Chapter 19

Biology of Tumors of the Peripheral Nervous System

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INTRODUCTION

Tumors of the peripheral nervous system include neuroblastomas, pheochromocytomas, and neuroepitheliomas. Neuroblastomas and pheochromocytomas are adrenergic in origin and share certain genetic features, whereas neuroepitheliomas are thought to be cholinergic and are characterized by distinct genetic features. Neuroblastomas are characterized by deletion of the short arm of chromosome 1 (1p), amplification of the N-*myc* proto-oncogene, and hyperdiploidy in subsets of tumors. All three of these genetic features have prognostic value in subsets of patients. Allelic loss of 14q also occurs with increased frequency, but the prognostic importance of this abnormality is not known yet. Pheochromocytomas have not been studied as extensively, but allelic loss for 1p appears to be a frequent change, and no clear examples of oncogene activation have been identified. Neuroepitheliomas are characterized by translocation between chromosomes 11 and 22. Although they have a characteristic pattern of proto-oncogene expression, it is not clear that any of these oncogenes are activated specifically, and no sites of allelic loss have been identified to date. Thus, cytogenetic and molecular analysis of neuroblastomas, pheochromocytomas, and neuroepitheliomas is useful in distinguishing them from each other and from other tumors in selected cases. Furthermore, certain genetic markers help predict a tumor's clinical behavior, especially for neuroblastoma.

NEUROBLASTOMA

Considerable progress has been made in recent years in advancing our knowledge of human neuroblastoma at the cellular and molecular level (Brodeur, 1990a; Brodeur and Fong, 1989; Evans et al., 1988, 1991; Pochedly, 1990). These advances have led to the identification of specific genetic abnormalities that are characteristic of subsets of tumors. These abnormalities include amplification of the *MYCN* (N-*myc*) proto-oncogene, deletion of the short arm of chromosome 1 (1p), and hyperdiploid or near-triploid tumor cell DNA content. In addition, other abnormalities have more recently been identified whose clinical significance is not yet known. These observations have contributed to our understanding of tumor heterogeneity, malignant transformation, and progression. In this chapter, the biologic and clinical significance of these genetic changes are reviewed.

Cytogenetic Analysis and Assessment of DNA Content

Neuroblastomas are characterized cytogenetically by 1p deletion, double minute chromatin bodies (dmins), and homogeneously staining regions (HSRs) (Brodeur et al., 1981; Gilbert et al., 1984). The former is thought to be the site of a suppressor gene that is lost or inactivated in neuroblastomas, whereas the latter two abnormalities are cytogenetic manifestations of gene amplification. No other cytogenetic abnormality has emerged as yet that is characteristic of neuroblastomas. The majority of neuroblastomas have a near-diploid chromosome number or DNA content, as detected by flow cytometric analysis, whereas some are clearly hyperdiploid, near-triploid or even tetraploid.

Flow cytometric analysis of the DNA content of human neuroblastoma cells was first reported by Look and colleagues (1984) in a series comprised of infants. In this analysis, abnormally high DNA content was associated with lower stages of tumor and a response to initial chemotherapy, whereas those with a "normal" DNA content were likely to have advanced stages of tumor (especially stage 4) and a poor response to this chemotherapy. This latter group of tumors likely had subtle genetic abnormalities that were not detectable by flow cytometry, such as dmins or 1p

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deletions. Subsequent studies by Look et al. (1991) and others have confirmed the prognostic importance of hyperdiploidy by flow cytometric measurement of DNA content (Bourhis et al., 1991; Cohn et al., 1990; Taylor and Locker, 1990) or by karyotype analysis (Christiansen and Lampert, 1988; Hayashi et al., 1988; Kaneko et al., 1987), although the utility of this marker may be limited to patients less than one or two years of age (Look et al., 1991).

Loss of heterozygosity and mapping of putative suppressor genes

Deletion of 1p has emerged as the most characteristic cytogenetic abnormality in primary human neuroblastomas and tumor-derived cell lines (Brodeur et al., 1981; Brodeur and Fong, 1989; Gilbert et al., 1984). These deletions usually lead to partial 1p monosomy. The region most commonly deleted in these tumors is between 1p32 and 1pter (Brodeur et al., 1981; Brodeur and Fong, 1989). A panel of polymorphic DNA probes for 1p was used to assess chromosome deletion or somatic loss of heterozygosity (LOH). Based on our analysis of a panel of normal and tumor DNA pairs from individual patients (Fong et al., 1989), the common region of LOH is between 1p36.1 and proximal 1p36.3 (Fig. 1).

