

SMP: Cell

Project: Alternative Splicing of disease relevant genes

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Introduction

Protein variants as a result of alternative splicing have been shown to be associated with human diseases, such as cancer (1) and several neurodegenerative disorders (2). Therefore the correlation of splice form variation and its protein function with relevance to human diseases is of great importance.

In this project we clone and analyse the alternative splice isoforms of disease relevant genes. We will focus on genes that fulfil both of the following conditions:

First, supposing that variations of the gene might contribute to tumour formation we will preferentially concentrate on genes which are involved in cancer-relevant pathways (Fig.1).

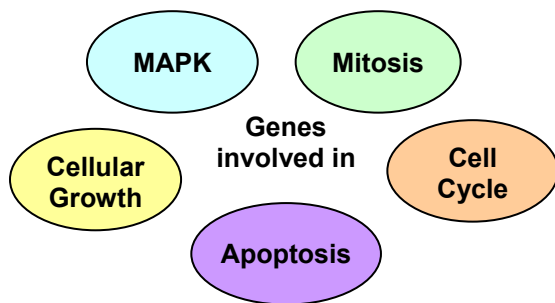


Fig 1: Focus on genes that are involved in these cancer-relevant pathways.

Second, the splice variant exists in the coding region and shows at least one exon difference (Fig.2).

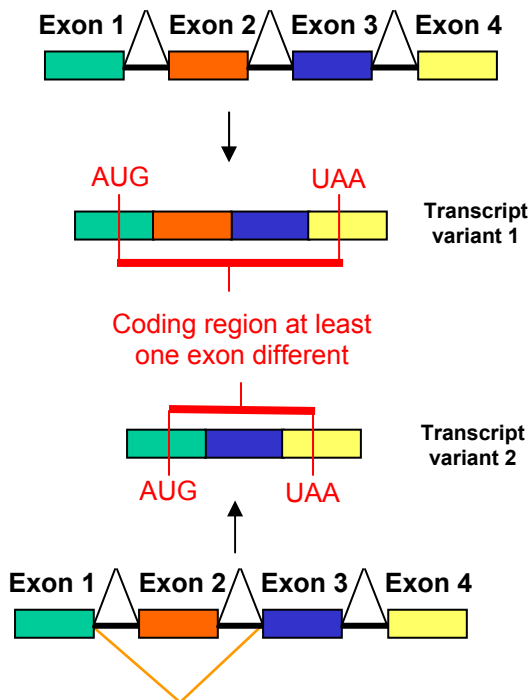


Fig 2: Transcript variants that show at least one exon difference and thus encode variant proteins.

Our aim is the amplification of the complete open reading frame (ORF) of splice variants of these genes and their cloning into a shuttle vector system (Gateway®). This allows us to express the ORFs of the splice variants N- and C-terminally fused to the yellow fluorescent protein (YFP). The YFP-tagged splice isoforms will then be analysed by the high throughput functional assay (3) within SMP-Cell.

Project Status

Search for genes with splice isoforms

We started with all Unigene clusters and selected those clusters which contained more than one validated mRNA (e.g. Refseq). This means that there exists at least two splice variants for the respective gene. Moreover, we considered only those validated mRNAs which were represented by a full length clone. From these selected genes we mapped the encoded proteins on pathways and selected specifically such that were identified to be involved in those pathways described in Fig. 1. In the next selection step we focused only on those genes of which different functions of the splice variants have not been published yet.

