

TYRROLE OF PLOIDY, CHROMOSOME 1p, AND SCHWANN CELLS IN THE MATURATION OF NEUROBLASTOMA

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Abstract Background. Neuroblastoma is a heterogeneous disease, with manifestations ranging from spontaneous regression to lethal spread. Sometimes the tumor spontaneously differentiates toward a benign ganglioneuroma (maturing neuroblastoma). The prognosis is frequently related to ploidy, deletions in the short arm of chromosome 1, and amplifications of the *N-myc* oncogene. Maturing neuroblastomas consist of both neuronal cells and Schwann cells. We investigated the genetic composition of both cell types in maturing neuroblastomas, to determine the relation between genetic abnormalities and maturation.

Methods. We studied 20 maturing and mature neuroblastomas by in situ hybridization to count the chromosomes and evaluate possible deletions in the short arm of chromosome 1 in neuronal and Schwann cells. The DNA content of the cells was measured by flow cytometry.

Results. Neuroblastic and ganglionic cells showed aberrations in the number of chromosomes. In situ hy-

bridization and flow cytometry demonstrated near-triploidy in 18 of 19 tumors and pentaploidy in the remaining tumor. The Schwann cells in all 20 neuroblastomas contained normal numbers of chromosomes. In 18 tumors studied, there were no chromosome 1 deletions in either type of cell.

Conclusions. The Schwann cells in maturing neuroblastomas differ genetically from the neuronal cells. The normal number of chromosomes in Schwann cells and the abnormal number in neuroblastic and ganglionic cells suggest that Schwann cells are a reactive population of normal cells that invade the neuroblastoma. Near-triploidy of neuroblastoma cells and intact chromosome 1 are presumably genetic prerequisites for spontaneous organoid maturation, because we found no diploidy or chromosome 1 deletions in the neuronal cells of spontaneously maturing neuroblastomas. (N Engl J Med 1996; 334:1505-11.)

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NEUROBLASTOMAS are the most common extracerebral solid tumors of infancy and childhood. These embryonal tumors of the sympathetic nervous system are unique among pediatric tumors because of their biologic complexity. Neuroblastoma is often lethal, but up to 30 percent of children have favorable outcomes. In this group, neither widespread disease (stage IVS disease)¹⁻³ nor the presence of residual tumor after surgery influences the prognosis.^{4,5} Mass screening of infants during the first months of life was reported to increase the detection of neuroblastoma in the screened population as compared with an unscreened population.⁶ These unusual phenomena can be explained by two biologic features of neuroblastoma: spontaneous regression and the capacity of neuroblastoma cells to undergo maturation.

Deletions in the short arm of chromosome 1 (1p36), the most common structural aberrations of chromosomes in neuroblastomas, identify patients with a poor prognosis.⁷⁻¹³ Amplification of the *N-myc* oncogene, a less frequent abnormality, almost always occurs together with 1p deletions. Amplification of *N-myc* was the first molecular abnormality to be associated with advanced stages of neuroblastoma and a rapidly progressive course of the tumor.^{11,14-17} The prognostic effect of ploidy (the DNA content of neuroblastoma cells) alone is controversial. However, diploid and tetraploid neuroblastomas are associated with structural aberrations of

chromosomes and *N-myc* amplification more often than nearly triploid neuroblastomas.^{11,17}

The spontaneous maturation of a neuroblastoma starts from a morphologically undifferentiated or poorly differentiated neuroblastic tumor, passes through transitional states, and ends with the formation of a mature ganglioneuroma. Together with the degree of neuronal differentiation, the number of Schwann cells increases dramatically during the maturation of a neuroblastoma. In the most commonly used histologic grading system for neuroblastomas, the presence of Schwann-cell stroma has a central role.¹⁸ The "stroma-rich" group, characterized by extensive Schwann-cell stroma, is divided into two subgroups: one with a favorable prognosis, in which both intermixed and well-differentiated tumors are present, but there are no grossly visible nodules containing immature neuroblastic cells; and one with an unfavorable prognosis, in which there are nodules of neuroblasts. Among stroma-rich tumors, the terms "intermixed" and "well differentiated" roughly correspond to the terms "ganglioneuroblastoma" and "ganglioneuroma" of other classifications.

The Schwann cells in neuroblastomas are thought to arise from neuroblastoma cells and therefore to be of neoplastic origin. This idea, which is based on the coexistence of ganglionic cells and Schwann cells in maturing neuroblastomas and the appearance of "Schwann-cell-like cells" in neuroblastoma-cell cultures,^{19,20} led to the hypothesis that neuroblastomas originate from a pluripotent neural-crest cell.

Since conventional cytogenetic investigation requires proliferating cells, it is not useful in the study of maturing neuroblastomas, which have virtually no proliferation. However, in situ hybridization applied to paraffin sections^{21,22} can detect aberrations in the number and structure of specific chromosomes in differentiating or

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mature neuroblastomas. This technique of genetic analysis can be combined with morphologic and immunohistochemical investigations of individual cells. In this study, we used in situ hybridization and immunohistochemical analysis to study changes in the number of chromosomes and the integrity of 1p in individual neuroblastic, ganglionic, and Schwann cells. The DNA content of neuronal cells and Schwann cells was determined by flow cytometry.

We used these methods to address the following questions. Do tumor-cell ploidy and the integrity of chromosome 1p have a role in the maturation of neuroblastomas? Do favorable histopathological patterns in the stroma-rich group of neuroblastomas correlate with favorable cytogenetic findings? Are the Schwann cells and neuronal cells in differentiated neuroblastomas derived from the same progenitor cell?

