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Original Articles

Myofibroblasts in Soft Tissue Sarcomas

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Summary. A series of 129 soft tissue sarcomas was examined ultrastructurally to determine in which neoplasms and to what extent myofibroblasts could be demonstrated. Twenty cases of fibromatosis and fasciitis served as controls.

Myofibroblasts were identified in all 30 cases of malignant fibrous histiocytoma and all 4 cases of well-differentiated sclerosing liposarcoma. Though most numerous in areas of desmoplasia, in no instance did myofibroblasts constitute the dominant cellular constituent of either neoplasm. Myofibroblasts were identified with lesser frequency and in smaller numbers in fibrosarcoma, synovial sarcoma, malignant hemangiopericytoma and neuroblastoma. None were observed in a wide assortment of diverse sarcomas in which desmoplasia was not a feature. In comparison each lesion judged by light microscopy to represent either fibromatosis or fasciitis was composed principally of myofibroblasts.

The demonstration of abundant myofibroblasts within a soft tissue lesion which has been subjected to wide sampling strongly suggests a benign proliferative process as opposed to a malignant neoplasm. It is hypothesized that myofibroblasts observed within collagenized regions of soft tissue sarcomas may constitute an expression of host response to neoplasia.

Key words: Soft tissue sarcoma – Myofibroblasts – Ultrastructure – Host response.

Introduction

Recent pathological studies of human soft tissue sarcomas have been principally focused on histological classification, the delineation of specific entities and

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clinicopathological correlates in an attempt to provide both a better understanding of the natural biology of these neoplasms and a rational basis for therapy. Electron microscopic and tissue culture studies employed with increasing frequency have facilitated diagnostic accuracy and in some instances have elucidated the histogenetic origin of some neoplasms. In contrast to studies of human carcinoma (Ioachim 1976) scant attention has been accorded the cellular response of the host to malignant mesenchymal neoplasms.

A recent publication describes a peculiar to distinctive stromal response in carcinoma (Seemayer et al. 1979). Considerable numbers of myofibroblasts were noted within the stroma of diverse primary invasive and metastatic carcinomas, especially in neoplasms which demonstrated desmoplasia and retraction. In comparison, non-invasive intraepithelial carcinomas and normal tissues lacked stromal myofibroblasts. Similar findings were subsequently observed in the nodule-stromal interphase of nodular sclerosis Hodgkin's disease (Seemayer et al. 1980). It was suggested that the stromal myofibroblasts in these pathologic states constituted a unique expression of host response to neoplasia.

It was, therefore, decided to conduct a retrospective ultrastructural study of our soft tissue sarcomas to determine with what frequency and in which neoplasms stromal myofibroblasts might be demonstrable. The purpose of this paper is to document the results of this study and discuss the possible significance of myofibroblasts in lesions of mesenchymal soft tissue.

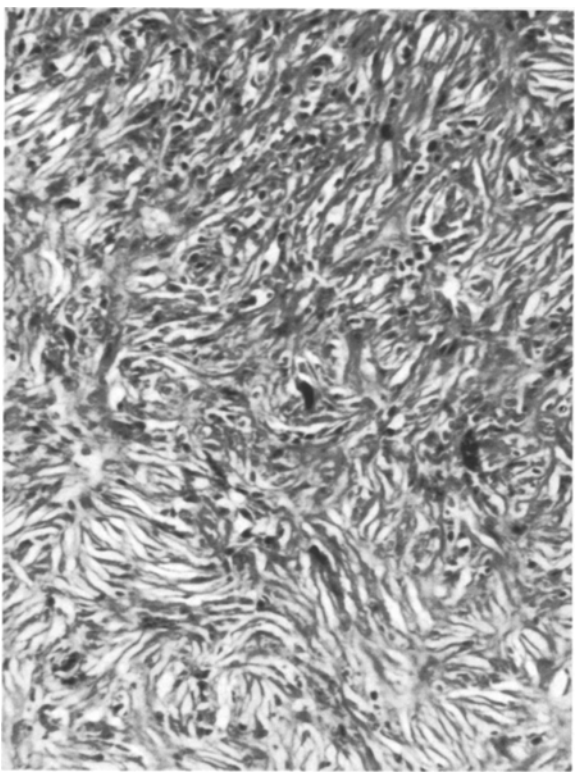


Fig. 1. Photomicrograph of a malignant fibrous histiocytoma (MFH) of the arm showing the storiform pattern within a dense collagenic area. (× 100)

