Chromosomal analysis of neuroblastoma

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Summary Ten children with abdominal neuroblastoma were included in the study. Biopsies from the neuroblastomas were taken during surgical operations, nine from the primary tumours and one from a metastasis. Histopathology was done for diagnosis. Chromosomal cultures of neuroblastoma cells and peripheral blood lymphocytes were performed.

The peripheral blood lymphocytes revealed normal chromosomal complements. The 10 tumours were in the peridiploid range with random gains or losses of chromosomes. Deletion of the short arm of chromosome number 1 distal to band p31 occurred in 6 tumours. Other structural changes and giant markers were found. It was concluded that a regulatory gene controlling the transformation gene of neuroblastoma, is present at or distal to lp31.

Neuroblastoma is a malignant tumour arising from the primitive neural crest cells that form the adrenal medulla and sympathetic nervous system. It is one of the most common malignancies of young children. The histologic composition of this tumour is mainly of primitive neuroblast cells.

Analysis of chromosomes in some malignancies such as chronic myelogenous leukaemia and retinoblastoma has been informative. The clustering of aberrations to specific chromosomes in neoplasms indicates that these changes are non-random and possibly related to malignant transformation (Miles 1974; Mitelman & Levan, 1976). The reported chromosome numbers in neuroblastomas vary considerably but are in the peridiploid range (Brewster & Garrett, 1965; Brodeur et al., 1977). Sandberg et al. (1972) reported double minute chromosomes in neuroblastoma, while Brodeur et al. (1977) could not confirm this. However, these were also found frequently in glial as well as other neurogenic tumours. Some workers reported deletion of the short arm of chromosome number 1 in some of their neuroblastomas (Brodeur et al., 1977, 1981; Haag et al., 1981; Gilbert et al., 1982 and Trent, 1983).

Since there is no previous report of clear and specific chromosomal abnormalities in neuroblastoma, we planned this cytogenetic study in order to search for any specific aberration.

Materials and methods

Ten children with abdominal neuroblastoma were included in the study. The clinical picture and stages are presented in Table I. No child had any congenital anomalies or dysmorphic features. Chromosomal studies were carried out on cultures of peripheral blood lymphocytes from the 10 children. Biopsies from the primary abdominal neuroblastomas in 9 cases and from a metastasis in the temporal bone in one case were taken during surgical operations in the Surgical Oncology and Pediatric Surgery Units, Mansoura University Hospital during the last 4 years (1980–84). Histopathology of tumour specimens was done for diagnosis. Direct preparations from tumour material was carried out (Harnden, 1974). Chromosomal analysis was performed with a modification of the technique of Moorhead et al. (1960). Slides were stained by the ASG technique for G-band identification of each chromosome (Sumner et al., 1971). At least 100 cells were counted and 20 photographed for each of the tumour explants and for peripheral blood lymphocytes.

Results

Histopathologic studies of the 10 tumour specimens revealed the classical picture of neuroblastoma. The tissue comprised primitive neuroblasts, which were rounded, with darkly stained nuclei, prominent nucleoli and scanty cytoplasm. The cells were poorly supported by stroma.
Table I  Clinical data on neuroblastoma patients

| Case no. | Age  | Sex | Site of primary | Site of biopsy | Stage | Treatment |
|----------|------|-----|-----------------|----------------|-------|-----------|
| 1        | 4/0  | F   | Suprarenal      | Primary        | III   | none      |
| 2        | 1/7  | M   | Suprarenal      | Primary        | II    | none      |
| 3        | 6/4  | F   | Suprarenal      | Primary        | III   | none      |
| 4        | 2/6  | F   | Suprarenal      | Primary        | IV    | none      |
| 5        | 3/4  | M   | Suprarenal      | Primary        | IV    | none      |
| 6        | 0/11 | F   | Suprarenal      | Primary        | III   | none      |
| 7        | 3/0  | F   | Suprarenal      | Primary        | IV    | none      |
| 8        | 1/0  | M   | Suprarenal      | metastases     | IV    | none      |
| 9        | 0/1  | M   | Suprarenal      | Primary        | IV    | none      |
| 10       | 8/0  | F   | Suprarenal      | Primary        | IV    | none      |

All patients were untreated at the time of biopsy.

Studies of the chromosomes of the peripheral blood lymphocytes revealed normal chromosomal complements in the 10 children. Chromosomes of the 10 tumours were examined and their analysis summarized as follows:

Numerical changes

The 10 tumours were in the peridiploid range with modes of 46, 47, 46, 49, 46, 47, 46, 48 and 49 respectively (Table II). A small percentage of cells were in the peritriploid range (2.9, 2.0, 2.8 and 2.2% in Case nos. 3, 4, 9 and 10 respectively) and in the peritetraploid range (18.3 and 3.0% in Case nos. 2 and 10 respectively). There were no bimodal lines, and cells with more or less than the modal number were found to have random gains or losses of chromosomes.

Structural changes

Deletion of the short arm of chromosome number 1 is present in all the cells examined in 6 (including the metastasis in case no. 8) of the 10 tumours. In all of these the deletion is distal to band p31 (Figures 1, 2, 3). Marker chromosomes are present in 3 cases (Figure 4). Their origin could not be ascertained. In Case no. 2, 21.1% cells show a marker chromosome with concomitant loss of number 1. In Case no. 5, 10.7% of metaphases show a giant marker with loss of number 3. In 20.1% cells of Case no. 7 also a giant marker chromosome is present along with a 1p− chromosome, and loss of number 2 in only 6.7% of cells (Table III).