METHODS

Tumor Tissue and Clinical Data

The tumors were classified according to the grading system of Shimada et al.¹⁸ Fifteen were of the stroma-rich, intermixed type, and five were well-differentiated, stroma-rich neuroblastomas (Table 1). All the patients had localized tumors, and the tumor stages (Table 1)

were defined according to the method of Evans et al.²³ The median age at diagnosis was 44 months (range, 7 months to 9 years). All the patients were well and disease-free a range of 27 to 154 months after surgery alone (17 patients), surgery plus chemotherapy (2), or radiotherapy and surgery (1). The median observation time was 73 months (range, 27 to 154).

In Situ Hybridization

The tumor tissues were fixed in 7.5 percent phosphate-buffered formalin before being embedded in paraffin. Thin (6- μ m) sections were cut for the experiments involving in situ hybridization. The pretreatment and hybridization were carried out according to the methods of Hopman et al.²¹ and Stock et al.²² Biotinylated DNA probes specific for the centromeres of chromosome 1 (D1Z1²⁴; kindly provided by Dr. T. Cremer, Heidelberg, Germany), chromosome 6 (p308),²⁵ chromosome 10 (D10Z1),²⁶ chromosome 12 (D1Z3),²⁷ and chromosome 17 (p17H8)²⁸ were used. DNA probes were obtained from the American Type Culture Collection, except for D1Z1 and p308. To determine the integrity of 1p, the tandem-repeat probe D1S32²⁹ (a gift from Dr. J. Giannakidis, Marburg, Germany) or D1Z2³⁰ was used. Both probes identify the subtelomeric region of the short arm of chromosome 1 (1p36.33).^{31,32} This chromosomal region is most often involved in deletion events in neuroblastomas.¹³ The hybridized sections or cytoplasmic preparations were washed and the biotinylated probes were detected as described elsewhere.²² In the peroxidase reaction, 3,3'-diaminobenzidine was used as a substrate. Tissue sections were counterstained with Mayer's hemalum. In eight tumors (Table 1), double-target fluorescence in situ hybridization was performed on isolated nuclei, as described by Stock et al.,²² with the probes D1Z1 and D1Z2.

Table 1. Clinical Data, Results of in Situ Hybridization, and Flow-Cytometric Measurements of 20 Stroma-Rich Neuroblastomas with a Favorable Prognosis.*

TUMOR No.	PATIENT'S		EVANS STAGE	HISTOLOGIC TYPE†	DISEASE-FREE SURVIVAL (MO)	IN SITU HYBRIDIZATION‡				FLOW CYTOMETRY	
	AGE (MO)/SEX	TUMOR LOCATION				NEURONAL CELLS		SCHWANN CELLS		NEURONAL CELLS	SCHWANN CELLS
						D1Z1	D1Z2	D1Z1	D1Z2		
						<i>no. of chromosomes</i>				<i>DNA index</i>	
1	7/F	Neck	II	Intermixed	129	3	ND	2	ND	1.5	1.0
2	19/F	Retroperitoneum	I	Intermixed	78	3	3	2	2	1.6	1.0
3	23/F	Retroperitoneum	I	Intermixed	68	3	3	2	2	1.5	1.0
4	24/M	Thorax	II	Intermixed	117	3	3	2	2	1.4	1.0
5	24/F	Thorax	II	Well differentiated	107	3	3	2	2	1.4	1.0
6	32/M	Thorax	II	Intermixed	31	3	3	2	2	1.6	1.0
7	34/F	Neck	II	Intermixed	117	2/3	2/3	2	2	ND	ND
8	35/M	Neck	I	Intermixed	62	3	3	2	2	1.5	1.0
9	37/M	Thorax	I	Intermixed	50	3	3	2	2	1.5	1.0
10	38/M	Adrenal gland	I	Intermixed	63	4	4	2	2	2.3	1.0
11§	50/M	Retroperitoneum	III	Intermixed	30	3	3	2	2	1.7	1.0
12¶	53/M	Sacrum	I	Intermixed	106	3	3	2	2	Single peak	
13	57/F	Adrenal gland	I	Intermixed	60	3	ND	2	ND	ND	ND
14	60/F	Retroperitoneum	II	Well differentiated	30	3	3	2	2	ND	ND
15§	62/M	Adrenal gland	I	Well differentiated	154	3	3	2	2	Single peak	
16	64/F	Pelvis	I	Intermixed	87	2/3	2/3	2	2	1.5	1.0
17	69/M	Neck	I	Intermixed	109	2	2	2	2	1.5	1.0
18	72/F	Thorax	II	Intermixed	90	3	3	2	2	1.5	1.0
19	97/M	Thorax	II	Well differentiated	27	3	3	2	2	1.5	1.0
20	108/M	Neck	I	Well differentiated	60	3	3	2	2	ND	ND

*D1Z1 denotes a DNA probe specific for the centromere of chromosome 1; D1Z2, a DNA probe specific for the telomere of the short arm of chromosome 1 (1p36.33); and ND, not determined because of insufficient tumor material.

†According to the classification system of Shimada et al.¹⁸

‡Results were obtained by double-target fluorescence in situ hybridization performed on nuclei isolated from paraffin blocks (tumors 2, 3, 4, 5, 7, and 12) or on fresh cells (tumors 6 and 19). The designation "2/3" indicates that a tumor contained a mixed population of disomic and trisomic tumor cells.

§This child received chemotherapy after surgery.

¶This child received radiotherapy before surgery.

||Flow-cytometric analysis revealed only one DNA peak, in the diploid range.

The number of hybridization spots per nucleus was counted according to the method of Hopman et al.,²¹ with additional criteria as described by Stock et al.²² Trisomy was considered present with respect to an individual chromosome when the mean statistical probability of finding nuclei with three signals on 6- μ m sections in a tumor-cell population was 15.6 percent among smaller nuclei of neuroblasts (7 to 12 μ m) and 4.7 percent among larger nuclei of ganglionic cells (13 to 20 μ m). The decision to consider a population of cells trisomic was made on the basis of these cutoff levels.

