

PO-159 EXPRESSION PROFILES OF P53/P73 ISOFORMS IN HUMAN MELANOMA CELL LINES

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Introduction *TP53* is the most frequently mutated gene in human cancer. However, in metastatic melanoma mutations of *TP53* occur infrequently and p53 fails to function as a tumour suppressor. The altered expression of p53 family members, including p53/p73 isoforms, as well as of the interactions among them could affect normal function of p53. Furthermore, somatic BRAF mutations have been found in 37%–50% of all melanomas, of which almost 90% harbour the activating V600E mutation. Although initial response to BRAF inhibitors is highly effective, the resistant clones frequently develop, and, in treated patients disease progression is observed within 6 to 8 months. To address this, a better understanding of the genetic basis of melanoma initiation and progression is needed.

Material and methods The expression profile of p53 and its potential interaction partners - p53 and p73 isoforms was determined in a panel of melanoma cell lines by western blot analysis and quantitative RT-PCR. We have determined the protein levels of p53/p73 isoforms in response to DNA damage treatment (γ -irradiation and etoposide) in cell lines with different *TP53* mutational status using western blot analysis. Furthermore, vemurafenib resistant cells are generated and resistance was confirmed by MTT assay. Expression of p53/p73 isoforms was determined in these cells.

Results and discussions Relative expression analysis of metastatic melanoma cell lines revealed that the most expressed p53 isoforms are p53 α and Δ 133p53 α , while Δ 40p53 β , Δ 40p53 γ and Δ 133p53 γ are least expressed. Also, interestingly, relative expression of full length TAp73 was higher than Δ Np73. Furthermore, the most expressed proteins were p53 α , Δ 40p53 α , Δ 133p53 γ and Δ 160p53 γ . Contrary to gene expression, the most expressed p73 isoform is oncogenic Δ Np73 β . γ -irradiation induced accumulation of all p53 α isoforms in p53 mutant melanoma cell lines, but not in p53 wild type cells. Levels of p53 beta isoforms remained the same, while gamma isoforms were undetectable. Upon γ -irradiation, accumulation of Δ Np73 isoforms was observed in p53 mutant cells. Treatment with etoposide induced expression of p53 α isoform, and both TAp73 and Δ Np73 isoforms in p53 wild type cells. Furthermore, in vemurafenib resistant clones the changes in p53/p73 protein expression were observed.

Conclusion Taken together, these analyses enabled us to detect p53/p73 isoforms in melanoma cell lines and gave us insight into their abundance in melanoma cell lines for further analyses of p53 interacting partners.

PO-160 INVESTIGATION OF UBIQUITIN-LIGASE HUWE1 IN THE MODULATION OF RAS PATHWAY IN LEUKAEMIA MODELS

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Introduction The RAS/RAF/MEK/ERK pathway is frequently hyperactivated in several tumours. In leukaemia, this activation can arise, among other mechanisms, from point mutations in the RAS genes, which are important in acute lymphoid leukaemia (ALL) and acute myeloid leukaemia (AML), or from chromosomal translocations such as the *BCR-ABL* gene, which is a driver mutation in chronic myeloid leukaemia (CML) and some cases of ALL. The hyperactivation of this pathway stimulates cell proliferation and, consequently, the production of reactive oxygen species (ROS), which is one of the main mechanisms involved with induction of cellular senescence in tumours. Thus, tumour cells that harbour the mutated RAS gene are critically dependent on feedback mechanisms to regulate pathway activation. Jang *et al.* demonstrated that the ubiquitin-ligase HUWE1 acts on a negative feedback mechanism that controls the activation of ERK1/2. Although widely studied in the context of tumorigenesis, the role of this molecule in events related to leukemogenesis has not yet been described.

Material and methods In this study, leukaemia cell lines and human hematopoietic stem and progenitor cells (HSPCs) with KRAS^{G12V} mutation were transduced with miR-E lentiviral particles for *HUWE1* knockdown. Cell proliferation, apoptosis, ROS production and analysis of gene and protein expression were performed in cell lines; cumulative growth analysis, cobblestones area formations, clonogenic capacity and differentiation profile analysis were performed in HSPCs.

Results and discussions In cell lines, it was observed that *HUWE1* knockdown reduced the proliferative capacity of Nalm-6, K562 and THP-1, but not of HL-60. Besides that, it caused a reduction in ROS production ($p < 0,05$), associated with reduction of apoptosis rates ($p < 0,01$), especially in K562 in which it also promoted activation of ERK1/2. In HSPCs, a reduction of the proliferative capacity was observed in cultures expressing KRAS^{G12V} in combination with *HUWE1* knockdown. In the same conditions, a drastic reduction of clonogenic capacity ($p < 0,001$), especially of erythroid burst forming units (BFU-E) colonies, was observed. *HUWE1* knockdown also changed HSPCs differentiation profile from the granulocytic to the monocytic lineage.

Conclusion Results suggest that HUWE1 might play a role in leukemogenesis process and differentiation of human HSPCs, acting in the modulation of RAS/RAF/MEK/ERK pathway.

PO-161 THE AMPK AND MEK/ERK SIGNALLING PATHWAYS REGULATE MITOCHONDRIAL FOXO3A IMPORT THROUGH PHOSPHORYLATION OF SERINE 12 AND SERINE 30

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Introduction FoxO3A is a well-known tumour suppressor transcription factor involved in the regulation of various metabolic and cell-death/survival genes. Its activity is finely modulated through specific post-translational modifications functioning as a ‘molecular FoxO code’. Recently, we described a novel mitochondrial arm of the AMPK-FoxO3A axis in normal cells upon

nutrient shortage. Here, we show that the MEK/ERK and AMPK pathways induce FoxO3A mitochondrial accumulation in cancer cells upon metabolic stress or chemotherapy treatment.

