assay and circulating experiments were performed with an experimental setup with a peristaltic pump.

**Results and discussions** In our studies, HOTAIR expression is found to be reversely correlated with c-Met expression and activation in HCC cell lines, for the first time. c-Met activation leads to down-regulation of IncRNA HOTAIR expression and also, inhibition of c-Met activation recovers HOTAIR expression. c-Met pathway is defined to be activated in ligand-dependent and independent manner. Reciprocal interaction of c-Met and HOTAIR was conserved in ligand-independent c-Met activation, too. Bioinformatics analysis of HOTAIR regulated genes highlighted some genes that are known to contribute to c-Met pathway via enabling its activation. The possible molecules that take part in reciprocal interaction of c-Met and HOTAIR are analysed by qPCR and western blot. Our findings are the first to show Caveolin-1, a membrane protein, is an important target of HOTAIR to regulate c-Met pathway and also Vimentin is regulated by HOTAIR expression to mediate epithelial/mesenchymal phenotype in HCC. To further understand the role of HOTAIR and c-Met interaction in metastasis; expression of HOTAIR, c-Met and related molecules were analysed in circulating HCC cells under shear stress.

**Conclusion** Reported data revealed that c-Met pathway related mesenchymal phenotype and adhesion-independent cell growth requires HOTAIR downregulation which leads to increase of Caveolin-1 expression. Here we report that HOTAIR downregulation is a requirement to survive in circulation, adhesion independent survival and growth and it is regulated by c-Met receptor tyrosine kinase activity. Our results contribute to the literature by mapping the interaction of HOTAIR and c-Met.

**Poster Presentation: Signalling Pathways**

**PO-146** **KNOCKOUT VALIDATION OF ANTIBODIES TO COMPONENTS OF THE NF-kB SIGNALLING PATHWAY**

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**Introduction** Nuclear factor-kB (NF-kB) plays a pivotal role in inflammation and innate immunity and aberrant regulation of NF-kB can influence cancer development and progression (Ref 1). As a result, a great deal of research is now focused on targeting this signalling pathway therapeutically. In order to accurately and reproducibly examine the NF-kB signalling cascade there is a need for highly specific antibodies. We, at Abcam, have developed knockout (KO)-validated antibodies targeted to distinct elements of the NF-kB signalling pathway to maximise experimental accuracy and reproducibility.

**Material and methods** Our KO validation program uses the extensive library of KO cell lines available from Horizon Discovery. Target genes are mutated via CRISPR-Cas9 within a haploid cell line, resulting in a frameshift and complete loss of gene expression. Target specificity is then confirmed via western blot, flow cytometry or immunocytochemistry. These KO cell lines function as true negative controls that can be used at a large scale to confirm antibody specificity to target proteins.

**Results and discussions** We present antibody validation data for seven targets in the NF-kB signalling pathway. Of the 30 Abcam products tested, we demonstrate that 22 antibodies are specific. The remaining 8 have since been removed from our catalogue due to specificity concerns. We have now validated over 1000 antibodies using this KO method, including many that are relevant to immunology and immuno-oncology research.

**Conclusion** Following validation with KO cell lines as a negative control, we can recommend 22 highly specific antibodies to proteins in the NF-kB signalling pathway. KO validation is one of many strategies that we use to ensure the specificity of our antibodies. By providing robust and specific antibodies which work the first time, we hope to help researchers to improve the experimental reproducibility and accuracy of their studies.

**REFERENCE**

1. Bastian Hoesel and Johannes A Schmid; Mol Cancer. 2013 12: 86. doi:10.1186/1476-4598-12-86
Conclusion 1. Taken together, the results of this study demonstrate that the hypoxia has an inhibiting effect on BRCA1 network and decreases hormonal stimulation of breast cancer cell growth. 2. The data obtained point to the different mechanisms of BRCA1 and steroid hormones expression regulation with phytoestrogens in adenocarcinoma breast cancer cells and open further perspectives for their complex investigation. (RFBR grants №№ 15-04-06991-a, 16-34-01049-mol-a and RSF grant 14-15-00362 (cell culture experiments)).

Introduction Malignant melanoma is the most aggressive form of skin cancer and resistant to available therapies, therefore new molecular approaches for better understanding of disease are needed. Although TP53 is rarely mutated in melanoma, it fails to function as a tumour suppressor. This may result from alterations in p53 family members, including the diverse isoforms of p53 and its homologue p73. Moreover, we assume that p53 functions in melanoma might be altered through interactions with small molecular weight variants of p53 and p73 isoforms, NME and GLI families of proteins. In this study, we conducted a gene/protein expression profiling for p53 and its potential interaction partners (p73/NME/GLI) in metastatic melanoma tissue.

Material and methods Metastatic melanoma and adjacent healthy skin tissues were obtained from 38 patients during surgery in the Sestre milosrdnice University Hospital Center, Zagreb. Expression of 9 TP53 isoforms, both N- (full-length, Δ40 and Δ133) and C-terminal (α, β and γ), 2 TP73 isoforms (TAP73 and ANN’p73), NME1, NME2, GLI1, GLI2, GLI3 and PTCCH1, was analysed by RT-qPCR. Expression of p53 (p53α, p53β, Δ40p53α, Δ133p53α, Δ133p53β and Δ160p53 isoforms), p73 (TAP53α, TAP53β, ΔNp73α and ΔNp73β), NME1, NME2, GLI1 (130 and 160 kDa isoforms), GLI2 (133 and 250 kDa) and GLI3 (activator/repressor forms) was analysed by western blot.

Results and discussions Relative expression of ‘short’ TP53 isoforms in tumour tissue was as follows: p53α>p53β>Δ40p53>Δ40p53γ>Δ40β>Δ40γ. Expression of ‘short’ TP53 isoforms was: Δ133α>Δ133β>Δ133γ. Only Δ40β and Δ40γ were significantly downregulated in tumours. Expression of full length TAP73 was higher than ΔNp73, and both were significantly downregulated in tumours. Significant downregulation in tumours was also observed for PTCCH1, GLI1 and GLI2; while NME1 and NME2 were generally the most expressed genes but without significant difference between healthy and tumour tissue. In addition, in metastatic melanomas the most expressed proteins were p53α and NME1, while ΔNp73β, GLI2 (250 kDa) and Δ133p53α showed lowest expression. Eight proteins showed significantly higher expression in tumours compared with healthy skin: 2 GLI1 isoforms (130 and 160 kDa), 133 kDa GLI2 isoform, NME1 and NME2, ΔNp73Δ and 2 p53 isoforms with shortest N- and longest C-terminus.

Conclusion We have shown that TP53/TP73/NME/GLI genes are generally downregulated in metastatic melanoma tissue compared with healthy skin, while, on the contrary, their protein products seem to be upregulated in tumours.