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Stromal cells of the fibroadenoma of the human breast*

An immunohistochemical and ultrastructural study

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Summary. Fourteen fibroadenomas of the human breast were examined by light and electron microscopy, and by immunohistochemistry for actin. They were classified into 3 groups according to their stromal patterns; myxoid, fibrous-cellular and sclerotic. Actin immunohistochemistry revealed that the stromal areas were strongly positive in the fibrous-cellular group and weakly positive in the myxoid and sclerotic groups. By electron microscopy the stromal cells in most cases of the myxoid and fibrous-cellular groups were fibroblasts, containing varying amounts of microfilaments, 5–7 nm in diameter (actin type filaments). However, a dense body was not usually present suggesting these stromal cells were variants of myofibroblasts. The amount of microfilaments in fibroblasts was greater in the fibrous-cellular group than in the myxoid group. This was consistent with the results of actin immunohistochemistry. In 3 cases of the fibrous-cellular group peculiar structures simulating Z-lines of striated muscles were noted in some stromal cells. Since no myosin filaments were detected, they were regarded as intermediate structures between Z-lines of striated muscles and dense bodies of smooth muscles. In the sclerotic group, stromal fibroblasts were sparse and had fewer organelles.

Key words: Fibroadenoma – Stromal cells – Actin – Ultrastructure – Myofibroblast

We have studied the occurrence of active fibroblasts and myofibroblasts in the stroma of invasive ductal carcinomas, considered to be a host reaction to carcinoma growth (cf Seemayer et al. 1980).

Our study was extended into fibroadenoma, a mixed connective tissue and epithelial tumor of the breast. The present study was focused on the

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Table 1. Summary of data

No.	Age	Type	Stroma	Actin Immuno-histo-chemistry	Amount of micro-filaments	Stromal cell count in 3,900 μm² (mean ± 1 SD)	
1	30	IC	M	+	+	4.8 ± 2.4	total of
2	33	IC	M	+	+	6.5 ± 2.7	myxoid group
3	27	IC	M	−	− ~ +	6.9 ± 2.2	8.2 ± 4.1
4	33	IC	M	+	+	7.4 ± 2.6	
5	19	IC	M	+	+	7.4 ± 2.6	
6	23	PC	M	−	+	7.5 ± 4.0	
7	39	IC	M ~ (F-C)	+	+	14.0 ± 3.7	
8	20	PC	F-C	+ +	+ +	15.6 ± 2.7	total of
9	20	PC	F-C	+ +	+ +	12.5 ± 3.4	fibrous-cellular
10	31	PC	F-C	not done	+ + *	11.4 ± 3.4	group
11	22	PC	F-C	+ +	+ + *	10.2 ± 3.3	11.9 ± 3.7
12	36	PC	F-C	+ +	+ + *	9.9 ± 2.8	
13	28	PC	S	+	−	5.6 ± 2.9	total of
14	36	IC	S	−	−	3.8 ± 2.3	sclerotic group
							4.7 ± 2.8

IC = intracanalicular type, PC = pericanalicular type; M = myxoid, F-C = fibrous-cellular, S = sclerotic; − = not detected, + = a few amount, + + = abundant; * = a case in which Z-line like structures were observed

There are statistically significant differences between mean values of each two groups ($p < 0.001$)

relationship between actin immunohistochemistry and cytoplasmic filaments of stromal cells, and concerned the occurrence of peculiar Z-line like structures. Readers should note that we use the term *fibroblast* in a wider sense, including myofibroblast, since myofibroblasts are usually a variant or subgroup of fibroblasts and a definite discrimination between the two cells is almost impossible (see Ohtani and Sasano 1983).

Materials and methods

Fibroadenomas of the breast obtained surgically from 14 female patients were used (Table 1).

