# HeLa Cells Research Literatures

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**Abstract:** HeLa (also Hela or hela) is a cell type in an immortal cell line used in scientific research. It is the oldest and most commonly used human cell line. The line was derived from cervical cancer cells taken on February 8, 1951 from Henrietta Lacks, a patient who died of her cancer on October 4, 1951. The cell line was found to be remarkably durable and prolific which warrants its extensive use in scientific research. The cells from Lacks's cancerous cervical tumor were taken without her knowledge or consent. Cell biologist George Otto Gey found that they could be kept alive, and isolated one specific cell, multiplied it, and developed a cell line. (Before this, cells cultured from other human cells would only survive for a few days; scientists spent more time trying to keep the cells alive than performing actual research on them. Cells from Lacks's tumor behaved differently.) As was custom for Gey's lab assistant, she labeled the culture 'HeLa', the first two letters of the patient's first and last name; this became the name of the cell line. These were the first human cells grown in a lab that were naturally "immortal", meaning that they do not die after a set number of cell divisions (i.e. cellular senescence). These cells could be used for conducting a multitude of medical experiments — if the cells died, they could simply be discarded and the experiment attempted again on fresh cells from the culture. This represented an enormous boon to medical and biological research. The stable growth of HeLa enabled a researcher at the University of Minnesota hospital to successfully grow polio virus, enabling the development of a vaccine, and by 1952, Jonas Salk developed a vaccine for polio using these cells. To test Salk's new vaccine, the cells were put into mass production in the first-ever cell production factory. In 1953, HeLa cells were the first human cells successfully cloned and demand for the HeLa cells quickly grew in the nascent biomedical industry. Since the cells' first mass replications, they have been used by scientists in various types of investigations including disease research, gene mapping, and effects of toxic substances and radiation on humans. Additionally, HeLa cells have been used to test human sensitivity to tape, glue, cosmetics, and many other products. Scientists have grown an estimated 20 tons of HeLa cells, and there are almost 11,000 patents involving these cells. The HeLa cell lines are also notorious for invading other cell cultures in laboratory settings. It is estimated that HeLa cells, at one point, contaminated millions of dollars' worth of biological research. 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

HeLa (also Hela or hela) is a cell type in an immortal cell line used in scientific research. It is the oldest and most commonly used human cell line. The line was derived from cervical cancer cells taken on February 8, 1951 from Henrietta Lacks, a patient who died of her cancer on October 4, 1951. The cell line was found to be remarkably durable and prolific which warrants its extensive use in scientific research. The cells from Lacks's cancerous cervical tumor were taken without her knowledge or consent. Cell biologist George Otto Gey found that they could be kept alive, and isolated one specific cell, multiplied it, and developed a cell line. (Before this, cells cultured from other human cells would only survive for a few days; scientists spent more time trying to keep the cells alive than performing actual research on them. Cells from Lacks's tumor behaved differently.) As was custom for Gey's lab assistant, she labeled the culture 'HeLa', the first two letters of the patient's first and last name; this became the name of the cell line. These were the first human cells grown in a lab that were naturally "immortal", meaning that they do not die after a set number of cell divisions (i.e. cellular senescence). These cells could be used for conducting a multitude of medical experiments — if the cells died, they could simply be discarded and the experiment attempted again on fresh cells from the culture. This represented an enormous boon to medical and biological research. The stable growth of HeLa enabled a researcher at the University of Minnesota hospital to successfully grow polio virus, enabling the development of a vaccine,

and by 1952, Jonas Salk developed a vaccine for polio using these cells. To test Salk's new vaccine, the cells were put into mass production in the first-ever cell production factory. In 1953, HeLa cells were the first human cells successfully cloned and demand for the HeLa cells quickly grew in the nascent biomedical industry. Since the cells' first mass replications, they have been used by scientists in various types of investigations including disease research, gene mapping, and effects of toxic substances and radiation on humans. Additionally, HeLa cells have been used to test human sensitivity to tape, glue, cosmetics, and many other products. Scientists have grown an estimated 20 tons of HeLa cells, and there are almost 11,000 patents involving these cells. The HeLa cell lines are also notorious for invading other cell cultures in laboratory settings. It is estimated that HeLa cells, at one point, contaminated millions of dollars' worth of biological research. 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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The following introduces recent reports as references in the related studies.

**2. Literatures**

The following gives some recent reference papers on.

Abnous, K., et al. (2013). "Synthesis and molecular modeling of six novel monastrol analogues: evaluation of cytotoxicity and kinesin inhibitory activity against HeLa cell line." Daru **21**(1): 70.

BACKGROUND AND THE PURPOSE OF THE STUDY: A common approach in cancer chemotherapy is development of drugs that interrupt the mitosis phase of cell division. Dimethylenastron is a known kinesin inhibitor. In this study, six novel dimethylenastron analogues (4a-f), in which 3-hydroxyphenyl substituent has been replaced with substituted benzylimidazolyl, were synthesized through Biginelli reaction. METHODS: Six novel Biginelli compounds (4a-f) were synthesized through one step Biginelli reaction of imidazole aldehydes (3a-c), dimedone and urea or thioura. In vitro cytotoxicities of prepared compounds were investigated using MTT assay. Furthermore the ELIPA kit was implemented to study inhibitory effects of synthesized compounds on ATPase activity of kinesin by measuring of organic phosphate. RESULTS: Our results indicated that analogue 4c is the most toxic and analogues 4f, 4b and dimethylenasteron were less cytotoxic in compare with other analogues. On the other hand, analogue 4a, 4b, 4c and 4e showed stronger Kinesin inhibition as compared with analogue 4f and dimethylenasteron. None of synthesized compounds were as potent kinesin inhibitor as Taxol. Docking analysis revealed that hydrogen bond formation and hydrophobic interactions were the key factors affecting inhibitory effects of these compounds. CONCLUSION: Newly synthesized compounds were found to have moderate to good cytotoxicity against HeLa cancer cell. Our results may be helpful in further design of dihydropyrimidine as potential anticancer agents.

Abu, N., et al. (2016). "Dual Regulation of Cell Death and Cell Survival upon Induction of Cellular Stress by Isopimara-7,15-Dien-19-Oic Acid in Cervical Cancer, HeLa Cells In vitro." Front Pharmacol **7**: 89.

The Fritillaria imperialis is an ornamental flower that can be found in various parts of the world including Iraq, Afghanistan, Pakistan, and the Himalayas. The use of this plant as traditional remedy is widely known. This study aims to unveil the anti-cancer potentials of Isopimara-7,15-Dien-19-Oic Acid, extracted from the bulbs of F. imperialis in cervical cancer cell line, HeLa cells. Flow cytometry analysis of cell death, gene expression analysis via cDNA microarray and protein array were performed. Based on the results, Isopimara-7,15-Dien-19-Oic acid simultaneously induced cell death and promoted cell survival. The execution of apoptosis was apparent based on the flow cytometry results and regulation of both pro and anti-apoptotic genes. Additionally, the regulation of anti-oxidant genes were up-regulated especially thioredoxin, glutathione and superoxide dismutase- related genes. Moreover, the treatment also induced the activation of pro-survival heat shock proteins. Collectively, Isopimara-7,15-Dien-19-Oic

Acid managed to induce cellular stress in HeLa cells and activate several anti- and pro survival pathways.

Adey, A., et al. (2013). "The haplotype-resolved genome and epigenome of the aneuploid HeLa cancer cell line." Nature **500**(7461): 207-211.

The HeLa cell line was established in 1951 from cervical cancer cells taken from a patient, Henrietta Lacks. This was the first successful attempt to immortalize human-derived cells in vitro. The robust growth and unrestricted distribution of HeLa cells resulted in its broad adoption--both intentionally and through widespread cross-contamination--and for the past 60 years it has served a role analogous to that of a model organism. The cumulative impact of the HeLa cell line on research is demonstrated by its occurrence in more than 74,000 PubMed abstracts (approximately 0.3%). The genomic architecture of HeLa remains largely unexplored beyond its karyotype, partly because like many cancers, its extensive aneuploidy renders such analyses challenging. We carried out haplotype-resolved whole-genome sequencing of the HeLa CCL-2 strain, examined point- and indel-mutation variations, mapped copy-number variations and loss of heterozygosity regions, and phased variants across full chromosome arms. We also investigated variation and copy-number profiles for HeLa S3 and eight additional strains. We find that HeLa is relatively stable in terms of point variation, with few new mutations accumulating after early passaging. Haplotype resolution facilitated reconstruction of an amplified, highly rearranged region of chromosome 8q24.21 at which integration of the human papilloma virus type 18 (HPV-18) genome occurred and that is likely to be the event that initiated tumorigenesis. We combined these maps with RNA-seq and ENCODE Project data sets to phase the HeLa epigenome. This revealed strong, haplotype-specific activation of the proto-oncogene MYC by the integrated HPV-18 genome approximately 500 kilobases upstream, and enabled global analyses of the relationship between gene dosage and expression. These data provide an extensively phased, high-quality reference genome for past and future experiments relying on HeLa, and demonstrate the value of haplotype resolution for characterizing cancer genomes and epigenomes.

Ahmad, S., et al. (2016). "Evaluation of Rumex hastatus D. Don for cytotoxic potential against HeLa and NIH/3T3 cell lines: chemical characterization of chloroform fraction and identification of bioactive compounds." BMC Complement Altern Med **16**: 308.

BACKGROUND: The importance of Rumex genus and the renowned ethnopharmacological and biological potentials of Rumex hastatus is evident from the previous reports. Recently the R. hastatus has been evaluated for anticancer potential against HepG2, MCF7 or LNCaP cell lines with considerable cytotoxicity. We also reported the anti-tumor and anti-angiogenic potentials of R. hastatus. The current study has been arranged to evaluate cytotoxic potential of this plant against HeLa and NIH/3T3 cell lines and sort out the most active fraction of R. hastatus along with the identification of bioactive compounds responsible for cytotoxicity. METHODS: The cytotoxic potential of methanolic extract and sub-fractions of R. hastatus was performed following (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) MTT calorimetric assay. Four concentrations (500, 250, 125 and 62.5 mug/ml) of each sample were used against both cell lines. Two cell lines i.e. HeLa and NIH/3T3 were used in the assay. Furthermore, chemical characterization of chloroform fraction was performed by GC-MS analysis. RESULTS: The current investigational study demonstrates that all the solvent fractions of R. hastatus were active against HeLa and NIH/3T3 cell lines. Among all the fractions, chloroform fraction was dominant in activity against both cell lines. The observed IC50 values of chloroform fraction were 151.52 and 53.37 mug/ml against HeLa and NIH/3T3 respectively. The GC-MS analysis of chloroform fraction revealed the identification of 78 compounds with the identification of bioactive ones like ar-tumerone, phytol, dihydrojasmone, sitostenone etc. CONCLUSION: It can be concluded from our results that Rumex hastatus D. Don possess strong cytotoxic potential. Moreover, the observed IC50 values and GC-MS analysis of chloroform fraction reveal that most of the bioactive compounds are in chloroform fraction. It can be further deduce that the chloroform fraction is a suitable target for the isolation of compounds having potential role in cancer therapy.

Ahn, J. C. and P. S. Chung (2012). "The activity of G-ROS and the predominant role of Type II reaction in the photodynamic therapy using 9-hydroxypheophorbide-alpha for HeLa cell lines." Gen Physiol Biophys **31**(3): 343-350.

Photodynamic therapy (PDT) is a treatment modality that destroys the tumor. It activates the photosensitizer with the light of a specific wavelength, where the light is well absorbed by the photosensitizer, thus causing a fatal injury and thereby leading to a tumor necrosis. To date, a hematoporphyrin-derived photosensitizer has been widely used. It is disadvantageous, however, in that it causes a long-term photo-toxicity and has a poor selectivity for the tumor. This had led to the development of a chlorophyll-derived photosensitizer. We conducted this study to elucidate the mechanisms by which the activity of ROS is involved in the PDT using a novel

type of chlorophyll-derived photosensitizer, 9-hydroxypheophorbide-alpha (9-HpbD-alpha), for the HeLa cell lines. Besides, we also attempted to determine which reaction plays a predominant role in the synthesis of ROS, either Type I reaction or Type II one, when both reactions are involved in the synthesis of ROS during the PDT using 9-HpbD-alpha. Our results showed not only that the activity of ROS is involved in the PDT using 9-HpbD-alpha in human uterine cervical cancer cell lines but also that the mechanisms of PDT are based on Type II reaction where the singlet oxygen is involved.

Al Haj, A., et al. (2014). "Thymosin beta4 inhibits ADF/cofilin stimulated F-actin cycling and hela cell migration: reversal by active Arp2/3 complex." Cytoskeleton (Hoboken) **71**(2): 95-107.

F-actin treadmilling plays a key part in cell locomotion. Because immunofluorescence showed colocalisation of thymosin beta4 (Tbeta4) with cofilin-1 and Arp2/3 complex in lamellipodia, we analyzed combinations of these proteins on F-actin-adenosine triphosphate (ATP)-hydrolysis, which provides a measure of actin treadmilling. Actin depolymerising factor (ADF)/cofilin stimulated treadmilling, while Tbeta4 decreased treadmilling, presumably by sequestering monomers. Tbeta4 added together with ADF/cofilin also inhibited the treadmilling, relative to cofilin alone, but both the rate and extent of depolymerization were markedly enhanced in the presence of both these proteins. Arp2/3 complex reversed the sequestering activity of Tbeta4 when equimolar to actin, but not in the additional presence of cofilin-1 or ADF. Transfection experiments to explore the effects of changing the intracellular concentration of Tbeta4 in HeLa cells showed that an increase in Tbeta4 resulted in reduced actin filaments bundles and narrower lamellipodia, and a conspicuous decrease of cell migration as seen by two different assays. In contrast, cells transfected with a vector leading to Tbeta4 knockdown by small interfering RNA (siRNA) displayed prominent actin filament networks within the lamellipodia and the leading lamella and enhanced migration. The experiments reported here demonstrate the importance of the interplay of these different classes of actin-binding proteins on cell behaviour.

Aldaghi, L., et al. (2016). "In Silico and In Vitro Evaluation of Cytotoxic Activities of Farnesiferol C and Microlobin on MCF-7, HeLa and KYSE Cell Lines." Drug Res (Stuttg) **66**(10): 532-538.

Background: Cancer is one of the leading causes of death worldwide. Despite certain advances in cancer therapy, still there is considerable demand for developing efficient therapeutic agents. Nowadays, there is a rising interest in the use of natural-based anti-cancer drugs. In this study, the cytotoxicity of farnesiferol C and microlobin isolated from Ferula szowitsiana was investigated against MCF-7, HeLa and KYSE cancer cell lines. In addition, the mechanism of binding of these compounds to apoptotic proteins (Bax, Bak and Bcl-2) was analyzed by an in silico method. Materials and methods: We used MTT assay in order to assess the cytotoxicity of compounds against cancer cell lines. For in silico study, the AutoDock 4 was adopted. Results and discussion: According to the in vitro findings, in general, farnesiferol C showed significant cytotoxicity at higher concentrations (>50 microM) following 48 and 72 h incubation with the selected cancer cells; however, microlobin exhibited almost no activity at concentrations up to 100 microM. The in silico results revealed that both compounds could bind to Bax more efficiently rather than to Bcl-2 or Bak proteins. Conclusion: The results obtained by our preliminary in vitro and in silico studies suggest that these compounds might induce apoptosis through Bax activation; however further studies, either in vitro or in vivo are needed to clarify these activities.

Aliomrani, M., et al. (2017). "Phytochemical Screening and Cytotoxic Evaluation of Euphorbia turcomanica on Hela and HT-29 Tumor Cell Lines." Adv Biomed Res **6**: 68.

BACKGROUND: Cancer is a term for a large group of different diseases, all involving uncontrolled cell growth. Many of Euphorbiaceae plants have been traditionally used for the treatment of ulcers, tumors, warts, and other diseases. In addition, in the last decade, there are studies showing cytotoxic effects of different species of Euphorbia on tumor cell lines. In this study, we attempted to determine if Euphorbia turcomanica possess any cytotoxic activity. MATERIALS AND METHODS: Solvents extracted the plant powder with various polarities by a maceration method, and qualitative phytochemical analyzes were carried out on them to identify the constituents. On the other hand, the possible cytotoxicity of different extracts on Hela and HT-29 tumor cells was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and 50% reduction in cell survival was considered as a cytotoxic effect. Analyze of variance followed by Student-Newman-Keuls test was used to see the differences among the groups. RESULTS: Phytochemical analysis of E. turcomanica showed the presence of flavonoid, alkaloid, anthraquinone and tannin in plant aerial parts. Methanol-water, acetone, dichloromethane, methanol, and heptane extracts of E. turcomanica significantly reduced viability of Hela cells ($P < 0.05$) with inhibitory concentration 50%

(IC50) of 50, 90, 230, 420, and 450 mug/ml, respectively. While methanol-water, dichloromethane, methanol, ethyl acetate, and heptane extracts were cytotoxic with IC50 of 43, 115, 125, 250, and 390 mug/ml, respectively ($P < 0.05$), on HT-29 cells. CONCLUSION: It can be concluded that E. turcomanica is a good candidate for further study toward cytotoxic agents.

Almada, M., et al. (2014). "Interaction and cytotoxic effects of hydrophobized chitosan nanoparticles on MDA-MB-231, HeLa and Arpe-19 cell lines." Curr Top Med Chem **14**(6): 692-701.

In this work, we investigate the effect of chitosan hydrophobization on the internalization and cytotoxic effect of chitosan-based nanoparticles (NPs) on breast cancer cells (MDA-MB-231), cervical cancer cells (HeLa) and noncancer cells (Arpe-19). We also analyzed the interaction of NPs with a phospholipid (DPPC) membrane model at the airwater interface. An alkylation procedure to insert 8 carbon chains along the chitosan macromolecule with final 10 and 30 % substitution degrees was used. Nuclear magnetic resonance (NMR) and infrared spectroscopes (IR) were used to evaluate the success and extent of the hydrophobization procedure. Size, shape, and charge of NPs were evaluated by dynamic light scattering (DLS), atomic force microscope (AFM), and zeta potential, respectively. The effect of hydrophobicity on NPs was the reduction of the NPs average size, the formation of slightly elongated structures and the enhancing of the interaction of NPs with a DPPC monolayer at the air-water interface. By using fluorescence images on fluorescein-chitosan NPs, we observed a higher internalization of hydrophobic chitosan NPs in cancer cells in comparison with a low internalization of these NPs in normal cells. Even when non modified chitosan NPs were highly internalized in all cell lines, hydrophobized chitosan NPs showed a significantly higher cytotoxic effect on cancer cells in comparison with a lower effect showed by non-modified chitosan NPs on these cells. The cytotoxic effect on the normal cell line used was low for native chitosan NPs and negligible for hydrophobized chitosan NPs.

Almahdy, O., et al. (2011). "Examination of the activity of camel milk casein against hepatitis C virus (genotype-4a) and its apoptotic potential in hepatoma and hela cell lines." Hepat Mon **11**(9): 724-730.

BACKGROUND: Hepatitis C is a global health concern that represents a major cause of liver disease and socioeconomic burden. Currently, there is no vaccine that protects against this infection or drug that treats it effectively. The current treatment for hepatitis C virus (HCV) infection does not produce a sustained virologic response. Therefore, discovery and identification of a new drug for HCV treatment is a high priority. Camel milk is a traditional medicine that could improve the control of HCV. OBJECTIVES: To assess the potential effect of casein purified from camel milk on HCV cellular infectivity in a tissue culture model. MATERIALS AND METHODS: Casein was purified from defatted camel milk to electrophoretic homogeneity. PBMCs and HepG2 and HeLa cell lines were used. Three kinds of experiments were conducted. HCV was directly interacted with casein and then mixed with different cell types, casein was incubated with the cells and then exposed to HCV, and the HCV pre-infected cells were treated with casein at different concentrations and time intervals. Non-infected cells were used to assess cytotoxicity and the apoptosis effect of casein. RESULTS: Direct interaction of casein (with or without alpha-lactalbumin) with neither the virus nor the cells prevented HCV cell entry. However, casein with alpha-lactalbumin induced a cytotoxic effect in HepG2 and HeLa cell lines but not in human naive leukocytes. At all concentrations tested, casein with alpha-lactalbumin could induce apoptosis in both infected and non-infected HepG2 cells. CONCLUSIONS: Camel milk casein (with or without alpha-lactalbumin) did not demonstrate any anti-HCV activity. However, the cellular apoptotic cascade was initiated in HepG2 and HeLa cells treated with casein (with alpha-lactalbumin) but not in naive leukocytes.

Alonso, E., et al. (2013). "Nanomolar ouabain elicits apoptosis through a direct action on HeLa cell mitochondria." Steroids **78**(11): 1110-1118.

The steroid Na (+)/K (+) ATPase (NKA) blocker ouabain has been shown to exhibit pro-apoptotic effects in various cell systems; however, the mechanism involved in those effects is unclear. Here, we have demonstrated that incubation of HeLa cells during 24h with nanomolar concentrations of ouabain or digoxin causes apoptotic death of 30-50% of the cells. Ouabain caused the activation of caspases-3/7 and -9; however, caspase-8 was unaffected. The fact that compound Z-LEHD-FMK reduced both apoptosis and caspase-9 activation elicited by ouabain, suggest a mitochondrially-mediated pathway. This was strengthened by the fact that ouabain caused ATP depletion and the release of mitochondrial cytochrome c into the cytosol. Furthermore, upon ouabain treatment mitochondrial disruption and redistribution into the cytosol were observed. A mitochondrial site of action for ouabain was further corroborated by tight co-localisation of fluorescent ouabain with mitochondria. Finally, in ouabain-treated cells the histamine-elicited elevation of cytosolic Ca (2+) concentration ([Ca (2+)]c) suggests an additional

effect on the endoplasmic reticulum (ER) leading to Ca (2+) store depletion. We conclude that fluorescent ouabain is taken up and tightly co-localises with mitochondria of HeLa cells. This indicates that apoptosis may be triggered by a direct action of ouabain on mitochondria.

Ascer, L. G., et al. (2015). "CDC42 Gtpase Activation Affects Hela Cell DNA Repair and Proliferation Following UV Radiation-Induced Genotoxic Stress." J Cell Biochem **116**(9): 2086-2097.

Cell division control protein 42 (CDC42) homolog is a small Rho GTPase enzyme that participates in such processes as cell cycle progression, migration, polarity, adhesion, and transcription. Recent studies suggest that CDC42 is a potent tumor suppressor in different tissues and is related to aging processes. Although DNA damage is crucial in aging, a potential role for CDC42 in genotoxic stress remains to be explored. Migration, survival/proliferation and DNA damage/repair experiments were performed to demonstrate CDC42 involvement in the recovery of HeLa cells exposed to ultraviolet radiation-induced stress. Sub-lines of HeLa cells ectopically expressing the constitutively active CDC42-V12 mutant were generated to examine whether different CDC42-GTP backgrounds might reflect different sensitivities to UV radiation. Our results show that CDC42 constitutive activation does not interfere with HeLa cell migration after UV radiation. However, the minor DNA damage exhibited by the CDC42-V12 mutant exposed to UV radiation most likely results in cell cycle arrest at the G2/M checkpoint and reduced proliferation and survival. HeLa cells and Mock clones, which express endogenous wild-type CDC42 and show normal activity, are more resistant to UV radiation. None of these effects are altered by pharmacological CDC42 inhibition. Finally, the phosphorylation status of the DNA damage response proteins gamma-H2AX and p-Chk1 was found to be delayed and attenuated, respectively, in CDC42-V12 clones. In conclusion, the sensitivity of HeLa cells to ultraviolet radiation increases with CDC42 over-activation due to inadequate DNA repair signaling, culminating in G2/M cell accumulation, which is translated into reduced cellular proliferation and survival.

Asgharian, A., et al. (2014). "Optimizing A Lipocomplex-Based Gene Transfer Method into HeLa Cell Line." Cell J **15**(4): 372-377.

One of the most significant steps in gene expression studies is transferring genes into cell cultures. Despite there are different methods for gene delivery such as viral and non-viral producers, some cationic lipid reagents have recently developed to transfect into mam- malian cell lines. The main aim of this study was optimizing and improving lipocomplex based transient transfection procedures into HeLa cell line which is being used widely as a typical cell in biological studies. This study was an experimental research. In this work, pCMV beta-Gal DNA plasmid was used as a reporter DNA for determining the rate of gene transfection into HeLa cells. To accomplish the highest gene delivery into HeLa cells, optimizing experiments were car- ried out in different volumes of FuGENE-HD, Lipofectamine (TM)2000 and X-tremeGENE. Also, we investigated tranasfection efficiency in presence of various cell densities of HeLa cells. Then, transfection efficiency and cell toxicity were measured by beta gal staining and trypan blue methods, respectively. Using FuGENE-HD in volume of 4microl along with 10(5) HeLa cells, transfection efficiency was higher (43.66 +/- 1.52%) in comparison with the cationic lipids Lipofectamine (TM)2000 and X-tremeGENE. In addition, the rate of cell toxicity in presence of FuGENE-HD was less than 5%. In summary, the cationic lipid FuGENE-HD indicates a suitable potential to transfer DNA into HeLa cells and it can be an efficient reagent for gene delivery for HeLa cells in vitro. Moreover, it is worth designing and optimizing gene transfer experiments for other cell lines with FuGENE-HD due to its low toxicity and high efficiency.

Avato, P., et al. (2017). "Activity of Saponins from Medicago species Against HeLa and MCF-7 Cell Lines and their Capacity to Potentiate Cisplatin Effect." Anticancer Agents Med Chem **17**(11): 1508-1518.

BACKGROUND: Saponins from Medicago species display several biological activities, among them apoptotic effects against plant cells have been evidenced. In contrast, their cytotoxic and antitumor activity against animal cells have not been studied in great details. OBJECTIVE: To explore the cytotoxic properties of saponin from Medicago species against animal cells and their effect in combination with the antitumoral drug cisplatin. METHOD: Cytotoxic activity of saponin mixtures from M. arabica (tops and roots), M. arborea (tops) and M. sativa (tops, roots and seeds) and related prosapogenins from M. arborea and M. sativa (tops) against HeLa and MCF-7 cell lines is described. In addition, cytotoxicity of soyasaponin I and purified saponins (1-8) of hederagenin, medicagenic and zanhic acid is also presented. Combination experiments with cisplatin have been also conducted. RESULTS: Saponins from M. arabica tops and roots (mainly monodesmosides of hederagenin and bayogenin) were the most effective to reduce proliferation of HeLa and MCF-7 cell lines. Among the purified saponins, the most cytotoxic was

saponin 1, 3-O-ss-D-glucopyranosyl (1-->2)-alpha-L-arabinopyranosyl hederagenin. When saponins, derived prosapogenins and pure saponins were used in combination with cisplatin, they all, to different extent, were able to potentiate cisplatin activity against HeLa cells but not against MCF-7 cell lines. Moreover uptake of cisplatin in these cell lines was significantly reduced. CONCLUSION: Overall results showed that specific molecular types of saponins (hederagenin glycosides) have potential as anti-cancer agents or as leads for anti-cancer agents. Moreover saponins from Medicago species have evidenced interesting properties to mediate cisplatin effects in tumor cell lines.

Aydin, A., et al. (2017). "Anticancer and Cytotoxic Activities of [Cu (C6H16N2O2)2] [Ni (CN)4] and [Cu (C6H16N2O2)Pd (CN)4] Cyanidometallate Compounds on HT29, HeLa, C6 and Vero Cell Lines." Anticancer Agents Med Chem **17**(6): 865-874.

BACKGROUND: In cancer, apoptosis relevant proteins-such as CaM kinase, Bcl-2 or P53, topoisomerase I, cell migration feature and DNA/BSA-macromolecules represent significant targets for current chemotherapeutics. OBJECTIVE: We recently reported two coordination compounds-[Cu (C6H16N2O2)2] [Ni (CN)4] (1) and [Cu (C6H16 N2O2)Pd (CN)4] (2)-together with their IR spectra, magnetic properties, thermal analyses and crystal structures. Herein, we describe the ability of these complexes to induce apoptosis in relevant proteins and stimulate topoisomerase I activity, cell migration velocity and DNA/BSA binding properties. METHOD: The in vitro antiproliferative effects and cell toxicity of both compounds were investigated through pharmacological measurement techniques, and interactions between both compounds and CT-DNA/BSA were studied with UV-Vis spectroscopy and fluorescence spectroscopy. Results & amp; Conclusion: Studies on cells revealed that 2 (i) demonstrated a high antiproliferative effect, which was higher toward HeLa and C6 cancer cells than toward healthy Vero cells; (ii) impaired the migration of HeLa cells; (iii) altered the P53-Bcl-2 ratio in favor of apoptosis; (iv) strongly bound to DNA/BSA macromolecules; and (v) inhibited human topoisomerase I and KpnI or BamHI restriction endonucleases. In conclusion, this preliminary information demonstrates that 2 may represent a promising antiproliferative agent and a potential candidate for a therapeutic approach against HeLa.

Bajgar, R., et al. (2014). "High oxygen partial pressure increases photodynamic effect on HeLa cell lines in the presence of chloraluminium phthalocyanine." Anticancer Res **34**(8): 4095-4099.

BACKGROUND: Photodynamic therapy (PDT) is linked with oxidative damage of biomolecules causing significant impairment of essential cellular functions that lead to cell death. It is the reason why photodynamic therapy has found application in treatment of different oncological, cardiovascular, skin and eye diseases. Efficacy of PDT depends on combined action of three components; sensitizer, light and oxygen. In the present study, we examined whether higher partial pressure of oxygen increases lethality in HeLa cell lines exposed to light in the presence of chloraluminium phthalocyanine disulfonate (ClAlPcS2). METHODS: ClAlPcS2-sensitized HeLa cells incubated under different oxygen conditions were exposed to PDT. Production of singlet oxygen ((1)O2) and other forms of reactive oxygen species (ROS) as well as changes in mitochondrial membrane potential were determined by appropriately sensitive fluorescence probes. The effect of PDT on HeLa cell viability under different oxygen conditions was quantified using the standard methylthiazol tetrazolium (MTT) test. RESULTS: At the highest oxygen concentration of 28 +/- 2 mg/l HeLa cells were significantly more sensitive to light-activated ClAlPcS2 (EC50=0.29 +/- 0.05 muM) in comparison to cells incubated at lower oxygen concentrations of 8 +/- 0.5 and 0.5 +/- 0.1 mg/l, where the half maximal effective concentration was 0.42 +/- 0.06 muM and 0.94 +/- 0.14 muM, respectively. Moreover, we found that the higher presence of oxygen is accompanied with higher production of singlet oxygen, a higher rate of type II photodynamic reactions, and a significant drop in the mitochondrial membrane potential. CONCLUSION: These results demonstrate that the photodynamic effect in cervical cancer cells utilizing ClAlPcS2 significantly depends on oxygen level.

Bakhtiari, E., et al. (2015). "Synergistic, cytotoxic and apoptotic activities of olmesartan with NF-kappaB inhibitor against HeLa human cell line." Toxicol Mech Methods **25**(8): 614-621.

CONTEXT: Over expression of renin-angiotensin system (RAS) and nuclear factor-kappaB (NF-kappaB) has a major role in many cancers. It has been suggested that some angiotensin receptor blockers (ARBs) could reduce the proliferation of cancer cells. The role of NF-kappaB pathway has been documented in cell proliferation. OBJECTIVE: In this study, the role of angiotensin II and NF-kappaB pathway in human cervical cancer cell line (HeLa) proliferation was studied using olmesartan (as a novel Ag II antagonist) and Bay11-7082 (as NF-kappaB inhibitor). MATERIALS AND METHODS: HeLa cells were treated with different concentrations of

olmesartan and Bay11-7082. Cell proliferation was determined after 24, 48, and 72 h by MTT assay. Synergistic activity of olmesartan with Bay11-7082 was analyzed with Compusyn software. Apoptotic cells were determined using PI staining of DNA fragmentation. RESULTS: Cell viability decreased with olmesartan and Bay11-7082 in HeLa cells by 24, 48 and 72 h. Olmesartan had synergistic activity with Bay11-7082 and combinations of olmesartan with Bay11-7082 decreased cell viability as compared with single agent treatments. Olmesartan and Bay11-7082 induced a sub-G1 peak in flow cytometry histogram of treated cells indicating that apoptotic cell death is involved in olmesartan and Bay11-7082-induced toxicity. DISCUSSION AND CONCLUSION: Results imply that olmesartan and Bay11-7082 inhibit the growth of HeLa cells as a concentration- and time-dependent mode and they have synergistic activity. Results show that RAS and NF-kappaB pathway blockade lead to significant cytotoxicity against tumor cell line. So, ARBs and NF-kappaB pathway inhibitors could be considered as good anti-cancer agents in cervix carcinoma after further studies.

Bakhtiari, E., et al. (2017). "The role of ROS and NF-kappaB pathway in olmesartan induced-toxicity in HeLa and mcf-7 cell lines." Biomed Pharmacother **93**: 429-434.

We have recently shown that olmesartan could induce toxicity in HeLa and MCF-7 cell lines. In this study we investigated toxicity mechanism of olmesartan in HeLa and MCF-7 cell lines. HeLa and MCF-7 cells were cultured in DMEM in optimum conditions. Cells were pretreated with rutin as an antioxidant and treated with olmesartan as a cytotoxic agent. Cell proliferation was determined by MTT assay. The role of ROS was determined using DCFH-DA by flow cytometry analysis. Also, cells were treated with olmesartan (5mM) and Bay 11-7-82 (25muM) for 24h, then expression of apoptotic proteins including Bax, caspase3 and IkappaB were investigated in both cell lines by western blotting. Cell viability decreased with olmesartan in malignant cell lines. Kinetic of ROS assay showed increment of ROS generation starting at 2h which peaked at 4h after treatment. Pretreatment with antioxidant rutin decreased ROS increment which was consistent with improved viability of olmesartan-treated cells. Apoptosis results showed that olmesartan and Bay 11-7082 increased expression of apoptotic proteins such as Bax, caspase3 and IkappaB. Results proposed ROS increment and apoptosis could be involving mechanisms in olmesartan-induced toxicity in HeLa and MCF-7 cell lines.

Banerjee, S., et al. (2017). "Synthesis and electronic properties of ester substituted 1,4-dicyanodibenzodioxins and evaluation of anti-proliferative activity of all isomeric 1,2-, 2,3- and 1,4-dicyanodibenzodioxins against HeLa cell line." Bioorg Med Chem Lett **27**(18): 4280-4284.

1,4-Dicyanodibenzodioxins bearing carboxy methyl ester groups were synthesized using our established one-step SNAr coupling reaction between ortho- and meta-ester substituted catechols and perfluorinated terephthalonitrile. These are the first examples of 1,4-dicyanodibenzodioxins substituted at both the benzene moieties. Optical spectra were similar to the earlier examples reported, with a marginal blue shift for the ester dibenzodioxins. Theoretical analysis of the molecular orbitals reveals modest destabilization of the frontier molecular orbitals of one carboxy methyl ester isomer over the other and overall higher HOMO-LUMO gap for both isomers when compared to the earlier published 1,4-dicyanodibenzodioxins. In vitro cytotoxicity against human cervical cancer HeLa cell line was evaluated for these two compounds and all other previously published dibenzodioxins from our laboratory (1,4-dicyano, 1,2-dicyano and 2,3-dicyano variants). A number of derivatives showed anti-tumor activity in muM ranges and also exhibited no cytotoxicity against normal HEK 293 cell line. Mechanistic investigation of cell death pathways indicated high levels of reactive oxygen species (ROS) in the dibenzodioxin treated tumor cell lines along with cellular nuclear fragmentation, both of which are markers of the apoptotic cell death pathway.

Bednarczyk-Cwynar, B., et al. (2016). "Oleanolic Acid A-lactams Inhibit the Growth of HeLa, KB, MCF-7 and Hep-G2 Cancer Cell Lines at Micromolar Concentrations." Anticancer Agents Med Chem **16**(5): 579-592.

Oleanolic acid ketones, oximes, lactams and nitriles were obtained. Complete spectral characterizations (IR, (1)H NMR, (13)C NMR, DEPT and MS) of the synthesized compounds are presented. The derivatives had oxo, hydroxyimino, lactam or nitrile functions at the C-3 position, an esterified or unmodified carboxyl group at the C- 17 location and, in some cases, an additional oxo function at the C-11 position. The new compounds were tested for cytotoxic activity on the HeLa, KB, MCF-7 and Hep-G2 cancer cell lines with the application of MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test. Among the tested compounds, some oximes and all lactams proved to be the most active cytotoxic agents. These triterpenes significantly inhibited the growth of the HeLa, KB, MCF-7 and

Hep-G2 cancer cell lines at micromolar concentrations.

Bednarczyk-Cwynar, B., et al. (2012). "Anticancer effect of A-ring or/and C-ring modified oleanolic acid derivatives on KB, MCF-7 and HeLa cell lines." Org Biomol Chem **10**(11): 2201-2205.

New A-ring or/and C-ring modified methyl oleanolate derivatives were prepared. New simple method of synthesis of 3,12-diketone (3) from methyl oleanonate (2) was worked out. The obtained new compounds were tested for cytotoxic activity on KB, MCF-7 and HeLa cell lines. The derivatives had acetoxy, oxo or hydroxyimino function at the C-3 position and in some cases oxo, hydroxyimino or acyloxyimino group at the C-12 position. Almost all of the compounds showed strong cytotoxic activity, higher than unchanged oleanolic acid. The most active substances turned out to be the derivatives with acyloxyimino function, especially 4 and 8d.

Ben Mabrouk, H., et al. (2017). "A New Protein Extract Inhibitor from Hypobranchial Purple Gland of Hexaplex trunculus, a Mediterranean Mollusk, Impairs the Motility of Human Glioblastoma U87 and the HeLa Cell Line of Cervical Carcinoma Cells." Nutr Cancer **69**(7): 1028-1035.

The aim of this study is to evaluate the effect of hypobranchial gland protein extracts (HGPEs) of Hexaplex trunculus on the viability, cell adhesion, and migration of human U87 glioblastoma cells and the HeLa cell line obtained from epithelial cervical carcinoma cells. Analysis of the HGPE on polyacrylamide gel (12%) shows a variety of proteins whose molecular weights vary between 12 and 1OO kDa. Chromatographic analysis shows 16 peaks obtained at various retention times. Cytotoxic effect was observed after 24 hours of incubation at the concentrations 20, 40, and 60 mug/ml in a dose-dependent manner. Concentrations giving 50% inhibition (IC50) are 22 mug/ml for U87 and 15 mug/ml for HeLa cells. Our results show inhibition of U87 and HeLa cancer cell adhesion at concentrations of 10 and 20 microg/ml, respectively. High-pressure liquid chromatography fractions did not show antiadhesive effect on both cancer cell lines. The presence of HGPEs completely blocked the migration of the two cancer cell lines at 10 microg/ml. This inhibition is dose-dependent. IC50 is about 2.5 mug/ml for both cancer cells. The HGPE of Hexaplex trunculus may have the potential to serve as a model for future anticancer drug development with probably a synergistic activity of the proteins of this extract.

Benitez-Rangel, E., et al. (2015). "DIDS (4,4'-Diisothiocyanatostilbene-2,2'-disulfonate) directly inhibits caspase activity in HeLa cell lysates." Cell Death Discov **1**: 15037.

Apoptosis is an important mechanism of cell demise in multicellular organisms and Cl (-) transport has an important role in the progression of the apoptotic volume decrease (AVD). DIDS (4,4'-Diisothiocyanatostilbene-2,2'-disulfonate) is one of the most commonly used Cl (-) transport inhibitors that eliminates or reduces different apoptotic hallmarks such as AVD, caspase-3 activity and DNA fragmentation. DIDS is also a protein crosslinker that alkylates either amino or thiol groups. Since caspases are thiol proteases, our aim was to study whether DIDS could directly inhibit the activity of these proteases. Here, we show that caspase activity induced by 4 h incubation with staurosporine was inhibited by DIDS in HeLa cells that were maintained in the absence of serum for 24 h. Interestingly, the caspase-inhibitory effect of DIDS is downstream to the inhibition of cytochrome c release, suggesting that DIDS might be also acting at the apoptosome. Moreover, DIDS was able to inhibit capase-3, -9, and -8 activities in cell lysates, implying that DIDS can react with and directly block caspases. Our data suggest that antiapoptotic activity of DIDS involves not only inhibition of the voltage-dependent anion channel (VDAC) at the mitochondria and Cl (-) channels at the plasma membrane, but also a third mechanism based on the direct inhibition of caspases.

Benito-Miguel, M., et al. (2015). "Assessment of sequential combination of 5-fluorouracil-loaded-chitosan-nanoparticles and ALA-photodynamic therapy on HeLa cell line." Photodiagnosis Photodyn Ther **12**(3): 466-475.

BACKGROUND: Natural polymers are used as components of nanoparticles (NPs) for drug delivery, as they provide targeted, sustained release and biodegradability. The purpose of this study was to increase the efficacy of the photodynamic therapy (PDT) by the combination of 5-aminolevulinic acid (ALA) with 5-fluorouracil-loaded-chitosan-nanoparticles (5-Fu-CNPs). METHODS: Nanoparticles based on chitosan (CNPs) were synthesized by the ionic crosslinking method via the TPP addition. 5-Fluorouracil (5-Fu), a first-line anticancer drug, was loaded into these 5Fu-CNPs, and they were assayed as controlled delivery formulation. HeLa cells were incubated in the presence of 5Fu-CNPs for 24h, next ALA was added to the culture medium and 4h later, to complete the PDT, light irradiation took place. Analysis of cell viability, reactive oxygen species (ROS) production, observation of the apoptosis by fluorescence microscopy followed by analysis of caspase-3 activity were carried out. RESULTS: Spherical 5Fu-CNPs

with a mean diameter of 324+/-43nm, were successfully synthesized and characterized by TEM and DLS. 5-Fu incorporation was achieved successfully (12.3mug 5Fu/mg CNP) and the maximum 5-Fu release took place at 2h. The combined administration of 5Fu-CNPs and PDT mediated by ALA (ALA-PDT) led to an improved efficacy of the antineoplastic treatment by generation of great cytotoxicity inducted through an increased ROS production. HeLa cells were destroyed by apoptosis through activation of caspase pathway. CONCLUSIONS: This study proves that combination therapy (photodynamic "ALA"+chemical "5-Fu"+immunoadjuvant "chitosan") may be an effective approach for the treatment of cancer.

Berrington, D. and N. Lall (2012). "Anticancer Activity of Certain Herbs and Spices on the Cervical Epithelial Carcinoma (HeLa) Cell Line." Evid Based Complement Alternat Med **2012**: 564927.

Acetone extracts of selected plant species were evaluated for their in vitro cytotoxicity against a noncancerous African green monkey kidney (Vero) cell line and an adenocarcinoma cervical cancer (HeLa) cell line. The plants studied were Origanum vulgare L. (Oregano), Rosmarinus officinalis L. (Upright and ground cove rosemary), Lavandula spica L. (Lavender), Laurus nobilis L. (Bay leaf), Thymus vulgaris L. (Thyme), Lavandula x intermedia L. (Margaret Roberts Lavender), Petroselinum crispum Mill. (Curly leaved parsley), Foeniculum vulgare Mill. (Fennel), and Capsicum annuum L. (Paprika). Antioxidant activity was determined using a quantitative DPPH (1,1-diphenyl-2-picryl hydrazyl) assay. The rosemary species exhibited effective radical scavenging capacity with 50% inhibitory concentration (IC (50)) of 3.48 +/- 0.218 mug/mL and 10.84 +/- 0.125 mug/mL and vitamin C equivalents of 0.351 g and 1.09 g for McConnell's Blue and Tuscan Blue, respectively. Cytotoxicity was measured using XTT (Sodium 3'-[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitro] benzene sulfonic acid hydrate) colorimetric assay. Only L. nobilis and O. vulgare exhibited pronounced effects on the HeLa cell line. Dose-dependent studies revealed IC (50) of 34.46 +/- 0.48 mug/mL and 126.3 +/- 1.00 mug/mL on the HeLa cells and on the Vero cells 124.1 mug/mL +/- 18.26 and 163.8 mug/mL +/- 2.95 for L. nobilis and O. vulgare, respectively. Light (eosin and haematoxylin staining) and confocal microscopy (Hoechst 33342, acridine orange, and propidium iodide staining) were used to evaluate the cytotoxic mechanism of action for L. nobilis and O. vulgare.

Bien, S., et al. (2010). "Doxorubicin-induced cell death requires cathepsin B in HeLa cells." Biochem Pharmacol **80**(10): 1466-1477.

The cysteine protease cathepsin B acts as a key player in apoptosis. Cathepsin B-mediated cell death is induced by various stimuli such as ischemia, bile acids or TNFalpha. Whether cathepsin B can be influenced by anticancer drugs, however, has not been studied in detail. Here, we describe the modulation of doxorubicin-induced cell death by silencing of cathepsin B expression. Previously, it was shown that doxorubicin, in contrast to other drugs, selectively regulates expression and activity of cathepsin B. Selective silencing of cathepsin B by siRNA or the cathepsin B specific inhibitor CA074Me modified doxorubicin-mediated cell death in Hela tumor cells. Both Caspase 3 activation and PARP cleavage were significantly reduced in cells lacking cathepsin B. Moreover, mitochondrial membrane permeabilization as well as the release of cytochrome C and AIF from mitochondria into cytosol induced by doxorubicin were significantly diminished in cathepsin B suppressed cells. In addition, doxorubicin associated down-regulation of XIAP was not observed in cathepsin B silenced cells. Lack of cathepsin B significantly modified cell cycle regulatory proteins such as cdk1, Wee1 and p21 without significant changes in G (1), S or G (2)M cell cycle phases maybe indicating further cell cycle independent actions of these proteins. Consequently, cell viability following doxorubicin was significantly elevated in cells with cathepsin B silencing. In summary, our data strongly suggest a role of cathepsin B in doxorubicin-induced cell death. Therefore, increased expression of cathepsin B in various types of cancer can modify susceptibility towards doxorubicin.

Birame, B. M., et al. (2014). "Potentiation of Apoptin-induced apoptosis by Cecropin B-like antibacterial peptide ABPs1 in human HeLa cervical cancer cell lines is associated with membrane pore formation and caspase-3 activation." J Microbiol Biotechnol **24**(6): 756-764.

Apoptin, a chicken anemia virus-encoded protein, induces apoptosis in chicken or human tumor cells, localizing in their nuclei as opposed to the cytoplasm of non-transformed cells. The present study was undertaken to investigate whether ABPs1 could potentiate apoptininduced apoptosis in HeLa cells. ABPs1 and the apoptin genes were successfully cloned into pIRES2-EGFP expression vector and expressed in HeLa cells. We report that ABPs1 augments apoptin cell growth inhibition in a concentration- and time-dependent manner. The DAPI staining and scanning electron microscopy observations revealed apoptotic bodies and plasma membrane pores, which were

attributed to apoptin and ABPs1, respectively. Further, ABPs1 in combination with apoptin was found to increase the expression of Bax and to decrease the expression of survivin compared with either agent alone or the control. The apoptotic rate of HeLa cells treated with ABPs1 and apoptin in combination for 48 h was 53.95%. The two-gene combination increased the caspase-3 activity of HeLa cells. Taken together, our study suggests that ABPs1 combined with apoptin significantly inhibits HeLa cell proliferation, and induces cell apoptosis through membrane defects, up-regulation of Bax expression, down-regulation of survivin expression, and activation of the caspase-3 pathway. Thus, the combination of ABPs1 and apoptin could serve as a means to develop novel gene therapeutic agents against human cervical cancer.

Borner, G. H. and A. B. Fielding (2014). "Isolating HeLa cell fractions enriched for clathrin-coated vesicles." Cold Spring Harb Protoc **2014**(11): 1184-1187.

HeLa cell lines can be experimentally manipulated using drugs or gene-silencing techniques such as RNA interference. Fractions enriched for clathrin-coated vesicles (CCVs) can be isolated from these cell lines and used to study the effects of these manipulations on the composition of CCVs. This protocol, originally developed in the laboratory of Margaret Robinson (Cambridge, United Kingdom), describes the preparation of a HeLa cell fraction that is enriched for a mixed population of CCVs and is suitable for analysis by mass spectroscopy, western blotting, or electron microscopy.