Flow Cytometry

Cell-separation procedures for the DNA analysis of paraffin-embedded tumor specimens by flow cytometry were performed according to the method of Heiden et al.³³ Tumor cells were isolated from the same areas of the tumor block that were used in the evaluation by in situ hybridization. These areas contained almost exclusively tumor cells and tumor-associated Schwann cells without adherent normal tissue except for one tumor (tumor 15), in which tissue from the adrenal cortex was also present. The DNA content of the tumor cells is expressed as the ratio of the modal G₀-G₁ peak of the aneuploid cells to the modal G₀-G₁ peak of the diploid cells in the tumor samples.

Immunohistochemical Analysis

Parallel sections of each tumor sample were stained for immunohistochemical analysis with an antibody against S-100 (Dakopatts, Glostrup, Denmark), a marker for Schwann cells. An indirect immunoperoxidase method of staining was used according to standard protocols.

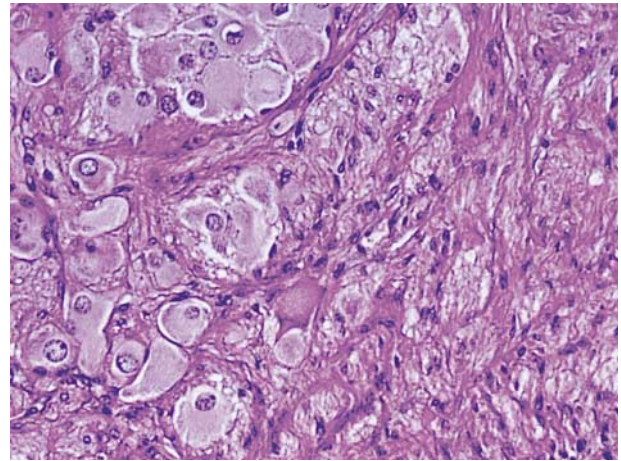
RESULTS

Histologic Analysis and S-100 Immunostaining

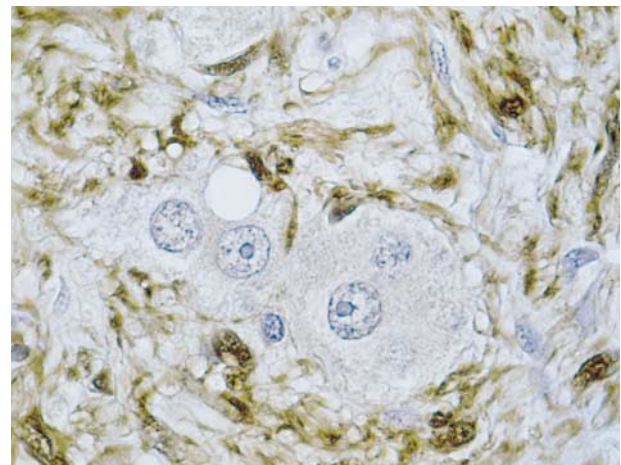
Fifteen stroma-rich, intermixed neuroblastomas and five stroma-rich, well-differentiated neuroblastomas were studied. The well-differentiated tumors consisted mainly of mature ganglionic cells and Schwann cells (Fig. 1A); a few immature, randomly distributed neuroblasts were found in each tumor. In the stroma-rich, intermixed tumors, the ganglioneuromatous tissue contained microscopic foci of neuroblastic cells of different sizes and variable degrees of differentiation. On immunohistochemical analysis, the Schwann cells stained strongly for the S-100 antigen (Fig. 1B).

Aberrations in the Number of Chromosomes in Neuroblastic and Ganglionic Cells

Trisomy 1 was detected in the nuclei of immature neuroblastic and ganglionic cells (Fig. 2A and Table 1) in 16 of the 20 tumors. The biotinylated DNA probes revealed three signals in the nuclei of 11 to 51 percent (mean, 26 percent) of the neuroblastic and ganglionic cells. The large, highly differentiated ganglionic-cell nuclei (13 to 20 μ m in diameter) had the lowest proportion of signals (11 to 15 percent in tumors 5, 7, and 14), because of the truncation of nuclei in the 6- μ m paraffin sections. In tumor 17, an intermixed, stroma-rich neuroblastoma, 32 percent of the neuroblastic and ganglionic cells had two signals per nucleus with the D1Z1 probe and only 2 percent had three signals, indicating the presence of disomy 1. In 2 of the 20 tumors, the smaller neuroblastic-cell nuclei (7 to 12 μ m in diameter) had three signals with the probe D1Z1 in 10 percent (tumor 7) and 11 percent (tumor 16) of the nuclei. These figures were below the cutoff level for the smaller nuclei and indicated that only a subpopulation of cells



A



B

Figure 1. Ganglionic Cells Embedded in a Dense Schwann-Cell Stroma.

The ganglionic cells in Panel A are highly differentiated (hematoxylin and eosin, $\times 200$). In the parallel section shown in Panel B ($\times 400$), immunohistochemical analysis revealed strong expression of the S-100 antigen in the Schwann cells.

had trisomy. Tetrasomic tumor cells, with four signals in 29 percent of the nuclei of neuroblastic and ganglionic cells, were found in tumor 10.

In situ hybridization with DNA probes specific for the centromeres of chromosomes 6, 10, 12, and 17 were performed in four tumors whose DNA content had not been determined by flow cytometry (tumors 7, 13, 14, and 20) or in which only one DNA peak was found (tumor 12). The neuroblastic and ganglionic cells had trisomy for at least three chromosomes, including chromosome 1 (Table 2). This observation indicates that the DNA content of these tumors was probably in the nearly triploid range.

