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# ULTRASTRUCTURE OF LIPID DROPLETS AND NUCLEAR INCLUSIONS IN A PLEOMORPHIC LIPOSARCOMA

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SUMMARY

We examined ultrastructures of a pleomorphic liposarcoma originating from the epicardium with special reference to lipid droplets and nuclear inclusions in the sarcoma cells. Consequently, four types of lipid droplets were discriminated in tumor cells. The most frequent type was mainly round lipid droplets with moderate electron density and diamaters of 0.7 to 2.4$\mu$m. The second most frequent type was round or elliptical lipid droplets with a halo or a central shaggy core of moderate electron density. Their size measured about 1.0$\mu$m in diameter. Droplets of the third type were irregular and highly osmiophilic and resembled lysosomes, though they lacked a limiting membrane. The size of these droplets ranged from 0.6 to 2.0$\mu$m in the largest diameter. Droplets of the fourth type were mainly round. Each had a large central vacuole and the diameter was from 0.6 to 2.0$\mu$m. The last two types were much less frequent than the former two.

These findings might indicate deviated lipogenesis of the sarcoma cells, compared with lipogenesis in normal fat cells.

Several types of nuclear inclusion bodies were found mainly in the mononuclear tumor cells. They were fundamentally round and multi-lamellar bodies, with diameters of about 400 to 800nm. In addition, some nuclear bodies contained small lipid droplets, an aggregate of microfilaments or a round dense central core, and they occasionaly showed vacuolation suggestive of their degeneration. Fusion of several kinds of the nuclear bodies was also found and resulted in an irregular large inclusion. The origin of the nuclear bodies is obscure but they seem to indicate high nuclear activities of the sarcoma cells.

## INTRODUCTION

Liposarcomas are uncommon neoplasms which principally occur in the retroperitoneum and lower extremities[2,3].

Their ultrastructural features are characterized by lipid inclusion in the cytoplasm. The lipid droplets in liposarcoma described to date have been regarded as a reflection of the wide cellular spectrum seen during the differentiation of adipose tissue[4,11].

This paper deals with ultrastructural characteristics of the lipid droplets which seem to include hitherto undescribed types and several kinds of intranuclear inclusion also not described previously in pleomorphic liposarcomas.

## MATERIALS AND METHODS

Case: A 79-year-old female with pleomorphic liposarcoma in the epicardium has been reported upon perviously[15]. Histological features of the liposarcoma showed mainly malignant fibrous histiocytoma-like, and partly myxoid or fibrosarcoma-like varieties. Most of the tumor cells, irrespective of their morphological floridness, were intensely stained with histochemical methods for lipids such as Sudan III, Sudan black B, oil red O and Nile blue.

Transmission electron microscopy: Tumor tissue obtained at autopsy was minced into small pieces, and immediately fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer at ph7.4, post-fixed with 1% osmium tetroxide in the same buffer, dehydrated and embedded in Epon 812.

The ultrathin sections were cut in an LKB ultrotome, and stained with uranyl acetate and lead citrate, and examined with a Hitachi HS-9 electron microscope (Hitachi Ltd., Tokyo).

## RESULTS

Lipid droplets: Lipid droplets observed with an electron microscope could be subdivided into four types according to their shapes and electron density. Each type of the lipid droplets in the order of frequency is as follows. Type I lipid droplets were the most frequent, and mainly round, showed moderate and homogeneous electron density (Fig. 1a). The droplets were not associated with a limiting membrane but an incomplete linear rim of high electron density was frequently found at their peripheral margin.

The size of the droplets ranged from 0.7 to 2.4$\mu$m in diameter. The nucleus of the cell which harbored this type of lipid droplets was very irregular in shape and had dense heterochromatin beneath the nuclear membrane. Type II lipid droplets were round and could be seen as elliptical inclusions with a halo or a central shaggy core of moderate electron density. They were encountered in the second highest frequency (Fig. 1b).

