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Malignant Mixed Osteogenic Tumours of the Breast

An Ultrastructural Study of Two Cases

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Summary. Two cases of malignant mesodermic tumours of the human mammary gland with osteogenic and chondrogenic structures were analysed by optical and electronic microscopical means. One of them was associated with an adenocarcinoma of the gland. The histological pattern was similar to that of those cases previously examined under the optical microscope in the mammary gland and in extraskeletal osteogenic sarcomas of soft tissues. When investigated under the electron microscope, the chondroblasts possessed a highly developed RER in active synthesis with an amorphous material which contributed to the building up of the ground substance matrix of the tumorous cartilage. Osteoid fields with scattered osteoblasts appear throughout the tumorous stroma and were associated with calcium deposits. They were continuous with fibroblasts and mesenchymal undifferentiated cells of a very immature character. Giant cells of osteoclastic type were included within the mononucleated mesenchymal cells mimicking bone osteoclastoma. The presence of all these cell types suggests the existence of a common malignant origin, the stem cell being differentiated into epithelial carcinomatous and mesenchymal sarcomatous chondral and osteogenic tissues.

Introduction

The present report deals with two cases of mixed malignant mesodermic tumours of the breast, which were studied under the optical and electron microscopes. In the first case an adenocarcinoma was associated with giant cells—malignant chondral and osseous metaplasia of the stroma. The second tumour was a fibroblastic sarcoma continuous with giant cells and chondro-sarcomatous structures.

Case Reports

Case 1 (M.J.H.). A woman aged 40 years, gravida II, para II, noticed a nodule on her right breast two months before medical consultation. Physical examination revealed a firm tumorous mass, about 6 cm in diameter, in the upper outer quadrant of the breast. The tumour was not fixed to the skin or to the chest wall. During radical mastectomy performed according to the method of Halstead, a biopsy was taken. This tumour was 4 cm in diameter and had a necrotic and hemorrhagic central zone surrounded by solid whitish elastic tissue of mesenchymal appearance, and through which peripherical fat was infiltrated.

Microscopic Study. The tumour was formed by a complex association of the following tissues: undifferentiated adenocarcinomatous tissue with solid, trabecular disposition; an angiomatous mesenchymal variety with osteoclastic multinucleated giant cells; cartilaginous tissue of a highly anaplastic nature giving a chondrosarcomatous image; areas of abundant osteoid tissue with atypical osteoblasts and foci of multiple calcification. No predominance of one tissue component was found over others. There was a continuous transition between the carcinomatous, chondromatous and sarcomatous osteoclastic zones, osteosarcomatous areas being differentiated within the latter two.

The adenocarcinoma formed masses and solid cords of cells which were separated by fine, well vascularized, walls. These epithelial-looking cells had rounded or oval nuclei with irregularly scattered chromatin and frequent mitosis. Their cytoplasm was vacuolated and lumpy (Fig. 1a). The osteoclastoma was in continuity with the carcinoma. The epithelial cords were distended by lacunae filled with blood. The walls of these lacunae were formed by mesenchymal cells of a dual nature; some, giant and multinucleated, broke loose and freely occupied the vascular sinus; while others of a fibroblastic character formed irregular trabeculae (Fig. 1c). The chondral metaplastic portions of the tumour contained atypical cells forming compact masses engulfed by an abundant amorphous basophilic matrix. The chondroblasts were clustered in groups of several cells. Mitosis and nuclear anaplasia were very common (Fig. 1b). These nodules were continuous with the osteoid and carcinomatous portions. There was transformation of solid carcinomatous trabeculae into chondromatous structures by means of the progressive interstitial flooding of mucoid basophilic material. The metaplastic epithelial cells were progressively enclosed in the chondral matrix and became calcified.

We did not find a direct transition from the osseous metaplasia of the tumour to the carcinomatous areas, the chondrosarcomatous and osteoclastic structures invariably being interposed. The bone therefore proceeded indistinctly from both matrixes and originated by forming osteoid reticular trabeculi, bordered by threads of atypical osteoblasts which were progressively included in them and calcified, resembling the osteosarcomas of bone (Fig. 1d).

Case 2 (J.P.G.). The patient, a 44 year-old woman, gravida IV, para I, complained 5 months before medical consultation of a fast growing tumorous mass in the left breast. Medical examination revealed a voluminous tumorous mass of firm consistency and plurinodular nature occupying the whole upper and lower inner quadrants of the left breast, fixed to the skin but not to the chest wall. No axillary nodes were present. During radical mastectomy, performed according to the method of Halstead, a biopsy was taken.

Macroscopically, the tumour was 12×10 cm in size, of a well delimited nodular nature and adhered to the skin. On being dissected there where foci of necrosis as well as portions of a cartilaginous consistency.

