Ultrastructure of Human Chordoma

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SUMMARY

Three sacrococcygeal chordomas, two of which were recurrent, were examined by electron microscopy. The three tumors, which were quite similar, consisted of cords, lobules, and clusters of cells embedded in a mucinous matrix. The so-called stellate and physaliferous cells represented the extremes of a developmental sequence of tumor cells and were separated by a spectrum of morphologically intermediate cells. The physaliferous cells appeared to arise from the stellate cells by a process of progressive cytoplasmic vacuolization. Some of the resulting vacuoles were so large that the nucleus and the cytoplasmic organelles were displaced to the cell periphery. Many vacuoles contained deposits of glycogen.

The most striking feature of the tumor cells was the consistent association of mitochondria and endoplasmic reticulum in a structurally ordered complex. In such complexes, single mitochondria alternated with single cisternae, each organelle being separated from its neighbor by an interval of constant width. The central portions of the mitochondria were so flattened that the limiting membranes of opposite sides almost met; at their ends, the mitochondria were bulbous and possessed a few angulated cristae. Beyond the lateral boundaries of the complex, the cisternae abruptly became smooth surfaced. The function of these unusual complexes is unknown.

INTRODUCTION

Chordoma, a tumor which usually appears in middle or late adult life, arises from remnants of the embryonic notochord (20, 27). The majority of these tumors occur at sites corresponding to the ends of the notochord, 45 to 56% of reported cases occurring in the sacrococcygeal region (25, 32). While of low malignancy, chordomas have a tendency to be locally invasive and destructive. A recent report by Higinbotham et al. (19) has shown that following surgical removal, local recurrences and metastases commonly occur.

Although the light microscopic characteristics of human chordomas have been described in numerous articles (6, 10, 12, 14, 17, 31), few studies of their ultrastructural organization have appeared. To date, only three such works have been published (5, 15, 29); each deals with a single case. The present article describes the fine structure of three sacrococcygeal chordomas, with special emphasis on a unique complex of cytoplasmic organelles which was observed in each case.

MATERIALS AND METHODS

Specimens of sacrococcygeal chordomas were obtained from three patients by aspiration biopsy. Their case histories are briefly summarized below:

Case 1. The patient was a 78-year-old white Jewish male who in 1950 underwent partial sacrectomy because of chordoma. He remained symptom-free until 1965, when recurrent tumor necessitated cobalt-60 therapy (6300 rads, tumor dose). The tissue specimen was obtained late in 1966 about three hours after a single treatment of 300 rads.

Case 2. The patient was a 60-year-old white Jewish female who had undergone a partial sacrectomy for chordoma after six months of rectal pain. The present specimen was from a recurrent lesion removed in 1966. No radiotherapy had been given.

Case 3. The patient was a 65-year-old white Jewish male who in 1966 underwent partial sacrectomy for chordoma after a single treatment of 300 rads. No antecedent radiotherapy had been given.

For electron microscopy, small blocks of tissue were fixed for one hour in a mixture of 2% acrolein and 6% glutaraldehyde (28) and postfixed for one hour in 2% osmium tetroxide (24) with added sucrose (7). Both fixing solutions were buffered with veronal acetate. After dehydration, the specimens were embedded in Maraglas-Dow Epoxy Resin 732 (11). Thin sections were doubly stained with methanolic uranyl acetate (30) and with lead citrate (26), and were examined in a Siemens Elmiskop I electron microscope.

Specimens for routine light microscopy were fixed in phosphate-buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Half-micron thick sections of epoxy-embedded tissue were stained with toluidine blue using the method of Björkman (2). Photomicrographs were taken with a Zeiss standard WL microscope.

OBSERVATIONS

Light Microscopy

In hematoxylin and eosin-stained paraffin sections, the tumors appeared to consist of two distinct cell types. The larger cell type contained prominent vacuoles, and it is because of this
feature that they are called "physaliferous cells." The smaller, spindly cells, which appeared to be nonvacuolated, are usually referred to as "stellate cells." Both cell types, intermixed, were arranged in cords, lobules, and clusters (Figs. 1, 2). The relative proportion of the two cell types varied from tumor to tumor and also in different sites within each specimen. The cell groupings were separated by a homogenous, mucinous matrix, and in one specimen they were surrounded by fibrous connective tissue as well as matrix.

