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mRNA expression and splice variant changes in the colorectal adenoma-carcinoma sequence

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Background

- Expressional changes in different cancer types are well known regulators of cancer development.
- Splicing variations however are less frequently studied regarding cancer formation.
- Alternative splicing may occur in cancer as a consequence of mutations at splicing regulatory sites or through changes considering splicing factors.

Aims

- Our main goal was to examine the extent of splicing events in adenoma and CRC tissue samples.
- We have also wished to determine the ratio between genes that show only alternative splicing differences only and those exhibiting alternative splicing and gene expression differences in parallel.
- In the case of genes with only alternative splicing differences, we wanted to examine the biological functions they are associated with.

Samples and methods

1. In a previous experiment, 20 normal, adenoma and CRC tissue samples were collected for expression profiling. Human Transcriptome Array 2.0 (HTA 2.0) microarrays were used to obtain expression results.
2. Raw .CEL files were analysed in the Transcriptome Analysis Console (TAC) software. Signal normalisation was performed with SST-RMA method. During the alternative splicing analysis only the exon probesets were used.
3. We considered a probeset significantly differentially expressed when the absolute log2 fold change (or Splice Index for exon probesets) was higher than or equal to 2 and the FDR corrected p-value was less then 0.05.
4. An alternative splicing event was defined in case at least one significantly differentially expressed exon was found in a given Transcript Cluster. Further analysis was done in R and IPA softwares.

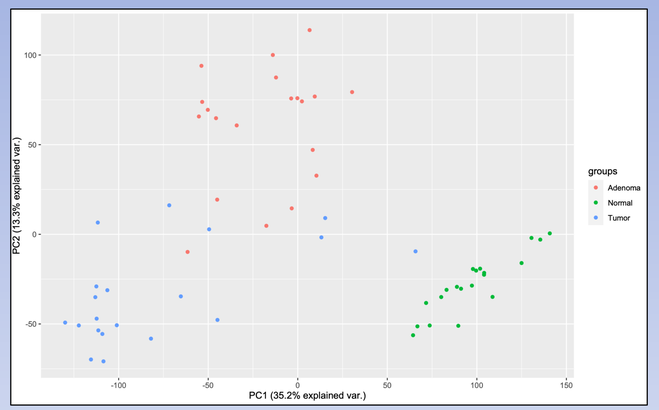
Conclusions

1. Large amounts of alternative splicing events can be found between normal and tumor or adenoma samples, indicating their significance in tumor development.
2. A large proportion of these events are not accompanied with differential expression, indicating their unique role in tumorigenesis and tumor progression.
3. Their significance is further emphasized by the fact that the genes that we found alternatively spliced but not differentially expressed were associated with tumor related biological functions.

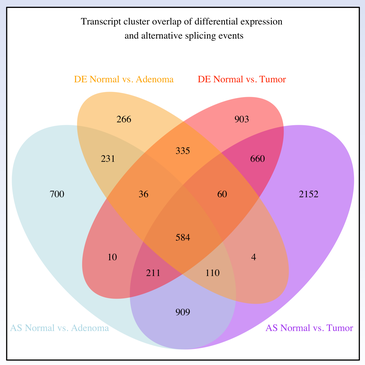
Results

1. 1088 Transcript Clusters showed higher expression in the adenoma group compared to the normal samples while 538 was significantly downregulated in this comparison. Considering the tumor samples compared to the normals we found 1549 upregulated and 1250 downregulated Transcript Cluesters.

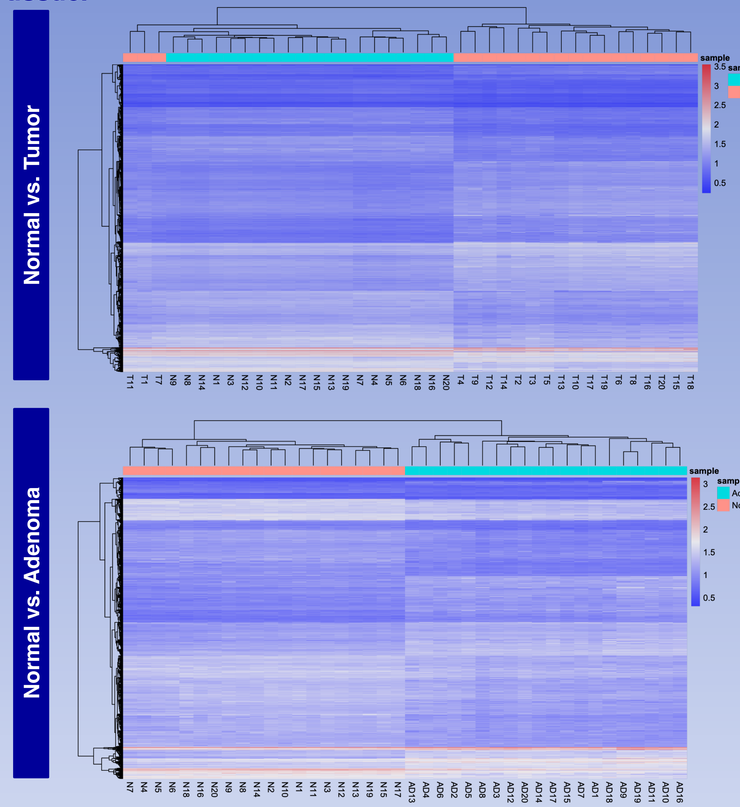
2. Regarding alternative splicing, we found 2791 Transcript Clusters exhibiting alternative splicing events at the adenoma and normal comparison and 4690 splicing events in the tumor samples compared to the normal ones. The principal component analysis of the significantly differentially expressed exon probesets showed clear separation of the normal samples and partially merged tumor and adenoma samples.



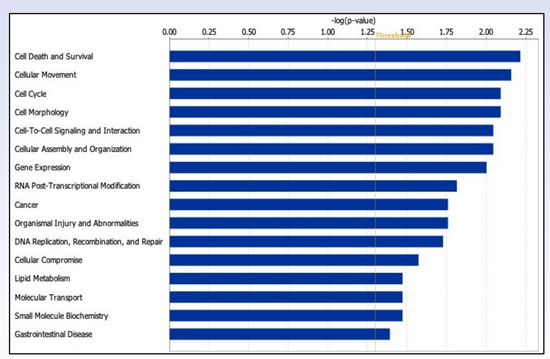
3. 961 and 1515 Transcript Cluster was differentially expressed as well, besides being alternatively spliced in the adenoma and tumor samples compared to normal, respectively.



4. Results of hierarchical clustering based on differentially expressed exon probesets clustered together the adenoma and tumor samples compared to healthy tissue.



5. Biological functions and disease terms that were significantly enriched among the genes that had only alternative splicing events at the tumor and normal comparison.



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