**CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET (1); INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES (2); UT SOUTHWESTERN MEDICAL CENTER (3)**

20-Hydroxyeicosatetraenoic acid (20-HETE), the product of 20-hydroxylation of arachidonic acid by cytochrome P450 isoforms (CYP4F2 and CYP4A11), has a role in the oncogenesis of several human tumors. Recently, the GPR75 receptor has been identified as the target for 20-HETE. We have shown that androgen independent prostate cancer cells (PC-3) express GPR75. The aim of this study was to assess in vitro if 20-HETE/GPR75 modify the metastatic features of PC-3 cells. Cells were incubated with 20-HETE or its stable analog 5,14-HEDGE (both 0.1 nM) in the presence or absence of two different antagonists of the 20-HETE receptor, AAA or 19-HEDE (both 5 or 10 uM). The following assays were performed: e-cadherin and vimentin protein expression (epithelial-mesenchymal transition), zymography (release of matrix metalloproteinase-2 (MMP-2)), immunofluorescence and p-FAK (changes of cytoskeleton), scratch wound healing (migration), and soft agar colony formation (anchorage-independent growth). Results were analyzed using one-way ANOVA followed by Dunnet's. 20-HETE (24 h) increased by 150 % the expression of vimentin ($p<0.0001$, n= 3) and diminished by 40 % the expression of e-cadherin ($p<0.0001$, n= 3), whereas these effects were reversed by AAA ($p<0.0001$ and $p<0.05$, respectively). 20-HETE increased by 52 % the release of MMP-2 ($p<0.05$, n= 3), and this was also inhibited by AAA ($p<0.001$). AAA disorganized the actin filaments throughout PC-3 cells, while tubulin filaments remained unchanged. Also, 20-HETE increased by 89 % FAK phosphorylation (Y397) ($p<0.0001$, n= 3). 20-HETE increased by 147 % cell migration rate ($p<0.0001$, n= 3) and this effect was reverted by both antagonists, AAA or 19-HEDE ($p<0.05$ and $p<0.0001$, respectively), or by knockdown of GPR75 ($p<0.0001$). Finally, 5,14-HEDGE (21 days) formed twice the number of colonies vs. control ($p<0.05$, n= 2) and this was abolished by AAA ($p<0.05$). These results strongly suggest a role for GPR75 in 20-HETE-mediated metastatic features in PC-3 cells.

## 0072 - INTRACELLULAR CL- MODULATION OF IL-1ß SECRETION AND THE NLRP3 INFLAMMASOME EXPRESSION/ACTIVITY REQUIRE SGK1

**Mariángeles CLAUZURE** | Ángel G. VALDIVIESO | Consuelo MORI | María Macarena MASSIP COPIZ | Verónica SOTOMAYOR | Cristian J. A. ASENSIO | Tomás SANTA COLOMA

**INSTITUTO DE INVESTIGACIONES BIOMEDICAS (UCA - CONICET)**

The impairment of the CFTR activity induces intracellular chloride $[Cl^-]_i$ accumulation and consequently, as a second messenger, stimulates the secretion of interleukin-1ß (IL-1ß). We have previously described that this secretion starts an autocrine positive feedback loop. Moreover, the expression of two subunits of the inflammasome complex: NLR family pyrin domain containing 3 (NLRP3) and caspase-1 (CASP1), that are involved in the IL-1ß maturation, are indirectly modulated by the $[Cl^-]_i$. On the other hand, cellular and mitochondrial ROS (reactive oxygen species) also are regulated by $[Cl^-]_i$. Recently, other authors found that differences in $[Cl^-]_i$ modulates SGK1 (serum-glucocorticoid kinase 1) phosphorylation and subsequently regulates NF-kB activation in airway epithelial cells. Therefore, we decided to study the effects of SGK1 on IL-1ß expression at different $[Cl^-]_i$. In this study we used IB3-1 cells (a bronchial cell line derived from a cystic fibrosis patient with a DF508/W1282X CFTR genotype) and Caco-2 cells (transfected with CFTR-shRNA). The cells were incubated for 1 h at 5 or 75 mM $Cl^-$, in presence of ionophores tributyltin (10 µM) and nigericin (5 µM) to equilibrate $[Cl^-]_e$ and $[Cl^-]_i$. To explore if SGK1 was also involved in the IL-1ß response to $[Cl^-]_i$, we used the SGK1 inhibitor GSK650394 at 0, 0.1, 1 and 10 µM. After, we determine IL-1ß expression by quantitative real-time RT-PCR and ELISA quantification in culture media. To analyze the ROS response, we determined DCF fluorescence and MitoSOX fluorescence by microplate reader and/or flow cytometry. The results showed that SGK1 inhibitor diminished the response of IL-1ß mRNA to changes in the $[Cl^-]_i$ from 5 to 75 mM; GSK650394, at 10 µM, completely abrogated the IL-1ß mRNA response to $Cl^-$ 75 mM ($p<0.05$, n= 3). Similar results were obtained on the secreted IL-1ß. On the other hand, SGK1 inhibitor, significatively reduced both, cellular and mitochondrial ROS levels at 75 mM $Cl^-$ ($p<0.05$, n =3), suggesting that both the IL-1ß loop and the ROS response to Cl- were blocked by GSK650394. Similar results were found in Caco-2 with CFTR-shRNA. The results suggest that $Cl^-$ effects are indirectly mediated by SGK1, which under $Cl^-$ modulation stimulates the secretion of mature IL-1ß, in turn responsible for the observed upregulation of ROS and CASP1, NLRP3, and IL-1ß itself. The exact point of SGK1 action is still unknown.

