Role of nm23 in neuroblastoma. From genetic aberrations to pathways abnormalities
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Summary and scope of this study

The aim of this study was to identify candidate neuroblastoma genes and define their functional role in neuroblastoma behavior. Neuroblastoma displays a broad range of clinical behavior as well as a wide variety of genomic abnormalities. Cytogenetic and molecular analysis of neuroblastoma has lead to the identification of regions with consistent chromosomal aberrations, e.g. loss of 1p, gain of 17q and amplification of MYCN.

We searched for small deletions on chromosome 1p to identify candidate tumor suppressor genes. Tumor series were analyzed by molecular tools, supplemented with cytogenetic techniques such as FISH and flow cytometry. We found no evidence for interstitial deletions in our panel (chapter 2).

Chromosome 17q gain occurs in 70% of all neuroblastomas. The region of gain involves a large region of ~50 Mb encompassing ~400 genes. As almost all MYCN amplified tumors also have gain of 17q we searched for MYCN regulated 17q genes. SAGE libraries of a MYCN transfected neuroblastoma cell line and a mock transfected control cell were analyzed. Both the nm23-H1 and H2 genes on chromosome 17q were found to be 10-fold up-regulated by MYCN. In a time course experiment we found early up-regulation of both genes by MYCN, suggesting that nm23-H1 and H2 are direct targets (chapter 3). Therefore, MYCN overexpression and 17q gain synergistically contribute to a strong overexpression of the nm23-H1 and -H2 genes.

To analyze whether up-regulation of nm23-H1 and H2 by MYCN also occurs in vivo we measured the protein expression on a tissue micro array in 113 neuroblastomas. We found frequent co-expression of nm23-H1 and H2 proteins. There was a strong correlation between expression of nm23 protein and MYCN expression. This suggests that MYCN also in vivo regulates nm23-H1 and H2. We found nm23-H1 and H2 expression in 26 out of the 29 (90%) evaluable cases with
chromosome 17q gain, suggesting that 17q gain contributes to the up-regulation of nm23-H1 and H2 as well. We also evaluated the prognostic value of the expression of nm23-H1 and H2 in this series of neuroblastomas. Expression of nm23-H1 and MYCN both predicted a poor prognosis (chapter 5).

To understand the role of nm23-H1 we speculated that the gene might modulate a function of MYCN. MYCN can induce proliferation but can also sensitize cells to drug induced apoptosis. We silenced the expression of nm23-H1 or H2 in neuroblastoma cell lines by siRNA and treated the cells with doxorubicin. We observed significantly more apoptosis in the nm23-H2 silenced cells, suggesting that nm23-H2 blocks apoptosis. We found that this nm23-H2 effect also functions in MYCN single copy cell lines. In addition, we demonstrated that nm23-H2, but not nm23-H1 blocks drug induced apoptosis through the mitochondrial mediated apoptosis pathway (chapter 4).