signalling has been linked with wide variety of malignancies, including lung tumours. However the association between co-expression profile of Notch downstream effector and lung cancer subtypes remains unclear. Therefore the aim of our study was to investigate functional gene co-expression networks to search for candidate biomarkers or therapeutic targets.

**Material and methods** In our analysis we used RNASeq expression and clinical data downloaded from The Cancer Genome Atlas (TCGA). Gene Set Enrichment Analysis (GSEA) performed for canonical pathways pointed to Notch pathway as one of the most significant. Subsequently we analysed expression of downstream Notch effectors, 2949 HES/HEY transcription factors targets. To this extent used Weighted Gene Co-expression Network Analysis (WGCNA) to find differences in gene expression profile between LUSC and LUAD.

**Results and discussions** The analysis of Notch pathway downstream targets which expression is regulated by HES/HEY transcription factors, identified 9 gene modules highly correlated with cancer type, with two of them as the most promising. Functional analysis revealed that among the differentially expressed genes were those involved in proliferation, cell cycle regulation, DNA repair, EMT, adhesion and metabolic processes, for example: TP63, PIK3CA, ADAM23, DLG1, FXR1, SENP3, TTK, BIRC5, KIF18A, KIF14, KIF4A, MCM4, MCM10. For one module the highly connected hub gene is TP63, acts as oncogene in many tumour types including squamous cell carcinomas, and for the second module is KIF4A. Interestingly, all listed genes were found to be overexpressed in LUSC and downexpressed in LUAD.

**Conclusion** Our analysis could be valuable for better understanding of the molecular mechanism of lung carcinoma as well as Notch signalling in lung cancer with emphasis of pathway gene expression as useful biomarker for differentiating cancer progression in lung cancer subtypes.

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**PO-154 GENE EXPRESSION PROFILE IN TUMOUR CELLS EXPOSED IN VITRO TO PHOTODYNAMIC THERAPY WITH A NOVEL PORPHYRINIC COMPOUND**

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**Introduction** Photodynamic therapy (PDT) has emerged as efficient and reasonably safe targeted therapeutic strategy in solid tumours. Briefly, a non-toxic photosensitizer is injected and then is activated at tumor-site only by exposure to tissue-penetrating red light. Cytotoxic singlet oxygen is formed, hence triggering massive death of tumour cells.

**Material and methods** A new porphyrinic compound (P2.2), previously described by us (Boscencu 2017) in Molecules 22.11:1815), was used as photosensitizer in U-87 MG human glioblastoma cells and HT-29 human colon carcinoma cells. A good concentration-dependent uptake of the fluorescent P2.2 compound by the investigated cells was demonstrated by flow cytometry. Cells loaded with 10 μM P2.2 were subjected to in vitro PDT using the MODULIGHT 6600 equipment at fluences of 5 to 10 J/cm². For mechanistic studies, cells were investigated at 3–6 hours post-PDT regarding viability, morphology and gene expression. The gene expression profile was assessed by pathway-focused PCR array (Stress and Toxicity Pathway Finder, QIAGEN), addressing 84 genes critically involved in oxidative, osmotic, hypoxic and inflammatory stress, in cell
death by apoptosis, necrosis and autophagy, in DNA damage and heat shock proteins/unfolded protein response.

Results and discussions Viability tests indicated that cell death arises within 6 hours post-PDT, but massive cell death occurs more than 48 hours post-PDT. The single oxygen burst triggered an early activation of some protective mechanisms against oxidative stress (full signature of the cytoprotective NRF2), hypoxia (SERPINE1) and osmotic stress (HSPA4L). Apoptosis (TNFα mediated), necrosis, autophagy and DNA damage (DDIT3) responses were highlighted. Interestingly, a complex inflammatory network was triggered by PDT (CXCL8, TNF, IL1), possibly associated to oxidative stress through NF-kB. These protective responses could be responsible for the delayed massive death of cells subjected to PDT.

Conclusion In response to PDT, glioblastoma and colon carcinoma cells developed in vitro various protective mechanisms against a complex web of stressors. Genes overexpressed in response to PDT might be candidates for co-therapies aiming to increase PDT efficacy. It seems reasonable that inhibition of endogenous NRF2-mediated antioxidant mechanisms could be an option for improving oxidative stress-mediated PDT.