Integrity of the Short Arm of Chromosome 1 in Neuroblastic and Ganglionic Cells

We assessed the presence or absence of 1p deletions with the 1p36.33 probe D1Z2. The number of signals obtained by in situ hybridization with this probe was

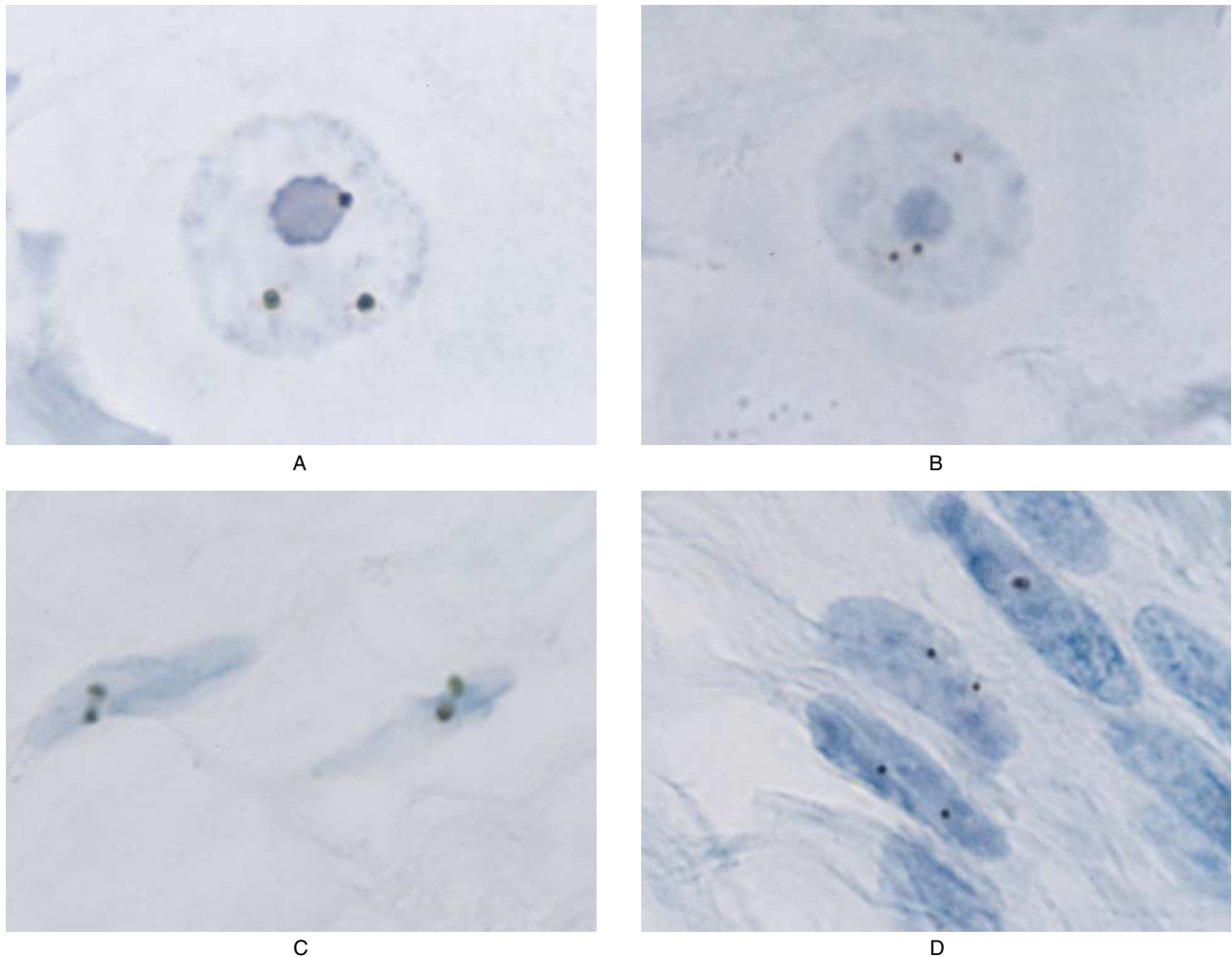


Figure 2. Results of in Situ Hybridization with Two Probes to Assess the Number of Copies of Chromosome 1 and the Presence of Chromosomal Deletions at 1p36 ($\times 1000$).

Three signals are visible when a mature ganglionic cell is hybridized with the probe D1Z1, indicating trisomy 1 (Panel A), and when a similar cell is hybridized with the probe D1Z2, which is specific for 1p36.3 (Panel B). Two signals are visible in the nuclei of Schwann cells hybridized with D1Z1 (Panel C) or D1Z2 (Panel D). When equal numbers of hybridization spots are visible with each probe in both cell types, the short arm of chromosome 1 is intact.

identical to the number obtained with the probe D1Z1 (Table 1), indicating that the short arms of chromosome 1 were intact (Fig. 2B). Two tumors (tumors 1 and 13) were not analyzed with D1Z2 because there was insufficient material in the sample.

Double-target fluorescence in situ hybridization using the probes D1Z1 and D1Z2 simultaneously was carried out in eight tumors (tumors 2 through 7, 12, and 19) on intact nuclei isolated from paraffin blocks or on touch preparations of fresh tumor samples. The results were compatible with the results of peroxidase-based in situ hybridization on thin sections, although the percentage of the isolated intact nuclei that had three signals when hybridized with each probe was much higher (57 to 76 percent).

Chromosome Count and Integrity of Chromosome 1 in Schwann Cells

The Schwann cells in all 20 tumors had exclusively disomic patterns on in situ hybridization with the cen-

tromere- and telomere-specific probes for chromosome 1 (Fig. 2C and 2D). The DNA probes specific for the centromeres of chromosomes 6, 10, 12, and 17 also revealed a disomic hybridization pattern in the five tumors analyzed (Table 2).

