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Human Neuroblastomas and Abnormalities of Chromosomes 1 and 17

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ABSTRACT

Structural rearrangements of chromosome 1p have been reported previously as a frequent finding in human neuroblastomas. In a review of karyotypes from 35 neuroblastomas (including 29 published cases and 6 unpublished tumors and cell lines), it was found that, in addition to the abnormalities of chromosome 1p (found in approximately 70% of cases), abnormalities involving only 2 other chromosome segments occurred with significant frequency (in 20% or more of cases) in this cancer. These abnormalities involved trisomies for the long arms of chromosomes 1 and 17. In addition, two novel cytogenetic aberrations, homogeneously staining regions and double minutes, were identified in two-thirds of the cases. It is postulated that the gene change(s) produced by the abnormalities of chromosome 1p in neuroblastoma play a primary role in the development of this cancer. The gene changes produced by the abnormalities of chromosomes 1q and 17q and by the homogeneously staining regions and double minutes are presumed to contribute to tumor progression.

INTRODUCTION

Since the first description (1960) of a specific cytogenetic abnormality in a particular cancer (the Philadelphia chromosome in chronic myelogenous leukemia), a number of human and animal cancers have been shown to contain characteristic marker rearrangements (20, 23). Among the human solid tumors in which a specific chromosome abnormality has been found is neuroblastoma (10). Several investigators have identified deletions or rearrangements of the short arm of chromosome 1 (1p), always involving the loss of structural material distal to band 1p31, in karyotypes prepared directly from neuroblastomas and from permanent neuroblastoma cell lines (4, 7, 8, 10, 14, 17). Abnormalities of other chromosome segments, notably 1q, 17q, and 22q, have also been reported in neuroblastoma (4, 8, 11). The frequency with which such abnormalities occur is unknown, as is their possible significance. We now present an analysis of the banded tumor cell karyotypes from 6 human neuroblastomas (4 previously unreported tumors and 2 permanent neuroblastoma cell lines). We will also summarize the cytogenetic data from our 8 previously published cases (2, 12, 14) and from 21 cases reported by other laboratories. Possible relationships between particular chromosome abnormalities, treatment history, and the form of the tumor, whether primary or metastatic, will be discussed.

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MATERIALS AND METHODS

The techniques for trypsin-Giemsa banding of metaphases from tumor tissue and permanent cell lines were as described in Ref. 14. In Case NB 82, chromosome preparations were made from minced tumor tissue after short-term culture (24 hr). In Case NB 108, chromosome preparations were made from overnight cultures of minced primary tumor and peritoneal fluid. In Case NB 80, the chromosome preparations were made from a bone marrow sample after 4 days in culture. Successful preparations from the marrow in Case NB 83 were obtained after 6 weeks in culture. Two permanent cell lines (NB4, CHP-166) were karyotyped from recently reconstituted samples of aliquots that had been frozen within months of receipt of the original tumor tissue.

To determine the modal chromosome number for each case, the chromosomes of between 20 and 100 metaphases were counted. The preparation of the consensus karyotype for each case was based on an analysis of between 5 and 20 metaphases.

RESULTS

For 4 tumors (NB80, NB82, NB83, NB108) and one permanent cell line (CHP-166), the age at diagnosis, sex of patient, treatment history, and modal chromosome number are as given in Table 1. For the permanent line from NB4, records are no longer available as to the age at diagnosis and the treatment status of the patient at the time the bone marrow was collected.

Representative karyotypes of the 6 cases are illustrated in Fig. 1. [An unbanded karyotype from CHP-166 was included in an earlier review (25).]

Abnormalities involving only 2 chromosomes, Nos. 1 and 17, were found in a significant fraction of cases (20% or greater). The particular abnormalities identified [deletions/rearrangements of 1p, trisomy for an intact chromosome 1 or for the long arm of chromosome 1 (1q), and trisomy 17 or iso(17q)] are listed in Tables 1, 2, and 3.

Selected clinical and cytogenetic information from 8 previously reported cases from this laboratory and 21 cases reported by other investigators is given in Tables 1 and 2. Only those cases for which complete banded karyotypes were given or adequately described were considered for inclusion in this review.

