Chromosome 22 abnormalities in Ewing's sarcoma

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SUMMARY A child with disseminated Ewing's sarcoma underwent cytogenetic investigations which showed different structural rearrangements of chromosome 22 at diagnosis (?ring22), and at relapse [t(10; 22)], but the classic translocation t(11; 22) was not detectable. This case provides further evidence of the importance of chromosome 22 in this disease, while raising some questions about the central importance of the translocation between chromosomes 11 and 22.

Ewing's sarcoma is one of the commonest primary malignancies of bone in children. It tends to spread to lungs, bone, and bone marrow. The morphological diagnosis of marrow infiltrate with Ewing's sarcoma cells may be difficult, but the presence of chromosomal abnormalities consistent with Ewing's sarcoma in bone marrow would strongly support the diagnosis of metastatic disease. For this reason, staging marrow samples are examined cytogenetically at this centre. The translocation t(11; 22) is well established as a clonal abnormality in Ewing's sarcoma.¹ We present a case of disseminated Ewing's sarcoma in which, both at diagnosis and relapse, abnormalities of chromosome 22 were found, but not the t(11; 22).

Case history

An 8 year old girl, one of identical twins, presented with a four month history of painful swelling of her left iliac crest, weight loss, anorexia, and fever. A hard mass was palpable in the left iliac fossa in continuity with the iliac crest. An x-ray picture of her pelvis showed spiculated new bone formation and mixed sclerotic and lytic lesions of the left ilium and a large soft tissue mass. A chest x-ray picture and a computed tomogram of her chest showed no evidence of metastases. Isotope bone scan showed one area of increased uptake at the site of the primary tumour, but no evidence of other bone disease. A biopsy specimen of the primary tumour showed anaplastic round malignant cells with focal areas of necrosis. The cells had regular round nuclei with a few tiny nucleoli, and had some poorly defined cytoplasm. There was a paucity of reticulin in the tumour and some cells were periodic and Schiff positive, but negative after diastase.

Antibodies to vimentin reacted positively with the tumour, but antibodies to neurone specific enolase, S100, cytokeratin, myosin and common leucocyte antigen were all negative.

Electron microscopic examination showed that the predominant population of cells had poorly defined margins, empty cytoplasm, irregular round nuclei and a few nucleoli. Some intracellular and extracellular glycogen was present. A smaller cell population was present which contained irregular amounts of rough cytoplasmic reticulin. These are the features typically described in Ewing's sarcoma.² Examination of bone marrow from the left iliac crest showed replacement with primitive cells which represented direct extension of the primary tumour. Marrow aspirated from the right iliac crest showed no infiltrate, but a trephine biopsy specimen from this site showed a single substantial deposit of tumour cells, establishing a diagnosis of disseminated disease.

Despite treatment with ifosfamide, vincristine, and adriamycin, followed by treatment with etoposide, melphalan, total body irradiation 10 months after diagnosis, and a marrow transplant from her identical twin, she relapsed with disseminated disease affecting bone and bone marrow, but not lungs. Bone marrow samples at diagnosis, at intervals during her treatment, and at relapse were examined cytogenetically.

CYTOGENETIC METHODOLOGY

Bone marrow samples were cultured overnight in RPMI medium supplemented with FCS, glutamine, and antibiotics. Colcemid (at a final concentration of 0.02 µg/ml), was added for the final 15 minutes of culture. Harvesting was carried out using standard methods and slides were aged overnight at 60°C before trypsin-Leishman G-banding.

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Table Cytogenic analysis at diagnosis and during progression of disease

Stage	No of cells	Karyotype
At diagnosis:		
Left iliac crest (primary tumour)	No meta-phases	
Right iliac crest	30	46,XX
	2	45,X,-7,-10,-17,+del(17)(p11)-21,-22,+r(22),+M ₁
Three months after diagnosis	20	46,XX
Six months after diagnosis	20	46,XX
At relapse (10 months after diagnosis)	30	51,XX,t(3;18)(q13;q12-3)t(10;22)(q14;q12),-1,+1p+,-del(1)(p33),-2,+2p+,-5,+5q-,-8,-17,+del(17)(p11),+t(17p:?),+M ₁ ,+M ₂

Results

The cytogenetic results are shown in the table. At diagnosis only two cells showed an abnormal

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karyotype, which, because of poor preparations, were difficult to interpret completely. There seemed to be a structural abnormality of chromosome 22, however, which was tentatively identified as a ring chromosome, a del(17)(p11) chromosome, and a marker chromosome which could not be identified. Over the next six months her marrow was examined twice but there was no evidence of the abnormal cell line. At relapse, all the metaphase spreads had a hyperdiploid karyotype (figure). There was no evidence of the structurally abnormal 22, but there was a t(10;22) translocation. The breakpoint in the 22 seemed to be at 22q12. The small marker chromosome (M₁) consisted of 17p but the long arm could not be identified; the larger marker chromosome (M₂), which was represented twice, was a translocation between Xp and 8q.

Discussion

Ewing's sarcoma shares with rhabdomyosarcoma, neuroblastoma, and some neuroectodermal tumours the propensity to disseminate to the bone marrow. The translocation t(11; 22)(q23; q12) is useful in differentiating this disease from rhabdomyosarcoma and neuroblastoma¹³ but because it is also associated with

Figure Karyotype at relapse of disease.

some primitive neuroectodermal tumours,⁴ it cannot be regarded as a diagnostic marker for Ewing's sarcoma. Detection of this translocation in an otherwise normal marrow of a patient with Ewing's sarcoma would provide convincing evidence of marrow infiltration. In the case described here, infiltration was morphologically detectable in the iliac crest opposite the primary tumour. It is therefore reasonable to assume that the two abnormal metaphases obtained from this site at diagnosis represented tumour cells. This is strengthened by the presence of the abnormal del(17)(p11) at diagnosis and relapse. All the cells were abnormal at relapse.

Rearrangements of 22q are over-represented in both the neoplasias and constitutional translocations. The translocations t(8; 22) in Burkitt's lymphoma t(9; 22) in chronic myeloid leukaemia, and the constitutional t(11; 22) translocations have all been shown to be proximal to the Ewing's sarcoma breakpoint.⁵ In the present case the breakpoint is thought to be at 22q12, which agrees with the published data.

The translocation t(11; 22) was not detected at diagnosis or relapse in the present case; the actual role of this translocation is still unclear. Neither the *c-sis* oncogene on 22q or the *c-ets* oncogene on 11q seem to be implicated. Chromosome 22 is also seen in translocation with other chromosomes in Ewing's sarcoma. Aurias *et al* reported three cases of primary Ewing's sarcoma with translocations t(2; 22), t(9; 22) and t(1; 22) respectively.³ The case with the t(1; 22) also had a typical t(11; 22). Bennett *et al* reported an i(11q) in a cell line established from a metastatic lesion,⁶ but the breakpoint on the chromosome 11 differed from that in the (11; 22). These reports, together with the present case, suggest that chromosome 22 may have a more important role than 11, and that the microscopically detectable translocation may not be central to the pathogenesis of Ewing's sarcoma. Further experience may show, as in chronic granulocytic

leukaemia, more subtle molecular changes which may precede and be of more importance than the complete translocation.

The further cytogenetic changes noted in our patient, have been reported previously in Ewing's sarcoma, but are also common in other neoplastic diseases. As more data accumulate it may be possible to relate these additional changes to progression of the malignancy, and further strengthen the case implicating genetic material of 22q12 in the development of Ewing's sarcoma.

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