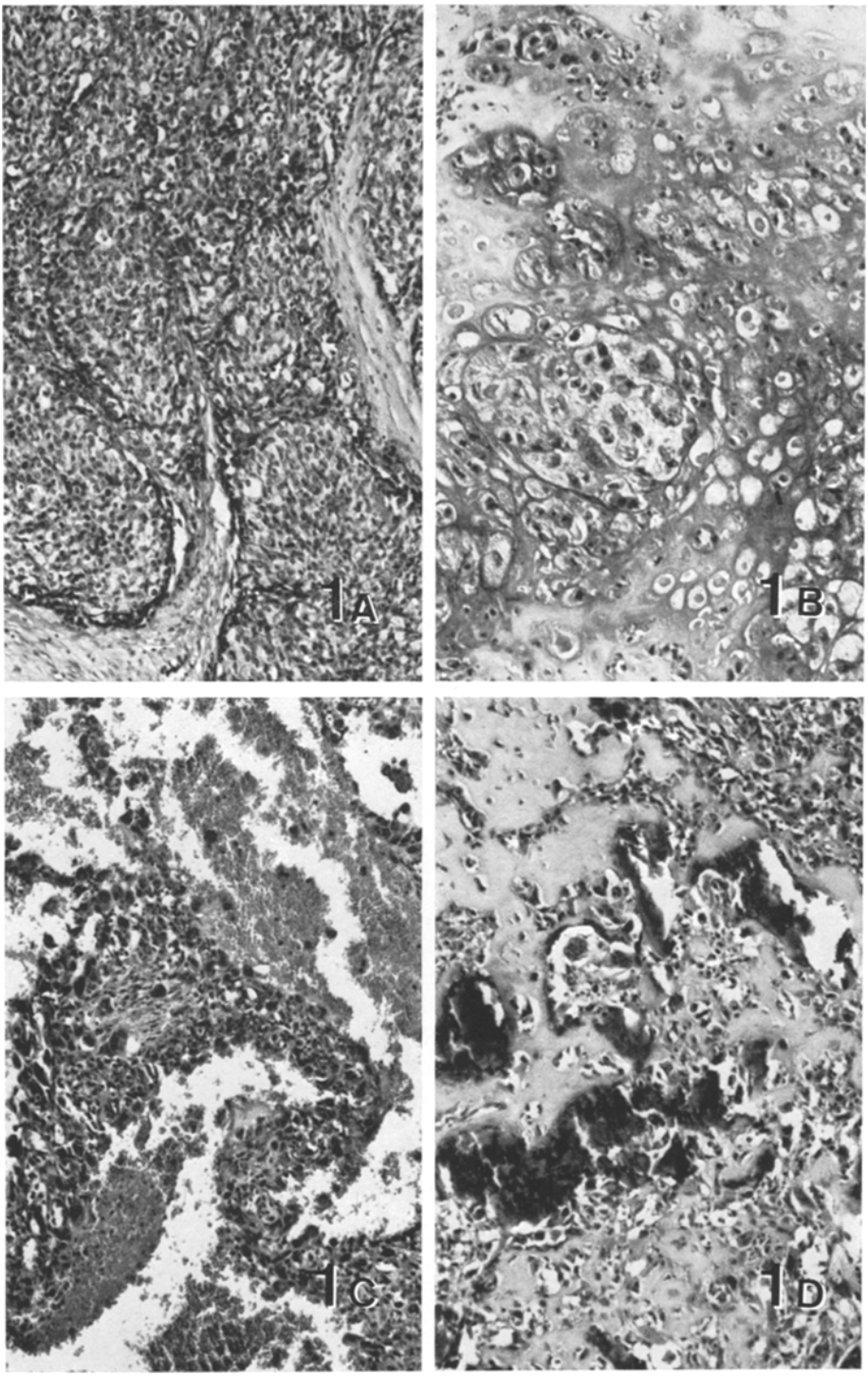
The microscopic study revealed a neoplasm of a malignant, mesenchymal nature but without evidence of epithelial participation. Fibroblastic and osteoclastic giant cells predominated with multiple foci of atypical chondral metaplasia organized in bands or compact fascicles. The fibroblasts were atypical and mitoses were frequently seen.

The chondrosarcoma was configurated by multiple confluent nodules within the sarcomatous mesenchyma (Fig. 2a). The cells forming them were atypical chondrocytes arranged in groups included in a basophilic mucoprotein matrix (Fig. 2b). There were some deposits of calcic salts within this mucoprotein substance. The osteoclasts accumulated in small clusters surrounded by chondrosarcomatous areas and were accompanied by undifferentiated mesenchymal mononucleated cells (Figs. 2c–d). There were haemorrhagic lacunae. No osseous metaplasia was present.

Ultrastructural Findings

Embedding and Cutting with Conventional Methods. Both tumours were highly compact and possessed abundant collagen fibres. The cells were grouped in irregular blocks and were close to the blood vessels. In the first case, numerous capillary lumina lined by endothelial cells appeared among the tumorous cells. These endothelial cells had a cytoplasm with filaments, micropinocytose vesicles and multiple microvilli toward the lumen; they also had continuous basal membrane. Furthermore, there existed sinusoids with badly defined basal membranes situated close to the tumorous cells of a mesenchymal character (Fig. 3a and 6).

Fig. 1 A—D. General view of the mammary tumour (case one) in which tissue of solid carcinomatous (a), chondrosarcomatous (b), atypical angioblastic fields coexist with giant cells (c) and bony trabeculae of malignant osteogenic nature (d). (H. and E., $\times$ 10)


1A
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I. Mesenchymatous Cells

Tumorous and Non-tumorous Fibroblasts. These cells were situated among dense networks of collagen and abundant amorphous fundamental substance. They were of immature appearance, with varying degrees of differentiation, having an elongated contour with tiny slender projections. The nucleus was centrally located and possessed a dense chromatin. The cytoplasm was well developed, having a frequently distended RER, filled with granular material. There were also bands of filaments in the perinuclear cytoplasm and among the cisternae of the RER.

