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Abstract Book

Melatonin: Its expanding universe

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When melatonin was identified in the bovine pineal gland in 1958, even those with the most vivid imagination could not envisage what the widely diverse functions of this critically-important molecule would be. Initially, it was presumed that melatonin was exclusively of pineal origin and, because it is uniquely synthesized and released at night, its major actions were thought to relate to circadian and circannual rhythm regulation, including the sleep-wake cycle. Soon thereafter, however, melatonin was identified in multiple organs in vertebrates and in invertebrates and unicells which lack a pineal gland; moreover, in 1995 melatonin was identified in land plants (1) and in a prokaryotic bacterium (2), the evolutionary precursors of mitochondria and chloroplasts of current-day animals and plants. Because of this, we speculated that mitochondria and chloroplasts of all animal and plant cells produce melatonin (3), as has now been confirmed (4,5). Beyond rhythm regulation, melatonin is a potent and diverse free radical scavenger (antioxidant) (6). Melatonin synthesis in mitochondria is especially fortuitous since numerous destructive reactive oxygen species are formed when electrons leak from the electron transport chain. This damage contributes to many free radial-mediated diseases (7), most of which melatonin has been shown to protect against (8), for example, neurodegenerative diseases, cancer initiation, progression and metastasis, and viral infections. In reference to cancer, melatonin also reverses the chemoresistance of cancer cells thereby re-sensitizing them to chemotherapies; this reversal in sensitivity involves melatonin's ability to inhibit Warburg-type metabolism and convert the cancer cells to mitochondrial glucose oxidation. For this to occur, melatonin inhibits hypoxia-inducible factor-1α which eventually leads to the downstream stimulation of pyruvate dehydrogenase complex allowing for the conversion of pyruvate to acetyl co-enzyme A in the mitochondrial matrix (9). In plants, melatonin also has many physiological functions and anti-pathological effects. Incubating seeds in a melatonin solution increases plant growth and crop yield, delays leaf senescence, makes plants more resistant to abiotic and biotic stressors and prolongs post-harvest fruit quality (10).

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Hallmarks of cancer: loss of TGF-β type V receptor (TβR-V) expression changing TGF-β from a tumor suppressor to a tumor promoter

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The TβR-V is a 600-kDa plasma-membrane glycoprotein and expressed in normal epithelial cells. It mediates growth inhibition by IGFBP-3 and TGF-β, which are known cytokine-tumor suppressors that prevent carcinogenesis or tumorigenesis by inhibiting growth in normal epithelial cells. TβR-V is the only known membrane receptor-tumor suppressor required for epithelial cells. Loss of TβR-V expression in epithelial tissues leads to development of carcinoma and more than 80% of human cancers are carcinoma. IGFBP-3 and TGF-β do not inhibit growth in cancer cells lacking TβR-V, such as Chinese hamster ovary carcinoma cells (CHO-LRP-1-/- cells) and H1299 human lung carcinoma cells. TGF-β acts as a tumor promoter toward these cells. Stable transfection of H1299 and CHO-LRP-1-/- cells with TβR-V cDNA restores squamous normal epithelial cell morphology and confers sensitivity to either TGF-β or IGFBP-3 growth inhibition. We recently demonstrated that, in epithelial cells, IGFBP-3 and TGF-β moderately inhibit growth by stimulating TβR-V-mediated IRS-2-dependent activation and cytoplasm-to-nucleus translocation of PP2Ac and PP1c, and dephosphorylation of p130/p107 and pRb, respectively1. TGF-β potently inhibits growth in epithelial cells by stimulating TβR-V-mediated tumor suppressor signaling (TβR-V/IRS-2/PP1c/pRb) in concert with canonical signaling mediated by TβR-I and TβR-II. TGF-β-stimulated signaling (TβR-I/TβR-II/Smad2/3/4) is required for potent TGF-β growth inhibition (100% at 1 pM) mediated by TβR-V via transcriptional activation of CDK inhibitors which activate the TβR-V-mediated tumor suppressor signaling cascade to maintain pRb unphosphorylated (active). The TβR-V-mediated signaling cascade should be an ideal target for developing a strategy to prevent and treat carcinoma cancers. Thus small molecule compounds, which enhance TGF-β-stimulated canonical signaling by recruiting TβR-I-TβR-II complexes from lipid rafts to non-lipid raft microdomains in the plasma membrane of cells, could be used to prevent and treat human cancer.

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Functions of a major nucleolar protein, nucleolin, in oncogenesis

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During tumorigenesis there is a tremendous remodeling of the nuclear architecture and particularly of nucleoli that reflects the increase of ribosome biogenesis that is required to sustain the high proliferation rate of cancer cells. This increase of ribosome biogenesis is partly due to an activation of RNA polymerase I activity since in cancer cells the RNA polymerase I activators are frequently over-expressed or over-activated and RNA polymerase I repressor are often inactivated. One major protein of the nucleoli, Nucleolin (NCL), is involved in the increase of ribosome biogenesis at the transcription level. NCL is a multifunctional protein with oncogenic properties. Anti-NCL drugs (N6L) show strong cytotoxic effects, including in triple-negative breast cancer (TNBC) models, and pancreatic ductal adenocarcinoma (PDAC). In order to better understand how the NCL antagonist, N6L, inhibits cancer cell growth, we carried out a translatome analysis in mPDAC cell lines (1). Using this approach, we showed that N6L induces translational reprogramming through the activation of the mTOR pathway during the treatment. Interestingly, we showed that N6L and mTOR inhibitors act synergistically to inhibit the proliferation of several PDAC and human PDX cell lines. In conclusion, we show that the NCL aptamer's strong inhibition of PDAC cell growth is coupled with the reprogramming of the translational machinery through the activation of the mTOR signaling pathway. We further demonstrate that NCL targeting by N6L treatment sensitizes PDAC cells to mTOR inhibitors. We propose a novel therapeutic strategy based on mTOR inhibition combined with NCL targeting by N6L to treat PDAC.

