Ultrastructural Studies on Congenital Generalized Fibromatosis Regressed Spontaneously

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KOBAYASHI, Y., WATANABE, H., SUZUKI, H., KONNO, T. and YAMAMOTO, T.Y. Ultrastructural Studies on Congenital Generalized Fibromatosis Regressed Spontaneously. Tohoku J. exp. Med., 1981, 134 (4), 431–445 — Cellular components of a subcutaneous nodule from a male infant with congenital generalized fibromatosis consisted of myofibroblasts, fibroblasts, and their various intermediate types. The myofibroblasts were characterized by the presence of bundles of microfilaments and dense bodies similar to those in smooth muscle cells. Membrane-bound vesicles containing dense material were abundant in the extracellular space. They were probably lysosomes released by disintegration of cellular components. A possible role of the myofibroblasts and extracellular lysosomes in the regression of nodular lesions was discussed.

Congenital generalized fibromatosis is an extremely rare disease characterized by multiple tumors of differentiated fibrous tissue in the skin, the musculoskeletal system, and various visceral organs. Its clinical result is not uniform ranging from fatal to spontaneously regressive.

In 1977, Benjamin et al. first revealed the presence of myofibroblasts in the nodule of this disease by electron microscopy. They emphasized the role of these contractile myofibroblasts in the spontaneous regression.

Recently, we had a 4-month-old male Japanese infant with congenital generalized fibromatosis which took a course of spontaneous regression. To our knowledge, it is the first case of this disease in Japan. This communication describes the fine structure of biopsy specimens of subcutaneous nodules from this patient and discusses the mechanism of spontaneous disappearance of the tumor.

CASE REPORT

A 4-month-old male Japanese infant was referred to Tohoku University Hospital because of a difference in length of the lower extremities and punched out lesions on general bone roentgenograms. The patient was the second child of healthy parents. There was no family history of neurofibromatosis. He was born
in the 41st week of gestation after uneventful pregnancy. The delivery was by cesarean section, and the birth weight was 2,420 g.

At 3 months of age, he received an examination for dislocation of the hip joint, and at that time the punched out lesions were found on roentgenograms of the hip bones. At 4 months of age, he consulted an orthopedic clinic, where multiple punched out lesions were discovered throughout the skeleton. The initial diagnosis was eosinophilic granuloma.

On admission to our Ward, physical examination revealed a well-developed, active infant, and we noted four subcutaneous nodules; one on the right scapula, one on the chest, and two on the abdomen. They were apparently indurated, not freely movable, nor tender.

The roentgenograms showed multiple lytic lesions of the skull, humeri, ulnae, femora, tibiae, thoracic vertebrae, and hip bones. A total of 19 bone lesions were counted. A chest roentgenogram revealed a pattern of interstitial fibrosis of the lung with multiple small nodules. Barium enema showed several filling defects, which were interpreted as intraluminal nodules. Examinations of the cardiovascular system disclosed no abnormalities. The intravenous pyelogram was normal. Needle biopsy of the liver showed no histopathological evidence. A $^{99m}$Tc sulphur colloid liver scan was normal. Biopsy was performed for a lesion in the left tibia, but no characteristic findings of eosinophilic granuloma were obtained by histological examination of the bone. One of the subcutaneous nodules on the abdomen was also examined by biopsy. This nodule was firmly adherent to the abdominal rectal muscle.

The patient was treated with corticosteroid (prednisolone, 2 mg/kg/day orally). However, this produced no beneficial effect, and was discontinued after one month. At 2 years of age, multiple lytic lesions of bones disappeared spontaneously and he is growing satisfactorily.

**Materials and Methods**

Under general anesthesia small pieces of tissue were removed from the subcutaneous nodule on the right upper abdomen. The specimens were immediately fixed in a mixture of 5% glutaraldehyde and 4% paraformaldehyde buffered at pH 7.4. After post fixation in 1% osmium tetroxide, they were dehydrated in graded concentrations of ethanol and embedded in epoxy resin. Ultrathin sections were stained with 1% uranyl acetate and lead citrate and examined with a JEM 100C electron microscope.