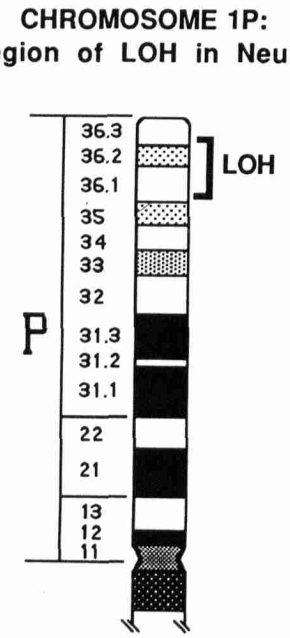

Fig. 1. Consistent region of LOH on chromosome 1p in neuroblastomas. Shown is a diagram of the short arm of chromosome 1, with the bands and subbands indicated on the left. Also shown is a bracket indicating the region that is consistently deleted in human neuroblastomas, based on molecular analysis with polymorphic probes (Fong et al., 1989; Weith et al., 1989). A neuroblastoma suppressor gene is probably encoded in this region. (Reproduced with permission from Brodeur, 1991b.)

Similar results have been obtained independently by other investigators (Weith et al., 1989). Loss or inactivation of a putative neuroblastoma suppressor gene at this site may be critical for the development or progression of neuroblastoma. Furthermore, 1p deletion or LOH may be an adverse prognostic marker (see below).

Mutation in the critical 1p36 locus on one chromosome, followed by deletion of the same region on the homologous chromosome (as manifested by LOH), could be an important mechanism in the malignant transformation or progression of some or all human neuroblastomas. Indeed, there is recent evidence that the introduction of 1p into a neuroblastoma cell line caused neural differentiation followed by cessation of cell growth and ultimately cell death (Bader et al., 1991). This provides functional evidence to support the structural evidence for a neuroblastoma suppressor gene on the short arm of chromosome 1.

There is recent evidence that LOH for the long arm of chromosome 14 also occurs with increased frequency in neuroblastomas (Suzuki et al., 1989). In this report by Suzuki, LOH for chromosome 14 was found in six of 12 informative cases, whereas LOH for chromosome 1p was found in two of nine cases. One patient had LOH for both regions. In addition, Suzuki and co-workers found LOH for chromosome 13q in two of 11 cases. Although the exact frequency and significance of these abnormalities is not yet clear, the finding of LOH on other chromosome arms besides 1p provides further evidence for genetic heterogeneity in this tumor.

Oncogene Activation

Oncogene amplification

A substantial number of neuroblastomas have either extrachromosomal dmins or chromosomally integrated HSRs (Brodeur et al., 1981; Brodeur and Fong, 1989). These two abnormalities are cytogenetic manifestations of gene amplification, but the nature of the amplified sequences was not known until recently. Schwab and colleagues (1983) identified an oncogene called N-*myc* that was amplified in all the neuroblastoma cell lines with dmins or HSRs, but not in other cell lines with these abnormalities. The amplified N-*myc* sequence was mapped to the HSRs on different chromosomes in neuroblastoma cell lines, and the normal single-copy locus was mapped to the distal short arm of chromosome 2 (Schwab et al., 1984).

We determined that N-*myc* was amplified from three- to 300-fold in a substantial number of primary neuroblastomas from untreated patients (Brodeur et al., 1984). N-*myc* amplification was highly correlated with advanced stages of disease, rapid progression, and a poor outcome (Brodeur et al., 1984; Seeger et al., 1985). These studies have been

extended to more than 1,200 patients with neuroblastoma enrolled in protocols of the Children's Cancer Study Group (CCSG) and the Pediatric Oncology Group (POG) (Brodeur, 1990a; Brodeur and Fong, 1989). Examples of N-*myc* amplification seen in some of the primary tumors are shown in Figure 2. The strong association between N-*myc* amplification and advanced stages of disease seen in our earlier studies were borne out by the larger study (Table 1). However, about 5–10% of patients with more localized disease have tumors with N-*myc* amplification. Tumors with amplification are associated with more rapid progression and a worse outcome than their nonamplified counterparts, and this was especially true for patients with lower stages of disease (Brodeur, 1990a; Brodeur and Fong, 1989). This association between N-*myc* amplification and a poor prognosis is supported by studies from Japan and Europe (Bartram and Berthold, 1987; Bourhis et al., 1991; Nakagawara et al., 1987; Tsuda et al., 1987).

TABLE 1. Correlation of N-*myc* Copy Number and Stage in 1,200 Patients with Neuroblastoma*

Stage at diagnosis	Frequency of N-*myc* amplification	
Benign ganglioneuromas	0/26	(0%)
Low stages (A, B; I, II)	10/309	(3%)
Stage D-S or IV-S	6/85	(7%)
Advanced stages (C, D: III, IV)	230/780	(31%)

*Modified from previous publications (Brodeur and Fong, 1989; Brodeur, 1990a,b).