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Gold nanoparticles from *Peganum harmala*, *Artimesia absinthium* and *Morus nigra*: biosynthesis, characterization and cytotoxic activity against cancer cell lines

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The aim of this study was to develop an ecofriendly, simple, cost effective and single step method for the synthesis of three different gold nanoparticles (AuNps), using plant extracts including *Peganum harmala* (Ph) seeds, *Morrus nigra* (Mn) fruits and *Artimesia absinthium* (Aa) aerial parts. Synthesis of plant AuNps were confirmed by relevant color change, DLS, Zeta potential and characterized by relevant surface plasmon resonance peak for AuNps between 500 to 600 nm. Under optimized formulation the average size of synthesized AuNps were 65.18, 69.13 and 217nm for Aa, Mn and Ph AuNps, respectively. Cytotoxic activity of synthesized AuNps was evaluated using MTT assay followed by flow cytometry to assess its mechanism. According to the obtained results Aa-AuNPs, Mn-AuNps and Ph-AuNps were cytotoxic against MCF-7, HT-29, OVCAR-3 and HeLa cells in a dose dependent manner. Results also revealed that Ph-AuNps were the most potent nanoparticles (IC50s of 7.7, 16.7, 30 and 40 against HeLa, HT-29, OVCAR3 and MCF-7, respectively) and HeLa cells were the most sensitive cell line toward all tested nanoparticles ($p < 0.05$). Flow cytometry results confirmed cytotoxic effects of AuNps were performed through apoptosis induction. The depicted biological activities of AuNps are not simply due to the capped gold atoms but also to their surface macromolecules. Thus the use of Ph, Mn and Aa as a reducing and capping agents will retain its biological activity even after the depletion of maintained gold. The rapidly synthesized AuNps could play a role in the field of nanotechnology and biomedical applications.

Keywords: Gold nanoparticles; *Peganum harmala; Morrus nigra; Artimesia absinthium;* Cytotoxicity

Association analysis of kruppel like factor 1 (*KLF1*) and secretion associated ras related GTPase-1A (*SAR1A*) genetic polymorphisms with hydroxyurea response inβ-Thalassemia patients

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Haemoglobinopathies, characterized by abnormal hemoglobin levels, including β thalassemia are the most common inherited genetic blood disorders worldwide. Incidences of β thalassemia are increasing in Pakistan with more than 5000 cases being diagnosed every year. Management of β thalassemia requires regular blood transfusions and iron chelation therapy causing major healthcare burden on resource limited clinical settings in Pakistan. Hydroxyurea is the only cost-effective drug approved by Food and Drug Administration (FDA) to treat haemoglobinopathies. Mechanistically, hydroxyurea decreases the disease severity by regulating fetal haemoglobin (HbF). However, significant variability in response to hydroxyurea therapy exists among patients due to proposed genetic factors known to regulate gamma globin protein expression, necessitating the importance of better patients' stratification.

The current study reports association analysis of single nucleotide polymorphisms (SNPs) in *KLF1* and *SAR1A* with hydroxyurea efficiency in patients with β thalassemia. To conduct this study 100, β thalassemia patients on hydroxyurea therapy and 100 patients without hydroxyurea therapy were recruited on the base of their basal HbF levels from different centers across Punjab, Pakistan. Blood samples were collected from these patients following standard procedures. Whole blood DNA extraction was performed, *KLF1* and *SAR1A* genes were amplified using polymerase chain reaction (PCR), followed by RFLP based genotyping to determine the association between SNPs (*KLF1*: rs2072597, rs11085824, *SAR1A*: rs9971030, rs3858169) of candidate genes and hydroxyurea response.

Findings of this study revealed no association between polymorphisms of *KLF1* gene (rs2072597 A>G and rs11085824 A>G) (p=0.96 and p=0.75 respectively) and hydroxyurea therapeutic effectiveness. Moreover, association between HbF levels and genotypes of *KLF1* polymorphisms rs2072597 A>G and rs11085824 A>G (p=0.45 and p=0.16 respectively) was not observed. Furthermore, association of *SAR1A* rs9971030:C>T and rs3858169: T>G polymorphisms with either hydroxyurea response (p=0.96 and p=0.26 respectively) or HbF levels (p=0.13 and p=0.34 respectively) was not found. Conversely, a strong association between physical activity (p=0.001) and hydroxyurea response was observed, suggesting that the hydroxyurea responders were physically active as compared to non-responders. In addition, significant association between rs9971030 genotypes with high HbF levels (p>0.05) was observed. On the whole, the present study suggests that these genetic polymorphisms are not valuable pharmacogenomic predictors of hydroxyurea response in β thalassemia patients in Pakistan.

Use of drug repositioning for chemosensitization of epithelial ovarian cancer cells

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Epithelial ovarian cancer (EOC) is a lethal neoplasm with non-specific symptoms, late diagnosis, and poor prognosis (5-year survival below 40%). The standard treatment for EOC is cytoreductive surgery and chemotherapy (cisplatin or carboplatin and paclitaxel). Unfortunately, resistance to chemotherapy is a usual phenomenon in patients with EOC, worsening their prognosis. Some molecules involved in the chemoresistance are ATP-binding cassette transporters (ABC), c-MYC, Acyl-CoA synthetase 4 (ACSL-4, an enzyme involved in arachidonic acid metabolism), or epithelial-mesenchymal transition (EMT) proteins. Drug repurposing, or using an affordable, cheap, safe, and widely available medication often indicated for another common condition, could be key in the development of new therapeutic strategies for EOC. Drugs such as metformin or non-steroidal anti-inflammatory drugs (NSAIDs) or metformin have shown anti-tumoral properties, including an increase in chemosensitivity in most kinds of cancer cells. Based on these antecedents, we test the combination of metformin and NSAIDs in cisplatin sensibility in both cisplatin sensible and resistant EOC cells. Our results indicate that both metformin and NSAIDs increase cisplatin cytotoxicity in EOC cells, suggesting that their combined use could be beneficial in patients with EOC.

