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M. J. Perez-Bacete · A. Llombart-Bosch

FU-3 monoclonal antibody: a specific marker for malignant fibrous histiocytoma? An analysis of 32 malignant soft tissue and bone sarcomas

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Abstract An immunohistochemical study on frozen sections was carried out on 51 malignant tumours of soft tissue and bone using the FU-3 monoclonal antibody. This antibody is claimed to be specific for malignant fibrous histiocytoma (MFH) and liposarcoma and for normal and tumour cells located in perivascular fields. The results show a lack of specificity in MFH staining: several malignant tumours such as synovial sarcoma, fibrosarcoma, rhabdomyosarcoma, osteogenic sarcoma, and including an anaplastic malignant melanoma, presented positive staining somewhat similar to that found in MFH. The value of this antibody in the differential diagnosis of MFH is doubtful. It might be useful to recognize a common pathway of terminal differentiation expressed by several pleomorphic sarcomatous neoplasms.

Key words Malignant fibrous histiocytoma
Soft tissue sarcomas · Immunohistochemistry
Monoclonal antibody

Introduction

Malignant fibrous histiocytoma (MFH) is a heterogeneous neoplasm displaying a pleomorphic histological picture and a debatable histogenesis. Facultative fibrohistiocytes of mesenchymal origin seem to constitute the cellular component of the tumour (Dehner 1988; Iwasaki et al. 1992a, b; Lawson et al. 1987; Roholl et al. 1985), but other cells may be associated. At present no specific immunomarkers have been recognized for MFH cells. A mesenchymal nature for the tumour (Greaves et al. 1985; Hirose et al. 1989; Iwasaki et al. 1992a, b; Miettinen et al. 1984; Wood et al. 1986), or a monocyte-macrophage phenotype (Du Boulay 1982; Strauchen and Dimitriu-Bona 1986), or both (Brooks 1986; Fletcher 1992; Genberg et al. 1989; Lentini et al. 1986; Roholl et al. 1988) are theories that have been suggested to explain the origin of this neoplasm, but there are no results that validate any of them, and conflicting results have been communicated. As a further complication, activated macrophages and reactive fibroblasts coexist within the tumour stroma in variable amounts (Brooks 1986; Hirose et al. 1989; Iwasaki et al. 1992a, b).

FU-3 antibody was originally raised from a cultured MFH cell line (Iwasaki et al. 1987), and was proposed to be a specific marker for MFH tumoral cells as well as for fibroblasts or mesenchymal cells of perivascular fields (Iwasaki et al. 1987, 1992a, b). No other variant of soft tissue sarcoma (except liposarcoma) stains positively with this antibody.

To confirm the potential value of this antibody in the histological assessment and differential diagnosis of MFH, we tested frozen sections of 50 primary and xenografted human sarcomas with two immunocytochemical techniques (avidin-biotin-peroxidase and immunogold methods). Our results do not confirm the tumour cell specificity for this antibody regarding MFH; other neoplasms express positivity with variable intensity.

Materials and methods

The present study comprises 51 human sarcomas of soft tissue and bone. These neoplasms were either original tumours or nude mice xenografts. The technique for transplants and follow-up of these neoplasms has been described elsewhere (Llombart-Bosch et al. 1988). Tumours were classified according to the standard criteria proposed by Enzinger and Weiss (1988), and the histological types of neoplasms and results are summarized in Table 1. Within the term MFH we have considered several variants (storiform, pleomorphic, myxoid, giant cell; but no angiomatoid sub-type was included). The synovial sarcomas tested were monophasic

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M. J. Perez-Bacete (✉) · A. Llombart-Bosch
Department of Pathology, University of Valencia Medical School, Av. Blasco Ibañez, 17 (46010), Valencia, Spain

Table 1 Classification of material and results of immunostaining with the FU-3 monoclonal antibody