We have sought evidence for amplification of a number of other oncogenes in a panel of neuroblastoma cell lines and primary tumors, but none was found (Brodeur and Fong, 1989). Thus, N-*myc* amplification of N-*myc* appears to be characteristic of a subset of neuroblastomas.

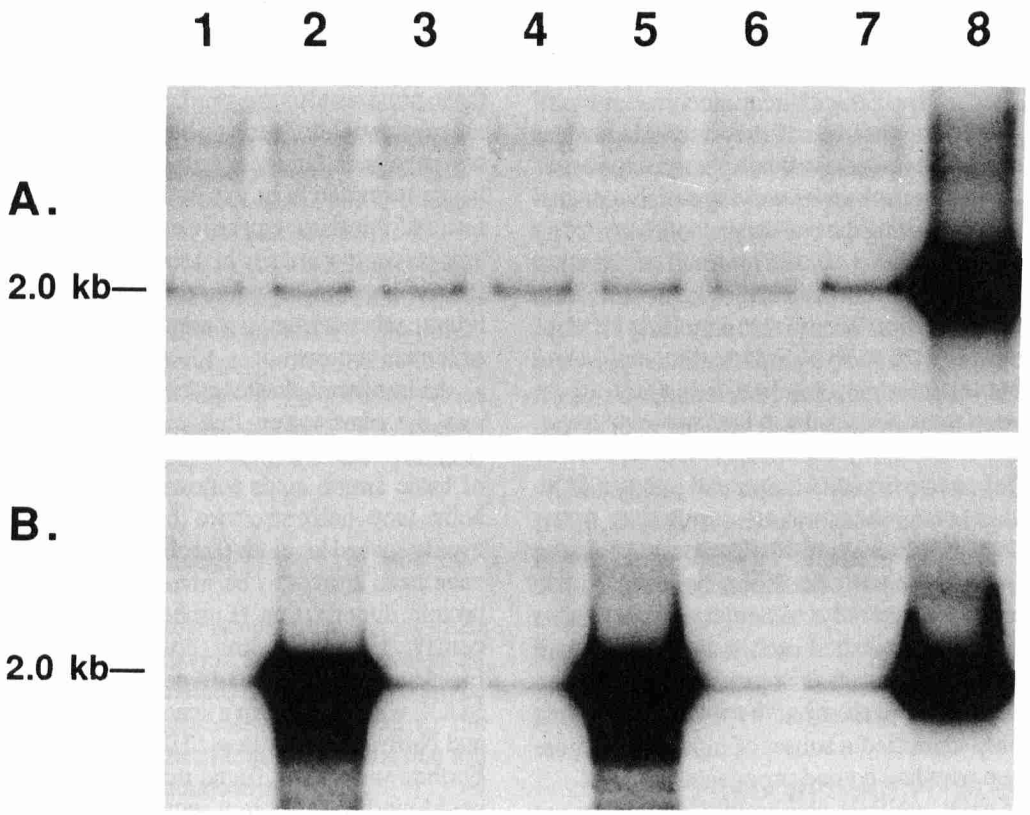

Fig. 2. Southern blots showing N-*myc* amplification. Equal amounts of DNA were digested to completion with the restriction enzyme EcoRI, electrophoresed, blotted, and hybridized to a radioactive probe for the N-*myc* oncogene. In both rows, **lane 1** represents DNA from a normal lymphoblastoid cell line as a single-copy control, and **lane 8** represents DNA from the NGP cell line, with 150 copies of N-*myc* per haploid genome, as determined by serial dilution and laser densitometry. **A: Lanes 2–7** represent six neuroblastomas with a single copy of N-*myc* per haploid genome. **B: Lanes 2 and 5** show examples of tumors with N-*myc* amplification, whereas the other tumors have the normal single-copy signal. (Reproduced with permission from Brodeur, 1990a.)

We analyzed the N-*myc* copy number in multiple simultaneous or consecutive samples of neuroblastoma tissue from 60 patients (Brodeur et al., 1987) to determine whether or not the presence or absence of N-*myc* was consistent in different tumor samples from a given patient, or if single-copy tumors ever developed amplification at the time of recurrence. Indeed, we found a consistent pattern of N-*myc* copy number (either amplified or unamplified) in different tumor samples taken from an individual patient, either simultaneously or consecutively (Brodeur et al., 1987).

These results suggest that N-*myc* amplification is an intrinsic biological property of a subset of aggressive neuroblastomas. Tumors that develop N-*myc* amplification generally do so by the time of diagnosis, and so far no cases of neuroblastoma with a single copy (per haploid genome) of N-*myc* at the time of diagnosis have developed amplification subsequently.

