

Short reports

Rapid differential diagnosis of myxoid liposarcoma by fluorescence in situ hybridisation on cytological preparations

A Mezzelani, G Sozzi, M A Pierotti, S Pilotti

Abstract

In two cases of suspected myxoid liposarcoma, where chromosomal metaphase preparations were not available, fluorescence in situ hybridisation was performed on interphase nuclei of cytological preparations for the detection of the specific translocation, t(12;16), characteristic of this tumour and of trisomy 8, which is the most frequent secondary chromosome aberration. Probes directed against chromosomes 12 and 16 and against the centromeres of chromosomes 12 and 8 were hybridised on cell brushings and cytocentrifuge preparations. The finding of three painting domains of both chromosomes 12 and 16 and of only two signals with the centromeric probe directed against chromosome 12, suggested the presence of t(12;16) in both cases. In one case trisomy 8 was inferred from the occurrence of three centromere 8 signals. This approach can be used to detect specific chromosomal abnormalities when an urgent differential diagnosis is requested or when chromosome preparations are not available, or both.

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diagnosis in uncertain cases or for use in retrospective studies.²

Methods

Cytological preparations from two sarcoma recurrences were studied. Histologically, the first recurrence was suggestive of typical myxoid liposarcoma, although the primary tumour had been categorised elsewhere as well differentiated. The second recurrence, the primary diagnosis of which was unknown, was suggestive of the cellular variant of myxoid liposarcoma.

The cytological preparations obtained from the tumour specimens were hybridised with painting and centromeric probes directed against chromosomes 12 and 16 in order to detect t(12;16)(q13;p11), which is characteristic of this tumour.³ As a positive control, the same probes were used on cytological preparations from a myxoid liposarcoma with a t(12;16)(q13;p11), as assessed by conventional cytogenetic and FISH analyses.

As trisomy 8 has been reported in about 18% of myxoid liposarcomas as an additional, non-random abnormality,⁴ the α satellite probe directed against chromosome 8 was also hybridised on the cytological preparations of these patients.

Cytological preparations were harvested using standard methods. Briefly, tumour cells were mounted on poly-L-lysine coated slides,⁵ either by brushing or by cytocentrifuging tumour cell suspensions in phosphate buffered saline (PBS) and PreservCyt at 400 rpm. Biotinylated whole chromosome painting DNA probes directed against chromosomes 12 and 16 (Cambio Probes (Cambridge, UK), distributed by Technogenetics) and α satellite probes directed against chromosomes 12 and 8 (Oncor, Gaithersburg, Maryland, USA) were denatured and hybridised on denatured slides as described by Lichter⁶ and according to the manufacturer's instructions.

The hybridisation signal was detected with two layers of avidin conjugated fluorescein isothiocyanate. The nuclei were counterstained

Keywords: fluorescence in situ hybridisation, chromosomal metaphase preparations, myxoid liposarcoma.

Most sarcomas are characterised by recurrent chromosomal abnormalities detectable by cytogenetics or fluorescence in situ hybridisation (FISH). Many of these chromosome aberrations are of diagnostic or prognostic, or both, importance.¹ Cytogenetic analyses give a complete morphological assessment of the total chromosomal complement but require living tumour cells and good quality metaphases and banding preparations.

Here, we describe the application of FISH to cytological preparations for rapid, differential

Divisione di Anatomia Patologica e Citologia, Istituto Nazionale dei Tumori, Milan, Italy
A Mezzelani
S Pilotti

Divisione di Oncologia Sperimentale A
G Sozzi
M A Pierotti

Correspondence to:
Alessandra Mezzelani,
Divisione di Anatomia Patologica e Citologia,
Istituto Nazionale dei Tumori, Via G. Venezian 1,
20133 Milan, Italy.

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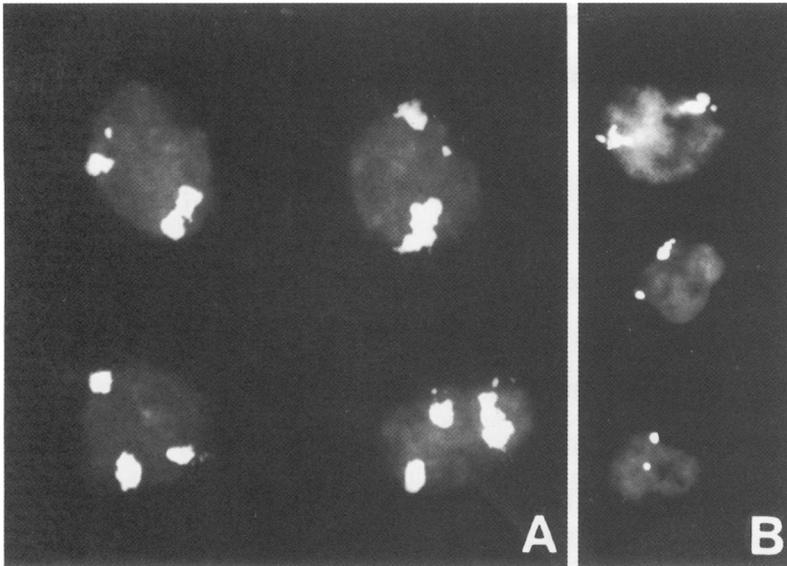


