 DNA-binding and transcriptional activating activities than do oncogene-transformed HMECs or human breast cancer cells, with a gradual decrease in AP-1 transactivating activity as cells progress through the carcinogenesis pathway (normal > immortal > oncogene-transformed > cancer cell lines). The AP-1 activity in normal or immortal cells was not modulated by growth factor supplementation or oncogene overexpression, as it is in breast cancer cells. However, the addition of suramin, a nonspecific growth factor antagonist, did inhibit AP-1 in these HMECs, suggesting that this high level of AP-1 present in normal HMECs may be due to autocrine stimulation of growth factor pathways. The differences in AP-1 activity in normal and malignant breast cells may indicate that normal cells are more dependent on AP-1-mediated signals for their growth than are breast cancer cells.

Soeur, J., et al. (2011). "Selective cytotoxicity of Aniba rosaeodora essential oil towards epidermoid cancer cells through induction of apoptosis." Mutat Res **718**(1-2): 24-32.

Essential oils are complex mixtures of odorous and volatile compounds derived from secondary plant metabolism. They can be isolated from many plants by mechanical pressing or hydro- and steam-distillation and are known to induce a wide

range of biological effects through their antibacterial, antifungal, cytotoxic, antioxidant and antimutagenic activities. In order to explore their beneficial properties on human skin cells, we investigated the effects of an essential oil from rosewood Aniba rosaeodora (REO) on the human epidermoid carcinoma cell line A431, on immortal HaCaT cells thought to represent an early stage of skin carcinogenesis, on transformed normal HEK001 keratinocytes and on primary normal NHEK keratinocytes. In a defined range of concentrations, REO selectively killed A431 and HaCaT cells. The same treatments had only a minor cytotoxic effect on HEK001 and NHEK cells. Preferentially in A431 and HaCaT cells, REO triggered the production of reactive oxygen species, induced depolarization of the mitochondrial membrane and caused caspase-dependent cell death characterized by phosphatidylserine externalization, an early marker of apoptosis. Both intrinsic and extrinsic apoptotic pathways were implicated in REO-induced cell death. The identification of selective induction of apoptosis in precancerous and cancerous skin cells by REO highlights the potential anticancer activity of this essential oil.

Sommers, C. L., et al. (1989). "Vimentin rather than keratin expression in some hormone-independent breast cancer cell lines and in oncogene-transformed mammary epithelial cells." Cancer Res **49**(15): 4258-4263.

To characterize differences in gene expression between hormone-dependent and hormone-independent mammary carcinoma, we cloned complementary DNAs of genes expressed in a hormone-independent breast carcinoma cell line that were not expressed in a hormone-dependent line. One clone, which was isolated in many copies, coded for the intermediate filament protein vimentin. A complementary DNA clone 1.8 kilobases long included the entire protein-coding region for vimentin. Vimentin was expressed by more than one-half of the hormone-independent breast carcinoma cell lines tested but not by the hormone-dependent cell lines. The cell lines which expressed vimentin expressed only low levels of cytokeratins. The correlation between vimentin expression and more advanced stages of mammary cell transformation was tested in a model system in which immortal, nontumorigenic human mammary epithelial cells or derivative lines transformed with v-ras-H or SV40 T-antigen were found not to express vimentin, whereas a derivative highly tumorigenic cell line transformed by both v-ras-H and T-antigen did express vimentin. Analysis of several other kinds of epithelial carcinoma cell lines showed only rare examples of vimentin expression.

Soria, J. C. and O. Rixe (1997). "[Telomeres, telomerase and cancer]." Bull Cancer **84**(10): 963-970.

The chromosome ends are specialized nucleoprotein structures called telomeres, which length predicts replicative capacity of cells. Activation of telomerase, the DNA polymerase that synthesizes telomeric repeats, seems to be necessary for cells to become immortal. Methods of measuring telomerase activity, now reliable and semiquantitative, have shown that telomerase is expressed in most human cancers, but not in normal somatic tissues. Research about regulation of telomere length and telomerase activity, highlights connexions between senescence and cancer. This article details diagnosis, prognosis and therapeutic prospects linked to the study of telomerase activity.

Sprung, C. N., et al. (1999). "Telomere instability in a human cancer cell line." Mutat Res **429**(2): 209-223.

Telomere maintenance is essential in immortal cancer cells to compensate for DNA lost from the ends of chromosomes, to prevent chromosome fusion, and to facilitate chromosome segregation. However, the high rate of fusion of chromosomes near telomeres, termed telomere association, in many cancer cell lines has led to the proposal that some cancer cells may not efficiently perform telomere maintenance. Deficient telomere maintenance could play an important role in cancer because telomere associations and nondisjunction have been demonstrated to be mechanisms for genomic instability. To investigate this possibility, we have analyzed the telomeres of the human squamous cell carcinoma cell line SQ-9G, which has telomere associations in approximately 75% of the cells in the population. The absence of detectable telomeric repeat sequences at the sites of these telomere associations suggests that they result from telomere loss. The analysis of telomere length by quantitative in situ hybridization demonstrated that, compared to the human squamous cell carcinoma cell line SCC-61 which has few telomere associations, SQ-9G has more extensive heterogeneity in telomere length and more telomeres without detectable telomeric repeat sequences. The dynamics of the changes in telomere length also demonstrated a higher rate of fluctuation in telomere length, both on individual telomeres and coordinately on all telomeres. These results demonstrate that telomere maintenance can play a role in the genomic instability seen in cancer cells.

Stefflova, K., et al. (2007). "Using molecular beacons for cancer imaging and treatment." Front Biosci **12**: 4709-4721.

Molecular beacons are essentially all probes

that illuminate particular cellular target or cells with similar characteristics. In this review we focus on those molecular beacons that use near-infrared fluorescence imaging (NIRF-I) to identify the unique cellular and metabolic markers characteristic of cancer. They employ various delivery and activation pathways, selectively or specifically targeting proliferating and immortal cancer cells. These beacons can either be used in an imaging step separate from therapy or they can intimately connect these two steps into a single process. Matching cancer therapy to NIRF-I is photodynamic therapy (PDT) that uses the light-triggered phototoxic properties of some porphyrin-based dyes. Guided by beacon's restored fluorescence, the PDT laser could be focused on affected sites, killing the cancer cells using the enhanced photoactivity of the same beacon. Or vice versa-the restored fluorescence from the cleaved beacon could be used as an indication of the beacon's own therapeutic success, imaging the post-PDT apoptotic cells.

Sternlicht, M. D. and S. H. Barsky (1997). "The myoepithelial defense: a host defense against cancer." Med Hypotheses **48**(1): 37-46.

The behavior of human tumors depends not only on the nature of the tumor cells themselves but also on the modifying effects of various normal host cells such as fibroblasts and endothelial cells. One cell type, however--the myoepithelial cell--has not been studied scientifically. Myoepithelial cells normally surround ducts and acini of glandular organs such as the breast and salivary glands and contribute to the synthesis of a surrounding basement membrane. This relationship suggests that myoepithelial cells may exert paracrine effects on glandular epithelium and also regulate the progression of ductal carcinoma in situ (DCIS) to invasive carcinoma. Myoepithelial tumors, in turn, tend to be benign or low-grade neoplasms that exhibit the rare property of accumulating rather than degrading extracellular matrix material. To better understand the nature of myoepithelial tumors, as well as the possible role of normal myoepithelial host cells in cancer, we have established immortal cell lines and a number of transplantable xenografts from various human myoepithelial tumors of the salivary gland and breast. The cell lines exhibit a normal myoepithelial phenotype and the xenografts continue to accumulate an abundant extracellular matrix. Further ultrastructural, immunocytochemical, molecular, and biochemical studies reveal that myoepithelial cells secrete relatively low levels of matrix-degrading proteinases but relatively high levels of maspin and various other anti-invasive proteinase inhibitors, that some of these inhibitors accumulate within the myoepithelial matrix, and that myoepithelial cells can induce epithelial morphogenesis (spheroid formation) and inhibit tumor-cell invasion in vitro. Myoepithelial cells, which surround normal breast ducts and DCIS, have also been found to selectively express maspin and certain proteinase inhibitors in situ. These inherent myoepithelial properties are likely to contribute to the low-grade nature of myoepithelial neoplasms and advance our hypothesis that host myoepithelial cells regulate the progression of in situ to invasive carcinoma by providing an important host defense against cancer invasion.

Stopsack, K. H., et al. (2017). "Common medications and prostate cancer mortality: a review." World J Urol **35**(6): 875-882.

PURPOSE: Most prostate cancer patients also have comorbidities that are treated with both prescription and nonprescription medications; furthermore, many use dietary supplements. We assess their association with prognosis after prostate cancer diagnosis, and we discuss methodological challenges and clinical implications. METHODS: We reviewed high-quality observational studies investigating the association of commonly used medications and supplements with prostate cancer-specific mortality. RESULTS: There is preliminary evidence that statins and metformin use may be associated with lower risk of cancer-specific mortality after prostate cancer diagnosis; conversely, high calcium and multivitamin supplementation may be associated with increased risk. Evidence is inconclusive for nonsteroidal anti-inflammatory drugs, acetylsalicylic acid (aspirin), insulin, antihypertensives such as angiotensin-converting enzyme inhibitors and beta-blockers, digoxin, and warfarin. Common limitations of the internal validity of studies examined include unmeasured confounding and confounding by indication, competing risks, and time-related biases such as immortal time bias. The majority of studies focused on Caucasian men with specific comorbidities, while heterogeneity among patients and tumors was mostly not assessed. CONCLUSIONS: Commonly prescribed medications and over-the-counter supplements may influence prognosis among prostate cancer patients. Further well-designed pharmacoepidemiologic studies and randomized controlled trials of selected medications in appropriate patient groups are necessary before these drugs can bear new indications for prostate cancer treatment. We discuss considerations when deciding about use of these drugs in clinical practice at the present time.

Stopsack, K. H., et al. (2016). "Metformin and prostate cancer mortality: a meta-analysis." Cancer Causes Control **27**(1): 105-113.