DNA Content of Neuroblastic and Ganglionic Cells and Schwann Cells

DNA content was determined in 16 tumors. In 14 tumors, flow cytometry revealed two DNA peaks (Fig. 3), a finding that corresponded with the results obtained by in situ hybridization. The DNA indexes of the tumors were in the diploid and the triploid range (1.4 to 1.7) in 13 tumors and in the pentaploid range (2.3) in 1 (Table 1). The nearly triploid tumors (tumors 1 through 6, 8, 9, 11, and 16 through 19) had three copies of chromosome 1, except for tumor 16, which had a mixed population of disomic and trisomic tumor cells, and tumor 17, which had only two copies of that chromosome in the neuronal cells. The nearly pentaploid

Table 2. Results of in Situ Hybridization of Five Stroma-Rich Neuroblastomas with a Favorable Prognosis with DNA Probes Specific to the Centromeres of Chromosomes 1, 6, 10, 12, and 17.*

TUMOR No.	CHROMOSOME 1		CHROMOSOME 6		CHROMOSOME 10		CHROMOSOME 12		CHROMOSOME 17		
	NEURONAL CELLS	SCHWANN CELLS	NEURONAL CELLS	SCHWANN CELLS	NEURONAL CELLS	SCHWANN CELLS	NEURONAL CELLS	SCHWANN CELLS	NEURONAL CELLS	SCHWANN CELLS	
	<i>no. of copies of chromosome</i>										
7	2/3†	2	3	2	3	ND	3	ND	ND	ND	
12	3	2	3	2	3	ND	3	ND	3	2	
13	3	2	3	2	3	2	ND	ND	3	2	
14	3	2	ND	ND	ND	ND	3	2	3	2	
20	3	2	3	2	ND	ND	3	2	ND	ND	

*For the detection of chromosome 1, the probe used was D1Z1; for chromosome 6, p308; for chromosome 10, D10Z1; for chromosome 12, D12Z3; and for chromosome 17, p17H8. ND denotes not determined because of insufficient tumor material.

†This tumor contained a mixed population of disomic and trisomic tumor cells.

tumor (tumor 10) showed tetrasomy 1. In two tumors (tumors 12 and 15), only one DNA peak was seen, in the diploid range, although aberrations in the chromosome count were found in the neuroblastic and ganglionic cells by in situ hybridization. In tumor 12 all five chromosomes tested (chromosomes 1, 6, 10, 12, and 17) had trisomic patterns on hybridization. In tumor 15, neither flow-cytometric analysis nor in situ hybridization with the centromere-specific probes for chromosomes 6, 10, 12, and 17 was performed, because of lack of material. In four other tumors (tumors 7, 13, 14, and 20), the limited quantity of tumor material in the sample did not permit analysis by flow cytometry.

DISCUSSION

Spontaneously differentiating or mature neuroblastomas contain two main populations of cells: neuronal cells and Schwann cells. We looked for structural and numerical aberrations of the chromosomes in these two kinds of cells. On in situ hybridization and flow cytometry, neuroblastic and ganglionic cells had numerical aberrations indicating near-triploidy in 18 of 19 tumors and indicating pentaploidy in the 19th tumor. By contrast, the Schwann cells in all 20 neuroblastomas had normal numbers of chromosomes.

Although triploidy is known to indicate a favorable prognosis in neuroblastoma,¹⁷ data on the DNA content of the stroma-rich subgroup, thought to have such a prognosis, are sparse and controversial. Taylor et al.³⁴ analyzed a series of ganglioneuromas by flow cytometry and found diploidy in a substantial number of them, as we did in two tumors in our series. However, in one of these two tumors (tumor 12), in situ hybridization with five centromeric chromosome probes revealed trisomy of chromosomes 1, 6, 10, 12, and 17 in the neuroblastic and ganglionic cells. The other tumor (tumor 15) also had trisomy of chromosome 1, but it was not analyzed with other chromosome probes because of lack of material. We attribute the diploidy that flow cytometry revealed in these tumors to the presence of numerous Schwann cells; the few trisomic neuroblastic and ganglionic cells escaped detection. Flow cytometry could thus assign diploidy (an unfavorable feature) to a tumor with a favorable prognosis. However, it is important to note that the number of copies of a single chro-

sosome in tumor cells does not necessarily reflect the ploidy of the tumor, as was demonstrated in tumors 7, 10, 16, and 17.

Deletions in the short arm of chromosome 1, the most frequently observed structural aberrations of chromosomes in neuroblastomas, are frequently associated with poor clinical outcomes.^{9-13,35} In the spontaneously differentiating, stroma-rich neuroblastomas we studied, the short arms of chromosome 1 were intact, as was true of 15 spontaneously regressing tumors in a previous study.¹² In contrast, the presence of 1p deletions in stage IVS neuroblastomas or localized neuroblastomas was associated with a fatal course.¹² Stock et al. found 1p deletions restricted to the yolk sac and the embryonal carcinoma component of mixed pediatric germ-cell tumors.³⁶ In the parts of the tumors consisting of mature teratoma, the short arms of chromosome 1 were

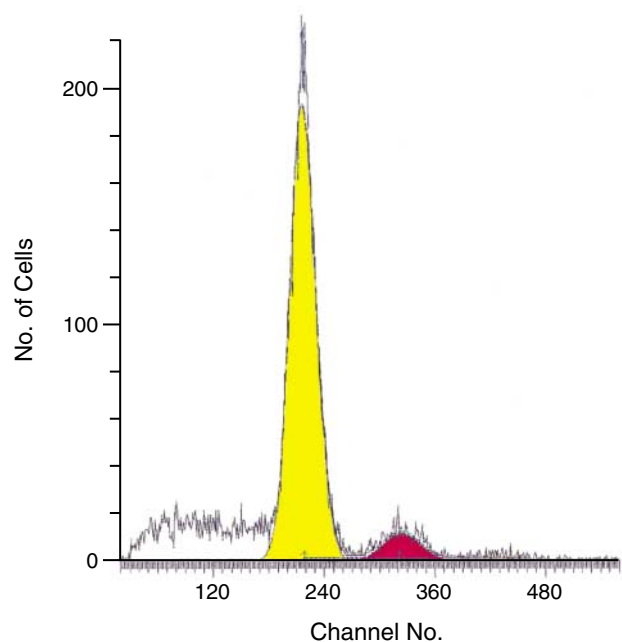


Figure 3. Flow-Cytometric Analysis of an Intermixed, Stroma-Rich Neuroblastoma, Showing Two DNA Peaks.