Bruce, A. and A. P. Rybak (2014). "CYB5D2 requires heme-binding to regulate HeLa cell growth and confer survival from chemotherapeutic agents." PLoS One **9**(1): e86435.

The cytochrome b5 domain containing 2 (CYB5D2; Neuferricin) protein has been reported to bind heme, however, the critical residues responsible for heme-binding are undefined. Furthermore, the relationship between heme-binding and CYB5D2-mediated intracellular functions remains unknown. Previous studies examining heme-binding in two cytochrome b5 heme-binding domain-containing proteins, damage-associated protein 1 (Dap1; Saccharomyces cerevisiae) and human progesterone receptor membrane component 1 (PGRMC1), have revealed that conserved tyrosine (Y) 73, Y79, aspartic acid (D) 86, and Y127 residues present in human CYB5D2 may be involved in heme-binding. CYB5D2 binds to type b heme, however, only the substitution of glycine (G) at D86 (D86G) within its cytochrome b5 heme-binding (cyt-b5) domain abolished its heme-binding ability. Both CYB5D2 and CYB5D2(D86G) localize to the endoplasmic reticulum. Ectopic CYB5D2 expression inhibited cell proliferation and anchorage-independent colony growth of HeLa cells. Conversely, CYB5D2 knockdown and ectopic CYB5D2(D86G) expression increased cell proliferation and colony growth. As PGRMC1 has been reported to regulate the expression and activities of cytochrome P450 proteins (CYPs), we examined the role of CYB5D2 in regulating the activities of CYPs involved in sterol synthesis (CYP51A1) and drug metabolism (CYP3A4). CYB5D2 co-localizes with cytochrome P450 reductase (CYPOR), while CYB5D2 knockdown reduced lanosterol demethylase (CYP51A1) levels and rendered HeLa cells sensitive to mevalonate. Additionally, knockdown of CYB5D2 reduced CYP3A4 activity. Lastly, CYB5D2 expression conferred HeLa cell survival from chemotherapeutic agents (paclitaxel, cisplatin and doxorubicin), with its ability to promote survival being dependent on its heme-binding ability. Taken together, this study provides evidence that heme-binding is critical for CYB5D2 in regulating HeLa cell growth and survival, with endogenous CYB5D2 being required to modulate CYP activities.

Burges, A., et al. (2011). "Inhibin-betaA and -betaB subunits in normal and malignant glandular epithelium of uterine cervix and HeLa cervical cancer cell line." Arch Gynecol Obstet **284**(4): 981-988.

INTRODUCTION: Inhibins, dimeric peptide hormones composed of an alpha-subunit and one of two possible beta-subunits (betaA or betaB), exhibit substantial roles in human reproduction and in endocrine-responsive tumors. However, it is still unclear if normal and cancerous cervical glandular epithelial cells as well as cervical cancer cell lines of glandular origin express the inhibin-betaA and -betaB subunits. MATERIALS AND METHODS: Normal cervical tissue samples and a total of 10 specimens of well-differentiated adenocarcinomas of the human cervix were analyzed for inhibin-betaA and -betaB subunit expression by immunohistochemical analysis. Additionally, the cervical carcinoma cell line HeLa was analyzed by immunofluorescence and RT-PCR analysis for the expression of inhibin subunits. RESULTS: Immunolabeling of normal and malignant glandular epithelium of human cervical tissue revealed a positive staining reaction for the inhibin-betaA and -betaB subunits. Additionally, the cancer cell line HeLa synthesized both inhibin subunits. When compared to the normal cervical glandular epithelium, the expression of the inhibin beta subunits became significantly reduced in cervical adenocarcinoma tissues. DISCUSSION: In conclusion, we demonstrated a strong, though differential expression pattern of inhibin-betaA and -betaB subunits in normal

and malignant glandular epithelial cells of the human uterine cervix. Although the physiological role of inhibins is still quite unclear in cervical tissue, the expression of inhibin-beta-subunits might play an important role in cervical cancer carcinogenesis, since they are significantly down-regulated during pathogenesis in cervical adenocarcinomas.

Camargo, R., et al. (2014). "Trypanosoma cruzi infection down-modulates the immunoproteasome biosynthesis and the MHC class I cell surface expression in HeLa cells." PLoS One **9**(4): e95977.

Generally, Trypanosoma cruzi infection in human is persistent and tends to chronicity, suggesting that the parasite evade the immune surveillance by down regulating the intracellular antigen processing routes. Within the MHC class I pathway, the majority of antigenic peptides are generated by the proteasome. However, upon IFN-gamma stimulation, the catalytic constitutive subunits of the proteasome are replaced by the subunits beta1i/LMP2, beta2i/MECL-1 and beta5i/LMP7 to form the immunoproteasome. In this scenario, we analyzed whether the expression and activity of the constitutive and the immunoproteasome as well as the expression of other components of the MHC class I pathway are altered during the infection of HeLa cells with T. cruzi. By RT-PCR and two-dimensional gel electrophoresis analysis, we showed that the expression and composition of the constitutive proteasome is not affected by the parasite. In contrast, the biosynthesis of the beta1i, beta2i, beta5i immunosubunits, PA28beta, TAP1 and the MHC class I molecule as well as the proteasomal proteolytic activities were down-regulated in infected-IFN-gamma-treated cell cultures. Taken together, our results provide evidence that the protozoan T. cruzi specifically modulates its infection through an unknown posttranscriptional mechanism that inhibits the expression of the MHC class I pathway components.

Canonico, B., et al. (2014). "Campylobacter jejuni cell lysates differently target mitochondria and lysosomes on HeLa cells." Apoptosis **19**(8): 1225-1242.

Campylobacter jejuni is the most common cause of bacterial gastroenteritis in humans. The synthesis of cytolethal distending toxin appears essential in the infection process. In this work we evaluated the sequence of lethal events in HeLa cells exposed to cell lysates of two distinct strains, C. jejuni ATCC 33291 and C. jejuni ISS3. C. jejuni cell lysates (CCLys) were added to HeLa cell monolayers which were analysed to detect DNA content, death features, bcl-2 and p53 status, mitochondria/lysosomes network and finally, CD54 and CD59 alterations, compared to cell lysates of C. jejuni 11168H cdtA mutant. We found mitochondria and lysosomes differently targeted by these bacterial lysates. Death, consistent with apoptosis for C. jejuni ATCC 33291 lysate, occurred in a slow way (>48 h); concomitantly HeLa cells increase their endolysosomal compartment, as a consequence of toxin internalization besides a simultaneous and partial lysosomal destabilization. C. jejuni CCLys induces death in HeLa cells mainly via a caspase-dependent mechanism although a p53 lysosomal pathway (also caspase-independent) seems to appear in addition. In C. jejuni ISS3-treated cells, the p53-mediated oxidative degradation of mitochondrial components seems to be lost, inducing the deepest lysosomal alterations. Furthermore, CD59 considerably decreases, suggesting both a degradation or internalisation pathway. CCLys-treated HeLa cells increase CD54 expression on their surface, because of the action of lysate as its double feature of toxin and bacterial peptide. In conclusion, we revealed that C. jejuni CCLys-treated HeLa cells displayed different features, depending on the particular strain.

Cao, J., et al. (2015). "Biocompatible ZnS:Mn (2+) quantum dots/SiO2 nanocomposites as fluorescent probe for imaging HeLa cell." J Mater Sci Mater Med **26**(9): 236.

ZnS:Mn (2+) quantum dots (QDs) were successfully embedded in SiO2 spheres by a reverse microemulsion method. The results showed that the monodispersed core/shell nanocomposites were uniform in size, with the majority of the SiO2 nanoparticles containing one QD in the center of the sphere. The shell thickness of SiO2 increased from 7 to 18 nm as the hydrolysis time of TEOS increased from 20 to 40 h. The quantum yield (QY) of the yellow-orange emission (coming from the Mn (2+) ions (4)T1-(6)A1 transition) for the ZnS:Mn (2+) (3 %) QDs and ZnS:Mn (2+) (3 %) QDs@SiO2 (when t = 40 h) nanocomposites was measured to be 34.5 and 22.4 %, respectively. All samples showed no significant cytotoxicity against the HeLa cells even at a high concentration of 500 mug/ml after incubation for 24 h. The red fluorescence can be observed in the cytoplasm of the HeLa cell, further proving its biolabeling applications.

Carey, M. F., et al. (2010). "G-less cassette in vitro transcription using HeLa cell nuclear extracts." Cold Spring Harb Protoc **2010**(3): pdb prot5387.

The G-less cassette is a 365-nucleotide (nt) segment of DNA lacking guanine (G) residues on the nontemplate strand. In principle, a full-length transcript can be generated in an in vitro reaction lacking GTP, an omission that leads to suppression of most random, nonspecific transcription throughout the

plasmid. This method, like runoff transcription, generates radiolabeled RNA products, directly bypassing the necessity and extra time required to perform primer extension or other indirect mRNA product measurements. Unlike runoff transcription, which requires a cleaved end, the G-less assay can be performed on a circular, supercoiled plasmid, which in many systems is a more efficient template. In practice, most crude systems, such as HeLa nuclear extracts, contain low amounts of contaminating GTP that lead to small amounts of background transcription and occasionally can cause random upstream transcription to read through the G-less cassette. To minimize these artifacts, the reaction generally contains 3'-O-Me-GTP, a chain-terminating analog of GTP that causes transcription to cease when it is incorporated into a growing transcript, much like the dideoxy analogs used in DNA sequencing. The reaction products are cleaved with T1 RNase, which cleaves RNAs at G residues, further reducing background transcription; the G-less mRNA remains intact, whereas small random RNAs are digested.

Carey, M. F., et al. (2010). "Purification of mediator from HeLa cell lines expressing a flag-tagged mediator subunit." Cold Spring Harb Protoc **2010**(7): pdb prot5451.

INTRODUCTION: The Mediator (Med) complex plays a key role in promoter-specific activation of transcription by RNA polymerase II (Pol II). Med is a major target of activators and can be used in many types of affinity binding and immobilized template studies to evaluate interactions with individual activators. Additionally, all of the Med subunits have been cloned and can be subjected to individual structure-function analyses to learn how a specific activator interacts with a particular subunit. This protocol presents a simple affinity-based method to purify Med complex from HeLa cells stably expressing the Flag-tagged Intersex protein.

Carrascoso, I., et al. (2014). "Genome-wide profiling reveals a role for T-cell intracellular antigens TIA1 and TIAR in the control of translational specificity in HeLa cells." Biochem J **461**(1): 43-50.

TIA (T-cell intracellular antigens)-knockdown HeLa cells show an increase in ribosomes and translational machinery components. This increase correlates with specific changes in translationally up-regulated mRNAs involved in cell-cycle progression and DNA repair, as shown in polysomal profiling analysis. Our data support the hypothesis that a concerted activation of both global and selective translational rates leads to the transition to a more proliferative status in TIA-knockdown HeLa cells.

Carson, S. D. and S. J. Pirruccello (2013). "HeLa cell heterogeneity and coxsackievirus B3 cytopathic effect: implications for inter-laboratory reproducibility of results." J Med Virol **85**(4): 677-683.

Concerns over cell line identities and contamination have led investigators to acquire fresh stocks of HeLa CCL-2 cells, but results with the HeLa CCL-2 cells do not always reproduce results with HeLa cells that have long history in the laboratory. When used for TCID (50) assays of Coxsackievirus B3/28 (CVB3/28), HeLa CCL-2 cells returned titers for CVB3/28 that were more than ten-fold lower than titers obtained using laboratory HeLa cells. The viral cytopathic effect was less distinct in the HeLa CCL-2 cultures, suggestive of a mixed population of cells with varied susceptibility to viral cytopathic effect. Analysis of short tandem repeat markers confirmed the identities of the cell lines as HeLa. Subpopulations in the HeLa CCL-2 culture, separated easily based on the speed with which they were released by trypsin-EDTA, differed in their susceptibilities to CVB3/28 cytopathic effect, and in their expression of the Coxsackievirus and adenovirus receptor (CAR). The distinctions between Lab HeLa and HeLa CCL-2 cells were less obvious when infected with CVB3/RD, a strain selected for growth in RD cells. Results that differ among laboratories may be due to the use of HeLa cell strains with different histories, and experiments using HeLa CCL-2 available from the American Type Culture Collection are probably incapable of reproducing many of the published studies of Coxsackievirus that have used HeLa cells with laboratory-dependent histories.

Cetkovic, G. S., et al. (2011). "Apple pomace: antiradical activity and antiproliferative action in HeLa and HT- 29 human tumor cell lines." J BUON **16**(1): 147-153.

PURPOSE: Apple pomace is an easily accessible source of bioactive compounds which can be used for various purposes in the food, pharmaceutical and cosmetic industry. Six types of apple pomace extracts were tested to study their health benefits, free radical scavenging and antiproliferative activities. METHODS: The radical scavenging activity was determined by electron spin resonance (ESR) spectroscopy. Antiproliferative action was measured using MTT [3-(4,5-dimethylthiazol- 2-yl)2,5-diphenyl tetrazolium bromide] colorimetric assay in cervix epithelioid carcinoma (HeLa) and colon adenocarcinoma (HT-29) human cancer cell lines. RESULTS: All extracts suppressed the formation of 2,2-diphenyl- 1-picrylhydrazyl (DPPHo) and hydroxyl-free radical in a dose-dependent manner. In the presence of 12.5 mg/ ml Pinova, Reinders and Nectar pomace extract, the ESR DPPHo signals

vanished. The oOH was completely scavenged in the presence of 45 mg/ml or higher concentration of the investigated extracts. Pinova and Braeburn pomace extracts showed the strongest antiproliferative activity against the investigated human cancer cell lines. Also, HeLa cells were found more sensitive than HT-29 cells to all extracts. CONCLUSION: Although the relationship between radical scavenging activities and phenolic contents or flavonol glycosides (R (2)>/=0.80) was high, there were no significant correlations between the total phenolic contents or individual phenolic compounds and the antiproliferative activity.

Chen, C. and R. L. Yang (2013). "A phthalide derivative isolated from endophytic fungi Pestalotiopsis photiniae induces G1 cell cycle arrest and apoptosis in human HeLa cells." Braz J Med Biol Res **46**(8): 643-649.

MP [4-(3',3'-dimethylallyloxy)-5-methyl-6-methoxyphthalide] was obtained from liquid culture of Pestalotiopsis photiniae isolated from the Chinese Podocarpaceae plant Podocarpus macrophyllus. MP significantly inhibited the proliferation of HeLa tumor cell lines. After treatment with MP, characteristic apoptotic features such as DNA fragmentation and chromatin condensation were observed in DAPI-stained HeLa cells. Flow cytometry showed that MP induced G1 cell cycle arrest and apoptosis in a dose-dependent manner. Western blotting and real-time reverse transcription-polymerase chain reaction were used to investigate protein and mRNA expression. MP caused significant cell cycle arrest by upregulating the cyclin-dependent kinase inhibitor p27(KIP1) protein and p21(CIP1) mRNA levels in HeLa cells. The expression of p73 protein was increased after treatment with various MP concentrations. mRNA expression of the cell cycle-related genes, p21(CIP1), p16(INK4a) and Gadd45alpha, was significantly upregulated and mRNA levels demonstrated significantly increased translation of p73, JunB, FKHR, and Bim. The results indicate that MP may be a potential treatment for cervical cancer.

Chen, Y. Q., et al. (2014). "Intracellular viscoelasticity of HeLa cells during cell division studied by video particle-tracking microrheology." J Biomed Opt **19**(1): 011008.

Cell division plays an important role in regulating cell proliferation and differentiation. It is managed by a complex sequence of cytoskeleton alteration that induces dividing cells to change their morphology to facilitate their division. The change in cytoskeleton structure is expected to affect the intracellular viscoelasticity, which may also contribute to cellular dynamic deformation during cell division. However, the intracellular viscoelasticity during cell division is not yet well understood. In this study, we injected 100-nm (diameter) carboxylated polystyrene beads into the cytoplasm of HeLa cells and applied video particle tracking microrheology to measure their intracellular viscoelasticity at different phases during cell division. The Brownian motion of the intracellular nanoprobes was analyzed to compute the viscoelasticity of HeLa cells in terms of the elastic modulus and viscous modulus as a function of frequency. Our experimental results indicate that during the course of cell division, both intracellular elasticity and viscosity increase in the transition from the metaphase to the anaphase, plausibly due to the remodeling of cytoskeleton and redistributions of molecular motors, but remain approximately the same from the anaphase to the telophase.

Cheng, H., et al. (2013). "[Effect and mechanism of polyphyllin I on human cervical cancer cell HeLa in vitro]." Zhong Yao Cai **36**(11): 1815-1819.

OBJECTIVE: To study the effect and mechanism of polyphyllin I on human cervical cancer cell HeLa. METHODS: The cell growth and proliferation effect of Polyphyllin I on HeLa cells were measured by MTT assay; Hoechst 33258 fluorescent staining was used to record changes in cell morphology and morphological changes in mitochondria of Polyphyllin I before and after treatment on He La cells. Annexin V-FITC/PI staining was used to detect the ratio of tumor cell apoptosis by flow cytometry. Release of intracellular re active oxygen species (ROS) generation level in HeLa cells was determined by flow cytometry, Caspase-3 activity was measured by fluorescent assay kits. RESULTS: MTT results showed that Polyphyllin I could significantly suppressed the proliferation of HeLa cells and in time-and concentration-dependence manner. The intracellular ROS levels were increased dramatically and the mitochondrial membrane was decreased consistently. Caspase-3 proteins expression levels were increased after Polyphyllin I treatment. CONCLUSION: Polyphyllin I could inhibit HeLa cells growth and proliferation and its mechanism may be related to inducing cell apoptosis.

Cheriyamundath, S., et al. (2018). "Safranal Inhibits HeLa Cell Viability by Perturbing the Reassembly Potential of Microtubules." Phytother Res **32**(1): 170-173.

Saffron, a spice from Crocus sativus, has been known for its health benefits and medicinal properties. Safranal is a component of saffron and is known for its antioxidant and anticancer properties. In this study, we elucidated a possible tubulin-targeted antiproliferative mechanism of action of safranal. In vitro, the compound perturbed secondary structure of tubulin

without altering net microtubule polymer mass. It inhibited HeLa cell viability in a concentration-dependent manner, with minimal damage to cellular microtubules. However, it strongly inhibited recovery of microtubule network after cold-induced disassembly, indicating its ability to interfere with the nucleation potential of tubulin. Further, as the acetylation pattern of the safranal-treated microtubules revealed, unlike many tubulin-targeted agents, the compound did not appear to induce persistent stabilization of microtubules. Our data shows an unusual, tubulin-targeted antiproliferative mechanism of safranal. Copyright (c) 2017 John Wiley & Sons, Ltd.

Chetty, N. K., et al. (2016). "Analysis of Current Pulses in HeLa-Cell Permeabilization Due to High Voltage DC Corona Discharge." IEEE Trans Nanobioscience **15**(6): 526-532.

Corona discharges are commonly utilized for numerous practical applications, including bio-technological ones. The corona induced transfer of normally impermeant molecules into the interior of biological cells has recently been successfully demonstrated. The exact nature of the interaction of the corona discharge with a cell membrane is still unknown, however, previous studies have suggested that it is either the electric fields produced by ions or the chemical interaction of the reactive species that result in the disruption of the cell membrane. This disruption of the cell membrane allows molecules to permeate into the cell. Corona discharge current constitutes a series of pulses, and it is during these pulses that the ions and reactive species are produced. It stands to reason, therefore, that the nature of these corona pulses would have an influence on the level of cell permeabilization and cell destruction. In this investigation, an analysis of the width, rise-time, characteristic frequencies, magnitude, and repetition rate of the nanosecond pulses was carried out in order to establish the relationship between these factors and the levels of cell membrane permeabilization and cell destruction. Results obtained are presented and discussed.

Chi, X., et al. (2018). "Lamprey immune protein-1 (LIP-1) from Lampetra japonica induces cell cycle arrest and cell death in HeLa cells." Fish Shellfish Immunol **75**: 295-300.

The lamprey (Lampetra japonica), a representative of the jawless vertebrates, is the oldest extant species in the world. LIP-1, which has a jacalin-like domain and an aerolysin pore-forming domain, has previously been identified in Lampetra japonica. However, the structure and function of the LIP-1 protein have not been described. In this study, the LIP-1 gene was overexpressed in HeLa cells and H293T cells. The results showed that the overexpression of LIP-1 in HeLa cells significantly elevated LDH release ($P<0.05$), phosphatidylserine exposure and ROS accumulation. The overexpression of LIP-1 also had remarkable effects on the organelles in HeLa cells, while it had no effect on H293T cell organelles. Array data indicated that overexpression of LIP-1 primarily upregulated P53 signaling pathways in HeLa cells. Cell cycle assay results confirmed that LIP-1 caused arrest in the G2/M phase of the cell cycle in HeLa cells. In summary, our findings provide insights into the function and characterization of LIP-1 genes in vertebrates and establish the foundation for further research into the biological function of LIP-1. Our observations suggest that this lamprey protein has the potential for use in new applications in the medical field.

Chinnakkannu Vijayakumar, C., et al. (2015). "Harmonizing HeLa cell cytoskeleton behavior by multi-Ti oxide phased nanostructure synthesized through ultrashort pulsed laser." Sci Rep **5**: 15294.

Knowledge about cancer cell behavior on heterogeneous nanostructures is relevant for developing a distinct biomaterial that can actuate cancer cells. In this manuscript, we have demonstrated a harmonized approach of forming multi Ti-oxide phases in a nanostructure (MTOP nanostructure) for its unique cancer cell controlling behavior. Conventionally, single phases of TiO2 are used for targeted therapy and as drug carrier systems. In this research, we have shown a biomaterial that can control HeLa cells diligently using a combination of TiO, Ti3O and TiO2 phases when compared to fibroblast (NIH3T3) cells. MTOP-nanostructures are generated by varying the ionization energy in the vapor plume of the ultrashort pulse laser; this interaction with the material allows accurate tuning and composition of phases within the nanostructure. In addition, the lattice spacing of MTOP-nanostructures was analyzed as shown by HR-TEM investigations. An FESEM investigation of MTOP-nanostructures revealed a greater reduction of HeLa cells relative to fibroblast cells. Altered cell adhesion was followed by modulation of HeLa cell architecture with a significant reduction of actin stress fibers. The intricate combination of MTOP-nanostructures renders a biomaterial that can precisely alter HeLa cell but not fibroblast cell behavior, filling a void in the research for a biomaterial to modulate cancer cell behavior.

Choe, S. W., et al. (2017). "Combinational light emitting diode-high frequency focused ultrasound treatment for HeLa cell." Comput Assist Surg (Abingdon) **22**(sup1): 79-85.

PURPOSE: Light sources such as laser and light emitting diode or ultrasound devices have been widely used for cancer therapy and regenerative medicines, since they are more cost-effective and less harmful than radiation therapy, chemotherapy or magnetic treatment. Compared to laser and low intensity ultrasound techniques, light emitting diode and high frequency focused ultrasound shows enhanced therapeutic effects, especially for small tumors. MATERIALS AND METHODS: We propose combinational light emitting diode-high frequency focused ultrasound treatment for human cervical cancer HeLa cells. Individual red, green, and blue light emitting diode light only, high frequency focused ultrasound only, or light emitting diode light combined with high frequency focused ultrasound treatments were applied in order to characterize the responses of HeLa cells. RESULTS: Cell density exposed by blue light emitting diode light combined with high frequency focused ultrasound (2.19 +/- 0.58%) was much lower than that of cells exposed by red and green light emitting diode lights (81.71 +/- 9.92% and 61.81 +/- 4.09%), blue light emitting diode light (11.19 +/- 2.51%) or high frequency focused ultrasound only (9.72 +/- 1.04%). CONCLUSIONS: We believe that the proposed combinational blue light emitting diode-high frequency focused ultrasound treatment could have therapeutic benefits to alleviate cancer cell proliferation.

Choi, J. S., et al. (2012). "PKCdelta promotes etoposide-induced cell death by phosphorylating Hsp27 in HeLa cells." Biochem Biophys Res Commun **426**(4): 590-595.

We investigated the regulation of Hsp27 phosphorylation by protein kinase C delta (PKCdelta) during etoposide-induced apoptosis. The phosphorylation of Hsp27 at Ser78 was temporally correlated with the proteolytic activation of PKCdelta during apoptosis. Hsp27 phosphorylation was dependent on the activity of PKCdelta since treatment with rottlerin, a chemical inhibitor of PKCdelta, or overexpression of a PKCdelta dominant negative mutant abolished the phosphorylation. In addition, recombinant PKCdelta phosphorylated Hsp27 at Ser78 in vitro. Moreover, caspase-3 was specifically activated following Hsp27 phosphorylation at Ser78. Pull-down assays using a phosphomimetic Hsp27 mutant revealed that binding between Hsp27 and cytochrome c was abolished by the phosphorylation. These results suggest that Hsp27 dissociates from cytochrome c following PKCdelta-mediated phosphorylation at Ser78, which allows formation of the apoptosome and stimulates apoptotic progression.

Choudhari, A. S., et al. (2013). "The aqueous extract of Ficus religiosa induces cell cycle arrest in human cervical cancer cell lines SiHa (HPV-16 Positive) and apoptosis in HeLa (HPV-18 positive)." PLoS One **8**(7): e70127.

Natural products are being extensively explored for their potential to prevent as well as treat cancer due to their ability to target multiple molecular pathways. Ficus religiosa has been shown to exert diverse biological activities including apoptosis in breast cancer cell lines. In the present study, we report the anti-neoplastic potential of aqueous extract of F. religiosa (FRaq) bark in human cervical cancer cell lines, SiHa and HeLa. FRaq altered the growth kinetics of SiHa (HPV-16 positive) and HeLa (HPV-18 positive) cells in a dose-dependent manner. It blocked the cell cycle progression at G1/S phase in SiHa that was characterized by an increase in the expression of p53, p21 and pRb proteins with a simultaneous decrease in the expression of phospho Rb (ppRb) protein. On the other hand, in HeLa, FRaq induced apoptosis through an increase in intracellular Ca (2+) leading to loss of mitochondrial membrane potential, release of cytochrome-c and increase in the expression of caspase-3. Moreover, FRaq reduced the migration as well as invasion capability of both the cervical cancer cell lines accompanied with downregulation of MMP-2 and Her-2 expression. Interestingly, FRaq reduced the expression of viral oncoproteins E6 and E7 in both the cervical cancer cell lines. All these data suggest that F. religiosa could be explored for its chemopreventive potential in cervical cancer.

Cichello, S. A., et al. (2016). "Proliferative activity of a blend of Echinacea angustifolia and Echinacea purpurea root extracts in human vein epithelial, HeLa, and QBC-939 cell lines, but not in Beas-2b cell lines." J Tradit Complement Med **6**(2): 193-197.

Echinacea is used for its immunostimulating properties and may have a role in modulating adverse immune effects of chemotherapy (i.e., use of 5-fluorouracil (5-FU); fluorouracil and its immunosuppressive effect). Patients may seek herbal remedies such as Echinacea (Echinacea angustifolia and Echinacea purpurea) for immune stimulation. Echinacea extracts have been prescribed to supplement cancer chemotherapy for their immune-supportive effects; however, the extracts may also influence tumourgenesis. Our study aimed to determine the proliferative effect of the ethanolic blend of E. angustifolia and E. purpurea on various cancer cervical and bile duct cell lines, including HELA and QBC-939. Various cancer cells (HeLa and QBC-939) and human vein epithelial cells (HUVEC) were treated

with the Echinacea blend sample that was evaporated and reconstituted in Dimethyl sulfoxide (DMSO). As the extract concentration of Echinacea was increased from 12.5 mug/mL to 25 mug/mL, there was an increase in cell inhibition up to 100%, which then reduced to 90% over the next three concentrations, 50 mug/mL, 100 mug/mL, and 200 mug/mL, in HeLa cells; further inhibitory effects were observed in QBC-939 cells, from 9% inhibition at a concentration of 25 mug/mL up to 37.96% inhibition at 100 mug/mL concentration. Moreover, this is the first study to report the growth-promoting effects of this Echinacea blend in HUVEC, up to 800% at a dose concentration of 200 mug/mL. Previous studies have suggested that chicoric acid of Echinacea spp. is responsible for the increased cell growth. The results of this study show that the hydroethanolic extract of Echinacea herbal medicine promotes the growth of HeLa cells and QBC-939 cancer cell proliferation, and may interfere with cancer treatment (i.e., chemotherapy drugs such as 5-fluorouracil and Cisplatin (DDP)). However, the Echinacea blend shows potential in neurodegenerative diseases with growth-promoting effects in HUVEC. Further animal trials (in vivo effect) measuring dose toxicology are necessary to demonstrate the interaction of this blend with body and tumor growth, and also any positive synergistic or adverse interaction with chemotherapeutic drugs listed, so as to confirm the current observation and epithelial tissue growth or regeneration in a neurodegenerative disease model.

Colombo, S. L., et al. (2011). "Molecular basis for the differential use of glucose and glutamine in cell proliferation as revealed by synchronized HeLa cells." Proc Natl Acad Sci U S A **108**(52): 21069-21074.

During cell division, the activation of glycolysis is tightly regulated by the action of two ubiquitin ligases, anaphase-promoting complex/cyclosome-Cdh1 (APC/C-Cdh1) and SKP1/CUL-1/F-box protein-beta-transducin repeat-containing protein (SCF-beta-TrCP), which control the transient appearance and metabolic activity of the glycolysis-promoting enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, isoform 3 (PFKFB3). We now demonstrate that the breakdown of PFKFB3 during S phase occurs specifically via a distinct residue (S (273)) within the conserved recognition site for SCF-beta-TrCP. Glutaminase 1 (GLS1), the first enzyme in glutaminolysis, is also targeted for destruction by APC/C-Cdh1 and, like PFKFB3, accumulates after the activity of this ubiquitin ligase decreases in mid-to-late G1. However, our results show that GLS1 differs from PFKFB3 in that its recognition by APC/C-Cdh1 requires the presence of both a Lys-Glu-Asn box (KEN box) and a destruction box (D box) rather than a KEN box alone. Furthermore, GLS1 is not a substrate for SCF-beta-TrCP and is not degraded until cells progress from S to G2/M. The presence of PFKFB3 and GLS1 coincides with increases in generation of lactate and in utilization of glutamine, respectively. The contrasting posttranslational regulation of PFKFB3 and GLS1, which we have verified by studies of ubiquitination and protein stability, suggests the different roles of glucose and glutamine at distinct stages in the cell cycle. Indeed, experiments in which synchronized cells were deprived of either of these substrates show that both glucose and glutamine are required for progression through the restriction point in mid-to-late G1, whereas glutamine is the only substrate essential for the progression through S phase into cell division.

Coombs, K. M. (2013). "HeLa cell response proteome alterations induced by mammalian reovirus T3D infection." Virol J **10**: 202.

BACKGROUND: Cells are exposed to multiple stressors that induce significant alterations in signaling pathways and in the cellular state. As obligate parasites, all viruses require host cell material and machinery for replication. Virus infection is a major stressor leading to numerous induced modifications. Previous gene array studies have measured infected cellular transcriptomes. More recently, mass spectrometry-based quantitative and comparative assays have been used to complement such studies by examining virus-induced alterations in the cellular proteome. METHODS: We used SILAC (stable isotope labeling with amino acids in cell culture), a non-biased quantitative proteomic labeling technique, combined with 2-D HPLC/mass spectrometry and reciprocal labeling to identify and measure relative quantitative differences in HeLa cell proteins in purified cytosolic and nuclear fractions after reovirus serotype 3 Dearing infection. Protein regulation was determined by z-score analysis of each protein's label distribution. RESULTS: A total of 2856 cellular proteins were identified in cytosolic fractions by 2 or more peptides at >99% confidence and 884 proteins were identified in nuclear fractions. Gene ontology analyses indicated up-regulated host proteins were associated with defense responses, immune responses, macromolecular binding, regulation of immune effector processes, and responses to virus, whereas down-regulated proteins were involved in cell death, macromolecular catabolic processes, and tissue development. CONCLUSIONS: These analyses identified numerous host proteins significantly affected by reovirus T3D infection. These proteins map to numerous inflammatory and innate immune pathways, and provide the starting point for more detailed kinetic studies and delineation of virus-modulated host signaling pathways.

Date, A. A., et al. (2015). "Development and validation of a simple and isocratic reversed-phase HPLC method for the determination of rilpivirine from tablets, nanoparticles and HeLa cell lysates." Biomed Chromatogr **29**(5): 709-715.

In the present investigation, a simple and isocratic HPLC-UV method was developed and validated for determination of rilpivirine (RPV) from dosage forms (tablets and nanoparticles) and biological matrices like HeLa cell lysates. The separation and analysis of RPV was carried out under isocratic conditions using (a) a Gemini reversed-phase C18 column (5 microm; 4.6 x 150 mm) maintained at 35 degrees C, (b) a mobile phase consisting of a mixture of acetonitrile and 25 m m potassium dihydrogen phosphate (in the ratio 50:50 v/v) at a flow rate of 0.6 mL/min and (c) atazanavir as an internal standard. The total run time was 17 min and the analysis of RPV and internal standard was carried out at 290 nm. The method was found to be linear (r (2) value > 0.998), specific, accurate and precise over the concentration range of 0.025-2 microg/mL. The lower limit of quantification was 0.025 microg/mL, the limit of detection was 0.008 microg/mL and the recovery of RPV was >90%. The stability of the RPV analytical method was confirmed at various conditions such as room temperature (24 h), -20 degrees C (7 days), three freeze-thaw cycles and storage in an autosampler (4 degrees C for 48 h). The method was successfully applied for the determination of RPV from conventional dosage forms like tablets, from polymeric nanoparticles and from biological matrices like HeLa cell lysates.

Deb, R. and P. P. Goswami (2011). "Coexpression of PPE 34.9 Antigen of Mycobacterium avium subsp. Paratuberculosis with Murine Interferon Gamma in HeLa Cell Line and Study of Their Immunogenicity in Murine Model." Biotechnol Res Int **2011**: 632705.

Mycobacterium avium subsp. paratuberculosis (Map) is the causative agent of johne's disease whose immunopathology mainly depends on cell mediated immuneresponse. Genome sequencing revealed various PPE (Proline-Proline-Glutamic acid) protein family of Map which are immunologically importance candidate genes In present study we have developed a bicistrionic construct pIR PPE/IFN containing a 34.9 kDa PPE protein (PPE 34.9) of Map along with a cytokine gene encoding murine gamma Interferon gene (IFNgamma) and a monocistrionic construct pIR PPE using a mammalian vector system pIRES 6.1. The construct were transfected in HeLa cell line and expression were studied by Western blot as well as Immunefluroscent assay using recombinant sera. Further we have compared the immunereactivity of these two constructs in murine model by means of DTH study, LTT, NO assay and ELISA. DTH response was higher in pIR PPE/IFN than pIR PPE group of mice, similar finding also observed in case of LTT and NO production assay. ELISA titer of the pIR PPE/IFN was less than that with PPE only. These preliminary finding can revealed a CMI response of this PPE protein of Map and IFNgamma having synergistic effect on this PPE protein to elicit a T cell based immunity in mice.

Delinasios, J. G., et al. (2015). "Proliferating fibroblasts and HeLa cells co-cultured in vitro reciprocally influence growth patterns, protein expression, chromatin features and cell survival." Anticancer Res **35**(4): 1881-1916.

AIM: to identify biological interactions between proliferating fibroblasts and HeLa cells in vitro. MATERIALS AND METHODS: Fibroblasts were isolated from both normal and tumour human tissues. Coverslip co-cultures of HeLa and fibroblasts in various ratios with medium replacement every 48 h were studied using fixed cell staining with dyes such as Giemsa and silver staining, with immunochemistry for Ki-67 and E-cadherin, with dihydrofolate reductase (DHFR) enzyme reaction, as well as live cell staining for non-specific esterases and lipids. Other techniques included carmine cell labeling, autoradiography and apoptosis assessment. RESULTS: Under conditions of feeding and cell: cell ratios allowing parallel growth of human fibroblasts and HeLa cells, co-cultured for up to 20 days, a series of phenomena occur consecutively: profound affinity between the two cell types and exchange of small molecules; encircling of the HeLa colonies by the fibroblasts and enhanced growth of both cell types at their contact areas; expression of carbonic anhydrase in both cell types and high expression of non-specific esterases and cytoplasmic argyrophilia in the surrounding fibroblasts; intense production and secretion of lipid droplets by the surrounding fibroblasts; development of a complex net of argyrophilic projections of the fibroblasts; E-cadherin expression in the HeLa cells; from the 10th day onwards, an increasing detachment of batches of HeLa cells at the peripheries of colonies and appearance of areas with many multi-nucleated and apoptotic HeLa cells, and small HeLa fragments; from the 17th day, appearance of fibroblasts blocked at the G2-M phase. Co-cultures at approximately 17-20 days display a cell-cell fight with foci of (a) sparse growth of both cell types, (b) overgrowth of the fibroblasts and (c) regrowth of HeLa in small colonies. These results indicate that during their interaction with HeLa cells in vitro, proliferating fibroblasts can be activated against HeLa. This type of activation is not observed if

fibroblast proliferation is blocked by contact inhibition of growth at confluency, or by omitting replacement of the nutrient medium. CONCLUSION: The present observations show that: (a) interaction between proliferating fibroblasts and HeLa cells in vitro drastically influences each other's protein expression, growth pattern, chromatin features and survival; (b) these functions depend on the fibroblast/HeLa ratio, cell topology (cell-cell contact and the architectural pattern developed during co-culture) and frequent medium change, as prerequisites for fibroblast proliferation; (c) this co-culture model is useful in the study of the complex processes within the tumour microenvironment, as well as the in vitro reproduction and display of several phenomena conventionally seen in tumour cytological sections, such as desmoplasia, apoptosis, nuclear abnormalities; and (d) overgrown fibroblasts adhering to the boundaries of HeLa colonies produce and secrete lipid droplets.

Diao, M. K., et al. (2015). "Integrated HPV genomes tend to integrate in gene desert areas in the CaSki, HeLa, and SiHa cervical cancer cell lines." Life Sci **127**: 46-52.

AIMS: The integration preferences of human papillomavirus (HPV) have been intensively studied and contested over recent years. To disclose the integration preferences of high-risk HPV in cervical cancer, HPV transcriptional sites and features in different cervical cancer cell lines were identified. MAIN METHODS: In this study, three cervical cancer cell lines (CaSki, HeLa, and SiHa) were subjected for HPV genome status determination by amplification of papillomavirus oncogene transcripts (APOT) assay. The numbers of viral copies in human genomes and numbers of viral-human fusion mRNAs in three HPV-integrated cervical cancer cell lines were measured and analysed. KEY FINDINGS: The results revealed that the gene desert region 8q24 of the HPV type 18 integrated HeLa cell line and the 13q21-22 region of the HPV type 16 integrated CaSki and SiHa cell lines were hotspots for HPV integration, and the numbers of viral copies in the human genomes of the three cell lines that we detected were not in accordance with those reported in previous studies. SIGNIFICANCE: Integration of the HPV genome into the host cell chromosome suggests that persistent HPV infection is vital for malignant cell transformation and carcinogenesis. This study provides information to benefit health care professionals seeking more comprehensive and accurate diagnostics for HPV-related disease"? Please check, and amend as necessary.

Ding, Y., et al. (2016). "Forced expression of Nanog with mRNA synthesized in vitro to evaluate the malignancy of HeLa cells through acquiring cancer stem cell phenotypes." Oncol Rep **35**(5): 2643-2650.

Nanog is a pluripotency-related factor. It was also found to play an important role in tumorigenesis. To date, the mechanisms underlying cervical tumorigenesis still need to be elucidated. In the present study, Nanog mRNA was synthesized in vitro and transfected into HeLa cells. After mRNA transfection, the forced expressed of Nanog in HeLa cells led to markedly increased invasion, migration, resistance to chemotherapeutic agents and dedifferentiation. In a subcutaneous xenograft assay, these cells had significantly increased tumorigenic capacity. Real-time PCR indicated that Nanoginduced dedifferentiation was associated with increased expression of endogenous Oct4, Sox2 and FoxD3. In addition, the dedifferentiated HeLa cells acquired features associated with cancer stem cells (CSCs), such as multipotent differentiation capacity, and expression of CSC markers such as CD133. These data imply that Nanog is a positive regulator of cervical cancer dedifferentiation.

Dong, X., et al. (2012). "p38-NF-kappaB-promoted mitochondria-associated apoptosis and G2/M cell cycle arrest in norcantharidin-treated HeLa cells." J Asian Nat Prod Res **14**(11): 1008-1019.

Previous study proved that norcantharidin (NCTD) could exert its anticancer activity in a variety of malignant cell lines, including human cervical carcinoma HeLa cells. In this study, we found that NCTD-activated p38 mitogen-activated protein kinase (p38 MAPK)-nuclear transcription factor kappa B (NF-kappaB) signaling pathway induced mitochondrial apoptotic pathway activation and G2/M cell cycle arrest in HeLa cells. NCTD-induced mitochondria-associated apoptosis was concomitant with the collapse of mitochondrial membrane potential (DeltaPsi (m)), translocation of Bax, down-regulation of Bcl-2 expression, and release of cytochrome c. NCTD-led G2/M cell-cycle arrest was associated with the up-regulated p21 and p-cdc25c expression and the down-regulated cyclin B and cdc2 expression. Treatment of the cells with p38 inhibitor SB203580 and NF-kappaB inhibitor pyrrolidine dithiocarbamate (PDTC) showed that p38 functioned upstream of NF-kappaB, while augmented apoptosis and cell cycle arrest were induced in response to NCTD with NF-kappaB activation. Intriguingly, NF-kappaB had a negative feedback regulatory effect on p38 activation. Moreover, NCTD-induced apoptosis and cell cycle arrest were significantly blocked by SB203580 and PDTC but not by pifithrin-alpha (p53 inhibitor). Therefore, p38-NF-kappaB induced mitochondrial apoptotic pathway and G2/M cell cycle arrest in NCTD-treated HeLa cells.

Elsayed, E. A., et al. (2015). "In vitro Evaluation of Cytotoxic Activities of Essential Oil from Moringa oleifera Seeds on HeLa, HepG2, MCF-7, CACO-2 and L929 Cell Lines." Asian Pac J Cancer Prev **16**(11): 4671-4675.

Moringa oleifera Lam. (Moringaceae) is widely consumed in tropical and subtropical regions for their valuable nutritional and medicinal characteristics. Recently, extensive research has been conducted on leaf extracts of M. oleifera to evaluate their potential cytotoxic effects. However, with the exception of antimicrobial and antioxidant activities, little information is present on the cytotoxic activity of the essential oil obtained from M. oleifera seeds. Therefore, the present investigation was designed to investigate the potential cytotoxic activity of seed essential oil obtained from M. oleifera on HeLa, HepG2, MCF-7, CACO-2 and L929 cell lines. The different cell lines were subjected to increasing oil concentrations ranging from 0.15 to 1 mg/mL for 24h, and the cytotoxicity was assessed using MTT assay. All treated cell lines showed a significant reduction in cell viability in response to the increasing oil concentration. Moreover, the reduction depended on the cell line as well as the oil concentration applied. Additionally, HeLa cells were the most affected cells followed by HepG2, MCF-7, L929 and CACO-2, where the percentages of cell toxicity recorded were 76.1, 65.1, 59.5, 57.0 and 49.7%, respectively. Furthermore, the IC50 values obtained for MCF-7, HeLa and HepG2 cells were 226.1, 422.8 and 751.9 mug/mL, respectively. Conclusively, the present investigation provides preliminary results which suggest that seed essential oil from M. oleifera has potent cytotoxic activities against cancer cell lines.

Erdem, M., et al. (2017). "Folic acid-conjugated polyethylene glycol-coated magnetic nanoparticles for doxorubicin delivery in cancer chemotherapy: Preparation, characterization and cytotoxicity on HeLa cell line." Hum Exp Toxicol **36**(8): 833-845.

Conventional chemotherapy is the most valid method to cope with cancer; however, it has serious drawbacks such as decrease in production of blood cells or inflammation of the lining of the digestive tract. These side effects occur since generally the drugs used in chemotherapy are distributed evenly within the body of the patient and cannot distinguish the cancer cells from the healthy ones. In this study, folic acid (FA)-conjugated, polyethylene-coated magnetic nanoparticles (FA-MNPs), and doxorubicin (Dox)-loaded formulation (Dox-FA-MNPs) were prepared. The cytotoxicity of these nanoparticles on HeLa and Dox-resistant HeLa cells was investigated. Magnetic nanoparticles (MNPs), polyethylene glycol (PEG)-coated MNPs (PEG-MNPs), and FA-MNPs were successfully synthesized and characterized by several methods. Dox loading of FA-MNPs and release profile of Dox from the nanoparticles were studied. Cytotoxic effects of FA-MNPs and Dox-FA-MNPs on HeLa cells were analyzed. MNPs, PEG-MNPs, and FA-MNPs all had small sizes and supermagnetic behavior. High amounts of Dox could be loded onto the nanoparticles (290 mugmL (-1)). In 24 h, 15.7% of Dox was released from the Dox-FA-MNPs. The release was increased in acidic conditions (pH 4.1). Internalization studies showed that FA-MNPs and Dox-FA-MNPs were taken up efficiently by HeLa cells. The investigation of cytotoxicity of the particles indicated that 38-500 mugmL (-1) Dox-FA-MNPs significantly decreased the proliferation of HeLa cells compared to FA-MNPs. Due to their size, magnetic properties, internalization, drug release, and cytotoxicity characteristics, the MNPs prepared in this study may have potential application as a drug delivery system in cancer chemotherapy.

Etcheverry, M. E., et al. (2016). "Photodynamic therapy of HeLa cell cultures by using LED or laser sources." J Photochem Photobiol B **160**: 271-277.

The photodynamic therapy (PDT) on HeLa cell cultures was performed utilizing a 637nm LED lamp with 1.06W power and m-tetrahydroxyphenyl chlorin (m-THPC) as photosensitizer and compared to a laser source emitting at 654nm with the same power. Intracellular placement of the photosensitizer and the effect of its concentration (CP), its absorption time (TA) and the illumination time (TI) were evaluated. It was observed that for CP>40mug/ml and TA>24h, m-THPC had toxicity on cells in culture, even in the absence of illumination. For the other tested concentrations, the cells remained viable if not subjected to illumination doses. No effect on cells was observed for CP<0.05mug/ml, TA=48h and TI=10min and they continued proliferating. For drug concentrations higher than 0.05mugml (-1), further deterioration is observed with increasing TA and TI. We evaluated the viability of the cells, before and after the treatment, and by supravital dyes, and phase contrast and fluorescence microscopies, evidence of different types of cell death was obtained. Tetrazolium dye assays after PDT during different times yielded similar results for the 637nm LED lamp with an illuminance three times greater than that of the 654nm laser source. Results demonstrate the feasibility of using a LED lamp as alternative to laser source. Here the main characteristic is not the light coherence but achieving a certain light fluence of the appropriate wavelength on cell cultures. We conclude that the efficacy was achieved satisfactorily and is essential for convenience, accessibility and safety.

Fale, P. L., et al. (2012). "Acetylcholinesterase inhibition, antioxidant activity and toxicity of Peumus boldus water extracts on HeLa and Caco-2 cell lines." Food Chem Toxicol **50**(8): 2656-2662.

This work aimed to study the inhibition on acetylcholinesterase activity (AChE), the antioxidant activity and the toxicity towards Caco-2 and HeLa cells of aqueous extracts of Peumus Boldus. An IC (50) value of 0.93 mg/mL, for AChE inhibition, and EC (50) of 18.7 mug/mL, for the antioxidant activity, was determined. This activity can be attributed to glycosylated flavonoid derivatives detected, which were the main compounds, although boldine and other aporphine derivatives were also present. No changes in the chemical composition or the biochemical activities were found after gastrointestinal digestion. Toxicity of P. boldus decoction gave an IC (50) value 0.66 mg/mL for HeLa cells, which caused significant changes in the cell proteome profile.

Fang, B. Y., et al. (2016). "Detection of adenosine triphosphate in HeLa cell using capillary electrophoresis-laser induced fluorescence detection based on aptamer and graphene oxide." Colloids Surf B Biointerfaces **140**: 233-238.