PURPOSE: Observational studies report conflicting results on the association between metformin exposure and prostate cancer outcomes. This meta-analysis summarizes studies reporting overall survival, prostate cancer-specific mortality, and biochemical recurrence. METHODS: PubMed and Embase were systematically reviewed to identify studies investigating the association between metformin use and clinical endpoints among men with prostate cancer while taking confounding by diabetes diagnosis into account. Pooled risk estimates (hazard ratios, HRs) and 95 % confidence intervals (CIs) were calculated using random-effects models. Sensitivity analyses for quality components and factors for heterogeneity were conducted. RESULTS: Of 549 articles identified, nine retrospective cohort studies representing 9,186 patients were included. There was significant heterogeneity between studies, and studies differed in quality. Metformin use was associated with improved overall survival in studies with clear risk window definition (HR 0.88, 95 % CI 0.86-0.90, $p < 0.001$) and in studies with potential immortal time bias (HR 0.52, 95 % CI 0.41-0.65, $p < 0.001$). No significant association with prostate cancer-specific mortality was detected (HR 0.76, 95 % CI 0.44-1.31, $p = 0.33$). Metformin use was associated with a decreased risk of biochemical recurrence (HR 0.79, 95 % CI 0.63-1.00, $p = 0.047$). CONCLUSIONS: This meta-analysis suggests a benefit of metformin in men with diabetes and prostate cancer. However, further carefully designed studies are needed to confirm findings and to assess potential generalization to non-diabetic, non-white, and less aggressively treated men with prostate cancer.

Stovall, D. B., et al. (2013). "The regulation of SOX7 and its tumor suppressive role in breast cancer." Am J Pathol **183**(5): 1645-1653.

Both epigenetic silencing and genetic deletion of tumor suppressors contribute to the development and progression of breast cancer. SOX7 is a transcription factor important to development, and its down-regulation has been reported in tumor tissues and cell lines of prostate, colon, and lung cancers. However, the regulation of SOX7 expression and its functional role in breast cancer have not been reported. The current study demonstrates that SOX7 mRNA and protein expression are down-regulated in breast cancer tissues and cell lines compared with adjacent normal tissues and nontumorigenic cells, respectively. The SOX7 promoter is hypermethylated in breast cancer cell lines compared with nontumorigenic cells, and the inhibition of DNA methylation increases SOX7 mRNA levels. With shRNA-mediated SOX7 silencing, nontumorigenic immortal breast cells display increased proliferation, migration, and invasion and form structures that resemble that of breast cancer cells in a three-dimensional culture system. Conversely, ectopic SOX7 expression inhibits proliferation, migration, and invasion of breast cancer cells in vitro and tumor growth in vivo. Importantly, we discovered that SOX7 transcript levels positively correlated with clinical outcome of 674 breast cancer patients. Overall, our data suggest that SOX7 acts as a tumor suppressor in breast cancer. SOX7 expression is likely regulated by multiple mechanisms and potentially serves as a prognostic marker for breast cancer patients.

Suissa, S. (2012). "Randomized Trials Built on Sand: Examples from COPD, Hormone Therapy, and Cancer." Rambam Maimonides Med J **3**(3): e0014.

The randomized controlled trial is the fundamental study design to evaluate the effectiveness of medications and receive regulatory approval. Observational studies, on the other hand, are essential to address post-marketing drug safety issues but have also been used to uncover new indications or new benefits for already marketed drugs. Hormone replacement therapy (HRT) for instance, effective for menopausal symptoms, was reported in several observational studies during the 1980s and 1990s to also significantly reduce the incidence of coronary heart disease. This claim was refuted in 2002 by the large-scale Women's Health Initiative randomized trial. An example of a new indication for an old drug is that of metformin, an anti-diabetic medication, which is being hailed as a potential anti-cancer agent, primarily on the basis of several recent observational studies that reported impressive reductions in cancer incidence and mortality with its use. These observational studies have now sparked the conduct of large-scale randomized controlled trials currently ongoing in cancer. We show in this paper that the spectacular effects on new indications or new outcomes reported in many observational studies in chronic obstructive pulmonary disease (COPD), HRT, and cancer are the result of time-related biases, such as immortal time bias, that tend to seriously exaggerate the benefits of a drug and that eventually disappear with the proper statistical analysis. In all, while observational studies are central to assess the effects of drugs, their proper design and analysis are essential to avoid bias. The scientific evidence on the potential beneficial effects in new indications of existing drugs will need to be more carefully assessed before embarking on long and expensive unsubstantiated trials.

Suissa, S. and L. Azoulay (2012). "Metformin and the risk of cancer: time-related biases in observational studies." Diabetes Care **35**(12): 2665-2673.

OBJECTIVE: Time-related biases in observational studies of drug effects have been

described extensively in different therapeutic areas but less so in diabetes. Immortal time bias, time-window bias, and time-lag bias all tend to greatly exaggerate the benefits observed with a drug. RESEARCH DESIGN AND METHODS: These time-related biases are described and shown to be prominent in observational studies that have associated metformin with impressive reductions in the incidence of and mortality from cancer. As a consequence, metformin received much attention as a potential anticancer agent; these observational studies sparked the conduction of randomized, controlled trials of metformin as cancer treatment. However, the spectacular effects reported in these studies are compatible with time-related biases. RESULTS: We found that 13 observational studies suffered from immortal time bias; 9 studies had not considered time-window bias, whereas other studies did not consider inherent time-lagging issues when comparing the first-line treatment metformin with second- or third-line treatments. These studies, subject to time-related biases that are avoidable with proper study design and data analysis, led to illusory extraordinarily significant effects, with reductions in cancer risk with metformin ranging from 20 to 94%. Three studies that avoided these biases reported no effect of metformin use on cancer incidence. CONCLUSIONS: Although observational studies are important to better understand the effects of drugs, their proper design and analysis is essential to avoid major time-related biases. With respect to metformin, the scientific evidence of its potential beneficial effects on cancer would need to be reassessed critically before embarking on further long and expensive trials.

Sullivan, R. (2007). "Policy challenges for cancer research: a call to arms." Ecancermedicalscience **1**: 53.

Research has delivered remarkable benefits for cancer patients and their families since James Watson and Francis Crick wrote the now immortal line, 'We wish to propose a structure for the salt of deoxyribonucleic acid' thus setting the molecular foundations for the modern era of cancer control. The pace of technological innovation from fundamental scientific discoveries to the policy impact of huge population studies has been breathtaking. One has only to contrast a paper on the treatment of solid epithelial cancers written by Henri Tagnon and colleagues in 1966 (Eur J Cancer2 51-7) with the myriad of chemotherapeutic approaches at the oncologists disposal today. Inevitably, as the tide of research has risen so it has bought the flotsam and jetsam of regulations and policies. Some have been helpful, many pointless and too many actually harmful. Naturally, some of these regulatory and general policies (by this I mean those concerned with funding, structure and organization) have been specifically targeted at cancer research, e.g. US National Cancer Act 1971, whilst others have been a product of the general regulatory environment with indirect consequences for cancer research, e.g. EU Data Protection Directive 1995. Policy issues thus cover a vast terrain criss-crossed by complex interdependencies between scientific areas, countries S&T policies and socio-political constructs. Unfortunately, there has been little attention paid to the consequences of these policy issues from which the research community has, by and large, been passenger rather than driver.Global investment in cancer research is now at unprecedented levels. The recently published report by the European Cancer Research Managers Forum has found some 14 billion euros being annually spent worldwide on cancer research (this figure includes industry but overall probably underestimates spend by at least one billion [2]). With the ageing demographics of developed countries and the catch-up effect in developing countries, the rising burden of cancer is driving research activity in cancer ever upwards. Opportunities for delivering even greater measures for preventing and controlling cancer abound, but the shackles of bureaucracy (stifling regulations and poor research policies) threaten this future more than ever-'Man is born free and everywhere he is in chains'. Jean-Jacques Rousseau's quote could equally be applied to spirit of research creativity in today's environment. So what are the main issues and what is to be done?

Sun, A. S. and M. Renaud (1989). "Enhancement of 5'-nucleotidase activity of human leukemic cells after fractionation: implications for cancer and aging." Mutat Res **219**(5-6): 295-302.

Previous studies reported that 5'-nucleotidase activity was undetectable or at much lower levels in the homogenate of human chronic lymphocytic leukemic (CCL) cells than in normal lymphocytes. In the present study, 5'-nucleotidase specific activity in acute myelocytic leukemia (AML), which varied in a range from undetectable to 1.4 (nmoles/min.mg protein), was enhanced by cell fractionation, from undetectable in the homogenate, up to 18.8 +/- 1.2, 6.4 +/- 0.7 and 0.68 +/- 0.12 in plasma membranes, microsomes, and cytosol fraction, respectively. In a further fractionation of the cytosol of various leukemic cells with ammonium sulfate, 5'-nucleotidase specific activity increased up to 14-fold in the 60% (NH4)2SO4 fraction, with a recovery of 1266 +/- 115%. These data suggest that 5'-nucleotidase activity in fractionated leukemic cells is higher than reported previously and that the sum of 5'-nucleotidase activity in subcellular compartments is higher than that detected in the homogenate. Furthermore, even when 5'-nucleotidase was undetectable in a homogenate, it

became detectable in the plasma membranes, suggesting that its ecto-enzyme function is still active in leukemic cells. The undetectable or low 5'-nucleotidase in the homogenate is indicative of (1) the enzyme itself being in an inactive form but becoming active after the fractionations, or (2) the presence of a factor(s) that prevents the enzyme from being detected but that is separated from the enzyme by the fractionations. In both cases, the rate of nucleotide catabolism by inactive 5'-nucleotidase in rapidly proliferating leukemic cells should be slower than when the enzyme is active. The present finding is consistent with our previous findings that during normal cell aging the high 5'-nucleotidase activity is associated with senescent non-proliferating cells but low or undetectable activity with rapidly proliferating immortal cells. The implications of 5'-nucleotidase for DNA synthesis in aging and cancer are discussed.

Sun, B., et al. (2005). "The minimal set of genetic alterations required for conversion of primary human fibroblasts to cancer cells in the subrenal capsule assay." Neoplasia **7**(6): 585-593.

Based on previous studies, a minimal set of genetic alterations that is required to convert normal human fibroblasts into cancer cells has been defined. Essential roles for telomere maintenance and alterations in phosphatase 2A activity were inferred from experiments in which tumorigenicity was tested by injecting cells under the skin of immunodeficient mice. However, in the present experiments, the combination of SV40 large T antigen and activated Ras, without hTERT or SV40 small t antigen, was sufficient to convert nine different primary human fibroblast cell strains to a fully malignant state. The malignant behavior of the cells was demonstrated by growth of the cells into invasive tumors when the cells were injected beneath the kidney capsule of immunodeficient mice. Lung metastases and circulating tumor cells were also detected. These tumors were not immortal; cells entered crisis, from which they could be rescued by expression of hTERT. However, the same cell populations were not tumorigenic when they were injected under the skin. In this site, tumorigenicity required the expression of hTERT and SV40 small t antigen as well as SV40 large T antigen and Ras. The cellular pathways targeted by SV40 large T antigen (p53 and pRb) and those targeted by activated Ras represent a minimal set of genetic alterations required for the conversion of normal human fibroblasts into cancer cells.