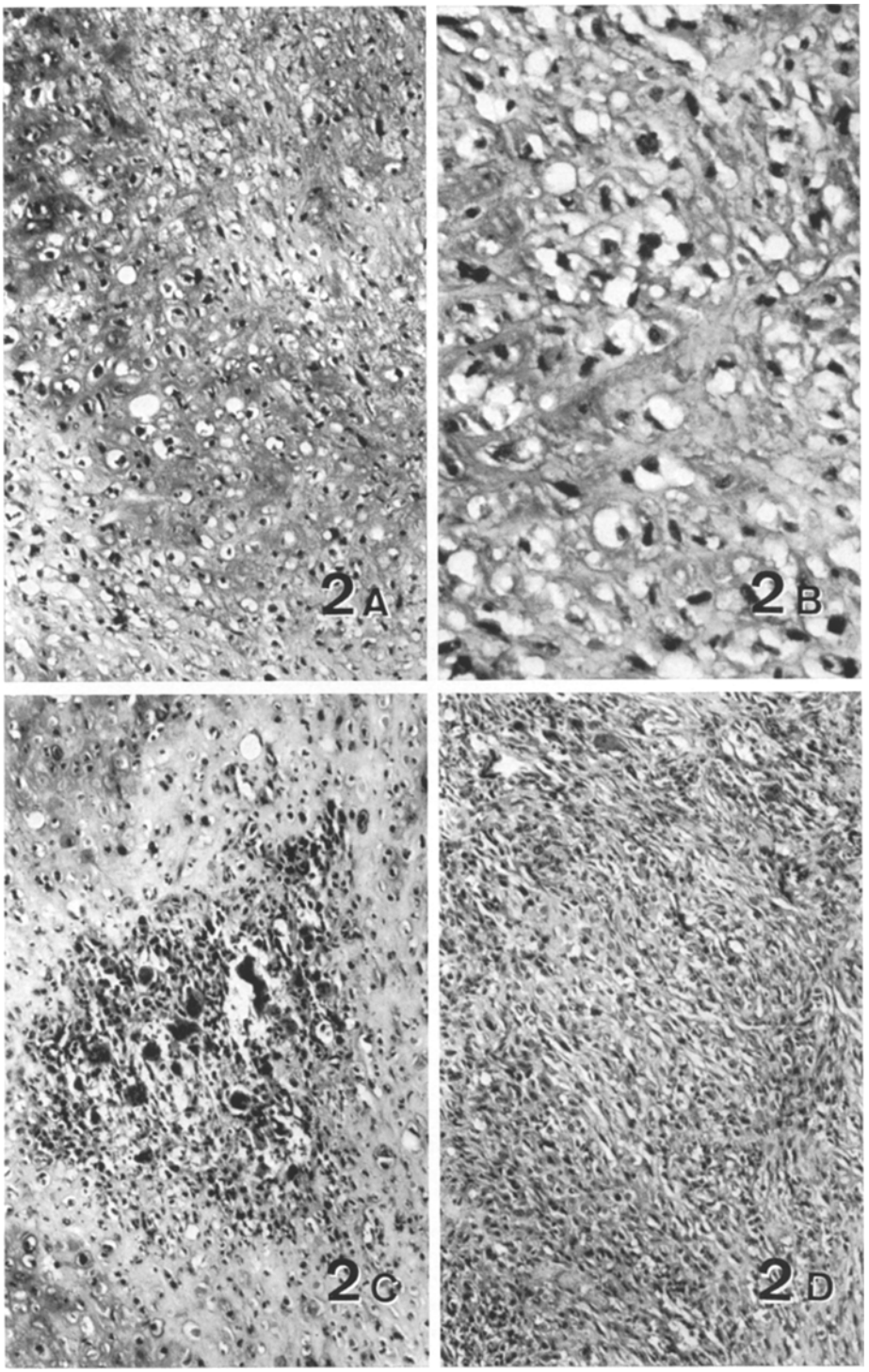
The greater their degree of maturity, the greater was their resemblance to normal fibroblasts. Moreover, adult fibrocytes of the kind habitually seen in the mammary gland were also present. These cells were similar in both neoplasms, but were more abundant in the second case.

Chondroblasts. These were very similar in both cases, and, in texture, resembled embryonic chondroblasts. The differentiation in the chondroblast-chondrocyte sense was more marked in the second tumour. The chondroblasts in the first case were more immature and often difficult to differentiate from osteoblasts. In both cases, there were also mesenchymal matrix cells with progressive simultaneous chondroblastic and osteoblastic differentiation (Scott's type A cells) (Fig. 3a).

The cellular contours of the chondroblast were blunt, rounded, polygonal or starred with short multiple projections. The more mature ones or those with greater functional activity possessed elongated cytoplasms arranged in nests and whose prolongations came into contact without attaining close unions of a desmosomic nature (Fig. 3a). The morphology of the cytoplasm was conditioned by the functional state of the cell and its secretory capacity. The more immature cells possessed large rounded nuclei with prominent central nucleoli and perinuclear chromatin (Fig. 5a). There were other chondroblasts whose nuclei were highly irregular and had numerous invaginations, infoldings and strangulations giving a polynuclear structure (Fig. 5). These were similar to those described as typical for the chondroblastoma of bone. An outstanding characteristic of all these cells was the intense development of the RER owing to a synthetic activity of fine granular material which condensed itself within the cisternae. Rounded or oval mitochondria were abundant, their matrix was vacuolated and of low density, containing isolated or multiple filamentous or granular deposits (Fig. 6). These intramitochondrial granules also appeared in the osteoblasts of Case one. The association between mitochondria and the RER was extremely intimate. There were lipid inclusions of a neutral type and lysosomes. Some of the cells presented a moderate amount of glycogen.

