extracellular matrix degradation, migratory capacity, 3D and in-vivo growth rate than controls. In order to identify possible molecular pathways that modulate p63 and that could eventually be drug molecular targets, UMUC14 BC cells were treated with inhibitors of different pathways SB20358 (p38 pathway), PD98059 (MAPK pathway), LY29002 (PI3K pathway). p63 expression was tested by Western blot. 2D growth was measured by MTS. UMUC14 cells were grown in 3D conditions during 30 days treated with or without inhibitors and spheroids diameter was measured. All of the inhibitors showed a decrease in p63 expression, being LY29002 the one that showed the best and most sustained effect over time. This decrease in p63 expression (52 % less expression, $p<0.05$ unpaired t test) was accompanied by a decrease in the growth capacity in 2D (33 % less viability, $p<0.0001$ two way ANOVA) as in 3D (60 % less spheroid surface at day 27, $p<0.001$ two way ANOVA/Tukey) of UMUC14 cells. These results demonstrate that inhibition of the PI3K pathway could be an interesting therapeutic target for p63 positive bladder tumors.

0211 - NOVEL INTERACTION OF 14-3-3 ZETA/DELTA AND HO-1 IN PROSTATE CANCER

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Abstract/Resumen: Proteomic signatures of primary prostate cancer (PCa) and associated metastases may aid in the identification of key player proteins involved in progression. We have previously showed that heme-oxygenase 1 (HO-1), encoded by the gene HMOX-1, the rate-limiting enzyme in heme degradation, has a strong anti-tumoral effect in PCa. In an effort to identify HO-1 molecular partners which might collaborate with its biological function, we undertook an in-depth mass spectrometry-based proteomics study to find HO-1 interactors. We constructed a recombinant GSTHO-1 protein. PC3 cells were transiently transfected with GSTHO-1 or the respective control and treated with the stressor agent H_2O_2. Immunoprecipitated protein complexes were subjected to LC-ESI MS/MS. The proteomics analysis of HO-1 interacting factors revealed a list of 44 proteins including 14-3-3 zeta/delta, a protein encoded by YWHAZ, an androgen-responsive gene that activates proliferation, cell survival, and androgen receptor transcriptional activity. These results were validated by co-immunoprecipitation analysis. Immunofluorescence assays provided evidence that HO-1 and 14-3-3 zeta/delta co-localize in the cell nuclei under H_2O_2 treatment. Bioinformatics analysis were performed to investigate the clinical relevance of these two proteins using public database repositories. The Ross-Adams (GSE70769) dataset showed a negative correlation between HMOX-1 and YWHAZ expression ($r= -0.3286$; $p<0.0001$). When analyzing the ratio YWHAZ/HMOX-1 in patients with PCa, the relapse free survival increased almost 4 times when the ratio was ‹1.466 (HR= 3.76; p= 0.00023), i.e. when HMOX-1 expression increased. Moreover, high expression of HMOX-1 increased relapse free survival in patients with high YWHAZ expression (HR= 0.4; p= 0.025). In summary, our results, may cast a new light on PCa treatment highlighting a multifaceted role for HO-1 in inhibiting 14-3-3 zeta/delta function.

0213 - CROSSTALK BETWEEN ADIPOCYTES AND BREAST CANCER CELLS: MODULATION OF NUCLEAR RECEPTOR COACTIVATOR 3 (RAC3) IN FAT CELLS

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Abstract/Resumen: Adipocytes account for the largest proportion among the cells that comprise breast tissue. Although they are considered to be a critical cell type in the tumor microenvironment of breast cancer, there is still unclear the molecular mechanisms that control their behavior in this context. We have demonstrated that the transcriptional coactivator RAC3 decreases during adipogenesis and its high expression in adipose tissue adjacent to breast tumors correlate with different markers of tumoral progression in patient samples. Therefore, the aim of this work was to further study the role of this molecule in an in vitro model. Conditioned media (CM) were collected from non-tumoral or tumoral breast human cell lines with the following RAC3 mRNA levels: MCF10A: 1.55 ± 0.73, MDA-MB-231: 167.20 ± 6.91, T47D: 571.00 ± 12.59. 3T3-L1 derived-murine adipocytes were stimulated with CM or serum-free medium. RAC3 expression levels measured by qPCR resulted significantly higher in adipocytes stimulated with CM from high RAC3 levels-expressing T47D cells (9.25 ± 1.02) compared to the other conditions: basal (1.00 ± 0.00), MCF10A (2.33 ± 0.96), MDA-MB-231 (2.80 ± 1.19) ($p<0.05$). As RAC3 is a NF-kB coactivator we studied by immunofluorescence the presence of its phosphorylated subunit p65. We observed a greater fluorescence intensity in nucleus when adipocytes were stimulated with CM from tumoral cell lines (MDA-MB-231 or T47D) compared to the other conditions. Even more, since TNF is a NF-kB target gene, we evaluated by dot plot its levels in CM from adipocytes post-stimulation. Adipocytes secreted more TNF after being stimulated with CM from T47D (1.41 ± 0.03) compared to all other conditions: basal (1.00 ± 0.00), MCF10A (1.08 ± 0.11), MDA-MB-231 (1.02 ± 0.04) ($p<0.05$). The values were relativized to basal condition and results are shown as the mean ± SEM. These results validate our previous findings and suggest RAC3 as a molecular key to understand changes in the adipokines pattern in the tumor context.

0219 - GENE EXPRESSION AND CHIP-SEQ DATA ANALYSES CONFIRM FOXM1 AS A PLAYER IN PROSTATE CANCER BIOLOGY

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Abstract/Resumen: Dysregulation of the androgen receptor (AR) and glucocorticoid receptor (GR) expression is responsible, at least in part, for the development and progression of prostate cancer (PCa). GR might also have oncogenic or tumor suppressor activities depending on the presence/absence of AR and other regulatory cofactors such as the Forkhead Box (FOX). We previously showed the involvement of FOXM1 in cell migration, proliferation, viability, morphology, apoptosis, and the modulation of the transcriptional activity of GR and AR in PCa cells. The aim of this study was to validate the potential use of FOXM1 as a biomarker for PCa and understand the molecular mechanisms involved. We took advantage of public repositories to study the association between FOXM1 expression and the clinico-pathological characteristics of patients with PCa. We downloaded raw transcriptome data from 3 datasets GSE54460, GSE94767 and PRAD-TCGA. The expression of FOXM1 and AR were positively correlated with Gleason score ($p<0.05$). We also observed significant higher expression of FOXM1 and AR in patients who relapsed ($p<0.02$ and $p<0.05$, respectively). Since FOXM1 modulates the transcriptional activity of AR and GR, we analyze publicly available data from ChIP-seq studies: 1) LNCaP cells treated with Dexamethasone + IP-GR, 2) LNCaP cells treated with Testosterone + IP-AR, and 3) MDA-MB-231 and MCF7 cells + IP-FOXM1. We identified 439 gene promoters having binding sites for all three abovementioned factors. Thirty-eight of these genes have been previously related to PCa; and 5/38 have strong associations with FOXM1, AR and GR: BMP7, NCOA3, SMAD2, FAM120B and AHR. Altogether, the in-vitro and bioinformatic analyses demonstrate the involvement of FOXM1 in PCa. In addition, the identification of genes regulated by AR, GR