PO-219 METHYLGLYOXAL-INDUCED DICARBONYL STRESS: ROLE IN MELANOMA PROGRESSION AND RESPONSE TO THERAPY

[1]J Bellier*, [1]MJ Nokin, [1]F Durieux, [2]F Journe, [2]G Ghanem, [1]V Castronovo, [1]A Bellahcène. [1]*University of Liège, Metastasis Research Laboratory- GIGA-Cancer, Liège, Belgium*; [2]*Free University of Brussels, Laboratory of Oncology and Experimental Surgery- Institut Jules Bordet, Brussels, Belgium*

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Introduction Methylglyoxal (MG) is an endogenous dicarbonyl spontaneously produced during glycolysis able to react with proteins, lipids and DNA, inducing a carbonyl stress. Glyoxalase 1 (GLO1) detoxifies MG into D-Lactate. High MG is notably associated with diabetes and cancer. Melanoma is the most deadly form of skin cancer. Therapy is notably based on the inhibition of the MAPK pathway, often over activated. Unfortunately, BRAF and MEK inhibitors are briefly efficient as tumours rapidly develop resistance mechanisms. Melanoma tumours are generally highly glycolytic and therefore inevitably produce high amounts of MG, accumulating Advanced Glycation End products (AGEs). Phenformin, a metformin analogue with MG-scavenging properties, improves the therapeutic effect of BRAF inhibition. This observation is in good accordance with our hypothesis that MG could be involved in progression and resistance of melanoma.
Material and methods This study aims to assess the metabolic profile of various human melanoma cell lines comprising BRAF and/or MEK inhibitors sensitive and resistant cells. First, we plan to explore thoroughly dicarbonyl stress status in these cell lines and in patient tissues. Next, we will subject melanoma cells to anti-carbonyl stress agents such as l-carnosine and aminoguanidine alone or in combination with MAPK pathway inhibitors and assess their effect on tumour cell survival.
Results and discussions IHC staining of argpyrimidines, MG-derived AGEs, in a collection of melanoma samples showed that MG carbonyl stress is a constant feature in melanoma. MG AGEs were detectable in both BRAF inhibitors sensitive and resistant cell lines: A375 and A2058, respectively. Interestingly, the treatment of these cells with exogenous MG induced different responses: A375 cells increased their MG detoxification system and their metabolic activity as assessed by their increased GLUT1 and GLUT3 glucose transporters and PGC1alpha mitochondrial regulator. Whereas, A2058 resistant melanoma cells showed a decrease of both GLO1 activity and the metabolic markers investigated.
Conclusion Ongoing experiments will help to understand these phenotypes and to further characterise a serie of WT and BRAF mutated melanoma cells. We plan to assess carbonyl stress in a larger human melanoma collection to investigate the correlation with tumour stage, metastasis, overall survival and resistance to therapy. Finally, we will test the efficacy of a combined therapy using BRAF and/or MEK inhibitors and MG scavenger molecules such as carnosine.

PO-220 RAN, A NOVEL AND PROMISING GENE FOR MALIGNANT PLEURAL MESOTHELIOMA

[1]I Dell'anno*, [1]E Barone, [1]I Lepori, [1]L Migliore, [1]S Agostini, [1]O Melaiu, [2]L Poliseno, [1]F Gemignani, [1]S Landi. [1]*University of Pisa, Department of Biology – Unit of Genetic, Pisa, Italy*; [2]*Institute of Clinical Physiology IFC – CNR, Oncogenomic Unit, Pisa, Italy*

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Introduction *RAN* is a member of RAS superfamily of GTPases involved in a varied range of cellular processes. Although it is widely demonstrated *RAN* is overexpressed in many human tumours having an essential role in malignant cell survival and cancer progression, little is known about its role in Malignant Pleural Mesothelioma (MPM). Previous studies showed the *RAN* gene is upregulated in mesothelioma tissues and cell lines, so it might be involved in carcinogenesis of MPM. We aimed to explore the functional role of *RAN* in MPM cell lines and its likely use as co-target in mesothelioma treatment.
Material and methods The role of *RAN* in MPM tumorigenesis was investigated through RNA interference, on a panel of one mesothelial cell line (Met-5A) and four MPM cell lines (Mero-14, Mero-25, Istmes-2 and NCI-H28). After monitoring gene knockdown, at both the mRNA and protein levels, a phenotypic study was performed through Caspase-3/7, Sulforhodamine B, Wound-Healing and Colony Formation assays. Flow cytometry was employed to monitor cell cycle. To validate data from siRNA experiments, two different siRNA were independently used to target *RAN*. The gene was also knocked-out using a lentiviral CRISPR/Cas9 system in Mero-14. Cas9 endonuclease and gRNA were transduced by two different lentiviral transfer vectors.The doxycycline-regulated Cas9 induction was followed by DNA, RNA and proteins extraction to confirm the occurrence of gene disruption. TIDE analysis was carried out to monitor targeted mutations triggered by the genome editing.
Results and discussions The siRNA-mediated knockdown was confirmed at both the mRNA and protein level in all cell lines. The silencing caused a statistically significant decrease of proliferation rate and clonogenicity in Mero-14, Mero-25 and Istmes-2.The migration ability was affected in Met-5A and Istmes-2. An increase in apoptosis was observed in all cell lines, being statistically significant only in the malignant ones. Flow cytometry analysis showed an increase of cells in G0/G1 phase and a decrease of cells in S phase, being significant in Mero-14 cell line only. *RAN* knock-out has been confirmed at both the mRNA and protein level, whereas the TIDE analysis is still ongoing.
Conclusion This study showed that MFAP5 is a novel myoepithelial cell marker that appears to be up-regulated in duct epithelium in DCIS and IC-NST during tumourogenesis and that its cytoplasmic expression in invasive tumours seems to have a poor prognostic role manifested by its association with poor prognostic parameters such as high grade, late stage, lymph node invasion and increased MVD.

PO-221 PROSTATE CANCER CELLS ARE ABLE TO USE FRUCTOSE AS A METABOLIC SOURCE

[1]D Carreño*, [1]N Corro, [1]C Schmidt, [2]P Sotomayor, [3]P Cisternas, [3]N Inestrosa, [4]F Nualart, [5]JC Cardenas, [1,6]AS Godoy. [1]*Pontificia Universidad Catolica de Chile, Physiology, Santiago, Chile*; [2]*Universidad Andres Bello, Center for Integrative Medicine and Innovative Science, Santiago, Chile*; [3]*Pontificia Universidad Catolica de Chile, Cell Biology, Santiago, Chile*; [4]*Universidad de Concepcion, Department of Cell Biology, Concepción, Chile*; [5]*Universidad de Chile, Institute of Biomedical Sciences, Santiago, Chile*; [6]*Roswell Park, Department of Urology, Buffalo, USA*

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Introduction The clinical localization of primary cancers and sites of metastasis by positron emission tomography (PET) is based on the enhanced cellular uptake of 2-deoxy-2-[^{18}F]-fluoro-d-glucose (FDG). In prostate cancer (CaP), however, FDG-PET