Notch pathway inhibition with DAPT, the expression of *TMPRSS2*, an androgen dependent gene, showed lower levels after 24 h of treatment (10 and 30 μM) by RT-qPCR (p=0.02; n=2). In turn, Enz treatment (30 and 50 μM) reduced the levels of *HES1,* a target gene of the Notch pathway*,* determined by RT-qPCR (n=3).
We observed significantly reduced viability of PC3 cells both with DAPT (p=0.0027; n=3) and Enz isolated treatments (p=0.0018; n=3) using MTS assay. Importantly, the combined treatment with DAPT and Enz significantly reduced PC3 viability at 48 and 72 h (p=0.0043; n=3). We observed reduced migratory abilities both with DAPT (p=0,0022; n=3) and Enzalutamide isolated treatment (p=ns; n=1.2), and also with the combined treatment (p=0,0526; n=3) using wound healing assay.
Our study suggests an interconnection between Notch and AR pathways in PCa. That would restrain in an alone or combined manner the viability and migratory abilities of PC3 cells. Therefore, a combined approach consisting of Notch and AR inhibitors could be more effective than isolated antiandrogen treatment, especially in patients with advanced and metastatic CRPC.

507. (537) **INVOLVEMENT OF RUNX1 IN DRUG RESISTANCE IN TNBC SUBTYPE**

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Triple negative breast cancer (TNBC) is associated with epithelial-mesenchymal transition (EMT) and an enrichment in cancer stem cell (CSC) population, which according to growing evidence, are involved in tumor chemoresistance. Our group has shown that RUNX1 could be involved in the aggressiveness of this breast cancer subtype. We reported that RUNX1 is able to promote cell migration and regulate tumor gene expression, such as the oncogene RSPO3 and the metastasis marker gene GJA1. ChIP assays done in our lab revealed that RUNX1 can regulate transcription factors involved in EMT. Moreover, RUNX1 protein expression in TNBC correlates with poor patient prognosis. Our aim was to evaluate RUNX1 relevance in drug treated human TNBC cell lines. Here we show that RUNX1, KLF4 (stemness marker) and GJA1 gene expression are significantly upregulated in doxorubicin-or paclitaxel-treated TNBC cell lines (all *p* values were at least <0,02). Interestingly, we observe that loss of RUNX1 transcriptional activity significantly enhances doxorubicin and paclitaxel toxicity in TNBC cell lines (all *p* values were <0,0001). In addition, we found a potential DNA binding site for glucocorticoid receptor (GR) in RUNX1 gene. TNBC cell lines show that *RUNX1* mRNA is significantly upregulated with dexamethasone (GR agonist) and downregulated with mifepristone (GR antagonist) (p=0,0037 on MDA-MB-453 and p<0,0001 on MDA-MB-468). Therefore, our data suggests that RUNX1 may be involved in TNBC chemoresistance and its expression could be externally regulated by GR activity modulation.

508. (573) **INVOLVEMENT OF RUNX1 IN DRUG RESISTANCE IN TNBC SUBTYPE**

Sofía María Sosa[1], Natalia Fernández[1], Facundo Couto[1], Ana Raimondi[2] & Natalia Rubinstein[1]
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509. (588) **RAC-FUNCTION MODULATES IN A DIFFERENT WAY PROLIFERATION OF NORMAL RENAL PROXIMAL AND CLEAR RENAL CARCINOMA CELLS: EFFECT OF ALKALOSIS AND ISOFORM 1 OF NA^+/H^+ EXCHANGER**