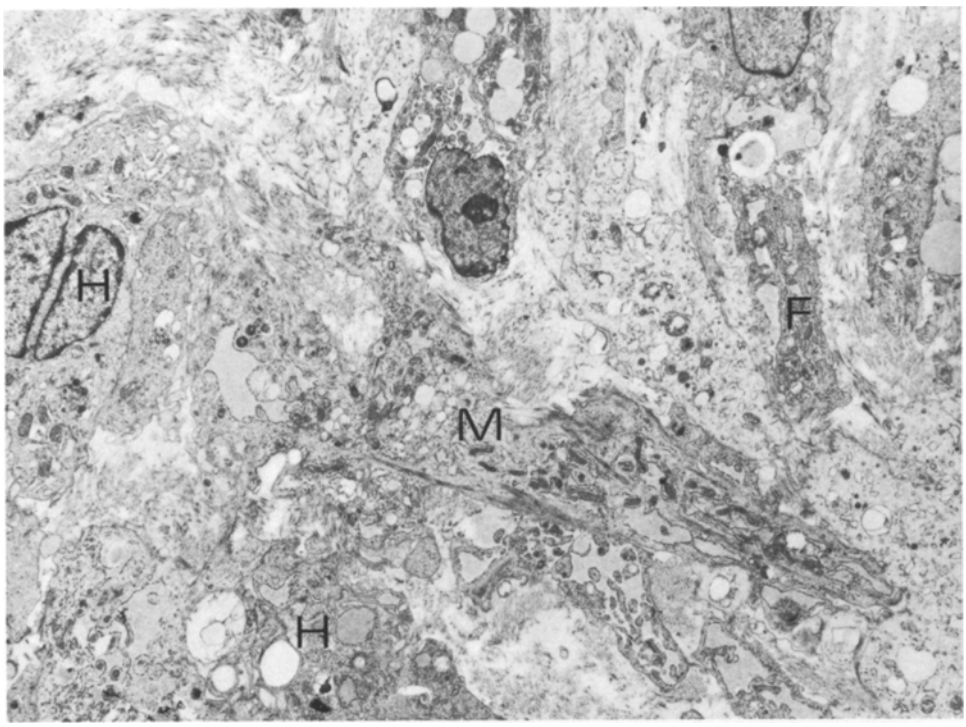
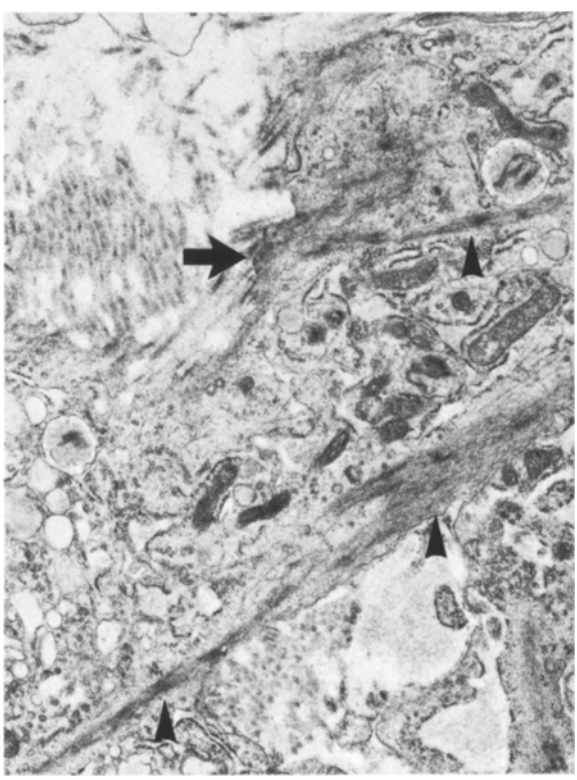


Fig. 2.
a Electron micrograph of the MFH illustrated in Fig. 1 demonstrating portion of tumor cells (Histiocytes *H*, Fibroblasts *F*) and a myofibroblast *(M)*. (×3,960)



b Higher magnification of Fig. 2a demonstrating the presence of bundles of microfilaments *(arrow-heads)* and the microtendons at the cell surface *(large arrow)*. (×13,200)

Materials and Methods

129 soft tissue sarcomas formed the basis of the study (Table 1). For purposes of comparison 20 examples of various expressions of fibromatosis and fasciitis were similarly examined.