There was no limiting membrane around the droplets. Their size was about 0.1$\mu$m in diameter. The nucleus of the cell which had these lipid droplets was rather smooth in

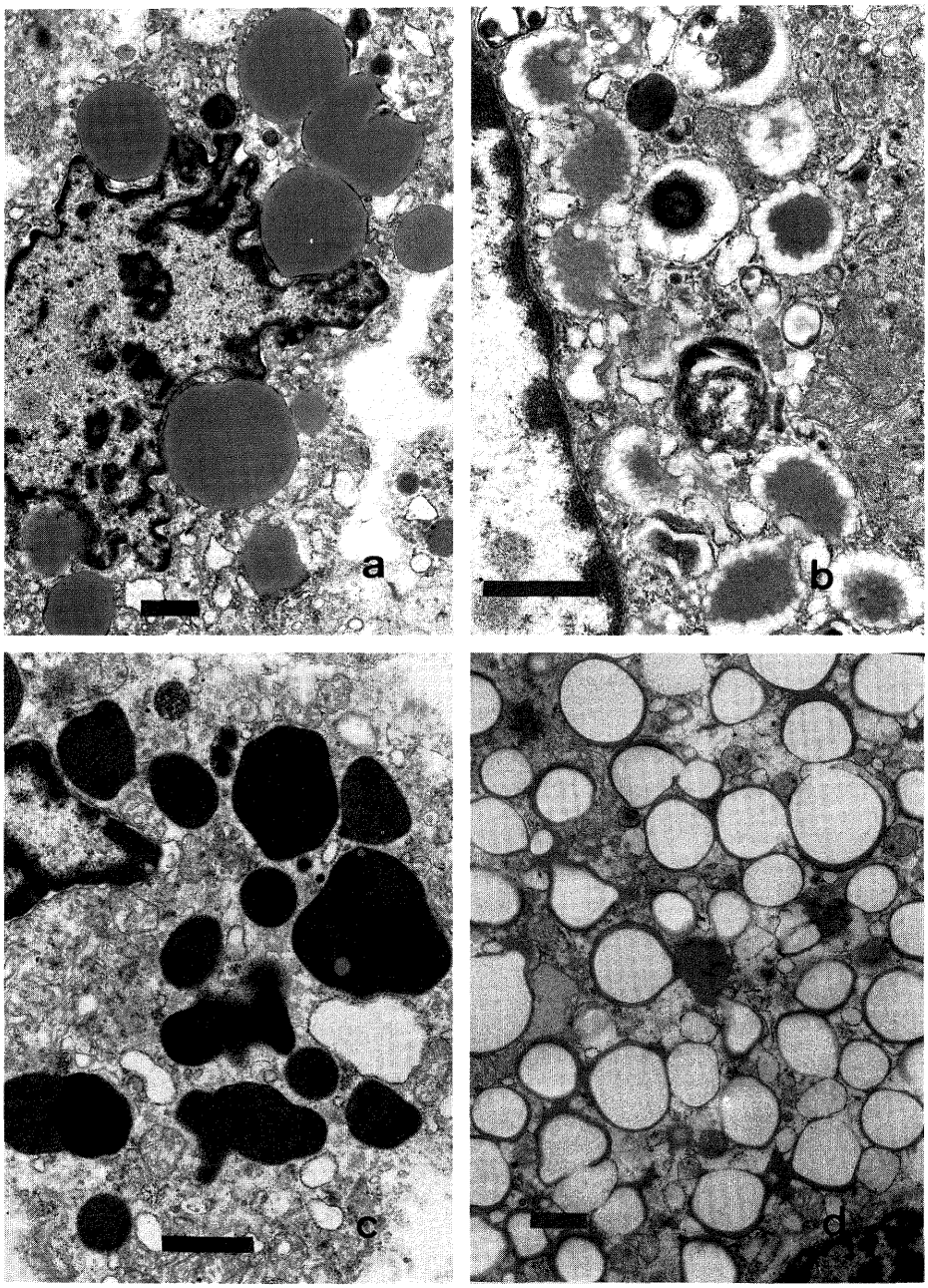


**Fig. 1.** Four types of lipid droplets observed in different tumor cells. Type I lipid droplets with moderate electron density are round and associated with an incomplete linear high electron dense margin (a), type II are round and have a halo or a central shaggy core with moderate electron density (b), type III are pleomorphic and highly osmiophilic (c) and type IV are round and have large central vacuolation (d). (a, d: × 7,200. b: × 15,000. c: 12,000. bar: 1$\mu$).