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PO-155 THERAPEUTIC EVALUATION OF HEDGEHOG-GLI SIGNALLING PATHWAY THROUGH MRNA AND MRNA PROFILING OF OVARIAN CARCINOMA

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Introduction Signalling pathway Hedgehog-Gli (Hh-Gli) is involved in embryonal ovarian development, but its atypical activation can lead to different types of ovarian tumours. Our previous studies showed aberrant Hh-Gli activity in some ovarian tumour types and hypermethylation in a promoter of tumour suppressor gene PTCH1 in the CpG islands near the GLI-binding site (Sabol et al. Int J Oncol 2012, 2017;Musani et al. Gene 2013; Maurac et al. Int J Gynecol Pathol 2012). We and other are aware that various genetic and epigenetic alterations contribute to aberrant Hh-Gli pathway activity in ovarian cancer, which could serve as interesting targets for cancer treatment and therapy, especially if can be used to bypass resistance and to target cancer in more efficient way.

We hypothesise that changes in the expression of miRNA molecules related to the Hh-Gli signalling pathway genes contribute to the development of high-grade serous ovarian carcinoma (HGSOC).

Material and methods We conducted a miRNA profiling of HGSOC and healthy Fallopian tube control samples with Agilent SurePrint G3 Human miRNA 8 × 60K Microarray Kit containing probes for 2549 human miRNAs. Gene expression profiling was performed using Agilent SurePrint G3 Human Gene Expression v3 8 × 60K Microarray Kit, which covers 37 756 known RefSeq coding transcripts. Data were analysed using R/Bioconductor packages AgiMicroRna and limma. Online DIANA Tools (TarBase, microT-CDS and mirPath) were used to find which Hh-Gli genes are targets of miRNAs differentially expressed in HGSOC.

Results and discussions Data filtration gave 55 miRNAs: 32 up- and 23 down-regulated in HGSOC. Out of 47 genes involved in Hh-Gli pathway in humans (KEGG Pathway hsa04340), 35 are known targets for 27 over-expressed miRNAs, 22 are known targets for 16 under-expressed miRNAs, while 28 genes are potential targets for 26 up-regulated miRNAs and 24 are potential targets for 19 down-regulated miRNAs in HGSOC. In addition, 1090 genes were significantly over-expressed and 1692 were under-expressed in HGSOC. Further analysis revealed a couple of potential combinations of miRNAs and their target genes which are members of Hh-Gli pathway: ADRBK2/hsa-miR-96–5 p, BCL2/hsa-miR-16–5 p and hsa-miR-96–5 p, BOC/hsa-miR-224–5 p, and IHH/hsa-miR-16–5 p, hsa-miR-107 and hsa-miR-103a-3p.

Conclusion Our results highlighted several candidate miRNAs targeting Hh-Gli signalling pathway genes, which, when additionally verified, could be potentially used for better therapeutic and early prevention approaches for HGSOC.

Abstracts

PO-156 TNFα-INDUCED CELL FUSION BETWEEN MDA-MB435-PFDR.1 AND M13SV1-CRE CELLS IS SUPPRESSED BY MINOCYCLINE THROUGH INHIBITION OF THE NF-KB PATHWAY

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Introduction Cell fusion is involved in a wide range of physiological processes like fertilisation or wound healing. But also diseases could arise by cell fusion, such as cancer. In a cancer context, cell fusion generates hybrid cells, which often show a more malignant phenotype as their parental cells. Here, we have recently demonstrated that the fusion of M13SV1-Cre cells and MDA-MB435-pFDR.1 cells is induced by TNFα and that the TNFα induced fusion could be blocked by minocycline.

Material and methods To quantify occurring cell fusion events, a Cre-LoxP recombination system was established, which allows the identification of hybrid cells originated from M13SV1-Cre breast epithelial cells and MDA-MB435-pFDR.1 breast cancer cells. The phosphorylation state of IKK, p38, Erk 1/2 and JNK was carried out by western blots. Transcriptional activity of NFκB was characterised by immunocytchemistry and by ChIP coupled with a downstream qPCR assay to measure the NF-κB yield of selected genes.

Results and discussions The TNFα signal transduction pathway of both cell types was investigated to clarify the TNFα and minocycline effect in detail. We looked upstream and saw that TNFα bound to TNFR1 to recruit the adaptor TRAF2, which further resulted in activation of the IKK signalosome and activation of the MAP kinases JNK, Erk1/2 and p38. It was found that minocycline downregulate the TRAF2-TNFR1 recruitment and activation of IKK in both cell types, while the MAPK mediators p38, JNK and Erk1/2 were differently affected by minocycline treatment. Next, the activation of NFκB in the nucleus was examined and it was seen that TNFα increased the NFκB level in M13SV1-Cre, which in turn was blocked by minocycline. Addition of several specific inhibitors that block NFκB and MAPK activation confirmed that the TNFα induced cell fusion depends on activation of the NFκB nexus. A ChIP assay was used to detect putative cell fusion genes, which are TNFα up and minocycline down regulated. Several targets were picked, inhibited and analysed.