The pertinent cytogenetic data are summarized in Table 3. A total of 35 cases have been collated; of this number, information on whether the material being analyzed was from a primary or metastatic tumor was available for 34. The most common cytogenetic finding was a deletion or rearrangement of chromosome 1p, always involving the loss of structural material distal to band 1p31; this was evident in 25 of the 35 cases (71%). The other chromosome abnormalities under consideration were: additional 1q material in 13 cases (37%) and additional 17q material in 8 cases (23%). In Case NB16, current analysis indicates that the previously described (14) abnormal chromosome 1 contains additional 1q material replacing all or part of the short arm. While

Table 1

Pertinent cytogenetic and clinical information from human neuroblastomas studied in the authors' laboratory

Karyotypes were prepared from tumor material or, in NB4 and CHP-166, from permanent cell lines.

Sample	Age (yr)	Sex	Site	D/L[a]	Treatment	Mode	Chromosome abnormalities			HSRs/DMS	Ref.
							1p–[b]	+1q	+17q		
NB4	NA	M	BM	L	NA	46	+[c]	–	–	–/+	
NB80	2	M	BM	D	CT	83	+	+	+17	–/–	
NB82	1/12	F	MET	D	CT	49	+	+	–	–/–	
NB83	45/12	M	BM	D	CT	46	+	+	(–17)	–/+	
CHP-166	2.5	M	BM	D	No	46, 92	+	–	–	–/+	
NB108	4	M	PRIM	D	No	47, 48	+	+	–	–/–	
			MET	D	No	47, 48	+	+	–	–/–	
NB16	3	F	BM	D	No	46	+	+	17q+	–/+	14
NB19	1	F	BM	D	CT	46	–	–	–	+/–	14
NB56	2	M	BM	D	CT/RT	47, 83	–	–	–	+/–	14
NB76	3	M	Ascites	D	No	46	+	–	–	–/+	14
NB9	22/12	M	PRIM	D	No	46	+	–	–	–/+	14
CHP-126	14/12	F	PRIM	L	No	45, 46	–	–	–	+/+	2
CHP-134	13/12	M	PRIM	L	CT/RT	46	+	–	–	+/–	2
NB69	16/12	M	PRIM	D	No	45, 84	+	–	–	–/–	12
	27/12		MET	D	CT	46	+	+	–	–/–	12

[a] D, direct preparation; L, established cell line; NA, information not available; BM, bone marrow; CT, chemotherapy; RT, radiotherapy; MET, metastasis; PRIM, primary tumor.
[b] 1p– and others, marker rearrangements described in Fig. 1 and the appropriate references.
[c] +, present; –, absent.

Table 2

Pertinent cytogenetic and clinical information from human neuroblastomas reported previously by ourselves or other investigators

Sample	Age (yr)	Sex	Site	D/L[a]	Treatment	Mode	Chromosome abnormalities				Ref.
							1p–[b]	+1q	+17q	HSRs/DMS	
SK-N-SH	4	F	BM	L	CT/RT	47	–[c]	+	+17q	–/–	10
SK-N-MC	12	F	MET	L	CT/RT	47	–	–	–	+/+	5
SK-N-BE (2)	2	M	BM	L	No	44	+	–	(–17)	+/–	5
LA-N-1	2	M	BM	L	CT	86, 89	–	+	+17q	+/–	10
MMH	3	M	BM	D, L	CT/RT	46, 47	+	–	+17	–/+	18
KO	17	F	BM	D	No	46	–	–	–	–/–	11
NMB (2)	2	M	BM	D	CT/RT	45	+	–	–	–/–	8
NKP	5	F	BM	D	No	53	+	+	–	–/–	8
NBB	2.5	M	BM	D	CT	46	–	–	–	–/+	8
NJF	4	F	BM	D	No	45	+	–	–	–/+	8
NGP	2.5	M	MET	L	CT	47	(1p+)	–	–	+/–	10
NWC	5	M	MET	D	No	82	+	–	(17p–)	–/+	8
NMB	10/12	F	BM	L	CT	89	+	–	–	+/+	7, 14
NAP	NA	F	NA	L	NA	45, 46	–	–	+17	+/+	4
NCG	NA	NA	PRIM	L	NA	45	+	+	–	–/–	8
NTP	2	F	PRIM	D	No	46	+	–	–	–/–	10
NCC	13/12	F	PRIM	D	No	46	+	–	–	–/–	10
SMS-KAN	3	F	PRIM	L	No	46	+	–	+17q	–/+	4
LA-N-2	3	F	PRIM	L	No	70	–	+	–	+/+	4
NRC	2	M	PRIM	D	CT	72	+	+	+	–/–	7
IMR32	13/12	M	PRIM	L	No	47	+	+	–	+/–	6, 14

[a] Abbreviations are as described in Table 1.
[b] 1p– and others, marker rearrangements described in Fig. 1 and the appropriate references.
[c] –, absent; +, present.

