B6

Alternative Splicing and Differential Gene Expression in Pancreatic NETs

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Background: Alternative splicing is an important mechanism to increase the diversity of the proteome in higher organisms. Protein isoforms resulting from differentially spliced mRNA may constitute peculiar drug targets or markers for cancer diagnosis. We here present a new DNA array that allows identification of splice variation and differential expression for 357 G protein-coupled receptors (GPCR) and 11 proteins of the extracellular matrix. GPCRs like somatostatin and dopamine receptors are established targets in neuroendocrine tumor disease. However, a considerable fraction of patients cannot be treated or diagnosed adequately using currently available agents. The G protein-coupled receptor superfamily is the most important hub for drug therapies. In addition to their widespread expression and their important regulatory properties, GPCRs are located on the plasma membrane which makes them easily accessible for both contrast agents and therapeutics. Aim of this work is to identify tumor specific splice variants and overexpressed genes in pancreatic NETs. After validation, such targets can be used to develop targeted therapeutics and contrast agents for nuclear medicine.

Methods: We designed an array with 15k probes which either bind to exon, junction or intron regions. Eight pancreatic NETs and eight pancreatic control tissues where hybridized. Expression results were validated by qPCR, while splice variants where amplified by RT-PCR and sequenced.

Results: In the expression level analysis overexpressed genes were found and validated. Most of the overexpressed genes were not known to be differential expressed in pancreatic NETs (e.g. GPR158) but familiar GPCRs were also found to be overexpressed (e.g. DRD2, SSTR2). By calculating the splicing index for all probes, genes with a high probability for differentially expressed splice variants were selected and validated in RT-PCR. Novel variants specific for tumor as well as for the control tissues were identified.

Conclusions: This novel DNA microarray enables parallel identification of splicing events and gene expression in complex samples, providing a tool to identify targets for therapy and diagnostic biomarkers.