9; MDA-MB231: 89 ± 2; and MDA-MB468: 44 ± 5 %; p<0.0001). NIC also increased the proliferation of non-tumorigenic mammary cells (MCF-10A: 45 ± 7; p<0.0001). In addition, treatment with PX reduced in a concentration-dependent manner the viability of all cell lines with Emax values close to 100 % (p<0.0001). In MDA-MB231 cells, derived from a triple negative tumor, the Emax of NIC was significantly reduced to the control value in the presence of mecamylamine (10^{-4} M) confirming the participation of nAChRs. Moreover, the addition of NIC 10^{-10} M or 10^{-9} M significantly reduced the Emax of PX to 30 ± 5 or 36 ± 6 % respectively (p<0.001). We conclude that NIC increases the proliferation of both tumor and non-tumor mammary cells, indicating that it could be promoting or inducing breast malignancy and reduces the efficacy of PX treatment in triple negative breast tumors.

0097 - GLYPICAN-3 (GPC3) INHIBITS METASTASIS DEVELOPMENT AND PROMOTES DORMANCY IN BREAST CANCER CELLS THROUGH P38 MAPK PATHWAY

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We previously showed that GPC3 prevents metastatic spread of breast cancer cells as well as it activates p38 pathway. We hypothesize that GPC3 acts as a metastasis suppressor. The aim of this study was to examine whether GPC3 is inhibiting metastasis through p38 activation. We used the murine mammary LM3 cancer cell line reexpressing GPC3. Since it has been proposed that metastasis suppressors promote dormancy, we evaluated dormancy markers. We showed that the pErk/pp38 ratio was lower in LM3-GPC3 cells, while p21, p27 and SOX2 protein levels were higher, suggesting a dormant phenotype. We did in vivo experimental metastasis assays, confirming that LM3-GPC3 cells reduced their metastatic ability (p<0.005 Kruskal-Wallis). Interestingly, the presence of LM3-GPC3 cells was demonstrated in primary cultures of lungs from mice inoculated with those sublines, despite that metastatic foci were not detected. The GPC3 role was specific to dormancy since it did not affect s.c. tumor growth, but lungs of LM3-GPC3 primary culture tumor bearing-mice had no metastasis. So, dormant LM3-GPC3 cells can reactivate their proliferative capacity, remain viable, tumorigenic, but they reenter in dormancy upon reaching secondary colonization site. We analyzed whether GPC3 inhibits metastasis through p38 activation. Cells were s.c. inoculated into mice, and the p38 inhibitor SB203580 (or DMSO) was i.p. administered. The in vivo inhibition of p38 did not affect the tumor growth, but it induced an increase in LM3-GPC3 tumors local invasion (p<0.05 chi-square), as well as in spontaneous metastatic dissemination (p<0.005, Kruskal-Wallis). We did experimental metastasis assays, where cells were simultaneously inoculated with SB203580, confirming that the treatment reverses the inhibition on the metastatic spread induced by GPC3 (p<0.05, Kruskal-Wallis). Our results prove that GPC3 inhibits the metastatic ability of breast cancer cells and induces dormancy at secondary site, through the p38 activation.

0261 - EFFECTS AND UNDERLYING MECHANISMS OF GENE THERAPY WITH SUICIDE SYSTEM CYTOSINE DEAMINASE/ 5-FLUOROCYTOSINE IN SPONTANEOUS CANINE MELANOMA

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Cancer occurs spontaneously in humans and dogs, and its progression is often similar in both species. As a pre-clinical study aimed to improve the local effects of suicide genes on oncologic veterinary patients, we explored the responses of different spontaneous canine melanoma cell lines to yeast Saccharomyces cereviseae cytosine deaminase/ uracil phosphoribosyl transferase fusion protein (Ycd::Yuprt) non-viral gene transfer in combination with 5-fluorocytosine (5-FC). Ycd catalizes the passage 5-FC to 5-fluorouracil (5-FU) that interferes with DNA replication, while Yuprt drives to the synthesis of 5-FUTP that inhibits rRNA and mRNA processing. We determined the survival rates of 3D cultures on agar coated wells 12 days after treatment by the acidic phosphatase assay. We found that the spheroids formation is inhibited and the survival rate decreased to 50 % (0.05 < p < 0.0001). We explored the mechanisms related to cytotoxicity by the colony formation assay, and the senescence-associated beta-galactosidase (SA-βgal) activity in monolayer cultures. Ycd::Yuprt/5-FC reduced colony formation, and with high concentration of 5-FC colony forming ability almost disappeared (0.01 < p < 0.0001) while senescent cells increased to 70 % (p<0.0001). Furthermore, 50 % of treated cells were in subGo fraction as analyzed by flow cytometry (0.01 < p < 0.001). Finally, the cytotoxicity produced inside the cells was able to diffuse into the extracellular environment, generating the same cytotoxicity in cells that were not exposed to gene transfer, indicating a strong bystander effect (0.01 < p < 0.0001). Our encouraging results support further research on the use of this suicide system for local treatment of melanoma tumors in companion animals.