Immunohistochemistry. Immunohistochemical stains for actin were performed on paraffin sections from 13 cases of the lesions fixed in 10% formalin. The peroxidase-antiperoxidase (PAP) immune complex method (Sternberger et al. 1970) was employed on serial sections, 2.5 μm in thickness. Rabbit antiserum against actin from plasmodium of a myxomycete, Physarum polycephalum, was generously supplied by Drs. Owaribe and Hatano, Institute of Molecular Biology, Faculty of Science, Nagoya University. The immunization and property of this antibody such as the cross-reactivity with actins in a wide variety of eukaryotic cells has been described previously (Owaribe et al. 1975 and 1979). Primary antiserum, swine antiserum against rabbit gammaglobulin (DAKO-Immunoglobulins, Ltd., Denmark) and PAP (DAKO) were reacted at dilutions of 1:80, 1:40 and 1:80, respectively. Positive controls were myoepithelial cells and vascular smooth muscle cells in each block. For negative control the primary antibody was replaced by normal rabbit serum at dilution of 1:20. Negative controls showed no specific reactions.

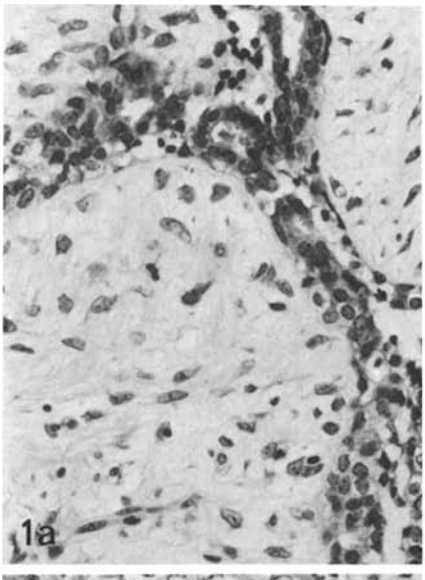
Electron microscopy. In all cases specimens were immediately fixed in 5% glutaraldehyde-4% paraformaldehyde or 2.5% glutaraldehyde in 0.8 M cacodylate buffer, pH 7.2–7.4. They were postfixed in 1% osmium tetroxide, dehydrated and embedded in Epon. Semi-thin sections were stained with toluidine blue to select proper areas for electron microscopy. Thin sections of silver-gold interference color were stained with uranyl acetate and lead citrate, and examined with a JEOL 100B electron microscope. For the control study of Z-line like structures 2 cases of rhabdomyosarcoma (embryonal type) were examined. Findings on invasive ductal carcinomas in our previous reports were used to compare with myofibroblasts.

Morphometry for cellularity of stromal cells. A square, 25 × 25 mm, was used in the ×400 fields, and stromal cells were counted within a square randomly obtained by the movement of microscopic stage. The area of one square was 3,900 μm^2. If epithelial components were present in the square, this square was not used for stromal cell counting. Total 30 squares in each case were studied. Leukocytes, plasm cells, macrophages and mast cells were excluded carefully. The mean values of stromal cell count per one square in the same group were compared.

Results

Histological and immunohistochemical findings. The 14 fibroadenomas examined were classified into 3 groups according to stromal patterns; myxoid (7 cases), fibrous-cellular (5 cases), and sclerotic (2 cases). The myxoid stroma was stained pale purple with haematoxylin with some stellate-shaped cells (Fig. 1a). The fibrous-cellular stroma had dense mature collagen bundles and fairly abundant stromal cells with plump nuclei (Fig. 2a). The sclerotic stroma had hyalinous collagen fibers with a few stromal cells with slender nuclei. The morphometric study showed higher cellularity in the fibrous-cellular group and lower in the sclerotic group (Table 1). Actin immunohistochemistry revealed a positive reaction in myoepithelial cells and vascular smooth muscle cells in every case. In 4 cases of the fibrous-cellular group, the most remarkable positive reaction was observed in stromal areas as a multiple short thread-like pattern (Fig. 2b). Since perinuclear cytoplasmic areas were positively stained, most of the positive reaction was presumed to be in the cytoplasmic areas. In 7 cases of the myxoid group and 2 cases of the sclerotic group, the stromal cells reacted either weakly or negatively (Fig. 1b).