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One of the hallmarks of cancer is tumor extracellular acidosis. Then, we hypothesize that extracellular pH (pHe) affects differently cancer or normal cells. Our previous studies showed that, in cells derived from renal cell carcinoma (RCC), the isoform 1 of the Na^+/H^+ exchanger (NHE1) function is antiproliferative in media at pH 7.4. Alkalosis reverts this effect. On the other hand, in normal proximal cells, while at pH 7.4 NHE1 favors cells proliferation, NHE1 is antiproliferative alkaline media. The aim of this study was to further investigate this process. We use three renal cell models: HK2, derived from normal human proximal epithelial cells, 786-O and Caki-1, both derived from human RCC. We exposed cells to media with 9.6 mM NaOH for 72h. Then, we estimated cell proliferation by BrdU experiments in the presence of IA116, a Rho GTPases Rac1 inhibitor and NHE1 expression by immunoblot studies. In normal HK2 cells at media 7.4 Rac inhibition enhanced proliferation (%BrdU + cells: +Rac: 36±3 vs -Rac= 71±4, p<0.001 n=11). This effect was reverted when NHE1 function was inhibited with 1µM HOE. Thus, Rac function potentiated NHE1-related proliferation. In 786-O RCC cells, at media 7.4 Rac inhibition inhibited NHE1-related antiproliferative effect (NHE1-induced proliferation: +Rac: 14±2 vs -Rac= -8±2, p<0.001 n=16). We also investigated if NHE1 expression could change with alkaline exposure. Our immunoblot studies showed that normal renal cells had higher NHE1 expression than RCC cells (NHE1 expression HK2 5.4±0.5 vs 786-O= 2.7±0.2, p<0.01 n=8). Exposure to alkaline media rose NHE1 expression only in RCC cells (NHE1 expression in 786-O pH7.4= 2.7±0.2 vs pH=7.5= 4.5±0.7, p<0.05 n=8). In summary, Rac function would reduce NHE1-related proliferation in normal renal proximal cells. In RCC cells, however, NHE-1 related antiproliferative effect would depend on Rac function. Finally, these effects would not be related to NHE1 expression.

510. (604) **TUMOR ASSOCIATED FIBROBLAST: IMPACT ON OSTEOSARCOMA PRIMARY AND METASTATIC TUMORAL MICROENVIRONMENT AND TREATMENT RESPONSE**

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Tumor associated fibroblast (TAF) have been implicated in almost every aspect of tumoral biology. Of relevance TAF could be modulating response to treatment and overall microenvironment development. Given that Osteosarcoma (OS) have the same 5-year survival rate for metastatic and treatment resistant patients since 1970´s, we dediced to investigate the role of TAF in OS primary and metastatic niches.
Aim: Evaluate TAF and human OS cell lines interaction in primary and pulmonary metastatic environments. To analyze if metastatic OS cell line has a higher inducing power than non-metastatic OS cell line analyzing the expression of different ABC transporters implicated in chemoresistance and the ability to exclude doxorubicin and rhodamine.
Methods: The expression of ABC transporters was analyzed by RT-qPCR on conditioned fibroblast. Rhodamine 123 exclution assay was used to determine the activity of P-glycoprotein (P-gp) mediated transport and doxorubicin (DOX) exclution was performed to analysis the overall ABC-related chemoresistant capacity. To evaluate the interaction of fibroblast with metastatic (LM7) and non-metastatic (SAOS2) OS human cells hetero – spheroid formation assays were performed.
Results: LM7 conditioned medium (CM) induced an overall upregulation of ABC transporters in comparison with SAOS2 CM. Conditioned fibroblast with LM7 CM showed lower levels of intracellular DOX and Rhodomine in comparison with SAOS2 CM fibroblast. Mixed spheroid compose of fibroblast ans OS cell lines display a lower area and more compact than single type aggregates.
OS has not changed the 5-year rate survival for metastatic patients since the 70', so the need to understand aspects of OS metastatic biology and chemoresistance could be helpful to develop new treatments to this group. Knowing aspects of the assocaited stroma and in particular TAF, could allow the development of new therapeutic possibilities targeting the tumoral associated stroma.