Impact of the hormone microenvironment on human endometrial function

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The endometrium of diverse species, including the human, constitutes an essential tissue for a successful reproduction. In women, the regulation of endometrial physiology is dependent on steroid action, like estrogens that promote proliferation and progesterone that induce differentiation of endometrial cells. Also, the energetic homeostasis of the endometrium is closely related to a normal reproductive function. Therefore, an abnormal hormone microenvironment affects endometrial physiology leading to reproductive failures. In fact, in women having Polycystic Ovarian Syndrome (PCOS), besides their ovulatory dysfunction, the observed endocrine/metabolic endometrial alterations conduct to a high probability to develop hyperplasia/endometrial cancer, where an imbalance of cell proliferation/apoptosis processes is detected, as we published previously. Additionally, since the insulin-pathway and the endometrial metabolic status (insulin resistance/hyperinsulinemia) are also compromised in PCOS, the expression and activity of important regulatory molecules are negatively regulated in this condition, as we have reported. In this regard, we have detected in PCOS-endometria a diminution in the levels and activity of molecules participating in the insulin pathway, like IRS-1, AS-160, PKCζ. Concomitantly, a defect in the synthesis and GLUT-4 translocation to cell surface is induced, with a decrease in glucose uptake. When metformin (insulin sensitizer) is orally administered to patients with insulin resistance-PCOS, endometrial GLUT-4 increases, improving their fertility, as previously published. Importantly, we recently reported that myo-inositol, an endogenous insulin sensitizer, improves GLUT-4 and p-AMPK levels and activity in endometrial cells. Another relevant feature is the high percentage of obesity in women with PCOS; adiponectin is an obesity marker and elicits an insulin-sensitizer action, being diminished in plasma of patients with obesity and PCOS, similar to its endometrial level. Moreover, obesity and PCOS can induce a pro-inflammatory environment (increase in macrophage number and TNF-α level in endometria), by exaggerating the alterations in the insulin pathway. Consequently, the evidences obtained in endometria from women with PCOS, clearly indicate that the molecular defects could partially explain the reproductive failures of these patients.
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Interplay between organophosphorous pesticides, acetylcholine, and estrogen receptor in breast carcinogenesis

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Breast cancer is a major health threat to women worldwide and the leading cause of cancer-related death. The use of organophosphorous pesticides (OPs) has increased in agricultural environments to control mosquito plagues. Furthermore, there is evidence that estrogen particularly 17β-estradiol, increases breast cancer risk in women. Estrogens have been demonstrated that drives the tumorigenic processes of breast cancer by binding to estrogen receptor alpha and regulating the expression of downstream genes. Previously, we showed that OPs decreased the levels of the enzyme acetylcholinesterase *in vivo*, increasing acetylcholine (ACh) serum levels. Such changes led to the formation of tumors in the mammary glands of rats. ACh is a neurotransmitter that regulates multiple functions in the nervous system. Emerging evidence indicates that ACh could play a role in cancer progression. Furthermore, we showed that ACh exposure induced overexpression of estrogen receptor alpha (ERα), in breast cancer cell lines a key protein described as the master regulator in breast cancer. Therefore, we hypothesize that ACh promotes ERα activity by leading to epithelial-mesenchymal transition (EMT) in breast cancer cell lines. The results showed that the physiological concentration of ACh leads to the release of Ca+2 from the reticulum, which promotes the activity of MAPK and 2/ERK signaling pathways. These changes are associated with an induction of p-ERα and its recruitment to the nucleus. Finally, ACh promotes the loss of anoikis and overexpression of key regulators of EMT in breast cancer cell lines. In summary, our results show that ACh promotes ERα activity in a ligand-independent manner through the mAChR/Ca+ and independent 1/2/ERK signaling pathways, eliciting EMT and suggesting its putative role in breast cancer progression.
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The significance of MRD detection in multiple myeloma

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Despite recent advances in its treatment providing superior clinical outcomes, multiple myeloma (MM) remains an incurable hematological malignancy. Minimal residual disease (MRD) has emerged as an independent prognostic biomarker in MM, as its presence is associated with poor overall and progression-free survival. Next-generation flow cytometry (NGF) is the analytical method of choice for MRD monitoring, combining high sensitivity, applicability in all patients, as well as wide availability and reproducibility among laboratories. We analyzed bone marrow (BM) aspirates and peripheral blood (PB) samples of MM patients at diagnosis and post treatment with NGF, according to the guidelines of the EuroFlow Consortium. After bulk lysis, nucleated cells were stained with two 8-color panels (CD19-PE-Cy7, CD27-AmCyan, CD38-FITC, CD45-PerCP-Cy5.5, CD56-PE, CD138-PacBlue, CD117-APC, CD81-APC-Cy7, CyIgκ-APC, CyIgλ-APC-Cy7). At least 10 million events were recorded per sample, using a BD FACSCanto II flow cytometer, and data were analyzed with the Infinicyt software (Cytognos). High phenotypic heterogeneity and clonal evolution were observed, as assessed in the clonal plasma cell compartment of the BM. A comparison between BM data and clonal tumor plasma cells (CTCs) in the PB showed a matched phenotypic profile of aberrant plasma cell at the two sites in 86% of cases analyzed. MM patients with phenotypic discrepancies (14%) had significantly higher CTC levels than those with a phenotypic agreement at the two sites (P =0.008), together with signs of a more diffuse disease pattern, as evidenced by the imaging results. Altogether, NGF offers, through the phenotypic characterization of clonal plasma cells in both BM and PB, the option to study clonal heterogeneity and clonal evolution in MM. Moreover, the analysis of CTCs may serve as a new hallmark for the real-time evaluation of a patient's disease and/or immune status.

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Development of DeepSnap, a novel *in silico* technology for predicting toxicity from chemical structures

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Typically, experiments are used to determine a compound's toxicity. From the perspectives of time, money, and animal welfare, it is unfeasible to evaluate several chemicals in their entirety. A crucial in silico tool to address these problems is a quantitative structure–activity relationship (QSAR) study. Using chemical structures and bioactivities, QSAR analysis creates mathematical models. Chemical structures are typically converted into hundreds of numerical values, such as molecular weight and lipophilicity, using this method. As it is challenging pattern recognition, artificial intelligence, such as deep learning, is currently used. To perform QSAR studies using molecular photographs rather than conventional chemical descriptors, we created a novel method called DeepSnap. DeepSnap is a deep learning technique for building a QSAR model using numerous three-dimensional structural images of low-molecular-weight compounds taken from 360° angles. We used the DeepSnap to forecast the activity of stress response pathways and nuclear receptors that were disclosed in Tox21. Models created using conventional descriptors and the DeepSnap prediction results' performance were compared. As a result, most toxicological targets' performances were on par with the best outcomes from conventional approaches. Additionally, we developed a feature region highlighter for molecular images. These aspects of the DeepSnap approach are anticipated to be used for various toxicity forecasts.