Figure 1 (A) Nuclei from cytological preparations hybridised with a painting probe directed against chromosome 12 showing three unequally sized signals. The smallest signal corresponds to the size of the segment translocated to chromosome 16. The differences in size among the four nuclei is related to the difficulty in finding three painting signals in the same plane of focus. (B) Nuclei showing two centromeres of chromosome 12.

with 4',6-diamidino-2-phenylindole dihydrochloride hydrate (DAPI) and viewed through a Zeiss Axioscop fluorescence microscope. Image detection was performed with a cooled CCD camera (Photometrics, Tucson, Arizona, USA), coupled to the microscope and controlled by a Power Macintosh 7100/80 PC. The IPLAB Spectrum software (Signal Analytics, Vienna, Virginia, USA) was used to acquire images that were then pseudocoloured and merged using Gene Join software (Yale University).

Results

Hybridisation of the painting probes directed against chromosomes 12 and 16 showed, both in the patients and positive control, three signals of differing sizes: the largest domain corresponded to the normal chromosome and the other two to its derivatives (fig 1A).⁷ Interestingly, in some of the whole chromosome painting signals the centromere was appreciable as a little nick. This is because painting probes do not contain the α satellite sequence corresponding to the centromeric region. As the detection of two centromeric signals for chromosome 12 excluded trisomy, t(12;16) was the most probable occurrence. In one case trisomy 8 was inferred from the occurrence of three centromere 8 signals.

Discussion

As expected, an average of 40% of whole nuclei carried two normal painting domains for chromosomes 12 and 16. Two chromosome 8 and 12 centromere signals related to the presence of non-tumour cells infiltrating the specimen.

As probes to very large targets, such as painting probes, produce large hybridisation domains and increase the chance for domain

overlap, this approach can be useful in detecting major translocations in near-diploid cells. For this reason, painting probes cannot be used successfully in the analysis of solid tumours with a karyotypically heterogeneous population or with a high DNA index. Indeed, the presence of many additional chromosomes of one type produces domains with considerable overlap.⁸

Compared with complexity and labour intensiveness of and length of time required for conventional cytogenetic procedures, this method is particularly useful for the detection of specific chromosomal aberrations associated with cancer.

This method could also be useful for those institutions lacking a conventional cytogenetic laboratory. It also avoids the need for living tumour cells, overcomes the problem that metaphases may not be representative of the cancer cell population as a whole and is quick and easy to use.

With regard to retrospective analyses, FISH carried out on paraffin wax embedded tissue is difficult to interpret. Thin (4–6 μ m) sections do not provide complete nuclei, whereas FISH on intact nuclei obtained through disaggregation of 40–60 μ m sections is not always reproducible. The latter depends on how the tissue responds to digestion and denaturation. The ability to store smears or to use fresh cytological samples suspended in PBS/PreservCyt will facilitate cytogenetic analysis by FISH.⁹

In conclusion, the use of painting probes, along with study of their respective centromeres, is an additional method for the detection of translocations in those cases lacking specific probes adjacent to or spanning the relevant breakpoints or when probes are not available.

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- Ladanyi M. The emerging molecular genetics of sarcoma translocations. *Diagn Mol Pathol* 1995;4:162–73.
- Abati A, Sanford JS, Fetsch P, Marincola FM, Wolman SR. Fluorescence in situ hybridization (FISH): a guide to optimal preparation of cytologic specimens. *Diagn Cytopathol* 1995;13:486–92.
- Aman P, Ron D, Mandahl N. Rearrangement of the transcription factor gene CHOP in myxoid liposarcomas with t(12;16)(q13;p11). *Genes Chromosom Cancer* 1992;5: 278–85.
- Sreekantaiah C, Ladanyi M, Rodriguez E, Chaganti RSK. Chromosomal aberration in soft tissue tumors. *Am J Pathol* 1994;144:1121–32.
- Huang WM, Gibson SJ, Facer P, Gu J, Polak JM. Improved section adhesion for immunocytochemistry using high molecular weight polymers of L-lysine as a slide coating. *Histochemistry* 1983;77:275–9.
- Lichter P, Yang CJC, Call K, Hermanson G, Evans GA, Housman D, et al. High resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science* 1991;247:64–9.
- Lee W, Han K, Harris CP, Shim S, Kim S, Meisner LF. Use of FISH to detect chromosomal translocations and deletions. *Am J Pathol* 1993;143:15–19.
- Tkachuk DC, Pinkel D, Kuo WL, Weier HU, Gray JW. Clinical applications of fluorescence in situ hybridization. *GATA* 1991;8:67–74.
- Xiao S, Renshaw A, Cibas ES, Hudson TJ, Fletcher JA. Novel fluorescence in situ hybridization approaches in solid tumors. Characterization of frozen specimens, touch preparations, and cytological preparations. *Am J Pathol* 1995;147:896–904.