For light microscopy tissue was fixed in 10% neutral formalin, embedded in paraffin, cut and stained in conventional fashion. For electron microscopy thin slices from freshly excised surgical material were placed in chilled 4% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, diced and fixed for 2 h. The fragments were then rinsed in buffer, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols and embedded in Epon. From multiple blocks one micron sections were cut, stained with toluidine blue and examined. From each case several blocks were selected for study. After appropriate trimming ultrathin sections were cut, stained with uranyl acetate and lead citrate and examined with a transmission electron microscope. More than 50 photographs representing different fields were taken in all cases.

The histopathological classification of sarcoma was usually based on light microscopic criteria, although, in some instances ultrastructural findings established the diagnosis.

Cells judged to represent myofibroblasts illustrated many of the following characteristics: a fusiform notched nucleus, prominent nuclear bodies, elongated cytoplasmic extensions, well-developed Golgi zones and rough endoplasmic reticulum, microtubules and abundant bundles of cytofilaments (40 to 60 Å) often peripherally distributed and condensed to form dense bodies. Attachment sites, either cell-cell or cell-stroma, basement-membrane-like material and micropinocytotic vesicles were noted rarely. Although these collective ultrastructural features were not demonstrable in every cell considered to represent a myofibroblast, those interpreted as myofibroblasts contained prominent Golgi zones, well-developed rough endoplasmic reticulum and abundant bundles of peripheral cytofilaments with dense bodies.

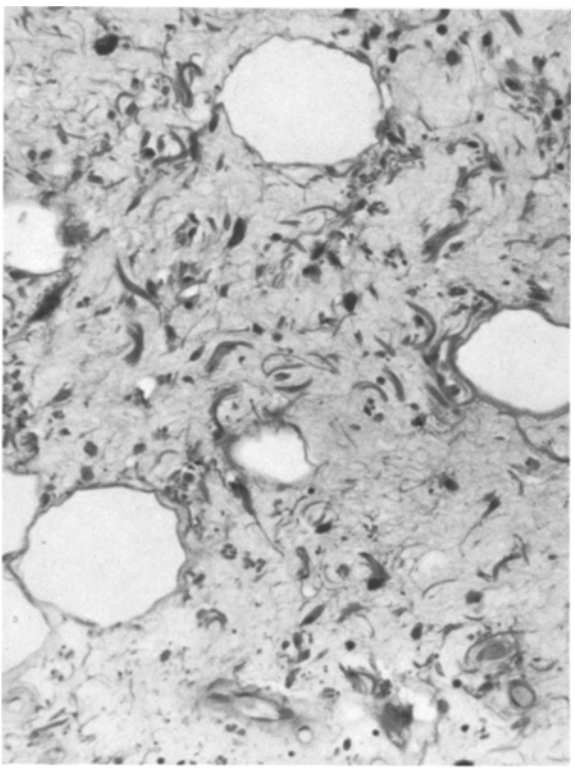


Fig. 3. Photomicrograph of a recurrent well differentiated sclerosing type liposarcoma of the inguinal region showing the presence of rare lipoblasts, round and spindle cells within bundles of collagen fibers. ($\times$ 100)

Results

The findings are divided into three groups: 1. Sarcomas in which myofibroblasts were constantly observed. 2. Sarcomas in which myofibroblasts were occasionally present. 3. Sarcomas in which myofibroblasts were not identified. In no instance did myofibroblasts constitute the major cellular component of the sarcoma. Indeed, when present, myofibroblasts represented but one of several cell types within the neoplasm. In contrast myofibroblasts represented the dominant cellular constituent of all 20 cases of fibromatosis and fasciitis.

