The Neuroblastoma Genome and Epigenome - Patient Stratification and Identification of Candidate Genes

AKADEMISK AVHANDLING

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av

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Avhandlingen baseras på följande arbeten:

I. **Carén H**, Ejeskär K, Fransson S, Sjöberg R-M, Krona C, Hesson L, Latif F, Martinsson T. A cluster of genes located in 1p36 are down-regulated in neuroblastomas with poor prognosis, but not due to CpG island methylation. *Mol Cancer. 2005 Mar 1;4(1):10.*

II. **Carén H**, Fransson S, Ejeskär K, Kogner P, Martinsson T. Genetic and epigenetic changes in the common 1p36 deletion in neuroblastoma tumours. *Br J Cancer. 2007 Nov 19;97(10):1416-24. Epub 2007 Oct 16.*

III. **Carén H**, Djos A, Nethander M, Sjöberg R-M, Enström C, Nilsson S, Martinsson T. Identification of epigenetically regulated genes that predict patient outcome in neuroblastoma. *2009, submitted*

IV. **Carén H**, Erichsen J, Enerbäck C, Olsson L, Sjöberg R-M, Abrahamsson J, Kogner P, Martinsson T. High-resolution array copy number analyses for detection of deletion, gain, amplification and copy-neutral LOH in primary neuroblastoma tumors; Four cases of homozygous deletions of the *CDKN2A* gene. *BMC Genomics. 2008 Jul 29;9(1):353.*

V. **Carén H,** Kryh H, Nethander M, Sjöberg R-M, Nilsson S, Abrahamsson J, Kogner P, Martinsson T. High-risk neuroblastoma without *MYCN* amplification; Characterization of the 11q deletion tumors reveal a poor prognostic chromosome instability phenotype with later onset. *2009, submitted*

UNIVERSITY OF GOTHENBURG

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Neuroblastoma (NB) is a tumor of the sympathetic nervous system, and the most common extracranial tumor of childhood. The prognosis for high-stage NBs is still poor, with survival rates of about 35%. Side-effects of treatment in these young children can also be severe. It is therefore important to develop better tools for improved patient stratification as well as to identify new targets for therapy.

Aims: Using genetic and epigenetic approaches, this thesis aimed to analyze candidate genes with potential involvment in the initation/progression of NB and to identify genes that can be used for improved patient stratification.

Results: The six candidate genes located in chromosome region 1p36.22 were down-regulated in tumors from patients with an unfavorable outcome compared with a favorable. DNA methylation was shown not to be involved in the down-regulation of gene transcripts.

In a more comprehensive analysis of 1p36, four genes, *ERRFI1*, *PIK3CD*, *RBP7* and *CASZ1*, were up-regulated by epigenetic treatment. Bisulfite sequencing revealed that DNA methylation most likely was not involved, suggesting for the potential involvement of other epigenetic mechanisms such as histone deacetylation. Missense mutations were identified in *PIK3CD* and *ERRFI1* and the down-regulated mRNA expression of *PIK3CD* and *CASZ1* was detected in high-stage NB. *CASZ1* plays a role in neural development and is therefore an interesting candidate for further study.

In a genome-wide analysis of DNA methylation, a group of methylated genes for which we showed gene expression was affected by epigenetic treatment was selected for further analysis. A selected group, e.g. *SCNN1A, PRKCDBP and KRT19* could be used to distinguish between patients with an unfavorable outcome from those with a favorable one.

Whole-genome copy number analysis of NB tumors identified homozygous deletions in the *CDKN2A* and *RBMS3* genes. Moreover, copy neutral loss of heterozygosity was rare, but could be detected in three chromosomal regions. Tumors with *MYCN* amplification and those with 11q deletion displayed very different genomic profiles. The 11q-deletion group had significantly more chromosomal breaks than the other group, indicative of an 11q localized chromosomal instability gene (CIN). This group also had a significantly higher age at diagnosis. The groups defined by 11q deletion, *MYCN* amplification and 17q gain were the only groups associated with poor patient outcome.

Conclusions: Whole-genome profiles add valuable information about genomic aberrations, which are important prognostic factors in NB. Aberrant DNA methylation can be a very early event in tumor development as well as in tumor progression. It is therefore of great importance to learn more about both the genetic and epigenetic profiles of NB. This thesis has added to the current knowledge in these regards and has also identified important genetic aberrations, as well as aberrantly methylated genes. In the future, these aberrations could possibly be used in patient stratification, as biomarkers or as targets for therapy.

Keywords: tumor, embryonal, neural crest, neuroblastoma, tumor suppressor gene, DNA methylation, epigenetics, bisulfite sequencing, microarray, 1p36, 11q, MYCN, CASZ1, PIK3CD, PRKCDBP, SCNN1A, TGFBI, DHRS3, KRT19, DUSP23, APITD1, H2AFX

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