Osteoblasts. These had a very irregular polygonal or stellated form with fine, elongated projections. The cell was enclosed in a ground substance containing

Fig. 2A—D. Histological structure of case two. The poorly differentiated cartilage (a) (H. and E., × 10), shows atypical chondroblasts (b), (H. and E., × 40), which are continuous with the osteoblastic fields (c), (H. and E., × 10). There exist fibrosarcomatous structures among the chondrosarcomatous nodules (d), (H. and E., × 10)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a filamentous or granular matrix and calcium deposits in intimate proximity. The calcium deposits enveloped the fine and ramified cytoplasmic digitations. The cell nucleus was very irregular, showing some strangulations and dense, granular chromatin. The mitochondria were round with vacuolated crests and poor matrix. There were isolated lipid deposits, and the Golgi complex was well developed (Fig. 3b).

It was sometimes difficult to differentiate between chondroblasts and osteoblasts when the latter had a more undifferentiated blastematous character and mimicked the very immature osteoblasts seen in both post-fracture osseous reparation tissue and malignant osteosarcoma.

Giant Multinucleated Cells were present in both cases. In the first tumour, they formed part of the highly vascularised tissue complex resembling the walls of osseous aneurysmal cysts. In the second, the giant cells appeared inside a more dense stroma. In addition the multinucleated nature was remarkable. These multiple nuclei had prominent nucleoli, with the chromatin localized in perinuclear lumps. There were huge quantities of small, round or elongated type mitochondria with a dense or finely granular matrix. Moreover, there were abundant vesicles and small vacuoles located among the mitochondria. The cytoplasm was dense, with the frequent occurrence of lysosomes. The cellular surface was irregular and had sparse microvilli. On the whole, these cells were similar to those described in the giant cell tumour of bone.

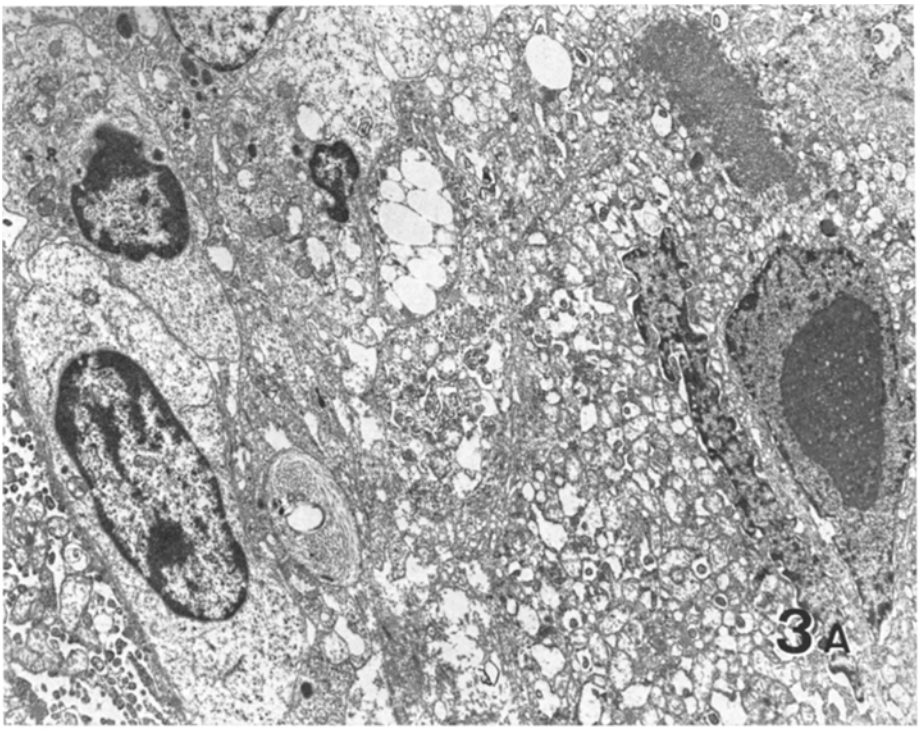
II. Epithelial Cells

These were only present in the first of the tumours, in which there also existed an association between the carcinoma and sarcomatous structures. These cells were difficult to differentiate from the matrix elements of the tumour, even though they possessed some characteristics peculiar to the epithelium, forming groups surrounded by incomplete basal membranes. The cells were densely associated among themselves, their membranes being joined not only by digitations and club-like projections but also by rudimentary desmosomes. They were linked with one another and formed alveoli with central lumina partly filled with microvilli. The cell nucleus was round with regular surface; the chromatin was fine and granular, and had a well developed nucleolus. In the cytoplasm, abundant, small and rounded mitochondria appeared arranged in groups. In association with these mitochondria, there were poor profiles of RER and some Golgi complexes. Some free ribosomes were present. We did not observe lysosomes, filamentous bundles or secretory material.