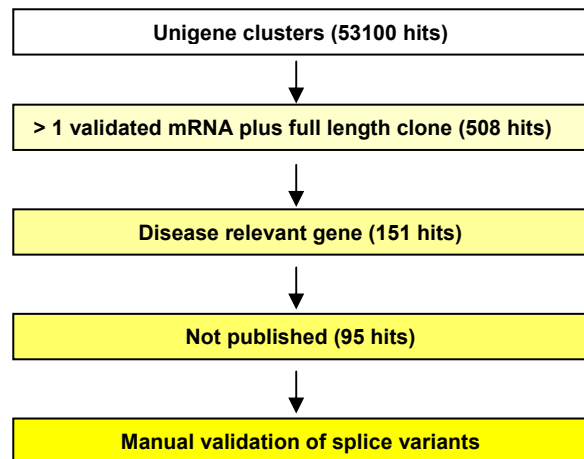


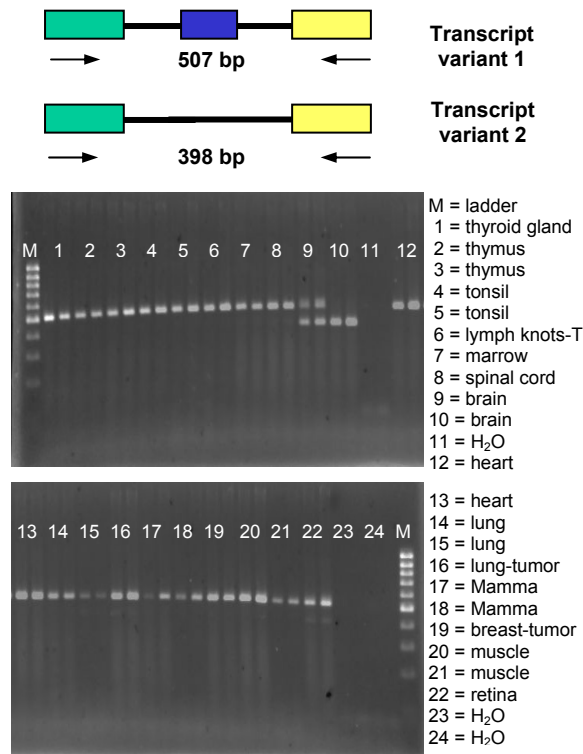
Fig 3: Selection process for disease relevant genes with splice isoforms.

Validation of splice variants

This project relies on the analytical characterisation of splice isoforms within human tissues by RT-PCR (4, 5) as shown in Fig.4.

PCR primers were generated on either side of alternative splice events. These primers were then used for RT-PCR experiments on 40 different tissue samples of our tissue resources.

Currently, we validate the splice variants amongst others with the help of the UCSC Gene Browser (<http://genome.ucsc.edu>) and following RT-PCR.



**Fig 4:** Example for the validation of splice variants in different human tissues by RT-PCR.

**Outlook**

This subproject describes for the first time the cloning of complete ORFs that carry the alternative splice isoforms and therefore provides access to the transcriptional isoforms for downstream functional analysis.

Our final goal is to identify splice variants that show different effects in functional assays. These variants will then be further characterised. One interesting question will be whether these splice variants bind to different interaction partners. This could be analysed by yeast-two-hybrid-interactions and co-immunoprecipitation. Furthermore, the expression profile of these splice variants in different tissues (also control tissue versus disease tissue) might be of central interest.

*Lit.: 1. Roy M et al. Evidence that public database records for many cancer-associated genes reflect a splice form found in tumors and lack normal splice forms. Nucleic Acids Res. 2005 Sep 7;33(16):5026-33. 2. Lee CJ and Irizarry K. Alternative splicing in the nervous system: an emerging source of diversity and regulation. Biol Psychiatry. 2003 Oct 15;54(8):771-6. 3. Arlt D et al. Functional Profiling: From microarrays via cell-based assays to novel tumor relevant modulators of the cell cycle. Cancer Res. 2005 Sep 1;65(17):7733-42 4. Gupta S et al. Strengths and weakness of EST-based prediction of tissue-specific alternative splicing. BMC Genomics. 2004 Sep 28;5(1):72 5. Gupta S. et al. Genome wide identification and classification of alternative splicing based on EST data. Bioinformatics. 2004 Nov 1;20(16):2579-85*