Identification of targets for diagnosis and therapy in pancreatic neuroendocrine tumors

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Introduction

Alternative splicing is an important mechanism to increase diversity of the proteome in higher eukaryotic organisms. Alternative proteins and variants are of special interest in tumor biology as they may provide a selective drug target or serve as a marker for cancer diagnosis1. While conventional identification of splice variants is labor intensive and generally targeted individual genes, we present here a custom DNA microarray that allows identification of splice variation and differential expression of 357 G-protein coupled receptors 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 therapies2. 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.

Methods

We designed approximately 15k 60-mer oligonucleotide probes covering 358 genes (357 GPCRs and 11 surface proteins) and their known variants isoforms on a custom Agilent DNA microarray. These probes were either homologous to exonic, exon-exon junction or intron regions (Figure 1). By calculating the splicing index of each gene, targets with high probability of specific alternative splicing were identified3. A total number of 16 chips were made, hybridized with 8 pancreatic neuroendocrine tumor and 8 pancreatic control tissue samples. Gene expression results were validated by qRT-PCR, immunohistochemistry or Western blot, while splice variants were amplified by RT-PCR and sequenced afterwards.

Results

At the level of the complete chip, the control tissue samples are homogenous whereas the different tumor samples are more divergent. This effect is clearly visible in a correlation plot of the raw data since the control samples form a homogeneous block (Figure 3). The results of the gene expression analysis is visualized in a double volcano plot (Figure 4). The following two figures are examples for validations of the gene expression results. In figure 5 an immunohistochemistry staining against Tubulin beta 3 (TUBB3) on control pancreas and pancreatic neuroendocrine tumor cryosections is shown. Quantitative real-time PCR results for six selected genes are shown in Figure 5.

Melanocortin Receptor 1 was one of the validated targets. It could be shown that the neuropeptide cell line LCC-18 is expressing the MCR1 receptor by functional cAMP assays (Figure 6). Splice variants of four selected genes are represented in Figure 7.

Conclusions

The DNA microarray presented here enables the parallel identification of alternative splicing events and gene expression in complex biological samples, providing a powerful tool to identify novel targets for therapy and diagnostic biomarkers.

In the expression level analysis several overexpressed genes were found and validated. While most of them were not known to be differentially expressed in pancreatic NETs (e.g. GPR115, TUBB3, MC1R or GIPR) also familiar G-protein coupled receptors were found to be overexpressed (e.g. SST2R or D2R2).

By calculating the Splicing Index for all probe sets on the array, 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.

References


Acknowledgements

This project was supported by grant 03P14 from the German Ministry of research (BMBF)