P30 Transcriptomic alterations of RRM genes in clear cell renal cell carcinoma (ccRCC) as a basis for new anticancer therapy.

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Introduction & Objectives: Renal cell carcinoma (RCC) represents 2-3% of all malignancies, while clear cell renal cell carcinoma (ccRCC) corresponds to about 80% of all RCC cases. Around 30% of ccRCC patients in time of diagnosis already developed metastasis what correlates with poor prognosis. Targeted therapy, including immunotherapy, exists to treat metastatic ccRCC, however this tumor is genetically diversified. Therefore, it is critical to develop a wide diagnostic, prognostic, and predictive biomarkers, that may help to guide the precise ccRCC treatment.

Materials & Methods: The data obtained from Gene Omnibus Database from ccRCC and healthy kidney microarray were reanalyzed using Genespring software. Results were correlated with stage, Fuhrman grade, and other available clinical and molecular data. ccRCC cell lines were treated with 70 and 250 nM mitomycin C. qRT-PCR for cell cycle, DNA repair and epithelial to mesenchymal transition marker genes was performed. Cell growing test as well as clonogenic assay were executed.

Results: The data analysis of GSE6344 and GSE36895 datasets revealed the 1579 specifically miss regulated genes in ccRCC stage I and 447 in stage II. 1680 differentially expressed genes were common for both stages. The subsequent Gene Ontology (GO) classification of genes upregulated specifically in stage II ccRCC resulted in the significant enrichment of the DNA replication related class. Among 226 genes belonging to this class the RRM1 and RRM2 genes were found. Next, we demonstrated that RRMs transcription level depends on PBRM1 mutation. The mitomycin C treatment of ccRCC cell lines caused significant reduction in cancer cell growth and inhibition of their clonogenic potential. Moreover, the overexpression of RRM genes correlated with poor prognosis for patients. The qRT-PCR analysis of glucose metabolic genes indicated the strong downregulation of all examined genes after mitomycin C treatment in both A498 and 786-O primary tumor cell lines although in metastatic cell line CAKI-1 these genes were upregulated. The strong decrease in transcription of BRCA1 and CTCF (DNA repair genes) in all examined lines was observed.

Conclusions: Our study showed that the biggest global transcriptomic changes appear at the very early phase of the ccRCC development. In ccRCC stage II replication related genes (RRM1 and RMM2) were strongly overexpressed what in consequences increased the proliferation potential of tumor cells. Consistently, all tested by us ccRCC lines exhibited dramatic sensitivity to treatment with low concentration of mitomycin C, a commonly used anticancer drug. Although, the mitomycin C differentially affected gene expression in primary and metastatic ccRCC cell lines. This strongly suggest different molecular mechanism of cancer cells growth inhibition.

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