0378 - EFFECT OF VASOPRESSIN ANALOGS ON ANGIOGENESIS AND NEUROENDOCRINE DIFFERENTIATION IN AGGRESSIVE PROSTATE CANCER

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Prostate cancer (PC) is the second cause of cancer-related death in males worldwide. Invariably after treatment, disease progresses into a castration-resistant PC (CRPC) which implies no therapy response and poor prognosis. This tumor progression correlates with a loss of epithelial characteristics (EMT), promoting metastatic progression and development of cell foci with neuroendocrine differentiation (NED). Angiogenesis is a key factor for PC progression and has been linked with NED and androgen deprivation therapy. For years, our group has studied desmopressin (dDAVP), a first generation vasopressin (AVP) analog, agonist of V_2 receptor (AVPR2) and [V4Q5]dDAVP, a second generation analog with enhanced cytostatic activity. Both peptides showed antiproliferative effects in vitro and in vivo on several tumor models including aggressive PC and, interestingly, reduced the expression of NE markers. Given this evidence, and the increasing incidence of aggressive PC with NE features, this work aims to evaluate the effect of AVP analogs on key processes related to cancer progression on aggressive NE PC-3 model. The cells were treated with AVP analogs for 7 days, subsequently studying its effect on the expression of NE markers and genes associated to EMT by RT-qPCR, and the sensitivity to the chemotherapeutic agent Cisplatin was measured by MTS assays. Sustained exposure to analogs reduced the NE markers expression, and modulated the expression of genes associated to EMT in vitro. Furthermore, we assessed angiogenesis in vivo with Matrigel® plug modified assay in nude mice. Treatment with each analog reduced PC-3 cell-induced angiogenic response by nearly 50 % versus control. These results position AVP analogs as potential and interesting angiostatic agents, with the ability to modulate aggressiveness for CRPC, a disease with few therapeutic alternatives.

0392 - DOWNREGULATION OF MUSCARINIC RECEPTORS GENE EXPRESSION IN HUMAN BREAST CANCER CELLS REGULATES ANCHORAGE-INDEPENDENT CELL GROWTH IN VITRO AND ANGIOGENESIS IN VIVO.

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Muscarinic receptors (M) expression, activation and signalling play important roles in regulating many cellular process and cancer progression. It has been reported that human breast cancer MCF-7 cells express muscarinic receptors M3 and M4 subtypes and its activation promotes tumoral progression. We previously reported that the silencing of both M3 and M4 in MCF-7 cells significantly reduced neovascularization capacity of tumoral cells in vivo. The aim of this work was to evaluate the specific contribution of each M receptor on different tumoral progression parameters like anchorage-independent cell growth and angiogenesis in vivo. Here, we silenced M3 or M4 subtypes in MCF-7 cells by specific RNAi. After 5 days we used the different experimental groups (siM3, siM4 and MCF-7 cells with and without carbachol (Carb, -8M)) in the following assays. Briefly, for soft agar colony assay we seeded $2X10^4$ cells of each group into medium with soft agar. After 2 weeks, the colonies larger than 60 µm in diameter were counted. We observed that cholinergic stimulation of siM cells showed a significant reduction in colony number when compared with MCF-7+Carb, however this effect was greater in siM3 cells than in siM4 cells (siM3: 99.97 ± 9.50 %, siM4: 289.4 ± 5.3 % vs. MCF-7: 509.1 ± 11.8 %; p<0.0001). Angiogenesis was measured by inoculation of $2x10^5$ cells in female nude mice. After 5 days, the animals were sacrificed and angiogenesis was quantified in the sites of inoculation as vessel density. We found that silencing of both M receptors decreased the neovascular response in vivo of siM cells treated with Carb compared with MCF-7+Carb (siM3: 3.6 ± 0.1, siM4: 3.7 ± 0.3 vs. MCF-7: 6.4 ± 0.7; p<0.0001). According to our results, M receptors expression downregulation can modulate the malignant phenotype of MCF-7 cells, having a high inhibitory effect on anchorage-independent cell growth and angiogenesis.

