Virchows Arch (1995) 426:519–522

CASE REPORT

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A case report of synovial sarcoma with translocation (X;18). Application of fluorescence in situ hybridization to paraffin-embedded tissue

Received: 4 January 1995 / Accepted: 30 March 1995

Abstract A 57-year-old female patient with synovial sarcoma in her right foot had a chromosome abnormality defined as translocation (X;18). The tumour was located in the subcutis, and histological investigation showed monophasic proliferation of oval to spindle-shaped cells with a fascicular arrangement lacking an epithelial component. Immunostaining disclosed no cytokeratin or epithelial membrane antigen in tumour cells. Karyotypic analysis revealed translocation (X;18) in addition to other nonspecific aberrations. Fluorescence in situ hybridization was carried out on paraffin-embedded tissue, using DNA probes for the centromeres of chromosomes X and 18 with whole chromosome painting probes for X and 18. The free nuclei showed two signals at a rate of 83–85% with the X and 18 centromeric probes, in contrast to three signals at a rate of 68–70% with the X and 18 painting probes.

Key words Synovial sarcoma · X;18 translocation · Fluorescence in situ hybridization (FISH) · Paraffin-embedded tissue

Introduction

Synovial sarcoma is a highly malignant tumour, mainly occurring in adolescents and young adults [1]. Diagnosis of synovial sarcoma may pose a considerable challenge to the pathologist owing to the lack of an epithelial component. Additional diagnostic aids, such as histochemical staining, electron microscopy or immunohistochemistry, have been tried in cases with predominant monophasic features, in order to discriminate them from fibrosarcoma, leiomyosarcoma and neurogenic tumours.

A chromosome rearrangement, translocation (t(X;18)), has been reported in several cases of synovial sarcoma as a specific chromosomal abnormality [2–7], but it remains unclear whether this translocation is present in all cases and is thus useful for diagnosis. The breakpoints of t(X;18) have been defined as mapping to Xp11.2 and 18q11.2 [8–11]. We report a case of synovial sarcoma in which we investigated the occurrence of t(X;18) by both conventional cytogenetic analysis and fluorescence in situ hybridization (FISH) with chromosome-specific DNA probes on nuclei isolated from paraffin-embedded sections.

Case report

The patient, a 57-year-old woman, had a soft, solid tumour on the inner side of the right foot, which had first been noticed in November 1993. She was admitted to hospital because it was gradually increasing in size. A mass lesion was detected in the deep cutis of the inner side of the right foot on physical examination. Radiological examination revealed an ill-defined, homogeneous tumour shadow in the deep cutis on computerized tomography scan and magnetic resonance imaging examination (Fig. 1). Laboratory data were within normal limits. Needle biopsy was carried out to determine the histological type of the tumour and to decide on the best surgical approach. Synovial sarcoma was suspected on the basis of the histological findings. The limb was amputated below the knee after preoperative chemotherapy. Since then (10 months to the time of writing), the patient has shown neither recurrence nor metastasis.

Materials and methods

For light microscopy, paraffin sections were stained with periodic acid-Schiff (PAS), colloidal iron, Masson's trichrome and silver impregnation for reticulin in addition to haematoxylin and eosin stain.

To eliminate other types of spindle cell sarcomas from the diagnosis of synovial sarcoma, immunostaining was carried out on paraffin-embedded sections, employing the avidin-biotin peroxidase complex method after reaction with the following antibodies:

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vimentin (monoclonal, 1:100, Dakopatts, Copenhagen, Denmark), S-100 (polyclonal, 1:150, Dakopatts), desmin (monoclonal, 1:50, Nichirei, Tokyo), alpha smooth muscle actin (monoclonal, 1:50, Nichirei), and also cytokeratin (polyclonal, 1:50, Nichirei) and EMA (monoclonal, 1:50, Nichirei).