Our recent studies showed a very strong correlation between N-*myc* amplification and chromosome 1p LOH, indicating that 1p LOH was common in patients with amplification (Fong et al., 1989). Both N-*myc* amplification (Brodeur, 1990a; Brodeur et al., 1984; Bourhis et al., 1991; Seeger et al., 1985) and deletion of chromosome 1p (as detected by cytogenetic analysis) (Christiansen and Lampert, 1988; Hayashi et al., 1989; Kaneko et al., 1987) appear strongly correlated with a poor clinical outcome and with each other, but it is not yet clear if they are independent prognostic variables. Nevertheless, they appear to characterize a genetically distinct subset of very aggressive neuroblastomas. Our data showing the consistency of N-*myc* copy number over time (Brodeur et al., 1987) would suggest that N-*myc* amplification is an intrinsic biologic property of a subset of tumors, so it must occur relatively early in these cases. Because cases with N-*myc* amplification represent a subset of patients with chromosome 1p deletion, we suspect that the 1p deletion may precede the development of amplification.

Recent studies have compared tumor cell ploidy and N-*myc* amplification in neuroblastomas (Bourhis et al., 1991; Cohn et al., 1990; Look et al., 1991; Taylor and Locker, 1990). There was a general correlation between N-*myc* amplification and near-diploidy, although the correlation was not absolute. It appears that each is the predominant prognostic factor for a given subset of patients. The majority of infants with localized disease had hyperdiploid tumors, and hyperdiploidy identified a subset of infants with more advanced disease who had a good prognosis.

On the other hand, while N-*myc* amplification does not identify all patients who are destined to fail, it does identify a substantial number of infants, as well as older patients with nonmetastatic disease, who have a very poor prognosis. Thus, analysis of N-*myc* copy number, as well as tumor cell ploidy, provides complementary genetic information that is extremely useful in predicting response to therapy and outcome.

Oncogene expression

About 25–30% of the children with neuroblastoma have N-*myc* amplification in their tumors, and virtually all of these children have rapidly progressive and fatal disease. However, in patients with single-copy tumors, there is not yet a biological marker or explanation why half of the remaining do not survive. A general correlation has been demonstrated between N-*myc* copy number and expression (Bartram and Berthold, 1987; Grady-Leopardi et al., 1986; Nisen et al., 1988; Seeger et al., 1988; Slavc et al., 1990). Also, it has been shown that a substantial number of tumors without N-*myc* amplification overexpress this gene, but single-copy tumors with high levels of N-*myc* expression do not appear to have a particularly poor outcome (Bartram and Berthold, 1987; Grady-Leopardi et al., 1986; Nisen et al., 1988; Seeger et al., 1988; Slavc et al., 1990). It is still possible that activation of N-*myc* by mechanisms other than amplification or overexpression may play an important role. In addition, it is likely that either activation of other oncogenes, deletion of suppressor genes, or other genetic lesions may contribute to the poor outcome in these patients.

The N-*myc* oncogene was identified first in 1983 by virtue of its homology to v-*myc* and c-*myc*. The structural homology between c-*myc* and N-*myc* proteins suggests that these proteins also are similar functionally. Indeed, to date no substantial differences have been found between these two myc gene family members, except that N-*myc* is more tissue-restricted in its expression than c-*myc*. Probably as a result of this tissue-specific expression, amplification of N-*myc* is found primarily in tumors of neuroectodermal origin (neuroblastoma, retinoblastoma, and small-cell lung carcinoma), whereas c-*myc* is amplified sporadically in a variety of human tumors.

An important clue to understanding myc protein function was the observation that myc proteins shared common structural motifs with several transcription factors: a stretch of basic amino acids followed by a domain with a basic helix–loop–helix structure (bHLH motif) and a number of evenly spaced leucines (leucine zipper motif). These motifs have been shown to be involved in DNA binding and in protein dimerization (Luscher and Eisenman, 1990). Recently, Eisenman and co-workers (Blackwood and Eisenman, 1991) isolated a gene coding for a protein named MAX, which can form a specific complex with both c-*myc* and N-*myc* through these bHLH and leucine zipper motifs. Furthermore, it was found that the myc/MAX heterodimer could bind to DNA in a sequence-specific fashion. These data strongly suggest that myc proteins act by altering the expression of certain key cellular genes, the altered expression of which is responsible for the myc-induced changes in cellular phenotype.