III. Undifferentiated Stem Cells

These were present in both cases, but were more frequent in the first tumour. Nevertheless, their ultrastructure was similar. They were arranged in nests or

Fig. 3A and B. Ultrastructural aspects of an atypical undifferentiated mesenchymal field within the tumour, with some cells initiating secretory activity at the RER (case one, (a) $\times$ 4000). A close-up view of an osteoblast with widely distended RER in secretory activity. Calcium deposits are visible around the cytoplasmic projections of the cells and among the reticular fibrillar matrix (case one, (b), $\times$ 8000)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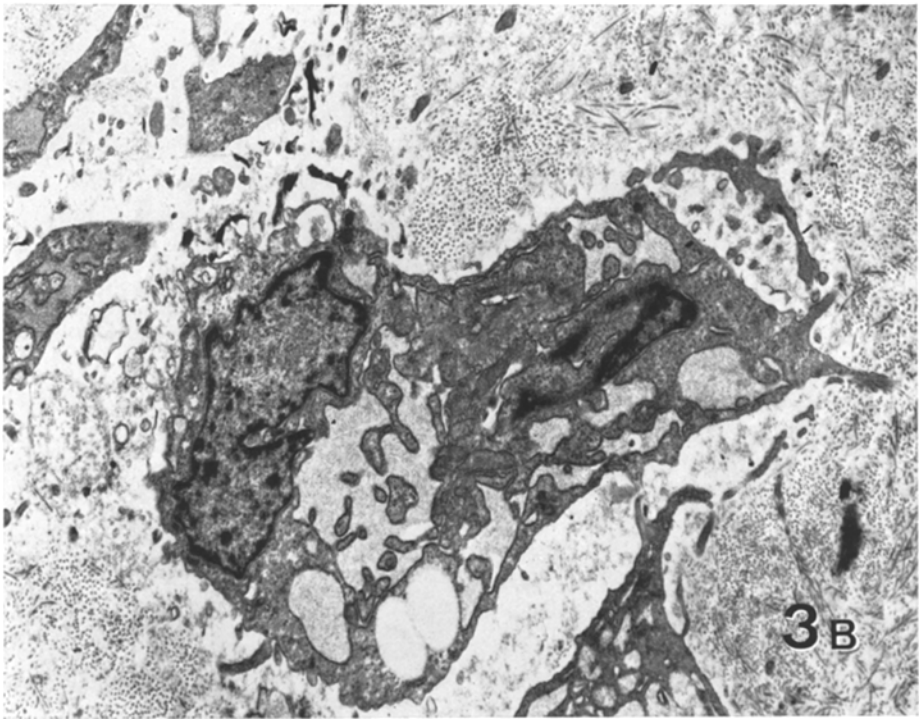
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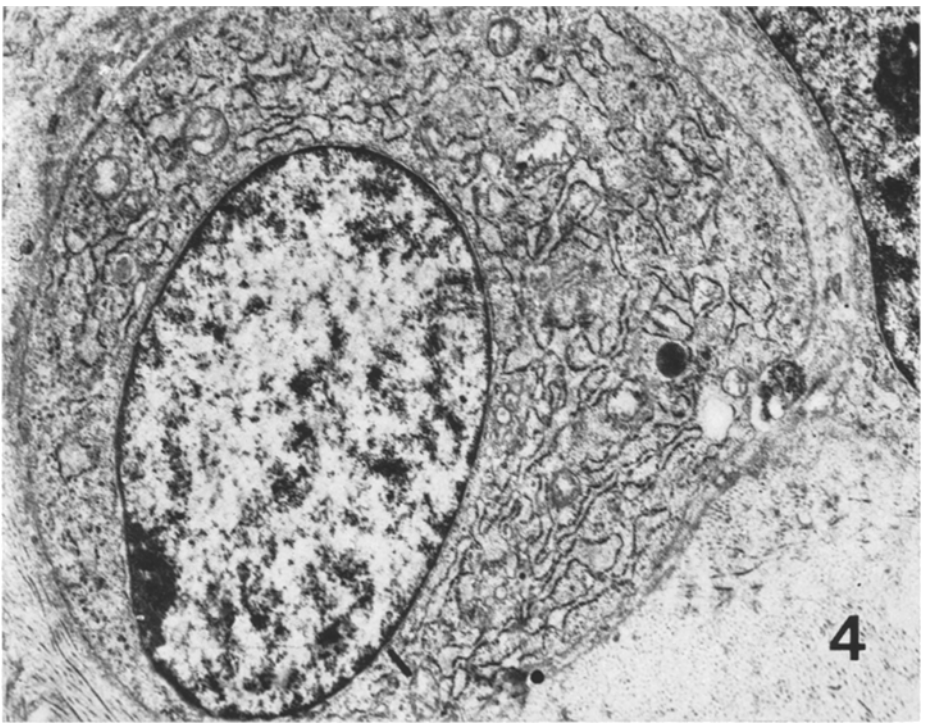
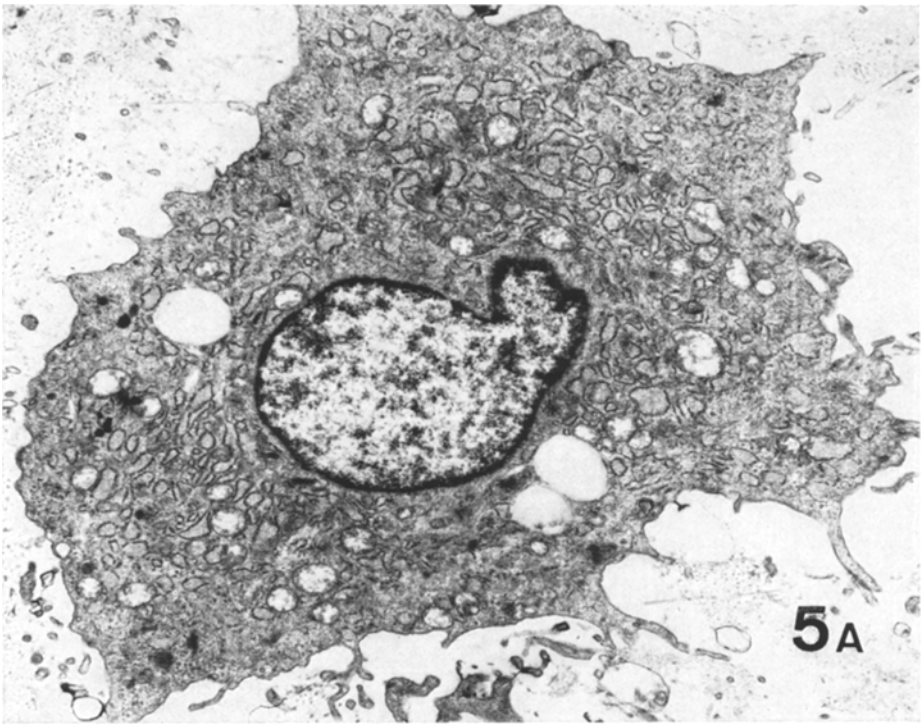


