Conclusion In conclusion, TAT-Cx43$_{266–283}$ exerts an important antitumor effect by inhibiting c-Src and up-regulating PTEN with the subsequent reduction in FAK phosphorylation required to establish appropriate focal adhesions for migration, proliferation and survival. This inhibition is mediated by the recruitment of the c-Src intrinsic inhibitors, PTEN and Csk. All together, these results reinforce the relevance of this sequence of Cx43 that interacts with c-Src for the development of new therapies against glioblastoma.

PO-292 BREAST CANCER STEM CELL REPROGRAMMING: DECIPHERING THE IMPACT OF GLUCOSE AND THE CONTRIBUTE OF TUMOUR MICROENVIRONMENT

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Introduction Diabetes-associated hyperglycemia is linked to poorer prognosis and survival in breast cancer (BC). Indeed, glucose can affect both tumour and tumour-surrounding cells. BC cells are embedded in an adipocyte-rich microenvironment, which, beside adipocytes, contains Stromal-Vascular Fraction Cells (SVFCs). In this *scenario*, epithelial and stromal compartments communicate through the release of soluble factors and establish an intricate crosstalk. Here, we analysed whether glucose could directly affect the phenotype of ER$^+$ MCF7 BC cells and interfere with their interaction with adipose-derived SVFCs, thereby promoting tumour progression.

Material and methods MCF7 cell stemness markers were measured by qReal-Time PCR. Adipose-derived (Ad-)SVFCs were obtained by mammary adipose tissue specimens of women undergoing plastic surgery. The trascriptome of MCF7 exposed to either low (LG-5.5 mM) or high glucose concentration (HG-25 mM) was obtained by RNA-Sequencing (Illumina HiSeq3000).

Results and discussions HG exposure of MCF7 determined a significant increase of SOX2 mRNA levels as compared to LG, suggesting the induction of stemness programming. Co-culture with Ad-SVFCs in HG increased SOX2, NANOG and OCT4 mRNA levels in MCF7, as compared to isolated culture, indicating the involvement of SVF-produced soluble factors in BC stem cell reprogramming. Moreover, in presence of Ad-SVFCs and HG, MCF7 produced a higher number of mammospheres, which also displayed larger size. However, both in LG and in HG, conditioned media (CM) obtained from Ad-SVFCs produced no relevant effect on MCF7 stemness. Nevertheless, when Ad-SVFCs were pre-incubated with CM obtained from HG-treated MCF7, their CM very effectively increased OCT4, NANOG and SOX2 mRNA levels in MCF7. Thus, HG likely perturbs MCF7, which produce soluble factors leading Ad-SVFCs to release, in turn, reprogramming factors for BC cell stemness. In this regard, we have observed that HG modification of MCF7 transcriptome includes deregulation of 17 genes (pval=0.05) encoding for secreted proteins involved in cancer progression-related pathways, which may potentially play a role in tumour-stroma interactions.

Conclusion Glucose affects BC stem cell reprogramming both directly and through Ad-SVFCs. Deciphering the mechanisms that govern this intricate crosstalk will pave the way to new targeted strategies to improve BC control in conditions of metabolic derangement.

PO-293 WITHAFERIN-A: A CANDIDATE ANTI-TUMOUR NATURAL COMPOUND FOR NON-SMALL CELL LUNG CANCER AND STEM CELLS

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Introduction Withaferin-A(WFA) is a natural compound extracted from Wihania somnifera plant which showed tumour growth inhibiting effects in both *in vitro* and *in vivo* models.of various cancers such as colorectal cancer, ovarian cancer, breast cancer, gastric cancers, glioma and hepatocellular carcinoma. Current commonly used therapeutic agents such as cisplatin (CDDP) are effective on cancer cells however there is a lack of efficiency on cancer stem cells (CSCs) which are considered to be responsible of recurrence and metastasis. Although anti-proliferative effect of WFA on non-small cell lunger cancer (NSCLC) cells was shown in previous studies, its effect on NSCLC CSCs remains unclear. Based on this fact, the aim of this study was to evaluate the *in vitro* effect of WFA on CD133 +NSCLC CSCs.

Material and methods A549 NSCLC cell line was incubated in DMEM supplemented with 10% FBS, 1% L-Glutamine, 1% Penicilline/Streptomycin at 5% CO_2 and 37°C. Cells were incubated with WFA 24, 48, 72 hours of 2.5,10,20 and 50 µM WFA and 75,100 µM CDDP and their combinations. Cell viability was determined by the WST-1 assay.CD133$^+$ A549 cells were isolated with magnetic beads. The purity of CD133 positivity was assessed by flow cytometry. A549 cells and CD133$^+$ A549 cells were treated with 50 µM WFA, 100 µM CDDP and incombination for 24 hours. Apoptosis was evaluated by flow cytometry with annexin V and PI staining. Furthermore DNA damage and cell proliferation of CD133$^+$ A549 cells were determined by flow cytomerty with BrdU and H2AX stainings.

Results and discussions WFA caused 18,6% early apoptosis (EA), 36,2% late apoptosis (LA), 56% anti-proliferation while CDDP caused 34,3% EA, 2,5% LA,%31,3% anti-proliferation and WFA+CDDP caused 45,6% EA, 34,5% LA 63,8% anti-proliferation on A549 cells. Also WFA 21,6% EA 38,3% LA, 6,6% DNA damage while CDDP caused 30% EA, 4,4% LA, 7,0% DNA damage and WFA+CDDP caused 21,9% EA, 42,9% LA,6,1% anti-proliferation on A549 CSCs.

Conclusion WFA showed anti-proliferative and apoptosis inducing effects of both NSCLCs and their CD133 +CSCs. In addition WFA did not change anti-tumour effect of CDDP and their combination showed more anti-proliferative and apoptosis inducing effect than their administration alone. In addition, WFA caused DNA damage in NSCLC CSCs. Although CDDP has critical anti-tumour effects, it might lead to adverse effects such as ototocity, neprotoxicity, peripheral neuropathy. Therefore, systemic effects of the natural compound WFA are suggested to be evaluated in animal models in future studies.