Rapid and specific diagnosis of t(11;22) translocation in paediatric Ewing's sarcoma and primitive neuroectodermal tumours using RNA-PCR

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Abstract

One case of paediatric Ewing's sarcoma and two peripheral primitive neuroectodermal tumours/extra-osseous Ewing's sarcoma were studied for the characteristic t(11;22) translocation, using a recently described RNA-polymerase chain reaction method (RNA-PCR). PCR products of the expected sizes were obtained from RNA derived from the Ewing's sarcoma and the peripheral primitive neuroectodermal tumours, but not from other paediatric malignancies. Direct sequencing of the RNA-PCR products confirmed the presence of the EWS-FLI-1 fusion transcript. In one case the presence of the translocation was confirmed by cytogenetic analysis. These results highlight the potential use of PCR for the rapid demonstration of diagnostically important tumour specific chromosomal rearrangements.

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Ewing's sarcoma is a bone tumour of childhood, which together with other peripheral primitive neuroectodermal tumours, often has a characteristic chromosome translocation; t(11;22) (q24;q12).^{1,2} The translocation can sometimes be demonstrated cytogenetically, but this relies on the availability of viable tumour tissue. Recently the Ewing's sarcoma translocation breakpoint has been cloned.³ As a result of this rearrangement a fusion mRNA transcript is formed between the 5' end of a novel gene, EWS, and the 3' end of the FLI-1 oncogene.⁴ Using primers within the EWS and FLI-1 sequences, RNA-polymerase chain reaction (RNA-PCR) products can be amplified from Ewing's sarcoma and peripheral primitive neuroectodermal tumour RNA, which are specific for this class of malignancy.⁴ In this report we demonstrate the potential use of the technique in the rapid diagnosis of paediatric malignancies.

Case reports

The tumour in case 1 arose in a 15 year old boy, who presented with a six month history of occasional febrile episodes, sometimes associated with frequency, urgency, and dysuria, which responded to antibiotic treatment. After one such episode, ulceration and

bruising of the glans penis were noted, associated with leg pains and numbness in the buttock area. Rectal examination revealed a large pelvic mass, which on computed tomogram, was shown to extend throughout the pelvis, with no obvious site of origin. There was a mass of iliac lymph nodes on the left side and multiple pulmonary metastases. The patient was receiving chemotherapy at the time of writing.

Histological examination (fig 1) of the tumour biopsy specimen showed sheets of cells separated by a fairly vascular fibrous stroma. The cells had scanty, faintly eosinophilic cytoplasm, with ill defined borders. The nuclei were large and round, with a coarse chromatin pattern and some had a small nucleolus. Mitoses were frequent and there was no necrosis, although there were scattered apoptotic cells. Some glycogen was present. Immunohistochemistry showed that some tumour cells were positive for vimentin and O13 (Signet Laboratories, Dedham, Massachusetts, USA), an antibody that recognises the HBA17 Ewing's sarcoma cell surface antigen.⁵ Occasional cells were also positive for both high and low molecular weight keratins (antibodies MNF116 and CAM5.2). Neurone specific enolase, chromogranin, and S100 were negative, as were CD45, CD20, CD45R0, epithelial membrane antigen and desmin. Electron microscopy showed the presence of glycogen, some of which was present as small lakes. Cell junctions could be identified in occasional cells, but no true desmosomes were present. Cytogenetic analysis revealed an abnormal karyotype: 49XY, + 8, t(11;22) (q24;q12), +der(11), +14. PNET/extra-osseous Ewing's

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Figure 1 High power view of tumour from case 1 (haematoxylin and eosin).

Figure 2 RNA-PCR of the Ewing's sarcoma fusion transcript. Lane 1, case 1; lane 2, Askin tumour (degraded RNA); lane 3, case 2; lane 4, osteosarcoma; lane 5, no RNA; lane 6, no RNA; lane 7, case 2; lane 8, osteosarcoma; lane 9, case 3; lane 10, rhabdoid tumour; lane 11, fetal kidney. Lanes 1–5 and 6–11 are from separate gels. Positions of molecular weight markers (in base pairs) are shown on the left.

sarcoma was diagnosed, in view of the tumour's extra-osseous location, focal cytokeratin positivity, and karyotype.

Case 2 was a 7 year old boy, who presented with a swelling on the left side of the chest. An x ray picture showed that the left 10th rib was destroyed, and a computed tomogram confirmed the presence of a large intrathoracic mass arising from the rib. No metastases were detected, and the patient was treated with chemotherapy, then resection of the entire affected rib, followed by a course of radiotherapy, as some viable cells were found in the resected rib. The patient remains alive and well, and is now seven years on from diagnosis.