A method for ATP quantification based on dye-labeled aptamer/graphene oxide (aptamer/GO) using capillary electrophoresis-laser induced fluorescence (CE-LIF) detecting technique has been established. In this method, the carboxyfluorescein (FAM)-labelled ATP aptamers were adsorbed onto the surface of GO, leading to the fluorescence quenching of FAM; after the incubation with a limited amount of ATP, stronger affinity between ATP aptamer and ATP resulted in the desorption of aptamers and the fluorescence restoration of FAM. Then, aptamer-ATP complex and excess of aptamer/GO and GO were separated and quantified by CE-LIF detection. It was shown that a linear relation was existing in the CE-LIF peak intensity of aptamer-ATP and ATP concentration in range of 10-700 muM, the regression equation was F=1.50+0.0470C (ATP) (R (2)=0.990), and the limit of detection was 1.28 muM (3S/N, n=5), which was one order magnitude lower than that of detection in solution by fluorescence method. The approach with excellent specificity and reproducibility has been successfully applied to detecting concentration of ATP in HeLa cell.

Farooqui, A., et al. (2018). "Glycyrrhizin induces reactive oxygen species-dependent apoptosis and cell cycle arrest at G0/G1 in HPV18(+) human cervical cancer HeLa cell line." Biomed Pharmacother **97**: 752-764.

Cervical cancer is the fourth most common cancer among women worldwide and is a major cause of morbidity and mortality. High-risk Human Papilloma Virus (mostly type 16 & 18) infection is the primary risk factor for the development of cervical carcinoma. The quest for strong, safe and cost effective natural antiproliferative agents that could reduce cervical cancer have been focussed now a day. Recently, glycyrrhizin, a triterpene glycoside (saponin) from licorice (Glycyrrhiza glabra Linn.), has been shown to exhibit potent antiproliferative and anticancer properties in a few preliminary studies. However, potential of this compound in cervical cancer has not been elucidated yet. Therefore the objective of this study was to analyze the antiproliferative and apoptotic properties of glycyrrhizin in human cervical cancer HeLa cells. Our results showed that glycyrrhizin exposure significantly reduced the cell viability of HeLa cells with a concomitant increase in nuclear condensation and DNA fragmentation in a dose dependent manner. The intracellular ROS generation assay showed dose-related increment in ROS production induced by glycyrrhizin. Glycyrrhizin also induced apoptosis in cervical cancer cells by exerting mitochondrial depolarization. Cell cycle study showed that glycyrrhizin induced cell cycle arrest in G0/G1 phase of cell cycle in a dose dependent manner. Thus, this study confirms the efficacy of glycyrrhizin in cervical cancer cells which could be an adjunct in the better prevention and management of cervical cancer worldwide.

Fattahi, S., et al. (2014). "Total Phenolic and Flavonoid Contents of Aqueous Extract of Stinging Nettle and In Vitro Antiproliferative Effect on Hela and BT-474 Cell Lines." Int J Mol Cell Med **3**(2): 102-107.

Phenolic compounds including flavonoids and phenolic acids are plants secondary metabolites. Due to their ability to act as antioxidant agents, there is a growing interest to use those components in traditional medicine for cancer prevention or treatment. The aim of this study was to measure the amounts of total phenolics and flavonoids as well as anti-proliferative effect of aqueous extract of Stinging nettle on BT-474 and Hela cell lines. The amounts of phenolics content and total flavonoids were determined by folin ciocalteu and aluminium chloride methods, respectively. The free radical scavenging activity was measured by using diphenyl - picrylhydrazyl (DPPH). The reducing power of the extract was measured in the presence of potassium hexacyanoferrate and its antiproliferative activity was assessed on BT-474 and Hela cell lines using MTT assay. Total phenolic content was 322.941+/- 11.811 mg gallic acid/g

extract. Total flavonoid content was 133.916+/-12.006 mg Catechin/g. The IC50 of DPPH radical was 1.2 mg/ ml and the reducing power was 218.9+/- 15.582 mug ascorbic acid/ g. Cell viability of BT-474 cells decreased to less than half of the control (no added extract) at the presence of 3 mg/ ml extract while no significant changes were detected for Hela cells at similar conditions. There was no significant difference in the percentage of surviving cells between consecutive days (day 1, 2 and 3) for both BT-474 and Hela cells (P>0.05). Although the relatively high amount of phenolic and flavonoid contents of the aqueous extract make this plant a promising candidate for diseases treatment; however, there is not a direct relationship between the amounts of these antioxidant components and the efficiency in in vitro cancer treatment.

Felisbino, M. B., et al. (2011). "Chromatin remodeling, cell proliferation and cell death in valproic acid-treated HeLa cells." PLoS One **6**(12): e29144.

BACKGROUND: Valproic acid (VPA) is a potent anticonvulsant that inhibits histone deacetylases. Because of this inhibitory action, we investigated whether VPA would affect chromatin supraorganization, mitotic indices and the frequency of chromosome abnormalities and cell death in HeLa cells. METHODOLOGY/PRINCIPAL FINDINGS: Image analysis was performed by scanning microspectrophotometry for cells cultivated for 24 h, treated with 0.05, 0.5 or 1.0 mM VPA for 1-24 h, and subjected to the Feulgen reaction. TSA-treated cells were used as a predictable positive control. DNA fragmentation was investigated with the TUNEL assay. Chromatin decondensation was demonstrated under TSA and all VPA treatments, but no changes in chromosome abnormalities, mitotic indices or morphologically identified cell death were found with the VPA treatment conditions mentioned above, although decreased mitotic indices were detected under higher VPA concentration and longer exposure time. The frequency of DNA fragmentation identified with the TUNEL assay in HeLa cells increased after a 24-h VPA treatment, although this fragmentation occurred much earlier after treatment with TSA. CONCLUSIONS/SIGNIFICANCE: The inhibition of histone deacetylases by VPA induces chromatin remodeling in HeLa cells, which suggests an association to altered gene expression. Under VPA doses close to the therapeutic antiepileptic plasma range no changes in cell proliferation or chromosome abnormalities are elicited. The DNA fragmentation results indicate that a longer exposure to VPA or a higher VPA concentration is required for the induction of cell death.

Fernandes, M. C., et al. (2011). "Trypanosoma cruzi trypomastigotes induce cytoskeleton modifications during HeLa cell invasion." Mem Inst Oswaldo Cruz **106**(8): 1014-1016.

It has been recently shown that Trypanosoma cruzi trypomastigotes subvert a constitutive membrane repair mechanism to invade HeLa cells. Using a membrane extraction protocol and high-resolution microscopy, the HeLa cytoskeleton and T. cruzi parasites were imaged during the invasion process after 15 min and 45 min. Parasites were initially found under cells and were later observed in the cytoplasm. At later stages, parasite-driven protrusions with parallel filaments were observed, with trypomastigotes at their tips. We conclude that T. cruzi trypomastigotes induce deformations of the cortical actin cytoskeleton shortly after invasion, leading to the formation of pseudopod-like structures.

Flores-Perez, A. and G. Elizondo (2018). "Apoptosis induction and inhibition of HeLa cell proliferation by alpha-naphthoflavone and resveratrol are aryl hydrocarbon receptor-independent." Chem Biol Interact **281**: 98-105.

Human papilloma viruses 16 and 18 express E6 and E7 oncoproteins. E6 activates and redirects E6-associated protein (E6AP), an E3 ubiquitin ligase. E6AP interacts with Ube2l3, an E2 ubiquitin conjugating enzyme protein (also known as UbcH7), to promote p53 ubiquitination and degradation by the 26S proteasome. Therefore, blocking E6-mediated p53 degradation might be an alternative treatment for cervical cancer. In addition, activation of the aryl hydrocarbon receptor (AHR) induces Ube2l3 expression, resulting in p53 ubiquitination and degradation. The aim of the present study was to determine whether inhibition of AHR in HeLa cells resulted in an increase in p53 and apoptosis along with a decrease in cell proliferation. The results demonstrate that two AHR antagonists, alpha-naphthoflavone (alpha-NF) and resveratrol, decreased cell proliferation, arrested cells in the gap 1/synthesis (G1/S) phases, and increased p53 levels and apoptosis. However, knocking out the Ahr gene did not abrogate the effects of alpha-NF and resveratrol. Moreover, Ahr-null cells presented similar cell proliferation rates and apoptosis levels when compared to control HeLa cells. Taken together, the results indicate that alpha-NF's and resveratrol's cytostatic and cytotoxic actions, respectively, occur through an AHR-independent mechanism, and that AHR is not required for HeLa cell proliferation.

Furusawa, Y., et al. (2012). "TGF-beta-activated kinase 1 promotes cell cycle arrest and cell survival of

X-ray irradiated HeLa cells dependent on p21 induction but independent of NF-kappaB, p38 MAPK and ERK phosphorylations." Radiat Res **177**(6): 766-774.

Transforming growth factor-beta-activated kinase 1 (TAK1) appears to play a role in inhibiting apoptotic death in response to multiple stresses. To assess the role of TAK1 in X-ray induced apoptosis and cell death, we irradiated parental and siRNA-TAK1-knockdown HeLa cells. Changes in gene expression levels with and without TAK1-knockdown were also examined after irradiation to elucidate the molecular mechanisms involved. After X-ray irradiation, cell death estimated by the colony formation assay increased in the TAK1-knockdown cells. Apoptosis induction, determined by caspase-3 cleavage, suggested that the increased radiosensitivity of the TAK1-knockdown cells could be partially explained by the induction of apoptosis. However, cell cycle analysis revealed that the percentage of irradiated cells in the G (2)/M-phase decreased, and those in the S- and SubG (1)-phases increased due to TAK1 depletion, suggesting that the loss of cell cycle checkpoint regulation may also be involved in the observed increased radiosensitivity. Interestingly, significant differences in the induction of NF-kappaB, p38 MAPK and ERK phosphorylation, the major downstream molecules of TAK1, were not observed in TAK1 knockdown cells compared to their parental control cells after irradiation. Instead, global gene expression analysis revealed differentially expressed genes after irradiation that bioinformatics analysis suggested are associated with cell cycle regulatory networks. In particular, CDKN1A (coding p21(WAF1)), which plays a central role in the identified network, was up-regulated in control cells but not in TAK1 knockdown cells after X-ray irradiation. Si-RNA knockdown of p21 decreased the percentage of cells in the G (2)/M phase and increased the percentage of cells in the S- and SubG (1)-phases after X-ray irradiation in a similar manner as TAK-1 knockdown. Taken together, these findings suggest that the role of TAK1 in cell death, cell cycle regulation and apoptosis after X irradiation is independent of NF-kappaB, p38 MAPK, and ERK phosphorylation, and dependent, in part, on p21 induction.

Gendaszewska-Darmach, E. and M. Szustak (2016). "Thymidine 5'-O-monophosphorothioate induces HeLa cell migration by activation of the P2Y6 receptor." Purinergic Signal **12**(2): 199-209.

ATP, ADP, UTP, and UDP acting as ligands of specific P2Y receptors activate intracellular signaling cascades to regulate a variety of cellular processes, including proliferation, migration, differentiation, and cell death. Contrary to a widely held opinion, we show here that nucleoside 5'-O-monophosphorothioate analogs, containing a sulfur atom in a place of one nonbridging oxygen atom in a phosphate group, act as ligands for selected P2Y subtypes. We pay particular attention to the unique activity of thymidine 5'-O-monophosphorothioate (TMPS) which acts as a specific partial agonist of the P2Y6 receptor (P2Y6R). We also collected evidence for the involvement of the P2Y6 receptor in human epithelial adenocarcinoma cell line (HeLa) cell migration induced by thymidine 5'-O-monophosphorothioate analog. The stimulatory effect of TMPS was abolished by siRNA-mediated P2Y6 knockdown and diisothiocyanate derivative MRS 2578, a selective antagonist of the P2Y6R. Our results indicate for the first time that increased stability of thymidine 5'-O-monophosphorothioate as well as its affinity toward the P2Y6R may be responsible for some long-term effects mediated by this receptor.

Geng, X. X., et al. (2011). "[Effects of As2O3 and all-trans retinoic acid on the growth of HeLa cell line and their relation with gene NDRG1]." Zhonghua Zhong Liu Za Zhi **33**(1): 8-12.

OBJECTIVE: To study the effect of arsenic trioxide (As2O3) and all-trans retinoic acid (ATRA) on human cervical carcinoma HeLa cell line. METHODS: HeLa cells were treated with As2O3 and ATRA. The cell proliferation was evaluated by MTT assay. The expressions of NDRG-1 protein and mRNA were determined by Western blot and RT-PCR analysis. RESULTS: MTT assay showed that As2O3 and ATRA inhibited the growth of human cervical carcinoma HeLa cells in vitro in a dose- and time-dependent manner. Western blot and RT-PCR techniques showed that As2O3 and ATRA down-regulated the expressions of NDRG-1 protein and mRNA ($P < 0.05$). CONCLUSION: As2O3 and ATRA can significantly inhibit the growth and proliferation of HeLa cells. The reason of these changes may be related with the down-regulation of expression of NDRG-1.

Geryani, M. A., et al. (2016). "Ctotoxic and apoptogenic effects of Perovskia abrotanoides flower extract on MCF-7 and HeLa cell lines." Avicenna J Phytomed **6**(4): 410-417.

OBJECTIVE: Perovskia abrotanoides Karel, belongs to the family Lamiaceae and grows wild alongside the mountainous roads inarid and cold climate of Northern Iran. The anti-tumor activity of P. abrotanoides root extract has been shown previously. This study was designed to examine in vitro anti-proliferative and pro-apoptotic effects of flower extract of P. abrotanoides on MCF-7 and Hela cell lines. MATERIALS AND METHODS: Cells were

cultured in DMEM medium with 10% fetal bovine serum, 100 units/ml penicillin and 100 microg/ml streptomycin and incubated with different concentrations of plant extracts. Cell viability was quantified by MTT assay. Apoptotic cells were determined using propidium iodide (PI) staining of DNA fragmentation by flow cytometry (sub-G1 peak). RESULTS: P. abrotanoides extract inhibited the growth of malignant cells in a time and dose-dependent manner and 1000 microg/ml of extract following 48h of incubation was the most cytotoxic dose against Hela cell in comparison with other doses; however, in MCF-7 cells,1000 and 500 microg/ml PA induced toxicity at all time points but with different features. Analysis of flowcytometry histogram of treated cells compared with control cells indicated that the cytotoxic effect is partly due toapoptosis induction. CONCLUSION: Hydro-alcoholic extract of P. abrotanoides flowers inhibits the growth of MCF-7 and HeLa cell lines, partly via inducing apoptosis. Their inhibitory effect was increased in a time and dose-dependent manner, especially in MCF7 cells. However, further studies are needed to reveal the mechanisms of P. abrotanoides extract-induced cell death.

Ghosh, S., et al. (2017). "Biological evaluation of a halogenated triterpenoid, 2alpha-bromo-dihydrobelulonic acid as inhibitor of human topoisomerase IIalpha and HeLa cell proliferation." Chem Biol Interact **268**: 68-76.

BACKGROUND: The pentacyclic lupane-type (6-6-6-6-5 type) triterpenoid, Betulinic acid (BA) is a potent inhibitor of topoisomerases and is of immense interest as anticancer drugs. However, the compound being highly lipophilic, has limited in vivo uptake capacity. BA derivatives with halogen substituent at C-2 have improved membrane permeability and cytotoxicity against cancer cells. AIM: The halogenated triterpenoid, 2alpha-bromo-dihydrobetulonic acid (B1) was synthesized from betulinic acid (BA) isolated from Bischofia javanica. Aim of the study was to determine whether B1 could act as a more efficient inhibitor of Topo IIalpha activity and HeLa cell proliferation, in comparison to BA. RESULT: B1 displayed efficient inhibition of DNA relaxation activity of topoisomerase IIalpha and the inhibitory effect was markedly improved upon pre-incubation of the compound with enzyme. Topoisomerase IIalpha inhibition by B1 was relieved in presence of increasing concentrations of DNA suggesting the compound as a reversible catalytic inhibitor. Subsequent UV and fluorescence spectroscopy studies indicated that B1 interacts and intercalates with DNA at concentrations signicantly greater than that required for topoisomerase IIalpha inhibition. The compound showed cytotoxic activity against HeLa cells with significantly lower IC50 value (7.5 muM) as compared to that of BA (30 muM) and had very low damaging/cytotoxic effect on normal cells. Treatment of B1 impaired HeLa cell proliferation by inducing Go-G1 arrest through lowered expression of cyclin D1 and PCNA polypeptides, and enhanced expression of p21. B1 treatment also increased the accumulation of early and late apoptotic cells in a concentration dependent manner as indicated by annexin V-FITC/PI binding assay.

Giliano, N. Y., et al. (2011). "Dinitrosyl iron complexes with thiol-containing ligands and apoptosis: studies with HeLa cell cultures." Nitric Oxide **24**(3): 151-159.

No pro-apoptotic effect of dinitrosyl iron complexes (DNIC) with glutathione, cysteine or thiosulfate was established after incubation of HeLa cells in Eagle's medium. However, DNIC with thiosulfate manifested pro-apoptotic activity during incubation of HeLa cells in Versene's solution supplemented with ethylene diamine tetraacetate (EDTA) known to induce the decomposition of these DNIC. The water-soluble small o, Cyrillic-phenanthroline derivative bathophenanthroline disulfonate (BPDS) had a similar effect on DNIC with glutathione during incubation of HeLa cells in Eagle's medium. It was assumed that EDTA- or BPDS-induced pro-apoptotic effect of DNIC with thiosulfate or glutathione is coupled with the ability of decomposing DNIC to initiate S-nitrosylation of proteins localized on the surface of HeLa cells. Presumably, the pro-apoptotic effect of S-nitrosoglutathione (GS-NO) on HeLa cells preincubated in Eagle's medium is mediated by the same mechanism, although the pro-apoptotic effect based on the ability of GS-NO to initiate the release of significant amounts of NO and its oxidation to cytotoxic peroxynitrite in a reaction with superoxide should not be ruled out either. No apoptotic activity was found in the presence of bivalent iron and glutathione favoring the conversion of GS-NO into DNIC with glutathione. It is suggested that interaction of HeLa cells with intact DNIC with glutathione or thiosulfate results in the formation of DNIC bound to cell surface proteins.

Goto, T., et al. (2015). "Visualizing cell-cycle kinetics after hypoxia/reoxygenation in HeLa cells expressing fluorescent ubiquitination-based cell cycle indicator (Fucci)." Exp Cell Res **339**(2): 389-396.

Hypoxia induces G1 arrest in many cancer cell types. Tumor cells are often exposed to hypoxia/reoxygenation, especially under acute hypoxic

conditions in vivo. In this study, we investigated cell-cycle kinetics and clonogenic survival after hypoxia/reoxygenation in HeLa cells expressing fluorescent ubiquitination-based cell cycle indicator (Fucci). Hypoxic treatment halted cell-cycle progression during mid-S to G2 phase, as determined by the cell cycle-regulated E3 ligase activities of SCF (Skp2) and APC/C (Cdh1), which are regulators of the Fucci probes; however, the DNA content of the arrested cells was equivalent to that in G1 phase. After reoxygenation, time-lapse imaging and DNA content analysis revealed that all cells reached G2 phase, and that Fucci fluorescence was distinctly separated into two fractions 24h after reoxygenation: red cells that released from G2 arrest after repairing DNA double-strand breaks (DSBs) exhibited higher clonogenic survival, whereas most cells that stayed green contained many DSBs and exhibited lower survival. We conclude that hypoxia disrupts coordination of DNA synthesis and E3 ligase activities associated with cell-cycle progression, and that DSB repair could greatly influence cell-cycle kinetics and clonogenic survival after hypoxia/reoxygenation.

Gurses, N. and M. Topcul (2013). "The effect of abraxane on cell kinetic parameters of HeLa cells." Asian Pac J Cancer Prev **14**(7): 4229-4233.

Abraxane (nab-paclitaxel) is a member of the group of nano chemotherapeutics. It is approved for metastatic breast cancer and non small cell lung cancer. Trials for several cancer types including gynecological cancers, head and neck, and prostatic cancer are being studied. In this study, the antiproliferative and apoptotic effect of abraxane was evaluated on HeLa cell line originated from human cervix carcinoma. Three different doses (D1=10 nM, D2=50 nM, D3=100 nM) were administered to HeLa cells for 24, 48 and 72 h. The 50 nM dose of abraxane decreased DNA synthesis from 4.62-0.08%, mitosis from 3.36-1.89% and increased apoptosis from 10.6-30% at 72 h. Additionally, tripolar metaphase plates were seen in mitosis preparations. In this study, abraxane effected cell kinetic parameters significantly. This results are consistent with other studies in the literature.

Gustin, E., et al. (2017). "Lipid Droplet Formation in HeLa Cervical Cancer Cells Depends on Cell Density and the Concentration of Exogenous Unsaturated Fatty Acids." Acta Chim Slov **64**(3): 549-554.

Cytosolic lipid droplets (LDs) store excess fatty acids (FAs) in the form of neutral lipids and prevent starvation-induced cancer cell death. Here we studied the ability of mono- and polyunsaturated FAs to affect LD formation and survival in HeLa cervical cancer cells. We found that the LD content in HeLa cells increases with cell density, but it decreases in MDA-MB-231 breast cancer cells. Exogenously-added unsaturated FAs, including oleic (OA), linoleic (LA), arachidonic (AA), eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) displayed a similar ability to alter LD formation in HeLa cells. There was a dual, concentration-dependent effect on neutral lipid accumulation: low micromolar concentrations of LA, AA and DHA reduced, while all FAs induced LD formation at higher concentrations. In serum starved He-La cells, OA stimulated LD formation, but, contrary to expectations, it promoted cell death. Our results reveal a link between cell population density and LD formation in HeLa cells and show that unsaturated FAs may both suppress or stimulate LD formation. This dynamic regulation of LD content must be accounted for when studying the effects of lipids and lipid metabolism-targeting drugs on LD metabolism in HeLa cells.

Habryka, A., et al. (2015). "Cell type-dependent modulation of the gene encoding heat shock protein HSPA2 by hypoxia-inducible factor HIF-1: Down-regulation in keratinocytes and up-regulation in HeLa cells." Biochim Biophys Acta **1849**(9): 1155-1169.

HSPA2 belongs to the multigene HSPA family, whose members encode chaperone proteins. Although expression and function of HSPA2 is mainly associated with spermatogenesis, recent studies demonstrated that in humans, the gene is active in various cancers, as well as in normal tissues, albeit in a cell type-specific manner. In the epidermis, HSPA2 is expressed in keratinocytes in the basal layer. Currently, the mechanisms underlying the regulation of HSPA2 expression remain unknown. This study was aimed at determining whether HIF-1 and its binding site, the hypoxia-response element (HRE) located in the HSPA2 promoter, are involved in HSPA2 regulation. As a model system, we used an immortal human keratinocyte line (HaCaT) and cervical cancer cells (HeLa) grown under control or hypoxic conditions. Using an in vitro gene reporter assay, we demonstrated that in keratinocytes HSPA2 promoter activity is reduced under conditions that facilitate stabilization of HIF-1alpha, whereas HIF-1 inhibitors abrogated the suppressive effect of hypoxia on promoter activity. Chromatin immunoprecipitation revealed that HIF-1alpha binds to the HSPA2 promoter. In keratinocytes, hypoxia or overexpression of a stable form of HIF-1alpha attenuated the expression of endogenous HSPA2, whereas targeted repression of HIF-1alpha by RNAi increased transcription of HSPA2 under hypoxia. Conversely, in HeLa cells, HSPA2 expression increased under conditions that stimulated HIF-1alpha activity,

whereas inhibition of HIF-1alpha abrogated hypoxia-induced up-regulation of HSPA2 expression. Taken together, our results demonstrate that HIF-1 can exert differential, cell context-dependent regulatory control of the HSPA2 gene. Additionally, we also showed that HSPA2 expression can be stimulated during hypoxia/reoxygenation stress.

Haddaji, N., et al. (2015). "Acid stress suggests different determinants for polystyrene and HeLa cell adhesion in Lactobacillus casei." J Dairy Sci **98**(7): 4302-4309.

Adhesion has been regarded as one of the basic features of probiotics. The aim of this study was to investigate the influence of acid stress on the functional properties, such as hydrophobicity, adhesion to HeLa cells, and composition of membrane fatty acids, of Lactobacillus probiotics strains. Two strains of Lactobacillus casei were used. Adhesion on polystyrene, hydrophobicity, epithelial cells adhesion, and fatty acids analysis were evaluated. Our results showed that the membrane properties such as hydrophobicity and fatty acid composition of stressed strains were significantly changed with different pH values. However, we found that acid stress caused a change in the proportions of unsaturated and saturated fatty acid. The ratio of saturated fatty acid to unsaturated fatty acids observed in acid-stressed Lactobacillus casei cells was significantly higher than the ration in control cells. In addition, we observed a significant decrease in the adhesion ability of these strains to HeLa cells and to a polystyrene surface at low pH. The present finding could first add new insight about the acid stress adaptation and, thus, enable new strategies to be developed aimed at improving the industrial performance of this species under acid stress. Second, no relationship was observed between changes in membrane composition and fluidity induced by acid treatment and adhesion to biotic and abiotic surfaces. In fact, the decrease of cell surface hydrophobicity and the adhesion ability to abiotic surface and the increase of the capacity of adhesion to biotic surface demonstrate that adhesive characteristics will have little relevance in probiotic strain-screening procedures.

Halder, R., et al. (2012). "Bisquinolinium compounds induce quadruplex-specific transcriptome changes in HeLa S3 cell lines." BMC Res Notes **5**: 138.

BACKGROUND: Guanosine rich sequences capable of forming G-quadruplex (G4) motifs are enriched near the gene transcription start site (TSS) in the human genome. When probed at the single gene level, G-quadruplex motifs residing in promoter regions show substantial effects on gene transcription. Moreover, further changes in transcription levels are noticed when G4-motifs are targeted with G-quadruplex-specific small molecules. RESULTS: Global studies concerning general changes of the transcriptome via targeting promoter-based G-quadruplex motifs are very limited and have so far only been carried out with compounds displaying weak selectivity for quadruplex sequences. Here we utilize two G-quadruplex-specific bisquinolinium derivatives PhenDC3 and 360A and investigate their effects on the expression of the HeLa S3 transcriptome. Our results show wide-spread changes in the transcriptome with specificity for genes with G-quadruplex motifs near their transcription start sites (TSS). Using real-time PCR we further confirmed the specificity of PhenDC3 and 360A as potent molecules to target G-quadruplex-regulated genes. CONCLUSIONS: Specific effects on quadruplex-containing genes have been observed utilizing whole-transcriptome analysis upon treatment of cultured cells with quadruplex-selective bisquinolinium compounds.

Halpert, M., et al. (2011). "Rac-dependent doubling of HeLa cell area and impairment of cell migration and cell cycle by compounds from Iris germanica." Protoplasma **248**(4): 785-797.

Plants are an infinite source of bioactive compounds. We screened the Israeli flora for compounds that interfere with the organization of the actin cytoskeleton. We found an activity in lipidic extract from Iris germanica that was able to increase HeLa cell area and adhesion and augment the formation of actin stress fibers. This effect was not observed when Ref52 fibroblasts were tested and was not the result of disruption of microtubules. Further, the increase in cell area was Rac1-dependent, and the iris extract led to slight Rac activation. Inhibitor of RhoA kinase did not interfere with the ability of the iris extract to increase HeLa cell area. The increase in HeLa cell area in the presence of iris extract was accompanied by impairment of cell migration and arrest of the cell cycle at G1 although the involvement of Rac1 in these processes is not clear. Biochemical verification of the extract based on activity-mediated fractionation and nuclear magnetic resonance analysis revealed that the active compounds belong to the group of iridals, a known group of triterpenoid. Purified iripallidal was able to increase cell area of both HeLa and SW480 cells.

Hammad Aziz, M., et al. (2016). "Photodynamic Effect of Ni Nanotubes on an HeLa Cell Line." PLoS One **11**(3): e0150295.

Nickel nanomaterials are promising in the biomedical field, especially in cancer diagnostics and targeted therapy, due to their distinctive chemical and

physical properties. In this experiment, the toxicity of nickel nanotubes (Ni NTs) were tested in an in vitro cervical cancer model (HeLa cell line) to optimize the parameters of photodynamic therapy (PDT) for their greatest effectiveness. Ni NTs were synthesized by electrodeposition. Morphological analysis and magnetic behavior were examined using a Scanning electron microscope (SEM), an energy dispersive X-ray analysis (EDAX) and a vibrating sample magnetometer (VSM) analysis. Phototoxic and cytotoxic effects of nanomaterials were studied using the Ni NTs alone as well as in conjugation with aminolevulinic acid (5-ALA); this was performed both in the dark and under laser exposure. Toxic effects on the HeLa cell model were evaluated by a neutral red assay (NRA) and by detection of intracellular reactive oxygen species (ROS) production. Furthermore, 10-200 nM of Ni NTs was prepared in solution form and applied to HeLa cells in 96-well plates. Maximum toxicity of Ni NTs complexed with 5-ALA was observed at 100 J/cm2 and 200 nM. Up to 65-68% loss in cell viability was observed. Statistical analysis was performed on the experimental results to confirm the worth and clarity of results, with p-values = 0.003 and 0.000, respectively. Current results pave the way for a more rational strategy to overcome the problem of drug bioavailability in nanoparticulate targeted cancer therapy, which plays a dynamic role in clinical practice.

Hasanpourghadi, M., et al. (2016). "Targeting of tubulin polymerization and induction of mitotic blockage by Methyl 2-(5-fluoro-2-hydroxyphenyl)-1H-benzo [d]imidazole-5-carboxylate (MBIC) in human cervical cancer HeLa cell." J Exp Clin Cancer Res **35**: 58.

BACKGROUND: Microtubule Targeting Agents (MTAs) including paclitaxel, colchicine and vinca alkaloids are widely used in the treatment of various cancers. As with most chemotherapeutic agents, adverse effects and drug resistance are commonly associated with the clinical use of these agents. Methyl 2-(5-fluoro-2-hydroxyphenyl)-1H- benzo [d]imidazole-5-carboxylate (MBIC), a benzimidazole derivative displays greater toxicity against various cancer compared to normal human cell lines. The present study, focused on the cytotoxic effects of MBIC against HeLa cervical cancer cells and possible actions on the microtubule assembly. METHODS: Apoptosis detection and cell-cycle assays were performed to determine the type of cell death and the phase of cell cycle arrest in HeLa cells. Tubulin polymerization assay and live-cell imaging were performed to visualize effects on the microtubule assembly in the presence of MBIC. Mitotic kinases and mitochondrial-dependent apoptotic proteins were evaluated by Western blot analysis. In addition, the synergistic effect of MBIC with low doses of selected chemotherapeutic actions were examined against the cancer cells. RESULTS: Results from the present study showed that following treatment with MBIC, the HeLa cells went into mitotic arrest comprising of multi-nucleation and unsegregated chromosomes with a prolonged G2-M phase. In addition, the HeLa cells showed signs of mitochondrial-dependant apoptotic features such as the release of cytochrome c and activation of caspases. MBIC markedly interferes with tubulin polymerization. Western blotting results indicated that MBIC affects mitotic regulatory machinery by up-regulating BubR1, Cyclin B1, CDK1 and down-regulation of Aurora B. In addition, MBIC displayed synergistic effect when given in combination with colchicine, nocodazole, paclitaxel and doxorubicin. CONCLUSION: Taken together, our study demonstrated the distinctive microtubule destabilizing effects of MBIC against cervical cancer cells in vitro. Besides that, MBIC exhibited synergistic effects with low doses of selected anticancer drugs and thus, may potentially reduce the toxicity and drug resistance to these agents.

Hemaiswarya, S. and M. Doble (2013). "Combination of phenylpropanoids with 5-fluorouracil as anti-cancer agents against human cervical cancer (HeLa) cell line." Phytomedicine **20**(2): 151-158.

Combination therapy is the most effective treatment strategy in cancer to overcome drug toxicity and drug induced resistance. The effect of eight phenylpropanoids in combination with 5-fluorouracil against the cervical cancer cells (HeLa) is reported here. The cytotoxic activity of these phenylpropanoids against HeLa cells is in the order of eugenol>ferulic>cinnamic>caffeic>chlorogenic>p-coumaric>3,4-dimethoxycinnamic>2,4,5-trimethoxycinnamic acids. Eugenol, ferulic and caffeic acids interacted synergistically with the drug, in bringing about a reduction in the amount of the latter. Flow cytometry results indicated that the combination of eugenol and 5-fluorouracil increased the number of cells in the S and G2/M phases when compared to treatment with the individual compounds alone. This indicates that they possess different cell cycle targets and induce apoptosis in the cancer cells. In vitro hemolytic activity of phenylpropanoids on human erythrocytes showed that the compounds possessed minimum amount of hemolytic activity, indicating that they can be used as drugs without causing adverse toxicity. 3D-quantitative structure activity relationship studies indicate the importance of electrostatic region near the substitutions present in the benzene ring and near the double bond of the compounds for anticancer

and hemolytic activities, respectively. The models derived had good statistical predictive capability.

Hembram, D. S., et al. (2013). "An in-cell NMR study of monitoring stress-induced increase of cytosolic Ca2+ concentration in HeLa cells." Biochem Biophys Res Commun **438**(4): 653-659.

Recent developments in in-cell NMR techniques have allowed us to study proteins in detail inside living eukaryotic cells. The lifetime of in-cell NMR samples is however much shorter than that in culture media, presumably because of various stresses as well as the nutrient depletion in the anaerobic environment within the NMR tube. It is well known that Ca (2+)-bursts occur in HeLa cells under various stresses, hence the cytosolic Ca (2+) concentration can be regarded as a good indicator of the healthiness of cells in NMR tubes. In this study, aiming at monitoring the states of proteins resulting from the change of cytosolic Ca (2+) concentration during experiments, human calbindin D9k (P47M+C80) was used as the model protein and cultured HeLa cells as host cells. Time-resolved measurements of 2D (1)H-(15)N SOFAST-HMQC experiments of calbindin D9k (P47M+C80) in HeLa cells showed time-dependent changes in the cross-peak patterns in the spectra. Comparison with in vitro assignments revealed that calbindin D9k (P47M+C80) is initially in the Mg (2+)-bound state, and then gradually converted to the Ca (2+)-bound state. This conversion process initiates after NMR sample preparation. These results showed, for the first time, that cells inside the NMR tube were stressed, presumably because of cell precipitation, the lack of oxygen and nutrients, etc., thereby releasing Ca (2+) into cytosol during the measurements. The results demonstrated that in-cell NMR can monitor the state transitions of stimulated cells through the observation of proteins involved in the intracellular signalling systems. Our method provides a very useful tool for in situ monitoring of the "healthiness" of the cells in various in-cell NMR studies.

Henrich, B., et al. (2017). "Validation of a novel Mho microarray for a comprehensive characterisation of the Mycoplasma hominis action in HeLa cell infection." PLoS One **12**(7): e0181383.

Mycoplasma hominis is the second smallest facultative pathogen of the human urogenital tract. With less than 600 protein-encoding genes, it represents an ideal model organism for the study of host-pathogen interactions. For a comprehensive characterisation of the M. hominis action in infection a customized Mho microarray, which was based on two genome sequences (PG21 and LBD-4), was designed to analyze the dynamics of the mycoplasma transcriptome during infection and validated for M. hominis strain FBG. RNA preparation was evaluated and adapted to ensure the highest recovery of mycoplasmal mRNAs from in vitro HeLa cell infection assays. Following cRNA hybridization, the read-out strategy of the hybridization results was optimized and confirmed by RT-PCR. A statistically robust infection assay with M. hominis strain FBG enabled the identification of differentially regulated key effector molecules such as critical cytoadhesins (4 h post infection (pI)), invasins (48 h pI) and proteins associated with establishing chronic infection of the host (336 h pI). Of the 294 differentially regulated genes (>2-fold) 128 (43.5%) encoded hypothetical proteins, including lipoproteins that seem to play a central role as virulence factors at each stage of infection: P75 as a novel cytoadhesin candidate, which is also differentially upregulated in chronic infection; the MHO_2100 protein, a postulated invasin and the MHO_730-protein, a novel ecto-nuclease and domain of an ABC transporter, the function of which in chronic infection has still to be elucidated. Implementation of the M. hominis microarray strategy led to a comprehensive identification of to date unknown candidates for virulence factors at relevant stages of host cell infection.

Ho, K. K., et al. (2015). "Engineering artificial cells by combining HeLa-based cell-free expression and ultrathin double emulsion template." Methods Cell Biol **128**: 303-318.

Generation of artificial cells provides the bridge needed to cover the gap between studying the complexity of biological processes in whole cells and studying these same processes in an in vitro reconstituted system. Artificial cells are defined as the encapsulation of biologically active material in a biological or synthetic membrane. Here, we describe a robust and general method to produce artificial cells for the purpose of mimicking one or more behaviors of a cell. A microfluidic double emulsion system is used to encapsulate a mammalian cell-free expression system that is able to express membrane proteins into the bilayer or soluble proteins inside the vesicles. The development of a robust platform that allows the assembly of artificial cells is valuable in understanding subcellular functions and emergent behaviors in a more cell-like environment as well as for creating novel signaling pathways to achieve specific cellular behaviors.

Honda-Uezono, A., et al. (2012). "Unusual expression of red fluorescence at M phase induced by anti-microtubule agents in HeLa cells expressing the fluorescent ubiquitination-based cell cycle indicator (Fucci)." Biochem Biophys Res Commun **428**(2): 224-229.

Plinabulin (NPI-2358) is a novel microtubule-depolymerizing agent. In HeLa cells, plinabulin arrests the cell-cycle at M phase and subsequently induces mitotic catastrophe. To better understand the effects on this compound on the cell-cycle, we used the fluorescent ubiquitination-based cell cycle indicator (Fucci), which normally enables G1 and S/G2/M cells to emit red and green fluorescence, respectively. When HeLa-Fucci cells were treated with 50 nM plinabulin, cells began to fluoresce both green and red in an unusual pattern; most cells exhibited the new pattern after 24 h of treatment. X-irradiation efficiently induced G2 arrest in plinabulin-treated cells and significantly retarded the emergence of the unusual pattern, suggesting that entering M phase is essential for induction of the pattern. By simultaneously visualizing chromosomes with GFP-histone H2B, we established that the pattern emerges after nuclear envelope breakdown but before metaphase. Pedigree assay revealed a significant relationship between the unusual expression and mitotic catastrophe. Nocodazole, KPU-133 (a more potent derivative of plinabulin), and paclitaxel also exerted similar effects. From these data, we conclude that the unusual pattern may be associated with dysregulation of late M phase-specific E3 ligase activity and mitotic catastrophe following treatment with anti-microtubule agents.

Hong, E. S., et al. (2017). "Effect of Phosphodiesterase in Regulating the Activity of Lysosomes in the HeLa Cell Line." J Microbiol Biotechnol **27**(2): 372-379.

The transport of lysosomal enzymes into the lysosomes depends on the phosphorylation of their chains and the binding of the phosphorylated residues to mannose-6-phosphate receptors. The efficiency of separation depends more on the phosphodiesterases (PDEs) than on the activity of the phosphorylation of mannose residues and can be determined in vitro. PDEs play important roles in regulation of the activation of lysosomes. The expression of proteins was confirmed by western blotting. All PDE4 series protein expression was reduced in high concentrations of rolipram. As a result of observing the fluorescence intensity after rolipram treatment, the lysosomal enzyme was activated at low concentrations and suppressed at high concentrations. High concentrations of rolipram recovered the original function. Antimicrobial activity was not shown in either 10 or 100 micro concentrations of rolipram in treated HeLa cells in vitro. However, the higher anticancer activity at lower rolipram concentration was shown in lysosomal enzyme treated with 10 micro of rolipram. The anticancer activity was confirmed through cathepsin B and D assay. Tranfection allowed examination of the relationship between PDE4 and lysosomal activity in more detail. Protein expression was confirmed to be reduced. Fluorescence intensity showed decreased activity of lysosomes and ROS in cells transfected with the antisense sequences of PDE4 A, B, C, and D. PDE4A showed anticancer activity, whereas lysosome from cells transfected with the antisense sequences of PDE4 B, C, and D had decreased anticancer activity. These results showed the PDE4 A, B, C, and D are conjunctly related with lysosomal activity.

Hopfe, M., et al. (2013). "Host cell responses to persistent mycoplasmas--different stages in infection of HeLa cells with Mycoplasma hominis." PLoS One **8**(1): e54219.

Mycoplasma hominis is a facultative human pathogen primarily associated with bacterial vaginosis and pelvic inflammatory disease, but it is also able to spread to other sites, leading to arthritis or, in neonates, meningitis. With a minimal set of 537 annotated genes, M. hominis is the second smallest self-replicating mycoplasma and thus an ideal model organism for studying the effects of an infectious agent on its host more closely. M. hominis adherence, colonisation and invasion of HeLa cells were characterised in a time-course study using scanning electron microscopy, confocal microscopy and microarray-based analysis of the HeLa cell transcriptome. At 4 h post infection, cytoadherence of M. hominis to the HeLa cell surface was accompanied by differential regulation of 723 host genes (>2 fold change in expression). Genes associated with immune responses and signal transduction pathways were mainly affected and components involved in cell-cycle regulation, growth and death were highly upregulated. At 48 h post infection, when mycoplasma invasion started, 1588 host genes were differentially expressed and expression of genes for lysosome-specific proteins associated with bacterial lysis was detected. In a chronically infected HeLa cell line (2 weeks), the proportion of intracellular mycoplasmas reached a maximum of 10% and M. hominis-filled protrusions of the host cell membrane were seen by confocal microscopy, suggesting exocytotic dissemination. Of the 1972 regulated host genes, components of the ECM-receptor interaction pathway and phagosome-related integrins were markedly increased. The immune response was quite different to that at the beginning of infection, with a prominent induction of IL1B gene expression, affecting pathways of MAPK signalling, and genes connected with cytokine-cytokine interactions and apoptosis. These data show for the first time the complex, time-dependent reaction of the host directed at mycoplasmal clearance and the counter measures of this pestering pathogen.

Horbach, S. and W. Halffman (2017). "The ghosts of HeLa: How cell line misidentification contaminates the scientific literature." PLoS One **12**(10): e0186281.

While problems with cell line misidentification have been known for decades, an unknown number of published papers remains in circulation reporting on the wrong cells without warning or correction. Here we attempt to make a conservative estimate of this 'contaminated' literature. We found 32,755 articles reporting on research with misidentified cells, in turn cited by an estimated half a million other papers. The contamination of the literature is not decreasing over time and is anything but restricted to countries in the periphery of global science. The decades-old and often contentious attempts to stop misidentification of cell lines have proven to be insufficient. The contamination of the literature calls for a fair and reasonable notification system, warning users and readers to interpret these papers with appropriate care.

Horvat, T., et al. (2012). "Epigenetic modulation of the HeLa cell membrane N-glycome." Biochim Biophys Acta **1820**(9): 1412-1419.

BACKGROUND: Epigenetic changes play a role in all major events during tumorigenesis and changes in glycan structures are hallmarks of virtually every cancer. Also, proper N-glycosylation of membrane receptors is important in cell to cell and cell-environment communication. To study how modulation of epigenetic information can affect N-glycan expression we analyzed effects of epigenetic inhibitors on HeLa cell membrane N-glycome. METHODS: HeLa cells were treated with DNA methylation (zebularin and 5-aza-2-deoxycytidine) and histone deacetylation (trichostatin A and Na-butyrate) inhibitors. The effects on HeLa cell membrane N-glycome were analyzed by hydrophilic interaction high performance liquid chromatography (HILIC). RESULTS: Each of the four epigenetic inhibitors induced changes in the expression of HeLa cell membrane N-glycans that were seen either as an increase or a decrease of individual glycans in the total N-glycome. Compared to DNA methylation inhibitors, histone deacetylation inhibitors showed more moderate changes, probably due to their higher gene target selectivity. CONCLUSIONS: The results clearly show that composition of HeLa cell membrane N-glycome can be specifically altered by epigenetic inhibitors. GENERAL SIGNIFICANCE: Glycans on the cell membrane are essential elements of tumor cell's metastatic potential and are also an entry point for nearly all pathogenic microorganisms. Since epigenetic inhibitors used in this work are registered drugs, our results provide a new line of research in the application of these drugs as anticancer and antimicrobial agents. This article is part of a Special Issue entitled Glycoproteomics.

Hosseini, A., et al. (2017). "Kelussia odoratissima potentiates cytotoxic effects of radiation in HeLa cancer cell line." Avicenna J Phytomed **7**(2): 137-144.

OBJECTIVE: Cervical cancer is the second most common cause of death from cancer in women throughout the world. The aim of this study was to evaluate the cytotoxic activity of Kelussia odoratissima (K. odoratissima) extract associated with radiotherapy in cervical cancer cells (HeLa cell line). MATERIALS AND METHODS: Different concentration of the extract (25-500microg/ml) was tested in HeLa cell lines. Cell cytotoxicity of the extract and the effects of the extract on radiation (2Gy/min)-induced damages were assessed by MTT assay. Apoptosis was assessed using flow cytometric analysis. RESULT: K. odoratissima decreased cell viability in HeLa cell line in a concentration and time-dependent manner. When compared to the control, K. odoratissima induced a sub-G1 peak in the flow cytometry histogram of treated cells, indicating that apoptotic cell death is involved in K. odoratissima-induced toxicity. It was also shown that K. odoratissima sensitizes cells to radiation-induced toxicity. CONCLUSION: Our result showed the extract increased the radiation effect. This observation may be related to the presence of active compounds such as phthalides and ferulic acid.

Hussain, S. (2017). "Comparative efficacy of epigallocatechin-3-gallate against H2O2-induced ROS in cervical cancer biopsies and HeLa cell lines." Contemp Oncol (Pozn) **21**(3): 209-212.

Aim of the study: Antioxidants play an important role in maintaining physiological homeostasis. Recent literature emphasises the potential therapeutic effects of natural antioxidants that play anti-inflammatory and antioxidant effects applicable in preventing oxidative stress-induced injury, which characterises their pathogenesis. The goal of this study was to evaluate the protective role of EGCG on the HeLa cell line and cancerous cells. Material and methods: The HeLa cell line and cervical cancer biopsies (CCB) were treated with varying doses of antioxidants to determine their effects. Thereafter, hydrogen peroxide (0-10 nM) - an ROS-generating compound - was co-cultured with varying doses of epigallocatechin-3-gallate (EGCG). The effect of this compound on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity was assessed. Result: The activity of SOD and GPx was protected significantly in the treatment of EGCG in cervical cancer biopsies and HeLa cell line. Hypothesis: It is hypothesised that EGCG has free

radical scavenging properties. Conclusions: EGCG protected the activity SOD and GPx equally in cervical cancer biopsies (CCB) and HeLa cell line.

Ishida, S., et al. (2014). "Phospholipase C-beta1 and beta4 contribute to non-genetic cell-to-cell variability in histamine-induced calcium signals in HeLa cells." PLoS One **9**(1): e86410.

A uniform extracellular stimulus triggers cell-specific patterns of Ca (2+) signals, even in genetically identical cell populations. However, the underlying mechanism that generates the cell-to-cell variability remains unknown. We monitored cytosolic inositol 1,4,5-trisphosphate (IP3) concentration changes using a fluorescent IP3 sensor in single HeLa cells showing different patterns of histamine-induced Ca (2+) oscillations in terms of the time constant of Ca (2+) spike amplitude decay and the Ca (2+) oscillation frequency. HeLa cells stimulated with histamine exhibited a considerable variation in the temporal pattern of Ca (2+) signals and we found that there were cell-specific IP3 dynamics depending on the patterns of Ca (2+) signals. RT-PCR and western blot analyses showed that phospholipase C (PLC)-beta1, -beta3, -beta4, -gamma1, -delta3 and -epsilon were expressed at relatively high levels in HeLa cells. Small interfering RNA-mediated silencing of PLC isozymes revealed that PLC-beta1 and PLC-beta4 were specifically involved in the histamine-induced IP3 increases in HeLa cells. Modulation of IP3 dynamics by knockdown or overexpression of the isozymes PLC-beta1 and PLC-beta4 resulted in specific changes in the characteristics of Ca (2+) oscillations, such as the time constant of the temporal changes in the Ca (2+) spike amplitude and the Ca (2+) oscillation frequency, within the range of the cell-to-cell variability found in wild-type cell populations. These findings indicate that the heterogeneity in the process of IP3 production, rather than IP3-induced Ca (2+) release, can cause cell-to-cell variability in the patterns of Ca (2+) signals and that PLC-beta1 and PLC-beta4 contribute to generate cell-specific Ca (2+) signals evoked by G protein-coupled receptor stimulation.