Sun, J. L., et al. (2004). "[Expression and structure of BNIP3L in lung cancer]." Ai Zheng **23**(1): 8-14.

BACKGROUND & OBJECTIVE: Bcl-2/E1B 19kDa interacting protein3-like (BNIP3L) gene is a tumor suppressor gene cloned from a human fetal liver cDNA library, which is located at 8p21, one of the high frequent regions of loss of heterozygosity (LOH) in lung carcinoma. BNIP3L protein can interact with antiapoptotic proteins, such as Bcl-2, Bcl-x(L), E1B19K, which promotes apoptosis. This study was designed to explore the correlation of alteration of expression and structure of BNIP3L gene with the progression of lung cancer. METHODS: The expression and structure of BNIP3L gene in 4 lung cancer cell strains and 30 tissues were determined by SP immunohistochemistry, immunoblot, semi-quantitative reverse transcription-PCR (RT-PCR), PCR-single strain conformation polymorphism (PCR-SSCP). RESULTS: (1) In 4 lung cancer cell strains, BNIP3L protein was not detected in A549, NCI-H460, NCI-H446, except for NCI-H520, in which the protein expression level was slightly lower than that in immortal bronchial epithelial cell strain HBE4-E6/E7. BNIP3L protein was observed in 46.7% (14/30) lung cancer tissues, while 100% (12/12) in normal lung tissues. The difference was significant in statistics ($P< 0.05$). (2) BNIP3L mRNA was detected in 4 lung cancer cell strains; and there existed no obvious discrepancy of the amount between these cell strains and HBE4-E6/E7. Absence or decrease of BNIP3L mRNA was observed in 26.7%(8/30) of lung cancer tissues. The average quantity of BNIP3L mRNA was 0.404+/-0.070 in lung cancer tissues, while 0.575+/-0.065 in paired normal lung tissues. The difference was significant in statistics ($P< 0.05$). In all the cancerous cell strains and tissues with BNIP3L mRNA, the products of RT-PCR were as long as those from their control samples in size, including the entire coding region, and no variation of BNIP3L gene structure such as absence, rearrangement, aberrant splicing were detected.(3) No point mutation was detected in all 6 exons of BNIP3L gene in 4 lung cancer cell strains and 30 tissues. CONCLUSION: BNIP3L protein expression was down-regulated in lung cancer, which might be involved in the occurrence and/or development of lung cancer. The down-regulation of BNIP3L protein expression in lung cancer was partly caused by the down-regulation of its transcription. The variation of gene structure may be not the reason of BNIP3L inactivity in lung cancer.

Sun, X., et al. (2018). "Upregulation of microRNA-3129 suppresses epithelial ovarian cancer through CD44." Cancer Gene Ther **25**(11-12): 317-325.

The purpose of this work is to evaluate whether human microRNA-3129 (hsa-miR-3129) may functionally regulate cancer development, possibly through downstream target CD44 in human epithelial ovarian cancer (EOC). Direct targeting of hsa-miR-3129 on human CD44 transcript was evaluated using a

dual-luciferase reporter assay. Gene expression of hsa-miR-3129 in immortal EOC cell lines was evaluated by qRT-PCR. Lentivirus-mediated hsa-miR-3129 upregulation or downregulation was conducted in SK-OV-3 and CAOV-3 cells, in which endogenous hsa-miR-3129 and CD44 expressions were then measured. In hsa-miR-3129 upregulated or downregulated EOC cells, functional assays were applied to evaluate EOC proliferation, bufalin chemoresistance in vitro, or xenotransplantation in vivo. Moreover, CD44 was ectopically overexposed in hsa-miR-3129 upregulated EOC cells to functionally evaluate the correlation between hsa-miR-3129 and CD44 in EOC. Dual-luciferase reporter assay confirmed hsa-miR-3129 directly binds CD44. QRT-PCR revealed that hsa-miR-3129 was substantially downregulated in EOC cell lines. In SK-OV-3 and CAOV-3 cells, lentivirus-induced hsa-miR-3129 upregulation downregulated CD44 whereas hsa-miR-3129 downregulation did not affect CD44 expression. Hsa-miR-3129 upregulation had significant anti-cancer effects by inhibiting EOC proliferation, increasing bufalin chemoresistance, and suppressing xenotransplantation. On the other hand, overexpressing CD44 reversed the anti-cancer functions by hsa-miR-3129 upregulation in EOC cells. In conclusion, Has-miR-3129 is a functional regulator, possibly through reverse targeting on CD44, in EOC.

Tainsky, M. A., et al. (1995). "Genomic instability due to germline p53 mutations drives preneoplastic progression toward cancer in human cells." Cancer Metastasis Rev **14**(1): 43-48.

Cells heterozygous for mutations in p53 demonstrate extreme genomic instability and develop mutations detectable at the chromosome level as well as the molecular level. This genomic instability causes initially nontumorigenic ras-expressing immortal LFS cells to progress to a tumorigenic state presumably due to additional mutational events. It is not surprising that LFS families with these p53 mutations develop the additional mutations necessary for cancer to occur at such high frequencies. This observation is consistent with increased cancer rates in these families being due to abrogation of a rate limiting step rather than a rate expected for one less step in a multistep carcinogenic process. Although p53 has been shown to be able to function as a transcription factor, mutations in p53 appear to affect genomic stability in LFS fibroblasts with double minutes and telomeric associations being prominent early events. One possibility is that p53 controls the expression of genes required for fidelity of replication or telomerase activity. Alternatively p53 may itself be a replication factor like the transcription factor CTF. In the future, we plan to investigate whether p53 plays a direct role in replication.

Thirthagiri, E., et al. (2007). "Spindle assembly checkpoint and centrosome abnormalities in oral cancer." Cancer Lett **258**(2): 276-285.

Like many solid tumours, oral squamous cell carcinomas (OSCC) invariably exhibit chromosomal instability (CIN) leading to aneuploidy. The mechanisms responsible for CIN in OSCC, however, are largely unknown. This study examined the fidelity of the spindle checkpoint, together with the number, structure and function of centrosomes in a series of well-characterised aneuploid immortal OSCC-derived cell lines that harbour p53 and p16(INK4A) defects. The spindle checkpoints were fully functional in 2 of 7 cell lines and attenuated in the remaining 5 cell lines. Overexpression of the spindle checkpoint protein, Cdc20, was observed in 2 of the cell lines with attenuated checkpoints. Defects in centrosome number, size and localisation were detected in 5 of the cell lines. Clonal cell populations contained cells with both normal and abnormal numbers of centrosomes, suggesting that the control of centrosome number may be inherently unstable in OSCC-derived cell lines. Centrosomal abnormalities were then examined in tissue samples of oral epithelial dysplasias and carcinomas. Abnormal centrosomes were detected in all the tissues examined albeit in a low percentage of cells (<1% to >5%). The percentage of cells containing centrosome abnormalities was significantly higher in the carcinomas than in the dysplasias ($p<0.02$) and in the poorly differentiated SCCs relative to their moderately differentiated ($p<0.04$) and well-differentiated ($p<0.01$) counterparts. We suggest that the genetic alterations associated with the development of the immortal phenotype, together with spindle checkpoint and centrosome defects, are responsible, albeit in part, for the complex karyotypes observed in OSCC. The presence of centrosome abnormalities in oral dysplasias raises the possibility that such defects might contribute to malignant progression.

Thorkildsen, J., et al. (2021). "Chondrosarcoma local recurrence in the Cancer Registry of Norway cohort (1990-2013): Patterns and impact." J Surg Oncol **123**(2): 510-520.

BACKGROUND: There appears to be an association between local recurrence (LR) and risk of metastasis and death in central conventional chondrosarcoma (CCCS) of bone, but this has not been quantified in modern cohorts at a subtype level. METHODS: We identified nonmetastatic cases of CCCS (N = 180) from the Cancer Registry of Norway. We present prognostic analysis of LR accounting for immortal time bias by descriptive statistics and multivariable Cox models. RESULTS: Of 40 LR, one case demonstrated upgrading while two

dedifferentiation. LR was associated with increased risk of metastasis (hazard ratio [HR] = 4.1 [confidence interval, 1.5-10.7]) and death (HR = 9.3 [5.0-17.5]) overall. LR was associated with significant increased risk of metastasis for those with a soft tissue component, axial location, malignancy grade 2, but not atypical cartilaginous tumor's, appropriately treated curettage patients, intramedullary tumors, grade 1 histology, extremity location or "Oslo low risk" group status. We found an increased risk of death for all groups except for those treated by appropriate curettage or belonging to the "Oslo low risk" group. About 50% of LR CCCS were asymptomatic and revealed by routine follow-up. CONCLUSIONS: Upgrading of LR for CCCS was a seldom event. LR was associated with significant increased risk of metastasis and death overall, but not for appropriately treated curettage patients or "Oslo low risk" status.

Tian, F. J., et al. (2004). "[Inhibition of anti-sense human telomerase reverse transcriptase (hTERT) retroviral vector on lung cancer cells]." Ai Zheng **23**(5): 545-549.

BACKGROUND & OBJECTIVE: Inhibition of telomere length can be achieved through suppression of telomerase activity, which may result in the inhibition of immortal cell proliferation. In order to explore the possibility of the telomerase as a target for lung cancer therapy, we investigated the effects of anti-sense human telomerase reverse transcriptase (hTERT) on telomerase activity and cell proliferation of A549 lung cancer cell line. METHODS: The anti-sense hTERT cDNA, an 835 bp in the 5' region of hTERT mRNA was amplified by reverse transcription polymerase chain reaction (RT-PCR), before cloning into pLXSN retroviral vector in sense and anti-sense orientations. A549 cells, a human lung cancer cell line, were infected with recombinant virus obtained after transfection into packaging cell PT67. The expression of hTERT protein was determined by Western blot analysis. The telomerase activity was measured by telomerase repeat amplification protocol (TRAP). The cell proliferation was depicted by cell morphology under inverted microscopy as well as cell growth curve. Apoptosis was analyzed by flow cytometry and DNA electrophoresis. RESULTS: Compared with sense hTERT transduction, hTERT expression and telomerase activity significantly decreased in A549 cells after anti-sense hTERT transduction. The cell proliferation was markedly inhibited with evidence of apoptosis. CONCLUSION: Anti-sense hTERT exhibited significant inhibition of telomerase activity and cell proliferation, in addition to acceleration of apoptosis. This implied the possibility of hTERT as the potential target for gene therapy of lung cancer.

