Abstracts

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 UPRREGULATION MEDIATED BY P63 PROMOTES MIGRATION AND INVASION OF ORAL CANCER CELLS

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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 cell lines 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 cell lines 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

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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 ANN‘p73), NME1, NME2, GLI1, GLI2, GLI3 and PTCH1, 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 ‘long’ TP53 isoforms in tumour tissue was as follows: p53α>Δp53β > Δ40α>Δp53γ > 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 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 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.