Ishii, T., et al. (2010). "Proliferating cell nuclear antigen-dependent rapid recruitment of Cdt1 and CRL4Cdt2 at DNA-damaged sites after UV irradiation in HeLa cells." J Biol Chem **285**(53): 41993-42000.

The licensing factor Cdt1 is degraded by CRL4(Cdt2) ubiquitin ligase dependent on proliferating cell nuclear antigen (PCNA) during S phase and when DNA damage is induced in G (1) phase. Association of both Cdt2 and PCNA with chromatin was observed in S phase and after UV irradiation. Here we used a micropore UV irradiation assay to examine Cdt2 accumulation at cyclobutane pyrimidine dimer-containing DNA-damaged sites in the process of Cdt1 degradation in HeLa cells. Cdt2, present in the nucleus throughout the cell cycle, accumulated rapidly at damaged DNA sites during G (1) phase. The recruitment of Cdt2 is dependent on prior PCNA chromatin binding because Cdt2 association was prevented when PCNA was silenced. Cdt1 was also recruited to damaged sites soon after UV irradiation through its PIP-box. As Cdt1 was degraded, the Cdt2 signal at damaged sites was reduced, but PCNA, cyclobutane pyrimidine dimer, and XPA (xeroderma pigmentosum, complementation group A) signals remained at the same levels. These findings suggest that Cdt1 degradation following UV irradiation occurs rapidly at damaged sites due to PCNA chromatin loading and the recruitment of Cdt1 and CRL4(Cdt2), before DNA damage repair is completed.

Jafari, N., et al. (2017). "CRISPR-Cas9 Mediated NOX4 Knockout Inhibits Cell Proliferation and Invasion in HeLa Cells." PLoS One **12**(1): e0170327.

Increased expression of NOX4 protein is associated with cancer progression and metastasis but the role of NOX4 in cell proliferation and invasion is not fully understood. We generated NOX4 knockout HeLa cell lines using the CRISPR-Cas9 gene editing system to explore the cellular functions of NOX4. After transfection of CRISPR-Cas9 construct, we performed T7 endonuclease 1 assays and DNA sequencing to generate and identify insertion and deletion of the NOX4 locus. We confirmed the knockout of NOX4 by Western blotting. NOX4 knockout cell lines showed reduced cell proliferation with an increase of sub-G1 cell population and the decrease of S/G2/M population. Moreover, NOX4 deficiency resulted in a dramatic decrease in invadopodium formation and the invasive activity. In addition, NOX4 deficiency also caused a decrease in focal adhesions and cell migration in HeLa cells. These results suggest that NOX4 is required for both efficient proliferation and invasion of HeLa cells.

Jager, W., et al. (2013). "Hiding in plain view: genetic profiling reveals decades old cross contamination of bladder cancer cell line KU7 with HeLa." J Urol **190**(4): 1404-1409.

PURPOSE: KU7 is a popular urothelial carcinoma cell line that was isolated from the bladder of a patient at Keio University in 1980. It has subsequently been widely used in laboratories around the world. We describe how routine cell line authentication revealed that KU7 was cross contaminated almost 30 years ago with HeLa, a cervical carcinoma cell line. MATERIALS AND METHODS: Presumed KU7 clones dating from 1984

to 1999 were provided by M.D. Anderson Cancer Center, Vancouver Prostate Centre, Kyoto University, Tokyo Medical University and Keio University. HeLa was obtained from ATCC. Genomic DNA was isolated and short tandem repeat analysis was performed at the M.D. Anderson Cancer Center Characterized Cell Line Core Facility, Johns Hopkins University Fragment Analysis Facility and RIKEN BioResource Center, Ibaraki, Japan. Comparative genomic hybridization was performed on a platform (Agilent Technologies, Santa Clara, California) at Vancouver Prostate Centre. RESULTS: The short tandem repeat profile of all KU7 clones was an exact match with that of HeLa. Comparative genomic hybridization of all samples revealed an abundance of shared chromosomal aberrations. Slight differences in some genomic areas were explained by genomic drift in different KU7 clones separated by many years. CONCLUSIONS: Our analysis identified that cross contamination of KU7 with HeLa occurred before 1984 at the source institution. All KU7 clones in the urological literature should be considered HeLa and experimental results should be viewed in this light. Our results emphasize the need to authenticate cell lines in oncological research.

Jakubowicz-Gil, J., et al. (2012). "Cell death in HeLa cells upon imperatorin and cisplatin treatment." Folia Histochem Cytobiol **50**(3): 381-391.

There is growing evidence that commonly applied chemotherapy regimens can be improved by introducing new, specific, active and low side-effect drugs, or by combining substances to obtain the required clinical effect. The aim of the present study was to investigate the effects of imperatorin and cisplatin, applied separately or in combination, on apoptosis, necrosis and autophagy induction in the human cervical carcinoma cell line (HeLa). Imperatorin appeared to be a potent autophagy inducer, rather than a necrotic or apoptotic one. In contrast, cisplatin induced mainly apoptosis and necrosis after 6 h and 24 h, while longer incubation resulted only in necrosis induction. When HeLa cells were incubated with both drugs, autophagy appeared most frequently, although to a smaller extent than that observed after imperatorin administered alone. At the molecular level, autophagy was correlated with the presence of the cleaved form of microtubule-associated protein 1 light chain LC3 - LC3II. It was also accompanied by the inhibition of heat shock proteins Hsp27 and Hsp72 expression. Our results indicate that imperatorin alone, or in combination with cisplatin, is mainly an autopahgy inducer in HeLa cells.

Janaki Ramaiah, M., et al. (2017). "Scriptaid cause histone deacetylase inhibition and cell cycle arrest in HeLa cancer cells: A study on structural and functional aspects." Gene **627**: 379-386.

Scriptaid (SCR), a well-known histone deacetylase inhibitor, cause various cellular effects such as cell growth inhibition and apoptosis. In this study, we have evaluated the anti-cancer effects of Scriptaid in HeLa cells, IMR-32 and HepG2 cells. Scriptaid inhibited the growth of HeLa cells with IC50 of 2muM at 48h in a dose-dependent manner. Flow-cytometric analysis indicated that SCR induced apoptosis. Scriptaid was found to inhibit HDAC-8 effectively than other HDAC inhibitor such as TSA as observed by HDAC-8 assay, Western blotting and modelling study. This observation was further strengthened by an artificial neuronal network (ANN) model.

Janas, T., et al. (2012). "Human tRNA (Sec) associates with HeLa membranes, cell lipid liposomes, and synthetic lipid bilayers." RNA **18**(12): 2260-2268.

We have shown previously that simple RNA structures bind pure phospholipid liposomes. However, binding of bona fide cellular RNAs under physiological ionic conditions is shown here for the first time. Human tRNA (Sec) contains a hydrophobic anticodon-loop modification: N (6)-isopentenyladenosine (i (6)A) adjacent to its anticodon. Using a highly specific double-probe hybridization assay, we show mature human tRNA (Sec) specifically retained in HeLa intermediate-density membranes. Further, isolated human tRNA (Sec) rebinds to liposomes from isolated HeLa membrane lipids, to a much greater extent than an unmodified tRNA (Sec) transcript. To better define this affinity, experiments with pure lipids show that liposomes forming rafts or including positively charged sphingosine, or particularly both together, exhibit increased tRNA (Sec) binding. Thus tRNA (Sec) residence on membranes is determined by several factors, such as hydrophobic modification (likely isopentenylation of tRNA (Sec)), lipid structure (particularly lipid rafts), or sphingosine at a physiological concentration in rafted membranes. From prior work, RNA structure and ionic conditions also appear important. tRNA (Sec) dissociation from HeLa liposomes implies a mean membrane residence of 7.6 min at 24 degrees C (t (1/2) = 5.3 min). Clearly RNA with a 5-carbon hydrophobic modification binds HeLa membranes, probably favoring raft domains containing specific lipids, for times sufficient to alter biological fates.

Jani, B. and R. Fuchs (2012). "In vitro transcription and capping of Gaussia luciferase mRNA followed by HeLa cell transfection." J Vis Exp (61).

In vitro transcription is the synthesis of RNA transcripts by RNA polymerase from a linear DNA template containing the corresponding promoter sequence (T7, T3, SP6) and the gene to be transcribed (Figure 1A). A typical transcription reaction consists of the template DNA, RNA polymerase, ribonucleotide triphosphates, RNase inhibitor and buffer containing Mg (2+) ions. Large amounts of high quality RNA are often required for a variety of applications. Use of in vitro transcription has been reported for RNA structure and function studies such as splicing (1), RNAi experiments in mammalian cells (2), antisense RNA amplification by the "Eberwine method"(3), microarray analysis (4) and for RNA vaccine studies (5). The technique can also be used for producing radiolabeled and dye labeled probes (6). Warren, et al. recently reported reprogramming of human cells by transfection with in vitro transcribed capped RNA (7). The T7 High Yield RNA Synthesis Kit from New England Biolabs has been designed to synthesize up to 180 mug RNA per 20 mul reaction. RNA of length up to 10kb has been successfully transcribed using this kit. Linearized plasmid DNA, PCR products and synthetic DNA oligonucleotides can be used as templates for transcription as long as they have the T7 promoter sequence upstream of the gene to be transcribed. Addition of a 5' end cap structure to the RNA is an important process in eukaryotes. It is essential for RNA stability (8), efficient translation (9), nuclear transport (10) and splicing (11). The process involves addition of a 7-methylguanosine cap at the 5' triphosphate end of the RNA. RNA capping can be carried out post-transcriptionally using capping enzymes or co-transcriptionally using cap analogs. In the enzymatic method, the mRNA is capped using the Vaccinia virus capping enzyme (12,13). The enzyme adds on a 7-methylguanosine cap at the 5' end of the RNA using GTP and S-adenosyl methionine as donors (cap 0 structure). Both methods yield functionally active capped RNA suitable for transfection or other applications (14) such as generating viral genomic RNA for reverse-genetic systems (15) and crystallographic studies of cap binding proteins such as eIF4E (16). In the method described below, the T7 High Yield RNA Synthesis Kit from NEB is used to synthesize capped and uncapped RNA transcripts of Gaussia luciferase (GLuc) and Cypridina luciferase (CLuc). A portion of the uncapped GLuc RNA is capped using the Vaccinia Capping System (NEB). A linearized plasmid containing the GLuc or CLuc gene and T7 promoter is used as the template DNA. The transcribed RNA is transfected into HeLa cells and cell culture supernatants are assayed for luciferase activity. Capped CLuc RNA is used as the internal control to normalize GLuc expression.

Jantova, S., et al. (2016). "Pro-apoptotic effect of new quinolone 7- ethyl 9-ethyl-6-oxo-6,9-dihydro [1,2,5]selenadiazolo [3,4-h]quinoline-7-carboxylate on cervical cancer cell line HeLa alone/with UVA irradiation." Toxicol In Vitro **33**: 35-44.

7- ethyl 9-ethyl-6-oxo-6,9-dihydro [1,2,5]selenadiazolo [3,4-h]quinoline-7-carboxylate (E2h) is a new synthetically prepared quinolone derivative, which in our primary study showed cytotoxic effects towards tumor cells. The aim of the present study was to examine the antiproliferative and apoptosis inducing activities of E2h towards human cervical cancer cell line HeLa with/without the presence of UVA irradiation. Further, the molecular mechanism involved in E2h-induced apoptosis in HeLa cells was investigated. Our results showed that both non-photoactivated and photoactivated E2h caused morphological changes and inhibited the cell growth of HeLa cells in a time- and dose-dependent manner. Irradiation increased the sensitivity of HeLa cells to E2h. Quinolone induced S and G2/M arrest and apoptosis in HeLa cells, as characterized by DNA fragmentation and flow cytometry. In addition, E2h elevated the level of reactive oxygen species and activated caspases 3. In conclusions, E2h alone/in combination with UVA irradiation induced apoptosis in HeLa cells through the ROS-mitochondrial/caspase 3-dependent pathway.

Jovanovic, K. K., et al. (2016). "Cell cycle, apoptosis, cellular uptake and whole-transcriptome microarray gene expression analysis of HeLa cells treated with a ruthenium (II)-arene complex with an isoquinoline-3-carboxylic acid ligand." J Inorg Biochem **163**: 362-373.

Ruthenium (II)-arene complexes are promising drug candidates for the therapy of solid tumors. In previous work, seven new compounds of the general formula [Ru (eta (6)-p-cymene) (L (1-7))Cl] were synthesized and characterized, of which the complex with L=isoquinoline-3-carboxylic acid (RuT7) was two times as active on HeLa cells compared to normal cell line MRC-5, as indicated by IC50 values determined after 48h of incubation (45.4+/-3.0 vs. 84.2+/-5.7muM, respectively). In the present study, cell cycle analysis of HeLa cells treated with RuT7 showed S phase arrest and an increase in sub-G1 population. The apoptotic potential of the title compound was confirmed with the Annexin V-FITC/PI assay together with a morphological evaluation of cells using fluorescent microscopy. Analysis of the intracellular accumulation of ruthenium showed 8.9ng Ru/10(6) cells after 6h of

incubation. To gain further insight in the molecular mechanism of action of RuT7 on HeLa cells, a whole-transcriptome microarray gene expression analysis was performed. Analysis of functional categories and signaling and biochemical pathways associated with the response of HeLa cells to treatment with RuT7 showed that it leads the cells through the intrinsic (mitochondrial) apoptotic pathway, via indirect DNA damage due to the action of reactive oxygen species, and through direct DNA binding of RuT7. Statistical analysis for enrichment of gene sets associated with known drug-induced toxicities identified fewer associated toxicity profiles in RuT7-treated cells compared to cisplatin treatment. Altogether these results provide the basis for further development of RuT7 in animal and pre-clinical studies as a potential drug candidate.

Jung, J. H., et al. (2010). "Quercetin suppresses HeLa cell viability via AMPK-induced HSP70 and EGFR down-regulation." J Cell Physiol **223**(2): 408-414.

Quercetin, an anti-oxidant flavonoid that is widely distributed in the plant kingdom, has been suggested to have chemopreventive effects on cancer cells, although the mechanism is not completely understood. In this study, we found that quercetin increased the phosphorylation of AMP-activated protein kinase (AMPK) and downstream acetyl-CoA carboxylase (ACC) and suppressed the viability of HeLa cells. AICAR, an AMPK activator, and quercetin down-regulated heat shock protein (HSP)70 and increased the activity of the pro-apoptotic effector, caspase 3. Knock-down of AMPK blocked quercetin-mediated HSP70 down-regulation. Moreover, knock-down of HSP70 enhanced quercetin-mediated caspase 3 activation. Furthermore, quercetin sustained epidermal growth factor receptor (EGFR) activation by suppressing the phosphatases, PP2a and SHP-2. Finally, quercetin increased the interaction between EGFR and Cbl, and also induced the tyrosine phosphorylation of Cbl. Together, these results suggest that quercetin may have anti-tumor effects on HeLa cells via AMPK-induced HSP70 and down-regulation of EGFR.

Kaida, A. and M. Miura (2012). "Visualizing the effect of hypoxia on fluorescence kinetics in living HeLa cells using the fluorescent ubiquitination-based cell cycle indicator (Fucci)." Exp Cell Res **318**(3): 288-297.

Fluorescent proteins are widely used for the direct visualization of events such as gene expression and subcellular localization in mammalian cells. It is well established that oxygen is required for formation of functional chromophore; however, the effect of hypoxia on fluorescence emission has rarely been studied. For this purpose, under hypoxic conditions, we investigated the kinetics of red and green fluorescence in HeLa cells from two fluorescent proteins, monomeric Kusabira Orange 2 (mKO2) and monomeric Azami Green (mAG), respectively, using the fluorescent ubiquitination-based cell cycle indicator (Fucci). In this system, cells in G1 or other phases emit red or green fluorescence, respectively. We found that hypoxia abrogated both red and green fluorescence about ~10h after the treatment, although their protein levels were almost maintained. The treatment did not significantly affect fluorescence in cells constitutively expressing the same fluorescent proteins lacking the ubiquitin ligase-binding domains. The abrogation of fluorescence resulted from a combination of ubiquitination-dependent degradation of pre-existing functional proteins during specific cell cycle phases, and the expression of newly synthesized non-fluorescent proteins containing non-oxidized chromophore during hypoxic treatment. Indeed, non-fluorescent cells after hypoxic treatment gradually developed fluorescence after reoxygenation in the presence of cycloheximide; kinetics of recovery were much faster for mAG than for mKO2. Using the Fucci system, we could clearly visualize for the first time the effect of hypoxia on the fluorescence kinetics of proteins expressed in living mammalian cells.

Kaida, A. and M. Miura (2013). "Visualizing the effect of tumor microenvironments on radiation-induced cell kinetics in multicellular spheroids consisting of HeLa cells." Biochem Biophys Res Commun **439**(4): 453-458.

In this study, we visualized the effect of tumor microenvironments on radiation-induced tumor cell kinetics. For this purpose, we utilized a multicellular spheroid model, with a diameter of approximately 500 mum, consisting of HeLa cells expressing the fluorescent ubiquitination-based cell-cycle indicator (Fucci). In live spheroids, a confocal laser scanning microscope allowed us to clearly monitor cell kinetics at depths of up to 60 mum. Surprisingly, a remarkable prolongation of G2 arrest was observed in the outer region of the spheroid relative to monolayer-cultured cells. Scale, an aqueous reagent that renders tissues optically transparent, allowed visualization deeper inside spheroids. About 16 h after irradiation, a red fluorescent cell fraction, presumably a quiescent G0 cell fraction, became distinct from the outer fraction consisting of proliferating cells, most of which exhibited green fluorescence indicative of G2 arrest. Thereafter, the red cell fraction began to emit green fluorescence and remained in prolonged G2 arrest. Thus, for the first time, we visualized the prolongation of radiation-induced G2 arrest in spheroids and the

differences in cell kinetics between the outer and inner fractions.

Kaida, A., et al. (2011). "Fluorescence kinetics in HeLa cells after treatment with cell cycle arrest inducers visualized with Fucci (fluorescent ubiquitination-based cell cycle indicator)." Cell Biol Int **35**(4): 359-363.

Fucci (fluorescent ubiquitination-based cell cycle indicator) is able to visualize dynamics of cell cycle progression in live cells; G1- and S-/G2-/M-phase cells expressing Fucci emit red and green fluorescence, respectively. This system could be applied to cell kinetic analysis of tumour cells in the field of cancer therapy; however, it is still unclear how fluorescence kinetics change after various treatments, including exposure to anticancer agents. To explore this, we arrested live HeLa cells expressing the Fucci probes at various cell cycle stages and observed the fluorescence, in conjunction with flow cytometric analysis. X-irradiation, HU (hydroxyurea) and nocodazole arrest cells at G2/M boundary, early S-phase and early M-phase, respectively. Although X-irradiation and HU treatment induced similar accumulation kinetics of green fluorescent cells, nocodazole treatment induced an abnormal red fluorescence at M phase, followed by accumulation of both red and green fluorescent cells with 4N DNA content. We conclude that certain agents that disrupt normal cell cycle regulation could cause unexpected fluorescence kinetics in the Fucci system.

Kaji, T., et al. (2010). "ASK3, a novel member of the apoptosis signal-regulating kinase family, is essential for stress-induced cell death in HeLa cells." Biochem Biophys Res Commun **395**(2): 213-218.

Apoptosis signal-regulating kinase 1 (ASK1) and ASK2 are both members of mitogen-activated protein kinase kinase kinase (MAP3K) family that are implicated in apoptotic cell death, stress responses, and various diseases. We have determined that NT2RI3007443, TESTI4031745, SGK341, and human MAP3K15 are all transcribed from the same genomic locus, which we designate "ASK3 gene" based on sequence homology to ASK1 and ASK2. NT2RI3007443, TESTI4031745, and SGK341 displayed distinct expression profiles among human tissues. TESTI4031745 was expressed in relatively high levels. The expression of TESTI4031745 was increased in rectum tumor and Alzheimer's disease hippocampus and decreased in kidney tumor and Alzheimer's disease frontal lobe. NT2RI3007443 showed moderate levels of ubiquitous expression in normal adult tissues. They did not drastically change in diseases except for increase in cirrhosis liver. Expression of SGK341 was restricted. It was highly expressed in fetal brain, and moderately expressed in normal hippocampus, pancreas, spleen, lung, and kidney. Further, its expression was dramatically increased in hepatic cirrhosis and decreased in lung tumor. Target proteins encoded by NT2RI3007443 and TESTI4031745 were translated in cell-free protein synthesis system. They exhibited protein kinase activity indicated by ATP consumption and phosphorylation of Syntide 2 as a substrate. We demonstrated that knockdown of ASK3 protected HeLa cells against cytotoxicity induced by anti-Fas monoclonal antibody, TNF-alpha, or oxidative stress. These findings suggest that "ASK3 gene" is a novel member of apoptosis signal-regulating kinases and that it plays a pivotal role in the signal transduction pathway implicated in apoptotic cell death triggered by cellular stresses. It can be a putative therapeutic drug target for multiple human diseases.

Kamalipooya, S., et al. (2017). "Simultaneous application of cisplatin and static magnetic field enhances oxidative stress in HeLa cell line." In Vitro Cell Dev Biol Anim **53**(9): 783-790.

In this study, we reported the effects of simultaneous application of static magnetic field (SMF) and cisplatin as an anticancer drug on the oxidative stress in human cervical cancer (HeLa) cell line and normal skin fibroblast cells (Hu02). The cells were exposed to different SMF intensities (7, 10, and 15 mT) for 24 and 48 h. IC50 concentrations of cisplatin were obtained by MTT assay. The cytotoxic effects of combined treatment were studied by measuring the intracellular reactive oxygen species content using flow cytometric method and estimation of membrane lipid peroxidation by spectrophotometry. Statistical analysis was assessed using one-way repeated measures analysis of variance (ANOVA) followed by Tukey's test. Based on the obtained results, the highest and lowest death rate, respectively, in HeLa and Hu02 cell lines was observed at the intensity of 10 mT. Also, we found that membrane lipid peroxidation in cancer cells is higher than that of normal counterparts. SMF potently sensitized human cervical cancer cells to cisplatin through reactive oxygen species (ROS) accumulation while it had small effects on normal cells. The combination of both treatments for 48 h led to a marked decrease in the viability percentage of HeLa cells by about 89% compared to untreated cells. This study suggests that conjugation of both physical and chemical treatments could increase the oxidative stress in HeLa cell line and among three optional intensities of SMF, the intensity of 10 mT led to the higher damage to cancer cells in lower doses of drug.

Kaminaga, K., et al. (2015). "Visualisation of cell cycle modifications by X-ray irradiation of single HeLa cells using fluorescent ubiquitination-based cell cycle indicators." Radiat Prot Dosimetry **166**(1-4): 91-94.

To explore the effects of X-ray irradiation on mammalian cell cycle dynamics, single cells using the fluorescent ubiquitination-based cell cycle indicator (Fucci) technique were tracked. HeLa cells expressing Fucci were used to visualise cell cycle modifications induced by irradiation. After cultured HeLa-Fucci cells were exposed to 5 Gy X-rays, fluorescent cell images were captured every 20 min for 48 h using a fluorescent microscope. Time dependence of the fluorescence intensity of S/G2 cells was analysed to examine the cell cycle dynamics of irradiated and non-irradiated control cells. The results showed that irradiated cells could be divided into two populations: one with similar cell cycle dynamics to that of non-irradiated cells, and another displaying a prolonged G2 phase. Based on these findings, it is proposed in this article that an underlying switch mechanism is involved in cell cycle regulation and the G2/M checkpoint of HeLa cells.

Kandile, N. G., et al. (2012). "Antiproliferative effects of metal complexes of new isatin hydrazones against HCT116, MCF7 and HELA tumour cell lines." J Enzyme Inhib Med Chem **27**(3): 330-338.

New hydrazone ligands (HL) derived from 5-substituted isatins and 1-(4-(2-methoxybenzyl)-6-arylpyridazin-3-yl)hydrazines and its complexes with Co (II) and Cu (II) were synthesized. The new hydrazones and their complexes were characterized by means of elemental, spectral analyses and magnetic studies. Primary cytotoxicity evaluation of HL 5a and the new complexes showed that these complexes could act as anticancer agents since they reduced the growth of samples of human tumour cell lines (HCT116((Colon)), MCF7((Breast)) and HELA ((Cervix))) to </=18.5 mug/mL for the new complexes.

Kathirvel, P. and S. Ravi (2012). "Chemical composition of the essential oil from basil (Ocimum basilicum Linn.) and its in vitro cytotoxicity against HeLa and HEp-2 human cancer cell lines and NIH 3T3 mouse embryonic fibroblasts." Nat Prod Res **26**(12): 1112-1118.

This study examines the chemical composition and in vitro anticancer activity of the essential oil from Ocimum basilicum Linn. (Lamiaceae), cultivated in the Western Ghats of South India. The chemical compositions of basil fresh leaves were identified by GC-MS: 11 components were identified. The major constituents were found to be methyl cinnamate (70.1%), linalool (17.5%), beta-elemene (2.6%) and camphor (1.52%). The results revealed that this plant may belong to the methyl cinnamate and linalool chemotype. A methyl thiazol tetrazolium assay was used for in vitro cytotoxicity screening against the human cervical cancer cell line (HeLa), human laryngeal epithelial carcinoma cell line (HEp-2) and NIH 3T3 mouse embryonic fibroblasts. The IC (50) values obtained were 90.5 and 96.3 microg mL (-1), respectively, and the results revealed that basil oil has potent cytotoxicity.

Khazaei, S., et al. (2017). "Flower extract of Allium atroviolaceum triggered apoptosis, activated caspase-3 and down-regulated antiapoptotic Bcl-2 gene in HeLa cancer cell line." Biomed Pharmacother **89**: 1216-1226.

Cervical cancer accounts for the second most frequent cancer and also third leading cause of cancer mortality (15%) among women worldwide. The major problems of chemotherapeutic treatment in cervical cancer are non-specific cytotoxicity and drug resistance. Plant-derived products, known as natural therapies, have been used for thousands of years in cancer treatment with a very low number of side effects. Allium atroviolaceum is a species in the genus Allium and Liliaceae family, which could prove to have beneficial effects on cancer treatment, although there is a lack of corresponding attention. The methanolic extract from the A.atroviolaceum flower displayed marked anticancer activity on HeLa human cervix carcinoma cells with much lower cytotoxic effects on normal cells (3T3). The A.atroviolaceum extract induced apoptosis, confirmed by cell cycle arrest at the sub-G0 (apoptosis) phase, characteristic morphological changes, evident DNA fragmentation, observed by fluorescent microscope, and early and late apoptosis detection by Annexin V. Furthermore, down-regulation of Bcl-2 and activation of caspase-9 and -3 strongly indicated that the mitochondrial pathway was involved in the apoptosis signal pathway. Moreover, combination of A.atroviolaceum extract with doxorubicin revealed a significant reduction of IC50 and led to a synergistic effect. In summary, A.atroviolaceum displayed a significant anti-tumour effect through apoptosis induction in HeLa cells, suggesting that the A.atroviolaceum flower might have therapeutic potential against cervix carcinoma.

Khodarahmi, E., et al. (2015). "Cytotoxic evaluation of volatile oil from Descurainia sophia seeds on MCF-7 and HeLa cell lines." Res Pharm Sci **10**(2): 169-176.

Descurainia sophia is a plant widely distributed and used as folk medicine throughout the world. Different extracts of aerial parts and seeds of this plant

have been shown to inhibit the growth of different cancer cell lines in vitro. In this study, cytotoxic activity of D. sophia seed volatile oil was evaluated. D. sophia seed powder was mixed with distilled water and left at 25 degrees C for 17 h (E1), 23 h (E2) and 28 h (E3) to autolyse. Then, the volatile fractions of E1, E2, and E3 were collected after steam distillation for 3 h. Cytotoxic effects of the volatile oils alone or in combination with doxorubicin (mixture of E1 or E2 at 50 mug/ml or E1 at 100 mug/ml with doxorubicin at 0.1, 1, 10 muM) against MCF-7 cell line were determined using MTT assay. Cytotoxic effect of E1 volatile oil was also determined on HeLa cell line. The results indicated that 1-buten-4-isothiocyanate was the major isothiocyanate found in the volatile oils. The results of cytotoxic evaluations showed that volatile constituents were more toxic on MCF-7 cells with IC50< 100 mug/ml than HeLa cells with IC50> 100 mug/ml. No significant differences were observed between cytotoxic activities of E1, E2 and E3 on MCF-7 cell line. Concomitant use of E1 and E2 (50 mug/ml) with doxurubicin (1 muM) significantly reduced the viability of MCF-7 cells compared to the negative control, doxorubicin alone, or each volatile fraction. The same result was obtained on HeLa cells, when E1 (100 mug/ml) was concurrently used with doxorubicin (1 muM).

Khodarahmi, G. A., et al. (2011). "Cytotoxic Effects of Different Extracts and Latex of Ficus carica L. on HeLa cell Line." Iran J Pharm Res **10**(2): 273-277.

It has been reported that latex and extracts of different species of Ficus are cytotoxic to some human cancerous cell lines. In this study, cytotoxicity of fruit and leaf extracts as well as the latex of Ficuscarica L. on HeLa cell line were evaluated. ethanolic extracts of leaves and fruits were prepared through percolation and ethyl acetate and dichloromethane extracts were prepared by reflux method. Cytotoxic effects of these extracts and latex against HeLa cell line were then examined. Briefly, He Lacells were seeded at 2 x 10(4) cells/mL in 96-well plates. After 24 h incubation at 37( degrees )C, the cells were treated with different concentrations of the extracts or latex. The viability of the cells was determined by the reduction of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) from formazan following 48 h incubation and the absorbance was measured at 540 nm using an ELISA plate reader. The results indicated that the latex and different extracts of Ficus carica could reduce the viability of the He Lacells at concentrations as low as 2 microg/mL in a dose dependent manner. The approximate IC50 values of the ethanolic, ethyl acetate and dichloromethane extracts of the leaves and fruits were 10, 19, 12 microg/mL and 12, 12, 11.5 microg/mL, respectively. The IC50 for the latex was about 17 microg/mL.

Khodarahmi, G. A., et al. (2012). "Synthesis and cytotoxic evaluation of some new 4(3H)-quinazolinones on HeLa cell line." Res Pharm Sci **7**(2): 119-125.

Quinazolinone backbone is present in a large number of bioactive substances. Since remarkable cytotoxic activity is associated with some 4(3H)-quinazolinones, in this study some 4(3H)-quinazolinone were synthesized and screened against HeLa cells. The synthesis was performed via reaction of anthranilic acid with dicarboxylic anhydrides to produce carboxylic acids derivatives. The products were heated in acetic anhydride to produce benzoxazinones. Finally, 4(3H)-quinazolinones were synthesized by reaction between benzoxazinones and primary amines. The assessment of the structure of the synthesized compounds was based on spectral data (FT-IR, Mass and (1)HNMR). Subsequently, cytotoxic activity of compounds 3, 6, 9 and 13 (individually and in combination with doxorubicin) was evaluated on HeLa cell line using MTT assay. The results indicated that the tested compounds did not show significant cytotoxicity alone and in combination with doxorubicin (1 and 20 muM).

Khoshgard, K., et al. (2017). "Radiation dose rate affects the radiosensitization of MCF-7 and HeLa cell lines to X-rays induced by dextran-coated iron oxide nanoparticles." Int J Radiat Biol **93**(8): 757-763.

BACKGROUND AND PURPOSE: The aim of radiotherapy is to deliver lethal damage to cancerous tissue while preserving adjacent normal tissues. Radiation absorbed dose of the tumoral cells can increase when high atomic nanoparticles are present in them during irradiation. Also, the dose rate is an important aspect in radiation effects that determines the biological results of a given dose. This in vitro study investigated the dose-rate effect on the induced radiosensitivity by dextran-coated iron oxide in cancer cells. MATERIALS AND METHODS: HeLa and MCF-7 cells were cultured in vitro and incubated with different concentrations of dextran-coated iron oxide nanoparticles. They were then irradiated with 6 MV photons at dose rates of 43, 185 and 370 cGy/min. The MTT test was used to obtain the cells' survival after 48 h of irradiations. RESULTS: Incubating the cells with the nanoparticles at concentrations of 10, 40 and 80 mug/ml showed no significant cytotoxicity effect. Dextran-coated iron oxide nanoparticles showed more radiosensitivity effect by increasing the dose rate and nanoparticles concentration. Radiosensitization enhancement factors of MCF-7 and HeLa cells at a dose-rate of 370 cGy/min and nanoparticles'

concentration of 80 mug/ml were 1.21 +/- 0.06 and 1.19 +/- 0.04, respectively. CONCLUSION: Increasing the dose rate of 6 MV photons irradiation in MCF-7 and HeLa cells increases the radiosensitization induced by the dextran-coated iron nanoparticles in these cells.

Kitai, Y., et al. (2015). "New Sesquiterpene Lactone Dimer, Uvedafolin, Extracted from Eight Yacon Leaf Varieties (Smallanthus sonchifolius): Cytotoxicity in HeLa, HL-60, and Murine B16-F10 Melanoma Cell Lines." J Agric Food Chem **63**(50): 10856-10861.

Uvedafolin, 1, a new sesquiterpene lactone dimer, was isolated from the leaves of Smallanthus sonchifolius with five related compounds, 2-6, and their cytotoxicity was assessed against three tumor cell lines (HeLa, HL-60, B16-F10 melanoma). The stereostructure of 1 was newly elucidated by ESI-TOF-MS, 1D/2D NMR, and single-crystal X-ray diffraction. Dimers 1 and 2 had the most effective IC50 values, 0.2-1.9 muM, against the three tumor cell lines when compared with monomers 3-6 (IC50 values 0.7-9.9 muM) and etoposide (IC50 values 0.8-114 muM). The ester linkages of two sets of monomers, uvedalin, 5, and sonchifolin, 6, for 1, and enhydrin, 4, and sonchifolin, 6, for 2, as well as the acetyl group at the C-9 position, were essential for the high cytotoxicity. Dimers 1 and 2 would have potential as anticancer agents.

Kniss, D. A. and T. L. Summerfield (2014). "Discovery of HeLa Cell Contamination in HES Cells: Call for Cell Line Authentication in Reproductive Biology Research." Reprod Sci **21**(8): 1015-1019.

Continuous cell lines are used frequently in reproductive biology research to study problems in early pregnancy events and parturition. It has been recognized for 50 years that many mammalian cell lines contain inter- or intraspecies contaminations with other cells. However, most investigators do not routinely test their culture systems for cross-contamination. The most frequent contributor to cross-contamination of cell lines is the HeLa cell isolated from an aggressive cervical adenocarcinoma. We report on the discovery of HeLa cell contamination of the human endometrial epithelial cell line HES isolated in our laboratory. Short tandem repeat analysis of 9 unique genetic loci demonstrated molecular identity between HES and HeLa cells. In addition, we verified that WISH cells, isolated originally from human amnion epithelium, were also contaminated with HeLa cells. Inasmuch as our laboratory did not culture HeLa cells at the time of HES cell derivations, the source of contamination was the WISH cell line. These data highlight the need for continued diligence in authenticating cell lines used in reproductive biology research.

Kobayashi, D., et al. (2017). "Cesium reversibly suppresses HeLa cell proliferation by inhibiting cellular metabolism." FEBS Lett **591**(5): 718-727.

The aim of the present study was to investigate the influence of Cs (+) on cultured human cells. We find that HeLa cell growth is suppressed by the addition of 10 mm CsCl into the culture media. In the Cs (+) -treated cells, the intracellular Cs (+) and K (+) concentrations are increased and decreased, respectively. This leads to a decrease in activity of the glycolytic enzyme pyruvate kinase, which uses K (+) as a cofactor. Cs (+) -treated cells show an intracellular pH shift towards alkalization. Based on these results, CsCl presumably suppresses HeLa cell proliferation by inducing an intracellular cation imbalance that affects cell metabolism. Our findings may have implications for the use of Cs (+) in cancer therapy.

Kolar, P., et al. (2013). "The effect of photodynamic treatment on the morphological and mechanical properties of the HeLa cell line." Gen Physiol Biophys **32**(3): 337-346.

High resolution imaging of biological structures and changes induced by various agents such as drugs and toxins is commonly performed by fluorescence and electron microscopy (EM). Although high-resolution imaging is possible with EM, the requirements for fixation and staining of samples for image contrast severely limits the study of living organisms. Atomic force microscopy (AFM), on the other hand, is capable of simultaneous nanometer spatial resolution and piconewton force detection, allowing detailed study of cell surface morphology and monitoring cytomechanical information. We present a method that images and studies mechanically characterized cells using AFM. We used a HeLa cell line (cervix carcinoma cell), which is sensitive to photodynamic treatment (PDT); growth media as a scanning surrounding; atomic force microscopy NT-MDT Aura for cytomechanical measurement; and scanning electron microscope Hitachi Su 6600 for control images of the cells. The modulus of elasticity for intact and photodynamically damaged cells can indicate mechanical changes to the main properties of cells. Cell elasticity changes can provide information on the degree or value of cell damage, for example after PDT. Measurements were carried out on approximately sixty cells, including three independent experiments on a control group and on sixty cells in a photodamaged group. Cells before PDT show higher elasticity: the median of Young s modulus on the nucleus was 35.283 kPa and outside of the nucleus

107.442 kPa. After PDT, the median of Young's modulus on the nucleus was 61.144 kPa and outside of the nucleus was 193.605 kPa.

Koley, D. and A. J. Bard (2010). "Triton X-100 concentration effects on membrane permeability of a single HeLa cell by scanning electrochemical microscopy (SECM)." Proc Natl Acad Sci U S A **107**(39): 16783-16787.

Changes in HeLa cell morphology, membrane permeability, and viability caused by the presence of Triton X-100 (TX100), a nonionic surfactant, were studied by scanning electrochemical microscopy (SECM). No change in membrane permeability was found at concentrations of 0.15 mM or lower during an experimental period of 30 to 60 min. Permeability of the cell membrane to the otherwise impermeable, highly charged hydrophilic molecule ferrocyanide was seen starting at concentrations of TX100 of about 0.17 mM. This concentration level of TX100 did not affect cell viability. Based on a simulation model, the membrane permeability for ferrocyanide molecules passing though the live cell membrane was 6.5 +/- 2.0 x 10(-6) m/s. Cells underwent irreversible permeabilization of the membrane and structural collapse when the TX100 concentration reached the critical micelle concentration (CMC), in the range of 0.19 to 0.20 mM. The impermeability of ferrocyanide molecules in the absence of surfactant was also used to determine the height and diameter of a single living cell with the aid of the approach curve and probe scan methods in SECM.

Kong, W., et al. (2014). "Quantitative and real-time effects of carbon quantum dots on single living HeLa cell membrane permeability." Nanoscale **6**(10): 5116-5120.

The interaction between carbon quantum dots (CQDs) and a single living cell was explored in real time. Here, we provide the quantitative data on the permeability of the HeLa cell membrane in the presence of CQDs with different surface functional groups (CQDs terminated with -OH/-COOH (CQD-OH), -PEG (CQD-PEG), and -NH2 (CQD-NH2)). Although these CQDs have very low toxicity towards HeLa cells, they still increase the cell membrane permeability by 8%, 13%, and 19% for CQD-PEG, CQD-OH, and CQD-NH2, respectively, and this kind of permeability was irreversible. These observations are valuable for promoting the bio-applications of carbon nanostructures in living systems.

Kopp, M., et al. (2017). "Delivery of the autofluorescent protein R-phycoerythrin by calcium phosphate nanoparticles into four different eukaryotic cell lines (HeLa, HEK293T, MG-63, MC3T3): Highly efficient, but leading to endolysosomal proteolysis in HeLa and MC3T3 cells." PLoS One **12**(6): e0178260.

Nanoparticles can be used as carriers to transport biomolecules like proteins and synthetic molecules across the cell membrane because many molecules are not able to cross the cell membrane on their own. The uptake of nanoparticles together with their cargo typically occurs via endocytosis, raising concerns about the possible degradation of the cargo in the endolysosomal system. As the tracking of a dye-labelled protein during cellular uptake and processing is not indicative of the presence of the protein itself but only for the fluorescent label, a label-free tracking was performed with the red-fluorescing model protein R-phycoerythrin (R-PE). Four different eukaryotic cell lines were investigated: HeLa, HEK293T, MG-63, and MC3T3. Alone, the protein was not taken up by any cell line; only with the help of calcium phosphate nanoparticles, an efficient uptake occurred. After the uptake into HeLa cells, the protein was found in early endosomes (shown by the marker EEA1) and lysosomes (shown by the marker Lamp1). There, it was still intact and functional (i.e. properly folded) as its red fluorescence was detected. However, a few hours after the uptake, proteolysis started as indicated by the decreasing red fluorescence intensity in the case of HeLa and MC3T3 cells. 12 h after the uptake, the protein was almost completely degraded in HeLa cells and MC3T3 cells. In HEK293T cells and MG-63 cells, no degradation of the protein was observed. In the presence of Bafilomycin A1, an inhibitor of acidification and protein degradation in lysosomes, the fluorescence of R-PE remained intact over the whole observation period in the four cell lines. These results indicate that despite an efficient nanoparticle-mediated uptake of proteins by cells, a rapid endolysosomal degradation may prevent the desired (e.g. therapeutic) effect of a protein inside a cell.

Krajewski, W. A. (2014). "Isw1a does not have strict limitations on the length of extranucleosomal DNAs for mobilization of nucleosomes assembled with HeLa cell histones." J Biomol Struct Dyn **32**(4): 523-531.

The Saccharomyces cerevisiae Isw1a and Isw2 ATP-dependent chromatin-remodeling complexes have important roles in vivo in the regulation of nucleosome positioning and modulation of gene activity. We studied the ability of the Isw1a- and Isw2-remodeling enzymes to reposition nucleosomes in mono- and dinucleosomes templates with variably positioned histone octamers (in the center or at the ends of the DNA fragment). To compare the Isw1a and Isw2 nucleosome-mobilizing activities, we utilized mono- and dinucleosome templates reconstituted with purified HeLa cell histones and

DNA containing one or two copies of the "601" nucleosome high-affinity sequence used to specifically position nucleosomes on the DNA. The obtained data suggest that Isw1a is able to mobilize HeLa cell histone-assembled mononucleosomes with long (more than 30 bp) extranucleosomal DNAs protruding from both sides, which contrasts to the previously reported inability of Isw1 to mobilize similar nucleosomes assembled with recombinant yeast histones. The results also suggest that Isw1a and Isw2 can mobilize nucleosomes with unfavorably short linker DNA lengths, and the presence of internucleosomal interactions promotes mobilization of nucleosomes even when the linkers are short.

Kuang, W., et al. (2013). "[Inhibition of Jumi extraction on growth of human cervical cancer cell line HeLa]." Zhongguo Ying Yong Sheng Li Xue Za Zhi **29**(3): 275-279.

OBJECTIVE: To explore the inhibition of Jumi (traditional Chinese medicine) extraction on the growth of human cervical cancer cell line HeLa. METHODS: Nude mouse model of human cervical cancer HeLa cell transplantation was established. The nude mice bearing cancer were randomly divided into control group and Jumi treated groups with different concentration (0.001, 0.002, 0.005, 0.01 mg/ml). The growth of cervical cancer cell in experimental mice were measured. Cultured HeLa cells were incubated in culture media with or without Jumi extract for 48 hours. Cell proliferation rate, cell apoptosis, caspase-3/7 and caspase-6 activity were determined by MTT colorimetric assay, flow cytometry analysis and spectrophotometric detection, respectively. RESULTS: With the increase of the concentration of Jumi extract, tumor-bearing mice tumor inhibition rate gradually increased. The proliferation of cultured HeLa cells were significantly inhibited by Jumi extract in a dose-dependent manner. IC50 was 0.004 mg/ml. Apoptosis rates in the cells treated with Jumi extract were higher than those of the control group. Compared with the control group, except for lower Jumi treated group (0.001 mg/ml), caspase-3/7 and caspase-6 activity were significantly increased in the all Jumi treated groups. CONCLUSION: Jumi extract can inhibit the proliferation of human cervical cancer cell line HeLa in vitro in a dose-dependent manner and promote cell apoptosis through caspase-3, caspase-7 and caspase-6 pathway.

Kumar, R. and A. B. Tiku (2018). "Galangin induces cell death by modulating the expression of glyoxalase-1 and Nrf-2 in HeLa cells." Chem Biol Interact **279**: 1-9.

The present study was designed to understand the anticancer property and molecular mechanisms associated with chemo preventive effects of galangin. The anticancer effect was evaluated in vitro using human cervical cancer cell line (HeLa). Galangin was found to be effective in inducing cell death and inhibiting proliferation & migration significantly. The inhibitory effect of galangin could be correlated with the increase in ROS production & induction of apoptosis. Besides this the activity of glyoxalase-1, an enzyme important for the detoxification of cytotoxic metabolite methy glyoxal and Nrf-2 (a trascription factor), involved in redox signalling were found to be decreased. We concluded that galangin exerts its chemo preventive effect via redox signalling by inhibiting glyoxalase-1 & increasing oxidative & carbonyl stress.

Kumari, B., et al. (2016). "Structurally Characterized Zn2+ Selective Ratiometric Fluorescence Probe in 100 % Water for HeLa Cell Imaging: Experimental and Computational Studies." J Fluoresc **26**(1): 87-103.

Fluorescence recognition of Zn2+ in 100% aqueous medium using 2-((1, 3 dihydroxy-2-(hydroxymethyl)propan-2 ylimino) methyl) phenol (SALTM) as ratiometric probe is reported. Moreover, SALTM can discriminate Zn2+ from Cd2+very effectively. The binding constant and detection limit of the probe for Zn2+ is 2.2x10(4) M (-1/2) and 2.79x10(-8) M respectively. Interestingly, corresponding naphthalene derivative (HNTM) having less water solubility fails to be a ratiometric sensor. SALTM can detect intracellular Zn2+ in HeLa cervical cancer cells under fluorescence microscope. Moreover, DFT and TD-DFT studies support experimental findings.

Kuo, J. H. and C. W. Lin (2013). "Cellular uptake on N- and C-termini conjugated FITC of Rath cell penetrating peptides and its consequences for gene-expression profiling in U-937 human macrophages and HeLa cervical cancer cells." J Drug Target **21**(9): 801-808.

Rath peptide has been introduced as a delivery vector that transports various membrane-impermeable cargoes in a non-covalent fashion. In this paper, we present a study on Rath peptide conjugated with fluorescein-5-isothiocynate (FITC) differing in its N- and C-termini. We conducted cellular toxicity and uptake experiments in U-937 and HeLa cells to analyze biocompatibility profiles and translocation efficiencies of Rath peptide with FITC serving as both a cargo and a fluorescent marker. We found that the conjugation of FITC on Rath peptide at N-terminus (FITC-Rath) led to more rapid cellular uptake in U-937 cells and significantly higher cellular uptake in HeLa cells than that which occurred at C-terminus.

From DNA microarray analysis, FITC-Rath induced gene expression changes in both U-937 and HeLa cells. Five overlapping regulated genes were identified, and this overlap indicated that FITC-Rath displayed some degree of generality regarding gene responses in the two cell lines used. A real-time quantitative reverse transcriptase-polymerase chain reaction was used to confirm which regulated genes were affected by FITC-Rath. Cell communication, signal transduction, cell surface receptor signaling pathway, signal transducer activity and cellular process, were identified as overlapping biological themes. These data provide useful information on molecular mechanisms for using Rath-based delivery systems.

Kwon, H. R., et al. (2010). "Requirement of T-lymphokine-activated killer cell-originated protein kinase for TRAIL resistance of human HeLa cervical cancer cells." Biochem Biophys Res Commun **391**(1): 830-834.