The diploid peak (DNA index, 1.0) indicates the ploidy of the Schwann cells. The small peak in the triploid range (DNA index, 1.45) identifies the neuronal cells.

invariably intact. These observations suggest that there are differentiation-associated genes in this chromosomal region.

Our results indicate that a favorable morphologic pattern, such as the presence of ganglionic cells and abundant Schwann-cell stroma, correlates closely with favorable genetic findings. Conversely, none of the diploid or tetraploid tumors, accounting for 43 percent of all neuroblastomas studied, and none of the tumors with 1p deletions analyzed so far (28 percent of neuroblastomas studied) showed spontaneous development of Schwann-cell stroma (unpublished data).

To date, no evidence has been advanced to refute the view that the Schwann cells in neuroblastomas are neoplastic. If Schwann cells and ganglionic cells in differentiating neuroblastomas develop from a common neo-

plastic precursor cell, the Schwann cells should have the same clonal genetic changes as the ganglionic cells. Our results, however, show that trisomy is restricted to neuroblastic and ganglionic cells. In all the tumors we investigated, the Schwann cells had a disomic hybridization pattern with the DNA probes we used and had diploid DNA content. No evidence of aneuploidy was detected, a finding consistent with the hypothesis that these cells originated from normal cells rather than the neoplastic neuronal clone. It is implausible that a tumor initially consisting of triploid neuroblastic cells would differentiate into triploid ganglionic cells and diploid Schwann cells during maturation. We therefore favor the hypothesis that Schwann cells arrive in neuroblastomas by migrating into the tumor. In some poorly differentiated, stroma-poor neuroblastomas, a considerable number of Schwann cells are not intermingled with neuroblastoma cells in the way that would be expected if they had originated from the neuroblastoma cells; instead, they are restricted to the septal portion of the tumor. With maturation, the fibrovascular septa become more prominent and the Schwann cells, now increased in number, remain restricted to the septal portion of the tumor. In mature variants of neuroblastoma, the Schwann cells surround the ganglionic cells; ensheath the axons, forming well-organized nerve bundles; and cannot be distinguished histologically from normal Schwann cells. They bear no histologic similarity to neoplastic Schwann cells — that is, to schwannoma cells.

We hypothesize that differentiating neuroblastoma cells produce potent chemotactic, mitogenic, and differentiating factors for Schwann cells, reiterating the physiologic processes that occur during the formation of ganglia and the regeneration of peripheral nerves. It is known that mitogenic signals from axons of normal neuronal cells can provoke the proliferation of Schwann cells in vitro.³⁷⁻⁴² Several soluble growth factors that act on Schwann cells have been described.⁴³⁻⁴⁷ Schwann cells, by contrast, are known for their ability to inhibit proliferation and to induce the differentiation of neuronal cells. They express neurotrophins, such as nerve growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, and glia maturation factor β .⁴⁸⁻⁵¹ Most triploid neuroblastomas that have no other genetic changes express the high-affinity and low-affinity nerve growth factor receptors (p140^{trkA} and p75^{NGFR}, respectively).^{52,53} Schwann cells may provide a source of nerve growth factor, an essential molecule for the differentiation of sympathetic neuronal cells, and thus they may play a part in the self-limited process of some neuroblastomas. Figure 4 shows our view of the interactions between Schwann cells and neuronal cells in the maturation of neuroblastomas.

Triploidy and the absence of 1p deletions in neuroblastoma cells appear to be prerequisites for spontaneous maturation. Identifying the neuroblastoma-associated Schwann cell as a normal cell makes it necessary to reevaluate the concept of organoid differentiation in neuroblastomas. A better understanding of the "cross-

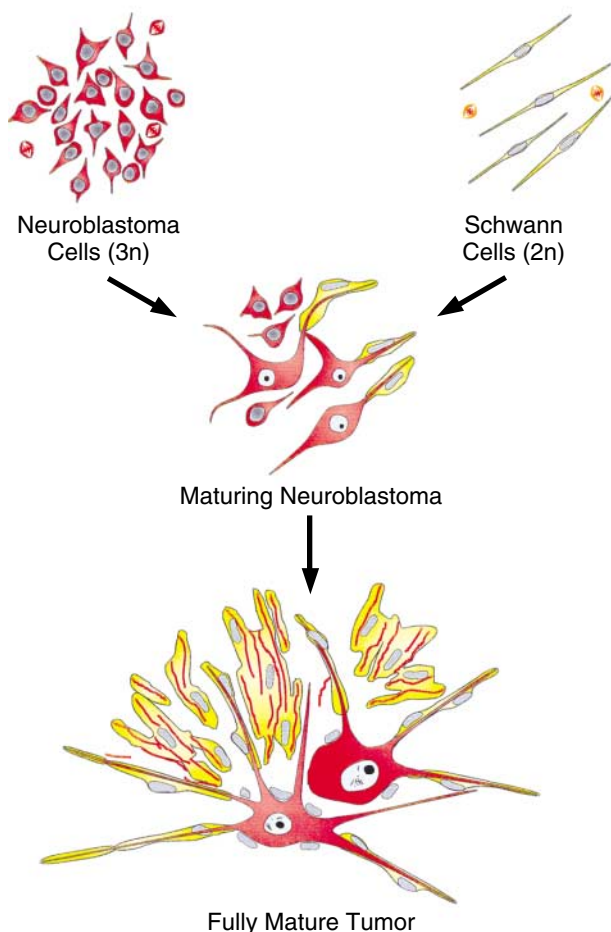


Figure 4. A Model of Maturation in a Neuroblastoma.