We have demonstrated that the N-*myc* protein can form a specific complex with the product of the retinoblastoma gene, $pp105^{Rb}$ (Rustgi et al., 1991). Because the

retinoblastoma protein generally is thought to be a negative regulator of cell growth, it is possible that $pp105^{Rb}$ suppresses the activity of the N-*myc* protein through direct binding. Amplification of N-*myc* would result in a large excess of N-*myc* protein relative to $pp105^{Rb}$, leaving much of the N-*myc* protein free to disrupt cellular gene expression. We have studied the effects of N-*myc* expression on neuroblastoma phenotype by transfecting a rat neuroblastoma cell line with the N-*myc* oncogene. We found that N-*myc* elicits a number of responses in these cells that are reminiscent of advanced stage human neuroblastoma, such as increased growth rate and increased metastatic ability. We compared the pattern of cellular gene expression in the parental rat neuroblastoma cells with that of the N-*myc*-transfected derivatives and found that N-*myc* suppresses the expression of major histocompatibility comples (MHC) class I antigens (Bernards et al., 1986), neural cell adhesion molecule (NCAM) (Akeson and Bernards, 1990), and the δ isoform of protein kinase C (Bernards, 1991) in these cells. These data suggest that N-*myc* is a pleiotropic factor that can perturb a variety of cellular processes in neuroblastoma, including immune recognition, cell adhesion, and signal transduction.

We have sought evidence for amplification or overexpression of a number of other oncogenes in a panel of neuroblastoma cell lines and primary tumors, but none was found (Brodeur and Fong, 1989). Although N-*ras* was first identified as the transforming gene of a human neuroblastoma cell line, subsequent studies of primary neuroblastomas by ourselves and others (Ballas et al., 1988; Ireland, 1989; Moley et al., 1991) indicate that ras activation by mutation of codons 12, 13, 59, or 61 is rare. Recent studies of H-*ras* and c-*src* expression in neuroblastomas suggest that higher levels of expression were associated with differentiated histology and with a good prognosis (Matsunaga et al., 1991; Tanaka et al., 1988). Thus, in the patients whose tumors lack N-*myc* amplification, patterns of oncogene expression may identify a subset with a more favorable outcome.

Summary

Neuroblastomas are heterogeneous in terms of their genotype and their clinical behavior. Recent studies suggest that these two features are related, and that the genotype frequently is predictive of response to treatment or the outcome of the patient. The genetic abnormalities that are characteristic of certain neuroblastomas include: (1) LOH for the short arm of chromosome 1, including band 1p36; (2) amplification of the N-*myc* proto-oncogene; and (3) hyperdiploidy, or near-triploidy determined by flow cytometry or karyotype. Hyperdiploidy is associated with lower stages of disease and with a favorable outcome in infants. However, LOH for 1p36 and N-*myc* amplification are more common in patients over one year of age with advanced stages of disease.

TABLE 2. Clinical/Genetic Types of Neuroblastoma*

Feature	Type 1	Type 2	Type 3
Ploidy	Hyperdiploid Near-triploid	Near-diploid Near-tetraploid	Near-diploid Near-tetraploid
1p LOH	Absent	±Present	±Present
N-*myc* Copy	Normal	Normal	Amplified
Age	<12 months	Any age	Any age
Stage	1, 2, 4S	3, 4	Any stage
Outcome	Good	Intermediate	Bad

*Modified from previous publications (Brodeur, 1990a,b).

Indeed, patterns are emerging, based on cytogenetic, molecular, and flow cytometric analysis, that suggest that neuroblastomas may be assigned to three genetically distinct groups (Table 2). The first comprises those with hyperdiploid or triploid modal karyotypes (or compatible DNA content by flow cytometry). LOH of 1p and N-*myc* amplification are rarely seen. These patients are more likely to be infants with low stages of disease (stages 1, 2, or 4-S by the International Neuroblastoma Staging System) (Brodeur et al., 1988), and they generally have a very favorable prognosis. The second group consists of tumors that generally have a near-diploid or tetraploid modal chromosome number or DNA content. These tumors lack 1p LOH and N-*myc* amplification, but the patients are more likely to be over one year of age and have advanced stages of disease (stages 3 or 4). While their overall outcome is intermediate, their tumors generally respond to initial treatment, and they may survive for years before succumbing to their disease. The third group consists of tumors that are also generally near-diploid or tetraploid, with 1p LOH and/or N-*myc* amplification. The patients also are more likely to be over one year of age and have advanced stages of disease (stages 3 or 4). In contrast to the second group, they respond to treatment only transiently, if at all, before they have rapid tumor progression and die within six to 12 months. It remains to be determined if tumors in one group ever evolve or "progress" into a less unfavorable group, but current evidence would suggest that they are distinct genetically. Thus, genetic analysis of neuroblastoma cells provides prognostic information beyond that provided by age and stage alone. Consideration of the genotype of neuroblastomas can assist in making the most appropriate choice of treatment for a given patient.

PHEOCHROMOCYTOMAS

Pheochromocytoma is a rare tumor derived from chromaffin cells (Carney et al., 1976). This tumor generally occurs in the adrenal medulla, but may also arise in the organ of Zuckerkandl or in the retroperitoneal area. About 20% of pheochromocytomas occur in the pediatric age group, and about one-third have multiple primary tumors. Since this

tumor excretes urinary catecholamines, some patients have hypertension due to excessive catecholamine secretion. Since urinary catecholamine metabolites are almost always increased, the tumor must be distinguished from neuroblastoma by clinical, radiographic, and histological features (Bravo and Gifford, 1984). Although the majority of pheochromocytomas are benign, some are locally invasive and metastatic. The distinction between malignant and benign tumors is based on clinical behavior.