T-lymphokine-activated killer cell-originated protein kinase (TOPK) appears to be highly expressed in various cancer cells and to play an important role in maintaining proliferation of cancer cells. However, the underlying mechanism by which TOPK regulates growth of cancer cells remains elusive. Here we report that upregulated endogenous TOPK augments resistance of cancer cells to apoptosis induced by tumor necrosis factor-related apoptosis inducing ligand (TRAIL). Stable knocking down of TOPK markedly increased TRAIL-mediated apoptosis of human HeLa cervical cancer cells, as compared with control cells. Caspase 8 or caspase 3 activities in response to TRAIL were greatly incremented in TOPK-depleted cells. Ablation of TOPK negatively regulated TRAIL-mediated NF-kappaB activity. Furthermore, expression of NF-kappaB-dependent genes, FLICE-inhibitory protein (FLIP), inhibitor of apoptosis protein 1 (c-IAP1), or X-linked inhibitor of apoptosis protein (XIAP) was reduced in TOPK-depleted cells. Collectively, these findings demonstrated that TOPK contributed to TRAIL resistance of cancer cells via NF-kappaB activity, suggesting that TOPK might be a potential molecular target for successful cancer therapy using TRAIL.

Lad, N. P., et al. (2017). "Piperlongumine derived cyclic sulfonamides (sultams): Synthesis and in vitro exploration for therapeutic potential against HeLa cancer cell lines." Eur J Med Chem **126**: 870-878.

A novel modification of piperlongumine is designed, bearing a cyclic sulphonamide (sultam) and its synthesis is described. For the first time herein we report the synthesis and biological evaluation of the natural product derived cyclic sulfonamides using Grubbs second generation catalyst (Grubbs II) via ring closing metathesis approach. Synthesis of a series of piperlongumine derived sultams is done in a moderate to good yield using Wittig reaction, Ring-Closing Metathesis (RCM) and, amide synthesis by using mixed anhydride, approach. All synthesized compounds were evaluated for anticancer activity and some demonstrated dose dependent reduction in HeLa cell growth. Of these 7, 10 and 14 significantly reduced the cell growth. Consequently their calculated GI50 values were found to be 0.1 or <0.1 muM.

Landry, J. J., et al. (2013). "The genomic and transcriptomic landscape of a HeLa cell line." G3 (Bethesda) **3**(8): 1213-1224.

HeLa is the most widely used model cell line for studying human cellular and molecular biology. To date, no genomic reference for this cell line has been released, and experiments have relied on the human reference genome. Effective design and interpretation of molecular genetic studies performed using HeLa cells require accurate genomic information. Here we present a detailed genomic and transcriptomic characterization of a HeLa cell line. We performed DNA and RNA sequencing of a HeLa Kyoto cell line and analyzed its mutational portfolio and gene expression profile. Segmentation of the genome according to copy number revealed a remarkably high level of aneuploidy and numerous large structural variants at unprecedented resolution. Some of the extensive genomic rearrangements are indicative of catastrophic chromosome shattering, known as chromothripsis. Our analysis of the HeLa gene expression profile revealed that several pathways, including cell cycle and DNA repair, exhibit significantly different expression patterns from those in normal human tissues. Our results provide the first detailed account of genomic variants in the HeLa genome, yielding insight into their impact on gene expression and cellular function as well as their origins. This study underscores the importance of accounting for the strikingly aberrant characteristics of HeLa cells when designing and interpreting experiments, and has implications for the use of HeLa as a model of human biology.

Lane, K. R., et al. (2013). "Cell cycle-regulated protein abundance changes in synchronously proliferating HeLa cells include regulation of pre-mRNA splicing proteins." PLoS One **8**(3): e58456.

Cell proliferation involves dramatic changes in DNA metabolism and cell division, and control of DNA replication, mitosis, and cytokinesis have received the greatest attention in the cell cycle field. To catalogue a wider range of cell cycle-regulated

processes, we employed quantitative proteomics of synchronized HeLa cells. We quantified changes in protein abundance as cells actively progress from G1 to S phase and from S to G2 phase. We also describe a cohort of proteins whose abundance changes in response to pharmacological inhibition of the proteasome. Our analysis reveals not only the expected changes in proteins required for DNA replication and mitosis but also cell cycle-associated changes in proteins required for biological processes not known to be cell-cycle regulated. For example, many pre-mRNA alternative splicing proteins are down-regulated in S phase. Comparison of this dataset to several other proteomic datasets sheds light on global mechanisms of cell cycle phase transitions and underscores the importance of both phosphorylation and ubiquitination in cell cycle changes.

Le Bihan, T., et al. (2010). "Quantitative analysis of low-abundance peptides in HeLa cell cytoplasm by targeted liquid chromatography/mass spectrometry and stable isotope dilution: emphasising the distinction between peptide detection and peptide identification." Rapid Commun Mass Spectrom **24**(7): 1093-1104.

We present the application of a targeted liquid chromatography/mass spectrometry (LC/MS) approach developed on a linear ion trap for the evaluation of the abundance of cytoplasmic proteins from a HeLa cell extract. Using a standard data-dependent approach, we identified some specific peptides from this extract which were also commercially available in their AQUA form (use for absolute quantitation). For some of the peptides, we observed a non-linear response between the intensity and the added quantity which was then fitted using a quadratic fit. All AQUA peptides spiked into a mix of 3 microg of the HeLa cell digest extract were detected down to 16 fmol. We placed an emphasis on peptide detection which, in this study, is performed using a combination of properties such as three specific Q3-like ion signatures (for a given Q1-like selection) and co-elution with the AQUA peptide counterparts. Detecting a peptide without necessarily identifying it using a search engine imposes less constraint in terms of tandem mass (MS/MS) spectra purity. An example is shown where a peptide is detected using those criteria but could not be identified by Mascot due to its lower abundance. To complement this observation, we used a cross-correlation analysis approach in order to separate two populations of MS/MS fragments based on differences in their elution patterns. Such an approach opens the door to new strategies to analyse lower intensity peptide fragments. An in silico analysis of the human trypsinosome allows the evaluation of how unique are the sets of features that we are using for peptide detection.

Lee, J. W., et al. (2014). "HeLa human cervical cancer cell migration is inhibited by treatment with dibutyryl-cAMP." Anticancer Res **34**(7): 3447-3455.

Cyclic AMP (cAMP) activates both protein kinase A (PKA) and guanine-nucleotide exchange factor exchange protein directly activated by CAMP (EPAC)-mediated Ras-related Protein1 (RAP1) GTPase that regulates various cellular functions including cell migration. Herein, we investigated whether cAMP-mediated PKA and EPAC1/RAP1 pathways differentially control HeLa cervical cancer cell migration. Although HeLa cell migration was reduced by dibutyryl-cAMP, we observed an increase in cAMP/PKA, cAMP/EPAC1/RAP1-GTPase, and RAC1-GTPase. HeLa cell migration and RAC1-GTPase were increased by treatment with 8-(4-chloro-phenylthio)-2'-O-methyladenosine-3',5'-cAMP analogue to activate EPAC-specific signaling pathways. When HeLa cells were treated with H-89, a PKA inhibitor, cell migration was enhanced but RAC1-GTPase was inhibited. In addition, cell migration induced by dibutyryl-cAMP was reversed but the activity of Rac1-GTPase was inhibited by H-89 treatment. Taken together, these data demonstrate that cAMP/PKA and cAMP/EPAC1/RAP1-GTPase might inversely control cervical cancer cell migration, although both signaling pathways may up-regulate RAC1-GTPase. It also suggests that cAMP-mediated cancer cell migration was independent of RAC1-GTPase activation.

Liu, L., et al. (2012). "[Differential expression of miR-21, miR-126, miR-143, miR-373 in normal cervical tissue, cervical cancer tissue and Hela cell]." Sichuan Da Xue Xue Bao Yi Xue Ban **43**(4): 536-539.

OBJECTIVE: To investigate the differential expression of miR-21, miR-126, miR-143 and miR-373 in normal cervical tissue, cervical cancer tissue and Hela cell. METHODS: The expressions of miR-21, miR-126, miR-143 and miR-373 were detected by real-time PCR in cervical cancer tissue, cervical tissue of benign uterine tumor and Hela cell. RESULTS: High expression of miR-21 was observed in cervical cancer and Hela cell, while low expression was observed in normal cervical tissue. The relative quantification of miR-21 in cerveical cancer was 11.3196 times that of miR-21 in normal cervical tissue ($P < 0.05$). The expression levels of miR-143 and miR-373 in cervical cancer and Hela cell were lower than those of normal cervical tissue. The relative quantification of miR-143 in cerveical cancer was 0.1553 times that of normal cervical tissue ($P < 0.05$), and the relative quantification of miR-373 in cerveical cancer was 0.4907 times that of normal cervical tissue ($P < 0.05$). The expression of miR-126 had no

significant difference among cervical cancer tissue, Hela cell and normal cervical tissue ($P > 0.05$). CONCLUSION: miRNAs are closely related to the occurrence and regulation of cervical cancer. The high expression of miR-21 in cervical cancer and Hela cell indicate that it may play a possible role of oncogenes, while miR-143 and miR-373 with low expression may play the role of tumor suppressor genes.

Luo, L., et al. (2017). "Biosynthesis of reduced graphene oxide and its in-vitro cytotoxicity against cervical cancer (HeLa) cell lines." Mater Sci Eng C Mater Biol Appl **78**: 198-202.

The present work proposed a simple, one pot, and green approach for the deoxygenation of graphene oxide (GO) using pyrogallol as reducing and stabilizing agent. This synthetic strategy prevents the utilization of toxic reducing reagents during synthesis. The characterization results of Ultra violet visible (UV-Vis), X-ray diffraction (XRD), X-ray photo electron spectroscopy (XPS), Transmission electron microscopy (TEM) for the synthesized GO and reduced graphene oxide (RGO) indicated the strong removal of oxygen groups after reduction which followed by stabilization with oxidized form of pyrogallol. TEM analysis showed the thin transparent silk like sheets of graphene. FTIR analysis confirmed the stabilization of graphene sheets with oxidized pyrogallol molecules. XRD and XPS analysis represented the deoxygenation of GO to RGO. The in-vitro cytotoxicity of RGO towards HeLa cells is dose dependant. The prepared RGO also exhibited the percent cell viability of about 80% even at higher concentrations indicating the less toxic nature of the RGO stabilized with pyrogallol. These results have represented that this synthetic approach is effective for the preparation of bulk scale RGO in a simple, less expensive and eco-friendly method. Since this method avoids the use of chemical reagents that are toxic in nature, the produced graphene are likely to offer several potential biomedical applications.

Ma, C., et al. (2014). "Nickel nanowires induce cell cycle arrest and apoptosis by generation of reactive oxygen species in HeLa cells." Toxicol Rep **1**: 114-121.

Nickel nanowires (Ni NWs) have great potential to be used as a living cell manipulation tool and developed into an anticancer agent. However, their candidacy as biomedical appliances need detailed human cell studies, such as study of the interaction between Ni NWs and tumor cells. The present study investigated the cytotoxicity of Ni NWs in HeLa cells. A dose-dependent inhibition of cell growth was observed by using the MTT assay. We demonstrated that Ni NWs induced oxidative stress by generation of reactive oxygen species (ROS). Apoptosis induction was evidenced by flow cytometry, annexin V binding assay and DAPI staining. DNA flow cytometric analysis indicated that Ni NWs significantly increased the percentages of cells in S phase compared with control cells. This process was accompanied by the loss of mitochondrial membrane potential. These results revealed that Ni NWs induced apoptosis in HeLa cells via ROS generation and cell cycle arrest.

Ma, M., et al. (2017). "High-Throughput Identification of miR-596 Inducing p53-Mediated Apoptosis in HeLa and HCT116 Cells Using Cell Microarray." SLAS Technol **22**(6): 636-645.

miRNAs play a key role in the regulation of gene networks in mammalian cells. However, little is known about their roles and functions in the apoptosis pathway. Here, we conducted a whole-genome miRNA screening for apoptosis and identified more than 100 miRNAs as apoptosis inducers. To further explain the roles of these mRNAs in apoptosis, a second round of screening was conducted between p53 +/+ and -/- cells. Among the hits, miR-596 was identified as a regulator of p53. The overexpression of miR-596 significantly increased p53 at the protein level, thereby inducing apoptosis. We also demonstrated that Smurf1 was the direct target of miR-596. Previously, Smurf1 was reported to attenuate the level of p53 through binding and stabilizing MDM2, a p53 inhibitor. Consequently, by targeting Smurf1, miR-596 indirectly increased the p53 level in mammalian cells. Moreover, our study demonstrated that miR-596 had other antitumor characteristics, such as inhibiting migration and proliferation. The data from the GEO dataset revealed that the high expression of miR-596 contributed to survival benefits among cancer patients. These results make miR-596 a potential antitumor factor for future biomedical applications.

Magan, N., et al. (2012). "Treatment with the PARP-inhibitor PJ34 causes enhanced doxorubicin-mediated cell death in HeLa cells." Anticancer Drugs **23**(6): 627-637.

Adjuvant therapies can incorporate a number of different drugs to minimize the cardiotoxicity of cancer chemotherapy, decrease the development of drug resistance and increase the overall efficacy of the treatment regime. Topoisomerase IIalpha is a major target of many commonly used anticancer drugs, where cell death is brought about by an accumulation of double-strand DNA breaks. Poly (ADP-ribose) polymerase (PARP)-1 has been extensively studied for its role in the repair of double-strand DNA breaks, but its ability to add highly negative biopolymers (ribosylation) to target proteins provides a vast number

of pathways where it can also be important in mediating cell death. In this study, we combine the classical topoisomerase IIalpha poison doxorubicin with the PARP inhibitor PJ34 to investigate the potentiation of chemotherapeutic efficiency in HeLa cells. We demonstrate that PJ34 treatment has the capacity to increase endogenous topoisomerase IIalpha protein by about 20%, and by combining doxorubicin treatment with PJ34, we observed a 50% improvement in doxorubicin-mediated cell death in HeLa cells. These results were correlated with the ribosylation of transcription factor specificity factor 1 after doxorubicin treatment, thereby altering its affinity for binding to known regulatory elements within the human topoisomerase IIalpha promoter. Taken together, these results highlight the synergistic potential of combining PARP inhibitors with classical topoisomerase IIalpha-targeting drugs.

Maghsoudi, N., et al. (2010). "Targeting enteroviral 2A protease by a 16-mer synthetic peptide: inhibition of 2Apro-induced apoptosis in a stable Tet-on HeLa cell line." Virology **399**(1): 39-45.

Enteroviridae such as coxsackievirus are important infectious agents causing viral heart diseases. Viral protease 2A (2Apro) initiates the virus life cycle, and is an excellent target for developing antiviral drugs. Here, to evaluate the validity of the 2Apro as a proper therapeutic target, and based on the existing information and molecular dynamics, a 16-mer peptide was designed to specifically target the active site of protease 2Apro in order to block the activity of CVB3 2Apro. We showed that the peptide could compete with endogenous substrate in a concentration-dependent manner. Further, we established a HeLa cell line that expressed 2Apro. Expression of 2Apro resulted in significant morphological alteration and eventual cell death. Western blot and viability assay showed that the 16-mer peptide (200 microg/ml) could significantly block 2Apro activity and its cytotoxic effect. Future modification of the 16-mer peptide can improve its affinity for 2Apro and therefore develop effective antiviral drug.

Mahamid, J., et al. (2016). "Visualizing the molecular sociology at the HeLa cell nuclear periphery." Science **351**(6276): 969-972.

The molecular organization of eukaryotic nuclear volumes remains largely unexplored. Here we combined recent developments in cryo-electron tomography (cryo-ET) to produce three-dimensional snapshots of the HeLa cell nuclear periphery. Subtomogram averaging and classification of ribosomes revealed the native structure and organization of the cytoplasmic translation machinery. Analysis of a large dynamic structure-the nuclear pore complex-revealed variations detectable at the level of individual complexes. Cryo-ET was used to visualize previously elusive structures, such as nucleosome chains and the filaments of the nuclear lamina, in situ. Elucidation of the lamina structure provides insight into its contribution to metazoan nuclear stiffness.

Malohlava, J., et al. (2016). "Effect of Porphyrin Sensitizer MgTPPS4 on Cytoskeletal System of HeLa Cell Line-Microscopic Study." Cell Biochem Biophys **74**(3): 419-425.

Metalloporphyrins are an important group of sensitizers with a porphyrin skeleton. Their photophysical properties are significantly affected by the nature of the central ion. In this work, we focus on the mechanical properties of a cervix carcinoma cell line which underwent photodynamic treatment (PDT) with MgTPPS4 photosensitzer. Atomic force microscopy alongside confocal microscopy was used to quantify and qualify the structural characteristics before and after PDT. Cells before PDT showed a fine actin network and higher elasticity with the median of Young modulus 12.2 kPa. After PDT, the median of Young modulus was 13.4 kPa and a large redistribution in the actin network was observed.

Manosroi, J., et al. (2012). "Anti-proliferative activities on HeLa cancer cell line of Thai medicinal plant recipes selected from MANOSROI II database." J Ethnopharmacol **142**(2): 422-431.

ETHNOPHARMACOLOGICAL RELEVANCE: The Thai/Lanna medicinal plant recipe database "MANOSROI II" contained the medicinal plant recipes of all regions in Thailand for the treatment of various diseases including anti-cancer medicinal plant recipes. AIM OF THE STUDY: To investigate anti-proliferative activity on HeLa cell lines of medicinal plant recipes selected from the Thai/Lanna medicinal plant recipe database "MANOSROI II". MATERIALS AND METHODS: The forty aqueous extracts of Thai/Lanna medicinal plant recipes selected from the Thai/Lanna medicinal plant recipe database "MANOSROI II" were investigated for anti-proliferative activity on HeLa cell line by SRB assay. The apoptosis induction by caspase-3 activity and MMP-2 inhibition activity by zymography on HeLa cell line of the three selected aqueous extracts, which gave the highest anti-proliferative activity were determined. Phytochemicals and anti-oxidative activities including free radical scavenging activity, inhibition of lipid peroxidation and metal chelating inhibition activities were also investigated. RESULT: Sixty percentages of the medicinal plant recipes selected from "MANOSROI II" database showed anti-proliferative activity on HeLa cell line. The recipes of

N031(Albizia chinensis (Osbeck) Merr, Cassia fistula L., and Dargea volubilis Benth.ex Hook. etc.), N039 (Nymphoides indica L., Peltophorum pterocarpum (DC.), and Polyalthia debilis Finet et Gagnep etc.) and N040 (Nymphoides indica L. Kuntze, Sida rhombifolia L., and Xylinbaria minutiflora Pierre. etc.) gave higher anti-proliferative activity than the standard anti-cancer drug, cisplatin of 1.25, 1.29 and 30.18 times, respectively. The positive relationship between the anti-proliferative activity and the MMP-2 inhibition activity and metal chelating inhibition activity was observed, but no relationship between the anti-proliferative activity and apoptosis induction, free radical scavenging activity and lipid peroxidation inhibition activity. Phytochemicals found in these extracts were alkaloids, flavonoids, tannins and xanthones, but not anthraquinones and carotenoids. The recipe N040 exhibited the highest anti-proliferative and MMP-2 inhibition on HeLa cancer cell line at 30 and threefolds of cisplatin, respectively ($p<0.05$), while recipe N031 gave the highest caspase-3 activity (1.29-folds over the control) ($p<0.05$). CONCLUSION: This study has demonstrated that recipe N040 selected from MANOSROI II database appeared to be a good candidate with high potential for the further development as an anti-cancer agent.

Mao, J., et al. (2012). "Cell cycle-dependent subcellular distribution of ClC-3 in HeLa cells." Histochem Cell Biol **137**(6): 763-776.

Chloride channel-3 (ClC-3) is suggested to be a component and/or a regulator of the volume-activated Cl (-) channel in the plasma membrane. However, ClC-3 is predominantly located inside cells and the role of intracellular ClC-3 in tumor growth is unknown. In this study, we found that the subcellular distribution of endogenous ClC-3 varied in a cell cycle-dependent manner in HeLa cells. During interphase, ClC-3 was distributed throughout the cell and it accumulated at various positions in different stages. In early G1, ClC-3 was mainly located in the nucleus. In middle G1, ClC-3 gathered around the nuclear periphery as a ring. In late G1, ClC-3 moved back into the nucleus, where it remained throughout S phase. In G2, ClC-3 was concentrated in the cytoplasm. When cells progressed from G2 to the prophase of mitosis, ClC-3 from the cytoplasm translocated into the nucleus. During metaphase and anaphase, ClC-3 was distributed throughout the cell except for around the chromosomes and was aggregated at the spindle poles and in between two chromosomes, respectively. ClC-3 was then again concentrated in the nucleus upon the progression from telophase to cytokinesis. These results reveal a cell cycle-dependent change of the subcellular distribution of ClC-3 and strongly suggest that ClC-3 has nucleocytoplasmic shuttling dynamics that may play key regulatory roles during different stages of the cell cycle in tumor cells.

Marin-Hernandez, A., et al. (2017). "Hypoglycemia Enhances Epithelial-Mesenchymal Transition and Invasiveness, and Restrains the Warburg Phenotype, in Hypoxic HeLa Cell Cultures and Microspheroids." J Cell Physiol **232**(6): 1346-1359.

The accelerated growth of solid tumors leads to episodes of both hypoxia and hypoglycemia (HH) affecting their intermediary metabolism, signal transduction, and transcriptional activity. A previous study showed that normoxia (20% O2 ) plus 24 h hypoglycemia (2.5 mM glucose) increased glycolytic flux whereas oxidative phosphorylation (OxPhos) was unchanged versus normoglycemia in HeLa cells. However, the simultaneous effect of HH on energy metabolism has not been yet examined. Therefore, the effect of hypoxia (0.1-1% O2 ) plus hypoglycemia on the energy metabolism of HeLa cells was analyzed by evaluating protein content and activity, along with fluxes of both glycolysis and OxPhos. Under hypoxia, in which cell growth ceased and OxPhos enzyme activities, DeltaPsim and flux were depressed, hypoglycemia did not stimulate glycolytic flux despite increasing H-RAS, p-AMPK, GLUT1, GLUT3, and HKI levels, and further decreasing mitochondrial enzyme content. The impaired mitochondrial function in HH cells correlated with mitophagy activation. The depressed OxPhos and unchanged glycolysis pattern was also observed in quiescent cells from mature multicellular tumor spheroids, suggesting that these inner cell layers are similarly subjected to HH. The principal ATP supplier was glycolysis for HH 2D monolayer and 3D quiescent spheroid cells. Accordingly, the glycolytic inhibitors iodoacetate and gossypol were more effective than mitochondrial inhibitors in decreasing HH-cancer cell viability. Under HH, stem cell-, angiogenic-, and EMT-biomarkers, as well as glycoprotein-P content and invasiveness, were also enhanced. These observations indicate that HH cancer cells develop an attenuated Warburg and pronounced EMT- and invasive-phenotype. J. Cell. Physiol. 232: 1346-1359, 2017. (c) 2016 Wiley Periodicals, Inc.

Martinez-Torres, A. C., et al. (2018). "IMMUNEPOTENT CRP induces cell cycle arrest and caspase-independent regulated cell death in HeLa cells through reactive oxygen species production." BMC Cancer **18**(1): 13.

BACKGROUND: Regulated cell death (RCD) is a mechanism by which the cell activates its own machinery to self-destruct. RCD is important for the

maintenance of tissue homeostasis and its deregulation is involved in diseases such as cervical cancer. IMMUNEPOTENT CRP (I-CRP) is a dialyzable bovine leukocyte extract that contains transfer factors and acts as an immunomodulator, and can be cytotoxic to cancer cell lines and reduce tumor burden in vivo. Although I-CRP has shown to improve or modulate immune response in inflammation, infectious diseases and cancer, its widespread use has been limited by the absence of conclusive data on the molecular mechanism of its action. METHODS: In this study we analyzed the mechanism by which I-CRP induces cytotoxicity in HeLa cells. We assessed cell viability, cell death, cell cycle, nuclear morphology and DNA integrity, caspase dependence and activity, mitochondrial membrane potential, and reactive oxygen species production. RESULTS: I-CRP diminishes cell viability in HeLa cells through a RCD pathway and induces cell cycle arrest in the G2/M phase. We show that the I-CRP induces caspase activation but cell death induction is independent of caspases, as observed by the use of a pan-caspase inhibitor, which blocked caspase activity but not cell death. Moreover, we show that I-CRP induces DNA alterations, loss of mitochondrial membrane potential, and production of reactive-oxygen species. Finally, pretreatment with N-acetyl-L-cysteine (NAC), a ROS scavenger, prevented both ROS generation and cell death induced by I-CRP. CONCLUSIONS: Our data indicate that I-CRP treatment induced cell cycle arrest in G2/M phase, mitochondrial damage, and ROS-mediated caspase-independent cell death in HeLa cells. This work opens the way to the elucidation of a more detailed cell death pathway that could potentially work in conjunction with caspase-dependent cell death induced by classical chemotherapies.

Martucci, H., et al. (2017). "Naphthablins B and C, Meroterpenoids Identified from the Marine Sediment-Derived Streptomyces sp. CP26-58 Using HeLa Cell-Based Cytological Profiling." J Nat Prod **80**(3): 684-691.

HeLa cell-based cytological profiling (CP) was applied to an extract library of marine sediment-derived actinomycetes to discover new cytotoxic secondary metabolites. Among the hit strains, Streptomyces sp. CP26-58 was selected for further investigation to identify its cytotoxic metabolites. CP revealed that the known ionophore tetronasin (1) was responsible for the cytotoxic effect found in the extract. Furthermore, three naphthoquinone meroterpenoids, naphthablin A (2) and two new derivatives designated as naphthablins B (3) and C (4), were isolated from other cytotoxic fractions. The structures of the new compounds were elucidated based on analysis of their HRESIMS and comprehensive NMR data. The absolute configurations of the new compounds were deduced by simulating ECD spectra and calculating potential energies for the model compounds using density function theory (DFT) calculations. Compound 1 showed a significant cytotoxic effect against HeLa cells with an IC50 value of 0.23 muM, and CP successfully clustered 1 with calcium ionophores.

Mendoza-Nava, H., et al. (2013). "Laser heating of gold nanospheres functionalized with octreotide: in vitro effect on HeLa cell viability." Photomed Laser Surg **31**(1): 17-22.

OBJECTIVE: The aim of this study was to assess the effect of laser heating a well-characterized gold nanoparticle (AuNP)-octreotide system on HeLa cell viability, to evaluate its potential as a suitable agent for plasmonic photothermal therapy. BACKGROUND DATA: Octreotide is a synthetic peptide derivative of somatostatin with an effect on the survival of HeLa cells. Peptides bound to AuNPs are biocompatible and stable multimeric systems with target-specific molecular recognition. METHODS: Octreotide was conjugated to AuNPs ( approximately 20 nm) by spontaneous reaction with the thiol groups. The nanoconjugate was characterized by transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), ultraviolet visible spectroscopy (UV-Vis), X-ray photoelectron spectroscopy (XPS), and Raman spectroscopy. Irradiation experiments were conducted using an Nd:YAG laser pulsed for 5 ns at 532 nm with a repetition rate of 10 Hz for up to 6 min while delivering an average irradiance of 0.65 W/cm (2). HeLa cells were incubated at 37 degrees C (1) with AuNP-citrate, (2) with AuNP-octreotide, or (3) without nanoparticles. RESULTS: After laser irradiation, the presence of AuNP caused a significant increase in the temperature of the medium (48 degrees C vs. 38.3 degrees C of that without AuNP). The AuNP-octreotide system resulted in a significant decrease in cell viability of up to 6 % compared with the AuNP-citrate system (15.8+/-2.1%). Two possible mechanisms could be at play: (1) octreotide alone exerts an effect on survival HeLa cells, or (2) the release of heat ( approximately 727 degrees C per nanoparticle) in the membranes or cytoplasm of the cells caused by the interaction between AuNP-octreotide and somatostatin receptors reduced viability. CONCLUSIONS: The AuNP-octreotide system exhibited properties suitable for plasmonic photothermal therapy in the treatment of cervical cancer.

Meng, L., et al. (2014). "Highly sensitive determination of copper in HeLa cell using capillary

electrophoresis combined with a simple cell extraction treatment." Talanta **121**: 205-209.

A new separation system of capillary electrophoresis (CE1) for the highly sensitive determination of copper was established by using ethylenediaminetetraacetic acid (EDTA) as a complexing agent and employing cetyltrimethylammonium chloride (CTAC) as a capillary inner wall modifier. Benefitted from the combination of field-enhanced sample injection (FESI) method, a limit of detection (LOD) of 2.7 nM was obtained, which was much lower than that of the conventional methods. This made it possible to determine trace copper in HeLa cell only by a simple cell extraction (CE2) treatment. Two copper-extraction methods-acid-hydrolysis and freeze-thaw-were compared. Limited by the requirement of low ion strength in FESI, only the extract using freeze-thaw could be successfully applied in the determination. The effectiveness assessment of this CE (2)-FESI method was adopted by inductively coupled plasma-atomic emission spectrometry (ICP-AES) as a gold standard.

Merenyi, G., et al. (2011). "Cellular response to efficient dUTPase RNAi silencing in stable HeLa cell lines perturbs expression levels of genes involved in thymidylate metabolism." Nucleosides Nucleotides Nucleic Acids **30**(6): 369-390.

dUTPase is involved in preserving DNA integrity in cells. We report an efficient dUTPase silencing by RNAi-based system in stable human cell line. Repression of dUTPase induced specific expression level increments for thymidylate kinase and thymidine kinase, and also an increased sensitization to 5-fluoro-2'-deoxyuridine and 5-fluoro-uracil. The catalytic mechanism of dUTPase was investigated for 5-fluoro-dUTP. The 5F-substitution on the uracil ring of the substrate did not change the kinetic mechanism of dUTP hydrolysis by dUTPase. Results indicate that RNAi silencing of dUTPase induces a complex cellular response wherein sensitivity towards fluoropyrimidines and gene expression levels of related enzymes are both modulated.

Messina, A., et al. (2014). "Live cell interactome of the human voltage dependent anion channel 3 (VDAC3) revealed in HeLa cells by affinity purification tag technique." Mol Biosyst **10**(8): 2134-2145.

In higher eukaryotes three different VDAC genes encode three homologous proteins which do not show the same activity. VDAC1 and VDAC2 isoforms have been characterized while VDAC3 isoform is still elusive. To explore VDAC3 protein interactions, we have established a stable cell line expressing a fluorescent and dual-tagged construct. This clone expresses a stable amount of VDAC3. Live cell imaging shows that fluorescent VDAC3 localizes in the mitochondria. Proteins interacting with VDAC3 have been separated by tandem-affinity purification and 2-D gel electrophoresis and identified by mass spectrometry. In the list of putative interacting proteins, there are cytosolic, mitochondrial, cytoskeletal and ER proteins. Coherent pathways like cell redox homeostasis, response to stress, formation/rearrangement of disulfide bonds, response to unfolded proteins or protein folding have been found to be related to clusters of proteins identified in this experiment. The list of associated proteins has been validated by immunoprecipitation experiments utilizing specific antibodies. Likely biological and pathological processes have been analyzed. Cytosolic proteins associated with VDAC3 include tubulins and cytoskeletal proteins, stress sensors, chaperones and proteasome components, redox-mediating enzymes such as protein disulphide isomerase. The overall picture points to a role for VDAC3 as mediator for the organization of protein complexes and regulator of the traffic of misfolded or non-folded proteins evoked from different stimuli.

Milani, S., et al. (2016). "Suppressive Effect of Constructed shRNAs against Apollon Induces Apoptosis and Growth Inhibition in the HeLa Cell Line." Iran Biomed J **20**(3): 145-151.

BACKGROUND: Cervical cancer is the second most common female cancer worldwide. Inhibitors of apoptosis proteins (IAPs) block apoptosis; therefore, therapeutic strategies targeting IAPs have attracted the interest of researchers in recent years. Apollon, a member of IAPs, inhibits apoptosis and cell death. RNA interference is a pathway in which small interfering RNA (siRNA) or shRNA (short hairpin RNA) inactivates the expression of target genes. The purpose of this study was to determine the effect of constructed shRNAs on apoptosis and growth inhibition through the suppression of apollon mRNA in HeLa cell line. METHODS: Three shRNAs with binding ability to three different target sites of the first region of apollon gene were designed and cloned in pRNAin-H1.2/Neo vector. shRNA plasmids were then transfected in HeLa cells using electroporation. Down-regulation effects of apollon and the viability of HeLa cells were analyzed by RT-PCR, lactate dehydrogenase assay, and MTT assay, respectively. Also, the induction and morphological markers of apoptosis were evaluated by caspase assay and immunocytochemistry method. RESULTS: The expression of shRNA in HeLa cells caused a significant decrease in the level of apollon mRNA1. In addition, shRNA1 effectively increased the mRNA

level of Smac (as the antagonist of apollon), reduced the viability of HeLa cells and exhibited immunocytochemical apoptotic markers in this cell line. CONCLUSION: Apollon gene silencing can induce apoptosis and growth impairment in HeLa cells. In this regard, apollon can be considered a candidate therapeutic target in HeLa cells as a positive human papillomavirus cancer cell line.

Minorics, R., et al. (2015). "A molecular understanding of D-homoestrone-induced G2/M cell cycle arrest in HeLa human cervical carcinoma cells." J Cell Mol Med **19**(10): 2365-2374.

2-Methoxyestradiol (ME), one of the most widely investigated A-ring-modified metabolites of estrone, exerts significant anticancer activity on numerous cancer cell lines. Its pharmacological actions, including cell cycle arrest, microtubule disruption and pro-apoptotic activity, have already been described in detail. The currently tested D-ring-modified analogue of estrone, D-homoestrone, selectively inhibits cervical cancer cell proliferation and induces a G2/M phase cell cycle blockade, resulting in the development of apoptosis. The question arose of whether the difference in the chemical structures of these analogues can influence the mechanism of anticancer action. The aim of the present study was therefore to elucidate the molecular contributors of intracellular processes induced by D-homoestrone in HeLa cells. Apoptosis triggered by D-homoestrone develops through activation of the intrinsic pathway, as demonstrated by determination of the activities of caspase-8 and -9. It was revealed that D-homoestrone-treated HeLa cells are not able to enter mitosis because the cyclin-dependent kinase 1-cyclin B complex loses its activity, resulting in the decreased inactivation of stathmin and a concomitant disturbance of microtubule formation. However, unlike 2-ME, D-homoestrone does not exert a direct effect on tubulin polymerization. These results led to the conclusion that the D-homoestrone-triggered intracellular processes resulting in a cell cycle arrest and apoptosis in HeLa cells differ from those in the case of 2-ME. This may be regarded as an alternative mechanism of action among steroidal anticancer compounds.

Mishra, R. R., et al. (2011). "A novel human TPIP splice-variant (TPIP-C2) mRNA, expressed in human and mouse tissues, strongly inhibits cell growth in HeLa cells." PLoS One **6**(12): e28433.

Alternative splicing of mRNAs is known to involve a major regulation of gene expression at RNA level in mammalian cells. The PTEN (Phosphatase and TENsin homologue deleted from the human chromosome 10), TPTE (Transmembrane Phosphatase with TEnsin homology) and TPIP (TPTE and PTEN homologous Inositol lipid Phosphatase) belong to a family of dual-specific lipid and protein phosphatases. PTEN is a well characterized tumor suppressor, which plays crucial role in cell survival, cell cycle regulation, cell proliferation as well as adhesion, motility and migration of cells. The C2-domain of PTEN is essential for PTEN-functions. We have isolated a novel 1019 bp human TPIP cDNA (TPIP-C2) from a human testis cDNA library. In silico analysis of the cDNA revealed that it is produced from the TPIP-locus on the human chromosome 13 by alternative RNA-splicing. It has a unique 5'-Alu sequence, a LINE sequence followed by a 582 bp Open Reading Frame (ORF) encoding a 193 aa polypeptide with a partial phosphatase domain and a C2-domain. TPIP-C2 mRNA is expressed in human testis and in mouse tissues. Mouse testis and brain showed higher levels of TPIP-C2 mRNA in comparison to the heart, liver and kidney under normal physiological conditions. TPIP-C2 mRNAs from human and mouse testes show extensive sequence identity. Over-expression of TPIP-C2 cDNA in HeLa cells strongly (up to 85%) inhibited cell growth/proliferation and caused apoptosis in a caspase 3-dependent manner. These findings suggest for the first time that a TPIP splice-variant mRNA with a partial phosphatase domain and a C2-domain is expressed in cells and tissues of human and murine origins under normal physiological conditions. Inhibition of cell growth/proliferation and induction of apoptosis by overexpression of TPIP-C2 mRNA in HeLa cells suggest that it may be involved in negative regulation of cell growth/proliferation.

Molouki, A., et al. (2010). "Newcastle disease virus infection promotes Bax redistribution to mitochondria and cell death in HeLa cells." Intervirology **53**(2): 87-94.

BACKGROUND/AIMS: Newcastle disease virus (NDV) is an avian paramyxovirus that has gained a lot of interest in cancer viro-therapeutic applications because of its ability to selectively induce apoptosis in human cancer cells. However, the underlying mechanisms by which NDV induces apoptosis in human cancer cells are still not entirely understood. METHODS: In this study we examined the effect of a Malaysian velogenic strain of NDV, known as AF2240, on some elements of the intrinsic pathway of apoptosis. RESULTS: We show that NDV infection leads to conformational change of Bax protein. This is associated with the translocation of Bax from the cytoplasm to mitochondria and the release of cytochrome c into the cytoplasm. Interestingly, the level of Bcl-2 protein was not affected by NDV treatment. CONCLUSION: We have shown that Bax conformational change and subcellular distribution is

involved in the intrinsic pathway of apoptosis induced by NDV.

Moravcik, R., et al. (2014). "Diquat-induced cytotoxicity on Vero and HeLa cell lines: effect of melatonin and dihydromelatonin." Interdiscip Toxicol **7**(4): 184-188.

Diquat dibromide is a moderately toxic contact herbicide belonging to the bipyridyl group of redox-active compounds that induce a strong oxidative damage. Melatonin (MEL) can protect against oxidative damage under in vivo conditions, probably through its anti-oxidative capacity and ability to induce expression of anti-oxidative enzymes. The objective of this study was to investigate effects of diquat on viability of Vero and HeLa cells and possible protective effects of MEL and its analogue 2,3-dihydromelatonin (DMEL). Cell viability was evaluated with the MTT test. First, we analyzed dose-dependent effects of diquat on cell viability using the concentration range of 0.1-100 muM. Second, we used the diquat dose which reduced cell viability by 50% and treated cells with either MEL or DMEL (both in the concentration range of 1-100 muM) in the presence or absence of diquat. In addition, effects of both diquat and MEL on oxidative stress in HeLa cells were measured by flow cytometry using 2',7'-dichlorofluorescin diacetate. We confirmed the expected negative effects of diquat on viability of Vero and HeLa cells. Melatonin and DMEL were able to prevent diquat reduced viability of Vero cells in rather low concentrations (1 muM) and DMEL exerted substantially stronger protective effects than MEL. However in HeLa cells, we did not find the same effects and MEL even reduced their viability. Moreover, treatment of HeLa cells with high concentrations of MEL (100 muM) exaggerated the pro-oxidative effects of diquat. The results suggest that in addition to the expected anti-oxidative effects, MEL exerts a pro-oxidative action which is cell type and dose dependent.

Morotomi-Yano, K., et al. (2013). "Nanosecond pulsed electric fields induce poly (ADP-ribose) formation and non-apoptotic cell death in HeLa S3 cells." Biochem Biophys Res Commun **438**(3): 557-562.

Nanosecond pulsed electric fields (nsPEFs) have recently gained attention as effective cancer therapy owing to their potency for cell death induction. Previous studies have shown that apoptosis is a predominant mode of nsPEF-induced cell death in several cell lines, such as Jurkat cells. In this study, we analyzed molecular mechanisms for cell death induced by nsPEFs. When nsPEFs were applied to Jurkat cells, apoptosis was readily induced. Next, we used HeLa S3 cells and analyzed apoptotic events. Contrary to our expectation, nsPEF-exposed HeLa S3 cells exhibited no molecular signs of apoptosis execution. Instead, nsPEFs induced the formation of poly (ADP-ribose) (PAR), a hallmark of necrosis. PAR formation occurred concurrently with a decrease in cell viability, supporting implications of nsPEF-induced PAR formation for cell death. Necrotic PAR formation is known to be catalyzed by poly (ADP-ribose) polymerase-1 (PARP-1), and PARP-1 in apoptotic cells is inactivated by caspase-mediated proteolysis. Consistently, we observed intact and cleaved forms of PARP-1 in nsPEF-exposed and UV-irradiated cells, respectively. Taken together, nsPEFs induce two distinct modes of cell death in a cell type-specific manner, and HeLa S3 cells show PAR-associated non-apoptotic cell death in response to nsPEFs.

Morre, J., et al. (2010). "Omega-3 but not omega-6 unsaturated fatty acids inhibit the cancer-specific ENOX2 of the HeLa cell surface with no effect on the constitutive ENOX1." J Diet Suppl **7**(2): 154-158.

Epidemiological and laboratory studies suggest that dietary fatty acids (oleic acid (in olive oil), eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) (in fish oil)) play important roles in carcinogenesis. The most potent antitumor effects of all fatty acids are given by fatty acid conjugated linoleic acid (CLA). The antitumor effects of CLA may be mediated through enhanced apoptosis. While CLA, EPA, and DHA (omega-3 polyunsaturated fatty acids) have inhibitory effects on cancer cells, omega-6 fatty acids have often shown negative or potentiating effects on cancer cells. Linoleic acid (an omega-6) is desaturated in the cell by delta 6 and 5 destaturases to form arachidonic acid. COX 1 and 2 isoforms then act on arachidonic acid to form prostaglandins and other related regulatory molecules. It is normally thought that what is important to the development of the cancerous phenotype is some balance of these various metabolites. In experiments with surface NOX proteins released from HeLa cells, spectrophotometric measurements of the oxidation of NADH revealed inhibition of the cancer-specific ENOX2 activity by CLA and the omega-3 fatty acids, eicosapentaenoic, docosahexaenoic, and alpha-linolenic acids. The constitutive ENOX1 activity was not inhibited. In contrast, the omega-6 fatty acids, linoleic acid, and arachidonic acid inhibited neither ENOX1 nor ENOX2. The findings indicate the possibility that a direct effect of CLA and omega-3 fatty acids on ENOX2 may be responsible for the potent activity of CLA and omega-3 fatty acids in cancer prevention and therapy.

Motevaseli, E., et al. (2016). "The Effect of Lactobacillus crispatus and Lactobacillus rhamnosusCulture Supernatants on Expression of Autophagy Genes and HPV E6 and E7 Oncogenes in The HeLa Cell Line." Cell J **17**(4): 601-607.

OBJECTIVE: The aim of this study was to clarify the mechanism by which lactobacilli exert their cytotoxic effects on cervical cancer cells. In addition, we aimed to evalu- ate the effect of lactobacilli on the expression of human papilloma virus (HPV) onco- genes. MATERIALS AND METHODS: In this experimental study, using quantitative real-time polymer- ase chain reaction (PCR), we analyzed the expression of CASP3 and three autophagy genes [ATG14, BECN1 and alpha 2 catalytic subunit of AMPK (PRKAA2)] along with HPV18 E6 and E7 genes in HeLa cells before and after treatment with Lactobacillus crispatus and Lactobacillus rhamnosus culture supernatants. RESULTS: The expression of CASP3 and autophagy genes in HeLa cells was de- creased after treatment with lactobacilli culture supernatants. However, this de- crease was not significant for PRKAA2 when compared with controls. In addition, expression of HPV E6 was significantly decreased after treatment with lactobacilli culture supernatants. CONCLUSION: Lactobacilli culture supernatants can decrease expression of ATG14 and BECN1 as well as the HPV E6 oncogene. It has been demonstrated that the main changes occurring during cervical carcinogenesis in cell machinery can be reversed by suppression of HPV oncogenes. Therefore, downregulation of HPV E6 by lacto- bacilli may have therapeutic potential for cervical cancer. As the role of autophagy in cancer is complicated, further work is required to clarify the link between downregula- tion of autophagy genes and antiproliferative effects exerted by lactobacilli.

Na, M., et al. (2017). "Optimizing In Vitro Pre-mRNA 3' Cleavage Efficiency: Reconstitution from Anion-Exchange Separated HeLa Cleavage Factors and from Adherent HeLa Cell Nuclear Extract." Methods Mol Biol **1507**: 179-198.

Eukaryotic RNA processing steps during mRNA maturation present the cell with opportunities for gene expression regulation. One such step is the pre-mRNA 3' cleavage reaction, which defines the downstream end of the 3' untranslated region and, in nearly all mRNA, prepares the message for addition of the poly (A) tail. The in vitro reconstitution of 3' cleavage provides an experimental means to investigate the roles of the various multi-subunit cleavage factors. Anion-exchange chromatography is the simplest procedure for separating the core mammalian cleavage factors. Here we describe a method for optimizing the in vitro reconstitution of 3' cleavage activity from the DEAE-sepharose separated HeLa cleavage factors and show how to ensure, or avoid, dependence on creatine phosphate. Important reaction components needed for optimal processing are discussed. We also provide an optimized procedure for preparing small-scale HeLa nuclear extracts from adherent cells for use in 3' cleavage in vitro.

Nakamura, K. and O. Muraoka (2018). "Effect of electrolyzed water produced using carbon electrodes on HeLa cell proliferation." Biosci Trends **11**(6): 688-693.

We developed electrolyzed water (EW) using carbon electrodes and investigated the ability of the developed EW to inhibit the proliferation of human cervical carcinoma HeLa cells. We observed that EW-containing media inhibited HeLa cell proliferation. Many very small black dots were produced in EW and these were associated with the inhibitory effect on the cell proliferation. Furthermore, the very small black dots that could inhibit cell proliferation were produced only at pH 3 to 3.5 of EW. Additional experiments showed that this inhibition of proliferation is reversible. These results suggest that the effect of EW on HeLa cells is cytostatic and not cytotoxic. Thus, our results indicate that the EW developed in this study may be used to inhibit cell proliretation.

Nakayama, M., et al. (2011). "Radiosensitivity of early and late M-phase HeLa cells isolated by a combination of fluorescent ubiquitination-based cell cycle indicator (Fucci) and mitotic shake-off." Radiat Res **176**(3): 407-411.

The mitotic shake-off method revealed the remarkable variation of radiosensitivity of HeLa cells during the cell cycle: M phase shows the greatest radiosensitivity and late S phase the greatest radioresistance. This method harvests all M-phase cells with a round shape, making it impossible to further subdivide M-phase cells. Recently, the fluorescent ubiquitination-based cell cycle indicator (Fucci) was developed; this system basically causes cells in G (1) to emit red fluorescence and other cells to emit green fluorescence. Because the green fluorescence rapidly disappears at late M phase, two-dimensional flow cytometry analysis can usually detect a green (high)/red (low) fraction including S-, G (2)- and early M-phase cells but not a transitional fraction between green (high)/red (low) and green (low)/red (low) including late M-phase cells. However, combining the shake-off method concentrated the transitional fraction, which enabled us to separate early and late M-phase cells without using any drugs. Here we demonstrate for the first time that cells in early M phase are more radiosensitive than those in late M phase, implying

that early M phase is the most radiosensitive sub-phase during the cell cycle.

Narita, A., et al. (2015). "Real-time observation of irradiated HeLa-cell modified by fluorescent ubiquitination-based cell-cycle indicator using synchrotron X-ray microbeam." Radiat Prot Dosimetry **166**(1-4): 192-196.

Fluorescent ubiquitination-based cell-cycle indicator (FUCCI) human cancer (HeLa) cells (red indicates G1; green, S/G2) were exposed to a synchrotron X-ray microbeam. Cells in either G1 or S/G2 were irradiated selectively according to their colour in the same microscopic field. Time-lapse micrographs of the irradiated cells were acquired for 24 h after irradiation. For fluorescent immunostaining, phosphorylated histone proteins (gamma-H2AX) indicated the induction of DNA double-strand breaks. The cell cycle was arrested by irradiation at S/G2. In contrast, cells irradiated at G1 progressed to S/G2. The foci were induced in cells irradiated at both G1 and S/G2, suggesting that the G1-S (or S) checkpoint pathway does not function in HeLa cells due to the fact that the cells are functionally p53 deficient, even though X-ray microbeam irradiation significantly induces double-strand breaks. These results demonstrate that single FUCCI cell exposure and live cell imaging are powerful methods for studying the effects of radiation on the cell cycle.