Diagnostic		Type of immunostaining						Number of cases
		Cellular			Perivascular			
		Focal	Diffuse	Negative	0	+	++	
MFH	Human	6	2	2	0	4	6	10
	Xenografts	4	2	1	0	6	1	7
Melanoma	Human	1	0	0	0	0	1	1
Fibrosarcoma	Human	1	2	1	0	1	3	4
Osteosarcoma	Human	4	1	1	0	2	4	6
	Xenografts	3	0	1	1	1	2	4
Synovial Sarcoma	Human	1	0	1	0	0	2	2
	Xenografts	5	0	2	0	3	4	7
Rhabdosarcoma	Human	1	1	1	0	0	3	3
	Xenografts	4	2	1	1	4	2	7

and the 10 rhabdomyosarcomas were of embryonal, alveolar, solid-alveolar and pleomorphic types. For differential diagnosis we included in this study a group of osteogenic sarcomas primary to bone, some of which showed fibro-histiocytoid patterns, but all possessed osteogenic foci. Finally a pleomorphic melanoma with scarce melanin production was analysed. All tumours were kept frozen in liquid nitrogen and stored at −80° C until use. Cryostat sections 5 μm thick were obtained from each case, mounted in poly-L-lysine coated slides and dried at room temperature.

For immunohistochemistry the sections were fixed in cold acetone over 10 min. Hydrogen peroxide 3% in methanol was applied to avoid endogenous peroxidase activity. Incubation in normal horse serum 20% was made to abolish non-specific cross reactions. Primary antibody FU-3 (1/10 pre-diluted ascites kindly supplied by Dr. H. Iwasaki, Japan) was applied at 1/150 (final dilution 1/1500) for 1 h at room temperature. Secondary antibody and ABC complexes were used as recommended by suppliers (Vector, Burlingame, Calif., USA). Peroxidase was revealed with 3'3-diamino benzidine (Sigma, St. Louis) over 4–5 min in a dark-room. Slides were counterstained with Harris haematoxylin. Blocking of non-specific endogenous biotin was carried out (using an avidin-biotin blocking kit from Vector), between primary and secondary antibody incubations.

A second set of slides was stained with the immunogold technique: after incubation with FU-3 monoclonal antibody at the same dilution and time of incubation, secondary antibody (goat anti-mouse immunoglobulins 10 nm gold conjugated) (Biocell, Cardiff, UK) was applied over 30 min at 1/100 dilution. We used a silver enhancing kit (Biocell) to visualize the gold precipitates. Slides were counterstained with Harris haematoxylin, dehydrated and mounted in synthetic resin (Entellan, Merck, Darmstadt).

Positive and negative controls were used to assess the specificity of immunostaining with both methods. Microphotographs were obtained from gold-stained slides, due to their better resolution for black and white prints.

In Table 1, with the term "cellular staining", we describe positive immunoreactivity that is located at cellular or cytoplasmic level in tumour cells. This positivity may appear in isolated cells (focal staining), or in cells grouped in large or small nodules (diffuse), or when the majority of cells was immunostained. Perivascular staining gives information about tumour microvasculature. This pattern is expressed in endothelial cells and undifferentiated normal or tumour cells surrounding vascular spaces.

Results

Forty out of the 51 tumours proved positive for the FU-3 antibody. Positivity was not restricted to MFH cells, being positive in all mesenchymal neoplasms examined, including the anaplastic melanoma. Table 1 summarizes results, for tumour and perivascular cells as well as some reactive fibroblasts or tumour cells that appeared intermingled. Endothelial cells of the intratumour vessels and of the capsule were positive.

MFH original tumours and nude mice xenografts stained positive in 14 cases out of 17. Positivity appeared both in the perivascular fields and/or within the tumour cells (Fig. 1B, D). Only 3 cases were negative, probably due to a poor degree of preservation of the tissue, as was confirmed with the haematoxylin-eosin (HE) control stain. The results varied widely from case to case, being mainly diffuse in the cytoplasm, but occasionally focally located within the Golgi fields. Even within the same tumour the intensity of the staining distribution varied from field to field. The morphology of the positive cells was either stellate or fusiform and with a fibrohistiocytic appearance. In all tumours at least some isolated cells were negative.

These results have been validated in fibrosarcomas which stained positive in perivascular fields as well as in the sarcomatous areas, in a way similar to that observed in MFH. Similar results appeared in various fields of all fibrosarcomas analysed.