Electron microscopy. The stromal fibroblasts in the myxoid and fibrous-cellular groups had a nucleus with dispersed chromatin and occasionally prominent nucleoli (Figs. 3, 4, and 6). Their cytoplasm had moderately developed rough endoplasmic reticulum, Golgi complex, and mitochondria. These findings suggested that they were in an active state. Short basal laminae were observed, and in rare occasions most part of a stromal cell was enveloped by a continuous basal lamina. In most cases stromal fibroblasts contained varying amounts of microfilaments, 5–7 nm in diameter, in the periphral cytoplasm. These filaments were remarkable in the fibrous-cellular group, rather sparse in the myxoid group and rare in the sclerotic group. This pattern was consistent with the actin immunohistochemistry. Dense bodies (focal densities) in bundles of microfilaments were generally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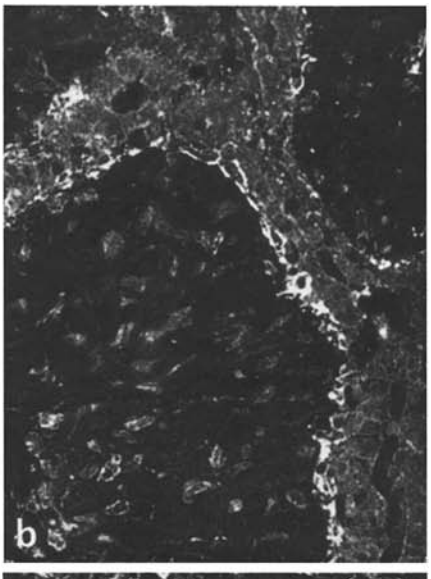
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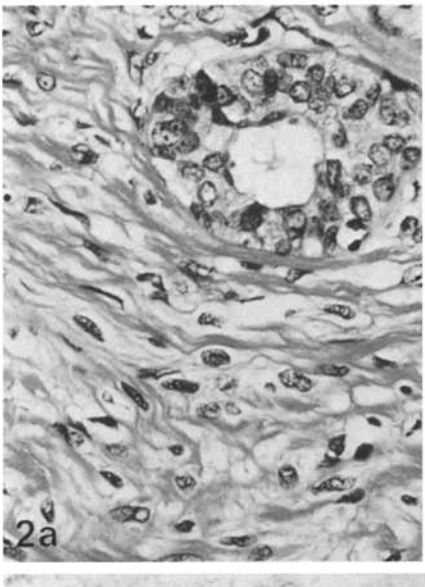
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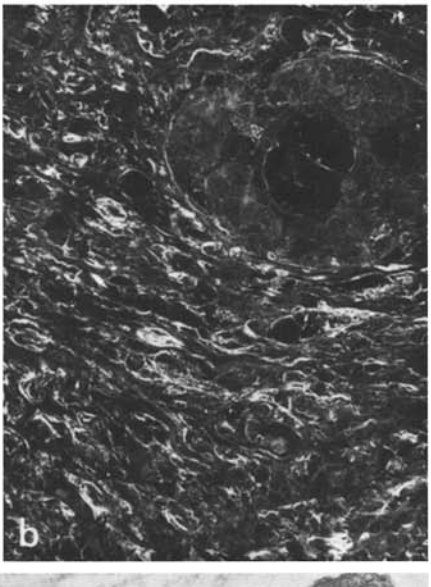
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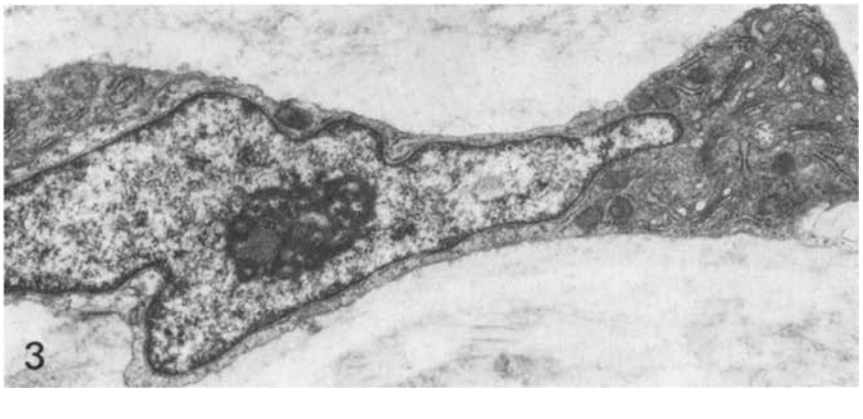
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undetectable except in a few cells in 3 cases of the myxoid group. This absence of a dense body was one of the significant differences from the microfilaments of myofibroblasts reported so far. Intermediate-sized filaments, 8–10 nm in diameter, were found more centrally in the cytoplasm (Fig. 5b).