Tiwari, A. K. and H. K. Roy (2012). "Progress against cancer (1971-2011): how far have we come?" J Intern Med **271**(4): 392-399.

'The big C', a common euphemism for cancer, has loomed large on the collective psyche of the mankind for centuries, not least because of the relative dearth of effective treatment against this disease but its ability to relentlessly evade them and come back to haunt us. However, the struggle against cancer took a decisive turn in 1971 when a relentless campaigning by health activists eventually led to signing of the National Cancer Act in the United States, an unprecedented event in the history of diseases. As we commemorate the 40th anniversary of the signing of that historic legislation, an assessment of the progress against cancer would naturally help us understand how we have fared so far in this struggle and guide us in our efforts to re-strategize and re-deploy our limited resources to their best use against this immortal enemy.

Toriola, A. T., et al. (2020). "Metformin Use and Pancreatic Cancer Survival among Non-Hispanic White and African American U.S. Veterans with Diabetes Mellitus." Cancer Epidemiol Biomarkers Prev **29**(1): 169-175.

BACKGROUND: The effect of metformin use on survival among patients with pancreatic ductal adenocarcinoma (PDAC) is controversial. Furthermore, there are no data on African American patients. To address these, we analyzed data from the United States Veterans Health Administration (VHA). METHODS: A population-based retrospective cohort study evaluating overall survival among 3,811 patients with PDAC with preexisting diabetes mellitus, diagnosed with PDAC within the VHA between 1998 and 2013. We calculated HRs and 95% confidence intervals (CI) using multivariable adjusted time-varying Cox proportional hazards regression to control for immortal time bias and confounders. RESULTS: Metformin use was not associated with overall survival in the complete analyses (HR = 1.05; 95% CI, 0.92-1.14; P = 0.28). However, among patients who were metformin naive at the time of PDAC diagnosis (N = 1,158), metformin use was associated with improved overall survival in non-Hispanic white patients (HR = 0.78; 95% CI, 0.61-0.99; P = 0.04), but not African American patients (HR = 1.20; 95% CI, 0.75-1.93; P = 0.45). The survival benefit among non-Hispanic whites was limited to patients with metastatic disease (HR = 0.67; 95% CI, 0.44-1.01; P = 0.06). Among African American patients with metastatic disease, HR was 1.30 (95% CI, 0.77-2.53; P = 0.28). There was a suggestion of heterogeneity by race in patients with metastatic disease (P heterogeneity = 0.05). CONCLUSIONS: We observed no associations between metformin use and survival in patients with

PDAC, but there appears to be a survival benefit among non-Hispanic white patients who were metformin naive at the time of PDAC diagnosis. IMPACT: If confirmed in other studies, our findings suggest that metformin as an adjunctive treatment for PDAC may not improve survival among African American patients.

Tseng, C. H. (2014). "A review on thiazolidinediones and bladder cancer in human studies." J Environ Sci Health C Environ Carcinog Ecotoxicol Rev **32**(1): 1-45.

There is a concern of an increased risk of bladder cancer associated with the use of thiazolidinediones, a class of oral glucose-lowering drugs commonly used in patients with type 2 diabetes with a mechanism of improving insulin resistance. Human studies on related issues are reviewed, followed by a discussion on potential concerns on the causal inference in current studies. Pioglitazone and rosiglitazone are discussed separately, and findings from different geographical regions are presented. Randomized controlled trials designed for primarily answering such a cancer link are lacking, and evidence from clinical trials with available data for evaluating the association may not be informative. Observational studies have been reported with the use of population-based administrative databases, single-hospital records, drug adverse event reporting system, and case series collection. Meta-analysis has also been performed by six different groups of investigators. These studies showed a signal of higher risk of bladder cancer associated with pioglitazone, especially at a higher cumulative dose or after prolonged exposure; however, a weaker signal or null association is observed with rosiglitazone. In addition, there are some concerns on the causal inference, which may be related to the use of secondary databases, biases in sampling, differential detection, and confounding by indications. Lack of full control of smoking and potential biases related to study designs and statistical approaches such as prevalent user bias and immortal time bias may be major limitations in some studies. Overlapping populations and opposing conclusions in studies using the same databases may be of concern and weaken the reported conclusions of the studies. Because randomized controlled trials are expensive and unethical in providing an answer to this cancer issue, observational studies are expected to be the main source in providing an answer in the future. Furthermore, international comparison studies using well-designed and uniform methodology to clarify the risk in specific sexes, ethnicities, and other subgroups and to evaluate the interaction with other environmental risk factors or medications will be helpful to identify patients at risk.

Tu, Y., et al. (2008). "Rhodiola crenulata induces death and inhibits growth of breast cancer cell lines." J Med Food **11**(3): 413-423.

Diverse compounds from many different chemical classes are currently targeted in preclinical analyses for their ability to act as both chemopreventive and chemotherapeutic agents. Phenolic phytochemicals from Rhodiola crenulata has such potential. This Rhodiola species is a perennial plant that grows in the Tundra, Siberia, and high-elevation regions of Tibet. The phenolic secondary metabolites isolated from R. crenulata were recently analyzed in a preclinical setting for their ability to treat lymphosarcomas and superficial bladder cancers. However, the effects of R. crenulata have yet to be examined for its implications in breast cancer prevention or for its chemotherapeutic abilities. Therefore this study investigated the effects of R. crenulata on breast cancer both in vivo and in vitro. Experiments using aggressive human-derived MDA-MB-231 and mouse-derived V14 breast cancer cell lines demonstrated that phenolic-enriched R. crenulata extract was capable of inhibiting the proliferation, motility, and invasion of these cells. In addition, the extracts induced autophagic-like vesicles in all cell lines, eventually leading to death of the tumor cell lines but not the immortal or normal human mammary epithelial cells. Finally, an in vivo experiment showed that phenolic-enriched dietary R. crenulata is effective in preventing the initiation of tumors and slowing down the tumor growth in mice bearing tumor grafts, thereby further demonstrating its possible potential for treatment of breast cancer progression and metastasis.

Untergasser, G., et al. (2002). "Characterization of epithelial senescence by serial analysis of gene expression: identification of genes potentially involved in prostate cancer." Cancer Res **62**(21): 6255-6262.

Evasion of cellular senescence is required for the immortal phenotype of tumor cells. The tumor suppressor genes p16(INK4A), pRb, and p53 have been implicated in the induction of cellular senescence. To identify additional genes and pathways involved in the regulation of senescence in prostate epithelial cells (PrECs), we performed serial analysis of gene expression (SAGE). The gene expression pattern of human PrECs arrested because of senescence was compared with the pattern of early passage cells arrested because of confluence. A total of 144,137 SAGE tags representing 25,645 unique mRNA species was collected and analyzed: 157 mRNAs (70 with known function) were up-regulated and 116 (65 with known function) were down-regulated significantly in senescent PrECs ($P < 0.05$; fold difference >2.5). The differential regulation of an exemplary set of genes

during senescence was confirmed by quantitative real-time PCR in PrECs derived from three different donors. The results presented here provide the molecular basis of the characteristic changes in morphology and proliferation observed in senescent PrECs. Furthermore, the differentially expressed genes identified in this report will be instrumental in the further analysis of cellular senescence in PrECs and may lead to the identification of tumor suppressor genes and proto-oncogenes involved in the development of prostate cancer.

Urquidi, V., et al. (1998). "Telomerase in cancer: clinical applications." Ann Med **30**(5): 419-430.

The biology of telomeres and telomerase has been the subject of intensive investigative effort since it became evident that they play a significant role in two important biological processes, the loss of cellular replicative capacity inherent to organismal ageing and the unrestricted cell proliferation characteristic of carcinogenesis. Telomere shortening in normal cells is a result of DNA replication events, and reduction beyond a critical length is a signal for cellular senescence. One of the cellular mechanisms used to overcome proliferative restriction is the activation of the enzyme telomerase, which replaces the loss of telomeric DNA that occurs at each cell division. Studies have demonstrated that tumours have shorter telomeres than normal tissue and that telomerase is activated in up to 90% of all human cancers while it is present only in a limited range of normal adult tissues. The role of telomerase in the extension of the cellular replicative lifespan has recently been shown by ectopic expression of the enzyme, being consistent with the oncogenesis model whereby the acquisition of an 'immortal' phenotype is a requirement for advanced tumour progression. In this article we review the present knowledge of telomeres and telomerase in cancer and discuss the potential use of this enzyme as a diagnostic and prognostic tumour marker and as a target for cancer therapy.

van Uden, D. J. P., et al. (2020). "Better survival after surgery of the primary tumor in stage IV inflammatory breast cancer." Surg Oncol **33**: 43-50.

INTRODUCTION: Information regarding the effects of resection of the primary tumor in stage IV inflammatory breast cancer (IBC) is scarce. We analyzed the impact of resection of the primary tumor on overall survival (OS) in a large stage IV IBC population. MATERIALS AND METHODS: Patients diagnosed with stage IV IBC between 2005 and 2016 were selected from the Netherlands Cancer Registry, excluding patients without any treatment. To correct for immortal time bias, we performed a landmark analysis including patients alive at least six months after diagnosis. With propensity score matching, patients undergoing surgery of the primary tumor were matched to patients not receiving surgery. Multivariable Cox proportional hazard analyses were performed to determine the association between treatment strategy and OS in the non-matched and matched cohort. RESULTS: Of the 580 included patients after landmark analysis, 441 patients (76%) received only non-surgical treatments and 139 (24%) underwent surgery (96% mastectomy). Median follow-up was 28.8 and 20.0 months in the surgery and no surgery group, respectively. Surgery in the non-matched cohort was independently associated with better survival (HR0.56[95%CI:0.42-0.75]). In the matched cohort (n = 202), surgically treated patients had improved survival over nonsurgically treated patients ($p < 0.005$). Multivariable analysis of the matched cohort revealed that surgery was still associated with better survival (HR0.62[95%CI:0.44-0.87]). CONCLUSION: Although residual confounding and confounding by severity cannot be ruled out, this study suggests that surgery of the primary tumor is associated with improved OS and should be considered as part of the treatment strategy in stage IV IBC.