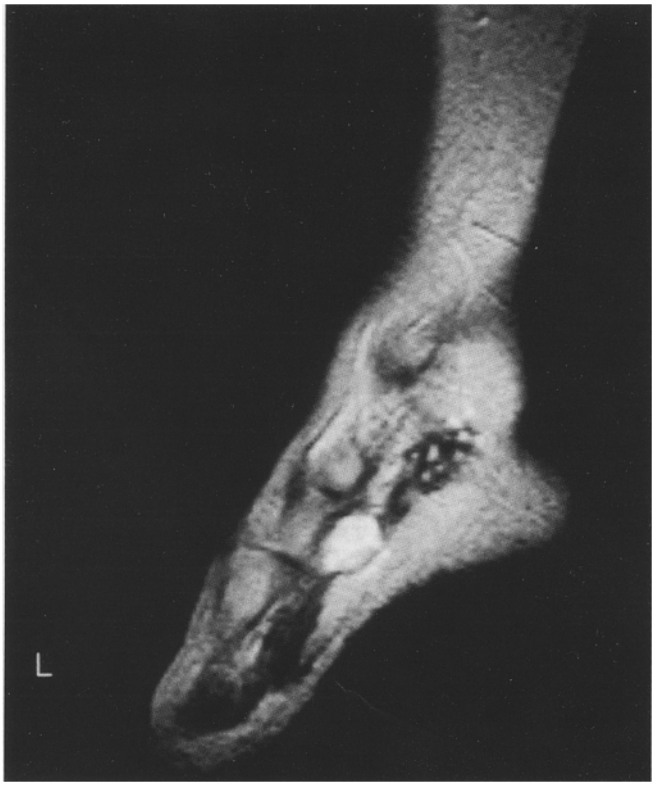

Fig. 1 A mass lesion in the subcutis of the inner side of the right foot was detected by MRI examination

FISH (fluorescence in situ hybridization) analysis was performed on formalin-fixed, paraffin-embedded tissue. The tissues were cut to a thickness of 50 μm from paraffin blocks and disaggregated according to the method reported by Schutte et al. [12]. Biotinylated alpha satellite centromeric probes for chromosomes X and 18 (DXZ1, D18Z1), and whole chromosome painting probes (Coatasome X, Coatasome 18) were purchased from Oncor. Both probes and DNA of the tumour cell nuclei on the slides were denatured at 70–75°C for 3–10 min, and hybridized at 37°C for 17 h. Detection and amplification were performed according to the manufacturer's instructions [13]. Hybridization signals were observed under a fluorescent microscope (Nikon, Tokyo, Japan) (Fig. 3). The number of signals in 100–200 nuclei was counted for evaluation of the possible translocation.

Fresh tumour tissue was used for karyotypic analysis within 30 min after surgical removal. The tissue was cross-minced with opposing scalpel blades and disaggregated overnight in 200 U/ml collagenase solution (Sigma, St. Louis, Mo.) [14]. After short-term culture for 7 days, cells in metaphase were harvested. Chromosomes in G band were prepared according to conventional techniques and stained by the trypsin-Giemsa method [15]. Analysis was conducted on appropriate numbers of cells in metaphase.

Results

Pathological findings

The tumour measured 2×1.5×1.5 cm, and appeared as an ill-defined mass located in the subcutis of the right foot. It was yellowish-white and soft with a small necrotic area on its cut surface.

Microscopic findings

The tumour consisted mainly of a packed cell proliferation of round to plump, spindle-shaped cells in a some-