Group 1. Myofibroblasts were observed in all thirty cases of malignant fibrous histiocytoma and were most numerous in collagenous zones showing a storiform pattern (Figs. 1 and 2). Myofibroblasts were identified with considerably less frequency in the cellular pleomorphic areas of conventional malignant fibrous histiocytoma and in the myxoid zones of the myxoid variant of malignant fibrous histiocytoma. Well-differentiated sclerosing liposarcoma represented a second entity in which myofibroblasts were constantly observed. They were not seen in "loose areas" composed of lipoblasts, blood vessels and mature fat but rather in zones showing a desmoplastic reaction. (Figs. 3 and 4).

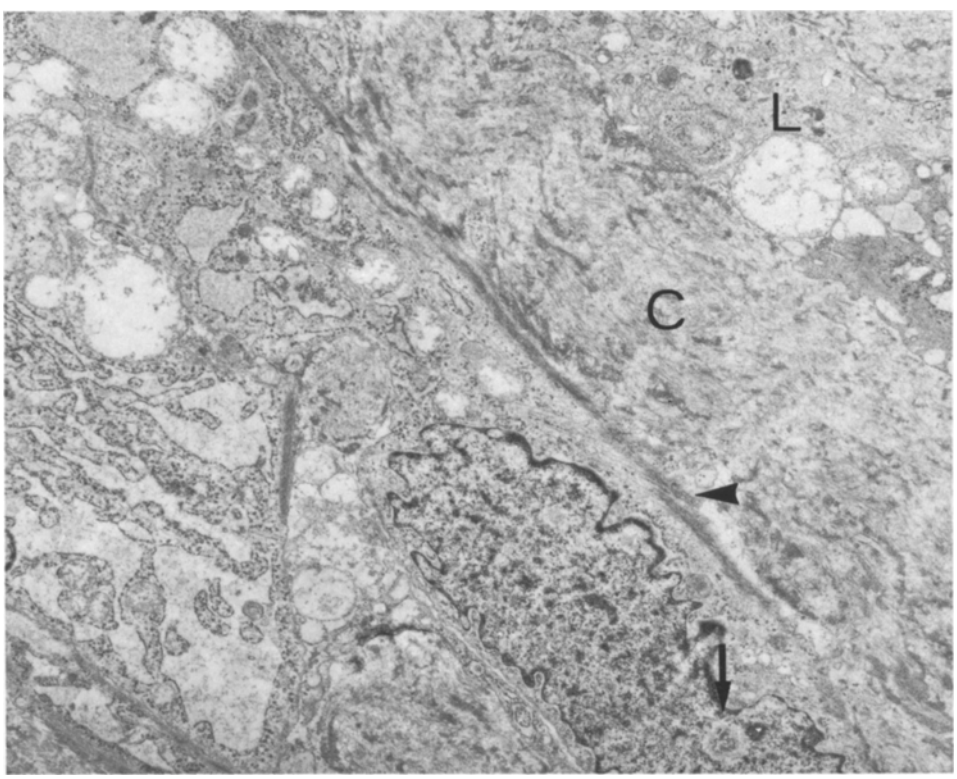


Fig. 4. Photomicrograph of an area of the tumor shown in Fig. 3 demonstrating a tumor cell *(L)* and portion of a myofibroblast characterized by a typical notched nucleus with a nuclear body *(arrow)* and the presence of microtendons at the cell surface *(arrow-head)*. The intercellular stroma is rich in collagen *(C)*. (× 5,760)