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MicroRNAs as a new complementary therapy for ovarian cancer

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Ovarian cancer is the most lethal gynecological cancer in Chile and the world. Between 80 to 90% of ovarian cancer corresponds to epithelial ovarian cancer (EOC), with high angiogenesis. One of the most known angiogenesis factors is the Vascular Endothelial Growth Factor (VEGF), which increases during EOC progression, along with the Nerve Growth Factor (NGF) and its high-affinity receptor TRKA as seen in epithelial cells. We found in EOC cell lines that NGF increases the levels of VEGF. On the other hand, also the TRKA receptors are expressed in endothelial cells; therefore, NGF induces the proliferation of endothelial cells and induces angiogenesis in these cells. In addition, in EOC cell lines, NGF promotes the expression of many oncogenic proteins, such as c-Myc, COX-2, VEGF, and ADAM17; these oncoproteins increase during EOC progression. In order to know which microRNAs (miRs) could be involved with the mRNAs of these proteins, an insilico study was assessed. Six miRs were found concerning NGF/TRKA in EOC, two of them were important since the mRNAs of oncogenic proteins increased by NGF, were targets of the chosen miRs. These miRs (miR-23b and miR-145) decrease during EOC progression and NGF decreases these miRs in EOC cells lines. Next, we over-expressed miR-23b and miR-145 and, with a mix of these two miRNAs in EOC cells by transient transfection, a decrease in the proliferation, migration, and invasion was observed along with c-Myc, and VEGF proteins. Importantly, the antitumor effects were greater with the mixture of miRs. Furthermore, we found that metformin has an antitumor effect in EOC cell lines by decreasing the NGF/TRKA signaling pathway and also, increases miR-23b and miR-145 levels in these cell lines.
Subsequently, we grafted EOC cells transfected with miR-145 in immuno-compromised mice, and the tumor size and metastasis decreased. Besides, we prepared miR-145 in gold-nanoparticles functionalized with FSH to improve the entrance to the cells of GNP/miR-145, and we evaluated in vitro the viability and migration of EOC cells in 2D culture by GNP/miR-145; a decrease of this process was found. In addition, a diminution in the size of EOC clones and spheroids with GNP/miR-145 and GNP/miR-145 plus chemotherapy was observed. Therefore, these data highly suggest that using miR-145 or a mix of miRs may be helpful as adjuvant therapy for ovarian cancer.

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Molecular and clinical features of aggressive thyroid cancer

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Thyroid cancer (TC) usually has a good outcome, however some histotypes such as poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC) have a bad prognosis, and their treatment is very challenging. The knowledge of the molecular mechanism and alterations implicated in cancer growth have open the way to the development and application of targeted therapies. Several multi-targeted kinase inhibitors, such as sorafenib, lenvatinib, and cabozantinib have been approved for the therapy of aggressive radioiodine (RAI)-resistant papillary TC (PTC) or follicular TC (FTC); in fact they are able to act against different altered pathways implicated in the pathogenetic process of aggressive TC. Selpercatinib and pralsetinib inhibit mutant RET in medullary thyroid cancer, but they can also block the RET fusion proteins-mediated signaling found in PTC. Entrectinib and larotrectinib, can be used in patients with progressive RAI-resistant TC harboring TRK fusion proteins. The combination of Dabrafenib (BRAFV600E inhibitor) plus trametinib (MEK inhibitor) has been authorized for the treatment of BRAFV600E-mutated ATC. New therapies strategies are under investigations, with drugs acting against immune checkpoint inhibitors. These drugs not only can limit the cancer spread, but in some circumstance they are able to induce the re-differentiation of aggressive tumors, which can be again submitted to new attempts of RAI therapy. These new therapies strategies pave the way towards a personalized therapy, in fact knowing the molecular pattern of each patient could aid in the choice of right therapies avoiding the administration of ineffective drugs.

Agrimonia eupatoria L.: effective modulator of skin wound healing

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We have previously shown that the water extract of *Agrimonia eupatoria L.* (AE) is a valuable source of polyphenols with excellent antioxidant properties and has clinical potential for the prevention and/or adjuvant therapy of cardiovascular complications associated with diabetes 1. Inspired by our previously published data 2, in the present study we further examined whether AE improves skin wound healing in a series of *in vitro* and *in vivo* experiments. In detail, we investigated the cytotoxicity of aqueous, 50% methanol and metanol extracts of AE on fibroblasts, keratinocytes and endothelial cells. We also demonstrated the ability of the extract to induce fibroblast to myofibroblast conversion, extracellular matrix (ECM) deposition, and keratinocyte proliferation/differentiation, in vitro. The cytotoxicity assay revealed comparable concentration-dependent effects between studied extracts, thus we used the water extract for the animal study. Subsequently, in Sprague-Dawley rats, we measured wound tensile strength (TS) and assessed the progression of open wounds using basic histology and immunofluorescence. The AE extract induced the myofibroblast-like phenotype and enhanced ECM deposition, both in vitro and in vivo. Furthermore, the wound TS of skin incisions and the contraction rates of open excisions were significantly increased in the AE-treated group. The present data show that AE water extract significantly improves the healing of open and sutured skin wounds. Therefore, our data warrant further testing in animal models that are physiologically and evolutionarily closer to humans.

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Novel approaches to treat (infected) wounds

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Poor wound healing presents a significant burden to the health-care system and society. Active wound dressings are attracting extensive attention in wound treatment including bacteria-infected lesions. As the wide use of antibiotics leads to drug resistance we present here a new concept of wound dressing based on the polycaprolactone nanofiber scaffold (NANO) releasing second generation lipophosphonoxin (LPPO) as antibacterial agent 1. Firstly, we demonstrated in vitro that LPPO released from NANO exerted antibacterial activity while not impairing proliferation/differentiation of fibroblasts and keratinocytes. Secondly, using animal model we showed that NANO loaded with LPPO significantly reduced the Staphylococcus aureus counts in infected wounds. Furthermore, the rate of degradation and subsequent LPPO release in infected wounds was also facilitated by lytic enzymes secreted by inoculated bacteria. LPPO displayed negligible to no systemic absorption. In conclusion, the composite antibacterial NANO-LPPO-based dressing reduces the bacterial load and promotes skin wound healing, thus may present a novel product improving patient`s care in the near future.

