MYCN Amplification in Neuroblastoma: a Paradigm for the Clinical Use of an Oncogene*

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Increase of the dosage of cellular oncogenes by DNA amplification is a frequent genetic alteration of cancer cells. The presence of amplified cellular oncogenes is usually signalled by conspicuous chromosomal abnormalities, "double minutes" (DMs) or "homogeneously staining chromosomal regions" (HSRs). Some human cancers carry a specific amplified oncogene at high incidence. In neuroblastomas the amplification of MYCN has been found associated with aggressively growing cancers and is an indicator for poor prognosis. MYCN amplification is of predictive value for identifying neuroblastoma patients that require specific therapeutic regimens and for identifying patients that will not benefit from chemotherapy. (Pathology Oncology Research Vol 3, No 1, 3-7, 1997.)

Introduction

First observations indicating that cellular genes may play a central role in the development of cancer date back to the end of the last century. In particular the fact that certain families are more prone to cancers than others has led to the idea that hereditary factors play an important role in cancer development. Today, there is no doubt that genetic alterations play a key role in the development of cancers, and we are beginning to recognize that this is true for "sporadic" cancer as well as those thought to be "induced" by viruses or resulting from exposure to physical or chemical carcinogens.

There are two genomic levels at which genetic alterations can occur: one, in a somatic cell, and second in a germ cell. The genetic alterations in a somatic cell results in a sporadic cancer confined to the individual affected. This type of mutation is the most common one.

In case a genetic alteration occurs in a germ cell, the individual remains healthy, but all cells of the offspring carry the alteration. This type of mutation may result in a predisposition for a particular type of cancer. As the principle, one single genetic alteration usually does not lead to the development of cancer. The development of cancer is regarded as a multi-stage process, in which combination of various genetic events eventually converts a normal cell to become a cancer cell. One focus of present cancer research is the search for genetic defects in cancer cells.

A multi-disciplinary approach is increasingly gaining importance. Cytogenetics, genetics, molecular genetics, virology, and other research areas have all made specific contributions to define cancer as a genetic disease. Of particular interest is tumor cytogenetics, which has developed from a merely descriptive discipline to a field of science that has – together with molecular-genetic and virological approaches – contributed significantly to the understanding of the genetic principles of cancer development.

The combination of classical and molecular-cytogenetic approaches is not only likely to provide insight into the genetic mechanisms of cancer development; it may also lay the basis for the development of modern methods of early diagnosis and possibly for new therapeutic strategies.
1. Oncogenes – Tumor suppressor genes

Genetic alterations related to tumorigenesis may involve two groups of genes. One, oncogenes; and two, tumor suppressor genes. Oncogenes result from activation cellular growth control genes. They are specifically altered by mutation or translocation, or their expression is enhanced by insertion of a heterologous promoter or by amplification (Fig 1). In contrast, tumor suppressor genes act through functional inactivation, either by mutation or by deletion. Their presence suppresses, their functional absence allows tumor growth. Alterations of oncogenes and tumor suppressor genes often contribute in combination to tumorigenesis.

2. Neuroblastoma – a childhood nervous cell cancer

Pediatric cancers have been particularly productive settings that have provided the basis for developing general concepts about the role of genetic alterations in tumorigenesis. In neuroblastoma cells, chromosome examinations have shown that two genetic alterations are particularly common. The first is an increase in the number of copies of the MYCN oncogene. The second is the alteration, often deletion, of a defined region on chromosome 1, indicating the role of an as yet unidentified tumor suppressor gene.23,24

2.1 Amplification of the MYCN gene

Amplification represents a major pathway by which the oncogenic potential of cellular genes can be activated.12,23 The consequence is an abnormally high level of the protein encoded by the amplified gene, which appears to impart a selective advantage on the host cell as a prelude to tumorigenesis. Many types of tumors carry amplification of cellular genes only sporadically. In contrast, some cancers are characterized by recurrent amplification of the same gene, the prototype being human neuroblastoma, where MYCN has been found frequently amplified in both tumors and cell lines.2,39 MYCN amplification is correlated with aggressive tumor growth39 and is an independent predictor for clinical outcome of the patient.2,22 Also, determination of the MYCN status can serve to identify patients for whom chemotherapy is not beneficial.