Structural abnormalities probably involve also chromosome number 13 in Case nos. 6 and 7

Table II  Ploidy range of the chromosomes of the ten neuroblastomas.

| Ploidy range | 1 (106) | 2 (109) | 3 (102) | 4 (100) | 5 (102) | 6 (104) | 7 (104) | 8 (102) | 9 (102) | 10 (134) |
|--------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Hypodiploid  | n       | 10      | 22      | 23      | 15      | 13      | 15      | 12      | 5       | 3       |
|              | %       | 9.1     | 21.5    | 23.0    | 14.7    | 14.7    | 14.3    | 11.8    | 4.8     | 2.2     |
| Diploid      | n       | 106     | 30      | 62      | 25      | 87      | 91      | 15      | 78      | 32      |
|              | %       | 100     | 27.5    | 60.7    | 25.0    | 85.3    | 87.5    | 14.3    | 76.5    | 30.5    |
| Hyperdiploid | n       | 49      | 15      | 50      | —       | —       | 74      | 12      | 65      | 86      |
|              | %       | 44.9    | 14.7    | 50.0    | —       | —       | 71.2    | 11.8    | 62.9    | 64.1    |

Mode  

46  47  46  49  46  46  47  46  46  48  49

( ) = total number of metaphases examined.

n  = number of metaphases.

%  = percentage of metaphases.
Figure 1. G-banded Karyotype (49 chromosomes) of a metaphase from Case no. 4. Notice the deletion of short arm of chromosome no. 1 at p14, and extra chromosomes numbers 6, 9, 10, & 18.
Figure 2  G-banded Karyotype (45 chromosomes) of a metaphase from Case no. 6. Notice the deletion of 1p\(^-\) at p31, 13q\(^+\), extrachromosome 9, and lost 8 and 17.
Figure 3  G-banded Karyotype (48 chromosomes) of a metaphase from Case no. 7. Notice deletion of 1p− at p31, deletion long arm 13q−, an abnormal marker chromosome (M3), and extrachromosome number 8.
(Figures 2 & 3). Chromosome breaks were noticed in 12% and double minutes (dms) in 1% of the metaphases.

Discussion

The significant chromosomal abnormality found in the present study was a 1p− deletion in 6 out of 10 cases probably distal to band p31. In a review made of the literature pertaining to the cytogenetics of human neuroblastomas (Gagnon et al., 1962; Springs et al., 1962; Brewster & Garret, 1965; Cox et al., 1965; Makino et al., 1965; Cohen & Falk, 1967; Miles, 1967; Cox, 1968; Levan et al., 1968; Wakonig-Vaartaja, 1971; Schlesinger et al., 1976, Brodeur et al., 1977, 1981; Mitelman, 1983), 24 cases were found. Brodeur et al. (1977) observed deletion of the short arm of chromosome number one in 3/6 neuroblastoma cases examined. However the 6 cases were 2 primary explants (included in the 3 tumours with 1p−) and four established lines of human neuroblastoma preserved for 2 to 4 years. The same author and his associates in 1981 reported structural abnormalities of 1p− in 7/10 cases they investigated. However the partial 1p monosomy was confirmed by Haag et al. (1981), Gilbert et al. (1982) and Trent (1983). Other workers used conventional staining without the benefit of the banding techniques. Although none of them describe a 1p−, there were 4 cases in which there was a relative loss of a number 1 chromosome and a relative increase in C-group chromosomes (Cox et al., 1965; Whang-Peng & Bennett, 1968; Mark, 1970; Schlesinger et al., 1976). These may represent a 1p− aberration since a 1p− would appear like a C-group chromosome by conventional staining.

Most of the karyotypes of our ten tumours including the metastasis were diploid or peridiploid. The presence of the marker chromosomes in a fairly reasonable proportion would support the

**Table III** Selected cytogenetic findings from the neuroblastomas studied.

| Case no. | Total no. of cells examined | 1p− | Marker chromosome | dms |
|---------|-----------------------------|-----|------------------|-----|
| 1       | 106                         | +   |                  | +(1.8) |
| 2       | 109                         | −   | M1 (21.1)        | +1.8 |
| 3       | 102                         | +   |                  | +0.9 |
| 4       | 100                         | +   |                  | −   |
| 5       | 102                         | −   | M2 (10.7)        | −   |
| 6       | 104                         | +   |                  | +1.9 |
| 7       | 104                         | +   | M3 (20.1)        | −   |
| 8       | 102                         | +   |                  | +0.9 |
| 9       | 102                         | −   |                  | −   |
| 10      | 134                         | −   |                  | −   |

( )= Percentage of cells examined and showing the abnormal chromosome.
+ = present, − = absent.
theory of the clonal derivation of this tumour. However ~80% of the published karyotypes had modes that are in the periploid range (Brewster & Garrett, 1965; Wakonig-Vaartaja et al., 1971; Brodeur et al., 1977, 1981; Haag et al., 1981; Gilbert et al., 1982).

Double minutes were found in 1% of the metaphases of our neuroblastomas. On the other hand the dms were reported in 7/39 published cases (Cox et al., 1965; Cox, 1968; Leven et al., 1968; Sandberg et al., 1972; Schlesinger et al., 1976). They were present in ~70% of the cells but they varied in size and in number from one to >100 dms per cell.

Thus we agree with Brodeur et al. (1977, 1981) that deletion of the short arm of chromosome number 1 (del (1) (p31)) would appear to be relatively unique to neuroblastoma. On this assumption we can conclude that a regulatory gene that controls the transformation gene (Tr) of neuroblasts in present distal to the lp31 area. Deletion or mutation of such a gene may lead to the release of the Tr gene of neuroblastoma which may be present elsewhere on another chromosome.

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