The second case was diagnosed as Ewing's sarcoma and was morphologically similar to the first, but with more abundant glycogen. Immunohistochemistry showed positivity for O13 and weak focal positivity for neurone specific enolase. The tumour cells were negative for vimentin, chromogranin, S100, MNF116, CAM5-2, CD45, CD20, CD45R0, epithelial membrane antigen and desmin. Tissue was not examined ultrastructurally, and cytogenetics were not performed routinely when this case was diagnosed (1986).

Case 3 was a 9 year old girl who presented with muscle weakness and pain in her right leg. Investigations revealed a mass in the right psoas muscle and retroperitoneum which surrounded the right kidney. The right lateral processes of the lumbar vertebrae were also affected.

Histology showed a small blue round cell tumour similar to cases 1 and 2, and PNET/extra-osseous Ewing's sarcoma was diagnosed. The tumour cells contained some glycogen and they were positive for vimentin and O13. All other immunohistochemical markers were negative. Electron microscopy showed glycogen lakes. Cytogenetic analysis of this tumour proved technically difficult, but there was some indication of a rearrangement involving chromosome 11, which was not inconsistent with an 11;22 translocation.

RNA was extracted from snap frozen tumour tissue, as described before.⁶ RNA-PCR was performed as described by Brown *et al.*,⁷ using primer 11A for the reverse transcription reaction, followed by primers 11-3 and 22-3 for the cDNA amplification (primer sequences as detailed in Delattre *et al.*⁸). RNA-PCR conditions were 45 minutes at 50°C for the reverse transcription, followed by a three minute denaturation step at 94°C, and then 30 cycles consisting of 30 seconds at 94°C, one minute at 65°C and one minute at

72°C. PCR products were separated on 2% agarose/1% NuSieve gels, and bands were directly sequenced using a 'Sequenase' kit⁸ (USB).

RNA was successfully amplified from all three tumours described above and products of about 350 base pairs in size were obtained from case 1 (fig 2, lane 1) and case 2 (fig 2, lanes 3 and 7), and 650 base pairs from case 3 (fig 2, lane 9). These correspond to the type 1 and 3 fusion transcripts, respectively, as described by Delattre *et al.*⁴ Direct DNA sequencing of the RNA-PCR products from cases 1 and 2 confirmed that they represented EWS-FLI-1 type 1 fusion transcripts.

No RNA-PCR products were obtained using the Ewing's sarcoma translocation primers on intact RNA obtained from fetal kidney tissue (fig 2, lane 11), and osteosarcoma (fig 2, lanes 4 and 8), a rhabdoid tumour (fig 2, lane 10), or from a Wilms' tumour (data not shown). All these control RNAs were amplifiable with appropriate control primers (HPRT, hypoxanthine phosphoribosyl transferase; data not shown), showing that the negative results with the Ewing's sarcoma primers were true negative results. Degraded RNA from an Askin tumour (fig 2, lane 2) also gave no product.

Ewing's sarcoma and peripheral primitive neuroectodermal tumours have been regarded as morphologically similar but distinct tumours, partly on the basis that Ewing's sarcoma arises in bone whereas peripheral primitive neuroectodermal tumour is a soft tissue tumour. However, the situation is confused by the description of extra-osseous Ewing's sarcoma,⁹ peripheral primitive neuroectodermal tumours in bone,¹⁰ and the related small round cell tumour of the thoracopulmonary area (Askin tumour). In the 1980s both Ewing's sarcoma and peripheral primitive neuroectodermal tumours were shown to have an identical cytogenetic abnormality; t(11;22) (q24;q12).¹² This characteristic abnormality is found in 85–90% of Ewing's sarcoma and peripheral primitive neuroectodermal tumours, and it is now therefore considered that Ewing's sarcoma and peripheral primitive neuroectodermal tumours form a single group of related tumours of neuroectodermal origin.

The undifferentiated nature of this group of tumours may cause difficulties with histological diagnosis, and often Ewing's sarcoma/peripheral primitive neuroectodermal tumour is diagnosed more by the exclusion of other conditions than by the finding of positive features. This report shows that the

amplification of the fusion transcript caused by the t(11;22) translocation is a useful diagnostic tool. The procedure takes about two days at present, because RNA is purified using an ultracentrifugation method which requires an overnight run.⁶ The PCR method has clear advantages over cytogenetic analysis, as it is faster and does not require viable tissue, although frozen tissue is necessary at present. However, it is possible to perform RNA-PCR with impure preparations of RNA, and so simpler methods of RNA extraction could be used to speed up the procedure. They might also permit the use of very small tissue samples—for example needle biopsy specimens or bone marrow samples, or archival paraffin wax embedded tissue. Using in situ PCR, it may be possible to detect the Ewing's sarcoma fusion transcript on histological sections, which could be useful in detecting small metastatic deposits or residual disease in the bone marrow.

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