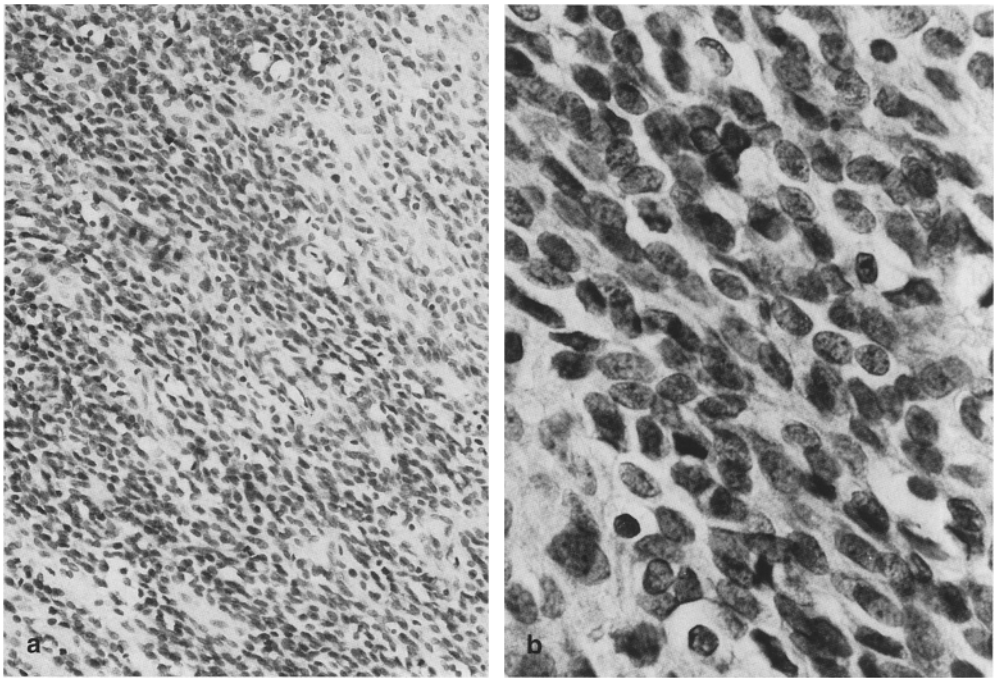

Fig. 2 The tumour consisted mainly of a packed cell aggregate of round to plump spindle-shaped cells in a fasciculated arrangement lacking an epithelial component (*left* ×100, *right* ×520)

Table 1 FISH analysis of chromosome X and 18

	Copy number of chromosome X					Copy number of chromosome 18				
	0	1	2	3	4≤	0	1	2	3	4≤
Centoromeric probe	2	12	85	1	0	4	7	83	5	1
Total probe	2	10	19	68	1	2	10	17	70	1

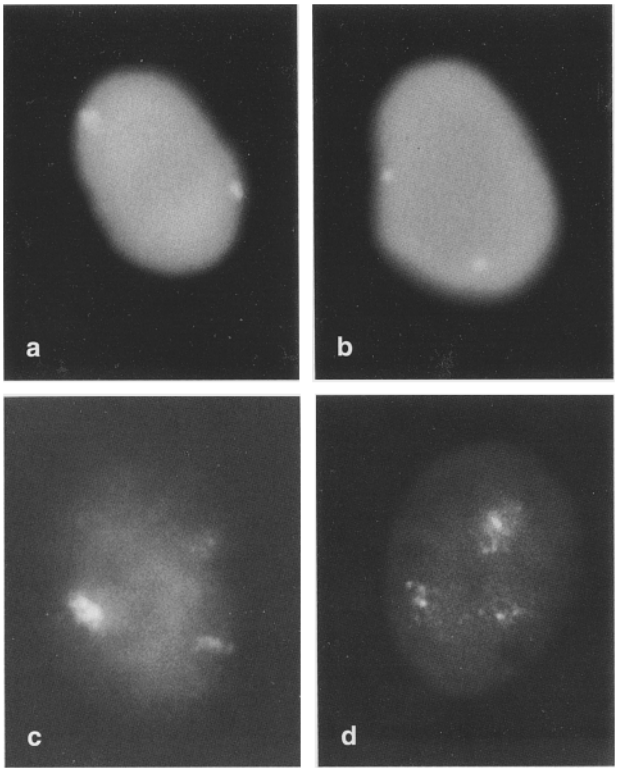

Fig. 3–d Fluorescent signals of chromosomes X and 18. The centromeric probes of **a** chromosome X and **b** chromosome 18 each showed two signals. The probes of **c** the entire chromosome X and **d** chromosome 18 each showed three painting signals

what fasciculated arrangement. Mitotic cells were scattered abundantly throughout the tumour. Fine vascular channels were easily recognized in the stroma, but collagen fibers were rarely found with Masson's trichrome stain. There was neither an epithelial component forming glandular structure at any place in the tumour tissue nor a myxoid stroma on colloidal iron staining (Fig. 2). Electron microscopy was performed on the tumour cells, showing fibroblastic features with well-developed rough endoplasmic reticulum, but neither microvilli nor a microtubular structure. Immunostaining revealed positive reactivity for vimentin but was negative for EMA, cytokeratin, S-100, desmin and alpha smooth muscle actin. Thus, the monophasic type of synovial sarcoma was strongly suggested by its location and the characteristics of the tumour cells negative for antibodies of differential antigens such as S-100, desmin and alpha smooth muscle actin. However, the final diagnosis remained uncertain after differentiation of the tumour from other soft-tissue sarcomas.