Undifferentiated or poorly differentiated triploid (3n) neuroblastoma cells recruit diploid (2n) Schwann cells and cause their proliferation by signals from axon-associated mitogens or soluble growth factors. The diploid Schwann cells migrate into the tumor, possibly using the slender neuritic processes of the tumor cells as guiding structures. There, they suppress the proliferation of neuroblastoma cells and stimulate their differentiation into ganglionic cells. Fully mature tumors consist of highly differentiated ganglionic cells, virtually no immature neuroblasts, numerous Schwann cells that form nerve bundles with axons, sustentacular cells, and perineurial cells.

talk" between neuroblasts and Schwann cells may ultimately provide the knowledge needed to induce differentiation processes in neuroblastomas therapeutically.

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REFERENCES

- D'Angio GJ, Evans AE, Koop CE. Special pattern of widespread neuroblastoma with a favourable prognosis. *Lancet* 1971;1:1046-9.
- Evans AE, Chatten J, D'Angio GJ, Gerson JM, Robinson J, Schnauffer L. A review of 17 IV-S neuroblastoma patients at the Children's Hospital of Philadelphia. *Cancer* 1980;45:833-9.
- Haas D, Ablin AR, Miller C, Zoger S, Matthay KK. Complete pathologic maturation and regression of stage IV-S neuroblastoma without treatment. *Cancer* 1988;62:818-25.
- Matthay KK, Sather HN, Seeger RC, Haase GM, Hammond GD. Excellent outcome of stage II neuroblastoma is independent of residual disease and radiation therapy. *J Clin Oncol* 1989;7:236-44.
- Kushner BH, Cheung N-KV, LaQualia MP, et al. Survival from locally invasive or widespread neuroblastoma without cytotoxic therapy. *J Clin Oncol* 1996;14:373-81.
- Bessho F, Hashizume K, Nakajo T, Kamoshita S. Mass screening in Japan increased the detection of infants with neuroblastoma without a decrease in cases in older children. *J Pediatr* 1991;119:237-41.
- Brodeur GM, Green AA, Hayes FA, Williams KI, Williams DL, Tsiatis AA. Cytogenetic features of human neuroblastomas and cell lines. *Cancer Res* 1981;41:4678-86.
- Gilbert F, Balaban G, Moorhead P, Bianchi D, Schlesinger H. Abnormalities of chromosome 1p in human neuroblastoma tumors and cell lines. *Cancer Genet Cytogenet* 1982;7:33-42.
- Kaneko Y, Kanda N, Maseki N, et al. Different karyotypic patterns in early and advanced stage neuroblastoma. *Cancer Res* 1987;47:311-8.
- Hayashi Y, Kanda N, Inaba T, et al. Cytogenetic findings and prognosis in neuroblastoma with emphasis on marker chromosome 1. *Cancer* 1989;63:126-32.
- Brodeur GM, Nakagawara A. Molecular basis of clinical heterogeneity in neuroblastoma. *Am J Pediatr Hematol Oncol* 1992;14:111-6.
- Ambros PF, Ambros IM, Strehl S, et al. Regression and progression in neuroblastoma: does genetics predict tumour behaviour? *Eur J Cancer* 1995;31A:510-5.
- Caron H, van Sluis P, de Kraker J, et al. Allelic loss of chromosome 1p as a predictor of unfavorable outcome in patients with neuroblastoma. *N Engl J Med* 1996;334:225-30.
- Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* 1984;224:1121-4.
- Seeger RC, Brodeur GM, Sather H, et al. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N Engl J Med* 1985;313:1111-6.
- Fong CT, Dracopoli NC, White PS, et al. Loss of heterozygosity for the short arm of chromosome 1 in human neuroblastoma: correlation with N-myc amplification. *Proc Natl Acad Sci U S A* 1989;86:3753-7.
- Look AT, Hayes FA, Shuster JJ, et al. Clinical relevance of tumor cell ploidy and N-myc gene amplification in childhood neuroblastoma: a Pediatric Oncology Group study. *J Clin Oncol* 1991;9:581-91.
- Shimada H, Chatten J, Newton WA Jr, et al. Histopathologic prognostic factors in neuroblastic tumors: definition of subtypes of ganglioneuroblastoma and an age-linked classification of neuroblastomas. *J Natl Cancer Inst* 1984;73:405-16.
- Sidell N, Sarafian T, Kelly M, Tsuchida T, Haussler M. Retinoic acid-induced differentiation of human neuroblastoma: a cell variant system showing two distinct responses. *Exp Cell Biol* 1986;54:287-300.
- Tsokos M, Scarpa S, Ross RA, Triche TJ. Differentiation of human neuroblastoma recapitulates neural crest development: study of morphology, neurotransmitter enzymes, and extracellular matrix proteins. *Am J Pathol* 1987;128:484-96.
- Hopman AHN, van Hooren E, van de Kaa CA, Vooijs PG, Ramaekers FCS. Detection of numerical chromosome aberrations using in situ hybridization in paraffin sections of routinely processed bladder cancers. *Mod Pathol* 1991;4:503-13.
- Stock C, Ambros IM, Mann G, Gadner H, Amann G, Ambros PF. Detection of 1p36 deletions in paraffin sections of neuroblastoma tissue. *Genes Chromosom Cancer* 1993;6:1-9.
- Evans AE, D'Angio GJ, Randolph J. A proposed staging for children with neuroblastoma. *Cancer* 1971;27:374-8.
- Cooke HJ, Hindley J. Cloning of human satellite III DNA: different components are on different chromosomes. *Nucleic Acids Res* 1979;6:3177-97.