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Comparative oncology as a feasible strategy for the discovery of biomarkers and molecular targets in osteosarcoma

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Osteosarcoma (OS) is the most common bone cancer in dogs and humans, with extensive similarities in genetics, biology and clinical presentation in these species, including the common occurrence of lung metastasis. There have been no significant advances in prognosis and treatment of OS in more than 30 years, for which it is imperative to find new avenues to improve patient outcome. The Dog Osteosarcoma Group: Biomarkers of Neoplasia (DOGBONe) is an interdisciplinary team at the University of Guelph aimed at accelerating advances in diagnosis, prognosis and treatment of OS through comparative oncology studies that begin with the analysis of dog samples. Here we present our recent study on extracellular vesicles (EVs), which are membrane-bound vesicles released by cells into their surroundings. Importantly, EVs circulate in biological fluids such as blood, carrying within them molecular signatures of their cell of origin. Therefore, we hypothesize that EVs derived from OS tumour tissue, could provide useful information about the biology of OS. To investigate this, we developed a pipeline to isolate, characterize, and profile EVs from normal bone and OS tissue explants from canine OS patients. Specifically, we performed uHPLC tandem mass spectrometry followed by label-free quantification to identify proteins of interest. A student's t-test was used to determine the differences between non-malignant bone and OS samples. This analysis revealed a protein signature related to protein metabolism. One molecule of interest, PSMD14/Rpn11, was explored further given its prognostic potential in human and canine OS, and its targetability with the drug capzimin. In vitro experiments demonstrated that capzimin induces apoptosis and reduces clonogenic survival, proliferation, and migration of metastases-derived canine OS cell lines. Capzimin also reduces the viability of metastatic human OS cells cultured under 3D conditions that mimic the growth of OS cells at secondary sites. This unique pipeline can improve our understanding of OS biology and identify new prognostic markers and molecular targets for both canine and human OS patients.

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An uncommon genetic landscape and clinical spectrum in italian patients referred for recurrent fever

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Autoinflammatory diseases (AIDs) are mostly monogenic disorders characterized by recurrent self-limiting flares of inflammation associated to periodic fever, systemic and localized serositis. Most patients carry mutations in genes of the innate immune system although in several patients no genetic changes are detected. Familial Mediterranean Fever (FMF) is the commonest among AIDs with high prevalence in the Mediterranean basin. It is caused by mutations in the *MEFV* gene and shows a prevalently autosomal recessive inheritance. FMF appears to be milder in non-middle eastern patients where amyloidosis, the most severe complication of FMF, is rarely present. Several criteria have been developed to increase diagnosis sensitivity and specificity. We performed gene testing and retrospectively investigated the clinical presentation in 260 patients referred on a suspect of AIDs, and all originating from Apulia in the south eastern Italy. A genetic change was identified in 51.1% of tested patients. Among the clinical manifestations, demographic info and ascertainment criteria, family history and Pras score were predictive of a genotype compatible with AID diagnosis. The variant R761H, which shows negligible frequencies among different Mediterranean populations and the Japanese, was the most common (19.1%) in our cohort. Further, it was prevalently present in compound heterozygosity with E148Q. The distribution of detected *MEFV* variants was non-random in chromosomes identified by three synonymous *MEFV* polymorphisms. Our results confirm that FMF is milder in non-middle eastern patients and suggest that chromosomes bearing the R202Q variant might not be evolutionarily neutral.

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Evaluation of potential biomarkers of therapy sensitivity and disease activity in non-infectious uveitis

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Non-Infectious Uveitis (NIU) is a leading cause of irreversible blindness in working-age population in the developed world. NIU is a group of disorders characterized by intraocular inflammation at different structures of the eye. Currently, the standard of care for NIU includes the administration of glucocorticoids (GC) as first line therapy agents. Nevertheless, some patients become refractory to GC treatment[1]. This clinical phenomenon has been associated with a change in the expression of glucocorticoid receptor (GR) isoforms[2]. Moreover, current methods of NIU show a suboptimal interobserver reproducibility due to a lack of objective measurements. We investigated the levels of the GRα isoform, IL-10, Mitogen-activated protein kinase phosphatase-1 (MKP-1) and inflammation related miRNAs in peripheral blood mononuclear cells (PBMC) and serum as potential biomarkers of disease activity in patients with NIU. This prospective cohort study included a total of 19 patients with NIU. A real-time quantitative PCR to measure the mRNA levels of GRα, MKP-1, IL-10, was carried out at baseline (before treatment initiation), one week and two weeks after GC treatment initiation. There were no significant differences in GRα and MKP-1 levels between baseline and after two weeks of prednisone treatment. However, the mRNA levels of IL-10 increased after two weeks of treatment. No significant differences were found in the expression of miRNAs obtained from PBMC in GC-sensitive and GC-resistant patients. However, patients with an active inflammatory state had significatively higher serum levels of miR-23a and miR-126 than inactive patients at baseline. We conclude that the evaluation of the change of IL-10 expression 14 days after GC treatment initiation could be a promising biomarker to determine the response to GC therapy, whereas the expression of serum miR-23a and miR-126 could constitute a biomarker of inflammatory activity in patients with uveitis.

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Immature granulocytes: Innovative biomarker for SARS-CoV-2 infection

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Background: Immature granulocytes (IGs) include metamyelocytes, myelocytes, and promyelocytes, and are the precursors of neutrophils. Increased IG counts found in peripheral blood indicate enhanced bone marrow activity. In addition, IGs have been evaluated in numerous clinical conditions, such as severe acute pancreatitis, systemic inflammatory response syndrome, and infectious complications following open-heart surgery under cardiopulmonary bypass. There has been limited research regarding the role of these neutrophil precursors in viral infections, including severe acute respiratory syndrome and coronavirus 2 infection. The present thus aimed to evaluate the role of the IG count in patients with COVID-19.
Methods: In the present study, adult patients who visited or were hospitalized at the COVID-19 Unit of Laiko General Hospital due to SARS-CoV-2 infection, were enrolled. The patients were predominantly infected with the alpha variant and were all unvaccinated. The following data were collected from all patients: i) Demographics; ii) The presence of comorbidities; iii) Outcomes (recovery, intubation, mortality); iv) The duration of hospitalization; v) The development of disease-related complications. The IG count was associated with disease severity, outcomes, the duration of hospitalization, and the development of complications.
Results: The IG count was significantly associated with the severity of COVID19 infection, with higher IG count values being detected in severe and critical cases. In addition, higher IG count values were associated with a longer duration of hospitalization. Furthermore, the IG count was found to be an independent prognostic biomarker of intubation and mortality in patients with COVID-19, according to multivariate logistic regression analysis, including age, the male sex, and the presence of comorbidities as confounders.
Conclusions: In conclusion, the present study demonstrates that the IG count is associated with the severity of COVID-19 and the duration of hospitalization. Furthermore, the IG count was found to be an independent prognostic indicator of intubation and mortality in patients with COVID-19.