In 3 out of 5 cases of the fibrous-cellular group, peculiar structures similar to the Z-lines of striated muscles were detected. Bundles of microfilaments, 5–7 nm in diameter, were arranged in arrays and inserted into electron dense zones situated perpendicularly to the long axis of microfilaments (Figs. 6, 8). We designated these structures "Z-line like". They were observed in the cytoplasm in certain cells near or among usual microfilaments. Two Z-line like structures were often arranged with intervals of 0.5–1 μm. No thick filaments of myosin type (approximately 15 nm in diameter) were observed. Fig. 9 indicated immature Z-lines in a rhabdomyosarcoma cell. Except for the presence of thick filaments, these immature Z-lines were similar to the Z-line like structures in the above stromal cells of fibroadenomas. Stromal cells had desmosome-like junctions (Fig. 5c). In addition to the central electron dense zones, subplasmalemmal electron dense zones were formed beneath apposed plasma membranes. Usually no converging filaments were found, but in some occasions several intermediate-sized filaments were inserted into these subplasmalemmal electron dense zones. These junctions were observed more frequently between cytoplasmic processes. The findings suggested network formation of stromal cells joined with each other by intercellular junctions.

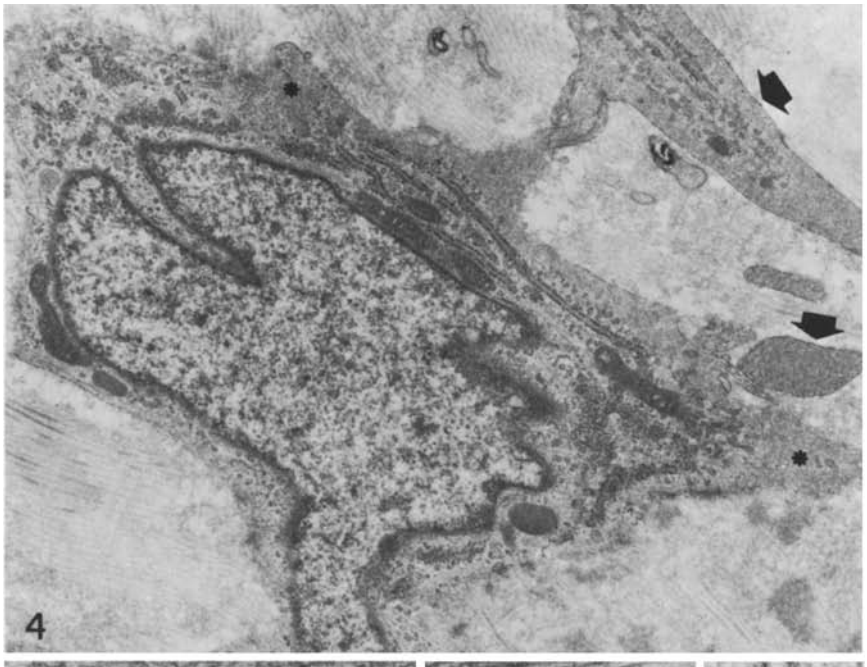
Discussion

Murad et al. (1967) reported that neoplastic cells in fibroadenoma of the breast were stromal cells and speculated that these cells were derived from pericytes on the grounds of the presence of cytoplasmic microfilaments and a basal lamina investing the cells. Their findings were, however, negated by Ozzello (1971). Carstens (1974), Fisher (1976), and Ahmed (1978) described the stromal cells as fibroblasts, while Horie (1981) regarded them