In the synovial sarcomas the perivascular cells coloured with this antibody appeared to be regularly distributed with dense staining in the original tumours and the xenografts. Most of the tumour cells were negative. The intensity of staining was similar to that seen in MFH, being both diffuse or weakly concentrated within the Golgi fields.

The group of rhabdomyosarcomas tested gave positive results both in the cells located in perivascular fields and in endothelial cells. Some neoplastic rhabdoblasts (embryonal, alveolar, pleomorphic) were positive with the antibody (Fig. 2B). Two cases which apparently became negative (one in the original tumour and a second in the xenografts) may have changed because of poor preservation of the frozen samples.

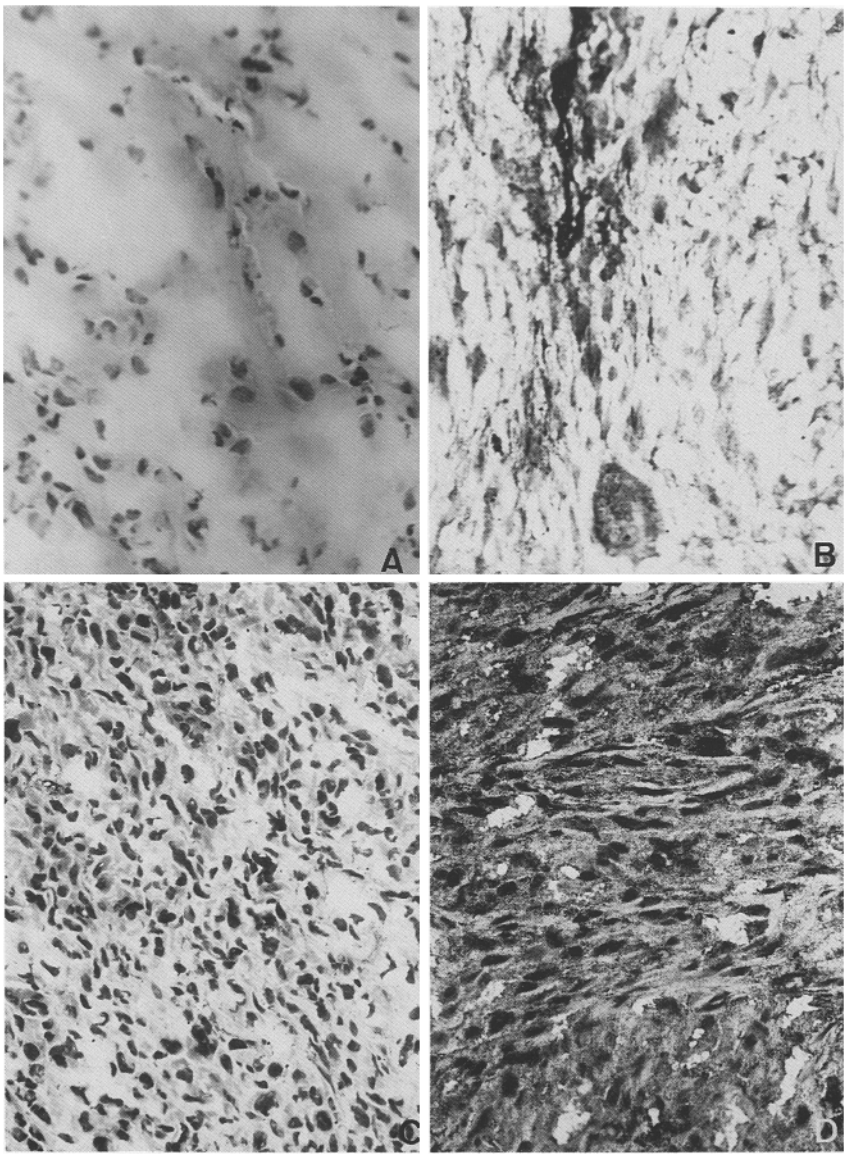

Fig. 1 **A** Malignant fibrous histiocytoma (frozen section, HE, 40X). In the centre 3 giant histiocytoid-like cells are seen. **B** Malignant fibrous histiocytoma (frozen section, immunogold, 60X). Amplification of **A** showing a stained vessel with a multinucleated histiocytoid-like cell. Some cells present a fine black cytoplasmic precipitation. **C** Malignant fibrous histiocytoma (frozen section, HE, 40X). Dense cellularity of mixed type. **D** Malignant fibrous histiocytoma (frozen section, immunogold, 60X). A diffuse immunoprecipitation is present in the cytoplasm of all the cells