0686 - ADENYLATE CYCLASE GENES ARE EXPRESSED IN BASAL CELL CARCINOMA AND NORMAL SURROUNDING SKIN OF NEVOID BASAL CELL CARCINOMA SYNDROME (NBCCS) PATIENTS

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NBCCS – also known as Gorlin-Goltz syndrome – is an autosomal dominant entity caused mainly by mutations in the PTCH1 gene. NBCCS is characterized by multiple basal cell carcinoma (BCC) development due to the Hedgehog (HH) pathway hyperactivation. We have previously described that the genes encoding components of the HH pathway are overexpressed in BCC and phenotypically normal skin of these patients (Martinez MF, et al. Cells, 2019). Taking into account that the HH pathway can be inhibited through proteolysis of its effectors by a cAMP-driven process that involved protein kinase A, we looked for the expression profile of the nine adenylate cyclase genes (ADCY 1 to 9). We performed quantitative RT-PCR in BCC and normal surrounding tissue (NST) of 4 NBCCS patients with PTCH1 mutations, and 3 control skin samples (CSS). We failed to detect ADCY6 mRNA in any tested samples. ADCY8 is only expressed in BCCs and the remaining ADCY genes are expressed in BCCs and NST of NBCCS patients. Any adenylate cyclase genes were expressed in the CSS. Additionally, we found a 2-fold increase in ADCY1 and a 10-fold decrease in ADCY5 mRNA levels in BCC compared to NST (p<0.05). These results reveal that adenylate cyclases are involved in NBCCS and suggest that the gene expression levels of cAMP pathway components could be modified directly or indirectly by the HH pathway hyperactivation. Our finding can improve the knowledge of phosphodiesterase inhibitors mechanism, another component of the cAMP pathway, in the treatment of BCCs and also be the initial study to delineate new ones.

0703 - HO1 PLAYS AN IMPORTANT ROLE IN IRON METABOLISM ALTERATION IN BREAST CANCER CELLS

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Heme Oxygenase-1 (HO1) catalyzes heme degradation, yielding biliverdin, carbon monoxide and iron. When iron is in excess produces oxidative stress through reactive oxygen species (ROS) generation. Since both HO1 and iron metabolism disruptions have been related to breast cancer progression, we sought to investigate how tumor cells regulate iron metabolism when HO-1 expression is altered. For this purpose, we first investigated the correlation of HO1 with several iron proteins by using in silico analyses and corroborated the strongest hits by using immunohistochemistry (IHC) performed on human biopsies (n= 33). In addition, a syngeneic model of LM3 and a xenograft model of MDA-MB-231 cells stably overexpressing HO1 were used to study these hits. We further performed in culture analyses using LM3 breast cancer cells treated with hemin (H), vehicle or the combination with an antioxidant, and studied iron storage (Prussian Blue), ROS levels (DFCA) and cell cycle progression (flow cytometry). In silico analyses showed that HO1 correlated with DMT1 (p= 9.8e-05), ZIP14 (p= 4.2e-06), Prohepcidin (p= 1.4e-12) and L-ferritin (p= 2.2e-16). In order to study the correlation between HO1 and DMT1 in breast cancer we analyzed by IHC their expression in biopsies. We observed an inverse correlation between DMT1 and HO1 expression (p<0.05). The IHC studies showed an increase in ZIP14 and prohepcidin expression and a slight decrease in L-ferritin and DMT1 expression in hemin-treated and HO1-overexpressing cells in both animal models. In culture studies showed that the iron storage was increased in hemin- treated LM3 cells and was associated to a decrease in cell viability (p<0.05), an increase in the apoptotic rate (p<0.05) and high ROS levels (p<0.01). NAC treatment reverted the apoptotic effect of H (p<0.05). Altogether these results indicate that HO1 induction plays a role in carcinogenesis through free iron accumulation, ROS production and oxidative stress.

0716 - BONE MARROW DERIVED MONOCYTES MEDIATE THE DELIVERY OF CONJUGATED POLYMER NANOPARTICLES IN A PLECLINICAL GLIOBLASTOMA ORTHOTOPIC MODEL

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Photodynamic therapy (PDT) has recently gain attention as alternative treatment of glioblastoma (GBM). Due to their superb light absorption and photostability, conjugated polymer nanoparticles (CPNs) are promising photosensitizers in PDT. However, GBM represent a challenge to current treatments due to the preferential location within Central Nervous System and the presence of the blood-brain barrier (BBB) hindering the arrival and accumulation of drugs into the tumor upon systemic administration. Trojan horse therapy, using cells with homing