Much greater detail could be discerned in the thinner, toluidine blue-stained epoxy sections (Figs. 3-5). Not only physaliferous and stellate cells were apparent, but a spectrum of morphologically intermediate cells as well. While the latter cells resembled stellate cells in form, they also possessed small cytoplasmic vacuoles. Many of the physaliferous cells contained such large vacuoles that they assumed a typical "signet ring" shape (Fig. 3). Some of the larger vacuoles contained a flocculent material (Figs. 4, 5).

The nuclei of the tumor cells frequently were highly irregular in size and contour. Many were multilobulated or had deep clefts. Occasional cells appeared to be binucleate. Intranuclear inclusions were present in many of the tumor cells, irrespective of cell type (Fig. 5). These inclusions consisted of either a dense spherical structure delimited by a light halo or a large mass of intermediate density surrounded by a very dense rim. The matrix, which appeared to be structureless, was stained pink by the metachromatic toluidine blue, a tinctorial response which was not shared by any other component of the tumors.

Electron Microscopy

Specimens from each of the three tumors studied were very similar at the ultrastructural level and are therefore described in composite. The electron microscope observations confirmed that stellate and physaliferous cells are not disparate cell types, but are related by a continuum of intermediate cells. Nevertheless, for the sake of convenience, this terminology is retained in this report.

The aggregations of tumor cells were easily recognized at low magnifications (Fig. 6). The cells were irregular in outline, frequently being locked together by interdigitating cellular processes (Fig. 6, 7). These processes often extended deep into adjacent cells. Desmosomes occasionally were present at such sites.

The nuclei of the tumor cells also were extremely irregular in form. Deep incursions of cytoplasm gave many of the nuclei a multilobulated appearance (Figs. 8-10). These cytoplasmic intrusions, when viewed in cross-section, appeared as intranuclear inclusions (Figs. 8, 9). Such pseudo-inclusions could be readily distinguished from true intranuclear inclusions for the following reasons: (a) They were bounded by the nuclear envelope, hence were delimited by two membranes. (b) They frequently were encompassed by condensed chromatin. (c) Nearly all contained cytoplasmic components such as mitochondria and endoplasmic reticulum. True intranuclear inclusions, on the other hand, were bound by a single membrane with no adjacent condensed chromatin and usually had an empty appearance (Fig. 10). Such inclusions were relatively rare.

The most striking feature of all the tumor cells was the consistent association of mitochondria and endoplasmic reticulum in a structurally ordered complex (Fig. 11). In such a complex, single mitochondria alternated with single ergastoplasmic cisternae, each organelle being separated from its neighbor by an interval of approximately 500 A. As many as 10 layers of mitochondria and 10 cisternae were observed in some arrays. The mitochondria usually were long (about 2.3 μ) and dumbbell shaped. In their central portions, they were so attenuated that membranes of opposite sides almost met. At such points the total thickness of the mitochondria measured as little as 340 A. The bulbous ends of the mitochondria were 10 times as thick, measuring about 3400 A. These end portions frequently contained a few angulated cristae. The cisternae were of the rough-surfaced variety (RER), but at the lateral boundaries of the complex, which can be defined as the point where the mitochondria end, the membranes abruptly became smooth (Figs. 13, 14). In many cells, especially the physaliferous cells, the smooth endoplasmic reticulum (SER) ramified throughout the cytoplasm (Figs. 7, 13).

Solitary mitochondria, as well as solitary elements of RER, were infrequently seen. In contrast, a single distended cisterna was frequently observed with several mitochondria in close relation (Fig. 12). In such cisternae, ribosomes were present primarily where mitochondria were apposed to the cisternal membrane; at other sites, the membranes usually were smooth.

Golgi complexes were present in many of the tumor cells, being most abundant in the physaliferous cells, where they were usually situated near to or contiguous with the cytoplasmic vacuoles (Fig. 15). The Golgi complexes consisted of closely packed, smooth cisternae showing many fenestrations. Small vesicles were numerous, and some were observed that were either budding or fusing with the Golgi cisternae (Fig. 16). Similar vesicles were sometimes joined to the limiting membrane of the vacuoles.