- Jabs EW, Wolf SF, Migeon BR. Characterization of a cloned DNA sequence that is present at centromeres of all human autosomes and the X chromosome and shows polymorphic variation. *Proc Natl Acad Sci U S A* 1984;81:4884-8.
- Devilee P, Kievits T, Waye JS, Pearson PL, Willard HF. Chromosome-specific alpha satellite DNA: isolation and mapping of a polymorphic aliphoid repeat from human chromosome 10. *Genomics* 1988;3:1-7.
- Looijenga LHJ, Smit VTHBM, Wessels JW, et al. Localization and polymorphism of a chromosome 12-specific alpha satellite DNA sequence. *Cytogenet Cell Genet* 1990;53:216-8.
- Waye JS, Willard HF. Structure, organization, and sequence of alpha satellite DNA from human chromosome 17: evidence for evolution by unequal crossing-over and an ancestral pentamer repeat shared with the human X chromosome. *Mol Cell Biol* 1986;6:3156-65.
- Giannakudis J, Vortkamp A, Kabisch R, Schmitt-Lingner E, Grzeschik KH. A hypervariable region located near 1pter. *Cytogenet Cell Genet* 1987;46:619-20. abstract.
- Buroker N, Bestwick R, Haight G, Magenis RE, Litt M. A hypervariable repeated sequence on human chromosome 1p36. *Hum Genet* 1987;77:175-81.
- Strehl S, Ambros PF. Fluorescence in situ hybridization combined with immunohistochemistry for highly sensitive detection of chromosome 1 aberrations in neuroblastoma. *Cytogenet Cell Genet* 1993;63:24-8.
- Van Roy N, Laureys G, Versteeg R, Opendakker G, Speleman F. High-resolution fluorescence mapping of 46 DNA markers to the short arm of human chromosome 1. *Genomics* 1993;18:71-8.
- Heiden T, Wang N, Tribukait B. An improved Hedley method for preparation of paraffin-embedded tissues for flow cytometric analysis of ploidy and S-phase. *Cytometry* 1991;12:614-21.
- Taylor SR, Blatt J, Costantino JP, Roederer M, Murphy RF. Flow cytometric DNA analysis of neuroblastoma and ganglioneuroma: a 10-year retrospective study. *Cancer* 1988;62:749-54.
- Mitelman F, ed. Catalog of chromosome aberrations in cancer. 5th ed. New York: Wiley-Liss, 1994.
- Stock C, Ambros IM, Lion T, et al. Detection of numerical and structural chromosome abnormalities in pediatric germ cell tumors by means of interphase cytogenetics. *Genes Chromosom Cancer* 1994;11:40-50.
- Wood PM, Bunge RP. Evidence that sensory axons are mitogenic for Schwann cells. *Nature* 1975;256:662-4.
- Salzer JL, Bunge RP. Studies of Schwann cell proliferation. I. An analysis in tissue culture of proliferation during development, Wallerian degeneration, and direct injury. *J Cell Biol* 1980;84:739-52.
- Idem. Studies of Schwann cell proliferation. II. Characterization of the stimulation and specificity of the response to a neurite membrane fraction. *J Cell Biol* 1980;84:753-66.
- Salzer JL, Bunge RP, Glaser L. Studies of Schwann cell proliferation. III. Evidence for the surface localization of the neurite mitogen. *J Cell Biol* 1980;84:767-78.
- Ratner N, Hong DM, Lieberman MA, Bunge RP, Glaser L. The neuronal cell-surface molecule mitogenic for Schwann cells is a heparin-binding protein. *Proc Natl Acad Sci U S A* 1988;85:6992-6.
- Nordlund M, Hong D, Fei X, Ratner N. Schwann cells and cells in the oligodendrocyte lineage proliferate in response to a 50,000 dalton membrane-associated mitogen present in developing brain. *Glia* 1992;5:182-92.
- Bressler JP, Grotendorst GR, Levitov C, Hjelmeland LM. Chemotaxis of rat brain astrocytes to platelet derived growth factor. *Brain Res* 1985;344:249-54.
- Weinmaster G, Lemke G. Cell-specific cyclic AMP-mediated induction of the PDGF receptor. *EMBO J* 1990;9:915-20.
- Davis JB, Stroobant P. Platelet-derived growth factors and fibroblast growth factors are mitogens for rat Schwann cells. *J Cell Biol* 1990;110:1353-60.
- Lemke GE, Brookes JP. Identification and purification of glial growth factor. *J Neurosci* 1984;4:75-83.
- Ridley AJ, Davis JB, Stroobant P, Land H. Transforming growth factors-beta 1 and 2 are mitogens for rat Schwann cells. *J Cell Biol* 1989;109:3419-24.
- Johnson EM Jr, Taniuchi M, DiStefano PS. Expression and possible function of nerve growth factor receptors on Schwann cells. *Trends Neurosci* 1988;11:299-304.
- Lim R, Miller JF, Zaheer A. Purification and characterization of glia maturation factor beta: a growth regulator for neurons and glia. *Proc Natl Acad Sci U S A* 1989;86:3901-5.
- Lim R, Liu YX, Zaheer A. Glia maturation factor beta regulates the growth of N18 neuroblastoma cells. *Dev Biol* 1990;137:444-50.
- Reynolds ML, Woolf CJ. Reciprocal Schwann cell-axon interactions. *Curr Opin Neurobiol* 1993;3:683-93.
- Nakagawara A, Arima M, Azar CG, Scavarda NJ, Brodeur GM. Inverse relationship between *trk* expression and N-myc amplification in human neuroblastomas. *Cancer Res* 1992;52:1364-8.
- Kogner P, Barbany G, Dominici C, Castello MA, Raschella G, Persson H. Coexpression of messenger RNA for *trk* protooncogene and low affinity nerve growth factor receptor in neuroblastoma with favorable prognosis. *Cancer Res* 1993;53:2044-50.