Verlaat, W., et al. (2018). "Host-cell DNA methylation patterns during high-risk HPV-induced carcinogenesis reveal a heterogeneous nature of cervical pre-cancer." Epigenetics **13**(7): 769-778.

Cervical cancer development following a persistent infection with high-risk human papillomavirus (hrHPV) is driven by additional host-cell changes, such as altered DNA methylation. In previous studies, we have identified 12 methylated host genes associated with cervical cancer and pre-cancer (CIN2/3). This study systematically analyzed the onset and DNA methylation pattern of these genes during hrHPV-induced carcinogenesis using a longitudinal in vitro model of hrHPV-transformed cell lines (n = 14) and hrHPV-positive cervical scrapings (n = 113) covering various stages of cervical carcinogenesis. DNA methylation analysis was performed by quantitative methylation-specific PCR (qMSP) and relative qMSP values were used to analyze the data. The majority of genes displayed a comparable DNA methylation pattern in both cell lines and clinical specimens. DNA methylation onset occurred at early or late immortal passage, and DNA methylation levels gradually increased towards tumorigenic cells. Subsequently, we defined a so-called cancer-like methylation-high pattern based on the DNA methylation levels observed in cervical scrapings from women with cervical cancer. This cancer-like methylation-high pattern was observed in 72% (38/53) of CIN3 and 55% (11/20) of CIN2,

whereas it was virtually absent in hrHPV-positive controls (1/26). In conclusion, hrHPV-induced carcinogenesis is characterized by early onset of DNA methylation, typically occurring at the pre-tumorigenic stage and with highest DNA methylation levels at the cancer stage. Host-cell DNA methylation patterns in cervical scrapings from women with CIN2 and CIN3 are heterogeneous, with a subset displaying a cancer-like methylation-high pattern, suggestive for a higher cancer risk.

Wang, Y., et al. (2020). "The Association between Metformin and Survival of Head and Neck Cancer: A Systematic Review and Meta-Analysis of 7 Retrospective Cohort Studies." Curr Pharm Des **26**(26): 3161-3170.

BACKGROUND: Metformin has been associated with improved survival outcomes in various malignancies. However, observational studies in head and neck cancer are inconsistent. OBJECTIVE: The study aimed to summarize and quantify the relationship between metformin use and the survival of head and neck cancer. METHODS: A meta-analysis based on cohort studies was systematically conducted (published up to Jan 18, 2020), identified from PubMed, Embase, Web of Science, Cochrane Library, Google Scholar, and Scopus databases. Summary hazard ratios (HR) and 95% confidence intervals (CI) were calculated using a random-effects model. RESULTS: Seven retrospective cohort studies including 3,285 head and neck cancer patients were included. The association between the use of metformin and cancer survival was not statistically significant: summarized HR of 0.89 (95% CI 0.66-1.18, P=0.413, I2=64.0%) for overall survival, summarized HR of 0.65 (95% CI 0.31-1.35, P=0.246, I2=60.3%) for disease-free survival, and summarized HR of 0.69 (95% CI 0.40-1.20, P=0.191, I2=73.1%) for disease-specific survival. CONCLUSION: In this meta-analysis of 7 retrospective cohort studies, there was not a statistically significant association between the use of metformin and better survival for head and neck cancer. However, the analysis may have been underpowered. More studies of prospective designs with larger sample sizes are needed to investigate the effect of metformin on the survival of head and neck cancer.

Wang, Y., et al. (2019). "No Effect of Metformin on Ovarian Cancer Survival: A Systematic Review and Meta-Analysis of Cohort Studies." Curr Pharm Des **25**(23): 2595-2601.

BACKGROUND: A number of observational studies examined the association between metformin therapy and ovarian cancer survival outcomes, but the results are inconsistent. OBJECTIVE: The study aimed to investigate the effect of metformin on survival for ovarian cancer patients. METHOD: PubMed, Embase and Web of Science databases were searched for relevant studies from the inception to June 11, 2019. The strength of the relationship was assessed using summary of hazard ratios (HRs) with corresponding 95% confidence intervals (CI). Statistical analyses were carried out using the random-effects model. RESULTS: Totally, 6 retrospective cohort studies involving 2,638 ovarian cancer patients were included. Metformin was not associated with improved overall survival (HR=0.78, 95% CI 0.54-1.12, P=0.175, I2= 61.6%) and disease- free survival (HR=0.49, 95% CI 0.20-1.17, P=0.106, I2=82.1%) in ovarian cancer patients compared to nonmetformin users. CONCLUSION: The current study provides preliminary evidence that metformin may not be associated with a survival benefit for ovarian cancer patients. More studies with rigorous designs are needed.

Wang, Y. B., et al. (2021). "Immortal time bias exaggerates the effect of metformin on the risk of gastric cancer: A meta-analysis." Pharmacol Res **165**: 105425.

High heterogeneity has been reported among epidemiological studies exploring the relationship between metformin and the risk of gastric cancer. Immortal time bias might be one of the vital factors causing heterogeneity because of its widespread existence in pharmacological observational studies and it could severely exaggerate the drug's effectiveness. Immortal time bias could occur in an observational study if exposure status is determined based on a measurement or event that occurs after baseline. In this study, we aimed to assess whether immortal time bias is responsible for the false assumption that metformin reduces the risk of gastric cancer. We searched PubMed, Embase, Web of Science and Cochrane Library databases for relevant studies from the inception to August 9, 2020. The strength of the relationship was assessed using pooled relative risks (RRs) with corresponding 95% confidence intervals (95% CIs). Statistical analyses were carried out using a random-effects model. Pooled RR from 6 cohort studies with immortal time bias found a clear 33% reduced risk associated with metformin use (RR = 0.67, 95% CI = 0.59, 0.77; P < 0.001; I(2) = 48.5%). However, pooled RR from 8 cohort studies without immortal time bias indicated no association between the use of metformin and gastric cancer risk (RR = 0.95, 95% CI = 0.85, 1.05; P = 0.317; I(2) = 64.5%). From a univariate meta-regression model, the presence of immortal time bias was associated with a significant reduction of 29% in the effect estimate of metformin on gastric cancer risk (ratio of RR = 0.71, 95% CI =

0.58, 0.86; P = 0.002). This meta-analysis indicates that metformin use has no protective effect on gastric cancer risk. The relationship between metformin use and gastric cancer risk has been exaggerated as a result of the presence of immortal time bias. Further studies are required to confirm the results by controlling for immortal time bias based on appropriate study designs and statistical methods.

Weberpals, J., et al. (2016). "Beta blockers and cancer prognosis - The role of immortal time bias: A systematic review and meta-analysis." Cancer Treat Rev **47**: 1-11.

BACKGROUND: Findings from experimental and observational studies have suggested beneficial effects of beta blocker (BB) use on cancer survival. Nevertheless, results have been inconclusive and there have been concerns that the observed associations might have resulted from immortal time bias (ITB). We conducted a systematic review and meta-analysis to summarize existing evidence, paying particular attention to this potential source of bias. METHODS: A systematic literature search was performed in PubMed and Web of Science. Studies investigating the association between BB use and overall or cancer-specific survival were included. Summary estimates were derived from meta-analyses using random effects models. The potential influence of ITB was investigated. RESULTS: We identified 30 eligible studies including 88,026 cancer patients in total. We deemed 11 studies to be at high or unclear risk of ITB. Including all studies in the meta-analysis, BB users had a significantly better overall (hazard ratio (HR) 0.88, 95% CI 0.79-0.97) and cancer-specific (HR 0.75, 95% CI 0.64-0.88) survival. Excluding the studies deemed to be prone to ITB resulted in HRs (95% CIs) of 1.00 (0.93-1.07) and 0.90 (0.83-0.98), respectively. Analyses on cancer site and BB type did not show beneficial associations besides overall survival among melanoma patients. However, melanoma-specific survival was not improved. CONCLUSION: We found no clinically meaningful evidence for an association between BB use and survival after excluding studies with a possible ITB. Our results support suggestions that the proposed beneficial effect of BBs on cancer survival might be based on ITB.

Weberpals, J., et al. (2018). "Comparative performance of a modified landmark approach when no time of treatment data are available within oncological databases: exemplary cohort study among resected pancreatic cancer patients." Clin Epidemiol **10**: 1109-1125.

Purpose: The Mantel-Byar method is the gold standard analytical approach to avoid immortal time bias, but requires information on the time between start of follow-up and exposure initiation. Alternatively, a modified landmark approach might be used to mitigate the amount of immortal time bias, which assumes exposure initiation at a predefined landmark time. In the context of an expected positive association between adjuvant chemotherapy (ACT) and overall survival among resected pancreatic cancer (PCa) patients, this study aims to empirically assess the performance of this approach relative to the Mantel-Byar method. Patients and methods: Data from resected PCa patients diagnosed between 2003 and 2014 and registered in the national cancer registries of Belgium, the Netherlands, and Slovenia were used to estimate the association between ACT and overall survival using a Cox proportional hazards model by country and overall. Results derived from the immortal time bias (misclassifying the time to ACT initiation), Mantel-Byar method, and conventional and modified landmark analyses with assumed cutoff times of ACT initiation at 9, 12 and 15 weeks post-diagnosis were compared. Results: In total, 5,668 patients with a total of 10,921 person-years of follow-up were eligible. All analytical approaches showed a significant survival benefit for resected PCa patients who received ACT, but immortal time bias analyses led to strong overestimation of ACT benefits compared to the Mantel-Byar method (immortal time bias: overall HR [95% CI] 0.68 [0.62-0.75] vs Mantel-Byar method: 0.82 [0.71-0.93]), whereas the conventional landmark approach generally provided rather conservative estimates (0.86 [0.75-1.00], 15 weeks landmark). HRs derived from modified landmark analyses depended on the cutoff time, but were similar compared to the Mantel-Byar method at 15 weeks (0.81 [0.70-0.94]). Conclusion: A modified landmark approach might be a valid alternative to the Mantel-Byar method if no time of treatment information is available. The performance depends on the chosen cutoff time.

Weberpals, J., et al. (2017). "Immortal time bias in pharmacoepidemiological studies on cancer patient survival: empirical illustration for beta-blocker use in four cancers with different prognosis." Eur J Epidemiol **32**(11): 1019-1031.

