Cytogenic investigations in the assessment of response to treatment in neuroblastoma

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SUMMARY In a patient with stage IV disseminated neuroblastoma treated by chemotherapy extensive cytogenetic investigations were performed on the residual primary tumour and bone marrow immediately before myeloablative treatment and autologous marrow rescue. Two abnormal clones both showing 1p⁺, a characteristic abnormality of neuroblastoma, were detected in cells from the residual primary tumour, providing direct evidence of persisting viable tumour. Such investigations should be a routine part of the assessment of response to treatment in patients with neuroblastoma, and could be extended to others in whom "second look" surgery is performed.

Most children over 1 year old with disseminated neuroblastoma will succumb to resistant disease. A prominent fibrous stromal background to tumour infiltrate in the bone marrow, both at diagnosis and subsequently, is characteristic of disseminated neuroblastoma. How much of this fibrous component is reactive and how much a product of the tumour itself is still unknown. With current histological techniques, it is difficult to determine whether residual tissue in marrow or primary site still contains viable tumour after chemotherapy. The presence of clonal karyotypic abnormalities characteristic of neuroblastoma in cells from residual scar tissue would provide evidence that viable tumour cells remained. We describe a child with stage IV neuroblastoma in whom intensive cytogenetic investigation of bone marrow and residual primary tissue was carried out immediately prior to myeloablative treatment and autologous marrow rescue.

Case report

Stage IV neuroblastoma was diagnosed in a 7 year old boy: this was based on infiltration of bone marrow with non-haemopoietic cells detected in marrow aspirate and a trephine biopsy specimen, a raised urinary vanillylmandelic acid (VMA) concentration, multiple

"hot" spots on bone scan, and the presence of a left adrenal primary tumour shown by ultrasound scanning. The cells in the marrow reacted with the monoclonal antibody UJ13A which detects cells of neuroectodermal origin.¹ Cytogenetic analysis of bone marrow cells at diagnosis showed apparently normal karyotypes in five metaphases. He received six courses of chemotherapy consisting of cisplatin and etoposide alternating with ifosphamide, vincristine, and adriamycin. On three occasions all four iliac crests were rebiopsied to assess response and showed a progressive decrease in frankly malignant cells but continued presence of fibrous tissue. Urinary VMA excretion returned to normal. Eight months after diagnosis he underwent a laparotomy to remove the residual shrunken primary tumour. Histological examination showed some normal adrenal tissue, fibrosis, calcification, ganglioneuroma and a few tiny foci of cells—possibly persisting neuroblastoma (fig 1). Some cells were shown by electron microscopy to contain neurosecretory granules. A decision was made to progress to myeloablative chemotherapy with high dose melphalan followed by autologous unpurged marrow rescue. At marrow harvest four further trephine biopsy specimens and aspirates of marrow were obtained for examination. No morphological evidence of continuing tumour infiltrate could be found. His marrow reconstituted and he remained well four months later.

Fig 1 Section of shrunken primary tumour showing residual adrenal tissue (bottom right) and scar tissue containing ganglion cells and smaller cells that may represent persisting neuroblastoma. (Haematoxylin and eosin.)

Material and methods

Bone marrow was cultured using standard techniques. The tumour tissue was cultured using direct and long term explant and suspension techniques. L-15 (Gibco) and Ham's F-10 (Gibco) media were used, supplemented with 15% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 µg/ml). Long term cultures were subcultured and harvested using conventional trypsinisation methods. Metaphase preparations were prepared after treatment with colcemid (0.08 µg/ml) and were stained using standard G-banding.

Results

BONE MARROW

Two hundred and fifty cells were analysed. All showed an apparently normal 46XY karyotype.

RESIDUAL PRIMARY TUMOUR

Results were obtained from the long term explant cultures (table). The direct and suspension cultures all failed. Representative partial karyotypes can be seen in fig 2. The precise nature of the 1p+ anomaly, common to both abnormal clones, is unclear. The abnormality of chromosomes 4 and 10 takes the form of an unbalanced translocation; examination of the 10q+ marker permitted precise description of 10pter → 10q23::4q11 → 4qter. The small acrocentric marker is assumed to represent the p arm and centromere of the deleted chromosome 4. The origin of the extra chromosomal material on chromosome 17p is unclear. Neither the t(2;12) nor the ?del 1p, + marker could be confirmed as clonal.

Discussion

On morphological, biochemical, and clinical grounds this patient responded to chemotherapy. The only histological abnormalities detectable before autotransplant was carried out consisted of patches of fibrous tissue in the marrow and the possibility of a few viable tumour cells in the shrunken primary. At the time of marrow harvest there had apparently been further improvement in the bone marrow histology.

Bone marrow obtained at the harvest was karyotypically normal, but the long term explant cultures of cells from the residual primary tumour showed the presence of two karyotypically abnormal clones. Three consistent cytogenetic anomalies have previously been described in association with neuroblastoma: double minute chromosomes; homogeneously staining regions; and rearrangements of distal 1p. Although the origin of the 1p anomaly in this case is not known, the chromosome is similar in appearance to that described by Cowell and Rup-

Table Results of cytogenetic analysis of metaphases derived from residual primary tumour

Method	No of cells	Karyotype
Ham's F-10 culture 1	29	46, XY
	3	46 XY, 1p +
Ham's F-10 culture 2	85	46, XY
	10	46, XY, 1p +, t(4; 10), 17p +
	1	46, XY, t(2; 12)
	1	46 XY, ? del 1p, + mar
L-15 culture 1	3	46, XY

Fig 2 Partial karyotypes of abnormal clones: (a) 1p+ as sole abnormality in culture 1; (b) 1p+, t(4;10), and 17p+ in culture 2.

niak,² who tentatively concluded that a homogeneously staining region was present distal to band 1p31. In the case reported here the 1p+ anomaly could equally well have resulted from the translocation on to 1p of other chromosomal material, but there may also have been a deletion of at least part of 1p32-1pter. Brodeur *et al* reported deletions of this region at a high frequency in neuroblastoma cells,³ which have subsequently been confirmed in other studies.⁴ The 10q breakpoint in the t(4;10) seems to be 10q23. This is known to be a common fragile site.⁵ Although there is also a common fragile site at 17p12, this could not be confirmed as the breakpoint in our 17p+ marker. No double minute chromosomes were observed in this study.

It is reasonable to assume that the 13 cells containing 1p+ represented viable neuroblastoma cells. The abnormalities were clonal and did not resemble those induced by chemotherapy. On the other hand, the absence of similar clonal abnormalities in the bone marrow can not be taken as evidence that the marrow was free of tumour. With the recovery of haemopoiesis, which usually accompanies chemotherapy in these children, there is considerable dilution of any persisting tumour cells. The analysis of 250 cells would exclude the presence of a clone at the 1% level with 95% confidence limits⁶ only if its members underwent mitoses as often as the haemopoietic cells.

This study provides direct evidence of the presence of viable tumour cells in a shrunken primary tumour and raises the possibility that similarly viable cells may be present in the residual fibrous tissue so often found in the bone marrow. When the primary tumour is removed in patients with neuroblastoma, cytogenetic investigation of any biopsy tissue should be

routinely performed. Information from such studies may make unequivocal the often ambiguous remission state of many children with disseminated neuroblastoma, permit better evaluation of their response to treatment, and provide the basis for a rational approach to further treatment. This approach could be extended to patients with other solid tumours in whom "second look" surgery is performed.

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