Negi, S., et al. (2015). "Intrinsic cell permeability of the GAGA zinc finger protein into HeLa cells." Biochem Biophys Res Commun **464**(4): 1034-1039.

We examined the intrinsic cell permeability of a GAGA zinc finger obtained from the Drosophila melanogaster transcription factor and analyzed its mechanism of cellular uptake using confocal microscopy and flow cytometry. HeLa cells were treated with the Cy5-labeld GAGA peptides (containing a fluorescent chromophore) to detect fluorescence signals from the fluorescent labeling peptides by confocal microscopy. The results clearly indicated that GAGA peptides possess intrinsic cell permeability for HeLa cells. Based on the results of the flow cytometry analysis and the theoretical net positive charge of the GAGA peptides, the efficiency of cellular uptake of the GAGA peptides was predicted to depend on the net positive charge of the GAGA peptide as well as the cationic component ratio of Arg residues to Lys residues.

Ng, W. K., et al. (2015). "Thymoquinone-loaded nanostructured lipid carrier exhibited cytotoxicity towards breast cancer cell lines (MDA-MB-231 and MCF-7) and cervical cancer cell lines (HeLa and SiHa)." Biomed Res Int **2015**: 263131.

Thymoquinone (TQ) has been shown to exhibit antitumor properties. Thymoquinone-loaded nanostructured lipid carrier (TQ-NLC) was developed to improve the bioavailability and cytotoxicity of TQ. This study was conducted to determine the cytotoxic effects of TQ-NLC on breast cancer (MDA-MB-231 and MCF-7) and cervical cancer cell lines (HeLa and SiHa). TQ-NLC was prepared by applying the hot high pressure homogenization technique. The mean particle size of TQ-NLC was 35.66 +/- 0.1235 nm with a narrow polydispersity index (PDI) lower than 0.25. The zeta potential of TQ-NLC was greater than -30 mV. Polysorbate 80 helps to increase the stability of TQ-NLC. Differential scanning calorimetry showed that TQ-NLC has a melting point of 56.73 degrees C, which is lower than that of the bulk material. The encapsulation efficiency of TQ in TQ-NLC was 97.63 +/- 0.1798% as determined by HPLC analysis. TQ-NLC exhibited antiproliferative activity towards all the cell lines in a dose-dependent manner which was most cytotoxic towards MDA-MB-231 cells. Cell shrinkage was noted following treatment of MDA-MB-231 cells with TQ-NLC with an increase of apoptotic cell population ($P < 0.05$). TQ-NLC also induced cell cycle arrest. TQ-NLC was most cytotoxic towards MDA-MB-231 cells. It induced apoptosis and cell cycle arrest in the cells.

Nginamau, E. S., et al. (2011). "An experimental protocol for the fractionation and 2DE separation of HeLa and A-253 cell lysates suitable for the identification of the individual antigenic proteome in Sjogren's syndrome." Autoimmunity **44**(8): 652-663.

Sjogren's syndrome (SS) is an autoimmune disease affecting exocrine glands, especially the salivary and lacrimal glands. Although most of the SS patients' sera have autoantibodies that can target a variety of antigens, it is not clear what determines which proteins will become autoantigens. The muscarinic receptor M3, an integral plasma membrane protein, has been proposed as a possible autoantigen in SS, and is endogenous in HeLa cells. The aim of this study was to develop a method that is able to separate and identify antigens recognised by sera from SS patients using lysates of HeLa and A-253 cells in 2D Western Blot (2DWB). The HeLa and A-253 cell lysates were fractionated in soluble and membrane-bound proteins, and the membrane-bound proteins were enriched for integral proteins. The fractions were tested using WB, confirming the presence of the main cell compartments. The rehydration solution containing ASB-14 performed better than the others in all three steps (active rehydration, focus and transfer), and efficiently separated the muscarinic receptor M3. The M3 receptor was also detected in lysates from A-253 cells. The presence of this receptor in this cell line

has not been proven earlier. This work develops a suitable protocol to perform a mapping of the autoantibodies present in the sera of single SS patients, using lysates from epithelial cell lines that represent the main cell compartments as an antigen source. It is our future aim to use this protocol to perform a mapping of the antibodies present in the sera of individual SS patients.

Nikoloff, N., et al. (2016). "Folic acid enhances the apoptotic and genotoxic activity of carboplatin in HeLa cell line." Toxicol In Vitro **37**: 142-147.

In human tumor cells, experimental and clinical evidence indicates that some factors involved in signal transduction and cell growth can also modulate the response to chemotherapeutic treatment. The aim of the present study was to investigate the role of folic acid (FA) as a modulator of carboplatin (CBDCA) activity. Genotoxicity and cytotoxicity induced by CBDCA alone and in combination with FA were assessed in cultured HeLa cells. We used comet assay, mitotic index analysis, MTT and NR assays, cytokinesis-block micronucleus cytome assay and annexin V-IP as different cytotoxicity and genotoxicity approaches for human cervical carcinoma cell line studies. The results showed that addition of 900nM FA together with 40.4mM CBDCA enhanced the activity of the platinum compound, increasing its effect on cell death by nearly 20%, as evidenced by the MTT and NR assays. Moreover, not only higher levels of DNA and chromosomal damage were reached but also the number of necrotic and apoptotic cells were significantly increased when cell cultures were treated with the combined procedure. This situation opens the possibility to explore the use of FA in platinum-based chemotherapy protocols to reduce the platinum doses for patient treatment and decrease the chance of developing the known side effects without losing biological activity.

Ning, B. T. and Y. M. Tang (2012). "Establishment of the cell line, HeLa-CD14, transfected with the human CD14 gene." Oncol Lett **3**(4): 871-874.

CD14 is the pivotal molecule in the diagnosis and therapy of CD14-associated diseases, and is important in bacteremia. The HeLa cell line is regarded as immortal due to its prolific character. The HeLa cell line is derived from human cervical cancer cells and has been widely used in cancer research and gene transfection. In the present study, we established the expression plasmid pcDNA3.1(+)-CD14, and transfected it into the human cervical cancer cell line HeLa to establish a stable cell line (HeLa-CD14) expressing human CD14 antigen on the membrane. After the human CD14 gene was cloned and sequenced through RT-PCR and T-A cloning techniques, the eukaryotic expression vector pcDNA3.1(+)-CD14 was constructed by cleaving with double restriction endonucleases and ligating with T4 ligase. HeLa cells were transfected with the pcDNA3.1(+)-CD14 recombinant plasmid using Superfect transfection reagent. The cells were selected using G418 and the expression of human CD14 on the transfectant was confirmed by RT-PCR and immunohistochemistry. The expression of CD14 mRNA was significantly different between the blank pcDNA3.1(+)-transfected cell group and the pcDNA3.1(+)-CD14-transfected cell group ($p<0.01$). The fluorescence was significantly stronger on the established stable cell line than on the transiently transfected HeLa cells, and no visible fluorescence was observed in blank pcDNA3.1(+)-transfected cells. In this study, the human CD14 transfectant, stable cell line HeLa-CD14, was successfully established, which may be used to study CD14 and cervical cancer in vitro and in vivo.

Notzold, L., et al. (2017). "The long non-coding RNA LINC00152 is essential for cell cycle progression through mitosis in HeLa cells." Sci Rep **7**(1): 2265.

In recent years, long non-coding RNA (lncRNA) research has identified essential roles of these transcripts in virtually all physiological cellular processes including tumorigenesis, but their functions and molecular mechanisms are poorly understood. In this study, we performed a high-throughput siRNA screen targeting 638 lncRNAs deregulated in cancer entities to analyse their impact on cell division by using time-lapse microscopy. We identified 26 lncRNAs affecting cell morphology and cell cycle including LINC00152. This transcript was ubiquitously expressed in many human cell lines and its RNA levels were significantly upregulated in lung, liver and breast cancer tissues. A comprehensive sequence analysis of LINC00152 revealed a highly similar paralog annotated as MIR4435-2HG and several splice variants of both transcripts. The shortest and most abundant isoform preferentially localized to the cytoplasm. Cells depleted of LINC00152 arrested in prometaphase of mitosis and showed reduced cell viability. In RNA affinity purification (RAP) studies, LINC00152 interacted with a network of proteins that were associated with M phase of the cell cycle. In summary, we provide new insights into the properties and biological function of LINC00152 suggesting that this transcript is crucial for cell cycle progression through mitosis and thus, could act as a non-coding oncogene.

Nouri, Z., et al. (2016). "Dual Anti-Metastatic and Anti-Proliferative Activity Assessment of Two Probiotics on HeLa and HT-29 Cell Lines." Cell J **18**(2): 127-134.

OBJECTIVE: Lactobacilli are a group of probiotics with beneficial effects on prevention of cancer. However, there is scant data in relation with the impacts of probiotics in late-stage cancer progration, especially metastasis. The present original work was aimed to evaluate the anti-metastatic and anti-proliferative activity of lactobacillus rhamnosus supernatant (LRS) and lactobacillus crispatus supernatant (LCS) on the human cervical and colon adenocarcinoma cell lines (HeLa and HT-29, respectively). MATERIALS AND METHODS: In this experimental study, the anti-proliferative activities of LRS and LCS were determined through MTT assay. MRC-5 was used as a normal cell line. Expression analysis of CASP3, MMP2, MMP9, TIMP1 and TIMP2 genes was performed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), following the cell synchronization. RESULTS: Supernatants of these two lactobacilli had cytotoxic effect on HeLa, however LRS treatment was only effective on HT-29 cell line. In addition, LRS had no side-effect on normal cells. It was shown that CASP3 gene expression has been reduced after treatment with supernatants of two studied lactobacilli. According to our study, LRS and LCS are efficacious in the prevention of metastasis potency in HeLa cells with decreased expression of MMP2, MMP9 and increased expression of their inhibitors. In the case of HT-29 cells, only LRS showed this effect. CONCLUSION: Herein, we have demonstrated two probiotics which have anti-metastatic effects on malignant cells and they can be administrated to postpone late-stage of cancer disease. LRS and LCS are effective on HeLa cell lines while only the effect of LRS is significant on HT-29, through cytotoxic and anti-metastatic mechanisms. Further assessments are required to evaluate our results on the other cancer cell lines, in advance to use these probiotics in other extensive trial studies.

Oskoueian, E., et al. (2012). "Phorbol esters from Jatropha meal triggered apoptosis, activated PKC-delta, caspase-3 proteins and down-regulated the proto-oncogenes in MCF-7 and HeLa cancer cell lines." Molecules **17**(9): 10816-10830.

Jatropha meal produced from the kernel of Jatropha curcas Linn. grown in Malaysia contains phorbol esters (PEs). The potential benefits of PEs present in the meal as anticancer agent are still not well understood. Hence, this study was conducted to evaluate the cytotoxic effects and mode of actions of PEs isolated from Jatropha meal against breast (MCF-7) and cervical (HeLa) cancer cell lines. Isolated PEs inhibited cells proliferation in a dose-dependent manner of both MCF-7 and HeLa cell lines with the IC (5) (0) of 128.6 +/- 2.51 and 133.0 +/- 1.96 microg PMA equivalents/mL respectively, while the values for the phorbol 12-myristate 13-acetate (PMA) as positive control were 114.7 +/- 1.73 and 119.6 +/- 3.73 microg/mL, respectively. Microscopic examination showed significant morphological changes that resemble apoptosis in both cell lines when treated with PEs and PMA at IC (5) (0) concentration after 24 h. Flow cytometry analysis and DNA fragmentation results confirmed the apoptosis induction of PEs and PMA in both cell lines. The PEs isolated from Jatropha meal activated the PKC-delta and down-regulated the proto-oncogenes (c-Myc, c-Fos and c-Jun). These changes probably led to the activation of Caspase-3 protein and apoptosis cell death occurred in MCF-7 and HeLa cell lines upon 24 h treatment with PEs and PMA. Phorbol esters of Jatropha meal were found to be promising as an alternative to replace the chemotherapeutic drugs for cancer therapy.

Ota, K., et al. (2010). "Interferon-alpha2b induces p21cip1/waf1 degradation and cell proliferation in HeLa cells." Cell Cycle **9**(1): 131-139.

Type I interferons (IFNs) are a family of cytokines that exhibit various biological activities. Besides their roles in immune response, IFNs have been known to modulate cell proliferation and to induce apoptosis. Thus, IFNs are used as an antitumor agent against certain types of cancer, but it is unclear why many other cancers are not influenced by IFNs. Here, we found that IFN-alpha2b, a subfamily of IFN-alpha, enhanced proliferation of HeLa cells, a cell line derived from human cervical cancer. IFN-alpha2b was rather inhibitory on the growth of other types of cervical cancer cells including those positive for HPV. Among the proliferation- and the apoptosis-related genes, p21(cip1/waf1) (p21) was upregulated by IFN-alpha2b, whereas p53, p27 or BCL-2 associated X protein (BAX) was not affected. IFN-alpha2b did not alter promoter activities of p21 but did prolong the decay of p21 mRNA. In contrast, the level of p21 protein was lowered by IFN-alpha2b, and half-life analysis of p21 protein revealed that IFN-alpha2b enhances p21 protein instability in HeLa cells. Pretreatment of the cells with MG132, a proteasome inhibitor, abolished the IFN-alpha2b-mediated p21 degradation, suggesting that IFN-alpha2b accelerated the ubiquitin-proteasome dependent degradation of p21. Consistent with these results, IFN-alpha2b increased S-phase cell cycle distribution in HeLa cells. In addition, IFN-alpha2b liberated the cells from G (1)-phase arrest by 5-fluorouracil (5-FU) and from G (2)-phase arrest by paclitaxel. These results provide a

novel role of Type I IFNs in cell cycle regulation and may define an importance of individualized IFN-based therapy against specific types of cancer.

Ozcan, G., et al. (2016). "Screening for antitumor activity of various plant extracts on HeLa and C 4-I cell lines." J BUON **21**(6): 1552-1560.

PURPOSE: Cancer is a long process that leads the organism to death and is associated with the normal cells acquiring the ability to divide permanently. Nowadays, the use of natural products in cancer therapy has a great importance. In addition, working with plants that are endemic to Turkey and determining the biological activities of these plant extracts, is extremely important due to the potential for new drug development. There is no comparative study available in the literature on the antitumor effects of Colchicum sanguicolle, a new found species of the genus Colchicum in Turkey, Crateagus microphylla, of the genus Crateagus and Centaurea antiochia of the genus Centaurea. In this study, we tried to demonstrate the antitumor effect of these plant extracts on HeLa and C 4-1 cells. METHODS: Five different doses (0.001, 0.01, 0.05, 0.25 and 0.5 mg/ml) of the three plant types were prepared and applied for 24, 48 and 72 hrs on the cervical cancer derived cell lines. Subsequently, the growth rate was evaluated with the mitochondrial dehydrogenase enzyme method. RESULTS: Colchicum sanguicolle extracts showed the most effective antitumor activity. For the Colchicum sanguicolle extract, the IC50 dose for HeLa cells was 0.01 mg/ml at 48 hrs, while for the C-4 I cells it was 0.001 mg/ml at 48 hrs. These results showed that C-4 I cells were more sensitive to the Colchicum sanguicolle extracts. Conclusion: The results of from this study regarding the antitumor effect of plant extracts of endemic varieties of Turkey may have an important place in design and development of anticancer drugs and would make contributions to other studies to be conducted in this area.

Ozsoylemez, O. D., et al. (2016). "The Effects of Colchicum baytopiorum on Regulatory Genes of Apoptotic and Autophagic Cell Death in HeLa Cells." Curr Pharm Biotechnol **17**(15): 1369-1376.

BACKGROUND: The natural products have increasing important for the development of anticancer agents. Colchicum baytopiorum C.D. Brickell (C. baytopiorum), an endemic species for Turkey, contains colchicine and its derivatives. Stimulation of apoptotic and autophagy-mediated cell deaths are effective strategy for anticancer therapies. AIM: The aim of the study is to determine the role of the extract on both apoptotic and autophagic cell death in HeLa cell line. METHODS: The cell viability of C. baytopiorum (0.1 mg/ml) was determined by MTT assay. Active caspase-3 and t-Bid expressions were evaluated by immunohistochemical method. The mRNA expression of apoptotic regulatory genes (Bcl-xL, Bid, Bad, PUMA, NOXA, Caspase-3, -8, -9, Fas, FADD, TRADD, TRAF2, TNF, TNFR1), autophagic cell death related genes (Atg5-12, Beclin-1, DAPK), and also both autophagic and apoptotic cell death regulatory genes (Bif-1 and BNIP-3) were investigated by qRT-PCR. RESULTS: We determined that the expressions of both apoptotic and autophagic regulatory genes were significantly increased in the treatment group compared to control group. Also, we showed that C. baytopiorum crude extract induces the cross-connection between apoptotic and autophagic cell deaths in HeLa cells. CONCLUSION: We suggested that this endemic plant extract seems to be a new promising therapeutic approach in cancer.

Palizban, A. A., et al. (2010). "Effect of cerium lanthanide on Hela and MCF-7 cancer cell growth in the presence of transferring." Res Pharm Sci **5**(2): 119-125.

The anti-cancer activity of metal ions in the lanthanide group is being considered recently. It has been reported that cerium salts might stimulate the metabolism and therefore, produce anti-cancer effects. However, little is known about the effects of protein-cerium complex in controlling cancer cell growth. The aim of the present study was to elucidate the possible pathways for the cytotoxic effect of cerium in the presence of apo-transferrin on two cancer cell lines (Hela and MCF-7), that express transferrin receptors 3-4 fold higher than normal cells. The effect of different concentrations of cerium (0.1, 1, 10, 100 muM) in the presence and absence of transferrin for 48 h and 72 h incubation periods (37 degrees C, 5% CO2 and 95% humidity) was studied using the MTT assay. The results showed that cerium has a cell-proliferation inhibitory activity which is significantly increased by transferrin protein. Compared with the direct treatment of cancer cells with cerium, the presence of transferrin assisted inhibition of cell proliferation by 20% and 40% in Hela and MCF-7 cells, respectively. Though apo-transferrin could lightly induce cell growth particularly in MCF-7 cells by itself, this phenomenon could not overcome the cerium-protein cell-proliferation inhibition activity. In conclusion, our results indicate that at a certain concentration, the cerium compounds could be possibly involved in the control of cell proliferation and inhibiting the growth of cancer cells.

Palma, A., et al. (2011). "(1)H-MRS can detect aberrant glycosylation in tumour cells: a study of the HeLa cell line." NMR Biomed **24**(9): 1099-1110.

Glycosylation is the most abundant and diverse form of post-translational modification of proteins. Two types of glycans exist in glycoproteins: N-glycans and O-glycans often coexisting in the same protein. O-glycosylation is frequently found on secreted or membrane-bound mucins whose overexpression and structure alterations are associated with many types of cancer. Mucins have several cancer-associated structures, including high levels of Lewis antigens characterized by the presence of terminal fucose. The present study deals with the identification of MR signals from N-acetylgalactosamine and from fucose in HeLa cells by detecting a low-field signal in one-dimensional (1D) spectra assigned to the NH of N-acetylgalactosamine and some cross peaks assigned to fucose in two-dimensional (2D) spectra. The increase of Golgi pH by treatment with ammonium chloride allowed the N-acetylgalactosamine signal assignment to be confirmed. Behaviour of MR peak during cell growth and comparison with studies from literature taken together made it possible to have more insight into the relationship between aberrantly processed mucin and the presence of non-processed N-acetylgalactosamine residues in HeLa cells. Fucose signals, tentatively ascribed to residues bound to galactose and to N-acetylglucosamine, are visible in both intact cell and perchloric acid spectra. Signals assigned to fucose bound to galactose are more evident in ammonium chloride-treated cells where structural changes of mucin-related Lewis antigens are expected as a result of the higher Golgi pH. A common origin for the N-acetylgalactosamine and fucose resonances attributing them to aberrantly processed mucin can be inferred from the present results.

Pan, S., et al. (2012). "[Study on proteomics of Hela cell apoptosis in bufalin-induced human cervical carcinoma]." Zhongguo Zhong Yao Za Zhi **37**(13): 1998-2004.

OBJECTIVE: To seek possible effect targets of bufalin in HeLa cells by studying the impact of bufalin on cell protein expression profile after treatment on human cervical carcinoma cell lines HeLa. METHOD: Bufalin's ICs0was measured by MTr assay. The apoptosis of cells was observed by FCM (flow cytometry) and Hoechst 33342 staining assay. Differentiated expression protein spots were founded and identified using proteomic techniques, which could induce HeLa cell apoptosis. RESULT: Bufalin showed remarkable cytotoxic effect on HeLa cells. IC50 (154 +/- 21.5) nmol X L (-1) indicated the possibility of inducing cell apoptosis. The protein expression profile showed 11 differentiated expression protein spots. Among the 11 proteins, nudix-type motif 5, vimentin, hnRNP C1/hnRNP C2 variant, HNRPK, HNRPK isoform a variant (two spots are the same protein), heat shock protein 27, macrophage-capping protein, SELENBP1 protein were down-regulated, while ribosomal protein, large, P0 and S-adenosylmethionine synthetase 2 were up-regulated by bufalin treatment. They may be effect targets of bufalin in HeLa cells. Western blotting showed consistent results in heat shock protein 27, vimentin and HNRPK between expression after treatment with bufalin and two-dimensional electrophoresis. CONCLUSION: Bufa-Lin can induce apoptosis in human cervical carcinoma cells HeLa and the effect of bufalin may be related to the joint intervention with multiple protein targets.

Panzarini, E., et al. (2017). "Glucose capped silver nanoparticles induce cell cycle arrest in HeLa cells." Toxicol In Vitro **41**: 64-74.

This study aims to determine the interaction (uptake and biological effects on cell viability and cell cycle progression) of glucose capped silver nanoparticles (AgNPs-G) on human epithelioid cervix carcinoma (HeLa) cells, in relation to amount, 2x10(3) or 2x10(4) NPs/cell, and exposure time, up to 48h. The spherical and well dispersed AgNPs (30+/-5nm) were obtained by using glucose as reducing agent in a green synthesis method that ensures to stabilize AgNPs avoiding cytotoxic soluble silver ions Ag (+) release. HeLa cells take up abundantly and rapidly AgNPs-G resulting toxic to cells in amount and incubation time dependent manner. HeLa cells were arrested at S and G2/M phases of the cell cycle and subG1 population increased when incubated with 2x10(4) AgNPs-G/cell. Mitotic index decreased accordingly. The dissolution experiments demonstrated that the observed effects were due only to AgNPs-G since glucose capping prevents Ag (+) release. The AgNPs-G influence on HeLa cells viability and cell cycle progression suggest that AgNPs-G, alone or in combination with chemotherapeutics, may be exploited for the development of novel antiproliferative treatment in cancer therapy. However, the possible influence of the cell cycle on cellular uptake of AgNPs-G and the mechanism of AgNPs entry in cells need further investigation.

Park, S. Y., et al. (2012). "Mimosine arrests the cell cycle prior to the onset of DNA replication by preventing the binding of human Ctf4/And-1 to chromatin via Hif-1alpha activation in HeLa cells." Cell Cycle **11**(4): 761-766.

Though the G (1) checkpoint in mammalian cells has been known for decades, the molecular targets that prevent S-phase entry remain unknown. Mimosine is a rare plant amino acid that arrests the cell cycle in the G (1) phase before entry into S phase. Here, we show that mimosine interrupts the binding of Ctf4 to

chromatin, which is essential for the initiation of DNA replication in HeLa cells, and this effect is mediated by the Hif-1alpha-dependent increase in the level of p27. Depletion of Hif-1alpha results in an increased binding of Ctf4 to chromatin and the entry of cells into S phase even in the presence of mimosine. These results suggest that the binding of Ctf4 to chromatin is the target of the Hif-1alpha-dependent checkpoint pathway for cell cycle arrest in G (1) phase. Although we observed Hif-1alpha-dependent arrest in mimosine-treated cells, it is possible that Ctf4 may act as a common target for G (1) arrest in various other checkpoint pathways.

Patathananone, S., et al. (2016). "Bioactive compounds from crocodile (Crocodylus siamensis) white blood cells induced apoptotic cell death in hela cells." Environ Toxicol **31**(8): 986-997.

Crocodile (Crocodylus siamensis) white blood cell extracts (WBCex) were examined for anticancer activity in HeLa cell lines using the MTT assay. The percentage viability of HeLa cells significantly deceased after treatment with WBCex in a dose- and time-dependent manner. The IC50 dose was suggested to be approximately 225 mug/mL protein. Apoptotic cell death occurred in a time-dependent manner based on investigation by flow cytometry using annexin V-FITC and PI staining. DAPI nucleic acid staining indicated increased chromatin condensation. Caspase-3, -8 and -9 activities also increased, suggesting the induction of the caspase-dependent apoptotic pathway. Furthermore, the mitochondrial membrane potential (DeltaPsim ) of HeLa cells was lost as a result of increasing levels of Bax and reduced levels of Bcl-2, Bcl-XL, Bcl-Xs, and XIAP. The decreased DeltaPsim led to the release of cytochrome c and the activation of caspase-9 and -3. Apoptosis-inducing factor translocated into the nuclei, and endonuclease G (Endo G) was released from the mitochondria. These results suggest that anticancer agents in WBCex can induce apoptosis in HeLa cells via both caspase-dependent and -independent pathways. (c) 2015 Wiley Periodicals, Inc. Environ Toxicol 31: 986-997, 2016.

Paul, A., et al. (2013). "Diarylheptanoid-myricanone isolated from ethanolic extract of Myrica cerifera shows anticancer effects on HeLa and PC3 cell lines: signalling pathway and drug-DNA interaction." J Integr Med **11**(6): 405-415.

OBJECTIVE: To test if myricanone (C21H24O5), a cyclic diarylheptanoid, has anticancer effects on two different cancer cell lines HeLa and PC3. The present study was conducted with a note on the drug-DNA interaction and apoptotic signalling pathway. METHODS: Several studies like cytotoxicity, nuclear damage, annexin-V-fluorescein isothiocyanate (FITC)/propidium iodide (PI)-labelled apoptotic assay and cell cycle arrest, immunoblot and reverse transcriptase-polymerase chain reaction (RT-PCR) were used following standard protocols. Circular dichroism (CD) spectroscopy was also done to evaluate whether myricanone effectively interacted with DNA to bring about conformational changes that could strongly inhibit the cancer cell proliferation. RESULTS: Myricanone showed a greater cytotoxic effect on PC3 cells than on HeLa cells. Myricanone promoted G0/G1 arrest in HeLa cells and S phase arrest in PC3 cells. Nuclear condensation and annexin V-FITC/PI studies revealed that myricanone promoted apoptotic cell death. CD spectroscopic data indicated that myricanone had an interaction with calf thymus DNA that changed DNA structural conformation. RT-PCR and immunoblot studies revealed that myricanone activated the apoptotic signalling cascades through down-regulation of transcription factors like nuclear factor-kappaB (NF-kappaB) (p65), and signal transducers and activators of transcription 3 (STAT3); cell cycle regulators like cyclin D1, and survivin and other signal proteins like Bcl-2 and up-regulation of Bax, caspase-9 and caspase-3. CONCLUSION: Myricanone induced apoptosis in both types of cancer cells by triggering caspase activation, and suppression of cell proliferation by down-regulation of NF-kappaB and STAT3 signalling cascades, which makes it a suitable candidate for possible use in the formulation of therapeutic agent for combating cancer.

Penjweini, R., et al. (2017). "Investigating the effect of poly-l-lactic acid nanoparticles carrying hypericin on the flow-biased diffusive motion of HeLa cell organelles." J Pharm Pharmacol.

OBJECTIVES: In this study, we investigate in human cervical epithelial HeLa cells the intracellular dynamics and the mutual interaction with the organelles of the poly-l-lactic acid nanoparticles (PLLA NPs) carrying the naturally occurring hydrophobic photosensitizer hypericin. METHODS: Temporal and spatiotemporal image correlation spectroscopy was used for the assessment of the intracellular diffusion and directed motion of the nanocarriers by tracking the hypericin fluorescence. Using image cross-correlation spectroscopy and specific fluorescent labelling of endosomes, lysosomes and mitochondria, the NPs dynamics in association with the cell organelles was studied. Static colocalization experiments were interpreted according to the Manders' overlap coefficient. KEY FINDINGS: Nanoparticles associate with a small fraction of the whole-organelle population. The organelles moving with NPs exhibit higher directed motion compared to those moving without them. The rate of the directed motion drops substantially after the application of

nocodazole. The random component of the organelle motions is not influenced by the NPs. CONCLUSIONS: Image correlation and cross-correlation spectroscopy are most appropriate to unravel the motion of the PLLA nanocarrier and to demonstrate that the rate of the directed motion of organelles is influenced by their interaction with the nanocarriers. Not all PLLA-hypericin NPs are associated with organelles.

Petrov Iu, P. (2013). "[Response of HeLa cells to mitomycine C. I. Cell division]." Tsitologiia **55**(12): 874-878.

Using light microscopy, time-lapse imaging, and digital image analysis, the effect of mitomycine C (10 mug/ml) on HeLa-M cells has been studied. It has been shown that, after a 2 h contact with mitomycine, the cells could be separated into 2 groups: M-1--the functional cells surviving after division but non-entering mitosis any more; M-II--the cells entering mitosis but incapable to finish it; they are lost. Mitomycine C is known to specifically block DNA replication being located in the DNA minor groove. It should inhibit PHK synthesis if one follows the standard hypothesis of a transcription bubble formation. However, increasing the cell and nucleolus area during the M-I cell growth suggests that RNA and protein synthesis is not blocked. The author concludes that the presented data confirm his hypothesis about RNA synthesis in the major DNA groove (Petrov, 2006).

Petrov Iu, P., et al. (2013). "[Vital measurement of optical density of HeLa cell line]." Tsitologiia **55**(9): 601-608.

Light absorption by the live intact HeLa cells during light microscopy was studied. The light absorption may be considered as a parameter analogous to optical density used in spectrophotometry. This parameter can be used as a quantitative characteristic of life cell as well as intracellular structures. It is shown that cells from one population but belonging to two different clones were differed by their optical density. Optical density correlation between the shadow peripheral regions of the cells and a actin localization in these regions was established.

Petrov Iu, P., et al. (2014). "[Colocalization of nucleoli in cell nuclei of HeLa line]." Tsitologiia **56**(3): 197-203.

The pattern of localization of nucleoli relative to each other and to cell nucleus was studied in M-HeLa cell line. For this puspose, the following morphometric parameters were introduced. For the two-nucleolar cells: 1) the ratio of the nucleus long axis to the length of a segment between the centers of the nucleoli, and 2) the angle between the segment connecting the centers of the nucleoli and a longitudinal axis of cell nucleus. For the three-nucleolar cells: the ratio perimeter of the nucleus to perimeter of a triangle with vertexes in the centre of nucleoli. We have shown that the values of these parameters are individual for each cell but their values remain constant for the cell in spite of the changes in cell shape. These results allow us to conclude that, on the one hand, the nucleoli colocalization is individual for each cell, and, on the other hand, location of nucleoli in relation to nucleus is not changed during interphase. Thereby, the distance between nucleoli increases proportionally with nucleus growth.

Pirkkanen, J. S., et al. (2017). "The CGL1 (HeLa x Normal Skin Fibroblast) Human Hybrid Cell Line: A History of Ionizing Radiation Induced Effects on Neoplastic Transformation and Novel Future Directions in SNOLAB." Radiat Res **188**(4.2): 512-524.

Cellular transformation assays have been utilized for many years as powerful in vitro methods for examining neoplastic transformation potential/frequency and mechanisms of carcinogenesis for both chemical and radiological carcinogens. These mouse and human cell based assays are labor intensive but do provide quantitative information on the numbers of neoplastically transformed foci produced after carcinogenic exposure and potential molecular mechanisms involved. Several mouse and human cell systems have been generated to undertake these studies, and they vary in experimental length and endpoint assessment. The CGL1 human cell hybrid neoplastic model is a non-tumorigenic pre-neoplastic cell that was derived from the fusion of HeLa cervical cancer cells and a normal human skin fibroblast. It has been utilized for the several decades to study the carcinogenic/neoplastic transformation potential of a variety of ionizing radiation doses, dose rates and radiation types, including UV, X ray, gamma ray, neutrons, protons and alpha particles. It is unique in that the CGL1 assay has a relatively short assay time of 18-21 days, and rather than relying on morphological endpoints to detect neoplastic transformation utilizes a simple staining method that detects the tumorigenic marker alkaline phosphatase on the neoplastically transformed cells cell surface. In addition to being of human origin, the CGL1 assay is able to detect and quantify the carcinogenic potential of very low doses of ionizing radiation (in the mGy range), and utilizes a neoplastic endpoint (re-expression of alkaline phosphatase) that can be detected on both viable and paraformaldehyde fixed cells. In this article, we review the history of the CGL1

neoplastic transformation model system from its initial development through the wide variety of studies examining the effects of all types of ionizing radiation on neoplastic transformation. In addition, we discuss the potential of the CGL1 model system to investigate the effects of near zero background radiation levels available within the radiation biology lab we have established in SNOLAB.

Piva, T. J., et al. (2012). "Increased activity of cell surface peptidases in HeLa cells undergoing UV-induced apoptosis is not mediated by caspase 3." Int J Mol Sci **13**(3): 2650-2675.

We have previously shown that in HeLa cells treated with a variety of agents there is an increase in cell surface peptidase (CSP) activity in those cells undergoing apoptosis. The increase in CSP activity observed in UVB-irradiated cells undergoing apoptosis was unaffected when the cultures were treated with the aminopeptidase inhibitor bestatin, and matrix metalloprotease inhibitor BB3103, but greatly enhanced when treated with the caspase 3 inhibitor-DEVD, and reduced in the presence of the poly (ADP-ribose) polymerase (PARP) inhibitor-3-aminobenzamide (3AB). Neither 3AB nor DEVD had an effect on the gross morphology of the apoptotic cells observed under electron microscopy, nor did they have an effect on phosphatidylserine eversion on the cell membrane, or that of PARP cleavage. All the agents except for DEVD had no effect on the level of caspase 3 activity in the cells. The results suggest that other caspases may cleave PARP in these cells. Both 3AB and DEVD treatment reduced the level of actin cleavage seen in the apoptotic cells. The increase in CSP activity observed in cells undergoing UVB-induced apoptosis appears to involve PARP but not caspase 3.

Plate, M., et al. (2010). "Identification and characterization of CMTM4, a novel gene with inhibitory effects on HeLa cell growth through Inducing G2/M phase accumulation." Mol Cells **29**(4): 355-361.

Human CMTM is a novel gene family consisting of CKLF and CMTM1-8. CMTM4 is the most conserved gene and has three RNA splicing forms designated as CMTM4-v1, -v2 and -v3, but in many types of tissue and cell lines, only CMTM4-v1 and -v2 could be detected. CMTM4-v2 is the full length cDNA product, which has been highly conserved during evolution. CMTM4-v1 and -v2 are broadly expressed in normal types of tissue. They are distributed on the cell membrane and across the cytoplasm in a speckled pattern. Overexpression of CMTM4-v1 and -v2 can inhibit HeLa cell growth via G2/M phase accumulation without inducing apoptosis. Therefore, CMTM4 might be an important gene involved in cell growth and cell cycle regulation.

Plotkin, B. J., et al. (2016). "Herpes Simplex Virus (HSV) Modulation of Staphylococcus aureus and Candida albicans Initiation of HeLa 299 Cell-Associated Biofilm." Curr Microbiol **72**(5): 529-537.

Although herpes simplex virus type-1 (HSV-1), and type-2 (HSV-2), Staphylococcus aureus and Candida albicans co-habit the oral and genital mucosa, their interaction is poorly understood. We determined the effect HSV has on bacterial and/or fungal adherence, the initial step in biofilm formation. HeLa229 cells were infected with HSV-1 (KOS) gL86 or HSV-2 (KOS) 333gJ (-) at a multiplicity of infection (MOI) of 50 and 10. S. aureus (ATCC 25923) and/or C. albicans (yeast forms or germ tube forms) were co-incubated for 30 min (37 degrees C; 5 % CO2; 5:1 organism: HeLa cell ratio; n = 16) with virus-infected HeLa cells or uninfected HeLa cell controls. Post-incubation, the monolayers were washed (3x; PBS), lysed (RIPA), and the lysate plated onto Fungisel and/or mannitol salts agar for standard colony count. The level of HeLa-associated S. aureus was significantly decreased ($P < 0.05$) for both HSV-1- and HSV-2-infected cells, as compared to virus-free HeLa cell controls (38 and 59 % of control, respectively). In contrast, HSV-1 and HSV-2 significantly ($P < 0.05$) enhanced HeLa cell association of C. albicans yeast forms and germ tube approximately two-fold, respectively. The effect of S. aureus on germ tube and yeast form adherence to HSV-1- and HSV-2-infected cells was specific for the Candida phenotype tested. Our study suggests that HSV, while antagonist towards S. aureus adherence enhances Candida adherence. Furthermore, the combination of the three pathogens results in S. aureus adherence that is either unaffected, or partially restored depending on both the herpes viral species and the fungal phenotype present.

Pourgonabadi, S., et al. (2017). "Cytotoxic and apoptogenic effects of Bryonia aspera root extract against Hela and HN-5 cancer cell lines." Avicenna J Phytomed **7**(1): 66-72.

OBJECTIVE: Bryonia aspera (Stev. ex Ledeb) is a plant that grows in northeast of Iran. In the present study, cytotoxic and apoptogenic properties of B. aspera root extract was determined against HN-5(head and neck squamous cell carcinoma) and Hela (cervix adenocarcinoma) cell lines. MATERIALS AND METHODS: HN-5 and Hela cell lines were cultured in DMEM medium and incubated with different concentrations of B. aspera root extract. Cell viability was quantitated by MTT assay and the optical absorbance was measured at 570 nm (620 nm as the reference) by an ELISA reader, in each experiment.

Apoptotic cells were assessed using PI staining of DNA fragmentation by flow cytometry (sub-G1 peak). The B. aspera inhibited 50% growth (IC50) of Hela and HN-5 cell lines at 100+/-28 mug/ml and 12.5+/-4 mug/ml, respectively after 48 hr of incubation. RESULTS: Cell viability assay showed that inhibitory effects of B. aspera were time and dose-dependent in both cell lines, which were consistent with morphological changes, observed under light microscope. Apoptosis was investigated by flow cytometry in which percentage of apoptotic cells increased in a dose and time-dependent manner. CONCLUSION: Based on our data, B. aspera has cytotoxic effects in which apoptosis played an important role. Further evaluations are needed to assess the possible anti-tumor properties of this plant.

Pradines, B., et al. (2015). "Cell line-dependent cytotoxicity of poly (isobutylcyanoacrylate) nanoparticles coated with chitosan and thiolated chitosan: Insights from cultured human epithelial HeLa, Caco2/TC7 and HT-29/MTX cells." Int J Pharm **491**(1-2): 17-20.

Nanoparticles composed of poly (isobutylcyanoacrylate) core coated with a mixture of chitosan and thiolated chitosan have already shown promising results in terms of mucoadhesion and permeation enhancement properties of pharmaceutical active drugs delivered via mucosal routes. In the present work, the cytotoxicity of these nanoparticles was first investigated using direct contact assay on undifferentiated human cervix epithelial HeLa cells. The results showed strong toxicity in HeLa cells for the two investigated concentrations 25 and 50 mug/mL. The cytotoxic effect was mainly attributed to the poly (isobutylcyanoacrylate) core since no significant differences in nanoparticle cytotoxicity were reported when nanoparticle shell composition was modified by adding chitosan or thiolated chitosan. In contrast, lower nanoparticle toxicity was reported using human fully-differentiated enterocyte-like Caco-2/TC7, and fully-differentiated mucus-secreting HT-29/MTX cells forming monolayer in culture mimicking an intestinal epithelial barrier. This study demonstrated that the toxicity of poly (isobutylcyanoacrylate) nanoparticles is highly cell line-dependent.

Priyadarsini, R. V., et al. (2010). "The neem limonoids azadirachtin and nimbolide induce cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells." Free Radic Res **44**(6): 624-634.

Limonoids from the neem tree (Azadirachta indica) have attracted considerable research attention in recent years owing to their potent antioxidant and anti-proliferative effects. The present study was designed to investigate the cellular and molecular mechanisms by which azadirachtin and nimbolide exert cytotoxic effects in the human cervical cancer (HeLa) cell line. Both azadirachtin and nimbolide significantly suppressed the viability of HeLa cells in a dose-dependent manner by inducing cell cycle arrest at G0/G1 phase accompanied by p53-dependent p21 accumulation and down-regulation of the cell cycle regulatory proteins cyclin B, cyclin D1 and PCNA. Characteristic changes in nuclear morphology, presence of a subdiploid peak and annexin-V staining pointed to apoptosis as the mode of cell death. Increased generation of reactive oxygen species with decline in the mitochondrial transmembrane potential and release of cytochrome c confirmed that the neem limonoids transduced the apoptotic signal via the mitochondrial pathway. Altered expression of the Bcl-2 family of proteins, inhibition of NF-kappaB activation and over-expression of caspases and survivin provide compelling evidence that azadirachtin and nimbolide induce a shift of balance toward a pro-apoptotic phenotype. Antioxidants such as azadirachtin and nimbolide that can simultaneously arrest the cell cycle and target multiple molecules involved in mitochondrial apoptosis offer immense potential as anti-cancer therapeutic drugs.

Qi, Z., et al. (2014). "Phosphorylation of heat shock protein 27 antagonizes TNF-alpha induced HeLa cell apoptosis via regulating TAK1 ubiquitination and activation of p38 and ERK signaling." Cell Signal **26**(7): 1616-1625.

Tumor necrosis factor (TNF)-alpha is a potent cytokine that regulates critical cellular processes including apoptosis. TNF-alpha usually triggers both survival and apoptotic signals in various cell types. Heat shock protein 27 (HSP27), an important cellular chaperone, is believed to protect cells from apoptosis. HSP27 can be phosphorylated and changed its cellular function according to different stimuli. However, available reports on the role of HSP27 phosphorylation in apoptosis remain elusive. In this study, we investigated the role of HSP27 phosphorylation in TNF-alpha induced apoptosis in human cervical carcinoma (HeLa) cells. We found that TNF-alpha induced apoptosis was enhanced if we suppressed the TNF-alpha induced HSP27 phosphorylation by specific inhibitor CMPD1 or MAPKAPK2 (MK2) knockdown or by overexpression of non-phosphorylatable mutant HSP27-3A. Through co-immunoprecipitation and confocal microscopy, we observed that HSP27 associated with transforming growth factor-beta (TGF-beta)-activated kinase 1 (TAK1) in response to TNF-alpha stimulation. By blocking MK2 activity or overexpressing phospho-

mimetic mutant Hsp27-3D, we further showed that HSP27 phosphorylation facilitated the TNF-alpha induced ubiquitination and phosphorylation of TAK1 and the activations of p38 MAPK and ERK, the TAK1 downstream pro-survival signaling. In addition, we also found that increased HSP27 phosphorylation inhibited TRADD ubiquitination but did not influence the binding between TRADD and FADD in a pro-apoptotic complex. Taken together, our data indicated that HSP27 phosphorylation was involved in modulating the TNF-alpha induced apoptosis via interacting with TAK1 and regulating TAK1 post-translational modifications in HeLa cells. This study demonstrates that HSP27 phosphorylation serves as a novel regulator in TNF-alpha-induced apoptosis, and provides a new insight into the cytoprotective role of HSP27 phosphorylation.

Qin, H. X., et al. (2016). "[miR-143 inhibits cell proliferation through targeted regulating the expression of K-ras gene in HeLa cells]." Zhonghua Zhong Liu Za Zhi **38**(12): 893-897.

Objective: To explore the effect of microRNA miR-143 on the proliferation of cervical cancer HeLa cells through targeted regulating the expression of K-ras gene. Methods: The luciferase report carrier containing wild type 3'-UTR of K-ras gene (K-ras-wt) or mutated 3'-UTR of the K-ras (K-ras-mut) were co-transfected with iR-143 mimic into the HeLa cells respectively, and the targeting effect of miR-143 in the transfectants was verified by the dual luciferase report system. HeLa cells were also transfected with miR-143 mimic (miR-143 mimic group), mimic control (negative control group), and miR-143 mimic plus K-ras gene (miR-143 mimic+ K-ras group), respectively. The expression of miR-143 in the transfected HeLa cells was detected by real-time PCR (RT-PCR), and the expression of K-ras protein was detected by Western blot. The cell proliferation activity of each group was examined by MTT assay. In addition, human cervical cancer tissue samples (n=5) and cervical intraepithelial neoplasia tissue samples (n=5) were also examined for the expression of miR-143 and K-ras protein by RT-PCR and Western blot, respectively. Results: The luciferase report assay showed that co-transfection with miR-143 mimic decreased the luciferase activity of the K-ras-wt significantly, but did not inhibit the luciferase activity of the K-ras-mut. The expression of miR-143 in the HeLa cells transfected with miR-143 mimic was significantly higher than that in the HeLa cells transfected with the mimic control (3.31+/-0.45 vs 0.97+/-0.22, P<0.05). The MTT assay revealed that the cell proliferative activity of the miR-143 mimic group was significantly lower than that of the negative control group (P<0.05), and the cell proliferative activity of the miR-143 mimic+ K-ras group was also significantly lower than the control group (P<0.05) but higher than the miR-143 mimic group significantly (P<0.05). The expression levels of K-ras protein in the miR-143 mimic group, the negative control group and the miR-143 mimic+ K-ras group were lowest, moderate, and highest, respectively (115.27+/-34.08, 521.36+/-41.89, and 706.52+/-89.44, all P<0.05). In the tissue samples, the miR-143 expression in the cervical cancer group was significantly lower than that of the cervical intraepithelial neoplasia group (0.32+/-0.06 vs. 0.93+/-0.17, P<0.05); whereas the K-ras protein expression in the cervical cancer group was significantly higher than that in the cervical intraepithelial neoplasia group (584.39+/-72.34 vs. 114.23+/-25.82, P<0.05). Conclusions: In vitro, miR-143 can inhibit the proliferative activity of HeLa cells through targeted regulating the expression of K-ras gene. In human cervical cancer tissues of a small sample set, the expression of miR-143 is downregulated, and the expression of K-ras is upregulated.

Resendes, K. K. (2015). "Using HeLa cell stress response to introduce first year students to the scientific method, laboratory techniques, primary literature, and scientific writing." Biochem Mol Biol Educ **43**(2): 110-120.

Incorporating scientific literacy into inquiry driven research is one of the most effective mechanisms for developing an undergraduate student's strength in writing. Additionally, discovery-based laboratories help develop students who approach science as critical thinkers. Thus, a three-week laboratory module for an introductory cell and molecular biology course that couples inquiry-based experimental design with extensive scientific writing was designed at Westminster College to expose first year students to these concepts early in their undergraduate career. In the module students used scientific literature to design and then implement an experiment on the effect of cellular stress on protein expression in HeLa cells. In parallel the students developed a research paper in the style of the undergraduate journal BIOS to report their results. HeLa cells were used to integrate the research experience with the Westminster College "Next Chapter" first year program, in which the students explored the historical relevance of HeLa cells from a sociological perspective through reading The Immortal Life of Henrietta Lacks by Rebecca Skloot. In this report I detail the design, delivery, student learning outcomes, and assessment of this module, and while this exercise was designed for an introductory course at a small primarily undergraduate institution, suggestions for modifications at larger universities or

for upper division courses are included. Finally, based on student outcomes suggestions are provided for improving the module to enhance the link between teaching students skills in experimental design and execution with developing student skills in information literacy and writing.

Rimkute, L., et al. (2016). "The role of neural connexins in HeLa cell mobility and intercellular communication through tunneling tubes." BMC Cell Biol **17**: 3.