QUÍMICA MEDICINAL

511. (137) **A NEW MIXED LIGAND COPPER(II) COMPLEX OF NARINGENIN AS A PROSPECTIVE ANTICANCER AGENT**

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Objective: The flavonoids can be chemically modified by complexation with metals to be applied for adjuvant therapy. In this context, we evaluated the anticancer activity on the lung cancer cell line A549 of CuNarBatho. Nar: naringenin, Batho: bathophenanthroline. Their bovine serum albumin (BSA) binding properties have also been evaluated.
Methods: The effects of the compounds (CuNarBatho, CuNar, ligands and metal) on the A549 cell viability were measured by MTT assay. To evaluate the probable mechanism of action, morphological changes, intracellular reactive oxygen species ROS content (using CM-H_2DCFDA probe), mitochondrial membrane potential (MMP) (using $DIOC_6$ probe), and GSH depletion and cell viability in the presence of NAC were examined. The interaction between CuNarBatho and BSA was studied using tryptophan fluorescence quenching.
Results: CuNarBatho was more efficient than Batho, and Nar in inhibiting A549 cell viability (IC_{50} values 0.3, 12.1 and > 100 μM respectively at 24 h incubation). CuNar slightly inhibited cell viability. The probable mechanism of action of the ternary complex implies increased ROS levels, reduced GSH and GSH/GSSG ratio levels, and decreased MMP. Also, increment of cytoplasm condensation and presence of pycnotic nuclei were observed. Upon addition of NAC, the anticancer effect has been reverted. The binding constant values (K_b) for the CuNarBatho-BSA system are 36.2, 14.9 and 2.3 × 10^5 M^{-1} at 298, 303 and 310 K suggesting that the compound can be stored and carried by the protein. The number of binding sites was *ca.* 1.0 corresponding to the binding sites with high affinity. The negative ΔH and ΔS values (-177.03 and -0.47 KJ/mol) obtained for the interaction of CuNarBatho with BSA indicated that hydrogen bonding and Van der Waals forces played major roles during the interaction.
Discussion: The results suggest that the CuNarBatho complex could serve as a pharmacologically active compound for the treatment of lung cancer.

512. (501) **ENZYMES INVOLVED IN EXTRACELLULAR MATRIX PROTEOGLYCAN´S SYNTHESIS AS A POTENTIAL THERAPEUTIC TARGET FOR COLORECTAL CANCER STEM CELLS**

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Cancer Stem Cells (CSC) are characterized by self-renewal, differentiation, chemoresistance and phenotypic reversibility, which is associated with worse prognosis in tumors. Interaction with the microenvironment is one of the factors related with stemness. The aim of the present work is to analyze the expression of extracellular matrix proteoglycans in CSC derived from cell lines. CSC were enriched by scaffold free 3D culture (colonospheres) of human colorectal cancer cell line HCT116 in the presence of bFGF and EGF, employing ultra-low attachment plates in serum-free culture conditions. After 14 days of culture, microscopy studies were performed to assess colonosphere formation. Stemness was addressed by the expression of master genes SOX2, Nanog and CD44 by RT-PCR. Decorin and biglycan proteoglycan´s core protein expression was also analyzed by RT-PCR. Moreover, glycosaminoglycan and protein quantification was addressed by ionic exchange chromatography of the culture medium followed by colorimetric determination. RT-PCR was performed for the study of glycosaminoglycan synthesis enzyme expression. Bright light microscopy showed colonospheres around 50-100um. The expression of master genes was heterogeneous among cultures correlated with an heterogeneous expression of decorin (number of experiments, n =3). On the other hand, no biglycan expression was detected among different colonospheres (n=3). No differences were registered in glycosaminoglycan/protein ratio among spheres (0,274 ± 0.127). Nevertheless, Chondroitin-4-O-Sulfotransferase (C4ST) expression was detected in colonospheres while no expression was observed for Dermatan-4-O-Sulfotransferase (D4ST). The heterogeneity presented by 3D cultures represents the heterogeneity reported for CSC within tumors and C4ST and D4ST pattern suggests differences in GAG chain´s quality. Therefore, colonospheres are a suitable model for the study of GAG enzymes as potential therapeutic targets.

REPRODUCCIÓN

513. (008) **EXPRESSION OF TRANSGLUTAMINASE 2 AND HISTOMORPHOLOGICAL CHARACTERIZATION OF THE OVIDUCT OF ADVANCED PREGNANT COWS**

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The oviduct suffers changes during the estrous cycle in relation to its function in reproduction that are attributed in part to estrogen (E2) variations. Raise of E2 and E2 receptors expression occurs along pregnancy, thus we hypothesized that effects on the oviduct should be observed in advanced pregnancy (AP). In this work, we studied changes in the oviductal epithelium and fluid through the estrous cycle and AP in cows. In AP oviducts higher leaf-like folds were observed and the width of the mucosa and height of the epithelium