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 p53α and Δ133p53α, while Δ40p53ß, Δ40p53γ and Δ133p53γ are least expressed. Also, interestingly, relative expression of full length TAp73 was higher than △Np73. Furthermore, the most expressed proteins were p53α, Δ40p53α, Δ133p53γ and Δ160p53γ. Contrary to gene expression, the most expressed p73 isoform is oncogenic ΔNp73β. γ-irradiation induced accumulation of all p53α 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 ΔNp73 isoforms was observed in p53 mutant cells. Treatment with etoposide induced expression of p53α isoform, and both TAp73 and ΔNp73 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 progenitors 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