Molecular Characterization of Neuroblastoma Tumors

A Basis for Personalized Medicine

Akademisk avhandling

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av

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The thesis is based on the following papers:

I. Carén H, **Kryh H**, Nethander M, Sjöberg RM, Träger C, Nilsson S, Abrahamsson J, Kogner P, Martinsson T. High-risk neuroblastoma tumors with 11q-deletion display a poor prognostic, chromosome instability phenotype with later onset. Proc Natl Acad Sci U S A. 2010 Mar 2;107(9):4323-8.

II. **Kryh H**, Abrahamsson J, Jegerås E, Sjöberg RM, Devenney I, Kogner P, Martinsson T. MYCN amplicon junctions as tumor-specific targets for minimal residual disease detection in neuroblastoma. Int J Oncol. 2011 Nov;39(5):1063-71.

III. **Kryh H**, Carén H, Erichsen J, Sjöberg RM, Abrahamsson J, Kogner P, Martinsson T. Comprehensive SNP array study of frequently used neuroblastoma cell lines; copy neutral loss of heterozygosity is common in the cell lines but uncommon in primary tumors. BMC Genomics. 2011 Sep 7;12:443.

IV. **Kryh H**, Hedborg F, Øra I, Ambros P.F., Gartlgruber M, Kogner P, Martinsson T. Amplification of two regions on chromosome arm 12q defines a clinically distinct subgroup of high risk neuroblastoma patients. 2012 Manuscript

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Abstract:

Neuroblastoma is a very heterogeneous tumor, ranging from spontaneous regression to aggressive tumor growth. A proper stratification of the patients into different risk groups is therefore important in order to provide the most suitable treatment for each patient. The primary aim of this thesis was therefore to further characterize the genes and mechanisms important for neuroblastoma development using genome-wide copy number data from single nucleotide polymorphism (SNP)-arrays as a starting point for more detailed studies of interesting regions of the genome. Furthermore, we wanted to investigate the clinical usefulness of SNP-arrays, both directly as a prognostic tool, and indirectly as a starting point for the generation of patient specific assays.

As to the genes and mechanisms important for neuroblastoma development, we have identified a chromosomal instability phenotype in the 11q deleted subgroup, possibly caused by the DNA-repair gene *H2AFX* located in the commonly deleted region. Furthermore, we have identified and characterized a small subgroup of neuroblastoma with amplification of two regions on 12q, occasionally accompanied by 11q amplification. Gene expression analysis and siRNA knockdown of the genes included in these amplicons indicate that *CDK4* and *CCND1* are possible drivers of this subgroup and we therefore suggest that this group of neuroblastoma is characterized by a cell cycle de-regulation phenotype.

Regarding the clinical usefulness, our results show that SNP-arrays are powerful tools for the stratification of neuroblastoma patients into different treatment groups. Not only is it possible to detect known prognostic markers such as *MYCN* amplification and 11q deletion, but the genome-wide copy number profile in itself is also important, especially for the identification of patients with a favorable prognosis. Moreover, we show that the array-data can be used for detailed mapping of the rearrangement boundaries, which in combination with a multiplex PCR reaction makes it possible to detect tumor specific fragments that span the junction of the rearranged DNA. These junction PCR assays were also tested for the detection of minimal residual disease, and were found to be sensitive enough to detect very small amounts of tumor DNA in the blood or bonemarrow from patients during treatment or follow-up.

To conclude, genome-wide techniques, such as SNP-arrays are useful not only for research purposes but also as a clinical tool. These arrays give valuable information for the risk-group stratification of neuroblastoma patients, and provide a robust foundation for the development of a personalized treatment strategy for patients with neuroblastoma.

Keywords: Neuroblastoma, Cancer, Tumor, SNP-array, Microarray, DNA Copy number

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