Fig. 4. A detail of a mesenchymal stem cell with a round contour and a large nucleus in the tumour in case two. Some RER profiles appear inside the cytoplasm (× 10000)

among more differentiated mesenchymatous-looking chondroblastic or osteoid cells (Fig. 3a). The cell was round or polygonal in shape, and its surface was smooth and homogeneous without digitations or membranous projections. They possessed a large, rounded or oval, and centrally situated nucleus, as well as fine chromatin. In addition the nucleolus was well developed. In the cytoplasm, the RER was sparse and arranged in isolated profiles, its cisternae being distended or not. Many ribosomes were found. There also existed some irregularly distributed mitochondria with scant crests and vacuolated matrix. No lipid or glycogen deposits were seen (Fig. 4).

We consider this cell type to be the most undifferentiated of all those seen and regard it as the matrix, since it is the point of differentiation from the remaining tumorous cells within the mesenchymal group. This criterion of differentiation manifests itself with an increase of the RER profiles and the posterior distension of those cisternae which were filled with secretory material. At times, glycogen, lipids and lysosomes were in evidence. These more mature mesenchymal cells

Fig. 5A and B. Chondroblasts of typical [(a), × 4000] and atypical character [(b), × 6000]. The latter shows close similarities to the types of cell described as specific for chondroblastoma of bone. (Case two)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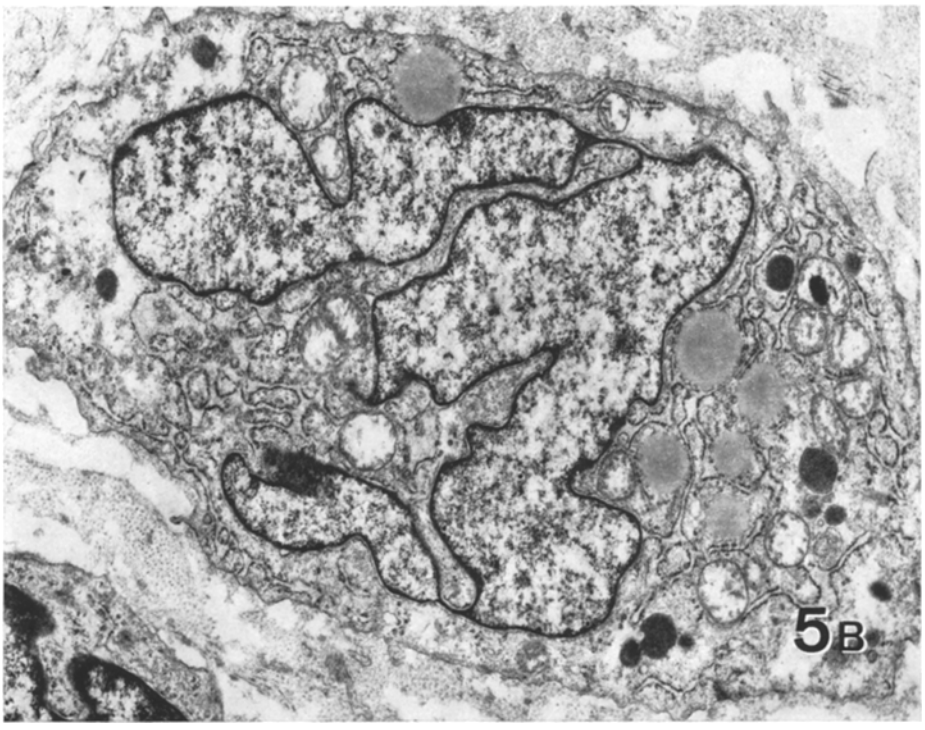
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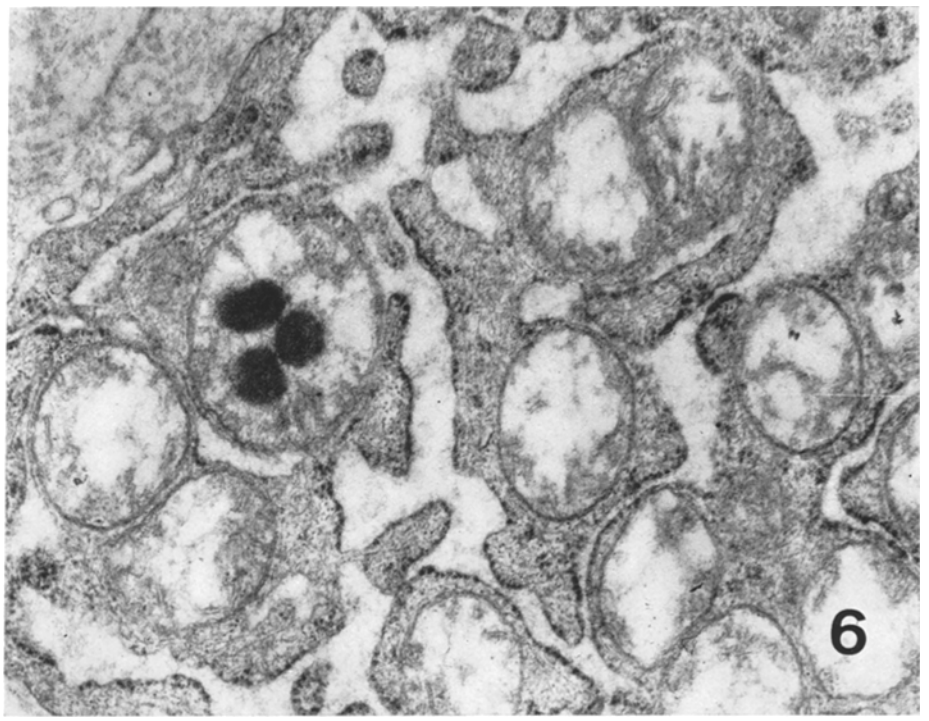