Pheochromocytomas can occur sporadically or as part of several genetic syndromes, such as multiple endocrine neoplasia (MEN) type 2A, MEN-2B, neurofibromatosis type 1 (NF-1), or von Hippel-Lindau (vHL). Interestingly, the predisposition locus for MEN-2A and -2B has been mapped to the pericentromeric region of chromosome 10 (Jackson et al., 1988; Mathew et al., 1987a; Simpson et al., 1987), whereas NF-1 has been mapped to chromosome 17q (Seizinger et al., 1987) and vHL has been mapped to 3p (Seizinger et al., 1988). This suggests that predisposition to develop this tumor is heterogeneous genetically. Thus, either there are several different genetic pathways to develop pheochromocytoma, or else what appears to be the same tumor histologically may actually be several different tumors genetically. Frequently it is difficult to predict the clinical behavior of these tumors based on the histologic appearance alone. A recent study showed a correlation between expression of neuropeptide Y benign clinical behavior of pheochromocytomas (Helman et al., 1989). However, this association was not confirmed in another report (Grouzmann et al., 1990). A better understanding of the genetic features of these different forms of neuroendocrine neoplasia may provide useful markers to predict clinical behavior.

Cytogenetic Analysis and Assessment of DNA Content

There is very limited information about genetic features of sporadic or hereditary pheochromocytomas. This is due in part to its infrequency, less malignant behavior, and its low mitotic rate. In one study of three pheochromocytomas in patients with vHL, one had a normal karyotype, one had trisomy 7, and a third had several clones, one of which had abnormalities of chromosome 3p (Kiechle-Schwartz et al., 1989). In another report there was partial monosomy for chromosome 22 (Szucs et al., 1989). However, no consistent karyotypic abnormalities have been identified to date, and there have not been any flow cytometric studies of the DNA content of pheochromocytomas.

Loss of heterozygosity and mapping of putative suppressor genes

In patients with hereditary retinoblastoma, all of the normal (or constitutional) cells of the body are heterozygous for the retinoblastoma locus on the long arm of chromosome 13. That is, they have one copy of a mutated gene, and one copy of the normal gene. The normal gene is expressed in a dominant fashion, so these cells do not exhibit malignant features. The retinoblastomas that develop in these patients, on the other hand, are homozygous for a mutated retinoblastoma gene. That is, they have lost the normal, wild-type gene, which allows expression of the retinoblastoma phenotype and formation of a tumor. However, LOH at the predisposing gene locus has not been found in tumors of the MEN-2A syndrome. Three recent reports noted allelic loss on chromosome 10 in only 5% of cases (4 of 84 informative tumors) (Landsvater et al., 1989; Nelkin et al., 1989; Okazaki et al., 1989). Therefore, malignant transformation in MEN-2A is probably caused by some mechanism other than homozygous loss or mutation of the predisposition locus, although homozygous submicroscopic mutations cannot be ruled out as yet.

Several studies using polymorphic probes for the short arm of chromosome 1 have noted LOH involving 1p in about half the cases studied (Khosla et al., 1991; Mathew et al., 1987b; Moley et al., 1992; Takai et al., 1987; Tsutsumi et al., 1989; Yang et al., 1990). In cases in which it was examined, these deletions were not noted in the constitutional (normal) DNA of the patients, indicating that the deletion does not reflect the site of the inherited mutation. LOH has been noted on other chromosomes also, including 3p, 11p, 17p, and chromosome 22q, but LOH for 1p has been the most consistent finding to date (Khosla et al., 1991; Mathew et al., 1987b; Moley et al., 1992; Takai et al., 1987; Tsutsumi et al., 1989; Yang et al., 1990). This suggests that an important suppressor gene resides on this chromosomal arm, and its loss or inactivation plays a role in the development or progression of pheochromocytomas, at least in a substantial subset of patients. It is possible that allelic loss occurs with increased frequency on some other chromosomal arm or arms, since most studies have involved small numbers of cases and/or polymorphic probes for a limited number of chromosomal arms. Nevertheless, 1p LOH has been seen frequently in every study in which it was examined.

Oncogene Activation

One group used *in situ* hybridization of paraffin-imbedded tissue sections and noted high levels of expression of the proto-oncogene N-*myc* in six of 21 medullary thyroid cancers (MTCs) analyzed (Boultwood et al., 1988). In addition, a recent study demonstrated expression (without gene amplification or rearrangement) of c-*myc* and c-*fos* in six pheochromocytomas, suggesting that these cells are in a state of growth stimulation (Goto et al., 1990). These studies suggest that increased expression of N-*myc*, c-*myc*, c-*fos*, and possibly other oncogenes may be characteristic of at

least some pheochromocytomas. Whether or not this increased expression represents activation of these oncogenes, or merely reflects their active state of growth, remains to be determined.