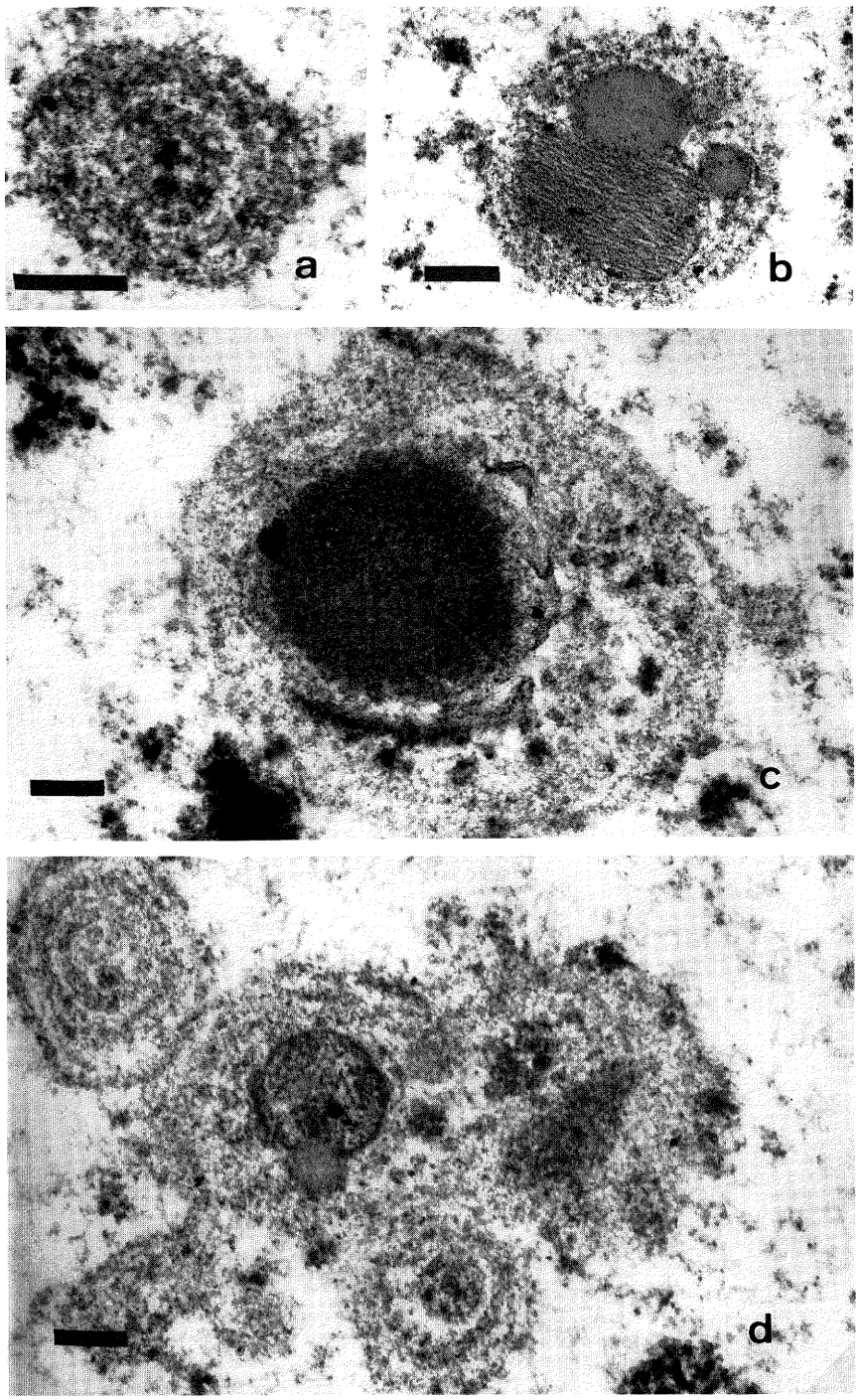


**Fig. 2.** Intranuclear inclusion bodies: round and vaguely lamellar (a), round and containing lipid droplets and filamentous bundle (b), round, vaguely lamellar cortical structures with a dense central core (c), and the coalescing type mentioned above (d).
(a: 72,000. b-d: × 48,000. bar: 1$\mu$).