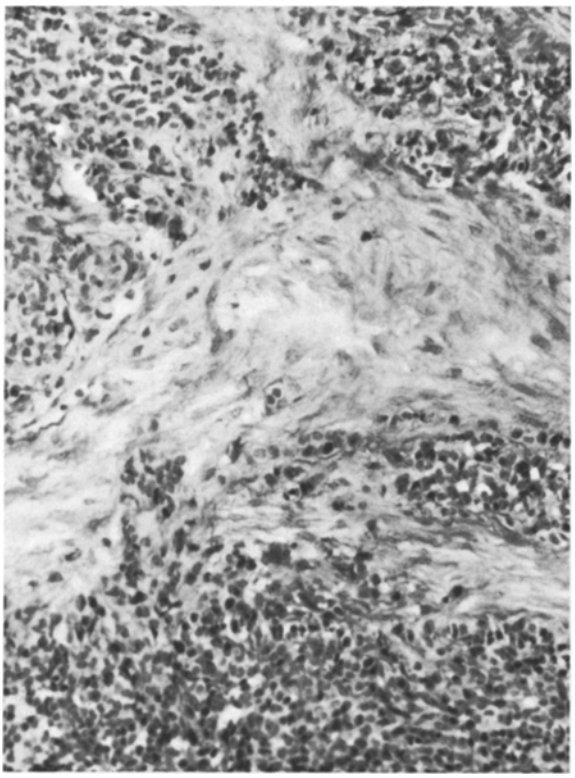


Fig. 5. Photomicrograph of a retroperitoneal neuroblastoma demonstrating clusters of tumor cells, in the vicinity of collagen fibers and spindle-shaped myofibroblasts. (× 400)

Group 2. Myofibroblasts were present in a variable frequency in a limited number of diverse soft tissue sarcomas; in each instance they were less numerous than in malignant fibrohistiocytic neoplasms. In one of eight fibrosarcomas, two of seven synovial sarcomas and one of three malignant hemangiopericytomas myofibroblasts were observed among the stromal component of these neoplasms. In two of five neuroblastomas a myofibroblastic population was identified in peripheral areas of the neoplasm in which a desmoplastic reaction was elicited (Figs. 5 and 6). None were present within the central nidus of the neoplasm.

Group 3. Myofibroblasts were not identified in the stroma of a mixed assortment of soft tissue sarcomas (Table 1).

Discussion

In the course of studying the mechanism of wound healing Majno and Gabbiani observed a unique cell with contractile properties in experimental granulation tissue (Majno et al. 1971). The biological nature of this cell, termed a myofibroblast, was subsequently delineated by morphological, pharmacological, biochemical and immunological methods (Gabbiani et al. 1972; Ryan et al. 1973). Studies

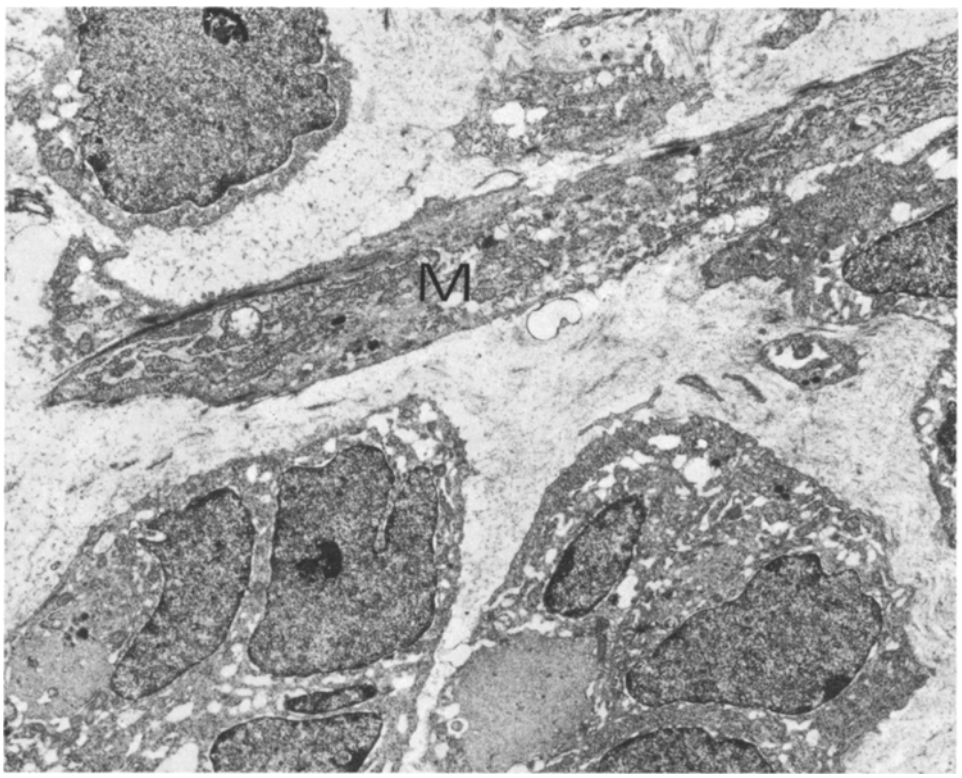


Fig. 6. Electron micrograph of the case illustrated in Fig. 5 showing clusters of neuroblasts recognizable by bundles of microfibrils transversally cut and occasional neurosecretory granules. The myofibroblast *(M)* demonstrate microfilaments with dense bodies. (×4,140)