Fig. 1a, b. Fibroadenoma with myxoid stroma. ×370. **a** H-E. **b** Actin immunohistochemistry on a successive serial section (PAP method, negative printing). Positive reactions are expressed as white in black back tone. Myoepithelial cells are clearly observed. Stromal cells reacted sporadically

Fig. 2a, b. Fibroadenoma with fibrous-cellular stroma. ×370. **a** H-E. **b** Actin immunohistochemistry on a successive serial section (PAP method, negative printing). The stromal area showed a strong reaction

Fig. 3. A stromal fibroblast in myxoid stroma. Microfilaments are not visible in this cell. ×11,000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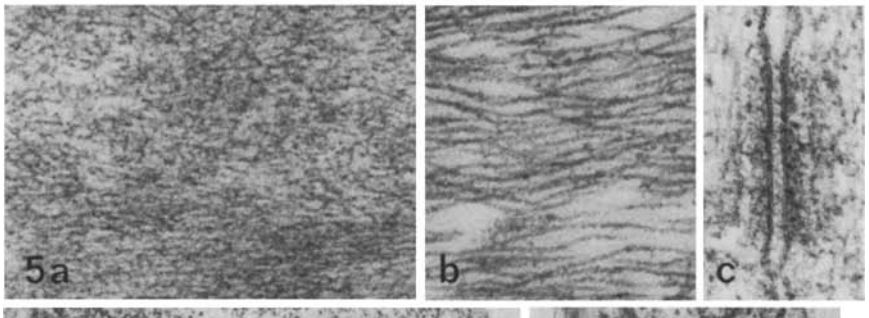
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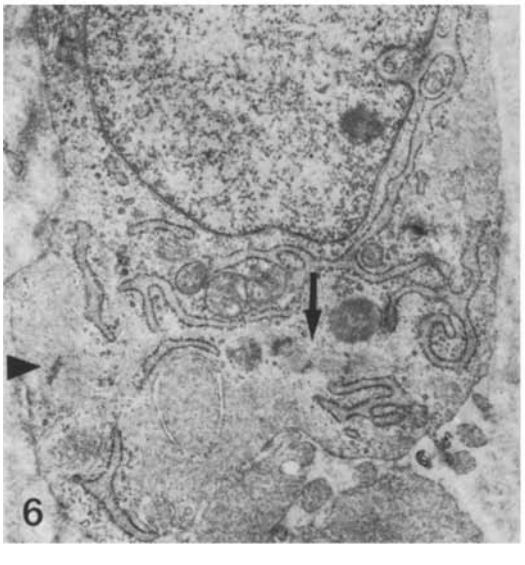
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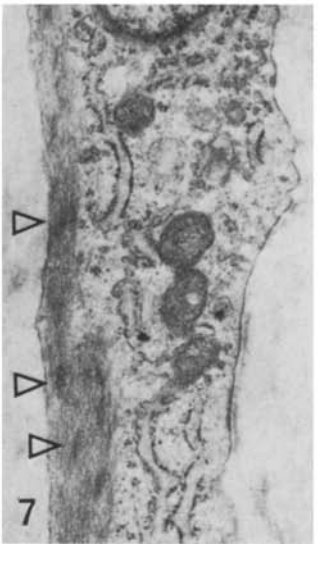
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as myofibroblasts. In this paper we partially corroborated the findings by Murad et al. and Horie since the stromal cells were basically fibroblasts occasionally containing fairly abundant microfilaments of actin type. Actin immunohistochemistry revealed their actin or actin-like properties, but no cell showed evidence of relationship with pericytes. We considered that the microfilaments in the stromal cells should be regarded as another type of actin filaments in fibroblasts. It is well known that fibroblasts are endowed with actin type microfilaments in certain circumstances and are then designated as myofibroblasts (reviewed by Liper et al. 1980; Seemayer et al. 1980). In these cells the microfilaments are arranged into narrow bundles beneath the plasma membranes with dense bodies (focal densities) (Fig. 7).

