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Over the last years, the incidence of melanoma, the deadliest form of skin cancer, has risen faster than any other cancer type. Considering that half of the patient's exhibit the BRAFV600E mutation, therapies with BRAF and MEK inhibitors (BRAFi/MEKi) showed an impressive success rate. Unfortunately, treatments are marginally effective since tumors quickly become resistant. In that regard, accumulating evidence supports that sphingosine-1-phosphate (S1P) is linked to multiple mechanisms leading to cancer progression and resistance. Thus, the aim of this study was to evaluate how vemurafenib (BRAFi) resistance affects the expression of sphingosine kinases (SphK) and S1P receptors (S1PR) in melanoma. To this end, we generated vemurafenib-resistant melanoma cells by continuous exposure of parental sensitive cells to increasing concentrations (0,01μM – 1μM) of the drug for 3 months. Previously, we showed that vemurafenib-resistant Lu1205 melanoma cell (Lu1205R) exhibit higher IC50 and pERK levels than their sensitive parents (Lu1205S). Here, we extended those studies to A375 melanoma cells. Certainly, A375R cells also showed increased resistance and pERK, but not pAKT levels. Expression of SphK and S1PR in both cells lines was evaluated by RT-qPCR. Surprisingly, the modulation of SphK1, S1PR1 and S1PR3 expression differ in Lu1205R and A375R cells. In addition, a bioinformatic analysis was performed using the public Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24862). This study showed a differential gene expression pattern in other cell lines, including A375, supporting the concept that melanoma heterogeneity may trigger different mechanisms of resistance to BRAFi therapy. In summary, although our results indicate that S1P signaling may have a role in vemurafenib resistance, further studies will be necessary to elucidate its importance.

446. (103) **PIN-POINTING THE KEY PLAYERS IN METABOLIC REWIRING OF PROSTATE TUMOR CELLS TOWARDS PROGRESSION IN THE BONE NICHE**

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Metabolic rewiring is associated with the metastatic cascade, and communication between tumor cells and the bone niche is determinant for tumor progression. Here, we sought to identify key metabolic genes that fuel prostate cancer (PCa) bone metastasis. By an indirect transwell co-culture system of PCa (PC3) and bone progenitor cells (MC3T3 or Raw264.7) we assessed the transcriptomic profile of PC3 cells modulated by soluble factors released from bone precursors. Strong activation of lipid metabolic pathways including PPAR and PI3K-Akt (P<0.05) was observed in PC3 cells. Next, we selected the altered metabolic genes for an unsupervised clustering analysis using transcriptomic data from human PCa and bone metastasic samples (GSE74685). Interestingly, those genes could cluster PCa patients in two defined groups: primary PCa and bone metastasis, highlighting that the early transcriptional metabolic alterations triggered in our co-culture model could discriminate primary tumors from bone metastatic samples. Further, the expression levels of four lipid associated genes (*VDR*, *PPARA*, *SLC16A1* and *GPX1*) could be independent risk-predictors of death (HR: 4.96, 2.85, 3.93 and 3.67, respectively; P<0.05), and that the combined expression of these four genes correlates with a worst outcome in metastatic patients (HR: 2.65, P<0.05) (SU2C-PCF data set). Further, we identified PKA as a master regulator of this lipid-associated signature (Ingenuity Pathway Analysis). Secretome analysis (ESI MS/MS) of conditioned media from these co-cultures revealed critical soluble factors secreted by bone progenitors (Col1a2, Fn1 and Cacna2d1) which could regulate PKA activity to promote the metabolic rewiring of PCa cells. Overall, we identified a novel lipid gene signature triggered during the communication between PCa and bone cells that appears to be critical for survival in PCa patients, pointing out to new attractive druggable targets for the disease.

447. (104) **MECHANISM OF ACTION OF A TRIAZOLYL PEPTIDYL PENICILLIN IN MELANOMA CELLS AND SYNERGISTIC ANTITUMOR EFFECT OF ITS COMBINATION WITH THAPSIGARGIN**

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In a previous study, we demonstrated that TAP7f, a synthetic triazolylpeptidyl penicillin, behaves as an effective antitumor agent and induces an apoptotic response in murine melanoma cells. In this work, we comparatively examined its mechanism of action in murine B16-F0 and human A375 melanoma cells. We first studied the contribution of an endoplasmic reticulum (ER) stress response to the apoptotic effect induced by the derivative. To this end, the expression levels of different ER stress-related proteins were evaluated by Western blot assays in both melanoma cell lines. A significant increase in the amount of ATF4 (≈1.5-2 fold), GADD153/CHOP (≈1.5-2.5 fold), Calnexin (≈2 fold) and GRP78/BIP (≈2-3.5 fold) was observed after incubating B16-F0 cells for 3 h or 6 h with a 20 μM concentration of TAP7f (p<0.05). A similar effect was observed in A375 cells for some of these ER markers. It was also demonstrated that TAP7f-induced activation of p38, JNK and PI3K-I/Akt signaling pathways occurred downstream ER stress. Based on the effectiveness of combined therapies for cancer treatment, we decided to investigate the *in vitro* antiproliferative effect of TAP7f with thapsigargin, a well-known ER stress activator. The simultaneous incubation of different concentrations of the two compounds showed a higher inhibition of cell growth with respect to the effect of each individual agent, both in B16-F0 and A375 melanoma cells. The quantitative analysis of dose-effect curves obtained by using Compusyn software rendered combination indexes lower than 1 (0.48-0.59 for B16-F0 and 0.41-0.76 for A375), indicating synergism (p<0.01). In conclusion, we showed that induction of ER stress and activation of p38, JNK and PI3K-I/Akt pathways are involved in the antitumor effect induced by TAP7f in melanoma cells. The efficacy of the combination of TAP7f with thapsigargin suggested that this therapy could be considered an auspicious tool for melanoma treatment.

448. (105) **SOLUBLE GUANYLYL CYCLASE BETA1 SUBUNIT OVEREXPRESSION DECREASES CELL CYCLE PROGRESSION, PROLIFERATION AND MIGRATION IN ECC-1 AND HELA TUMOR CELL LINE**

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Soluble guanylyl cyclase (sGC) is a heterodimeric enzyme constituted by two subunits, alpha1 (a1) and beta1 (b1). Previously we showed that a1 subunit promotes tumor cell growth, survival and migration in ECC-1 and HeLa tumor cell lines, while b1 role remains unclear. b1 subunit protein levels are slightly reduced in most biopsies from human hormone-dependent malignant tumors. Here we investigate the effects of b1 on cell cycle, migration and protein expression in ECC-1 and HeLa cells.