Lung function at 3 months after hospitalization due to COVID-19 pneumonia: comparison of alpha, delta, and omicron variant predominance

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Background: The coronavirus disease (COVID-19) pandemic has already affected millions of people, with increasing numbers of survivors. These data indicate that a myriad of people may be affected by the pulmonary sequelae of the infection. The aim of this study was to evaluate pulmonary function in patients hospitalized due to COVID-19 3 months after hospital discharge.
Methods: One hundred and seventeen patients, 34 females and 83 males, with a mean age of 57.92±11.52 years, who were hospitalized due to COVID-19 underwent pulmonary function testing 3 months after their hospital discharge.
Results: Eighty-four (71.8%) patients were hospitalized in the period of alpha variant predominance, 16 (13.7%) in the period of delta variant predominance, and 17 (14.5%) in the omicron variant predominance period. 88.9% were unvaccinated, and 11.1% were vaccinated. Sixty-five percent had comorbidities. 8 patients did not require oxygen, 71 (60.7%) patients required oxygen supply up to 60%, 33 (28.2%) required 100% supply or high flow nasal cannula or non-invasive mechanical ventilation, and 5 (4.3%) patients were intubated. The mean value of DLCO and TLC was statistically higher in patients affected by the omicron variant ($p<0.05$). Abnormal values (<80% pred) of DLCO and TLC were observed in 29.9% and 17.9% of the patients, respectively.
Age and female gender were independently associated with the occurrence of abnormal DLCO, while a greater need for oxygen administration during hospitalization was statistically associated with the occurrence of abnormal TLC ($p<0.05$).
Conclusions: Our data suggest that a significant percentage of individuals would develop abnormal pulmonary function after COVID-19, regardless of the SARS-CoV-2 variant.

Thyroid carcinoma: Germline DNA variant markers and cardiometabolic risk diseases

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Recent progress in the genome-wide association studies of thyroid cancer leads to the identification of several genetic variants conferring risk to this malignancy across different ethnicities. We set out to elucidate the impact of selected single nucleotide polymorphisms (SNPs) on papillary thyroid carcinoma risk and to evaluate the interactions of these genetic variants with associated diseases for the first time in the Slovak population. Six SNPs (rs966423, rs2439302, rs965513, rs116909374, rs1537424 and rs944289) were genotyped in 86 patients with PTC and 99 healthy control subjects. The association analysis and multivariable modelling of PTC risk by the genetic factors, supplemented with a rigorous statistical validation, were performed. One of the six SNPs rs966423 (DIRC3, OR=1.51, p=0.03) was significantly associated with PTC. Two SNPs rs965513 (PTCSC2, OR=1.34) and rs116909374 (MBIP, OR=0.44) showed a suggestive association. Haplotype TTC (SNPs located on chromosome 14q13) showed a suggestive association with PTC (p=0.07, OR=1.55). In the PTC group, significant associations were observed between rs966423 (DIRC3) and ischemic heart diseases (p=0.009), rs965513 (PTCSC2) and diabetes mellitus (p=0.04) and haplotype 14q13 and musculoskeletal diseases. Next associations rs966423 (DIRC3) and arterial hypertension; rs116909374 (MBIP) and other benign diseases; rs1537424 (MBIP) and disorder lipid metabolism, rs965513 (PTCSC2) and anti-Tg (thyroglobulin antibody) showed suggestive associations. These results indicate that germline variants not only predispose to PTC, but may also be related to other risk factors, including associated diseases. However, these associations were only moderate, and further multi-ethnic studies are required to evaluate the usefulness of these germline variants in the clinical stratification of PTC patients.
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Achievements in prevention, early detection and treatment of lung cancer

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Lung cancer remains one of the most frequently diagnosed malignancies in developed countries and the leading cause of cancer death, despite advances in understanding of risk factors, development, and treatment. Poor prognosis is due to the absence of early warning signals, which would allow the capture of this disease in its early stage. It is, therefore, necessary to seek for appropriate methods for prevention, early diagnosis and for the identification of predictive biomarkers allowing to predict the treatment response of the patients by targeted therapy and immunotherapy approaches. Implementation of the molecular and genomic profiling of the cancer tissue alterations either by single and multiplex gene detection or by more complex NGS analyses has become essential. We demonstrated panel of DNA repair gen´s polymorphisms associated with elevated risk for lung cancer development, micro RNAs potentially usable as biomarkers for early disease detection and currently we look for predictive biomarkers allowing to predict the treatment response using liquid biopsy.

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Cancer microenvironment in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive tumors. Despite the effort in pancreatic cancer research, the mortality to incidence ratio has not experienced remarkable revision over the last few decades thus, PDAC represents the fourth leading cause of cancer death. The five-year survival rate remains around 5 % and the one-year rate achieves a non-promising level of only 20%. Unfortunately, the incidence is rising worldwide. A typical histological attribute of this type of cancer is abundant stromal desmoplasia. The cancer microenvironment contains plentiful extracellular matrix fibres produced by cancer-associated fibroblasts (CAFs). The stroma is metabolic very potent and significantly influences tumour growth, metastatic spread and resistance to anticancer therapy. However, the tumor-stromal interactions between cancer cells and CAFs are still poorly understood. We isolated CAFs from PDAC donors, expanded them and *in vitro* evaluated their biological effect on PDAC cancer cell lines. Using the transcriptome analysis (ILLUMINA) we established genes differentially expressed in CAFs compare to control fibroblasts. Selected proteins may have effect to support tumor expanding and prolong survival of cancer cells. We conclude that our study supports the desmoplastic patient-specific character of cancer cell regulation by CAFs, which precludes development of an effective treatment strategy and rather requires establishment of an individualized tumor-specific treatment protocol for the use in human clinical practice.

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The efficacy of Pazopanib in the treatment of anaplastic thyroid cancer in primary culture

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Thyroid cancer (TC) is the most frequent endocrine tumor all over the world, with a growing incidence, and usually a good prognosis. However, anaplastic thyroid cancer (ATC) is a rare and rapidly fatal human cancer. The median survival of ATC, despite the usual treatment that includes surgery, external hyperfractionated radiation therapy, and chemotherapy, is of approximately 6-10 months. Therefore, it is challenging to predict the ATC patient clinical therapy responsiveness. The understanding of the molecular pathways involved in the pathogenesis of ATC led to the development of new drugs, such as small molecule inhibitors of tyrosine kinase (TKIs) pathways. Sorafenib, lenvatinib and cabozantinib have been approved for the therapy of recurrent or metastatic, radioactive iodine refractory DTC (RAIR-DTC), and cabozantinib and vandetanib for medullary TC. Dabrafenib plus trametinib have been approved for the treatment of ATC with V600EBRAF mutation. Pazopanib is a multitarget TKI of VEGF receptors, PDGF, and c-Kit, approved by FDA for the treatment of advanced renal cell carcinoma and advanced soft tissue sarcoma. We evaluated the antineoplastic effect of pazopanib in vitro in primary human ATC cells (pATC). We obtained surgical thyroid tissues from 5 patients with ATC, from thyroid biopsy at the moment of first surgical operation. We observed an inhibition of proliferation, migration and invasion, and an increase of apoptosis, in pATC cells treated with pazopanib ($P<0.05$). Furthermore, pazopanib was able to significantly decrease the VEGF expression in pATC cells ($P<0.05$). This study allow us to demonstrate the antineoplastic activity of the antiangiogenic inhibitor, pazopanib, in human pATC *in vitro*.