Immortal time bias (ITB) is still seen frequently in medical literature. However, not much is known about this bias in the field of cancer (pharmaco-)epidemiology. In context of a hypothetical beneficial beta-blocker use among cancer patients, we aimed to demonstrate the magnitude of ITB among 9876 prostate, colorectal, lung and pancreatic cancer patients diagnosed between 1998 and 2011, which were selected from a database linkage of the Netherlands Cancer Registry and the PHARMO Database Network. Hazard ratios (HR) and 95%

confidence intervals from three ITB scenarios, defining exposure at a defined point after diagnosis (model 1), at any point after diagnosis (model 2) and as multiple exposures after diagnosis (model 3), were calculated to investigate the association between beta-blockers and cancer prognosis using Cox proportional hazards regression. Results were compared to unbiased estimates derived from the Mantel-Byar model. Ignoring ITB led to substantial smaller HRs for beta-blocker use proposing a significant protective association in all cancer types [e.g. HR 0.18 (0.07-0.43) for pancreatic cancer in model 1], whereas estimates derived from the Mantel-Byar model were mainly suggesting no association [e.g. HR 1.10 (0.84-1.44)]. The magnitude of bias was consistently larger among cancer types with worse prognosis [overall median HR differences between all scenarios in model 1 and Mantel-Byar model of 0.56 (prostate), 0.72 (colorectal), 0.77 (lung) and 0.85 (pancreas)]. In conclusion, ITB led to spurious beneficial associations of beta-blocker use among cancer patients. The magnitude of ITB depends on the duration of excluded immortal time and the prognosis of each cancer.

Wei, M., et al. (2019). "Metformin and pancreatic cancer survival: Real effect or immortal time bias?" Int J Cancer **145**(7): 1822-1828.

High heterogeneity has been reported among cohort studies investigating the association between metformin and pancreatic cancer survival. Immortal time bias may be one importance source of heterogeneity, as it is widely present in previous cohort studies and may severely impair the validity. Our study aimed to examine whether metformin therapy improves pancreatic cancer survival, and to assess the impact of immortal time bias on the effect estimation of metformin in cohort studies. PubMed, EMbase and SciVerse Scopus were searched. Pooled relative risks (RRs) were derived using a random-effects model. Pooled RR from the six studies without immortal time bias showed no association between metformin and mortality in pancreatic cancer patients (RR 0.93, 95% CI 0.82, 1.05; p = 0.22 and I(2) = 75%). In contrast, pooled RR from the nine studies with immortal time bias showed a reduction of 24% in mortality associated with metformin (RR 0.76, 95% CI 0.69, 0.84; p < 0.001 and I(2) = 1%). From a meta-regression model, existence of immortal time bias was associated with a reduction of 18% in the effect estimate of metformin on pancreatic cancer survival (ratio of RR 0.82, 95% CI 0.70, 0.96; p = 0.02). In conclusions, cumulative evidence from cohort studies does not support a beneficial effect of metformin on pancreatic cancer survival. The association between metformin and pancreatic cancer survival has been greatly exaggerated in previous cohort studies due to the wide existence of immortal time bias. More rigorous designs and statistical methods are needed to account for immortal time bias.

Williams, A. C., et al. (1993). "In vitro models of human colorectal cancer." Cancer Surv **16**: 15-29.

Epithelial cell lines that differentiate in vitro have been isolated from hereditary and sporadic colorectal adenomas representing different stages in tumour progression, from small adenomas with a low malignant potential to large adenomas with a relatively high malignant potential. The majority of cell cultures derived from small adenomas senesced, whereas the larger adenomas were more likely to give rise to an immortal cell line. Karyotypic analysis has shown that specific abnormalities of chromosomes 1, 6, 7, 13, 14, 17, 18 and 22 occur in these adenoma cell lines. Abnormalities of chromosome 1 have been implicated in tumour progression and the in vitro immortalization of colorectal adenomas. Molecular and cellular changes involving abnormalities of chromosomes 1 and 18, TP53 and ras gene mutations and reduced response to the growth inhibitory effects of TGFB and sodium butyrate, which occur during tumour progression, suggest that the in vitro model has relevance to in vivo carcinogenesis.

Williams, S. (2018). "Surrogate endpoints in early prostate cancer research." Transl Androl Urol **7**(3): 472-482.

Clinical research into clinically-localized prostate cancer (PC) is a highly challenging environment. The protracted durations and large numbers required to achieve survival endpoints have placed much pressure on validating early surrogate endpoints. Further confounding is the predominance of deaths from causes other than PC. The analysis of multiple randomized clinical trials in early PC has shown MFS to be a robust surrogate for OS, using a contemporary analytic framework that identify patient-level and trial-level associations. This could potentially save around one year of trial follow-up in some therapies. Identification of a similarly robust surrogate at a substantially earlier timepoint remains a major challenge. Multiple biochemical indices based on PSA have been proposed in the literature, but all remain to be validated at the trial-level. Operationally, many of these indices have inherent biases such as immortal-time bias (ITB) and interval censoring that potentially weakens associations and the individual- or trial-level. The complexity of a failure definition can also impact the reliability of the derived outcomes. Confounding issues such as the impact of comorbidities leading to non-cancer deaths have been largely dealt with by their exclusion using cancer-specific endpoints and advanced statistical methods,

while issues such as PSA "bounce" and recovery from androgen deprivation therapy remain important to account for in cohorts treated with radiotherapy. Several potential surrogate endpoints based on serum prostate-specific antigen (PSA) levels show promising associations with PC-specific and overall survival (OS) in individual studies. Further large collaborative projects will continue to refine potential indices with these issues in mind, and explore the objective of an early surrogate of OS.

Wu, Y., et al. (2019). "ASF1a inhibition induces p53-dependent growth arrest and senescence of cancer cells." Cell Death Dis **10**(2): 76.

Anti-silencing function 1a (ASF1a) is a histone H3-H4 chaperone isoform involved in chromatin assembling and transcription regulation. Recently, ASF1a has been shown to be up-regulated in certain human malignancies and required for the expression of telomerase reverse transcriptase (TERT), a factor essential for the immortal phenotype of cancer cells; however, its role in oncogenesis remains poorly defined. In the present study, we determine whether ASF1a is required for the unlimited proliferation of cancer cells, a key cancer hallmark. Elevated ASF1a mRNA expression was observed in hepatocellular carcinoma (HCC) tumors. The overexpression of ASF1a was similarly found in 20 cancer types contained in TCGA and GTEx datasets. ASF1a knockdown led to growth arrest and senescence of wild-type (wt) p53-carrying HCC and prostate cancer cells. Cellular senescence mediated by ASF1a inhibition resulted from the robust up-regulation of p53 and p21(cip1) expression, but without detectable changes in TERT expression. p53 inhibition attenuated p21(cip1) induction caused by ASF1a depletion. Mechanistically, ASF1a-knocked down cells displayed widespread DNA damage. The TCGA dataset analysis revealed a negative correlation between ASF1a and p21(cip1) expression in multiple types of primary tumors, including HCC, prostate, gastric, and breast cancer. Higher ASF1a and lower p21(cip1) expression predicted a poor outcome in patients with HCC. Our results reveal that ASF1a overexpression is widespread in human malignancies and is required for the infinite proliferation of cancer cells, whereas its inhibition induces DNA damage and subsequent up-regulation of p53-p21(cip1) expression, thereby triggering cellular senescence. Thus, ASF1a may serve as a potential target in cancer therapy.

Xiao, L., et al. (2019). "Motor Neuron and Pancreas Homeobox 1 (MNX1) Is Involved in Promoting Squamous Cervical Cancer Proliferation via Regulating Cyclin E." Med Sci Monit **25**: 6304-6312.

BACKGROUND Cervical cancer is one of the most lethal gynecologic malignancies worldwide. The objective of this study was to assess the role of MNX1 in cervical cancer and its underlying mechanisms. MATERIAL AND METHODS The expression of motor neuron and pancreas homeobox 1 (MNX1) in immortal epithelial cervical cell line ECT, cervical cancer cell HeLa, and SiHa and cervical cancer, as well as in adjacent noncancer tissues, was detected and analyzed. CCK-8 and colony formation assays were performed to evaluate the effects of MNX1 overexpression on cervical cancer cell proliferation. Transwell assay was used to detect migration and invasion after MNX1 knockdown or overexpression. Real-time PCR and Western blotting were used to examine MNX1 and cell cycle regulator expression. RESULTS Data from our study indicated that MNX1 was upregulated both in cervical cancer cell lines and cervical cancer tissues. The high levels of MNX1 are related to advanced stages and lymph nodes metastasis. The overexpression of MNX1 promoted cervical cancer cells proliferation, migration, and invasion. Moreover, MNX1 upregulated 2 critical cell cycle regulators, CCNE1 and CCNE2. CONCLUSIONS These findings reveal MNX1 as a novel oncogene of cervical cancer and indicate MNX1 is a promising therapeutic and prognostic biomarker.

Xin, W., et al. (2018). "Effects of metformin on survival outcomes of pancreatic cancer patients with diabetes: A meta-analysis." Mol Clin Oncol **8**(3): 483-488.

Pancreatic cancer risk is reduced by metformin treatment in patients with diabetes. However, the effect of metformin on pancreatic cancer overall survival is unclear. The aim of the present study was to determine the association between metformin and clinical outcomes of pancreatic cancer patients with diabetes. An electronic and manual search was conducted using PubMed, Web of Science, Medline-Ovid and Cochrane Library databases between the beginning and March 31, 2017. A total of 8 studies consisting of 4,293 patients with pancreatic cancer with diabetes were included, comprising 2,033 patients who had received metformin and 2,260 patients who had not. The meta-analysis showed that metformin was associated with a relative survival benefit in pancreatic cancer patients [hazard ratio (HR), 0.81; 95% confidence interval (CI), 0.70-0.93]. These associations were also observed in subgroups of Asian countries 0.64 (95% CI, 0.52-0.80) and Western countries 0.88 (95% CI, 0.82-0.95), as well as diabetes (no indication of diabetes type). Excluding the studies considered as be prone to immortal time bias resulted in HRs (95% CIs) of 0.86 (0.69-1.07). The results of this study support the notion that the use of metformin may improve the overall survival of patients with

pancreatic cancer with concurrent diabetes. However, the proposed beneficial effect of metformin on pancreatic cancer survival may be based on immortal time bias. Further carefully designed studies with high quality are warranted to confirm this efficacy.

