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)).

PO-148 ACTIVIN A UPREGULATION MEDIATED BY P63 PROMOTES MIGRATION AND INVASION OF ORAL CANCER CELLS

D Mundhe*, R Mishra, T Teni. *Advanced Centre for Treatment Research and Education in Cancer- Tata Memorial Centre, Teni Laboratory, Kharghar, India*

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Introduction TGF-β family proteins, Activins are homo/heterodimers of β subunits βA, βB, βC and βE, while Inhibins are αβ heterodimers. Relative abundance of each monomer dictates which dimer is predominantly formed. Activins bind to typeII receptor on cell membrane and trigger their tetramerization with typeI receptor, ALK4 phosphorylating SMAD 2/3 which translocate to nucleus and regulate transcription. Activins are reported to be up regulated in various cancers including HNSCC; however, these are transcriptome-scale studies that have indicated altered βA expression, without considering other subunits' expression or their dimerization. An oncogenic role for Activins has been demonstrated in cancer; albeit, the precise mechanisms downstream of Activin or molecules regulating its expression/function are vaguely understood. This study evaluated the expression of Activins and phenotypes that are affected by its altered expression in oral cancer; with emphasis on Activin-SMAD 2/3 signalling as the downstream mechanism. We also tested the possible role of p53 family proteins in regulating Activin gene expression and functions.

Material and methods Activin subunits' expression was assessed in oral cancer celllines and tissues by qRT-PCR, IHC and Western blot. Wound healing, Matrigel Invasion, and MTT assays were performed post knockdown of Activin βA and SMAD 2/3 using siRNA and post treatment with recombinant Activin A. We tested the regulation of Activin βA expression by p53 and p63 using ChIP assay and analysed the effect of p63 knockdown in context of Activin expression on oral cancer cells.

Results and discussions Activin βA was significantly overexpressed in oral cancer cells and tissues compared to normal counterparts at transcript and protein level. Knockdown of βA resulted in decreased, while treatment with recombinant Activin A resulted in increased migration and invasion of oral cancer cells. Knockdown of SMAD 2/3 also led to similar effects, indicating that Activin A contributes to these phenotypes through SMAD2/3 signalling pathway. The ChIP experiments demonstrated that βA promoter could be bound by p63, a p53 family member with established oncogenic functions. Recombinant Activin A treatment could rescue the loss of migration upon p63 knockdown suggesting that p63 could be an upstream regulator of Activin expression and function in oral cancer.

Conclusion The study revealed a novel role of p63 in regulating Activin A expression. We also deciphered the mechanism downstream of p63-Activin A axis that contributes to migration and invasion of oral cancer.

PO-149 EXPRESSION PROFILING OF TP53, TP73, NME AND GLI FAMILIES OF GENES/PROTEINS IN METASTATIC MELANOMA

[1]P Ozretić*, [1]N Hanžić, [1]D Trnski, [1]B Proust, [1]M Radić, [1]M Sabol, [2]I Milas, [1]M Herak Bosnar, [1]S Levanat, [1]N Slade. *[1]Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia; [2]Sestre milosrdnice University Hospital Center, University Hospital for Tumors, Zageb, Croatia*

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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 Centre, 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 ΔNN'p73), *NME1*, *NME2*, *GLI1*, *GLI2*, *GLI3* and *PTCH1*, was analysed by RT-qPCR. Expression of p53 (p53α, p53β, Δ40p53α, Δ133p53α, Δ133p53β and Δ160p53α 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 'long' *TP53* isoforms in tumour tissue was as follows: p53α>p53β > △40α>p53γ > △40β > △40γ. Expression of 'short' *TP53* isoforms was: △133α > △133β > △133γ. Only △40β and △40γ were significantly downregulated in tumours. Expression of full length TAp73 was higher than △Np73, and both were significantly downregulated in tumours. Significant downregulation in tumours was also observed for *PTCH1*, *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 △Np73β, GLI2 (250 kDa) and △133p53α 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, △Np73△ 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.