14 of 24 metastatic tumors (58%) were from patients who had been pretreated (radio- and/or chemotherapy), trisomy for chromosome 1 or 1q was observed in 9 metastatic cases, of which 6 (66%) were pretreated, and abnormalities of 17q were noted in 5 cases, of which 4 (80%) were pretreated.

A deletion of chromosome 22q was reported previously as the only chromosome abnormality in neuroblastoma KO (11). Monosomy 22 was also noted in 2 other tumors [NB69 (12) and MMH (17)]. The loss of chromosome material from chromosome 22 was, therefore, found in 3 of the 35 cases (8.5%). In addition, reciprocal translocations involving chromosome 22 have been reported in 3 neuroblastoma cell lines, SK-N-SH, SK-N-MC, and NB82 [t(17;22)(q21;q13), t(21;22)(p11;q11), and t(9;22)(q34;q11), respectively] (Fig. 1*C*). This brings to 6 the number of tumors and cell lines in which structural or numerical abnormalities of chromosome 22 have been identified (17% of the 35 cases).

Two other cytogenetic abnormalities have been described in these tumors, HSRs[2] and DMS (6). Of the total of 35 tumors and cell lines listed in Tables 1 and 2, 23 had one or both of these abnormalities (66%); 7 had HSRs alone, 11 had DMS alone, and 5 contained both [any one cell in a tumor usually has one or the other abnormality, not both (14)]. In karyotypes prepared from permanent cell lines or directly from tumor tissue, 12 of 14 cell lines contained one or both abnormalities (86%), whereas HSRs/DMS were evident in only 8 of the 22 direct preparations (36%).

[2] The abbreviations used are: HSRs, homogeneously staining regions; DMS, double minute chromosomes.

Table 3

Summary of cytogenetic data from all cases

Lack of treatment information in every case and multiple samples from single patients results in discrepancies in overall totals.

Neuroblastoma	Case	Chromosome abnormalities			HSRs/DMS
		1p–[a]	+1q	+17q	
Total	35	25 (71)[b]	13 (37)	8 (23)	23 (66)
Site of tumor/treatment history					
Primary	12	10 (83)	5 (42)	2 (17)	6 (50)
Untreated	10	8	4	1	5
Treated	2	2	1	1	1
Metastases	24	17 (70)	9 (38)	5 (21)	15 (63)
Untreated	9	7	3	1	5
Treated	14	9	6	4	10
Karyotype preparation/site of tumor					
Direct	22	18 (82)	9 (41)	4 (18)	8 (36)
Primary	6	6	2	1	1
Metastases	16	12	7	3	7
Cell line	14	9 (64)	5 (36)	3 (21)	12 (86)
Primary	6	4	3	1	5
Metastases	8	5	2	2	7

[a] 1p– and others, marker rearrangements described in Fig. 1 and the appropriate references.
[b] Numbers in parentheses, percentage.

DISCUSSION

Neuroblastoma is a tumor of neural crest origin (28), occurring primarily in childhood; at least two-thirds of cases are reported in children younger than 5 years of age (16). The predisposition to tumor development can be transmitted in a dominant fashion; based on the pattern of recurrence and the epidemiology of the tumor, it has been argued that (as few as) 2 gene changes are sufficient for neuroblast transformation ("2-hit" hypothesis) (18).

The finding in tumor preparations from individuals whose constitutional karyotypes are normal of the preferential involvement of the short arm of chromosome 1 in structural rearrangements suggests that this segment contains one or more genes that play a role in neuroblast transformation (8, 10, 14).

The analysis of our cytogenetic data and the accumulated published experience of other investigators indicates that abnormalities involving 2 other chromosome segments, 1q and 17q, also occur frequently in neuroblastoma, though considerably less often than is the case for abnormalities of chromosome 1p. Additional 1q and 17q material were evident in greater than 20% of the cases; abnormalities of chromosome 1p, on the other hand, have been identified in greater than 70% of the cases.