Yang, X., et al. (2000). "KAI1 protein is down-regulated during the progression of human breast cancer." Clin Cancer Res **6**(9): 3424-3429.

The KAI1 gene was identified as a metastasis suppressor gene for human prostate cancer. Recently, we showed that KAI1 mRNA levels were higher in an immortal, normal-like breast epithelial cell line and nonmetastatic breast cancer cell lines but lower substantially in highly metastatic breast cancer cell lines. In this study, we examined KAI1 protein expression in breast cancer cell lines by Western blot and immunohistochemical study. KAI1 protein levels paralleled KAI1 mRNA levels and were inversely correlated with the metastatic potential of breast cancer cells. Furthermore, we examined KAI1 protein expression immunohistochemically in specimens from 81 patients with breast cancer and then correlated the findings with the clinical and histopathological parameters of the patients. High levels of KAI1 protein expression were found in normal breast tissues and noninvasive breast cancer (ductal carcinoma in situ). In contrast, KAI1 expression was reduced in most of the infiltrating breast tumors. We found that, in general, more malignant tumors demonstrated significantly lower KAI1 expression ($P = 0.004$). Additionally, among 29 specimens demonstrating multiple stages of malignancy within a single specimen, 23 demonstrated significant differences in KAI1 expression between benign breast tissue, ductal carcinoma in situ, and invasive carcinoma. The higher the incidence for malignancy within a given specimen, the lower the KAI1 expression ($P < 0.001$). These data suggest that in advanced breast cancer, KAI1 expression is down-regulated. Therefore, KAI1 may be a potentially useful indicator of human breast cancer progression.

Yang, X. L., et al. (2012). "Addressing different biases in analysing drug use on cancer risk in diabetes in non-clinical trial settings--what, why and how?" Diabetes Obes Metab **14**(7): 579-585.

Motivated by recent reports on associations between diabetes and cancer, many researchers have used administrative databases to examine risk association of cancer with drug use in patients with diabetes. Many of these studies suffered from major biases in study design and data analysis, which can lead to erroneous conclusions if these biases are not adjusted. This article discusses the sources and impacts of these biases and methods for correction of these biases. To avoid erroneous results, this article suggests performing sensitivity and specificity analysis as well as using a drug with a known effect on an outcome to ascertain the validity of the proposed methods. Using the Hong Kong Diabetes Registry, we illustrated the impacts of biases of drug use indication and prevalent user by examining the effects of statins on cardiovascular disease. We further showed that 'immortal time bias' may have a neutral impact on the estimated drug effect if the hazard is assumed to be constant over time. On the contrary, adjustment for 'immortal time bias' using time-dependent models may lead to misleading results biased towards against the treatment. However, artificial inclusion of immortal time in non-drug users to correct for immortal time bias may bias the result in favour of the therapy. In conclusion, drug use indication bias and prevalent user bias but not immortal time bias are major biases in the design and analysis of drug use effects among patients with diabetes in non-clinical trial settings.

Yeager, T. R., et al. (1998). "Overcoming cellular senescence in human cancer pathogenesis." Genes Dev **12**(2): 163-174.

Elevation of p16, the CDKN2/p16 tumor suppressor gene (TSG) product, occurs at senescence in normal human uroepithelial cells (HUC). Immortal HUCs and bladder cancer cell lines show either alteration of p16 or pRb, the product of the retinoblastoma (RB) TSG. In addition, many human cancers show p16 or pRb alteration along with other genetic alterations that we associated with immortalization, including +20q and -3p. These observations led us to hypothesize that p16 elevation plays a critical role in senescence cell cycle arrest and that overcoming this block is an important step in tumorigenesis in vivo, as well as immortalization in vitro. Using a novel approach, we tested these hypotheses in the present study by examining p16 and pRb status in primary culture (P0) and after passage in vitro of transitional cell carcinoma (TCC) biopsies that represented both superficial bladder tumors and invasive bladder cancers. We demonstrated that all superficial TCCs showed elevated p16 after limited passage in vitro and then senesced, like normal HUCs. In contrast, all muscle invasive TCCs contained either a p16 or a pRb alteration at P0 and all spontaneously bypassed senescence ($P = 0.001$). Comparative genomic hybridization (CGH) was used to identify regions of chromosome loss or gain in all TCC samples. The application of a statistical model to the CGH data showed a high probability of elevated alteration rates of +20q11-q12 (0.99) and +8p22-pter (0.94) in the immortal muscle invasive TCCs, and of -9q (0.99) in the superficial TCCs. Three myoinvasive TCCs lost 3p13-p14. In this study, four of six myoinvasive TCCs also showed TP53 mutation that

associated well with genome instability (P = 0.001), as previously hypothesized. Notably, TP53 mutation, which has been used as a marker of tumor progression in many human cancers, was less significant in associating with progression in this study (P = 0.04) than was p16 or pRb alteration (P = 0.001). Thus, these data support a new model in which overcoming senescence plays a critical role in human cancer pathogenesis and requires at least two genetic changes that occur in several combinations that can include either p16 or pRb loss and at least one additional alteration, such as +20q11-q12, -3p13-p14, or -8p21-pter.

Yoo, G. H., et al. (2000). "Progression of head and neck cancer in an in vitro model." Arch Otolaryngol Head Neck Surg **126**(11): 1313-1318.

OBJECTIVE: To identify alterations in angiogenesis and cell cycle regulation as preneoplastic cells progress to cancer in an in vitro model of head and neck tumor progression. METHODS: Immortal human gingival keratinocyte (IHGK) cells (preneoplastic) were derived from normal oral keratinocytes and were immortalized with human papillomavirus 16. Transformation of IHGK cells with a carcinogen (NNK, 4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone) gave rise to IHGKN cells. We determined the growth rates, cell cycle phase, expression of cell cycle regulators, and expression of vascular endothelial growth factor along with the organotypic features of these cells and compared them with characteristics of head and neck cancer cells. RESULTS: IHGK and IHGKN cells grown in raft culture were morphologically similar to severe dysplasia and carcinoma, respectively. The proportion of cells in G(0)/G(1) was similar between IHGK and IHGKN. However, the proportion of IHGK cells was 35% greater in S phase as compared with the IHGKN cells, while a greater percentage (40%) of IHGKN cells were in G(2)/M. The expression of the other cell cycle regulators tested was unchanged. IHGK cells secreted less vascular endothelial growth factor on day 1 when compared with IHGKN (50.6 vs 245.6 pg/mL), along with a lower overall production rate (79% vs 133%). CONCLUSIONS: Transformation of IHGK cells resulted in the activation of vascular endothelial growth factor associated with angiogenesis. Inactivation of the G(1) cell cycle regulation occurred during immortalization and before transformation, and was sustained after carcinogen exposure. These alterations correspond to changes observed in patients with head and neck squamous cell carcinoma. This model can be useful in testing novel therapeutic and preventive strategies.

Yoshida, K., et al. (1997). "Telomerase activity in bladder carcinoma and its implication for noninvasive diagnosis by detection of exfoliated cancer cells in urine." Cancer **79**(2): 362-369.

BACKGROUND: Telomerase is an enzyme that can reconstitute the ends (telomeres) of chromosomes after cell division and thus circumvent the cumulative damage that occurs in normal adult somatic cells during successive mitotic cycles. Recently, it has been proposed that this enzyme should, therefore, be detectable in immortal malignant cells but not in their normal counterparts, which stop dividing and senesce. Accordingly, telomerase activity has been reported in many types of malignant tumors, including those of the gastrointestinal tract, breast, and lung but little information was available regarding its status in bladder carcinoma or in exfoliated cancer cells. METHODS: In the current study, telomerase activity was examined by a polymerase chain reaction-based assay designated TRAP (telomeric repeat amplification protocol) in tissue samples from 56 bladder carcinomas, 17 nonneoplastic bladder lesions, and 2 dysplastic lesions of the urinary tract. The feasibility of identifying cancer patients by the detection of telomerase activity in exfoliated cancer cells in the urine was also investigated. Such activity was assayed in centrifuged urine cell pellets from 26 bladder carcinoma patients and from 83 patients with no evidence of malignant disease. RESULTS: Evidence of telomerase was detected in solid tissue specimens from 48 of the 56 bladder carcinomas (86%) regardless of tumor stage or differentiation, whereas it was not found in any normal bladder tissue specimen. However, it was present in the dysplastic bladder lesions as well as in nearly all Stage I well differentiated carcinomas, suggesting that its activation occurs for the early stages of carcinogenesis and could perhaps be a useful marker for the detection of early primary or recurrent bladder tumors. Telomerase activity was detected with various signal intensities in urine specimens from 16 of the 26 patients with bladder carcinoma (62% sensitivity), whereas only 3 of 83 nonmalignant urine samples showed any activity (96.4% specificity); this was very weak. CONCLUSIONS: These results suggest that telomerase could be a good diagnostic marker for the early noninvasive identification of patients with bladder carcinoma by facilitating the detection of exfoliated immortal cancer cells in their urine.

Yoshida, R., et al. (1999). "IFN-gamma- and cell-to-cell contact-dependent cytotoxicity of allograft-induced macrophages against syngeneic tumor cells and cell lines: an application of allografting to cancer treatment." J Immunol **163**(1): 148-154.

In allogeneic tumor or skin transplantation, the rejection process that destroys the allogeneic cells

leaves syngeneic cells intact by discrimination between self and nonself. Here, we examined whether the cells infiltrating into the allografts could be cytotoxic against syngeneic immortal cells in vitro and in vivo. The leukocytes (i.e., macrophages (Mphi; 55-65% of bulk infiltrates), granulocytes (20-25%), and lymphocytes (15-20%)) infiltrating into allografts, but not into autografts, in C57BL/6 mice were cytotoxic against syngeneic tumor cells and cell lines, whereas the cytotoxic activity was hardly induced in allografted, IFN-gamma-/- C57BL/6 mice. Among the leukocytes, Mphi were the major population of cytotoxic cells; and the cytotoxic activity appeared to be cell-to-cell contact dependent. When syngeneic tumor cells were s.c. injected into normal C57BL/6 mice simultaneously with the Mphi-rich population or allogeneic, but not syngeneic, fibroblastic cells, tumor growth was suppressed in a cell number-dependent manner, and tumor cells were rejected either with a Mphi:tumor ratio of about 30 or with an allograft:tumor ratio of approximately 200. In the case of IFN-gamma-/- C57BL/6 mice, however, the s.c. injection of the allograft simultaneously with tumor cells had no effect on the tumor growth. These results suggest that allograft or allograft-induced Mphi may be applicable for use in cancer treatment and that IFN-gamma induction by the allograft may be crucial for the treatment.