In our present material no dense body was observed except on rare occasions and the microfilaments were not arranged into well-oriented narrow bundles. These are the main differences between the cell types and therefore, the stromal cells of fibroadenoma, particularly those of the fibrous-cellular group, are regarded as variants of myofibroblasts. They are fiboblasts in an active form containing fairly abundant microfilaments of actin type without dense bodies.

Gibert et al. (1982) reported that stroma of fibroadenoma showed a strongly positive reaction with an antiactin antibody by the immunofluorescent method. Our results in the fibrous-cellular group are exactly the same as theirs but in the myxoid and sclerotic groups stromal areas did not show such strong reactions.

This may be the first report of the occurrence of Z-line like structures in the stromal cells of fibroadenoma. They resembled three kinds of structures of muscle cells (Fig. 10). First they resembled immature Z-lines such as observed in rhabdomyosarcoma cells, because of less well-ordered structures compared with normal Z-lines, but the lack of thick myosin filaments in Z-line like structures is an important difference. Second, they resembled dense bodies of smooth muscle cells, but the long axis of dense bodies was usually parallel to that of microfilaments, while the long axis of Z-line like structures was perpendicular to that of microfilaments. Finally they resembled leptomeric fibrils of striated muscles, which are composed of

Fig. 4. A stromal fibroblast in fibrous-cellular stroma. Microfilaments are abundant in peripheral cytoplasm (*asterics*). Arrows indicate cytoplasmic processes filled with microfilaments. × 14,300

Fig. 5. a Higher magnification of microfilaments, 5–7 nm in diameter. × 67,000. **b** Intermediate-sized filaments, 8–10 nm in diameter. × 67,000. **c** An intercellular junction between stromal cells. × 84,000

Fig. 6. A part of a stromal cell in the fibrous-cellular group with microfilaments. Notice two Z-line like structures (*arrow and triangle*). × 11,000

Fig. 7. A part of a "myofibroblast" in the stroma of invasive ductal carcinoma. Notice dense bodies in a bundle of microfilaments (*triangles*). × 16,000