The ras family of oncogenes (N-*ras*, H-*ras*, K-*ras*) has been implicated in the development of up to 30% of human tumors. Given the prevalence of ras mutations in human cancers, and the association of ras mutations with early changes and lower-grade malignancies, it was reasonable to suspect that ras mutations might occur in pheochromocytomas and MTCs. Two recent studies have examined the ras genes of pheochromocytomas and medullary thyroid cancers for mutations of codons 12, 13, 59, 60, and 61 (Moley et al., 1991; Okazaki et al., 1989). Both studies concluded that mutational activation of ras genes rarely if ever occurs in these tumors.

Insulinlike growth factor I (IGF-I) and IGF-II are polypeptide hormones that are structurally and functionally similar to insulin. There is some evidence that IGF-II is expressed at high levels in fetal tissues, and it stimulates growth of certain cells *in vitro*. The growth-stimulating activity of IGF-I and IGF-II is mediated by the IGF receptor (IGFR), which is a heterotetrameric protein that is similar to the insulin receptor (Rechler and Nissley, 1985). Increased levels of IGF-II (but not IGF-I) have been demonstrated in fetal adrenal medulla, compared to the adult adrenal (Brice et al., 1989). Interestingly, increased levels of IGF-II were detected in 11/11 pheochromocytomas from two studies (El-Badry et al., 1989; Haselbacher et al., 1987). A neuroblastoma cell line has been described that shows autonomous growth in serum-free medium mediated by IGF-II (El-Badry et al., 1989). Indeed, some other neuroblastoma cell lines show increased growth rates in response to IGF-II in the medium (El-Badry et al., 1991). These results suggest that the IGF-II/IGFR pathway may represent an autocrine or paracrine pathway of growth stimulation in neuroblastomas and possibly pheochromocytomas.

Summary

Pheochromocytomas may occur sporadically or in a variety of genetic backgrounds. The most characteristic genetic feature of these tumors is allelic loss for 1p, but other sites of allelic loss may occur as well. There have been several preliminary studies to detect activation of selected oncogenes and growth factors by amplification, gene rearrangement, overexpression, or base-pair mutation, but there are no unequivocal examples of genetic alterations leading to activation of a particular oncogene in these tumors. It is reasonable to examine more tumors for patterns of allelic loss or oncogene activation, but no particular oncogene or mechanism of activation has been identified so far that is characteristic of these tumors.

TABLE 3. Distinguishing Features of Neuroblastoma and Neuroepithelioma*

Characteristic	Neuroblastoma	Neuroepithelioma
Location	Adrenal, paraspinal	Extremity, Chest wall
Patient age	Usually <5 yrs	Usually >5 yrs
Neurotransmitter	Norepinephrine	Acetylcholine
HLA expression	Absent	Present
Cytogenetics	Del 1p; dmins, HSR	Trans (11;22)
Oncogene amplification	Common (N-*myc*)	Uncommon (c-*myc*)
Oncogene expression	N-*myc*	c-*myc*

*Modified from previous publication (Brodeur, 1991a).

NEUROEPITHELIOMAS

Peripheral neuroepithelioma (also known as primitive neuroectodermal tumor, or PNET) is an uncommon malignant tumor of the peripheral nervous system (Askin et al., 1979; Lagervist et al., 1969; Miser et al., 1987; Voss et al., 1984). While this tumor can resemble neuroblastoma histologically, there are distinguishing clinical and biological features (Table 3) (Brodeur, 1991a; Thiele et al., 1987a). These tumors tend to occur in older patients and arise more commonly in the chest wall or extremities from peripheral nerves. Biologically these tumors are characterized by a specific translocation between chromosomes 11 and 22 (see below), and have a cholinergic neurotransmitter enzyme phenotype. Recent evidence from chromosome analysis and oncogene expression pattern suggests that they may be related closely to Ewing's sarcomas.

Cytogenetic Analysis and Assessment of DNA Content

The majority of neuroepitheliomas that have been karyotyped have a reciprocal translocation involving chromosomes 11 and 22: t(11;22)(q24;q12) (Whang-Peng et al., 1984, 1986). However, they do not have 1p deletions, and they seldom have the cytogenetic manifestations of gene amplification (dmins, HSRs), which are characteristic of many neuroblastomas. Thus, the cytogenetic features serve to distinguish neuroepitheliomas from neuroblastomas (Table 3). There have not been any reports of analysis of tumor cell DNA content, but the majority of neuroepitheliomas have karyotypes that are pseudodiploid or hyperdiploid (47–53 chromosomes) (Whang-Peng et al., 1984, 1986).

