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Novel DNA methylation biomarkers of clear cell renal carcinoma

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Introduction & Objectives: Annually, renal cell carcinoma (RCC) affects nearly 300,000 people worldwide and is responsible for nearly 100,000 deaths. Clear cell RCC (ccRCC), the most common form of sporadic RCC, often presents with synchronous metastatic disease that correlates with poor prognosis. Lithuania is the 2nd in Europe according to RCC incidence and has the highest RCC mortality rate in the world. Despite of high mortality, neither genetic nor epigenetic studies have ever been performed on Lithuanian RCC samples so far. In this study, we investigated the genome-wide DNA methylation profile of ccRCC for identification of novel DNA methylation biomarkers for early tumor detection and prognosis of clinical course of the disease.

Materials & Methods: Genome-wide DNA methylation profiling was performed using two-color Human DNA Methylation 1 × 244K Microarrays. Samples (N=22) from indolent, progressive and metastatic ccRCC cases as well as paired non-cancerous tissue samples were analyzed. Selected genes were validated in a larger cohort (126 cancerous and 32 non-cancerous samples) by methylation-sensitive PCR (MSP). Statistical analysis was performed using GeneSpring GX v14.9 and STATISTICA8 software.

Results: The comparison of cancerous and non-cancerous renal tissue samples revealed significant methylation differences (fold-change ≥ 1.5; P ≤ 0.050) in >1000 of genes even at the initial non-aggressive stage of ccRCC. Biological pathway analysis showed significant enrichment of differentially methylated genes in the cell cycle, apoptosis, epithelial mesenchymal transition and other pathways. Eight protein-coding cancer-associated genes were selected for validation analysis by MSP. Methylation of all selected genes was cancer specific (P<0.050) with the frequency of 19-62% in tumor tissues and 0-11% in non-cancerous samples. Methylated promoter status of 6 genes was associated with the patient's gender (more frequent in males) and larger tumor size. Furthermore, aberrant *ZNF677* and *FLRT2* methylation was more frequent in tumors of higher stage (P = 0.008 and P = 0.011, respectively) having necrotic zones (P = 0.017 and P = 0.028, respectively), and invasive properties (*FLRT2* only, P = 0.003). Meanwhile, more frequent promoter methylation of *PCDH8* and *BMP7* was associated with a higher tumor differentiation grade (P = 0.009 and P = 0.006, respectively).

Conclusions: In conclusion, promoter methylation of a set of protein-coding genes, identified by genome-wide methylation profiling shows promising value as novel diagnostic and/or prognostic biomarkers of ccRCC.