combination of niclosamide (Wnt/β-catenin pathway inhibitor) and Valproic acid (VPA, histone deacetylase inhibitor) on breast CSCs.

Material and methods The effect of niclosamide (1 μM, 24 hour pre-treatment) and VPA (0.63–5 mM) combination on the viability of MCF-7s cells (CSCs-enriched population) were demonstrated by the ATP assay. Acetylated histone H3 levels at selected doses for the combination were assessed by ELISA. Protein levels associated with the Wnt/β-catenin signalling pathway, EMT, and histone modifications were shown by western blotting. Cell death mode was investigated via Hoechst 33342/PI double staining, M30 ELISA, real-time PCR (gene levels associated with apoptosis and autophagy) and western blotting (protein levels associated with apoptosis, autophagy and ER stress).

Results and discussions We found that combination therapy exhibited a marked decrease in cell viability by inducing extrinsic apoptosis along with the stronger Wnt inhibition and increased histone H3 acetylation in MCF-7s cells. Furthermore, it was found that mesenchymal markers (fibronectin, N-cadherin, and ZEB1) were decreased at 72 hour and cytokeratin 18 as an epithelial marker was re-expressed in which H3K9ac and H3K4me3 were also increased. In addition, ER stress and blockade of autophagic flux have also been shown to be involved in this process.

Conclusion In conclusion, the future success of this combination approach in targeting CSCs and converted CSCs to non-CSCs may hold significant promise for successful treatment of breast cancer.

PO-290 ETV7 REGULATES BREAST CANCER STEM CELLS CONTENT AND CHEMORESISTANCE

L Pezzè*, S Pontalti, K Badowska, Y Ciribilli. *Laboratory of Molecular Cancer Genetics-CIBIO, University of Trento, Povo, Italy*

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Introduction Cancer stem cells (CSCs) are considered the population of cells within the tumour able to drive tumorigenesis and known to be highly resistant to conventional chemotherapy. ETV7 is a poorly studied transcription factor member of ETS large family, known to be an interferon-stimulated gene. It has been recently found over-expressed in breast cancer (BC), with higher expression levels in the more aggressive BC subtypes. In this work, we investigated the effects of ETV7 increased expression on breast CSCs population and resistance to chemotherapy in BC cells.

Material and methods We generated MCF7 and T47D BC-derived cells stably over-expressing ETV7 and obtained ETV7 KO in MDA-MB-231 BC cells using CRISPR/Cas9 technology. We analysed breast CSCs content via CD44/CD24 staining and FACS analysis, as well as mammospheres formation assay. We measured expression of ABC transporters and anti-apoptotic proteins via RT-qPCR and western blot. We finally assessed sensitivity to Doxorubicin and 5-Fluorouracil (5-FU) via MTT assay and AnnexinV/PI staining at FACS.

Results and discussions We observed that the expression of ETV7 could be induced by various stimuli, particularly by chemotherapeutic drugs able to induce DNA damage. We then analysed the impact of ETV7 expression on the sensitivity to Doxorubicin and 5-FU and we could observe a significantly decreased sensitivity to these drugs upon ETV7 over-expression. We could also appreciate an increase in ABC transporters and BCL2 anti-apoptotic protein expression following ETV7 over-expression. We further observed that alteration of ETV7 expression could significantly affect the population of breast cancer stem cells ($CD44^{+}/CD24^{low}$ cells) in different BC cell lines.

Conclusion We propose a novel role for ETV7 in breast cancer stem cells plasticity and associated resistance to conventional chemotherapy. We finally suggest that an in-depth investigation of this mechanism could lead to novel breast CSCs targeted therapies and to the improvement of combinatorial regimens with the aim of avoiding resistance and relapse in breast cancer.

PO-291 A CELL-PENETRATING PEPTIDE BASED ON THE CONNEXIN43-SRC INTERACTING SEQUENCE REDUCES GLIOMA STEM CELL MIGRATION AND PROLIFERATION BY RECRUITING SRC, CSK AND PTEN

[1]M Jaraíz-Rodríguez*, [2]MD Tabernero, [2]M González-Tablas, [3]A Otero, [4]A Orfao, [1]JM Medina, [1]A Tabernero. *[1]Universidad de Salamanca, Instituto de Neurociencias de Castilla y León, Salamanca, Spain; [2]Universidad de Salamanca- Departamento de medicina, Instituto de Estudios de Ciencias de la Salud de Castilla y León IECSCYL- Instituto de Investigación Biomedicina de Salamanca IBSAL- Centre for Cancer Research CIC-IBMCC-CSIC/USAL, Salamanca, Spain; [3]Hospital Universitario de Salamanca and IBSAL, Neurosurgery Service, Salamanca, Spain; [4]Universidad de Salamanca- Departamento de medicina, Centre for Cancer Research CIC-IBMCC- CSIC/USAL- IBSAL, Salamanca, Spain*

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Introduction The expression of connexin43 (Cx43), the main gap junction channel-forming protein, is down-regulated in glioma stem cells (GSC). Our previous studies showed that a cell-penetrating peptide containing the region of Cx43 that interacts with the oncoprotein c-Src (TAT-$Cx43_{266–283}$), inhibits its oncogenic activity and up-regulates the tumour suppressor PTEN. Through this pathway, TAT-$Cx43_{266–283}$ reduces glioma cell proliferation and reverses the GSC phenotype. Our next goal was to uncover the mechanism of action and the effects of TAT-$Cx43_{266–283}$ on the migration and invasion of human GSCs.

Material and methods Human primary GSCs (G9, G12, G13, G15 and G16) were obtained from glioblastoma patients' biopsies from the Neurosurgery Service of the Hospital Universitario de Salamanca, Spain. The migration was analysed in these primary GSCs by tracking individual cell trajectories in cultures recorded by Time-Lapse Live-cell Imaging and the invasion with Matrigel-treated transwell inserts. The same tumour biopsies were used to study the effect of TAT-$Cx43_{266–283}$ on fresh undissociated tumour blocks by time-lapse microscopy. The molecular interactions were studied in G166 GSCs by pull-down assays of biotinylated TAT-$Cx43_{266–283}$ (TAT-$Cx43_{266–283}$-B) and Western blot analysis.

Results and discussions Our results confirmed that TAT-$Cx43_{266–283}$ strongly reduces the migration and invasion of human primary GSCs by inhibiting c-Src activity and up-regulating PTEN that consequently, lowers the levels of phosphorylation of focal adhesion kinase tyrosines 397, 576 and 577. In addition, fresh undissociated tumour blocks revealed a dramatic reduction on the survival of these glioblastoma cells when exposed to TAT-$Cx43_{266–283}$. By pull-down assays, we showed that TAT-$Cx43_{266–283}$-B recruits c-Src together with its inhibitors PTEN and Csk, confirming the proposed mechanism for Cx43.