## 0074 - INTRACELLULAR SIGNALING PATHWAYS TRIGGERED BY THE STIMULATION OF THE G-COUPLED PROTEIN RECEPTOR GPR75 BY 20-HYDROXYEICOSATETRAENOIC ACID (20-HETE) IN ANDROGEN INDEPENDENT PROSTATE CANCER CELLS.

**Sofia CARDENAS** (1) | Cecilia COLOMBERO(1) | Laura PANELO(2) | Rambabu DAKARAPU(3) | John FALCK(3) | Mónica COSTAS(2) | Susana NOWICKI(1)

**CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE)-CONICET (1); INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES (2); UT SOUTHWESTERN MEDICAL CENTER (3)**

20-HETE, the product of 20-hydroxylation of arachidonic acid by cytochrome P450 isoforms (CYP4F2 and CYP4A11), has a role in the oncogenesis of several human tumors. Recently, the GPR75 receptor has been identified as the target for 20-HETE. We have shown that androgen independent prostate cancer cells (PC-3) express GPR75. The aim of this study was to identify intracellular signaling molecules activated upon GPR75 stimulation by 20-HETE in PC-3 cells. Cells were incubated with 20-HETE (0.1 nM) in the presence or absence of the antagonist of the 20-HETE receptor, AAA (5 or 10 µM). Protein expression of the inducible focal adhesion protein Hydrogen Peroxide Inducible Clone-5 (HIC-5), the phosphorylated and total form of NF-kB, AKT, p38 MAP-Kinase (p38) and EGFR were assessed by Western blot. Intracellular localization of p-AKT, NF-kB and PKCa were determined by immunofluorescence and subcellular fractionation. Results were analyzed using one-way ANOVA followed by Dunnet's. Incubation with 20-HETE (2 h) increased the phosphorylation of EGFR, NF-kB and AKT by 146, 172 and 219 %, respectively (vs. control, $p<0.01$ for NF-kB, and $p<0.001$ for EGFR and AKT, n= 3), and this was inhibited by AAA (vs. 20-HETE alone, $p<0.05$ for NF-kB, $p<0.01$ for AKT and $p<0.001$ for EGFR). AAA alone increased p-38 phosphorylation by 248 % ($p<0.001$ vs. control, n= 3). 20-HETE (1 h) induced the translocation of p-AKT to the nuclei ($p<0.001$, n= 3) and promoted the redistribution of PKCa out of the nuclei ($p<0.05$, n= 3) to the plasma membrane ($p<0.001$). Both effects were inhibited by AAA (vs. 20-HETE, $p<0.01$ for AKT and $p<0.05$ for PKCa). AAA alone reduced the nuclear signal of p-AKT and NF-kB, usually activated in tumoral cells ($p<0.001$ for both, n= 3). Additionally, 20-HETE (12 h) increased by 150 % the protein expression of Hic-5 ($p<0.0001$, n= 5) and this was abolished by AAA ($p<0.001$). Our results show that 20-HETE modulates signaling pathways known to be deregulated in malignant cells through the GPR75-axis.

## 0076 - OPTIMIZATION OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR THE DETECTION OF TRITRICHOMONAS FOETUS

Mariana Paula MORERO(1) | Franco PARADISO LANGHOFF(1) | **Maria RAMIREZ** (2) | Jorge Anibal OYHENART(1)