BACKGROUND: Membranous tunneling tubes (TTs) are a recently discovered new form of communication between remote cells allowing their electrical synchronization, migration, and transfer of cellular materials. TTs have been identified in the brain and share similarities with neuronal processes. TTs can be open-ended, close-ended or contain functional gap junctions at the membrane interface. Gap junctions are formed of two unapposed hemichannels composed of six connexin (Cx) subunits. There are evidences that Cxs also play channel-independent role in cell adhesion, migration, division, differentiation, formation of neuronal networks and tumorigenicity. These properties of Cxs and TTs may synergetically determine the cellular and intercellular processes. Therefore, we examined the impact of Cxs expressed in the nervous system (Cx36, Cx40, Cx43, Cx45, and Cx47) on: 1) cell mobility; 2) formation and properties of TTs; and 3) transfer of siRNA between remote cells through TTs. RESULTS: We have identified two types of TTs between HeLa cells: F-actin rich only and containing F-actin and alpha-tubulin. The morphology of TTs was not influenced by expression of examined connexins; however, Cx36-EGFP-expressing cells formed more TTs while cells expressing Cx43-EGFP, Cx45, and Cx47 formed fewer TTs between each other compared with wt and Cx40-CFP-expressing cells. Also, Cx36-EGFP and Cx40-CFP-expressing HeLa cells were more mobile compared with wt and other Cxs-expressing cells. TTs containing Cx40-CFP, Cx43-EGFP, or Cx47 gap junctions were capable of transmitting double-stranded small interfering RNA; however, Cx36-EGFP and Cx45 were not permeable to it. In addition, we show that Cx43-EGFP-expressing HeLa cells and laryngeal squamous cell carcinoma cells can couple to the mesenchymal stem cells through TTs. CONCLUSIONS: Different Cxs may modulate the mobility of cells and formation of TTs in an opposite manner; siRNA transfer through the GJ-containing TTs is Cx isoform-dependent.

Rizvi, M. A., et al. (2015). "Nuclear blebbing of biologically active organoselenium compound towards human cervical cancer cell (HeLa): in vitro DNA/HSA binding, cleavage and cell imaging studies." Eur J Med Chem **90**: 876-888.

New pharmacophore organoselenium compound (1) was designed, synthesized and characterized by various spectroscopic methods (IR, ESI-MS, (1)H, (13)C and (77)Se NMR) and further confirmed by X-ray crystallography. Compound 1 consists of two 3,5-bis (trifluoromethyl)phenyl units which are connected to the selenium atom via the organometallic C-Se bond. In vitro DNA binding studies of 1 was investigated by absorption and emission titration methods which revealed that 1 recognizes the minor groove of DNA in accordance with molecular docking studies with the DNA duplex. Gel electrophoretic assay demonstrates the ability of 1 to cleave pBR322 DNA through hydrolytic process which was further validated by T4 religation assay. To understand the drug-protein interaction of which ultimate molecular target was DNA, the affinity of 1 towards HSA was also investigated by the spectroscopic and molecular modeling techniques which showed hydrophobic interaction in the subdomain IIA of HSA. Furthermore, the intracellular localization of 1 was evidenced by cell imaging studies using HeLa cells.

Rodamporn, S., et al. (2011). "HeLa cell transfection using a novel sonoporation system." IEEE Trans Biomed Eng **58**(4): 927-934.

Sonoporation has been shown to have an important role in biotechnology for gene therapy and drug delivery. This paper presents a novel microfluidic sonoporation system that achieves high rates of cell transfection and cell viability by operating the sonoporation chamber at resonance. The paper presents a theoretical analysis of the resonant sonoporation chamber design, which achieves sonoporation by forming an ultrasonic standing wave across the chamber. A piezoelectric transducer (PZT 26) is used to generate the ultrasound and the different material thicknesses have been identified to give a chamber resonance at 980 kHz. The efficiency of the sonoporation system was determined experimentally under a range of sonoporation conditions and different exposures time (5, 10, 15, and 20 s, respectively) using HeLa cells and plasmid (peGFP-N1). The experimental results achieve a cell transfection efficiency of 68.9% (analysis of variance, ANOVA, $p < 0.05$) at the resonant frequency of 980 kHz at 100 V (p-p) (19.5 MPa) with a cell viability of 77% after 10 s of insonication.

Roggers, R. A., et al. (2012). "Chemically reducible lipid bilayer coated mesoporous silica nanoparticles demonstrating controlled release and HeLa and normal mouse liver cell biocompatibility

and cellular internalization." Mol Pharm **9**(9): 2770-2777.

A controlled release system composed of mesoporous silica nanoparticles with covalently bound dipalmitoyl moieties supporting phosphorylated lipids has been successfully synthesized and characterized. This MSN system demonstrates controlled release of fluorescein molecules under disulfide reducing conditions. Flow cytometry analyses confirm increased biocompatibility of the resulting lipid bilayer MSNs (LB-MSNs) from nonfunctional MSNs. Fluorescently labeled LB-MSNs are examined via confocal fluorescent microscopy ex vivo and were found to enter both normal and cancer cell lines. The LB-MSNs presented here have potential to be used as rapid and diverse functionalized, stable liposome analogues for drug delivery.

Roomi, M. W., et al. (2015). "Effects of a nutrient mixture on immunohistochemical localization of cancer markers in human cervical cancer HeLa cell tumor xenografts in female nude mice." Exp Ther Med **9**(2): 294-302.

Although fully treatable in the early stages, once cervical cancer has metastasized, patient outcome is poor. The main objective of this study was to examine the effect of dietary supplementation with a nutrient mixture (NM) containing lysine, ascorbic acid, proline, green tea extract and other micronutrients on HeLa cell xenografts in nude female mice. Tumor growth was measured and immunohistochemical staining was evaluated for the following cancer markers: Ki67 (proliferation); matrix metalloproteinase (MMP)-2 and -9 (invasion/metastasis); vascular endothelial growth factor (VEGF) (angiogenesis); terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and B-cell lymphoma 2 (Bcl-2) (apoptosis); cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) (inflammation); and glutathione S-transferase pi (GSTpi) (a general cancer marker). Following housing for a week, 5/6-week-old female athymic nude mice (n=12) were inoculated subcutaneously with 3x10(6) HeLa cells in 0.2 ml phosphate-buffered saline and 0.1 ml Matrigel and randomly divided into two groups; control group mice were fed regular mouse chow and NM group mice the regular diet supplemented with 0.5% NM (w/w). After four weeks, the mice were sacrificed and their tumors were excised and processed for histology. The NM strongly inhibited the growth of HeLa xenografts in nude mice. The mean tumor weight was reduced to 59% (P=0.001) in the mice fed the NM compared with the tumor weight in the controlled diet mice. Ki67, MMP-2 and -9, VEGF, TUNEL, Bcl-2, COX-2, iNOS and GSTpi all showed a lower intensity and frequency of staining in the NM group compared with that in the control group. In conclusion, NM supplementation strongly inhibited tumor growth and cancer markers in female nude mice injected with HeLa xenografts.

Rupachandra, S. and D. V. Sarada (2014). "Induction of apoptotic effects of antiproliferative protein from the seeds of Borreria hispida on lung cancer (A549) and cervical cancer (HeLa) cell lines." Biomed Res Int **2014**: 179836.

A 35 KDa protein referred to as F3 was purified from the seeds of Borreria hispida by precipitation with 80% ammonium sulphate and gel filtration on Sephadex G-100 column. RP-HPLC analysis of protein fraction (F3) on an analytical C-18 column produced a single peak, detected at 220 nm. F3 showed an apparent molecular weight of 35 KDa by SDS PAGE and MALDI-TOF-MS analyses. Peptide mass fingerprinting analysis of F3 showed the closest homology with the sequence of 1-aminocyclopropane-1-carboxylate deaminase of Pyrococcus horikoshii. The protein (F3) exhibited significant cytotoxic activity against lung (A549) and cervical (HeLa) cancer cells in a dose-dependent manner at concentrations ranging from 10 microg to 1000 microg/mL, as revealed by the MTT assay. Cell cycle analysis revealed the increased growth of sub-G0 population in both cell lines exposed to a concentration of 1000 microg/mL of protein fraction F3 as examined from flow cytometry. This is the first report of a protein from the seeds of Borreria hispida with antiproliferative and apoptotic activity in lung (A549) and cervical (HeLa) cancer cells.

Saghaie, L., et al. (2013). "Synthesis, analysis and cytotoxic evaluation of some hydroxypyridinone derivatives on HeLa and K562 cell lines." Res Pharm Sci **8**(3): 185-195.

A range of iron bidentae ligands containing the chelating moiety 3- hydroxypyridin-4-ones (HPOs) have been synthesized via a single or a three-step synthetic pathway. In the single-step reaction, maltol was directly reacted by suitable primary amine and in the second synthetic method; benzylated maltol was reacted with related amines to give 1-substuted-2-methyl-3-benzyloxypyridin-4-one derivatives. Finally, removal of the benzyl group under acidic conditions was performed by catalytic hydrogenation to yield the favored bidentate chelators as HCl salt. The partition coefficient of the free ligands and their iron (III) complexes between an aqueous phase buffered at pH 7.4 and 1-octanol were also determined. The cytotoxic effects of these iron chelators against HeLa and K562 cell lines were evaluated using MTT assay and the results showed that cytotoxicity was closely related to the lipophilicity of compounds so that the most lipophilic compound (4g) revealed the highest activity

and compound 4e as a more hydrophilic agent (Kpart; 0.05) showed the lowest cytotoxic effect.

Saha, S., et al. (2015). "Isolation and Characterization of Enteropathogenic Escherichia coli (EPEC) in Paediatric Diarrhoeal Patients by Detection of bfpA gene by PCR and HeLa cell Adherence Assay." Mymensingh Med J **24**(4): 800-805.

This study has been undertaken to investigate the isolation and identification of EPEC strains from paediatric diarrhoeal patients. The study was carried out in the department of Microbiology, Sir Salimullah Medical College & Mitford Hospital, Bangladesh during January to December, 2011. Total 272 samples were studied. Samples from patients with diarrhoea were collected from two tertiary care hospital. At first Esch. coli were isolated from these specimens using standard microbiological techniques and then EPEC strains were identified on the basis of presence of bundle forming pilus (bfpA) gene. Virulence of EPEC strains were determined by detection of bfpA gene and observing localized adherence (LA) in HeLa cell adherence assay. Esch. coli was isolated and identified from all the 272 samples from patients using standard microbiological techniques. Among 272 samples 20(7.35%) isolates were identified as EPEC on the basis of presence of bfpA gene detected by polymerase chain reaction. EPEC strains were identified from those 240 samples, from which Esch. coli had been isolated only. Out of twenty EPEC strains, 17 strains (85%) showed a pattern of localized adherence in Hela cell adherence assay. EPEC strains can be identified by bfpA gene detection and by adherence assays. HeLa cell adherence assay is the most specific method for detection of EPEC strains which has bfpA gene, responsible for localized adherence (LA) in HeLa cell line. Rapid and reliable detection of EPEC is required for successful microbiological surveillance and for treatment of EPEC mediated diarrhoeal disease.

Saha, S. K. and A. R. Khuda-Bukhsh (2014). "Berberine alters epigenetic modifications, disrupts microtubule network, and modulates HPV-18 E6-E7 oncoproteins by targeting p53 in cervical cancer cell HeLa: a mechanistic study including molecular docking." Eur J Pharmacol **744**: 132-146.

Increased evidence of chemo-resistance, toxicity and carcinogenicity necessitates search for alternative approaches for determining next generation cancer therapeutics and targets. We therefore tested the efficacy of plant alkaloid berberine on human papilloma virus (HPV) -18 positive cervical cancer cell HeLa systematically-involving certain cellular, viral and epigenetic factors. We observed disruptions of microtubule network and changes in membrane topology due to berberine influx through confocal and atomic force microscopies (AFM). We examined nuclear uptake, internucleosomal DNA damages, mitochondrial membrane potential (MMP) alterations and cell migration assays to validate possible mode of cell death events. Analytical data on interactions of berberine with pBR322 through fourier transform infrared (FTIR) and gel migration assay strengthen berberines biologically significant DNA binding abilities. We measured cellular uptake, DNA ploidy and DNA strand-breaks through fluorescence activated cell sorting (FACS). To elucidate epigenetic modifications, in support of DNA binding associated processes, if any, we conducted methylation-specific restriction enzyme (RE) assay, methylation specific-PCR (MSP) and expression studies of histone proteins. We also analyzed differential interactions and localization of cellular tumor suppressor p53 and viral oncoproteins HPV-18 E6-E7 through siRNA approach. We further made in-silico approaches to determine possible binding sites of berberine on histone proteins. Overall results indicated cellular uptake of berberine through cell membrane depolarization causing disruption of microtubule networks and its biological DNA binding abilities that probably contributed to epigenetic modifications. Results of modulation in p53 and viral oncoproteins HPV-18 E6-E7 by berberine further proved its potential as a promising chemotherapeutic agent in cervical cancer.

Salazar-Aguilar, S., et al. (2017). "Sechium edule (Jacq.) Swartz, a New Cultivar with Antiproliferative Potential in a Human Cervical Cancer HeLa Cell Line." Nutrients **9**(8).

The Sechium edule Perla Negra cultivar is a recently-obtained biological material whose progenitors are S. edule var. nigrum minor and S. edule var. amarus silvestrys, the latter of which has been reported to have antiproliferative activity against the HeLa P-388 and L-929 cancer cell lines. The present study aimed to determine if the methanolic extract of the fruit of the Perla Negra cultivar had the same biological activity. The methanolic extract was phytochemically characterized by thin layer chromatography (TLC) and column chromatography (CC), identifying the terpenes and flavonoids. The compounds identified via high performance liquid chromatography (HPLC) were Cucurbitacins B, D, E, and I for the terpene fractions, and Rutin, Phlorizidin, Myricetin, Quercetin, Naringenin, Phloretin, Apigenin, and Galangin for the flavonoid fractions). Biological activity was evaluated with different concentrations of the methanolic extract in the HeLa cell line and normal lymphocytes. The methanolic extract inhibited the proliferation of HeLa cells (IC50 1.85 microg.mL (-1)), but the lymphocytes were affected by the extract

(IC50 30.04 microg.mL (-1)). Some fractions, and the pool of all of them, showed inhibition higher than 80% at a concentration of 2.11 microg.mL (-1). Therefore, the biological effect shown by the methanolic extract of the Perla Negra has some specificity in inhibiting tumor cells and not normal cells; an unusual feature among molecules investigated as potential biomedical agents.

Samudram, A., et al. (2016). "Passive permeability and effective pore size of HeLa cell nuclear membranes." Cell Biol Int **40**(9): 991-998.

Nuclear pore complexes in the nuclear membrane act as the sole gateway of transport of molecules from the cytoplasm to the nucleus and vice versa. Studies on biomolecular transport through nuclear membranes provide vital data on the nuclear pore complexes. In this work, we use fluorescein isothiocyanate-labeled dextran molecules as a model system and study the passive nuclear import of biomolecules through nuclear pore complexes in digitonin-permeabilized HeLa cells. Experiments are carried out under transient conditions in the time lapse imaging scheme using an in-house constructed confocal laser scanning microscope. Transport rates of dextran molecules having molecular weights of 4-70 kDa corresponding to Stokes radius of 1.4-6 nm are determined. Analyzing the permeability of the nuclear membrane for different sizes the effective pore radius of HeLa cell nuclear membrane is determined to be 5.3 nm, much larger than the value reported earlier using proteins as probe molecules. The range of values reported for the nuclear pore radius suggest that they may not be rigid structures and it is quite probable that the effective pore size of nuclear pore complexes is critically dependent on the probe molecules and on the environmental factors.

Sanchez-Sanchez, L., et al. (2015). "Evaluation of the antitumour activity of Rinvanil and Phenylacetylrinvanil on the cervical cancer tumour cell lines HeLa, CaSKi and ViBo." Eur J Pharmacol **758**: 129-136.

Capsaicin is a potent inducer of apoptosis in tumourreceptor potential vanilloid 1 (TRPV1). The present study determined the IC50 and cytotoxic and apoptotic activities of the Capsaicin analogues Rinvanil and Phenylacetylrinvanil (PhAR) on three cervical cancer cell lines: HeLa, CaSKi and ViBo. These analogues possess an increased affinity for TRPV1 receptors. The IC50 obtained proved to be cytotoxic for all three cell lines; however, in the cells treated with Capsaicin both active caspase-3 and nuclear fragmentation were present. Capsaicin and its analogues also inhibited the normal proliferation of lymphocytes, suggesting that they are non-selective antitumour compounds. Finally, we discuss the possible loss of the relation between apoptosis and affinity to TRPV1, and the need for other strategies to synthesise Capsaicin analogues that can be useful in cancer treatments.

Santos, C. M. A., et al. (2018). "Anti-inflammatory effect of two Lactobacillus strains during infection with Gardnerella vaginalis and Candida albicans in a HeLa cell culture model." Microbiology.

Lactobacilli are the dominant bacteria of the vaginal tract of healthy women and they play a major role in the maintenance of mucosal homeostasis, preventing genital infections, such as bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC). It is now known that one mechanism of this protection is the influence that lactobacilli can exert on host immune responses. In this context, we evaluated two Lactobacillus strains (L. plantarum 59 and L. fermentum 137) for their immunomodulatory properties in response to Gardnerella vaginalis (BV) or Candida albicans (VVC) infections in a HeLa cell infection model. G. vaginalis and C. albicans triggered the secretion of pro-inflammatory cytokines (TNF-alpha, IL-1beta, IL-6 and IL-8) and the activation of NF-kappaB in HeLa cells, in contrast to L. plantarum 59 and L. fermentum 137. Treatments with the Lactobacillus strains or their cell-free supernatants before (pre-treatment) or after (post-treatment) the challenge with the pathogens resulted in decreased secretion of pro-inflammatory cytokines and decreased activation of NF-kappaB. The treatments with Lactobacillus strains not only decreased the secretion of IL-8, but also its expression, as confirmed by gene reporter luciferase assay, suggesting transcription-level control by lactobacilli. In conclusion, L. plantarum 59 and L. fermentum 137 were confirmed to have an anti-inflammatory effect against G. vaginalis and C. albicans and they were able to influence signalling in NF-kappaB pathway, making them interesting candidates as probiotics for the prevention or treatment of BV and VVC.

Sarvmeili, N., et al. (2016). "Cytotoxic effects of Pinus eldarica essential oil and extracts on HeLa and MCF-7 cell lines." Res Pharm Sci **11**(6): 476-483.

Several attempts have so far been made in the search of new anticancer agents of plant origin. Some studies have reported that different species of Pine genus possess cytotoxic activities against various cancer cell lines. In the present study, we evaluated the cytotoxic effects of Pinus eldarica bark and leaf extracts or leaf essential oil on HeLa and MCF-7 tumor cell lines. Hydroalcoholic and phenolic extracts and the essential oil of plant were prepared. Total

phenolic contents of the extracts were measured using Folin-Ciocalteu reagent. Essential oil components were determined by gas chromatography-mass spectroscopy (GC-MS). Cytotoxic activity of the extracts and essential oil against HeLa and MCF-7 tumor cell lines were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The polyphenolic content of hydroalcoholic and phenolic extracts of the bark and hydroalcoholic extract of the leaf were 48.31%, 47.2%, and 8.47%, respectively. According to the GC-MS analysis, the major components of the leaf oil of P. eldarica were: beta -caryophyllene (14.8%), germacrene D (12.9%), alpha-terpinenyl acetate (8.15%), alpha -pinene (5.7%), and -alpha humulene (5.9%). Bark extracts and leaf essential oil of P. eldarica significantly reduced the viability of both HeLa and MCF-7 cells in a concentration dependent manner. However, leaf extract showed less inhibitory effects against both cell lines. The essential oil of P. eldarica was more cytotoxic than its hydroalcoholic and phenolic extracts. The terpenes and phenolic compounds were probably responsible for cytotoxicity of P. eldarica. Therefore, P. eldarica might have a good potential for active anticancer agents.

Sarzaeem, A., et al. (2012). "Cytotoxic effect of ICD-85 (venom-derived peptides) on HeLa cancer cell line and normal LK cells using MTT Assay." Arch Iran Med **15**(11): 696-701.

BACKGROUND: Cancer is the fifth leading cause of death worldwide. There are considerable efforts to identify naturally occurring substances for use as new drugs in cancer therapy. Some components of animal venoms have been identified that possess substantial anticancer properties. In our previous studies, the cytotoxic effects of ICD-85 (venom-derived peptides) have been reported on HL-60 and MDA-MB231 cell lines. This has prompted us to investigate the comparative cytotoxic effects of ICD-85 on the HeLa cell line and normal lamb kidney (LK) cells. METHODS: Cells were exposed to various concentrations (8 x 10-4 to 5.6 x 10 microg/ml) of ICD-85 at various incubation times (24, 48 and 72 hours). Cell viability was measured by the MTT assay. A morphological study was also carried out using an inverted microscope. Caspase-8 activity was assayed by the Caspase-8 Colorimetric Assay Kit in HeLa cells that were exposed to ICD-85 for 48 hours. RESULTS: Data analysis showed that ICD-85 has a dose-dependent cytotoxic effect on HeLa cells with an inhibitory concentration 50% (IC50) of 26.62 +/- 2.13 microg/ml at 24 hours, 27.33 +/- 2.35 microg/ml at 48 hours, and 28.13 +/- 2.52 microg/ml at 72 hours. Results also indicated that the cytotoxic effect of ICD-85, at 48 and 72 hours incubation times did not show significant alteration compared to 24 hours of exposure. Interestingly, the minimum concentration of ICD-85 which showed a cytotoxic effect on LK cells was found to be 3500-fold less than the minimum concentration that showed a cytotoxic effect on the HeLa cancer cells. While morphological analysis revealed a significant difference that included the characteristic rounding of dying cells by treatment with ICD-85 compared with untreated HeLa cells, this difference was not observed in normal cells. ICD-85 increased caspase-8 activity in HeLa cells after 48 hours of exposure. DISCUSSION: ICD-85 has a dose-dependent cytotoxic effect on HeLa cancer cells in contrast with its negligible effect on normal LK cells.

Sasaki, T., et al. (2017). "Live imaging reveals the dynamics and regulation of mitochondrial nucleoids during the cell cycle in Fucci2-HeLa cells." Sci Rep **7**(1): 11257.

Mitochondrial DNA (mtDNA) is organized in nucleoprotein complexes called mitochondrial nucleoids (mt-nucleoids), which are critical units of mtDNA replication and transmission. In humans, several hundreds of mt-nucleoids exist in a cell. However, how numerous mt-nucleoids are maintained during the cell cycle remains elusive, because cell cycle synchronization procedures affect mtDNA replication. Here, we analyzed regulation of the maintenance of mt-nucleoids in the cell cycle, using a fluorescent cell cycle indicator, Fucci2. Live imaging of mt-nucleoids with higher temporal resolution showed frequent attachment and detachment of mt-nucleoids throughout the cell cycle. TFAM, an mtDNA packaging protein, was involved in the regulation of this dynamic process, which was important for maintaining proper mt-nucleoid number. Both an increase in mt-nucleoid number and activation of mtDNA replication occurred during S phase. To increase mt-nucleoid number, mtDNA replication, but not nuclear DNA replication, was necessary. We propose that these dynamic and regulatory processes in the cell cycle maintain several hundred mt-nucleoids in proliferating cells.

Sato, S., et al. (2014). "Povidone-iodine-induced cell death in cultured human epithelial HeLa cells and rat oral mucosal tissue." Drug Chem Toxicol **37**(3): 268-275.

Although povidone-iodine (PVP-I) has been used as a gargle since 1956, its effectiveness and material safety have been remained controversial. The aim of this study was to investigate the toxicity of PVP-I to epithelial cells in a concentration range significantly lower than that used clinically. Study design was in vitro laboratory investigations and in vivo histological and immunologic analysis. We examined the effects of

PVP-I at concentrations of 1 x 10(-2) to 1 x 10(3) muM and 1 x 10(-4) to 1 x 10 muM on HeLa cells as a model of epithelial cells and rat oral mucosa, respectively, after 1 or 2 days of exposure. Annexin V/FLUOS was used to distinguish live, apoptotic and necrotic cells. The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method was also used to observe whether apoptotic epithelial cells exist in rat oral mucosa after 1 day of exposure of PVP-I. HeLa cells developed concentration-dependent cytotoxicity, and epithelium of rat oral mucosa was thinned in a concentration-dependent manner. HeLa cell apoptosis increased after 1 x 10(0) muM of PVP-I exposure for 2 days. In the TUNEL method, many apoptotic epithelial cells were observed in the rat oral mucosa after 1 day of exposure to diluted 1 x 10(-2) muM of PVP-I, but minimal apoptotic epithelial cells were observed using 1 x 10(-3) muM of PVP-I. Our findings suggest that exposure to PVP-I, of which concentrations are even lower than those used clinically, causes toxicity in epithelial cells. This knowledge would help us better understand the risk of the use of PVP-I against mucosa.

Setiawati, A. and A. Setiawati (2016). "Celecoxib, a COX-2 Selective Inhibitor, Induces Cell Cycle Arrest at the G2/M Phase in HeLa Cervical Cancer Cells." Asian Pac J Cancer Prev **17**(4): 1655-1660.

Celecoxib, a selective inhibitor of COX-2, showed cytotoxic effects in many cancer cell lines including cervical cancer cells. This study investigated the effect of celecoxib on cell cycle arrest in HeLa cervical cancer cells through p53 expression. In vitro anticancer activity was determined with the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) method. A double staining method was applied to investigate the mechanism of cell death, cell cycling was analyzed by flow cytometryand immunocytochemistry was employed to stain p53 expression in cells. Celecoxib showed strong cytotoxic effects and induced apoptosis with an IC50 value of 40 muM. It induced cell cycle arrest at G2/M phase by increasing level of p53 expression on HeLa cells.

Shan, Y., et al. (2012). "The study of single anticancer peptides interacting with HeLa cell membranes by single molecule force spectroscopy." Nanoscale **4**(4): 1283-1286.

To determine the effects of biophysical parameters (e.g. charge, hydrophobicity, helicity) of peptides on the mechanism of anticancer activity, we applied a single molecule technique-force spectroscopy based on atomic force microscope (AFM)-to study the interaction force at the single molecule level. The activity of the peptide and analogs against HeLa cells exhibited a strong correlation with the hydrophobicity of peptides. Our results indicated that the action mode between alpha-helical peptides and cancer cells was largely hydrophobicity-dependent.

Shi, C., et al. (2015). "Effect of bortezomib on migration and invasion in cervical carcinoma HeLa cell." Asian Pac J Trop Med **8**(6): 485-488.

OBJECTIVE: To explore the effect of bortezomib on migration and invasion of cervical carcinoma HeLa cell and specific molecular mechanism. METHODS: The effect of bortezomib on the viability of HeLa cell was measured by MTT assay. The effect of bortezomib on cell migration and invasion was measured by Transwell assay and invasion experiment respectively. The activation of Akt/mTOR signaling pathway and expression level of MMP2, MMP9 were assayed by western blot. RESULTS: MTT assay indicated bortezomib (2.5 muM, 5 muM, 10 muM) could inhibit HeLa cell viability, and the inhibitory rate was highest at 48 h. Transwell assay and invasion experiment results showed that bortezomib inhibited HeLa cell migration and invasion. Western blotting assays presented bortezomib could suppress the phosphorylation of Akt and mTOR, and down-regulate the expression of MMP2 and MMP9. CONCLUSIONS: These results suggested bortezomib could inhibit migration and invasion in cervical carcinoma HeLa cell, which might be related to Akt/mTOR signal pathway.

Shimada, H., et al. (2013). "Epiplakin modifies the motility of the HeLa cells and accumulates at the outer surfaces of 3-D cell clusters." J Dermatol **40**(4): 249-258.

Elimination of epiplakin (EPPK) by gene targeting in mice results in acceleration of keratinocyte migration during wound healing, suggesting that epithelial cellular EPPK may be important for the regulation of cellular motility. To study the function of EPPK, we developed EPPK knock-down (KD) and EPPK-overexpressing HeLa cells and analyzed cellular phenotypes and motility by fluorescence/differential interference contrast time-lapse microscopy and immunolocalization of actin and vimentin. Cellular motility of EPPK-KD cells was significantly elevated, but that of EPPK-overexpressing cells was obviously depressed. Many spike-like projections were observed on EPPK-KD cells, with fewer such structures on overexpressing cells. By contrast, in EPPK-KD cells, expression of E-cadherin was unchanged but vimentin fibers were thinner and sparser than in controls, and they were more concentrated at the peri-nucleus, as observed in migrating keratinocytes at wound edges in EPPK (-/-)

mice. In Matrigel 3-D cultures, EPPK co-localized on the outer surface of cell clusters with zonula occludens-1 (ZO-1), a marker of tight junctions. Our results suggest that EPPK is associated with the machinery for cellular motility and contributes to tissue architecture via the rearrangement of intermediate filaments.

Silva, V. C., et al. (2015). "A delay prior to mitotic entry triggers caspase 8-dependent cell death in p53-deficient Hela and HCT-116 cells." Cell Cycle **14**(7): 1070-1081.

Stathmin/Oncoprotein 18, a microtubule destabilizing protein, is required for survival of p53-deficient cells. Stathmin-depleted cells are slower to enter mitosis, but whether delayed mitotic entry triggers cell death or whether stathmin has a separate pro-survival function was unknown. To test these possibilities, we abrogated the cell cycle delay by inhibiting Wee1 in synchronized, stathmin-depleted cells and found that apoptosis was reduced to control levels. Synchronized cells treated with a 4 hour pulse of inhibitors to CDK1 or both Aurora A and PLK1 delayed mitotic entry and apoptosis was triggered only in p53-deficient cells. We did not detect mitotic defects downstream of the delayed mitotic entry, indicating that cell death is activated by a mechanism distinct from those activated by prolonged mitotic arrest. Cell death is triggered by initiator caspase 8, based on its cleavage to the active form and by rescue of viability after caspase 8 depletion or treatment with a caspase 8 inhibitor. In contrast, initiator caspase 9, activated by prolonged mitotic arrest, is not activated and is not required for apoptosis under our experimental conditions. P53 upregulates expression of cFLIPL, a protein that blocks caspase 8 activation. cFLIPL levels are lower in cells lacking p53 and these levels are reduced to a greater extent after stathmin depletion. Expression of FLAG-tagged cFLIPL in p53-deficient cells rescues them from apoptosis triggered by stathmin depletion or CDK1 inhibition during G2. These data indicate that a cell cycle delay in G2 activates caspase 8 to initiate apoptosis specifically in p53-deficient cells.

Simsek, E., et al. (2017). "Caspase-mediated Apoptotic Effects of Ebenus boissieri Barbey Extracts on Human Cervical Cancer Cell Line HeLa." Pharmacogn Mag **13**(50): 254-259.

BACKGROUND: Ebenus boissieri Barbey is an Antalya, Turkey-endemic plant belonging to Fabaceae family. The aerial parts and the roots of E. boissieri Barbey were used in this study. OBJECTIVE: In the present study, we have examined the apoptotic effects of hydroalcoholic extracts of E. boissieri Barbey in human cervical cancer cell line HeLa. MATERIALS AND METHODS: To determine the cytotoxic effect, cells were treated with various concentrations of extracts for 24, 48, and 72 h incubation periods. Cytotoxic effects were examined by Cell Titer 96 aqueous nonradioactive cell proliferation assay and the results were corrected by live/dead viability/cytotoxicity assay and trypan blue exclusion assay. Apoptotic effects were studied with multicaspase kit. Tumor necrosis factor-alpha (TNF-alpha) and interferon gamma (IFN-gamma) release were also measured by enzyme-linked immunosorbent assay. RESULTS: According to the results, E. boissieri Barbey extract caused significant increase in caspase levels. Thus, we suggest that the extract induces cells to undergo apoptosis. Especially, there was a sharp induction in caspase-3 activity. Levels of both TNF-alpha and IFN-gamma in extract-treated groups were significantly and dose dependently exalted as compared to their relative controls. CONCLUSION: The effects of the extract on caspase-3, TNF-alpha, and IFN-gamma levels mediate the plausible mechanism of apoptosis induction in HeLa. To the best of our knowledge, this is the first report indicating any pharmacological properties of E. Boissieri on HeLa cells. SUMMARY: HeLa cell viability was reduced in dose-dependent manner for 72 h with an IC50 of approximate 28.03 mug/mL for aerial and 41.02 mug/mL for rootHeLa cells, exposure to the aerial extract led to 1.9, 3.8, 1.2, 2.4, and 3.45 fold induction of all caspases activities (-2, -3, -6, -8, and -9, respectively)Both 30 mug/mL of aerial and 45 mug/mL of root extracts allowed the production of anticancer cytokines (TNFalpha; IFNgamma) in HeLa cell culture supernatants. Abbreviations used: Tumor necrosis factor-alpha (TNF-alpha); Interferon gamma (IFN-gamma); 3-(4, 5 dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfonyl)-2H-tetrazolium (MTS); Phosphate-Buffered Saline (PBS); Fetal Bovine Serum (FBS); para-Nitroanilin pNA; Enzyme-Linked ImmunoSorbent Assay (ELISA); Sodium Dodesyl sulphate -Polyacrilamide gel electrophoresis (SDS-PAGE); Tris-Buffered Saline (TBS); Hydocloric acid (HCl); Standart Error of Mean (SEM); National Cancer Institute (NCI); half maximal inhibitory concentration (IC50).

Slotta, C., et al. (2017). "CRISPR/Cas9-mediated knockout of c-REL in HeLa cells results in profound defects of the cell cycle." PLoS One **12**(8): e0182373.

Cervical cancer is the fourth common cancer in women resulting worldwide in 266,000 deaths per year. Belonging to the carcinomas, new insights into cervical cancer biology may also have great implications for finding new treatment strategies for other kinds of epithelial cancers. Although the transcription factor NF-kappaB is known as a key

player in tumor formation, the relevance of its particular subunits is still underestimated. Here, we applied CRISPR/Cas9n-mediated genome editing to successfully knockout the NF-kappaB subunit c-REL in HeLa Kyoto cells as a model system for cervical cancers. We successfully generated a homozygous deletion in the c-REL gene, which we validated using sequencing, qPCR, immunocytochemistry, western blot analysis, EMSA and analysis of off-target effects. On the functional level, we observed the deletion of c-REL to result in a significantly decreased cell proliferation in comparison to wildtype (wt) without affecting apoptosis. The impaired proliferative behavior of c-REL-/- cells was accompanied by a strongly decreased amount of the H2B protein as well as a significant delay in the prometaphase of mitosis compared to c-REL+/+ HeLa Kyoto cells. c-REL-/- cells further showed significantly decreased expression levels of c-REL target genes in comparison to wt. In accordance to our proliferation data, we observed the c-REL knockout to result in a significantly increased resistance against the chemotherapeutic agents 5-Fluoro-2'-deoxyuridine (5-FUDR) and cisplatin. In summary, our findings emphasize the importance of c-REL signaling in a cellular model of cervical cancer with direct clinical implications for the development of new treatment strategies.

Smith, C. M., et al. (2013). "Inhibition of clathrin by pitstop 2 activates the spindle assembly checkpoint and induces cell death in dividing HeLa cancer cells." Mol Cancer **12**: 4.

BACKGROUND: During metaphase clathrin stabilises the mitotic spindle kinetochore (K)-fibres. Many anti-mitotic compounds target microtubule dynamics. Pitstop 2 is the first small molecule inhibitor of clathrin terminal domain and inhibits clathrin-mediated endocytosis. We investigated its effects on a second function for clathrin in mitosis. RESULTS: Pitstop 2 did not impair clathrin recruitment to the spindle but disrupted its function once stationed there. Pitstop 2 trapped HeLa cells in metaphase through loss of mitotic spindle integrity and activation of the spindle assembly checkpoint, phenocopying clathrin depletion and aurora A kinase inhibition. CONCLUSIONS: Pitstop 2 is therefore a new tool for investigating clathrin spindle dynamics. Pitstop 2 reduced viability in dividing HeLa cells, without affecting dividing non-cancerous NIH3T3 cells, suggesting that clathrin is a possible novel anti-mitotic drug target.

Soica, C. M., et al. (2012). "Physico-chemical comparison of betulinic acid, betulin and birch bark extract and in vitro investigation of their cytotoxic effects towards skin epidermoid carcinoma (A431), breast carcinoma (MCF7) and cervix adenocarcinoma (HeLa) cell lines." Nat Prod Res **26**(10): 968-974.

Betulin and betulinic acid are pentacyclic triterpenes present in the bark of the birch tree and other vegetal sources. Quantitatively, in birch bark betulin is more significant than betulinic acid; therefore, birch can be a large and feasible source of raw material for betulin extraction. Betulin can be used as extracted or, after chemical modification, as a starting compound for its acid, betulinic acid, with both substances possessing various interesting pharmacological properties. The purpose of this study is to analyse the betulin and betulinic acid content of a birch tree bark extract, as well as its cytotoxic activity. The extraction was done using a Soxhlet extractor and chloroform/dichlormethane/methanol (1: 1: 1) as solvent. The betulin and betulinic acid content of the extract was estimated using standards of pure betulin and betulinic acid, by thermal analysis as opposed to pure substance (thermogravimetric and differential thermal analysis). The extract and the main compounds were also analysed by NMR. The results indicated a high amount of betulin in the final extract (up to 50%), and an important quantity of betulinic acid: over 3%. The cytotoxic activity indicated a high proliferation inhibition for the birch tree extract but was still comparable with betulinic acid and betulin.

Solano, J. D., et al. (2013). "The products of the reaction between 6-amine-1,3-dimethyl uracil and bis-chalcones induce cytotoxicity with massive vacuolation in HeLa cervical cancer cell line." Eur J Med Chem **60**: 350-359.

As a part of our research in the chemistry of chalcones we have prepared four pyrimidine monoadducts of bis-chalcones through the reaction with 6-amino-1,3-dimethyl uracil. These compounds displayed cytotoxicity with a massive vacuolation in different human cell lines in vitro. Compound 6 was the most cytotoxic inducer of vacuoles, this compound induced G1 phase cell cycle arrest, and their cytotoxicity went without morphological and biochemical evidence of apoptotic cell death in HeLa cells. In addition, our results showed that this vacuole formation does not require de novo protein synthesis and the content vacuolar is acidic. Compound 6 induce necrotic cell death with excessive vacuolation, similar to a process of autophagy. Spautin-1 an inhibitor of autophagy, decreased the transformation of microtubule-associated protein 1 light chain 3 (LC3B-I) to LC3B-II and the vacuolation induced by compound 6 in HeLa cells, both autophagy processes. These compounds could be of pivotal importance in the study of non-apoptotic cell death with vacuole formation and could be useful in research into new autophagy inhibitors agents.

Song, X., et al. (2014). "AMP-activated protein kinase is required for cell survival and growth in HeLa-S3 cells in vivo." IUBMB Life **66**(6): 415-423.

Activation of the AMP-dependent protein kinase (AMPK) is linked to cancer cell survival in a variety of cancer cell lines, particularly under conditions of stress. As a potent activator of AMPK, metformin has become a hot topic of discussion for its effect on cancer cell. Here, we report that AMPK activated by metformin promotes HeLa-S3 cell survival and growth in vivo. Our results show that metformin inhibited cell proliferation in MCF-7 cells, but not in LKB1-deficient HeLa-S3 cells. Re-expression of LKB-1 in HeLa-S3 cells restored the growth inhibitory effect of metformin, indicating a requirement for LKB-1 in metformin-induced growth inhibition. Moreover, AMPK activation exerted a protective effect in HeLa-S3 cells by relieving ER stress, modulating ER Ca (2+) storage, and finally contributing to cellular adaptation and resistance to apoptosis. Our findings identify a link between AMPK activation and cell survival in HeLa-S3 cells, which demonstrates a beneficial effect of AMPK activated by metformin in cancer cell, and suggests a discrete re-evaluation on the role of metformin/AMPK activation on tumor cell growth, proliferation, and on clinical application in cancer therapy.

Sood, S. and R. Srinivasan (2015). "Alterations in gene promoter methylation and transcript expression induced by cisplatin in comparison to 5-Azacytidine in HeLa and SiHa cervical cancer cell lines." Mol Cell Biochem **404**(1-2): 181-191.

Despite recent advances in treatment, cervical cancer still remains one of the leading causes of cancer related mortality among women worldwide including India. Chemoradiation treatment is the standard-of-care which involves administration of cisplatin, a radiosensitizer along with radiation. The epigenetic changes induced by cisplatin are not known and so we designed this in vitro experimental study. We evaluated the changes induced by cisplatin administration in gene promoter methylation and the transcript levels of set of 7 genes and compared it to the changes induced by 5-Azacytidine, a known demethylating agent in two cervical cancer cell lines: HeLa (adenocarcinoma derived) and SiHa (squamous cell carcinoma derived) cell lines. Overall, there was a pronounced cytotoxic and growth inhibitory effect of both the drugs alone and in combination for both the cell lines which was dose and time dependent. Cisplatin as well as 5-Azacytidine treatment affected gene promoter methylation status resulting in demethylation and re-expression of the genes under investigation which was more pronounced in case of SiHa cells as compared to HeLa cells. Further, both the drugs acted in synergism as evident from their combination treatment. Therefore, at the cellular level, cisplatin and 5-Azacytidine can induce epigenetic changes in gene promoter methylation with altered expression which can have implications for treatment of cervical cancer.

Soumyanarayanan, U., et al. (2012). "Monastrol mimic Biginelli dihydropyrimidinone derivatives: synthesis, cytotoxicity screening against HepG2 and HeLa cell lines and molecular modeling study." Org Med Chem Lett **2**(1): 23.

Biginelli dihydropyrimidinone derivatives as structural analogs of monastrol, a known human kinesin Eg5 inhibitor, were synthesized. IC50 values of the synthesized compounds against the proliferation of human hepatocellular carcinoma and human epithelial carcinoma cell lines were determined through MTT assay. Molecular docking study gave a clear insight into the structural activity relationship of the compounds in comparison with monastrol.

Sowmya, P. R., et al. (2015). "Role of different vehicles in carotenoids delivery and their influence on cell viability, cell cycle progression, and induction of apoptosis in HeLa cells." Mol Cell Biochem **406**(1-2): 245-253.

The objective of the present study was to determine the role of different vehicles in carotenoids delivery and their influence on cell viability, cell cycle progression and induction of apoptosis in HeLa cells. Cells (5 x 10(3)) were treated with different concentrations (25-100 microM) of beta-carotene (BC) or lutein (L) or astaxanthin (AST) dissolved in 0.5% of tetrahydrofuran (THF), dimethylsulfoxide (DMSO), and fetal bovine serum (FBS), respectively. The effect of delivery vehicle on carotenoids uptake, cytotoxicity, oxidative status, cell cycle distribution, and apoptosis was examined after 48 h of incubation. The results shown that, cell viability reduced significantly in a dose- and time-dependent manner irrespective of carotenoid delivered in vehicles. Cellular uptake of BC delivered in THF was higher by 49.1, 29.7% and L delivered through THF was higher by 41.7 and 37.5% than DMSO and FBS, respectively. While, AST delivered through DMSO was higher by 36.1 and 43.7% than the THF and FBS, respectively. In case of cells treated either with BC or L delivered through THF and AST in DMSO decreased the glutathione and increased the malondialdehyde levels. The net increase in the G 2/M phase percentage of cell cycle progression was observed in carotenoid-treated cells. The % induction of apoptosis by BC or L delivered with THF and AST in DMSO was higher than other treated groups. In conclusion, choice of suitable

vehicle for specific carotenoids delivery is essential that in turn may influence on cell proliferation and cell-based assays.

Srivastava, P., et al. (2014). "In vivo synthesis of selenium nanoparticles by Halococcus salifodinae BK18 and their anti-proliferative properties against HeLa cell line." Biotechnol Prog **30**(6): 1480-1487.

Nanoparticles synthesis by bacteria and yeasts has been widely reported, however, synthesis using halophilic archaea is still in a nascent stage. This study aimed at the intracellular synthesis of selenium nanoparticles (SeNPs) by the haloarchaeon Halococcus salifodinae BK18 when grown in the presence of sodium selenite. Crystallographic characterization of SeNPs by X-ray diffraction, Selected area electron diffraction, and transmission electron microscopy exhibited rod shaped nanoparticles with hexagonal crystal lattice, a crystallite domain size of 28 nm and an aspect ratio (length:diameter) of 13:1. Energy disruptive analysis of X-ray analysis confirmed the presence of selenium in the nano-preparation. The nitrate reductase enzyme assay and the inhibitor studies indicated the involvement of NADH-dependent nitrate reductase in SeNPs synthesis and metal tolerance. The SeNPs exhibited good anti-proliferative properties against HeLa cell lines while being non-cytotoxic to normal cell line model HaCat, suggesting the use of these SeNPs as cancer chemotherapeutic agent. This is the first study on selenium nanoparticles synthesis by haloarchaea.

Staff, P. O. (2015). "Correction: Enhanced cellular uptake of albumin-based lyophilisomes when functionalized with cell-penetrating peptide TAT in HeLa cells." PLoS One **10**(4): e0124465.

Stefanowicz-Hajduk, J., et al. (2016). "Securinine from Phyllanthus glaucus Induces Cell Cycle Arrest and Apoptosis in Human Cervical Cancer HeLa Cells." PLoS One **11**(10): e0165372.

BACKGROUND: The Securinega-type alkaloids occur in plants belonging to Euphorbiaceae family. One of the most widely distributed alkaloid of this group is securinine, which was identified next to allosecurinine in Phyllanthus glaucus (leafflower). Recently, some Securinega-type alkaloids have paid attention to its antiproliferative potency towards different cancer cells. However, the cytotoxic properties of allosecurinine have not yet been evaluated. METHODS: The cytotoxicity of the extract, alkaloid fraction obtained from P. glaucus, isolated securinine and allosecurinine against HeLa cells was evaluated by real-time xCELLigence system and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Apoptosis was detected by annexin V and 7-amino-actinomycin (7-AAD) staining and confirmed with fluorescent Hoechst 33342 dye. The assessment of mitochondrial membrane potential (MMP), reactive oxygen species (ROS) generation, the level of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), caspase-3/7 activity and cell cycle analysis were measured by flow cytometry. The enzymatic activity of caspase-9 was assessed by a luminometric assay. The expression of apoptosis associated genes was analyzed by real-time PCR. RESULTS: The experimental data revealed that securinine and the alkaloid fraction were significantly potent on HeLa cells growth inhibition with IC50 values of 7.02 +/- 0.52 mug/ml (32.3 muM) and 25.46 +/- 1.79 mug/ml, respectively. The activity of allosecurinine and Phyllanthus extract were much lower. Furthermore, our study showed that the most active securinine induced apoptosis in a dose-dependent manner in the tested cells, increased the percentage of ROS positive cells and depolarized cells as well as stimulated the activity of ERK1/2, caspase-9 and -3/7. Securinine also induced cell cycle arrest in S phase. Real-time PCR analysis showed high expression of TNFRSF genes in the cells stimulated with securinine. CONCLUSIONS: Securinine induces apoptosis and activates cell cycle checkpoints in HeLa cells which is associated with oxidative stress. The results indicate that the mitochondrial pathway is involved in the programmed cell death.

Stojanovic, I. Z., et al. (2014). "Effects of depsidones from Hypogymnia physodes on HeLa cell viability and growth." Folia Biol (Praha) **60**(2): 89-94.

The anti-proliferative activitiy of Hypogymnia physodes methanol extracts (ME) and its main constituents, physodalic acid (P1), physodic acid (P2), and 3-hydroxy physodic acid (P3), was tested on human cancer HeLa cell lines. Three lichen depsidones, P1, P2 and P3, were isolated from H. physodes ME using column chromatography and their structures were determined by UV, ESI TOF MS, 1H and 13C NMR. The content of P1, P2 and P3 in ME was determined using reversed-phase highperformance liquid chromatography with photodiode array detection. P1-3 represented even 70 % of the studied extract. The HeLa cells were incubated during 24 and 72 h in the presence of ME and depsidones P1, P2 and P3, at concentrations of 10-1000 mug/ml. Compounds P2 and P3 showed higher activity than compound P1. Half maximal inhibitory concentrations (IC50, mug/ml) of P1, P2, P3 and ME for 24-h incubation were 964, 171, 97 and 254 mug/ml, respectively, while for 72-h incubation they were 283, 66, 63 and 68 mug/ml. As far as we know, this is the first report on

the effect of H. physodes ME and their depsidones on HeLa cells.