to date suggest that the myofibroblast is derived from fibroblasts or more primitive mesenchymal cells (modulation) in response to diverse stimuli (Ryan et al. 1974). The life span of the myofibroblast appears finite except in processes of exuberant scarring in which they may persist indefinitely (Rudolph et al. 1977).

The myofibroblast is characterized by a fusiform shape, an elongated often serrated nucleus, well-developed Golgi zones and rough endoplasmic reticulum and a prominent microtubular and microfibrillary network, the latter composed of actin, myosin and intermediate filaments. Attachment sites, both cell-cell and cell-stroma, are occasionally demonstrable and probably facilitate the transmission of contractile forces (Gabbiani et al. 1972).

Myofibroblasts also possess synthetic properties since they have been shown to produce type III collagen (Gabbiani et al. 1976). Many are also observed in conditions with exuberant scar formation associated with more dense type I collagen (Baur et al. 1975). Cells closely related to myofibroblasts have been described in the aorta in relationship to the synthesis of elastin, collagen and matrix substance (Wissler 1967). It is, thus, possible that myofibroblasts possess synthetic capability for a variety of stromal components.

Table 1. Soft tissue sarcomas included in the study and relative frequency of myofibroblasts

Chondrosarcoma	(0/2)	
Malignant fibrous histiocytoma	(23/23)	+ + +
Myxoid variant of MFH	(7/7)	+ to + +
Rhabdomyosarcoma		
alveolar	0/4	
embryonal	0/3	
pleomorphic	0/2	
Liposarcoma		
well differentiated sclerosing type	(4/4)	+ + +
myxoid	(0/15)	
pleomorphic	(0/8)	
Alveolar soft part sarcoma	(0/4)	
Leiomyosarcoma	(0/15)	
Epithelioid leiomyosarcoma	(0/2)	
Hemangiopericytoma (malignant)	(1/3)	+
Neuroblastoma	(2/5)	+
Chordoma	(0/3)	
Synovial sarcoma	(2/7)	+
Epithelioid sarcoma	(0/9)	
Schwannosarcoma	(0/2)	
Fibrosarcoma	(1/8)	+
Chordoid sarcoma	(0/1)	
Extraosseous Ewing sarcoma	(0/2)	

( ) = Number of cases in which myofibroblasts were found on total number of cases examined; 0 = Absent; + to + + + = Increasing number of myofibroblasts

Since their demonstration in granulation tissue myofibroblasts have been observed in a wide variety of human pathologic conditions. They represent the principal cellular component of a variety of quasi-neoplastic proliferative conditions including fibromatoses of soft tissue (Bonenfant and Lagacé 1975) and bone (Lagacé et al. 1979), nodular fasciitis (Wirman 1976), digital fibroma of infancy (Bhawan et al. 1979), juvenile nasal angiofibroma (Taxy 1977), elastofibroma (Ramos et al. 1978) and the nodules of Dupuytren's, Ledderhose's and Peyronie's disease (Gabbiani and Majno 1972). Their dominance in these pathological settings suggests a fundamental basis of pathogenetic reactivity rather than neoplasia since myofibroblasts are pivotal components of pathobiological processes characterized by tissue injury and repair.

Myofibroblasts have been described in several soft tissue sarcomas. Churg and Kahn observed myofibroblasts and related cell types in fibroblastic regions which contained variable amounts of collagen in fibrosarcoma and malignant fibrous histiocytoma (Churg and Kahn 1977). Myofibroblasts, although illustrat-

ed in one case of fibrosarcoma of breast (Crocker and Murad 1969), were not identified in two cases of orbital fibrosarcoma (Jakobiec and Tannenbaum 1974). More recently they have been described admixed with various other cells in four malignant fibrous histiocytomas, three pleomorphic liposarcomas and two pleomorphic rhabdomyosarcomas (Reddick et al. 1979).