Material and methods We performed an extensive *in vitro* characterisation of the cleaved intra-mitochondrial form of FoxO3A, by analysing mitoplasts purified from several cancer cell lines and tumours. Then, after an *in silico* preliminary analysis, we generated FoxO3A mutants to identify the key residues required for its mitochondrial accumulation and we extended our *in vitro* analysis to define the involved kinases. Therefore, to dissect the impact of the MEK/ERK and AMPK pathways on FoxO3A mitochondrial import and functions, we expressed the previously generated mutants in FoxO3A-knock-out cancer cell lines obtained by using the CRISPR-Cas9 genome editing system.

Results and discussions In metabolically stressed cancer cells, activation of the MEK/ERK and AMPK pathways is required to phosphorylate, respectively, S12 and S30 on FoxO3A N-terminal domain, and promote FoxO3A mitochondrial translocation. Once into the mitochondria, FoxO3A is cleaved by MPPs (mitochondrial processing peptidases) to reach and bind to mitochondrial DNA in complex with TFAM, SIRT3 and mtRNAPol, activating its expression and supporting mitochondrial metabolism and cancer cell survival. Intriguingly, cancer cells treated with chemotherapeutic drugs only require the MEK/ERK pathway to accumulate FoxO3A into the mitochondria, through S12 phosphorylation, and promote resistance and cell survival. Finally, mitochondrial FoxO3A recruitment is necessary for metformin-induced apoptosis.

Conclusion The interplay between the MEK/ERK and AMPK pathways, which converge on the N-terminal domain of FoxO3A to eventually increase the expression of mitochondrial-encoded core subunits of the OXPHOS machinery, drives cancer cells towards survival or death. Further elucidation of the FoxO3A 'mitochondrial code' will be instrumental to devise personalised therapeutic strategies to selectively disable FoxO3A pro-survival activity.

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THE TRANSCRIPTION FACTOR GATA4 IS OVEREXPRESSED IN MALIGNANT MENINGIOMA ENHANCING PROLIFERATION VIA DOWNREGULATION OF MIR-15 FAMILY MEMBERS

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Introduction Meningioma is the most common primary brain tumour and is classified as benign (WHO I, 80%), atypical (WHO II, 15%–20%) and anaplastic (WHO III, 1%–3%). The 3 year recurrence rate in WHO I meningioma is ~50% and it is much greater in WHO II and III. Recent studies showed that cyclin D1 and E1 positively correlated with meningioma grade and higher recurrence rates, suggesting them as potential prognostic markers.

Here, we show that cyclin D1-D2 and E1 overexpression in malignant meningioma is driven by downregulation of the miR-15 family members via GATA4, a transcription factor overexpressed in WHO III meningioma cells and tissues.

Therefore, we propose GATA4 as a novel possible biomarker for monitoring meningioma progression.

Material and methods Meningioma (MN) specimens were collected from consented patients according to the ethical approval for this study. All cell lines and primary MN cells were isolated from tumour specimens and cultured following recommended conditions. Real Time PCR was conducted using TaqMan reagents (Applied Biosystems), according to the manufacturer's instructions following the 2^{-ΔΔC_T} method. *In silico* studies were conducted using TargetScanHuman7.1 to search for putative miRNA targets. P-values were calculated using the Student's t-Test or the ANOVA one-way analysis of variance, led by the GraphPad Prism 5.01 and MS Excel 2016 software (p values < 0.05 ± SEM).

Results and discussions Proteomic analysis showed an increase of cyclin D1 in Ben Men-1, primary WHO I and KT21-MG1 cells, and an increase of cyclin D2 in KT21-MG1 cells only. *In silico* studies identified cyclin D1-D2-D3 and E1 as targets of the miR-15 family members. QPCR showed that miR-195 and -497 are downregulated in WHO II and III samples compared to WHO I (3.38 and 6.02 folds, respectively; p=0.02); these results were consistent in cell culture exosomes.

Analysis of GATA4 revealed that the protein is highly overexpressed in KT21-MG1 cells but not in Ben Men-1 and primary meningioma cells (17940.19 and 4899.34 folds, respectively, p=0.03). These results were consistent in tissues (2640.53 folds).

Conclusion Our data show that members of the miR-15 family are downregulated in WHO III meningioma cells and tissues, suggesting their contribution to control tumour progression. In addition, in WHO III cells and tissues the transcription factor GATA4, involved in miR-15 family regulation, is overexpressed. Ongoing studies will address GATA4 role in the biological progression of meningioma. (DB and COH contributed equally).

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JUNCTIONAL ADHESION MOLECULE-A IS A NOVEL UPSTREAM REGULATOR OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR-3 SIGNALLING IN BREAST CANCER

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Introduction Junctional Adhesion Molecule-A (JAM-A) is a transmembrane protein with important physiological functions in regulating cell-cell adhesion. JAM-A levels have been reported to regulate HER2 expression (Brennan et al, Oncogene 2013;32(22):2799–804), thus we hypothesised that JAM-A also regulates expression of HER3 in breast cancer cells. HER3 is the most potent binding partner of HER2 in activating tumour growth signalling, and accumulating evidence suggests that HER3 plays an important role in resistance to anti-HER2 therapies. Furthermore HER3 is frequently overexpressed in HER2-negative breast cancers, and, along with other HER family members, may drive HER2-independent tumourigenic mechanisms.

Material and methods Using *in vitro* assays with a panel of breast cell lines, we are investigating whether JAM-A