Fig. 6. Cytoplasm of a chondroblast in case two with well-developed RER and dilated cisternae which are filled by amorphous or fine granular material. There exist intra-mitochondrial calcium deposits. (× 18000)

resembled the A type cells of Scott (matrix producing cells) seen in the earliest phases of ossification.

Comments

Malignant tumours in canine and feline breast often show chondroid or osteogenic metaplasia of the stroma (Smith and Langhanr, 1967). This is rare in the female human mammary gland in which sarcomas are very infrequent (Petracic *et al.*, 1970). The malignant mixed mesodermic tumour is basically a sarcoma or carcinosarcoma consisting of a multi-potent cellular stroma and indistinct areas of chondroblastic, osteoblastic, lipoblastic or rhabdomyoblastic differentiation. These tumours have been reported in several organs (thyroid, kidney, urinary bladder, uterus, testis, mammary gland and soft tissues) by different authors (Albertini, 1955; Willis, 1967). Extraskeletal malignant tumours, like the chondrosarcoma or myxoid chondrosarcoma, possess a definite clinical and pathological particularity. Nonetheless, their existence has not been reported in the human breast (Angerwall *et al.*, 1973) and the osteosarcoma is one of the rarest tumours of this gland (Anani and Baumann, 1972). The association of a chondroid or osteogenic sarcoma with a giant cell tumour (osteoclastoma) and a carcinoma

is virtually an extremely rare type of neoplasm in the breast of the human female (Gonzalez Licea *et al.*, 1967).

Seen under the optical microscope, the present cases show common structures of a mesenchymal nature, as they have an osteoclastic and chondroclastic sarcomatous pattern both continuous with each other. The histological criteria for giant cell tumour are fulfilled in the present cases (Jaffe, 1961), the multinucleated giant cells being found irregularly distributed among the stromatic cells which are mononucleated, spindle-shaped and show occasional mitoses. The presence of haemorrhage is predominant in the first case, but more conspicuous in the second. We have found continuity between the osteoclastic stroma and the more fibroblastic or chondroblastic kind. In both cases, a continuity of the reticular fibres was also found. This reticular network is coarse, a gradual transition existing toward areas of chondromatous structure in which the intercellular ground substance adopts a cartilaginous texture. The histological diagnosis of malignancy in the chondromatous metaplastic areas of the two tumours is based on criteria analogous to those employed for skeletal chondrosarcoma (Jaffe, 1961; Dahlin, 1969).

An osteoid ground substance also developed among the tumorous cells of mesenchymal appearance. They were enclosed in trabecular or reticular material and resembled atypical osteoblasts. The calcium deposits appeared inside the osteoid substance engulfing the tumorous cells which shrank like osteocytes. These structures were only visible in the first case. No osteogenic metaplasia existed in the second tumour. The first case presented also had a carcinomatous differentiation in close continuity with the osteoclastic and chondroblastic mesenchymal areas. The reticular fibres established a clear continuity between epithelial solid or trabecular cords, adopting a typical configuration of breast carcinoma for such a neoplasm (Hamperl, 1970). No secretory activity was detected in the present epithelial cells, but the close contacts and homogeneous disposition were more characteristic of carcinoma. Case two resembled case one, but lacked a carcinomatous and osteogenic stromatic metaplasia and had a spindle-shaped cell distribution. The cells were more or less differentiated and resembled to some extent embryonic fibroblasts. An abundant intercellular substance of homogeneous basophilic nature gave the tumour a myxoid aspect, but in other areas, broad collagen bundles were more prominent. Continuity also existed between the fibroblasts, the giant cells and chondroblastic atypical tumoral fields. The profiles observed were similar to those reported by several authors who have analysed by optical means similar mammary tumours of a mixed mesodermic malignant character (Kennedy and Biggart, 1967; Hamperl, 1970; Haagensen, 1971). There are some traits in common with the malignant mesenchymoma of the soft tissues analysed under the electron microscope by Adnett *et al.* (1971).