Thick sections were stained with toluidine blue for light microscopy. Some tissue pieces were fixed in formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin or van Gieson stain.

**Results**

*Light microscopic findings*

Light microscopically, the nodule consisted of spindle-shaped cells, fibroblasts, and collagen fibers arranged in a whorled pattern (Fig. 1). Its histological appearance showed a marked regional variation; some regions contained mostly cellular components, while others contained predominantly fibrous components.
There was no mitotic figure. Van Gieson-stained sections suggested the presence of smooth muscle cells and nerve fibers.

Electron microscopic findings

The cellular region

The region contained numerous myofibroblasts which possessed dense bundles of microfilaments accompanied with dense bodies (Fig. 2). The bundles of microfilaments run along the long axis of the cell and were particularly well-developed in the peripheral cytoplasm. Typical fibroblasts were only a few in this region.

In the axial cytoplasm of the myofibroblasts were cisternae of granular endoplasmic reticulum (GER). They were often markedly dilated and contained flocculent fibrillar precipitates (Figs. 2, 3). The amounts of microfilaments and GER varied from cell to cell, and the area occupied by the former was almost inversely proportional to that occupied by the latter (Figs. 4, 5).

Nuclei of the myofibroblasts had irregular contours with deep invaginations (Fig. 6). The inner layer of the nuclear envelope was closely associated with a layer of homogeneous nuclear material, "nuclear limiting zone".

Large vacuoles, lipid droplets and dense aggregations of glycogen granules were identified, especially in the axial cytoplasm (Figs. 4, 5). Some of these cells contained a number of dense bodies of various shapes surrounded by a limiting membrane (Fig. 5). They were probably lysosomes.

The external lamina surrounding the myofibroblast varied in appearance from cell to cell. Some cells had only discontinuous patches of laminal substance, while others had a well-defined continuous lamina measuring up to 1 μm (Figs. 4–7).

Among the myofibroblasts with various electron microscopic features, some showed a close similarity to the smooth muscle cells. In these cells a number of pinocytotic vesicles and dense bodies were formed on their cell membranes. Sometimes, two adjacent cells seemed to be connected with each other by facing closely their cell membranes lined with the dense body (Fig. 8).

The extracellular space in the cellular region contained abundant elastin and electron dense bodies surrounded by a limiting membrane (Figs. 4–7). The presence of these bodies suggested a degenerative process of the cellular components, especially myofibroblasts.

The fibrous region

This region was composed mostly of fibroblasts and abundant collagen fibers (Fig. 9), whereas elastin and extracellular dense bodies were scanty. A few blood vessels surrounded by typical smooth muscle cells were distributed here (Fig. 10).

Discussion

Since Stout (1954) defined and established the entity of congenital generalized fibromatosis, a few cases have been reported in the medical literature (Baer and
Radkowski 1973; Plaschkes 1974). Beatty (1962) was the first who pointed out the presence of smooth musculature in the tumor of this disease. A previous electron microscopic study on a lesion of the right atrium at necropsy showed fibroblasts, collagen fibers, and scattered smooth muscle cells embedded in an amorphous substance (Morettin et al. 1972). Benjamin et al. (1977) reported ultrastructural observations on a case of congenital generalized fibromatosis which took a course of complete spontaneous regression. They found many myofibroblasts in nodules and speculated that the fibrocontractive process caused by these myofibroblasts was responsible for shortening of connective tissue and resultant shrinkage of the nodules.