Amplified copies of MYCN are localized either in “double minutes” (DMs), which represent extrachromosomal elements, or in “homogeneously staining regions” integrated within derivative chromosomes (HSRs).4 HSRs have not been observed at the normal position of MYCN at 2p23-24,21 but the single copy MYCN is retained at its original site during amplification.4 Amplified MYCN is often regularly arranged assuming either a repetitive head-to-tail tandem.4 Recently it could be shown that in neuroblastoma cells lacking amplification the MYCN gene is often duplicated at 2p24.10

MYCN has been the only oncogene reported to date to be amplified in neuroblastoma cells. We have recently observed that an HSR on chromosome 12 in neuroblas-
oma cell line LS contains amplified chromosome 12 DNA, suggesting that chromosome 12 DNA was coamplified with MYCN.\textsuperscript{9,11} By using chromosomal fluorescence in situ hybridization we could show that the same chromosome 12 DNA is amplified in two additional cell lines, one carrying an HSR on 12q13-14, the other DMs. The amplified DNA encompasses the gene MDM2, which maps to 12q13-14. Amplification of MDM2 and MYCN are the result of independent genetic events in a clinically as yet undefined subgroup of neuroblastomas. Our results raise the possibility that MDM2 and MYCN may cooperate occasionally in neuroblastoma development.\textsuperscript{9}

### 2.2 Protein function

Amplification results in an increased synthesis of the protein encoded by MYCN. The MYCN protein is a phosphorylated protein located in the nucleus. Recent studies have shown that the MYCN protein possesses properties of a transcription factor.\textsuperscript{12} An interesting observation is the fact that it is able to specifically associate in vivo with another protein, Max, that we were able to identify recently by immunoprecipitation.\textsuperscript{10} This observation opens up new possibilities of analyzing the biochemical function of a nuclear oncoprotein. Of interest is also the neopreferredential expression of MYCN,\textsuperscript{9} although the molecular basis of expression has remained largely unknown.\textsuperscript{3,15,16}

### 2.3 Clinical significance of MYCN amplification

Important prognostic parameters for the neuroblastoma are the clinical stage and the age of the patient at the time of diagnosis. Patients with neuroblastomas of stages I and II mostly have favorable prognosis; 75 to 90 percent can expect to survive for at least two years. Patients with stage III and IV tumors have a poor prognosis. Significantly different prognosis for patient groups with tumors of stage III have been observed in the USA and in Germany. In the USA, the prognosis for stage III tumors is similar to that for stage IV tumors (10 to 30 percent), whereas a considerably more favorable prognosis is observed in Germany. The reason for this disagreement is not yet known.

Studies carried out independently by various groups have shown a significant correlation between amplification of MYCN and stages III and IV. This correlation was first shown within the framework of a study on 63 neuroblastomas; amplification was not detected in 15 stage I and stage II tumors, but was observed in 24 of 48 (50 percent) stage III and stage IV tumors.\textsuperscript{9} Subsequent studies by other authors have confirmed this correlation, though the incidence of amplification was somewhat smaller (between 20 and 30 percent).

A significant correlation between poor prognosis and MYCN amplification was also observed when comparing patients over 1 year of age with patients under one year. Prognosis for patients over one year of age, especially for those with stage III and stage IV tumors, is particularly unfavourable. One study showed MYCN amplification for more than 50 percent of the patients older than 1 year; for younger patients, the incidence of amplification was smaller. MYCN amplification thus seems to be an independent parameter for the evaluation of the prognosis of neuroblastoma.

Current therapy protocols for treatment of neuroblastoma depend on the prognosis for survival which is evaluated on the basis of the tumor stage, the degree of surgical resectability, and on an analysis of genetic alterations. The pilot study of the German Neuroblastoma Study Group recommends treatment according to protocols specific for each of four risk groups (Table 1). Risk group A includes patients with localized tumors of which at least 90 percent can be removed surgically (prognosis: 90 to 100 percent survival). Risk group B includes patients with localized tumors that extend beyond the organ of origin and which usually cannot be removed completely (prognosis: 65 to 80 percent). Risk group C includes patients with metastasizing tumors or localized tumors that do not regress after four cycles of chemotherapy

### Table 1. Risk groups in neuroblastoma and MYCN amplification (Criteria according to the pilot protocol of the German Neuroblastoma Study Group NB90P as of August 1, 1991 (head of study group: F. Berthold, Cologne). Patients with MYCN amplification were always classified into risk group C, independent of other parameters.