The fibroblasts were numerous and distributed in a haphazard fashion throughout the stroma. They has a structure very similar to that described for spindle-cell sarcoma or malignant synovioma (Hirohata and Miromoto, 1971). The cytoplasm showed a well-developed, rough-surfaced endoplastic reticulum, Golgi complexes and abundant vacuolated mitochondria. The cell shape was irregular, with multiple elongated processes whose borders were in very close relation with immature collagen fibres. Some of them had bizarre nucleus and much clumped

conglomerated chromatin localized in a perinuclear pattern. Some difficulties exist in distinguishing between malignant cells and juvenile activated fibroblasts; the latter is a common finding in the stroma of mammary carcinomas (Ozello, 1971) but sarcomatous fibroblasts are more undifferentiated, and their nuclei have more marked irregularities in their contours.

The fine structure of multinucleated giant cells is basically similar to that seen in the osteoclastoma of bone or reparative granuloma of the jaws (Anaoka *et al.*, 1970; Steiner *et al.*, 1972; Sapp, 1972). The cytoplasm possesses a large number of mitochondria and specific granules with the morphology of lysosomes, some of them in close correlation with the Golgi complex. Among the mitochondria, the cluster of ER adopts a vacuolar contour. Villous borders exist, extending throughout the membranous surface of the cell. We have not, however, observed any phagocytic activity. The origin of the giant cells in these tumours remains unexplained but they are in close contact, and contiguous with the undifferentiated mesenchymal type of cell. A stromatic origin from mononucleated cells has been admitted for giant cells in bone tumours (Steiner *et al.*, 1972). And it should be correlated to Scott's type B cells, or "cells unassociated with matrix production" in the fracture repair bone of the rat (Goethlin, 1973).

Undifferentiated, round or polygonal tumorous cells form groups or nests without contacts or desmosomes. They have poorly developed cytoplasmic organelles with some membranes of RER and round mitochondria. They adopt a more mesenchymatous character when appearing in proximity to chondroid, or osteoid tissue. Certain similarities exist with the immature mesenchymal cells present in osteosarcoma of bone (Hirohata and Miromoto, 1971).

Bone cell precursors of Scott's type A cells (1967) or matrix producing cells of Goethlin (1973) are present in the chondroblastic and osteoblastic areas of both tumours. Two kinds of chondral cells must be distinguished in the present cases. The first resembles the mesenchymatous chondroblastic matrix cell of Godman and Porter, 1960), being closely associated with the more mature chondral cells and mimicking cartilage in maturation (Weiß *et al.*, 1968). A second cell type of chondroblastic character is widespread in the second neoplasm. These cells resemble very closely those seen in the chondroblastoma of bone (Levine and Bensch, 1972). A particular feature is the nucleus with bizarre shapes, irregular contour, protrusions and chromatin bridges.

The osteogenic cells exist only in the first tumour and correspond to the osteoblasts described by Scott and Pease in 1956. They resemble both the matrix-producing cells seen by Goethlin (1973) during fracture repair and also principal cells of osteosarcoma (Kay, 1972; Peydro *et al.*, 1972) analysed under the electron microscope. There exist calcium deposits diffusely localized around the osteogenic cells. The calcium salts adopt a more trabecular pattern similar to that observed in normal bone formation. Both chondroblastic and osteoblastic tumorous cells possess so-called mitochondrial granules of an electronically dense nature associated with the cristae. Matthews and Martin (1971), have assumed that these granules are calcium deposits during the bone differentiation in the cells located in the vicinity of a calcified matrix.

Electron microscopy has been helpful in clarifying the nature of the epithelial cells seen in the first case. This cell type possesses characteristics similar to those

reported by other workers (Gonzalez Licea *et al.*, 1967). But desmosome junctions which were very well developed in that case show a poor differentiation in the present tumour. All the same, the existence of desmosomes must not be considered as indicative of an exclusively epithelial nature, since they are also present in mesenchymatous cells of a highly varied nature and function (Mori and Lennert, 1969; Hirohata and Miromoto, 1971). At the same time, it should be stressed that cells in adenocarcinoma of the breast do not always possess desmosomic junctions (Ozello, 1971) and that myo-epithelial tumours of the mammary gland are also lacking in desmosomes but show many orientated cytoplasmic filament bundles with dense zones. The ultrastructural criteria in support of an epithelial nature of what are considered by optical means as adenocarcinomatous fields in our tumour are as follows; presence of very close cytoplasmic processes forming nests of cells with a cubic or prismatic contour; and club-like projections and digitations along the plasma membrane of two neighbouring cells and the differentiation of sparse villosities in the apical surface of the cell leaning to the alveolar lumen. The cytoplasmic organelles such as Golgi complexes, cluster of RER and mitochondria with a dense matrix and poorly developed cristae have been seen in epithelial breast tumours under the electron microscope (Schäfer and Bässler, 1969, and Ozello, 1971) but are lacking in secretory membrane-bounded intracytoplasmic granules.

From the foregoing it is evident that we are dealing with a neoplasm with a distinctive histology and ultrastructural characteristics. Its histogenesis is still a matter of debate, and most of its various possibilities have been recently discussed (Anani and Baumann, 1972). We believe that all the cells in both these tumours, with or without adenocarcinomatous structure, have a unique and common origin. This "unitarian" approach has been also defended by Gonzalez Licea *et al.* (1967) and seems applicable to our observations. We have not found any support for an epithelial metaplasia to mesenchymal, osteoclastic, chondromatous or osteogenic patterns. The common point in both cases is the undifferentiated cell, which must be considered as the stem cell from which the other cell types derive.

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