The concept of myofibroblasts has been established rather recently. Myofibroblasts, a type of cell first identified in contractile granulation tissue (Gabbiani et al. 1971; Majno et al. 1971), have currently been found in a variety of neoplastic and non-neoplastic lesions (Gabbiani and Majno 1972; Madden et al. 1975; Stiller and Katenkamp 1975; Feiner and Kaye 1976; Bhawan et al. 1979; Ohtani and Sasano 1980). Majno et al. (1971) demonstrated that fibroblasts were capable of assuming structural and functional properties of smooth muscle cells in the process of granulation tissue contraction. They named these modified contractile fibroblasts "myofibroblasts". Thus, the fibroblast can be modified under certain conditions into a cell with contractile ability resembling the smooth muscle cell (Gabbiani et al. 1972; Ryan et al. 1974). Characteristic features of the myofibroblast described by Ryan et al. (1974) are as follows:

1. Bundles of intracytoplasmic microfilaments running in parallel to the long axis of the cell. In these bundles are formed many "dense bodies" similar to those in the smooth muscle cell.
2. Nuclear invaginations or deep folds of the nuclear surface.
3. Surface differentiation to a desmosome-like intercellular connection and the formation of external lamina.

The cells predominant in the cellular region of our material had all these properties, and therefore can be regarded as myofibroblasts. But, they were not uniform in structure; some were more similar to fibroblasts and some were akin to smooth muscle cells.

Benjamin et al. (1977) brought their attention to the fact that nodular lesions in the case of spontaneous regression contained abundant myofibroblasts. This fact suggests an important role of myofibroblasts in the regression process of this disease. Vasudev and Harris (1978) who recently reported a case of sarcoma of myofibroblasts stated that the presence of myofibroblasts in malignant tumors effected favorable clinical results. But, none of these reports could explain how the myofibroblast takes part in the disappearance of nodules.

The present study revealed abundant membrane-bound vesicles containing amorphous electron-dense material in the extracellular space as well as in the cytoplasm of myofibroblasts. They closely resembled in structure "matrix vesicles" in the hard tissue. The matrix vesicles have been regarded as initial loci of
calcification (Anderson 1969). Theyberg and Friberg (1970), Theyberg (1972) and Slavkin et al. (1972) demonstrated acid phosphatase and aryl sulphatase activity in such extracellular vesicles and considered them to be lysosomes. Riede and Staubesand (1977) suggested that these extracellular lysosomes exert their digestive function to degrade connective tissue, and thus the cells of mesenchymal nature renovate the extracellular substance. The extracellular lysosomes probably result from cell degeneration and disintegration.

We speculate that both contractile myofibroblasts and extracellular lysosomes play important roles in the disappearance of tumor and the spontaneous regression of the disease.

References


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Fig. 1. Light micrograph of the nodule showing spindle-shaped cells, fibroblasts, and collagen fibers. Toluidine blue stain. × 270.

Fig. 2. Myofibroblasts in the cellular region of the nodule. Note well-developed granular endoplasmic reticulum and bundles of microfilaments accompanied with dense bodies. × 8,300.
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Fig. 3. High magnification of well-developed granular endoplasmic reticulum in myofibroblast, which contained flocculent fibrillar precipitates. Gl, glycogen granules. $\times 44,000$.

Fig. 4. Lipid droplet (L) and dense aggregation of glycogen granules (Gl) of myofibroblasts. $\times 13,000$. 
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Fig. 5. A number of dense bodies of various shapes in the extracellular space as well as in the cytoplasm of myofibroblasts. $\times$ 12,500.

Fig. 6. Nucleus of the myofibroblast which had irregular contours with deep invaginations. The arrow indicates the nuclear limiting zone. $\times$ 8,900.
Fig. 7. Well-defined external lamina (arrow), measuring up to 1 μm, around the myofibroblast. × 11,000.

Fig. 8. Desmosome-like connection between myofibroblasts. × 54,500.
Fig. 9. The fibrous region of the nodule showing fibroblasts and abundant collagen fibers. \( \times 28,000 \).

Fig. 10. Typical smooth muscle cells surrounding blood vessels. \( \times 22,000 \).
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