Artemisinins and artesunate mitigate colo cell migration and reduce apoptosis

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Cancer is one of the deadliest diseases in the world with second-most leading death and approximately 9.6 million people have died of cancer in the year 2018 globally. Colorectal cancer is a very dangerous type of cancer with the third most diagnosis and deaths out of all cancer. Artemisinin(A1) and Artesunate(A2) derived from a type of sweet worm plant in china and have been widely used as a cancer treatment. A1 and A2 react with Fe^{2+} ions which are crucial for cancer cell mitosis. Upon activation, A1 and A2 produce free radicals that will attack the cancer cell membrane and cause a leakage of cellular material, ultimately killing the cell. Therefore, A1 and A2 have very strong effects on Colorectal cancer which goes through faster mitosis than other types of cancers. This study examined the effect of A1 and A2 on colorectal cancer cell proliferation and colony formation along with the cytotoxicity of the two chemicals. A1 and A2 have both shown a dose dependent decrease effect going from low to high concentration with A1 causing 110.93% decreased cell survival at 1 μM, 83.59% at 10 μM, and 78.47% at 100 μM whilst A2 showed 62.85% at 1 μM, 85.3% at 10 μM, and 71.7% at 100 μM ($p<0.05$). Furthermore, the two chemicals showed stronger effects when used in combined therapy where A1+A2 decreased cell survival by 64.21% at 1 μM, 59.64% at 10uM, and 59.61% at 100 μM ($p<0.05$), suggesting the two chemicals work synergistically along similar molecular pathways. A1 100 μM along with A2 and the combined treatment at all concentrations were statistically significant at $P<0.05$ in the MTT assay. The two chemicals also showed a dose decrease effect in cancer cell colony formation with stronger synergistic effects. In comparison to the control, A1 10uM showed a decrease of 5.1%, A1 100 μM 66.6%, A2 10uM 52.0%, A2 100 μM 28.5%, A2 10 μM 52.0% and A2 100 μM 25.9%. These results were statistically significant with the exception of A1 at 10 μM. The results indicated both A1 and A2 may induce apoptosis or autophagy. This study shows that A1 and A2 work synergistically along specific molecular pathways, future experiments could be conducted to find the specific pathway the two chemicals work through.

Cytotoxic effect of potassium bromate on human cells

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Potassium bromate (KBrO3) is a food additive to bread found in 130 companies across the United States (1). It is an inorganic chemical that has oxidizing properties due to the oxygen atoms and potassium ions bound to the outside of its structure (2). It is banned in Europe due to it cytotoxic and carcinogenic effects. This research aims to demonstrate if KBrO3 has cytotoxic effect at low concentrations toward several human cell models never tested before. The MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide), LDH (lactate dehydrogenase), and caspase (cysteine-aspartic proteases) assay were preformed to measure the chemical's effects. Our results shown KBrO3 1 day incubation diminished immune cell (U937) survivability by ~20 - 25% and on heathy gut cell (CCD-18), it decreases survivability by ~30% to ~50% at 0.03μM ($p<0.05$). Four-day incubation of KBrO3 had a significant increase in damage to U937 cells, (~25% to ~50%, $p<0.05$). Furthermore, KBrO3 at concentrations used showed it can increase cytotoxicity from 0.13% to 26% (at 0.3 μM; $p<0.05$). In addition, KBrO3 exhibited strong apoptotic effect on colon rectal cells (Colo-320), from 8% at 0.3 μM till 4300% at 300 μM; and on neuronal cell (HTB-11) from 167% to 333%. Interestingly KBrO3 at low concentrations increased apoptotic effect but showed negative effect at 30μM and higher concentration. This study demonstrated that KBrO3 can induce both apoptosis and necrosis at rather low concentration to most of the human cells exposure, especially towards human gut cells. It may have both short term and long-term impact on cells/tissue to induce pathological disorders.

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Metformin exhibits novel anti-amyloid beta peptides' pathological effects on human immune cells

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Alzheimer's Disease (AD) have notable neurological characteristics including the accumulation of amyloid-beta (Aβ) peptides, hyperphosphorylated tau proteins, and brian insulin resistance(1). Research has shown that there is a link between AD and Type 2 Diabetes Mellitus (T2DM)(2). Metformin is commonly used as an antihyperglycemic drug for T2DM(3).The aim of this research was to explore the effects of metformin on pathogenic Aβ using in vitro cell models and in silico molecular docking to determine its impact on formation of AD. MTT cell proliferation assay and Cell adhesion assay were used to determine metformin's effect on target cells. Metformin has exhibited strong binding affinity with macromolecules related to AD pathological processes. Metformin at 1 uM concentration significantly reduced Amyloid beta peptide-induced cell death by approximately 36% ($p<0.05$). Furthermore, Metformin at 1 uM concentration inhibited Aβ peptide-induced cell adhesion by 40% ($p<0.05$). Additionally, metformin at 1 uM reduced Aβ activation caspase significantly ($p<0.05$). In silico data showed metformin has strong binding affinity with Aβ peptide. These results suggest that Metformin at concentration used can provide strong protection on immune cells by mitigating Aβ peptide induced harmful impact on neural and immune cells.

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Neuroprotective effects of Naringenin against Parkinson's disease

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Recent research showed polyphenols as potential candidates for Parkinson's Disease (PD) treatment (1). The aim of study was to determine naringenin to have such effects against PD. Cell based assays, like MTT, LDH determined if naringenin can reduce rotenone induced cytotoxicity against HTB-11 and U937 model cell lines. Naringenin at 10 μM significantly mitigated rotenone induced neuronal cell (HTB-11) death, and at 0.1 μM had strong effects against rotenone induced immune cell (U937) survival ($p<0.05$). Furthermore, Naringenin at 10 uM reduced rotenone caused cytotoxicity by 9.37% (HTB-11), and at 0.1 μM reduced rotenone caused cytotoxicity by 7.6% ($p<0.05$). Moreover, Naringenin at 10 μM reduced rotenone caused apoptosis by 60% ($p<0.05$). *In silico* analysis revealed that naringenin may execute its ant-rotenone effect by targeting rotenone's binding site on mitochondrial complex 1. Lastly, Naringenin at 10 μM significantly reversed rotenone's effect on alpha-synuclein protein expression. Our study demonstrated that naringenin has strong neuro-immune protective effects, with the potential to be new candidate for the treatment of PD.