In all but one of the osteogenic sarcomas the endothelial and the perivascular cells were positive (Fig. 2D). This was also the case in the neoplastic fields which possessed MFH appearance. Furthermore some typical osteocytes immersed within the osteoid matrix expressed a diffuse cytoplasmic staining.

The melanoma tested gave positive staining both in the vascular fields and in several anaplastic tumour cells.

No differences were found when comparing ABC and immunogold methods with number of cases stained, or number of cells stained for each case. Intensity of immunostaining was strong with immunogold in all cases, independently of the cellular or perivascular pattern analysed. With ABC method significant differences in intensity were noted, also in cellular and perivascular patterns.

Discussion

The most common malignant tumour of soft tissues (and quite common in bone) in the human adult is MFH (Enzinger and Weiss 1988; Mazanet and Antman 1991; Weiss and Enzinger 1983). To date this tumour is considered to be of mesenchymal origin with a fibrohistiocytic phenotype (Fletcher 1992), but no specific markers have yet been described.

FU-3 monoclonal antibody was raised from cultured cell lines of MFH (SFT 7913); the molecular nature of the reacting antigen has still not been identified, but seems to precipitate a high-molecular weight protein (Iwasaki et al. 1987). This antibody shares antigenicity with a number of cells within in MFH and liposarcomas, as well as those fibroblasts located in the perivascular fields (Iwasaki et al. 1987, 1992a, b). Moreover this positivity extends to those malignant cells situated with-