Sun, H., et al. (2015). "Identification of HPV integration and gene mutation in HeLa cell line by integrated analysis of RNA-Seq and MS/MS data." J Proteome Res **14**(4): 1678-1686.

HeLa cell line, which was derived from cervical carcinoma, provides an idea platform to study both the integration of human papillomavirus and the massive mutations occurring on the cancer cell genome. Proteogenomics is a field with the intersection of proteomics and genomics to perform gene annotation and identify gene mutation. In this work, we first identified the SNV/INDEL, structural variation (SV), and virus infection/integration events from RNA-Seq data of HeLa cell line; then, by applying proteogenomics strategy, we were able to detect some of the genomic events with the tandem mass spectrometry (MS/MS) data from the same sample. Furthermore, some of the mutated peptides were experimentally validated using multiple reaction monitoring technology. The integrated analysis of the RNA-Seq and MS/MS data not only renders the discovery of HeLa cell genome variations more credible but also illustrates a practical workflow for protein-coding mutation discovery in cancer-related studies.

Suzuki, R., et al. (2015). "Spatiotemporal quantification of subcellular ATP levels in a single HeLa cell during changes in morphology." Sci Rep **5**: 16874.

The quantitative relationship between change in cell shape and ATP consumption is an unsolved problem in cell biology. In this study, a simultaneous imaging and image processing analysis allowed us to observe and quantify these relationships under physiological conditions, for the first time. We focused on two marginal regions of cells: the microtubule-rich 'lamella' and the actin-rich 'peripheral structure'. Simultaneous imaging and correlation analysis revealed that microtubule dynamics cause lamellar shape change accompanying an increase in ATP level. Also, image processing and spatiotemporal quantification enabled to visualize a chronological change of the relationships between the protrusion length and ATP levels, and it suggested they are influencing each other. Furthermore, inhibition of microtubule dynamics diminished motility in the peripheral structure and the range of fluctuation of ATP level in the lamella. This work clearly demonstrates that cellular motility and morphology are regulated by ATP-related cooperative function between microtubule and actin dynamics.

Talman, V., et al. (2014). "Evidence for a role of MRCK in mediating HeLa cell elongation induced by the C1 domain ligand HMI-1a3." Eur J Pharm Sci **55**: 46-57.

Diacylglycerol (DAG) is a central mediator of signaling pathways that regulate cell proliferation, survival and apoptosis. Therefore, C1 domain, the DAG binding site within protein kinase C (PKC) and other DAG effector proteins, is considered a potential cancer drug target. Derivatives of 5-(hydroxymethyl)isophthalic acid are a novel group of C1 domain ligands with antiproliferative and differentiation-inducing effects. Our previous work showed that these isophthalate derivatives exhibit antiproliferative and elongation-inducing effects in HeLa human cervical cancer cells. In this study we further characterized the effects of bis (3-trifluoromethylbenzyl) 5-(hydroxymethyl)isophthalate (HMI-1a3) on HeLa cell proliferation and morphology. HMI-1a3-induced cell elongation was accompanied with loss of focal adhesions and actin stress fibers, and exposure to HMI-1a3 induced a prominent relocation of cofilin-1 into the nucleus regardless of cell phenotype. The antiproliferative and morphological responses to HMI-1a3 were not modified by pharmacological inhibition or activation of PKC, or by RNAi knock-down of specific PKC isoforms, suggesting that the effects of HMI-1a3 were not mediated by PKC. Genome-wide gene expression microarray and gene set enrichment analysis suggested that, among others, HMI-1a3 induces changes in small GTPase-mediated signaling pathways. Our experiments revealed that the isophthalates bind also to the C1 domains of beta2-chimaerin, protein kinase D (PKD) and myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK), which are potential mediators of small GTPase signaling and cytoskeletal reorganization. Pharmacological inhibition of MRCK, but not that of PKD attenuated HMI-1a3-induced cell elongation, suggesting that MRCK participates in mediating the effects of HMI-1a3 on HeLa cell morphology.

Talman, V., et al. (2011). "C1 Domain-targeted isophthalate derivatives induce cell elongation and cell cycle arrest in HeLa cells." PLoS One **6**(5): e20053.

Diacylglycerol (DAG)-mediated signaling pathways, such as those mediated by protein kinase C (PKC), are central in regulating cell proliferation and apoptosis. DAG-responsive C1 domains are therefore considered attractive drug targets. Our group has designed a novel class of compounds targeted to the DAG binding site within the C1 domain of PKC. We have previously shown that these 5-(hydroxymethyl)isophthalates modulate PKC activation in living cells. In this study we investigated

their effects on HeLa human cervical cancer cell viability and proliferation by using standard cytotoxicity tests and an automated imaging platform with machine vision technology. Cellular effects and their mechanisms were further characterized with the most potent compound, HMI-1a3. Isophthalate derivatives with high affinity to the PKC C1 domain exhibited antiproliferative and non-necrotic cytotoxic effects on HeLa cells. The anti-proliferative effect was irreversible and accompanied by cell elongation. HMI-1a3 induced down-regulation of retinoblastoma protein and cyclins A, B1, D1, and E. Effects of isophthalates on cell morphology, cell proliferation and expression of cell cycle-related proteins were different from those induced by phorbol 12-myristate-13-acetate (PMA) or bryostatin 1, but correlated closely to binding affinities. Therefore, the results strongly indicate that the effect is C1 domain-mediated.

Tao, Y., et al. (2010). "Phosphorylation of vasodilator stimulated phosphoprotein is correlated with cell cycle progression in HeLa cells." Mol Med Rep **3**(4): 657-662.

Vasodilator stimulated phosphoprotein (VASP) is known as an actin-binding protein. The phosphorylation of VASP plays an important role in its function. In a previous study, serine 157 phosphorylated VASP (p-VASP S157) was shown to be co-localized with alpha-tubulin on the spindle of SGC-7901 cells. In the present study, we demonstrated that the level of p-VASP S157 increases and has a peak which coincides with serine 10 phosphorylated histone 3 (p-H3 S10) during mitotic progression in a human cervical cancer cell line (HeLa cells). Application of protein kinase A inhibitor H89, protein kinase G inhibitor KT5823 and protein kinase C inhibitor Go6983, or a combination of these inhibitors, caused a partial decrease in p-VASP S157 and a delay in G2/M progression. Depletion of p-VASP S157 by VASP siRNA resulted in an increase in binucleated cells and x4n cells, a further delay in G2/M progression and the inhibition of HeLa cell proliferation. These results suggest that p-VASP S157 may play an important role in the G2/M transition and the completion of cytokinesis in HeLa cells.

Thavamani, B. S., et al. (2013). "In vitro cytotoxic activity of menispermaceae plants against HeLa cell line." Anc Sci Life **33**(2): 81-84.

BACKGROUND: Menispermaceae, a family of flowering plants, is a medium-sized family of 70 genera totaling 420 extant species, mostly of climbing plants. It has various medicinal properties, which are used in the Ayurvedic system of medicine. Plants belonging to this family are rich in alkaloids, especially bisbenzylisoquinoline type. The hypothesis of this study is that the bisbenzylisoquinoline alkaloids present in the selected plants may exhibit in vitro cytotoxic property. AIM: The present study is aimed at estimating the total alkaloidal content of methanolic extract of Cocculus hirsutus and Cissampelos pareira and evaluating the in vitro cytotoxic activity of both the extracts on the HeLa cell line. SETTINGS AND DESIGN: Methanolic extracts of both the plants in the concentrations of 500, 250, 125, 62.5, and 31.25 mug/ml were assessed for its cytotoxic activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MATERIALS AND METHODS: Total alkaloidal content was studied for both the plants using ultraviolet-visible spectroscopy method. Methanol extracts of both the plants were tested for its inhibitory effect on HeLa cell line. Cytotoxicity of the plant extracts was evaluated by MTT assay. Nonlinear regression graph was plotted between % cell inhibition and Log10 concentration, and IC50 was determined using GraphPad Prism software. RESULTS: Preliminary phytochemical studies confirm the presence of alkaloids in both the plants. The total alkaloids present in C. hirsutus and C. pareira were found to be 0.252%w/w and 0.1656%w/w respectively. The IC50 values of C. hirsutus and C. pareria were found to be 111 mug/ml and 129.3 mug/ml respectively. CONCLUSION: From this study, it is observed that C. hirsutus and C. pareira have in vitro cytotoxic activity against HeLa cell line.

Thiede, B., et al. (2013). "High resolution quantitative proteomics of HeLa cells protein species using stable isotope labeling with amino acids in cell culture (SILAC), two-dimensional gel electrophoresis (2DE) and nano-liquid chromatograpohy coupled to an LTQ-OrbitrapMass spectrometer." Mol Cell Proteomics **12**(2): 529-538.

The proteomics field has shifted over recent years from two-dimensional gel electrophoresis (2-DE)-based approaches to SDS-PAGE or gel-free workflows because of the tremendous developments in isotopic labeling techniques, nano-liquid chromatography, and high-resolution mass spectrometry. However, 2-DE still offers the highest resolution in protein separation. Therefore, we combined stable isotope labeling with amino acids in cell culture of controls and apoptotic HeLa cells with 2-DE and the subsequent analysis of tryptic peptides via nano-liquid chromatography coupled to an LTQ-Orbitrap mass spectrometer to obtain quantitative data using the methods with the highest resolving power on all levels of the proteomics workflow. More than 1,200 proteins with more than 2,700 protein species were identified and quantified from 816 Coomassie Brilliant Blue G-250 stained 2-DE spots. About half of the proteins were identified and quantified only in

single 2-DE spots. The majority of spots revealed one to five proteins; however, in one 2-DE spot, up to 23 proteins were identified. Only half of the 2-DE spots represented a dominant protein with more than 90% of the whole protein amount. Consequently, quantification based on staining intensities in 2-DE gels would in approximately half of the spots be imprecise, and minor components could not be quantified. These problems are circumvented by quantification using stable isotope labeling with amino acids in cell culture. Despite challenges, as shown in detail for lamin A/C and vimentin, the quantitative changes of protein species can be detected. The combination of 2-DE with high-resolution nano-liquid chromatography-mass spectrometry allowed us to identify proteomic changes in apoptotic cells that would be unobservable using any of the other previously employed proteomic workflows.

Tomankova, K., et al. (2014). "Study of photodynamic, sonodynamic and antioxidative influence on HeLa cell line." Indian J Biochem Biophys **51**(1): 19-28.

Photodynamic treatment (PDT) in combination with sonodynamic treatment (SDT) can be used as suitable methods to treat malignant and benign diseases or combat resistant bacteria. Both methods affect the production of reactive oxygen species (ROS). On the other hand, antioxidants are useful for cell protection against ROS. This work was aimed to study the effect of PDT and SDT treatments on the HeLa cell line using antioxidant Pronalen Sensitive Skin as a protection from free radicals in the cells. We evaluated the effect of sensitizer ClAlPcS2 using battery of in vitro methods, including MTT assay, kinetic production of ROS, mitochondrial membrane potential change, type of cell death and microscopic analysis. Ultrasound treatment was observed to increase the production of ROS, only in combination with PDT, particularly at higher concentrations of ClAlPcS2. The added antioxidant acts as protection against free radicals and has potential as a dietary supplement against aging or free radicals. The results of study suggested that ClAlPcS2 could be used as a potential photosensitizer for treatment of a specific type of cancers.

Tsai, H. C., et al. (2012). "Two-photon confocal imaging study: cell uptake of two photon dyes-labeled PAMAM dendrons in HeLa cells." J Biomed Mater Res A **100**(3): 746-756.

A two-photon excitation difluoroboron dye activated in the near infrared region for biological image analysis was synthesized in this study. Cell affinity, membrane interaction, and the endocytosis pathway of PAMAM dendrons were investigated using only covalent two-photon dyes (TPD) at the periphery of the PAMAM dendrons. Generation 3 TPD-labeled PAMAM dendrons (BG3) exhibited multivalency binding on the HeLa cell membranes from the cell affinity study in the fixation of HeLa cells. Photo-stimulation on the membrane of the living HeLa cell was observed by confocal optical imaging in situ, using the two-photon model, when incubated with BG3. Analyses of cell membrane integrity via lactate dehydrogenase (LDH) assay confirmed membrane damage at two photon excitation model. However, no variation in the cell was observed using the one-photon excitation model. These results indicated a high degree of dendrons uptake by cells through binding to the cell membrane following the endocytotic pathway. Furthermore, the wide excitation fluorescence spectrum of difluoroboron dye provides dual imaging with which to study the endocytosis of TPD-labeled PAMAM dendrons using a single near infrared laser.

Tsai, J. H., et al. (2016). "1-(2-Hydroxy-5-methylphenyl)-3-phenyl-1,3-propanedione Induces G1 Cell Cycle Arrest and Autophagy in HeLa Cervical Cancer Cells." Int J Mol Sci **17**(8).

The natural agent, 1-(2-hydroxy-5-methylphenyl)-3-phenyl-1,3-propanedione (HMDB), has been reported to have growth inhibitory effects on several human cancer cells. However, the role of HMDB in cervical cancer remains unclear. Herein, we found that HMDB dose- and time-dependently inhibited growth of HeLa cervical cancer cells, accompanied with G1 cell cycle arrest. HMDB decreased protein expression of cyclins D1/D3/E and cyclin-dependent kinases (CDKs) 2/4/6 and reciprocally increased mRNA and protein levels of CDK inhibitors (p15, p16, p21, and p27), thereby leading to the accumulation of hypophosphorylated retinoblastoma (Rb) protein. HMDB also triggered the accumulation of acidic vesicles and formation of microtubule-associated protein-light chain 3 (LC3), followed by increased expression of LC3 and Beclin-1 and decreased expression of p62, suggesting that HMDB triggered autophagy in HeLa cells. Meanwhile, suppression of the expression of survivin and Bcl-2 implied that HMDB-induced autophagy is tightly linked to apoptosis. Exploring the action mechanism, HMDB induced autophagy via the modulation of AMP-activated protein kinase (AMPK) and mTOR signaling pathway rather than the class III phosphatidylinositol 3-kinase pathway. These results suggest that HMDB inhibits HeLa cell growth by eliciting a G1 arrest through modulation of G1 cell cycle regulators and by concomitantly inducing autophagy through the mediation of AMPK-mTOR

and Akt-mTOR pathways, and may be a promising antitumor agent against cervical cancer.

Tsai, Y. J., et al. (2012). "Ribosome distribution in HeLa cells during the cell cycle." PLoS One **7**(3): e32820.

In this study, we employed a surface-specific antibody against the large ribosome subunit to investigate the distribution of ribosomes in cells during the cell cycle. The antibody, anti-L7n, was raised against an expansion segment (ES) peptide from the large subunit ribosomal protein L7, and its ribosome-surface specificity was evident from the positive immuno-reactivity of ribosome particles and the detection of 60 S immune-complex formation by an immuno-electron microscopy. Using immunofluorescent staining, we have microscopically revealed that ribosomes are dispersed in the cytoplasm of cells throughout all phases of the cell cycle, except at the G2 phase where ribosomes show a tendency to gather toward the nuclear envelope. The finding in G2 cells was confirmed by electron microscopy using a morphometric assay and paired t test. Furthermore, further observations have shown that ribosomes are not distributed immune-fluorescently with nuclear envelope markers including the nuclear pore complex, the integral membrane protein gp210, the inner membrane protein lamin B2, and the endoplasm reticulum membrane during cell division we propose that the mechanism associated with ribosome segregation into daughter cells could be independent of the processes of disassembly and reassembly of the nuclear envelope.

Tshitenge, D. T., et al. (2017). "Gardenifolins A-H, Scalemic Neolignans from Gardenia ternifolia: Chiral Resolution, Configurational Assignment, and Cytotoxic Activities against the HeLa Cancer Cell Line." J Nat Prod **80**(5): 1604-1614.

From the tropical plant Gardenia ternifolia Schumach. and Thonn. (Rubiaceae), eight stereoisomeric 2,3-dihydrobenzo [b]furan neolignans, named gardenifolins A-H (1a-d and 2a-d), were isolated and fully structurally characterized. Reversed-phase chromatography of a stem bark extract afforded two peaks, viz. mixtures I and II, each one consisting of two diastereomers and their respective enantiomers. They were resolved and stereochemically analyzed by HPLC on a chiral phase coupled to electronic circular dichroism (ECD) spectroscopy, giving single ECD spectra of all eight stereoisomers. The double-bond geometries (E or Z) of the gardenifolins A-H and their relative configurations (cis or trans) at the stereogenic centers C-7 and C-8 in the dihydrofuran ring system were assigned by 1D and 2D NMR methods, in particular, using NOE difference experiments, whereas the absolute configurations of the isolated enantiomers were established by ECD spectroscopy by applying the reversed helicity rule. The individual pure gardenifolin isomers A-H showed the most different cytotoxic effects against the human cancer HeLa cell line, with 1d and 2a displaying the highest activities, with IC50 values of 21.0 and 32.5 muM, respectively. Morphological experiments indicated that gardenifolin D (1d) induces apoptosis of HeLa cells at 25 muM.

Tucker-Kellogg, L., et al. (2012). "Reactive oxygen species (ROS) and sensitization to TRAIL-induced apoptosis, in Bayesian network modelling of HeLa cell response to LY303511." Biochem Pharmacol **84**(10): 1307-1317.

BACKGROUND: The compound LY303511 (LY30) has been proven to induce production of ROS and to sensitize cancer cells to TRAIL-induced apoptosis, but the mechanisms and mediators of LY30-induced effects are potentially complex. Bayesian networks are a modelling technique for making probabilistic inferences about complex networks of uncertain causality. METHODS: Fluorescent indicators for ROS, reactive nitrogen species (RNS), and free calcium were measured in time-series after LY30 treatment. This "correlative" dataset was used as input for Bayesian modelling to predict the causal dependencies among the measured species. Predictions were compared against a separate "causal" dataset, in which cells had been treated with FeTPPS to scavenge peroxynitrite, EGTA-am to chelate calcium, and Tiron to scavenge O (2) (-). Finally, cell viability measurements were integrated into an extended model of LY30 effects. RESULTS: LY30 treatment caused a rapid increase of ROS (measured by DCFDA) as well as a significant increase in RNS and calcium. Bayesian modelling predicted that Ca (2+)was a partial cause of the ROS induced by short incubations with LY30, and that RNS was strongly responsible for the ROS induced by long incubations with LY30. Validation experiments confirmed the predicted roles of RNS and calcium, and also demonstrated a causal role for O (2) (-). In cell viability experiments, the additive effects of calcium and peroxynitrite were responsible for 90% of LY30-mediated sensitization to TRAIL-induced apoptosis. CONCLUSIONS: We conclude that LY30 induces interdependent pathways of reactive species and stress signalling, with peroxynitrite and calcium contributing most significantly to apoptosis sensitization.

Tugba Artun, F., et al. (2016). "In vitro anticancer and cytotoxic activities of some plant extracts on HeLa and Vero cell lines." J BUON **21**(3): 720-725.

PURPOSE: The aim of our study was to evaluate the effect of in vitro anticancer and cytotoxic activity of the methanolic extracts of 14 medicinal plants, 8 of which are endemic species in Anatolia, against the human HeLa cervical cancer cell line and to compare to the normal African green monkey kidney epithelial cell line (Vero) using the MTT colorimetric assay. METHODS: Values for cytotoxicity measured by MTT assay were expressed as the concentration that causes 50% decrease in cell viability (IC50, mug/mL). The degree of selectivity of the compounds can be expressed by its selectivity index (SI) value. High SI value (>2) of a compound gives the selective toxicity against cancer cells (SI = IC50 normal cell/IC50 cancer cell). RESULTS: Dose-dependent studies revealed IC50 of 293 mg/mL and >1000 mg/mL for Cotinus coggygria Scop., IC50 of 265 mug/mL and >1000 mg/mL for Rosa damascena Miller, IC50 of 2 mug/mL and 454 mg/mL for Colchicum sanguicolle K.M. Perss, IC50 of 427 mug/mL and >1000 mug/mL for Centaurea antiochia Boiss. var. praealta (Boiss & Bal) Wagenitz on the HeLa cells and the Vero cells, respectively. Four plants showed significant SI values which were 227 for Colchicum sanguicolle K.M. Perss (endemic species), >3.8 for Rosa damascena Miller, >3.4 for Cotinus coggygria Scop. and >2.3 for Centaurea antiochia Boiss. var. praealta (Boiss & Bal)Wagenitz (endemic species). CONCLUSION: According to our study, 4 methanolic extracts of 14 tested plants exhibit greater activity on the HeLa cell line and little activity on the Vero cell line, meaning that these plants can be evaluated for potential promising anticancer activity.

Uthaisang-Tanechpongtamb, W., et al. (2013). "Role of altholactone in inducing type II apoptosis signalling pathway and expression of cancer-related genes in cervical carcinoma HeLa cell line." Cell Biol Int **37**(5): 471-477.

Goniothalamus species (Annonaceae) is a shrub that grows in the rainforest of tropical Asia. Several compounds have been isolated and exhibit the potential use for cancer treatment. In this work, altholactone isolated from Goniothalamus macrophyllus was investigated for its cytotoxicity, apoptosis signalling and the expression of cancer-related genes in the cervical carcinoma HeLa cells. Cytotoxicity was evaluated by MTT assay. Apoptotic characteristics were evaluated by morphological studies. Caspase-3 activity was detected using a fluorogenic substrate. Cytochrome c release from mitochondria and protein Bid were determined by Western blotting and cancer-related genes expression by RT-PCR. The results demonstrated that altholactone was cytotoxic to HeLa (IC50 = 9.6 mug/mL), and apoptotic cell death was manifested by appearance of chromatin condensation and caspase-3 activation, which was inhibited by specific inhibitors of both caspase-8 and -9. Release into the cytosol of cytochrome and cleavage of Bid occurred. Altholactone also caused a decrease in bcl-2 and an increase in p53 expression. These unique properties of altholactone suggest a potential for cancer chemotherapy.

van Bracht, E., et al. (2014). "Enhanced cellular uptake of albumin-based lyophilisomes when functionalized with cell-penetrating peptide TAT in HeLa cells." PLoS One **9**(11): e110813.

Lyophilisomes are a novel class of biodegradable proteinaceous nano/micrometer capsules with potential use as drug delivery carrier. Cell-penetrating peptides (CPPs) including the TAT peptide have been successfully implemented for intracellular delivery of a broad variety of cargos including various nanoparticulate pharmaceutical carriers. In the present study, lyophilisomes were modified using CPPs in order to achieve enhanced cellular uptake. Lyophilisomes were prepared by a freezing, annealing, and lyophilization method and a cystein-elongated TAT peptide was conjugated to the lyophilisomes using a heterobifunctional linker. Fluorescent-activated cell sorting (FACS) was utilized to acquire a lyophilisome population with a particle diameter smaller than 1000 nm. Cultured HeLa, OVCAR-3, Caco-2 and SKOV-3 cells were exposed to unmodified lyophilisomes and TAT-conjugated lyophilisomes and examined with FACS. HeLa cells were investigated in more detail using a trypan blue quenching assay, confocal microscopy, and transmission electron microscopy. TAT-conjugation strongly increased binding and cellular uptake of lyophilisomes in a time-dependent manner in vitro, as assessed by FACS. These results were confirmed by confocal microscopy. Transmission electron microscopy indicated rapid cellular uptake of TAT-conjugated lyophilisomes via phagocytosis and/or macropinocytosis. In conclusion, TAT-peptides conjugated to albumin-based lyophilisomes are able to enhance cellular uptake of lyophilisomes in HeLa cells.

Vardhan, H., et al. (2010). "Higher expression of ferritin protects Chlamydia trachomatis infected HeLa 229 cells from reactive oxygen species mediated cell death." Biochem Cell Biol **88**(5): 835-842.

Apoptosis plays an important role in modulating the pathogenesis of a variety of infectious diseases. Chlamydial infection protects cells against different forms of apoptosis: extrinsic, intrinsic, and granzyme B mediated. Redox reactions are central to the life and death decision of cells and pathogens and an intimate relationship exists between oxidative stress and iron

metabolism. The link between redox status and ferritin was largely unexplored in chlamydia-infected cells. In the present study, we showed that Chlamydia trachomatis (CT) infection induced FHC protein in HeLa cells. FHC induction by CT-infected cells stably expressing FHC blunted ROS production compared with mock infected cells, and the infected cells were relatively resistant to apoptosis induced by H (2)O (2). We also demonstrated that endogenous FHC overexpression correlates well with the stabilization of the mitochondrial membrane potential in CT-infected cells. Increased expression of FHC is independent of iron supplementation (FAC) and depletion (DFO) in CT-infected cells. These data suggest that FHC up-regulation is an acute response of HeLa cells against CT infection and that FHC exerts anti-apoptotic activity against oxidative stress.

Vidya Priyadarsini, R., et al. (2010). "The flavonoid quercetin induces cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells through p53 induction and NF-kappaB inhibition." Eur J Pharmacol **649**(1-3): 84-91.

With increasing use of plant-derived cancer chemotherapeutic agents, exploring the antiproliferative effects of phytochemicals has gained increasing momentum for anticancer drug design. The dietary phytochemical quercetin, modulates several signal transduction pathways associated with cell proliferation and apoptosis. The present study was undertaken to examine the effect of quercetin on cell viability, and to determine the molecular mechanism of quercetin-induced cell death by investigating the expression of Bcl-2 family proteins (Bcl-2, Bcl-xL, Mcl1, Bax, Bad, p-Bad), cytochrome C, Apaf-1, caspases, and survivin as well as the cell cycle regulatory proteins (p53, p21, cyclin D1), and NF-kappaB family members (p50, p65, IkappaB, p-IkappaB-alpha, IKKbeta and ubiquitin ligase) in human cervical cancer (HeLa) cells. The results demonstrate that quercetin suppressed the viability of HeLa cells in a dose-dependent manner by inducing G2/M phase cell cycle arrest and mitochondrial apoptosis through a p53-dependent mechanism. This involved characteristic changes in nuclear morphology, phosphatidylserine externalization, mitochondrial membrane depolarization, modulation of cell cycle regulatory proteins and NF-kappaB family members, upregulation of proapoptotic Bcl-2 family proteins, cytochrome C, Apaf-1 and caspases, and downregulation of antiapoptotic Bcl-2 proteins and survivin. Quercetin that exerts opposing effects on different signaling networks to inhibit cancer progression is a classic candidate for anticancer drug design.

Vijayarathna, S., et al. (2017). "Polyalthia longifolia Methanolic Leaf Extracts (PLME) induce apoptosis, cell cycle arrest and mitochondrial potential depolarization by possibly modulating the redox status in hela cells." Biomed Pharmacother **89**: 499-514.

Medicinal plants have been accepted as a gold mine, with respect to the diversity of their phytochemicals. Many medicinal plants extracts are potential anticancer agents. Polyalthia longifolia var. angustifolia Thw. (Annonaceae) is one of the most significant native medicinal plants and is found throughout Malaysia. Hence, the present study was intended to assess the anticancer properties of P. longifolia leaf methanolic extract (PLME) and its underlying mechanisms. The Annexin V/PI flow cytometry analysis showed that PLME induces apoptosis in HeLa cells in dose-dependent manner whereas the PI flow cytometric analysis for cell cycle demonstrated the accumulation of cells at sub G0/G1, G0/G1 and G2/M phases. Investigation with JC-1 flow cytometry analysis indicated increase in mitochondria membrane potential depolarisation corresponding to increase in PLME concentrations. PLME was also shown to influence intracellular reactive oxygen species (ROS) by exerting anti-oxidant (half IC50) and pro-oxidant (IC50 and double IC50) affect against HeLa cells. PLME treatment also displayed DNA damage in HeLa cells in concentration depended fashion. The proteomic profiling array exposed the expression of pro-apoptotic and anti-apoptotic proteins upon PLME treatment at IC50 concentration in HeLa cells. Pro-apoptotic proteins; BAX, BAD, cytochrome c, caspase-3, p21, p27 and p53 were found to be significantly up-regulated while anti-apoptotic proteins; BCL-2 and BCL-w were found to be significantly down-regulated. This investigation postulated the role of p53 into mediating apoptosis, cell cycle arrest and mitochondrial potential depolarisation by modulating the redox status of HeLa cells.

Wan, Q., et al. (2011). "BnRCH gene inhibits cell growth of Hela cells through increasing the G2 phase of cell cycle." Hum Cell **24**(4): 150-160.

The ubiquitin-proteasome pathway (UPP) is an important protein degradation system universally existing in eukaryotic organisms from yeast to human. In this system, hundreds of E3 ubiquitin-protein ligases are most important because they provide the substrate specificity and control many cellular processes. UPP has been found to be relevant to cancer development. BnRCH, the protein product from a novel gene isolated from Brassica napus, also has E3 ubiquitin-protein ligase activity. In order to exploit its potential use, human cervical carcinoma cell Hela (Hela cells) was transiently and stably transfected with

BnRCH. The experimental results demonstrated: (1) in Hela cells, BnRCH inhibited the cell growth of Hela cells and increased their sensitivity to the anti-cancer chemotherapeutic drug cisplatin; and (2) the growth inhibition effect of BnRCH in Hela cells was found due to G2 phase cell cycle arrest with the transcriptional up-regulation of p21 (waf1/cip1), rather than apoptosis. This research suggests BnRCH has potential use in cancer therapy.

Wang, M. H., et al. (2011). "Single HeLa and MCF-7 cell measurement using minimized impedance spectroscopy and microfluidic device." Rev Sci Instrum **82**(6): 064302.

This study presents an impedance measurement system for single-cell capture and measurement. The microwell structure which utilizes nDEP force is used to single-cell capture and a minimized impedance spectroscopy which includes a power supply chip, an impedance measurement chip and a USB microcontroller chip is used to single-cell impedance measurement. To improve the measurement accuracy of the proposed system, Biquadratic fitting is used in this study. The measurement accuracy and reliability of the proposed system are compared to those of a conventional precision impedance analyzer. Moreover, a stable material, latex beads, is used to study the impedance measurement using the minimized impedance spectroscopy with cell-trapping device. Finally, the proposed system is used to measure the impedance of HeLa cells and MCF-7 cells. The impedance of single HeLa cells decreased from 9.55 x 10(3) to 3.36 x 10(3) Omega and the impedance of single MCF-7 cells decreased from 3.48 x 10(3) to 1.45 x 10(3) Omega at an operate voltage of 0.5 V when the excitation frequency was increased from 11 to 101 kHz. The results demonstrate that the proposed impedance measurement system successfully distinguishes HeLa cells and MCF-7 cells.

Wee, P. and Z. Wang (2017). "Cell Cycle Synchronization of HeLa Cells to Assay EGFR Pathway Activation." Methods Mol Biol **1652**: 167-181.

Progression through the cell cycle causes changes in the cell's signaling pathways that can alter EGFR signal transduction. Here, we describe drug-derived protocols to synchronize HeLa cells in various phases of the cell cycle, including G1 phase, S phase, G2 phase, and mitosis, specifically in the mitotic stages of prometaphase, metaphase, and anaphase/telophase. The synchronization procedures are designed to allow synchronized cells to be treated for EGF and collected for the purpose of Western blotting for EGFR signal transduction components.S phase synchronization is performed by thymidine block, G2 phase with roscovitine, prometaphase with nocodazole, metaphase with MG132, and anaphase/telophase with blebbistatin. G1 phase synchronization is performed by culturing synchronized mitotic cells obtained by mitotic shake-off. We also provide methods to validate the synchronization methods. For validation by Western blotting, we provide the temporal expression of various cell cycle markers that are used to check the quality of the synchronization. For validation of mitotic synchronization by microscopy, we provide a guide that describes the physical properties of each mitotic stage, using their cellular morphology and DNA appearance. For validation by flow cytometry, we describe the use of imaging flow cytometry to distinguish between the phases of the cell cycle, including between each stage of mitosis.

Winter, D. and H. Steen (2011). "Optimization of cell lysis and protein digestion protocols for the analysis of HeLa S3 cells by LC-MS/MS." Proteomics **11**(24): 4726-4730.

In order to maximize the number of proteins identified from Hela S3 cell lysate we tested various cell lysis, protein precipitation and digestion protocols. First, we compared three different lysis buffers, two mechanical cell disruption methods and two precipitation methods. Then, we tested six different in-solution digestion protocols, three different in-gel digestion protocols and ten different peptide extraction protocols. The result is a proposal for an optimized protocol to prepare the whole cell lysate samples from HeLa S3 cells.

Wu, P. P., et al. (2011). "Diallyl sulfide induces cell cycle arrest and apoptosis in HeLa human cervical cancer cells through the p53, caspase- and mitochondria-dependent pathways." Int J Oncol **38**(6): 1605-1613.

Diallyl sulfide (DAS), one of the main active constituents of garlic, causes growth inhibition of cancer cells in vitro and promotes immune responses in vivo in experimental settings. However, its effects on the induction of cell cycle and apoptosis in human cervical cancer cells are still unclear. The aims of this study were to explore the anti-cancer effects of DAS in HeLa human cervical cancer cells and to investigate the underlying mechanisms in vitro. Cytotoxicity and apoptosis in HeLa human cervical cancer cells were examined by the morphological changes, viability assay, 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) staining, comet assay, Western blotting and confocal microscopy examination. The results showed that DAS treatment for 24-72 h resulted in a marked decrease in cell viability time- and dose-dependently. Flow cytometric analysis showed that a 48-h treatment of 75 microM DAS induced G0/G1 cell cycle arrest

and sub-G1 phase (apoptosis) in HeLa cells. Typical apoptotic nucleus alterations were observed by fluorescence microscopy in HeLa cells after exposure to DAS using DAPI staining. Cells treated with different concentrations of DAS also showed changes typical of apoptosis such as morphological changes, DNA damage and fragmentation, dysfunction of mitochondria, cytochrome c release and increased expression of pro-caspase-3 and -9. DAS also promoted the release of AIF and Endo G from mitochondria in HeLa cells. In conclusion, DAS induced G0/G1 cell cycle arrest and apoptosis in HeLa cells through caspase- and mitochondria and p53 pathways providing further understanding of the molecular mechanisms of DAS action in cervical cancer. This study, therefore, revealed that DAS significantly inhibits the growth and induces apoptosis of human cervical cancer HeLa cells in vitro.

Yadavalli, R. and T. Sam-Yellowe (2015). "HeLa Based Cell Free Expression Systems for Expression of Plasmodium Rhoptry Proteins." J Vis Exp (100): e52772.

Malaria causes significant global morbidity and mortality. No routine vaccine is currently available. One of the major reasons for lack of a vaccine is the challenge of identifying suitable vaccine candidates. Malarial proteins expressed using prokaryotic and eukaryotic cell based expression systems are poorly glycosylated, generally insoluble and undergo improper folding leading to reduced immunogenicity. The wheat germ, rabbit reticulocyte lysate and Escherichia coli lysate cell free expression systems are currently used for expression of malarial proteins. However, the length of expression time and improper glycosylation of proteins still remains a challenge. We demonstrate expression of Plasmodium proteins in vitro using HeLa based cell free expression systems, termed "in vitro human cell free expression systems". The 2 HeLa based cell free expression systems transcribe mRNA in 75 min and 3 microl of transcribed mRNA is sufficient to translate proteins in 90 min. The 1-step expression system is a transcription and translation coupled expression system; the transcription and co-translation occurs in 3 hr. The process can also be extended for 6 hr by providing additional energy. In the 2-step expression system, mRNA is first transcribed and then added to the translation mix for protein expression. We describe how to express malaria proteins; a hydrophobic PF3D7_0114100 Maurer's Cleft - 2 transmembrane (PfMC-2TM) protein, a hydrophilic PF3D7_0925900 protein and an armadillo repeats containing protein PF3D7_1361800, using the HeLa based cell free expression system. The proteins are expressed in micro volumes employing 2-step and 1-step expression strategies. An affinity purification method to purify 25 microl of proteins expressed using the in vitro human cell free expression system is also described. Protein yield is determined by Bradford's assay and the expressed and purified proteins can be confirmed by western blotting analysis. Expressed recombinant proteins can be used for immunizations, immunoassays and protein sequencing.

Yahiro, K., et al. (2012). "Regulation of subtilase cytotoxin-induced cell death by an RNA-dependent protein kinase-like endoplasmic reticulum kinase-dependent proteasome pathway in HeLa cells." Infect Immun **80**(5): 1803-1814.

Shiga-toxigenic Escherichia coli (STEC) produces subtilase cytotoxin (SubAB), which cleaves the molecular chaperone BiP in the endoplasmic reticulum (ER), leading to an ER stress response and then activation of apoptotic signaling pathways. Here, we show that an early event in SubAB-induced apoptosis in HeLa cells is mediated by RNA-dependent protein kinase (PKR)-like ER kinase (PERK), not activating transcription factor 6 (ATF6) or inositol-requiring enzyme 1(Ire1), two other ER stress sensors. PERK knockdown suppressed SubAB-induced eIF2alpha phosphorylation, activating transcription factor 4 (ATF4) expression, caspase activation, and cytotoxicity. Knockdown of eIF2alpha by small interfering RNA (siRNA) or inhibition of eIF2alpha dephosphorylation by Sal003 enhanced SubAB-induced caspase activation. Treatment with proteasome inhibitors (i.e., MG132 and lactacystin), but not a general caspase inhibitor (Z-VAD) or a lysosome inhibitor (chloroquine), suppressed SubAB-induced caspase activation and poly (ADP-ribose) polymerase (PARP) cleavage, suggesting that the ubiquitin-proteasome system controls events leading to caspase activation, i.e., Bax/Bak conformational changes, followed by cytochrome c release from mitochondria. Levels of ubiquitinated proteins in HeLa cells were significantly decreased by SubAB treatment. Further, in an early event, some antiapoptotic proteins, which normally turn over rapidly, have their synthesis inhibited, and show enhanced degradation via the proteasome, resulting in apoptosis. In PERK knockdown cells, SubAB-induced loss of ubiquitinated proteins was inhibited. Thus, SubAB-induced ER stress is caused by BiP cleavage, leading to PERK activation, not by accumulation of ubiquitinated proteins, which undergo PERK-dependent degradation via the ubiquitin-proteasome system.

Yamaji, T. and K. Hanada (2014). "Establishment of HeLa cell mutants deficient in

sphingolipid-related genes using TALENs." PLoS One **9**(2): e88124.

Sphingolipids are essential components in eukaryotes and have various cellular functions. Recent developments in genome-editing technologies have facilitated gene disruption in various organisms and cell lines. We here show the disruption of various sphingolipid metabolic genes in human cervical carcinoma HeLa cells by using transcription activator-like effector nucleases (TALENs). A TALEN pair targeting the human CERT gene (alternative name COL4A3BP) encoding a ceramide transport protein induced a loss-of-function phenotype in more than 60% of HeLa cells even though the cell line has a pseudo-triploid karyotype. We have isolated several loss-of-function mutant clones for CERT, UGCG (encoding glucosylceramide synthase), and B4GalT5 (encoding the major lactosylceramide synthase), and also a CERT/UGCG double-deficient clone. Characterization of these clones supported previous proposals that CERT primarily contributes to the synthesis of SM but not GlcCer, and that B4GalT5 is the major LacCer synthase. These newly established sphingolipid-deficient HeLa cell mutants together with our previously established stable transfectants provide a 'sphingolipid-modified HeLa cell panel,' which will be useful to elucidate the functions of various sphingolipid species against essentially the same genomic background.

Yang, J., et al. (2010). "Protein tyrosine kinase pathway-derived ROS/NO productions contribute to G2/M cell cycle arrest in evodiamine-treated human cervix carcinoma HeLa cells." Free Radic Res **44**(7): 792-802.

A previous study indicated that reactive oxygen species (ROS) and nitric oxide (NO) played pivotal roles in mediating cytotoxicity of evodiamine in human cervix carcinoma HeLa cells. This study suggested that G2/M cell cycle arrest was triggered by ROS/NO productions with regulations of p53, p21, cell division cycle 25C (Cdc25C), Cdc2 and cyclin B1, which were able to be prevented by protein tyrosine kinase (PTK) activity inhibitor genistein or JNK inhibitor SP600125. The decreased JNK phosphorylation by addition of Ras or Raf inhibitor, as well as the increased cell viability by addition of insulin-like growth factor-1 receptor (IGF-1R), Ras, Raf or c-Jun N-terminal kinase (JNK) inhibitor, further demonstrated that the Ras-Raf-JNK pathway was responsible for this PTK-mediated signalling. These observations provide a distinct look at PTK pathway for its suppressive effect on G2/M transition by inductions of ROS/NO generations.

Yang, L., et al. (2011). "Mononaphthalimide spermidine conjugate induces cell proliferation inhibition and apoptosis in HeLa cells." Toxicol In Vitro **25**(4): 882-889.

Developing polyamine-drug conjugates that are capable of specific entry to tumor cells is attractive in improving chemotherapeutic efficacy. Currently, the exact cytotoxic mechanism of these conjugates is not well known. Here, our research revealed the effect of a mononaphthalimide-spermidine (MNISpd) conjugate on the growth and survival of HeLa cells and possible mechanisms. In characterizing the mechanism of MNISpd cytotoxicity, inhibition of proliferation is observed in the 0.5-6 muM range and there is evidence of apoptosis at equal or greater than 6 muM, but with less toxicity on HELF cell. The lower concentrations of MNISpd induced a cell cycle arrest correlated with enhanced p21 expression and decreased cdc2 but not Cdk2 expression. MNISpd-induced apoptosis was correlated with caspase-3 activation, decreased XIAP expression and a loss of mitochondrial membrane potential. Apoptosis but not cell cycle arrest was susceptible to N-acetyl-L-cysteine (NAC) treatment. It is proposed that MNISpd-induced apoptosis in HeLa cells is related to oxidative stress and that at lower exposure concentrations effects on cell proliferation predominate while at higher concentrations apoptosis develops.

Yang, L. L., et al. (2012). "CXCL10 enhances radiotherapy effects in HeLa cells through cell cycle redistribution." Oncol Lett **3**(2): 383-386.

Radiotherapy is a crucial treatment for cervical cancer, the second most common type of cancer in women worldwide. In this study, we investigated the effects of CXC chemokine ligand 10 (CXCL10) gene therapy combined with radiotherapy on cervical cancer using HeLa cells. TUNEL assay revealed that the apoptotic rate in the combined treatment of CXCL10 gene therapy and radiotherapy was greatly increased compared with that of CXCL10 or radiotherapy alone. Flow cytometry showed that CXCL10 overexpression in HeLa cells resulted in a prolonged G1 phase and shortened S phase at 72 h post-transfection. Western blot analysis revealed that p27(Kip1) was up-regulated in CXCL10-treated HeLa cells; however, cyclin E was down-regulated. These results indicate that the combination of CXCL10 gene therapy and radiotherapy is an effective strategy for the growth suppression of HeLa cells, and that CXCL10 enhances the radiotherapy effects through cell cycle redistribution. Our data provide new insight into the treatment of cervical carcinoma, involving an effective combination of gene therapy and radiotherapy against tumors.

Yang, X. L., et al. (2013). "Identification of complex relationship between protein kinases and substrates during the cell cycle of HeLa cells by phosphoproteomic analysis." Proteomics **13**(8): 1233-1246.

Each phase of eukaryotic cell cycle is tightly controlled by multicomponent regulatory networks based on complex relationships of protein phosphorylation. In order to better understand the relationships between kinases and their substrate proteins during the progression of cell cycle, we analyzed phosphoproteome of HeLa cells during G1, S, and G2/M phases of cell cycle using our developed quantitative phosphoproteomic approaches. A total of 4776 high-confidence phosphorylation sites (phosphosites) in 1177 proteins were identified. Bioinformatics analysis for predicting kinase groups revealed that 46 kinase groups could be assigned to 4321 phosphosites. The majority of phosphoproteins harboring two or more phosphosites could be phosphorylated by different kinase groups, in which nine major kinase groups accounted for more than 90% phosphosites. Further analyses showed that approximately half of the examined two phosphosite combinations were correlatively regulated, regardless of whether the kinase groups were same or not. In general, the majority of proteins containing correlated phosphosites had solely co-regulated or counter-regulated phosphosites, and co-regulation was significantly more frequent than counter-regulation, suggesting that the former may be more important for regulating the cell cycle. In conclusion, our findings provide new insights into the complex regulatory mechanisms of protein phosphorylation networks during eukaryotic cell cycle.

Yaoi, X., et al. (2017). "Taraxerol Induces Cell Apoptosis through A Mitochondria-Mediated Pathway in HeLa Cells." Cell J **19**(3): 512-519.

OBJECTIVES: Taraxerol acetate has potent anti-cancer effects via the induction of apoptosis, autophagy, cell cycle arrest, and inhibition of cell migration. However, whether taraxerol induced apoptosis and its underlying mechanisms of action is not clear. In the present study, we assess the effects of taraxerol on the mitochondrial apoptotic pathway and determine the release of cytochrome c to the cytosol and activation of caspases. MATERIALS AND METHODS: In this experimental study, we mainly investigated the effect of taraxerol on HeLa cells. We tested cell viability by the MTT assay and morphologic changes, analyzed apoptosis by DAPI staining and flow cytometry. We also determined reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) using a Microplate Reader. In addition, the apoptotic proteins were tested by Western blot. RESULTS: Taraxerol enhanced ROS levels and attenuated the MMP (Deltapsim) in HeLa cells. Taraxerol induced apoptosis mainly via the mitochondrial pathway including the release of cytochrome c to the cytosol and activation of caspases 9 and 3, and anti-poly (ADPribose) polymerase (PARP). Taraxerol could induce the down-regulation of the anti-apoptotic protein Bcl-2 and up-regulation of pro-apoptotic protein Bax. It suppressed the PI3K/ Akt signaling pathway. CONCLUSIONS: These results demonstrated that taraxerol induced cell apoptosis through a mitochondria-mediated pathway in HeLa cells. Thus, taraxerol might be a potential anticervical cancer candidate.

Ye, Y., et al. (2010). "[Effects of cryptotanshinone on proliferation and apoptosis of Hela cell line of cervical cancer]." Zhongguo Zhong Yao Za Zhi **35**(1): 118-121.

OBJECTIVE: To investigate the proliferation effects and apoptosis induction of cryptotanshinone on Hela cell line of cervical cancer. METHOD: The MTT assay was used to detect the growth inhibition rates of Hela cells at 24, 48, 72 h which cultured with cryptotanshinone in different concentrations. The cell cycle distribution and apoptosis were measured by flow cytometry. The protein expressions of E6, p53 and p21 were studied by Western blot. RESULT: The different concentrations (0.5-16 mg x L (-1)) of cryptotanshinone had cytotoxicity on Hela cells, which were clearly dose and time-dependent. The IC50 of 24, 48, 72 h were 17.8, 8.17, 6.55 mg x L (-1), respectively. Cells were treated with cryptotanshinone which had significant effects on cell cycle of Hela cell, and induced apoptosis. Western blot showed cryptotanshinone decreased expressions of HPV E6 and increased expressions of p53 and p21 proteins. CONCLUSION: Cryptotanshinone had significant cytotoic and radiosensitization effects on cervical cancer Hela cells. One of the mechanism may be that it might make significant G0/G1 phase arrest and induced apoptosis, a decrease in S phase, and restore the function of p53 to induce apoptosis in Hela cells to kill the tumor cell.

Zhurishkina, E. V., et al. (2015). "[Effect of Fucoidans Isolated from Seaweeds Laminaria Digitata and Fucus Vesiculosus on Cell Lines Hela G-63, Ecv 304 and Pc 12]." Tsitologiia **57**(10): 727-735.

The aim of the research was to investigate cytotoxicity of fucoidans on mammals cells. Three different samples of fucoidans were isolated from mechanically grounded brown algae Laminaria digitata and Fucus ve- siculosus. The sample F2 that differed from the others by higher sulfatation level and suppression of HeLa G-63 line culture growth was

taken for further study in cell lines HeLa G-63, ECV 304 and PC 12. We have shown that fucoidan preparation F2 inhibits proliferation and induces cell death in a dose- and time-dependent manner for all investigated cell lines. Neuroendocrine tumor rat cell line PC 12 appeared to be the most sensitive to fucoidan treatment whereas endothelial human cells ECV 304 were the least sensitive.

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