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Tumor</th>
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<tbody>
<tr>
<td>% of survival A (90-100%)</td>
<td>Tumor localized, almost completely resectable</td>
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<tr>
<td>Stage I: microscopic residual tumor acceptable</td>
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<tr>
<td>Stage II: small macroscopic residual tumor acceptable (&lt;10 percent); excluded: patients with amplification</td>
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</tr>
<tr>
<td>B (65-80%)</td>
<td>Tumor localized; incomplete resectability</td>
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<tr>
<td>Stage II: macroscopic residual tumor (&lt;10 percent);</td>
<td></td>
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<td>Stage IIIB: ipsilateral lymph node positive</td>
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<tr>
<td>Stage III: infiltration beyond median; excluded: patients with amplification</td>
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</tr>
<tr>
<td>C (20-30%)</td>
<td>Tumor metastatic</td>
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<tr>
<td>Risk groups A and B, if amplification is positive</td>
<td></td>
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<tr>
<td>D (75-80%)</td>
<td>Stage IV</td>
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(prognosis: 20 to 30 percent). Risk group D includes only patients with stage IV tumors that frequently show spontaneous regression (prognosis: 75 to 80 percent survival).

Patients of risk group C receive the most intensive therapeutic treatment. Amplification of MYCN is incompatible with being included in risk groups with favorable prognosis, even if the tumor is localized. All patients with amplification are included in risk group C and are submitted to the most intensive therapy. However, this only applies to tumors of stages I-III. In the case of stage IV tumors, no differences were observed with regard to amplification when comparing the patients’ prognoses. Thus, the amplification of MYCN is an independent parameter which allows us to classify a patient into a particular risk group and thus to decide on a specific treatment protocol.

3. Non-syntenic amplification of MYCN and MDM2 genes

Until recently it was thought that MYCN is the only oncogene amplified in human neuroblastoma cells. Through cytogenetic studies we have identified neuroblastoma cell lines that, in addition to amplified MYCN, carry “double minutes” (DMs) or “homogeneously staining chromosomal regions” (HSRs), not harbouring MYCN. In situ hybridization of biotinylated total genomic DNA to normal human lymphocytes (reverse genomic hybridization) revealed the amplified DNA to be derived from chromosome 12 band q13-14. Subsequent filter analyses showed 30- to 40-fold amplification of the MDM2 gene, located at 12q13-14, in both in 3 cell lines and in an original tumor, in addition to amplified MYCN.8 Abundant MDM2 protein was present, as the apparent consequence of amplification. Our result establish independent high level amplification of the non-syntenic oncogenes MYCN and MDM2 as a recurrent genetic alteration in human neuroblastoma cells and raise the possibility that the MDM2 protein cooperates with MYCN in a clinically as yet undefined subgroup of neuroblastomas.

4. Chromosome 1 alterations

When evaluating the significance of amplification in tumorigenesis, one has to take into account that tumor cells frequently have additional genetic alterations. In the case of neuroblastomas, the deletion of genetic material from the short arm of chromosome 1 seems to be of particular importance.25 Chromosome analyses have provided evidence for 1p deletion, which was recently specified to include bands 1p36.1-2 (Fig 5). By using polymorphic molecular probes, 1p deletion could be detected in almost all of the neuroblastomas examined, even in cases in which a deletion was too small to be detected by chromosome analysis.12,25 However, there does not seem to exist a direct correspondence between 1p deletion and MYCN amplification. Only two out of ten cases with deletion also showed amplification. Furthermore, MYCN is localized on another chromosome (2p23-24).21

The significance of 1p deletion still has to be clarified. It remains to be seen whether a certain gene in the region is lost by deletion. Only then will we be able to tell if there is altered or deleted genetic information in the sense of a “tumor suppressor gene”. Very recent progress has been made towards this goal by the identification of recurrent translocation breakpoints within bands 1p36.1-2.24,48 These breakpoints are well mapped on the chromosome and are expected to provide much better access to the cloning of a particular gene than the deletions, which are rather large for molecular analyses.

5. Outlook

Currently, molecular cancer research is pursued under three perspectives. First, it appears important to further identify the spectrum of genetic alterations in different tumor types and to clarify if non-random genetic alterations occur in specific tumor types. Second, it needs to be tested, if non-random alterations might be used as parameters in presymptomatic early detection of cancer diseases and whether they might be of help in deciding which of the proven therapies should be applied. And third, the biological function of genetic alterations in cancer cells has to be identified in order to lay the basis for causal therapeutic strategies.

References


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