Malignant fibrous histiocytoma, the most common malignant soft tissue neoplasm of adults (Weiss and Enzinger 1978), constitutes the largest group of soft tissue sarcomas in which myofibroblasts have been observed. (Alguacil-Garcia et al. 1978; Harris 1980; Limacher et al. 1978; Reddick et al. 1979; Taxy and Battifora 1977). In myxofibrosarcoma, a closely related entity, myofibroblasts were constantly present and were most numerous in fibroblastic areas of neoplasms judged to be of low grade malignancy (Kindblom et al. 1979). We have previously noted myofibroblasts in malignant fibrous histiocytomas, however, they were neither numerous nor did they constitute the predominant cellular component of the neoplasm (Limacher et al. 1978).

The present study demonstrates that the presence and relative abundance of myofibroblasts within soft tissue sarcomas are directly correlated with the amount of collagen within the neoplasm. Their constant occurrence in malignant fibrohistiocytic tumors, particularly in sclerotic areas, and in well-differentiated sclerosing liposarcoma provide substance for this interpretation. Their infrequent presence in rather limited numbers in other malignancies of soft tissue, i.e. malignant hemangiopericytoma, synovial sarcoma and neuroblastoma was also associated with the production of collagen within each neoplasm. Notably, only one of eight fibrosarcomas contained myofibroblasts, possibly a reflection of both the highly cellular nature and paucity of collagen in these neoplasms.

It has been suggested that myofibroblasts may constitute the major component of some soft tissue neoplasms and thus have a malignant counterpart (Vasudev and Harris 1978). Our study, to the contrary, suggests that myofibroblasts are present in very few soft tissue sarcomas and, when so, are most numerous in areas of desmoplasia. In no instance were myofibroblasts the principal cellular component of a neoplasm judged to represent a soft tissue sarcoma. In fact, the ultrastructural demonstration of large numbers of myofibroblasts within well studied lesions of soft tissue has forced us to reappraise (and amend) an original diagnosis of sarcoma. Thus, our experience suggests that soft tissue lesions which contain numerous myofibroblasts (from widely sampled regions) generally are indicative of one of several quasi-neoplastic proliferative conditions, i.e., fasciitis, fibromatosis, desmoid., etc.

The observation of stromal myofibroblasts in invasive and metastatic carcinoma, nodular sclerosis Hodgkin's disease and desmoplastic regions of several soft tissue sarcomas suggests a common biologic thread. The host commands several responses to neoplasia. Lymphocytes, plasma cells, macrophages and an assortment of humoral factors are cornerstones of immune surveillance against neoplasia (Ioachim 1976). A second response, tumor neovascularization, induced by a tumor angiogenesis factor (Folkman et al. 1971), has been demonstrated in a variety of epithelial neoplasms and may possibly account for the prominent vascularity which characterizes several soft tissue sarcomas, i.e., myxoid liposarcoma and the myxoid variant of malignant fibrous histiocytoma.

Myofibroblastic induction possibly represents a third mechanism of reactivity. It is plausible that neoplastic cells in soft tissue sarcomas, as in invasive and metastatic carcinomas and nodular sclerosis Hodgkin's disease, release factors which activate the mechanism of myofibroblast induction, proliferation and collagen synthesis. The resulting density and contractility of such tissue enveloping neoplastic cells might retard the process of invasion and limit the accessibility to vessels and/or perineural spaces, thus acting as a form of tumor containment. This might contribute to the relatively indolent course of a limited group of soft tissue sarcomas such as well-differentiated sclerosing liposarcoma and collagenized forms of malignant fibrous histiocytoma. The presence of blood vessels and the production of collagen constitute a stromal reaction common to a great number of malignant neoplasms. However, the observation of myofibroblasts within these neoplasms rather suggests and represents an unusual response.

Further studies are indicated to test this hypothesis. Factors which activate myofibroblast induction and proliferation might have considerable biologic significance in the control of neoplasia.

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