contour and had a smaller amount of heterochromatin. The third type of lipid droplets, types III, was irregular, highly osmiophilic and resembled lysosomal granules, but they had no limiting membrane (Fig. 1c). Their size was approximately 0.6 to 2.0$\mu$m in the largest diameter. The nucleus of the tumor cell with these lipid inclusions was rather irregular and had dense heterochromatin. The fourth type of lipid droplets, types IV, was mainly round and had a large central vacuole. Their size ranged from 0.6 to 2.0$\mu$m in diameter (Fig. 1d). The nucleus of the cell which contained these lipid droplets was round and had dense heterochromatin. The frequency of type III and IV of lipid inclusion was much less than the first two types.

Nuclear bodies: Several types of intranuclear inclusion bodies were also found mainly in the mononuclear fibroblastic tumor cells at a frequency rate of about 5% (Fig. 2). There was no limiting membrane around them. Fundamentally, they were round in shape and multi-lamellar in construction (Fig. 2a), measuring about 400 to 800nm in diameter. Some of them contained lipid droplets and aggregated filaments (Fig. 2b), or dense central core (Fig. 2c). Occasionally the nuclear bodies of differrent types joined with one another and formed an irregular large inclusion body (Fig. 2d).

## DISCUSSION

Ultrastructures of liposarcomas have been reported by over ten authors[1,4,6,7,8,10,11,12,13,15,19]. Almost all of them have pointed out the similarity of the lipid droplets in sarcoma cells to either white adipose or brown fat tissues.

In our case, type I lipid droplets which have been found in fibro- and myofibroblastic sarcoma cells are similar to brown fat vacuoles[16] and type II droplets in mesenchymal or early lipoblastic cells resembled lipid droplets in primitive white adipocytes[9]. It seems that type III and IV lipid inclusions have not been described in the literature.

These findings, however, suggest deviated lipogenesis in the tumor cells of a pleomorphic liposarcoma.

Membrane-bound lipid droplets reported by Lagaće et al.[8] and close association of lipid droplets to rough endoplasmic reticula described by Shimoda et al.[13] could not be detected in our study.

No intranuclear inclusion, except in studies of a pleomorphic liposarcoma by Desai et al.[1] and Fu et al.[4], has been pointed out, although some intracytoplasmic inclusions have been described in liposarcomas[7,12,19]. Desai et al.[1] have described a double-walled limiting membane-like structure completely encircling a nucleolus, and Fu at al.[4] have reported on a seemingly fibrous inclusion but the morphology is not clear because of the low magnification used. These nuclear bodies are not similar to the ones we found. The intranuclear inclusions presented in this study resemble, to some extent, those described in full-term human placenta (a concentrically arranged dense body), bronchial mucosa of a heavy smoker (an inclusion body with fibrillary cortex and some microtubules), chronic lymphocytic, leukemia (a fibrillogranular inclusion), liver of a phenobarbitone-treated

hamster (a nuclear body containing lipid droplet, numerous dense structures with paler center and a fibrillaly cortex)[5], and papillary thyroid cancer (an inclusion with fibrillary cortex and dense central core)[14], but are not identical with those found in pulmonary adenocarcinoma or alveolar cell carcinoma (an aggregate of tubular stuructures)[17,18].

We can not determine the origin of the nuclear bodies in our case, although Tsumuraya et al.[18] sugguest that intranuclear inclusions in a pulmonary papillary adenocarcinoma may be derived from an inner nuclear membrane. The function of these inclusions in our case is also not clear but the inclusion bodies might be a reflection of high nuclear activity or metabolism in the sarcoma cells.

In conclusion, we have presented here various shapes of lipid droplets and nuclear bodies in pleomorphic liposarcoma cells, these structures might imply that the tumor tissue is composed of functionally diversified sarcoma cells in accordance with their morphological pleomorphism.

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