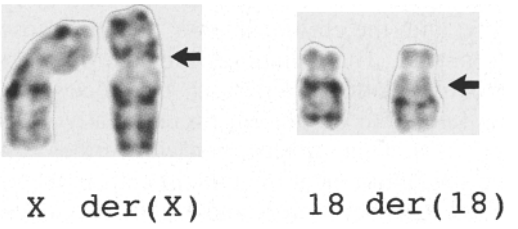

Fig. 4 Karyotype shows t(X;18)(p11.2;q11.2). The *arrows* indicate translocation breakpoints on the rearranged chromosomes

FISH analysis

The cumulative results of FISH analysis are shown in Table 1. The nuclei showed two signals at a rate of 85% with the X chromosome centromeric probe and at 83% with the chromosome 18 centromeric probe. Three fluorescent signals with additional small signals were defined at 68% with the X chromosome painting probe and at 70% with the chromosome 18 painting probe (Fig. 3).

Karyotypic analysis

Cytogenetic abnormality was confirmed in a population of 40 cultured cells, which included 37 tumour cells with a specific karyotypic change of 44, X, t(X;18)(p11.2;q11.2), -9, +dic(9;?)(p22;?), del(11)(p11), -22, -22. This change is shown in Fig. 4, the arrows indicating translocation t(X;18) breakpoints on the rearranged chromosomes. The remaining three tumour cells had other multiple changes.

Discussion

Synovial sarcoma accounts for 5–10% of all soft-tissue sarcomas and is characterized histologically as a biphasic tumour with distinct epithelial and spindle cells. It is difficult to distinguish from other spindle-cell sarcomas, such as fibrosarcoma, leiomyosarcoma and neurogenic tumours, even with the help of electron microscopy and immunohistochemistry, when there is a monophasic spindle cell proliferation. However, several studies on synovial sarcoma indicate a characteristic chromosome rearrangement defined as a specific translocation, t(X;18)(p11.2;q11.2) [16].

Our case was strongly suspected to be synovial sarcoma, but the tumour showed no histological evidence to distinguish it from other spindle-cell sarcomas; it

showed a monophasic proliferation of spindle-shaped cells lacking a glandular pattern. In addition, immunostaining for EMA and cytokeratin was negative. This raised the possibility of fibrosarcoma. FISH analysis, however, revealed two signals at a rate of 85% and at 83% with the chromosome X and chromosome 18 centromeric probes, while an additional small signal to large and small signals was observed at 68% and at 70%, respectively, with the chromosome X and chromosome 18 painting probes. These findings indicate the occurrence of the chromosomal aberration, translocation (X;18), which is known to be present preferentially in synovial sarcoma [2–7]. This translocation was further confirmed by karyotypic analysis of the tumour cells in metaphase.

Translocation between X and 18 has very occasionally been found in other tumours originally diagnosed as fibrosarcoma [17] and malignant fibrous histiocytoma [7]. Cin et al. [18] suggested that these two cases might represent histological variations of synovial sarcoma.

FISH with chromosome-specific DNA probes was developed by Lee et al. [19]. This technique can detect chromosomal aberrations on paraffin-embedded tissues, allowing screening for specific chromosomal rearrangements in synovial sarcoma as well as in other histological types of sarcomas, using a wide panel of DNA probes.

We detected the X;18 translocation in this case by two different methods: FISH analysis with chromosome-specific DNA probes and karyotypic detection in metaphase preparations of the fresh tumour cells. It is our conclusion that the final diagnosis of this tumour should be given as monophasic type of synovial sarcoma, and that the FISH technique is suitable for analysis in tumour cells from paraffin-embedded tissues. It is a useful aid to correct diagnosis of synovial sarcoma.

Acknowledgements This study was supported in part by a grant (no. 04151019) from the Ministry of Education, Tokyo, and also by a grant from the Vehicle Racing Commemorative Foundation, Tokyo, Japan.

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