While deletions/rearrangements of chromosome 1p, in the absence of trisomy for chromosome 1, appear to be associated with a single human neoplasm, neuroblastoma, extra 1q, and 17q material have been reported in a number of human tumors, particularly in lymphomas and leukemias (22). In certain lympho- and myeloproliferative disorders, trisomies for 1 (or 1q) and 17 [or iso(17q)] have also been observed to develop following the appearance of those marker rearrangements which are characteristic of the individual cancers (*e.g.*, the 22/autosome translocation seen in chronic myelogenous leukemia) (23). Each is among the cytogenetic changes which have been reported to accumulate as tumor cells from single cases are sampled over time ["clonal evolution" (21)].

As the abnormalities of chromosomes 1q and 17q described are clearly not limited to human neuroblastomas, it is unlikely that the gene changes each produces will be necessary steps in neuroblast transformation. Each may, in fact, represent a secondary event in tumor evolution (developing following tumorigenesis). (Their appearance in cell lines derived from primary tumors may, in fact, be a function of the number of divisions these cells have undergone following transformation.)

Support for the hypothesis that additional 1q material contributes primarily to tumor progression rather than to tumor initiation comes from 2 observations: (*a*) in karyotypes from Case MMH (17), it was noted that extra 1q marker chromosomes appeared over time in tissue culture; and (*b*) in case NB69 (12), while the primary tumor contained one normal chromosome 1 and one deleted 1p marker, the metastases obtained 11 months later contained 1 normal number 1 and 2 deleted 1p markers.

Pretreatment with radiation or chemotherapy could not unequivocally be associated with the appearance of abnormalities of chromosomes 1 or 17q. One cannot determine, then, whether drug or radiation resistance did or did not play a role in the selection or retention of these particular chromosome abnormalities.

In addition to abnormalities involving specific chromosome segments, a significant fraction of human neuroblastomas have been observed to contain 2 novel chromosome abnormalities, HSRs and DMS. Both HSRs and DMS are characterized by unusual staining properties (6); each has been identified in direct preparations from tumor material (1); one or both have been described in a wide range of human and animal tumors (15); and evidence has been presented that the breakdown of a HSR can give rise to DMS (3).

From studies of certain drug-resistant cell lines, it would appear that both HSRs and DMS contain multiple copies of one or a small number of genes (6, 24). Recent studies indicate that genes with limited homology to one of the retroviral *onc* genes (*v-myc*) are amplified in some (perhaps, all) of the neuroblastoma cell lines containing HSRs and DMS (19, 26). Amplification of these particular genes (designated *N-myc* and *c-myc*) is not evident in neuroblastomas without HSRs or DMS, however. An association has also been made between the length of an HSR and enhanced tumorigenicity (13), and our analysis indicates that permanent cell lines established from tumor material are more likely to contain HSRs/DMS than are preparations obtained

directly from the tumors or metastases (Table 3). In addition, preliminary evidence suggests that *onc* gene amplification may be limited to the more advanced clinical stages of neuroblastoma (those with the poorest prognosis) (9). The implication of these observations is that HSRs and DMS (and, therefore, gene amplification) are associated with, but not necessary for, the development of neuroblastoma; they probably develop in the course of tumor evolution and, again, are presumed to confer selective growth advantages on cells, thereby contributing to tumor progression.

Different leukemias and lymphomas have now been characterized by rearrangements, primarily reciprocal translocations, involving specific chromosomes (27). As the banded karyotypes from solid tumors other than neuroblastoma are analyzed, it should become clear whether the nonrandom involvement of small numbers of chromosomes in structural rearrangements is the exception or the rule in cancer. As more is learned about the single gene changes produced by, or producing, abnormalities of particular chromosomes, it should also become clear whether or not the distinction drawn between chromosome abnormalities which play primary or secondary roles in the development of specific cancers is correct.