Loss of heterozygosity and mapping of putative suppressor genes

There have been no studies documenting consistent deletions or LOH of any chromosomal regions. It is possible that

there is a suppressor gene whose expression is altered by the translocation between chromosomes 11 and 22. However, it seems more likely that this rearrangement results in activation of an oncogene, as is seen in most translocation breakpoints that have been cloned in leukemias. There have been very few molecular studies of neuroepitheliomas other than those focused on the 11;22 translocation. Thus, it is likely that more information will be obtained concerning LOH and putative suppressor gene loci once additional tumors are studied.

Oncogene Activation

There have been several studies of oncogene expression in neuroepitheliomas. In one study, patterns of oncogene expression served to distinguish this tumor from neuroblastomas, which it resembles histologically (Thiele et al., 1987a). In another study, the pattern of oncogene expression of neuroepitheliomas and Ewing's sarcoma, a tumor of bone, were indistinguishable (McKeon et al., 1988). Both had moderate expression of c-*myc* (and no expression of N-*myc*). Occasionally neuroepithelioma cell lines have c-*myc* amplification (Kohl et al., 1983; Reynolds et al., 1988). In addition, both had moderate expression of HLA class I antigens, whereas neuroblastomas generally have very low HLA antigen expression, low or absent c-*myc* expression, and moderate to high N-*myc* expression. Even though the c-*ets1* gene maps near the breakpoint on chromosome 11, and c-*sis* maps near the breakpoint on chromosome 22, neither appears to be activated by the 11;22 translocation in neuroepitheliomas or Ewing's sarcomas (Bechet et al., 1984; de Taisne et al., 1984; Emanuel et al., 1985; Thiele et al., 1987b). Thus, despite a characteristic pattern of oncogene expression in these tumors, no oncogene has been shown to be activated specifically or consistently. The most promising candidate at this time (other than the presumptive gene activated by the 11;22 translocation) is c-*myc*, which is expressed frequently and amplified occasionally.

Summary

Neuroepitheliomas resemble neuroblastomas histologically, although they are readily distinguishable on the basis of clinical and biological features. However, neuroepitheliomas do share a number of features with Ewing's sarcomas, such as a characteristic 11;22 translocation and similar patterns of expression of oncogenes, HLA antigens, and other histochemical markers. Although this translocation was identified more than seven years ago, the breakpoint has not yet been cloned, and no one has identified as yet a specific suppressor gene that is inactivated or an oncogene that is activated.

CONCLUSION

Neuroblastoma, pheochromocytoma, and neuroepithelioma are neuroectodermal tumors of the peripheral nervous system. Although the former two share a similar genetic lesion-deletion of 1p, it is not clear if the same gene or locus is involved in the pathogenesis of both diseases. Indeed, given the rather large deletions of 1p in most pheochromocytomas, it is possible that a more proximal locus is involved, or possibly two distinct loci on 1p. Furthermore, these two tumors have very different clinical behaviors. Neuroblastomas are more common in infants than in adults and frequently are metastatic at diagnosis, whereas pheochromocytomas are more common in adults, and have a much more benign clinical behavior.

Neuroepitheliomas resemble neuroblastomas histologically, but they can be distinguished readily based on histochemical, genetic, and clinical features. Although a characteristic translocation and pattern of oncogene expression have been identified, very little is known about the molecular genetic basis of this disease. It is not clear if the group of tumors that shares the 11;22 translocation (neuroepithelioma, Ewing's sarcoma, Askin's tumor, esthesioneuroblastoma) is identical, or if they have variations on a common rearrangement, as is seen in chronic myelogenous leukemia and acute lymphoblastic leukemia with a 9;22 translocation and different rearrangements of BCR and c-abl. Furthermore, there may be other genetic lesions (loss of suppressor genes, activation of oncogenes) that serve to distinguish these diseases. Finally, the clinical significance of genetic changes in these tumors remains to be determined.

Neuroblastomas have been characterized more thoroughly than the other two tumors by both cytogenetic and molecular analysis. Based on genetic features, these tumors can be categorized into at least three groups that have distinctive clinical behaviors. Hyperdiploidy, with whole chromosome gains, characterizes the first group, which has the least aggressive clinical behavior. Tumors that are near-diploid without N-*myc* amplification have an intermediate behavior, whereas tumors with N-*myc* amplification are very aggressive, regardless of other clinical or genetic features. The contribution of 1p LOH or 14q LOH to clinical behavior remains to be determined, although the former has been associated with a poor outcome. It is not clear if this is due to an adverse impact of 1p LOH itself or to its association with N-*myc* amplification. However, genetic analysis indicates clearly that neuroblastomas are more complex genetically than was thought originally.

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