P25 DNA methylation biomarkers of clear cell renal carcinoma

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Introduction & Objectives: Renal cell carcinoma (RCC) is the most lethal cancer of the urinary system and represents 2-3% of all cancers in adults. Clear cell RCC (ccRCC), the most common form of sporadic RCC, often presents with a 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. For molecular profiling of RCC and identification of biomarkers for early cancer detection and prognosis of clinical outcomes, genome-wide DNA methylation and gene expression analyses of ccRCC were performed. Cellular complexity of renal tumours was evaluated in a single cell level using droplet microfluidics.

Materials & Methods: Genome-wide DNA methylation profiling was performed using two-colour Human DNA Methylation 1 × 244K Microarrays, while SurePrint G3 Human GE 8 × 60K Microarrays was used for gene expression profiling. Samples (N=22) from indolent, progressive and metastatic ccRCC cases as well as paired non-cancerous tissue samples were analysed. Most recurrent DNA methylation changes were validated in a larger cohort (126 cancerous and 32 non-cancerous samples) by methylation-sensitive PCR (MSP). For the single cell analysis, dissociation of cancerous and non-cancerous tissue samples was performed and followed by single cells encapsulation in droplets with barcoding reagents, library preparation, and mRNA sequencing.

Results: The comparison of cancerous and non-cancerous renal tissue samples revealed significant methylation differences (fold-change ≥ 1.5; $P \leq 0.05$) 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 cellular pathways. Nine protein-coding cancer-associated genes were selected for validation analysis by MSP. DNA methylation changes of selected genes were cancer specific ($P<0.050$) with the frequency of the alterations in tumour tissues ranging from 20 to 60%. A set of biomarkers showed significant associations with a higher tumour grade, stage or the presence of necrotic zones. According to the single cell analysis the tumours were highly vascular and infiltrated by a plethora of immune cells.

Conclusions: Genome-wide ccRCC analysis identified a set of promising epigenetic biomarkers for further development of urine based diagnostic tests. However, cellular diversity of the tumours must be taken into account for proper selection of RCC-specific biomarkers.