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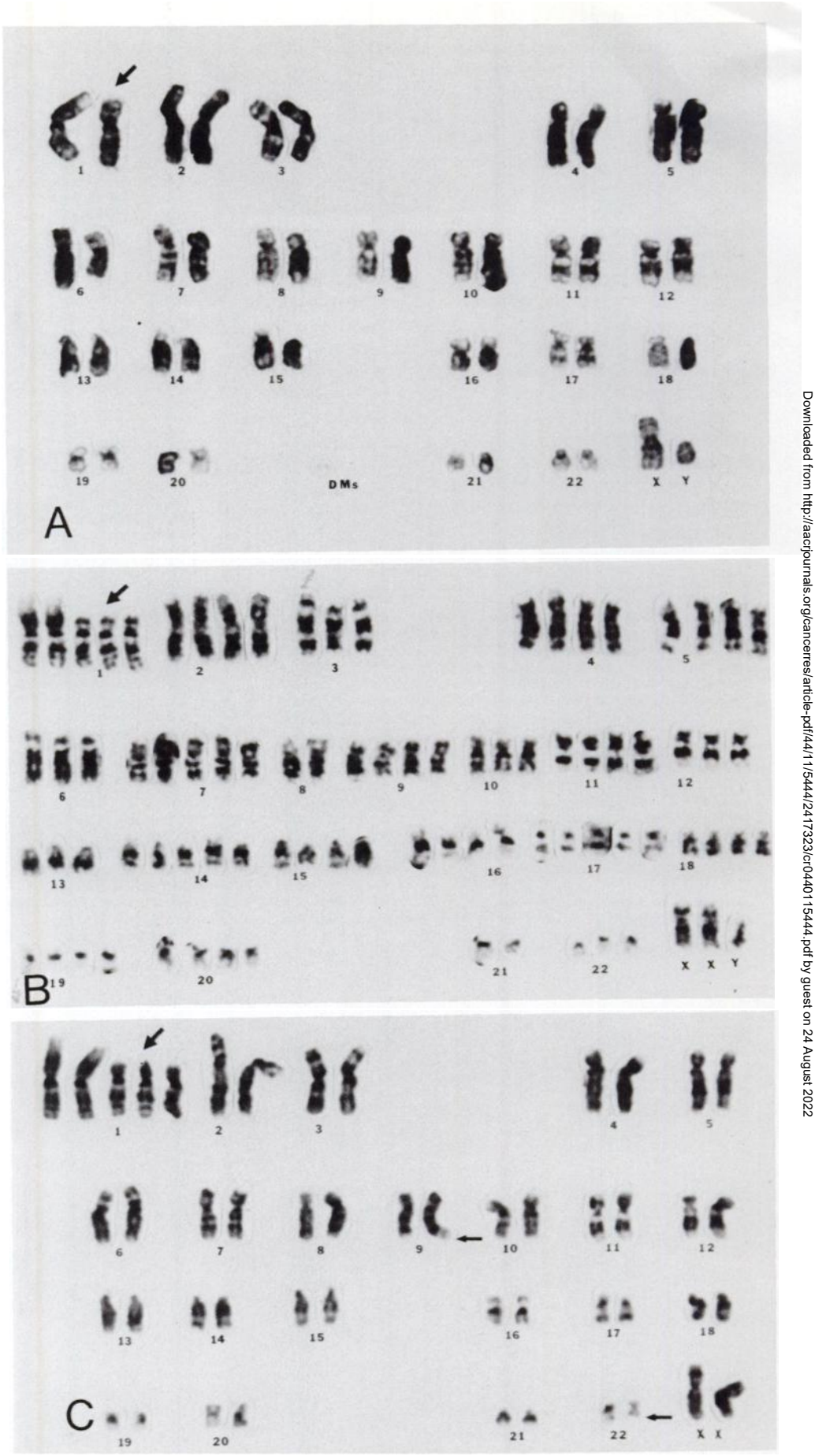

Fig. 1. Representative karyotypes from the previously unreported human neuroblastoma tumors and cell lines described in this report. Consistent chromosome abnormalities are identified by *arrows*. *A*, NB4. 46,XY; *arrow*, abnormal 1p [del(1)(:p22→qter)]. Also present: del(6)(pter→q21:) (note DMS). *B*, NB80. 85,XXY; *arrow*, chromosomes 1 containing abnormal p arms [either a translocation (origin of segment unknown) replacing entire 1p or del(1)(:p22→qter)] (note 5 chromosomes 1). *C*, NB82. 49,XX; *arrows*, chromosomes 1 with abnormal p arms [del(1)(:p31→qter)]; del(22)(pter→q11:); and t(9;22)(q34;q11) (note 5 chromosomes 1). *D*, NB83. 46,XY; *arrow*, abnormal 1p [nonreciprocal translocation (origin of segment unknown) replacing entire 1p] (note 3 chromosomes 1, monosomy 17, and DMS). *E*, CHP166. 92,XXYY; *arrow*, chromosome 1 containing abnormal p arms [nonreciprocal translocation (origin of segment unknown) to 1p22]. Also contains del(3)(pter→q12:) and DMS. *F*, NB108, 48,XY; *arrows*, t(1;11)(p13;q11); +5; t(8;?)(p33;?); and t(14;?)(q32;?).