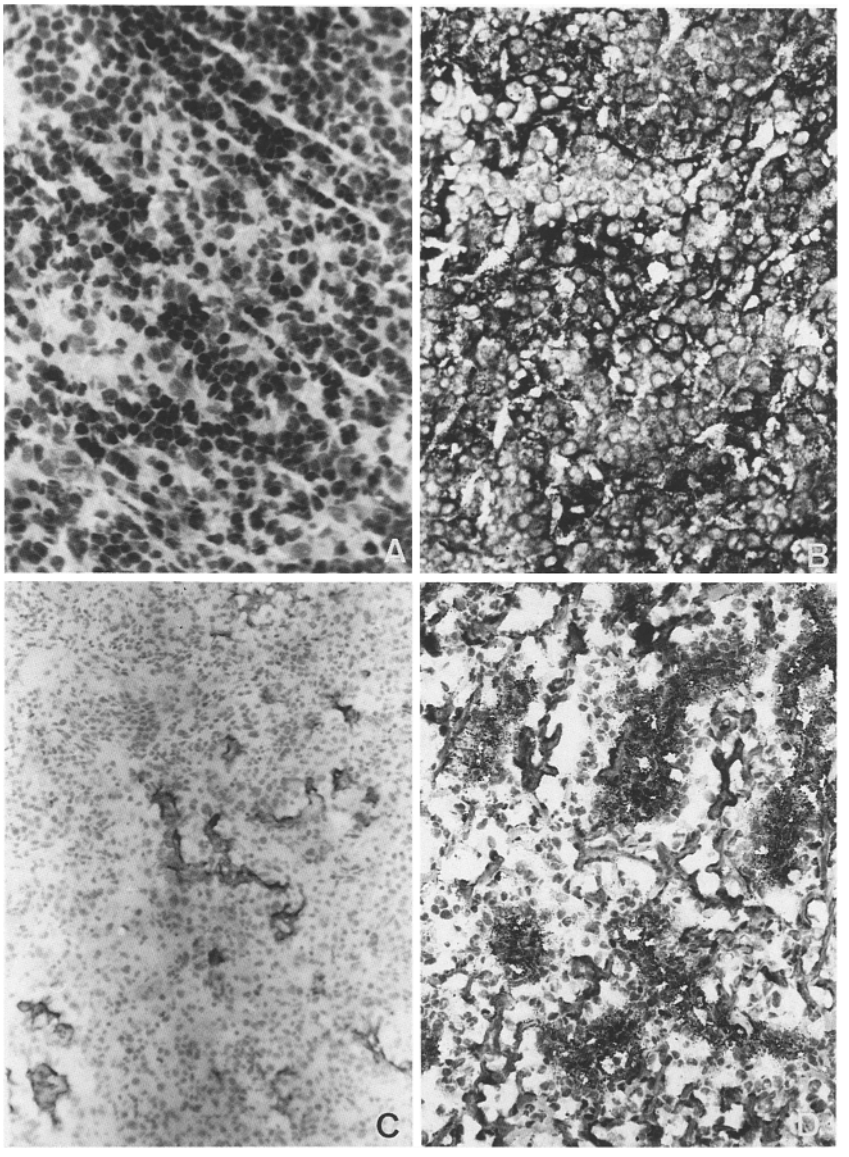

Fig. 2 **A** Rhabdomyosarcoma (frozen section, HE, 40X). This is an example of the embryonal subtype, with a large amount of small cells. **B** Rhabdomyosarcoma (frozen section, immunogold, 60X). Amplification of **A** with intense cellular staining in tumour cells, whereas others are negative. **C** Osteosarcoma (frozen section, HE, 20X). Presence of osteoid-like material surrounded by medium and large cells seen in the centre of the figure. **D** Osteosarcoma (frozen section, immunogold, 20X). Intense positive staining in endothelial and tumour cells close to vessels

in the neoplasms. This positivity seems to be expressed not only in the tumour cells but also in the non-neoplastic stromal component of the vessels (endothelium, pericytes).

Detailed analysis of the antigenicity was determined by electron-microscopy immunocytochemistry in human MFH and in cell lines (Iwasaki et al. 1992a) showing a clear staining location in the cell membrane, in the subplasmalemmal vesicles and in the Golgi areas.

The main objective of this study was to confirm whether FU-3 antibody expressed exclusively in MFH tumour cells and therefore should be considered to be a specific marker for this neoplasm. Unfortunately our results show that FU-3 antibody is non-specific for MFH other mesenchymal neoplasms, such as fibrosarcomas and synovial sarcomas also express this positivity. Moreover many tumour cells in osteogenic sarcomas of bone share the positivity for FU-3, and this occurs not only in the MFH appearing areas of these neoplasms but also within foci of osteogenesis. As an added non-specificity we have found occasional positivity for this antibody in xenografts and in original rhabdomyosarcomas (not only of pleomorphic type but also of alveolar and embryonal nature). This finding excludes the value of FU-3 as a specific MFH marker. Further doubts arose when anaplastic melanoma cells reacted positively with the antibody.

The immunogold technique provides more sensitivity than the ABC method. Usually all tumours stained with the ABC method were less reactive than with the immunogold technique, this being more evident when the perivascular pattern was analysed. Our results conclude that the immunogold method is more sensitive and appropriate for immunocytochemical purposes, as also described by others (Gu et al. 1981).

The intriguing nature and histogenesis of MFH has been pointed out by several authors: several morphological, immunohistochemical, biological and cytoge-

netic observations provide support to the multiclonality of this sarcoma which probably involves a number of divergent phenotypes (Brooks 1986; Dehner 1988; Fletcher 1992; Roholl et al. 1988; Vera-Sempere et al. 1989). This multiclonality could include a number of histologically poorly identifiable pleomorphic sarcomas, or even melanomas, representing, probably, a common ultimate pathway for all of them in advanced stages of their evolution. Based upon this currently acceptable hypothesis the FU-3 antibody could probably recognize an epitope in cells implicated in this final pathway, simultaneously expressed by all these neoplasms (mesenchymal, fibro-histiocytic, lipomatous, synovial, osteogenic, rhabdomyoblastic and even melanocytic) but not visible in their initial stages of transformation.

FU-3 should not be considered as a specific marker for MFH, although it might theoretically be related to, a final differentiation pathway common to all the tested neoplasms.

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