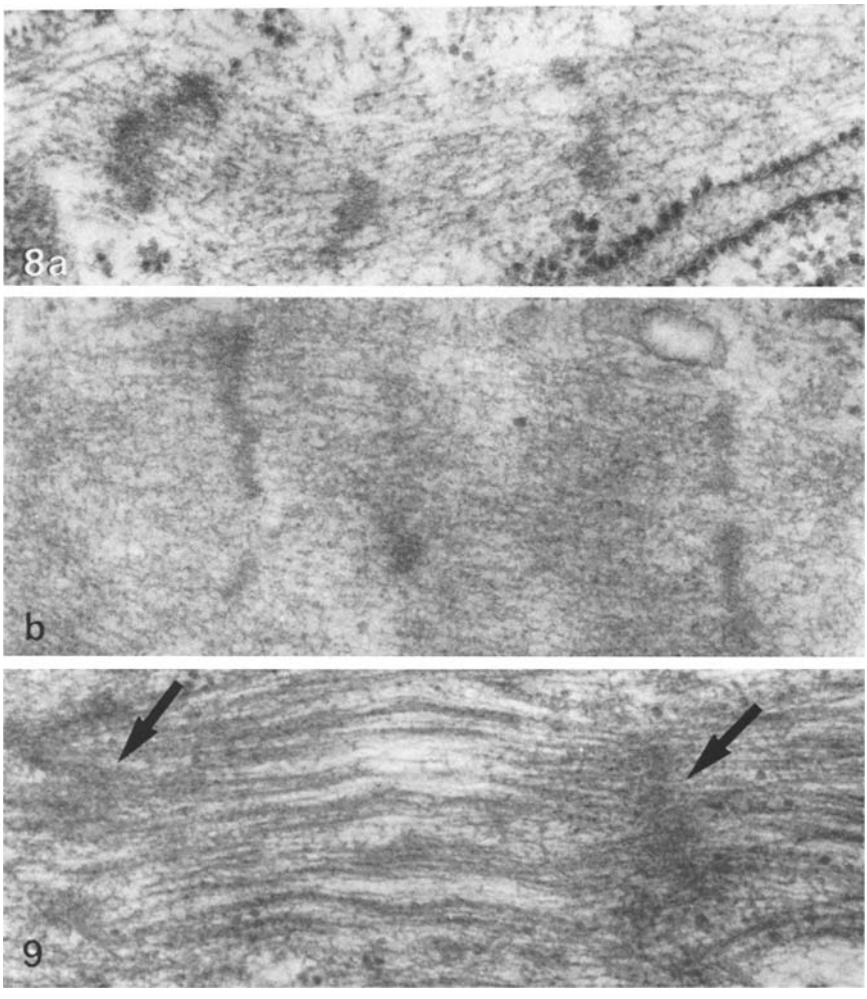

Fig. 8. a Higher magnification of Z-line like structures indicated by a long arrow in Fig. 6. × 67,000. **b** Other Z-line like structures in another cell. × 67,000

Fig. 9. Immature Z-lines in a rhabdomyosarcoma cell (*arrows*). × 52,000

a well-arranged bundle of microfilaments with several periodic transverse discs (Thoenes and Ruska 1960; Karlsson and Andersson-Cedergren 1968; Bogusch 1975). However, the interval of transverse discs was about 0.2 μm, shorter than that of the Z-line like structures. These facts suggested that the Z-line like structures were intermediate structures between Z-lines and transverse discs of leptomeric fibrils in striated muscles and dense bodies of smooth muscle cells. Since Z-line like structures were exclusively observed in stromal cells with abundant microfilaments in the fibrous-cellular group, they are apparently specialized structures of microfilaments. This occurrence of Z-line like structures seems to be one of the minifestations of multipoten-

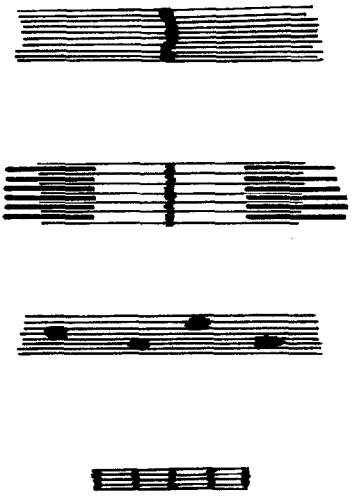

Fig. 10. A diagram of filamentous structures. **a** Z-line like structures and thin filaments, **b** Z-line, thin and thick filaments. **c** Dense bodies and thin filaments. **d** Leptomeric fibrils. (Thin filaments correspond to actin or actin-like filaments.)

tiality of stromal cells of fibroadenoma and may explain a rhabdomyosarcomatous change in malignant cystosarcoma phyllodes (Lester and Stout 1954; Barnes and Pietruszka 1978).

Our result that stromal cells in fibrous-cellular stroma have abundant microfilaments reminds us of the tissue contraction mechanism theory of myofibroblasts (Gabbiani et al. 1972; Ryan et al. 1974). But the fibroadenoma is usually a well circumscribed tumor and does not cause contraction of the neighboring tissue as invasive ductal carcinoma does (in the stroma of the latter "myofibroblasts" are observed). The true function of these contractile filaments of fibroblasts in fibroadenoma remains unclear.

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