(1) Zeng, W. et al., 10.1016/j.phrs.2018.08.002, 2018

Novel insights and recent discoveries on the genetics and pathogenesis of malignant mesothelioma

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Malignant mesothelioma is a highly aggressive malignancy arising from the serosal lining. While most cases originate in the pleural lining of the thoracic cavity, a subset of cases primarily involves the peritoneum or, rarely, the pericardium or the tunica vaginalis. Studies have suggested that malignant mesothelioma comprises several clinicopathologic subgroups, with distinct site predilections and pathogenetic mechanisms. Here, we provide an overview on the recent discoveries on the genetics and pathogenesis of malignant mesothelioma, as well as the implications of these findings on the diagnostic workup1. Ever since the seminal study by Wagner et al. on the mesothelioma epidemic in South African crocidolite miners in 1960, asbestos exposure has emerged as a significant risk factor for the development of mesothelioma. Various occupations associated with asbestos exposure, such as insulation workers and shipyard builders, demonstrate increased risks for developing malignant mesothelioma. Nonetheless, a subset of malignant mesothelioma, for instance peritoneal mesothelioma, is relatively more common in women, presents occasionally in young patients, and shows weaker to no association with asbestos exposure in epidemiologic studies. Given a latent period of several decades between asbestos exposure and mesothelioma development, the pathogenesis of mesothelioma in these patients has been hypothesized to be due to other mechanisms. For instance, a subset of patients who received prior therapeutic radiation for malignancies such as Hodgkin lymphoma developed malignant mesothelioma with a latency of 17–26 years. Compared to asbestos-related cases, radiation-associated malignant mesothelioma demonstrated unusual histology, including pleomorphic, myxoid, or signet-ring cell features, and showed better overall survival. In addition, aside from prior asbestos or radiation exposure, additional genetic mechanisms have been implicated in the development of malignant mesothelioma in a subset of cases, including: (I) germline BRCA1 associated protein-1 (*BAP1*) inactivation syndrome, (II) structural gene rearrangements in Ewing sarcoma breakpoint region 1 (*EWSR1*) or fused in sarcoma (FUS), and (III) anaplastic lymphoma kinase (*ALK*) rearrangements.

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TDO2-Kynurine pathway in CRPC

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Tumor dormancy is classically defined as the arrest of tumor growth in the primary site or in metastatic dissemination. The concept of dormancy is derived from clinical findings that cancer recurs several years or even decades after surgical resection of the primary tumor, especially in breast and prostate cancers. As tumor dormancy is one of the mechanisms of resistance against various cancer therapies, targeting dormant cancer cells should be considered for future treatment strategies. Although the phenomenon of dormancy is becoming increasingly recognized in the field of cancer research, the mechanisms for it are still largely unknown. Androgen deprivation therapy (ADT) is often used to treat high risk localized prostate cancer (PCa) and to prevent the progression towards metastasis. ADT-treated PCa, however, often regress to a dormant state that can last for available number of years. ADT eventually becomes ineffective due to the emergence of clinically evident castration resistant PCa (CRPC) or transdifferentiated neuroendocrine PCa (NEPC). Targeting ADT-induced Dormant Cancer Cells (DCCs) may be the key to preventing additional cascades of dormancy-relapse cycles and the emergence of CRPC and/or NEPC. However, little is known about the molecular mechanisms underpinning the emergence of ADT induced DCCs. Here we report that after ADT treatment of the androgen sensitive PCa cell line LNCaP, the level of Formyl kynurenine (KYN), a derivative metabolite of the amino acid tryptophan, was significantly increased. Interestingly, the protein expression of TDO2, a rate-limiting enzyme that converts tryptophan to KYN, is also upregulated and persistently increases as the disease progresses. We demonstrate that the ADT-induced TDO2 upregulation is required for the survival and maintenance of ADT-induced dormancy of prostate cancer and the persistent expression of TDO2 drives the recurrence of PCa tumors and therefore, leading to the failure of ADT treatment. This project will provide a new metabolic mechanism underpinning the ADT treatment-induced tumor dormancy as well as propose a novel treatment for PCa by combining ADT and targeting TDO2

PIN1 as a molecular target for glioblastoma treatment: Target validation, inhibitors rational design and biological activity determination

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The Peptidyl-prolyl isomerase PIN1 controls diverse cellular functions and participates in all the biological processes linked to tumor development and progression in several cancer types, including glioblastoma (GBM). GBM is a lethal disease with poor therapeutic resources. Thus, this research aims to assess PIN1 as a molecular target for a new therapeutic alternative. First, we developed a PIN1 knockout GBM cell line model (LN PIN1 KO) using CRISPR/Cas9 on LN229 cells. This new generated cell model was evaluated in diverse cellular process as migration, cell cycle progression, cell immortality and tumoral progression. Results showed a significant decrease in LN229 oncogenic features both *in vitro* and *in vivo* assays due PIN1 absence. Once PIN1 was confirmed as a relevant molecular target, we proceeded to search specific inhibitors by a docking-based virtual screening. We obtained a ranking of compounds from which we pre-selected 4 candidates that demonstrated the ability to bind to PIN1 *in vitro* in a Thermal Shift Assay. Following this, we evaluated the antiproliferative effect of selected compounds both in LN229 and LN PIN1 KO cells, to ensure that the effect was PIN1 dependent. Among all the candidates tested, compound 7 presented the greatest effect on LN229. This growth inhibition was also significantly reduced in absence of PIN1. Finally, we evaluated compound 7 on a panel of GBM cell lines which consistently inhibited proliferation among all the cell lines tested after 72hs. In conclusion, we demonstrated the key role of PIN1 in LN229 tumoral behavior, validating this protein as a relevant molecular target in GBM. Simultaneously, a list of candidate compounds was obtained with *in silico* and *in vitro* tests. From these candidates, a PIN1 inhibitor with specific biological activity was identified. This novel PIN1 inhibitor set the bases for a promising therapeutic alternative for GBM treatment.