Yusuf, M., et al. (2019). "Effects of adjuvant radiation therapy on survival for patients with resected primary tracheal carcinoma: an analysis of the National Cancer Database." Jpn J Clin Oncol **49**(7): 628-638.

OBJECTIVE(S): To identify predictors for receiving adjuvant radiation therapy (RT) and investigate the impact of adjuvant RT on survival for patients with resected primary tracheal carcinoma (PTC). METHODS: The National Cancer database was queried for patients with PTC diagnosed from 2004 to 2014 undergoing resection. Patients who died within 30 days of resection were excluded to minimize immortal time bias. Kaplan-Meier methods, Cox regression modeling and propensity score weighted (PSW) log-rank tests were considered to assess the relationship between adjuvant RT and overall survival (OS). Logistic regression was performed to identify predictors associated with receiving adjuvant RT. RESULTS: A total of 549 patients were identified with 300 patients (55%) receiving adjuvant RT. Squamous cell carcinoma (SCC) was the most common histology with 234 patients (43%). Adenoid cystic carcinoma (ACC) was second most frequent with 180 patients (33%). Adjuvant RT was not associated with OS by multivariable Cox analysis or PSW log-rank test (P values > 0.05). Patients with positive surgical margins (odds ratio (OR) 1.80, confidence interval (CI) 1.06-3.07) were more likely to receive adjuvant RT than those with negative surgical margins. Patients with ACC (OR 6.53, CI 3.57-11.95) were more likely to receive adjuvant RT compared with SCC. CONCLUSIONS: Adjuvant RT was not significantly associated with OS for patients with resected PTC in this analysis. Surgical margin status and tumor histology were associated with receiving adjuvant RT. Further investigations including prospective registry studies capturing radiation technique and treatment volumes are needed to better define which patients with resected PTC may benefit from adjuvant RT.

Zajchowski, D. A., et al. (1990). "Suppression of tumor-forming ability and related traits in MCF-7 human breast cancer cells by fusion with immortal mammary epithelial cells." Proc Natl Acad Sci U S A **87**(6): 2314-2318.

Somatic cell hybrids between MCF-7 human breast cancer cells and normal immortalized human mammary epithelial cells have been obtained by polyethylene glycol-mediated cell fusion. The hybrid cells are suppressed in their ability to form tumors in nude mice, as well as in traits specific to the tumorigenic MCF-7 parent: growth factor independence, tumor necrosis factor sensitivity, and pS2 gene expression. In addition, they display other characteristics of the "normal" parent, including increased expression relative to the MCF-7 cells of the genes for the extracellular matrix component fibronectin, the intermediate filament keratin 5, and the angiogenesis inhibitor thrombospondin. The levels of keratins 8 and 18 also resemble those of the nontumorigenic parent. These results provide evidence for the existence of tumor suppressor gene products in immortal mammary epithelial cells. We propose a characteristic "suppressed" tumor cell phenotype, which encompasses altered cytoarchitecture, angiogenesis capabilities, and growth factor requirements.

Zeng, J., et al. (2009). "FoxM1 is up-regulated in gastric cancer and its inhibition leads to cellular senescence, partially dependent on p27 kip1." J Pathol **218**(4): 419-427.

The FoxM1 transcription factor, a master regulator of mitotic gene expression, promotes the pathogenesis of several malignancies. However, little is known about its expression and function in gastric cancer. In the present study we determined whether FoxM1 is over-expressed in gastric cancer, and whether it is required to maintain an immortal phenotype of gastric cancer cells. The over-expression of FoxM1 was observed in 37/42 tumour specimens from patients with gastric cancer. When FoxM1 in gastric cancer cells was knocked-down, impaired

clonogenicity and cellular senescence occurred independently of p53 and p16 status. FoxM1 depletion led to the down-regulation of its target genes c-MYC and Skp2, coupled with the accumulation of the CDK inhibitor p27(kip1). Importantly, the FoxM1 inhibition-mediated cellular senescence and clonogenic defect was attenuated by the abolition of p27(kip1) induction. Telomerase reverse transcriptase, the key component of telomerase essential for cellular immortalization, was also inhibited in the FoxM1-depleted cells. Taken together, the FoxM1 gene is aberrantly activated in gastric cancer and its inhibition triggers p53- and p16-independent senescence of cancer cells by regulating the expression of p27(kip1) and other targets. These findings provide mechanistic insights into the role of FoxM1 in the pathogenesis of gastric cancer, which may have diagnostic and therapeutic implications in gastric cancer.

Zhang, W., et al. (1998). "Telomerase activity in prostate cancer, prostatic intraepithelial neoplasia, and benign prostatic epithelium." Cancer Res **58**(4): 619-621.

Telomerase is a ribonucleoprotein that synthesizes telomeric DNA on chromosomal ends. Telomerase activation has been seen in many immortal cell lines and cancers. Telomerase activity was analyzed in prostate carcinoma; in coexistent prostatic intraepithelial neoplasia (PIN), benign prostatic hyperplasia (BPH), atrophy and normal tissue; and in benign prostate glands. Telomerase activity was detected in 80 of 87 (92%) prostate cancers. Forty-one matched samples (from a total of 32 cases) were available for comparative analysis. The presence of telomerase activity in adjacent PIN, BPH, and normal tissue was correlated with telomerase activity in the malignant epithelium. In these adjacent tissues, telomerase activity was found in 11 of 15 (73%) PINs, 13 of 26 (50%) BPHs, and 1 of 6 (16%) atrophy and 4 of 11 (36%) normal tissues. In contrast to the BPH tissue from cancer-bearing glands, all 16 BPH specimens from patients only diagnosed with BPH were telomerase activity negative. In cancer samples, there was no correlation between telomerase activity and Gleason grade or preoperation prostate-specific antigen level. Our data indicate that telomerase activity is present in most prostate cancers. The high rate of telomerase activity in the benign-appearing areas of these glands may be attributed either to the presence of occult cancer cells or to early molecular alterations of cancer that were histologically inapparent.

Zhang, Z. J. (2019). "Metformin and Reduced Risk of Cancer in the Hong Kong Diabetes Registry: Real Effect or Immortal Time Bias?" J Gen Intern Med **34**(7): 1154-1157.

BACKGROUND: Whether metformin reduces cancer risk has been hotly debated. One common opinion is that the observed beneficial effects of metformin are the consequence of immortal time bias. OBJECTIVE: To examine whether the observed beneficial effects of metformin on cancer risk are the consequence of immortal time bias. DESIGN: Retrospective cohort study. PARTICIPANTS: A cohort of 3485 patients who started metformin before or at enrollment, 1226 patients who initiated metformin after enrollment, and an unexposed group of 1392 patients who never used metformin. MAIN MEASURES: Metformin users were categorized into 11 groups in terms of length of time between metformin initiation and enrollment. The percent changes in immortal person-time were calculated for each group. RESULTS: As the groups of current metformin users (n = 3485) were added sequentially to the metformin group with potential immortal time bias (n = 1226), the proportion of immortal person-time decreased gradually by 74%. As the immortal time decreased, the association between metformin and cancer risk remained statistically significant (uncorrected hazard ratio 0.54, 95% confidence interval 0.42-0.69, P < 0.0001). CONCLUSION: The change in the association between metformin and cancer is small compared with the changes in the proportion of immortal time, suggesting that immortal time bias does not account for the observed beneficial effect of metformin on cancer risk. Further studies are warranted to confirm this finding in other cohort studies.

Zhao, X., et al. (2008). "Cyclooxygenase-2 expression during immortalization and breast cancer progression." Cancer Res **68**(2): 467-475.

Identification of molecular aberrations in premalignant human mammary epithelial cells (hMEC), the precursors for breast cancers, is a central goal in breast cancer biology. Recent studies implicated expression of cyclooxygenase 2 (COX-2) as a marker to identify precursor cells for breast cancer. In this study, we analyzed COX-2 expression in preselection and postselection hMEC cells and observed similar COX-2 levels in both cells. Interestingly, immortalization of postselection cells using various methods leads to a dramatic decrease in COX-2 expression. Similar to immortal cells, the majority of breast cancer cell lines expressed low levels of COX-2 protein. Finally, analyses of COX-2 expression in a series of specimens from reduction mammoplasty, adenosis, ductal carcinoma in situ, and infiltrating ductal carcinoma showed down-regulation of COX-2 expression during tumor progression. Importantly, down-regulation of COX-2 using small

interfering RNA in cells showed no effect on cell proliferation, anchorage-independent growth, migration, or invasion. These results show that (a) COX-2 overexpression does not seem to predict a breast cancer precursor cell and does not provide advantage for the cell to be transformed; (b) inhibition of COX-2 does not affect hMEC growth and oncogenic behavior in the conditions analyzed; and (c) COX-2 expression is decreased in breast cancer cell lines and cancer specimens as compared with normal mammary epithelial cells.

Zhou, C. and J. Liu (2003). "Inhibition of human telomerase reverse transcriptase gene expression by BRCA1 in human ovarian cancer cells." Biochem Biophys Res Commun **303**(1): 130-136.

Human telomerase reverse transcriptase (hTERT), the catalytic subunit of human telomerase, is responsible for the synthesis and maintenance of the telomeric repeats at the distal ends of human chromosomes. Telomerase expression is repressed in normal human cells and is activated in immortal cells and during tumorigenesis, but the mechanism by which telomerase expression is regulated is not fully understood. Previous studies have shown that c-Myc stimulates hTERT transcription through the binding sites located on the hTERT promoter. In this study, we sought to determine whether BRCA1 inhibits hTERT transcription through its direct interaction with c-Myc. In ovarian cancer cells, c-Myc increased hTERT expression by threefold and BRCA1 completely abrogated this activity. A mutation in the c-Myc-binding site (E-box) of the hTERT promoter resulted in the loss of activation by c-Myc and in the loss of inhibition by BRCA1. Deletion of the c-Myc-binding domain in BRCA1 resulted in the loss of BRCA1's ability to inhibit transcription of the hTERT promoter. In addition, BRCA1 associates with c-Myc and inhibits the binding activity of c-Myc to the hTERT promoter. Our data indicate that BRCA1 is involved in regulating cellular immortalization through the modulation of c-Myc on the hTERT promoter.

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