Many of the cytoplasmic vacuoles, large and small, contained glycogen (Figs. 6, 11). Clusters of glycogen also were abundant in both intermediate and physaliferous cells in those cytoplasmic areas which were rich in SER (Fig. 7). Stellate cells contained little glycogen or SER, their cytoplasm being characterized instead by the presence of bundles of tonofilaments.

The extracellular matrix, at lower magnifications, appeared structureless, but it exhibited considerably greater electron density than the vacuolar contents, exclusive of the glycogen. At higher magnifications fine fibrils and granular material could be observed scattered throughout the matrix.

DISCUSSION

The mitochondrial-endoplasmic reticulum (MER) complexes described in the present study constitute the most prominent and striking ultrastructural feature of the cells of human chordoma. These structures, which consist of alternating layers of RER and attenuated mitochondria, have not been described previously in any vertebrate cell type. A similar-appearing complex was illustrated, but not discussed, in a report on the fine structure of the corpus allatum of the moth of the silk-worm, Bombyx mori (16).
While among vertebrates the MER complexes appear to be limited to chordoma cells, several examples of MER arrays, albeit of a somewhat different configuration, have been recognized in other cells. These occurred in the hepatic cells of rats subjected to several different experimental procedures, including prolonged starvation (22), copper intoxication (1), and partial hepatectomy (8). Like the MER complexes of chordoma, the hepatic arrays consist of elements of endoplasmic reticulum alternating with elongated mitochondria. Although they are in direct continuity with RER beyond the limits of the mitochondrial stack, the cisternal elements in the array are of the smooth-surfaced variety, the exact reverse of the situation in chordoma. Furthermore, instead of being separated from the mitochondria by an interval of fairly constant width, the smooth membranes are closely applied to the outer surface of both sides of the mitochondria. Since the mitochondria also possess longitudinally oriented cristae, the result is a densely packed stack of membranes in which the precise identity of individual membranes is not readily discernible.

At a simpler morphologic level, single cisternae of endoplasmic reticulum frequently have been observed in association with single mitochondria (13, 18). Such cisternae parallel the outer membrane of the mitochondria but are separated from the mitochondria by a gap equal to the intercisternal distance observed in parallel arrangements of RER, such as those found in exocrine cells. Fawcett (13) has suggested that in normal cells, closely related mitochondria and RER cisternae may signify a transient juxtaposition of the mitochondrion to a local site of energy utilization. However, the paucity of solitary mitochondria and solitary cisternae in chordoma cells strongly indicates that the MER complexes are not transient structures.

The functional significance of the MER complexes is not clear. It is obvious that stratification of the organelles brings the maximum surface area of RER membranes into juxtaposition with mitochondria. Fig. 12 demonstrates that the presence of attached ribosomes is directly related to mitochondrial propinquity. It is possible, though no direct proof exists, that in chordoma cells a high concentration of ATP is necessary to keep ribosomes attached to the membranes of the endoplasmic reticulum.

Whatever their functional status, the MER complexes constitute convenient morphologic markers. It is highly improbable that so unusual a structure could arise independently in unrelated cell types. Their presence in almost every cell in the three cases studied is compelling evidence that the different cell types are directly related and that they probably represent a developmental sequence.

The progressive increase in the degree of vacuolization also supports the concept of a single cell type in chordoma. Small vacuoles, which are present in the stellate cells, increase in dimension in the intermediate cells and may attain enormous size in the physaliferous cells. There is no doubt, as Cancilla et al. (5) have observed, that many of the larger so-called vacuoles apparent in routine light microscopic preparations are in reality large extracellular spaces demarcated by cell processes. However, the vacuoles examined by us in the electron microscope were predominantly cytoplasmic. That these vacuoles had no continuity with the extracellular matrix was demonstrated by the great difference in electron density between the two sites. In addition, the vacuoles remain unstained in 0.5-μ-thick sections stained with toluidine blue, while the matrix is stained an obvious pink.

Previous studies with the light microscope demonstrated the presence of glycogen in chordoma cell vacuoles by staining with Best's carmine (6, 9) or by the periodic acid-Schiff technic with diastase controls (9, 12). These observations were confirmed by the present study. In the electron microscope, the vacuolar glycogen is morphologically similar to the glycogen in the cytoplasmic ground substance. The latter glycogen is in close topographic relation to the abundant SER, but, as in liver cells (21), it is lodged in the interstices of the tubular reticulum and is not actually enclosed by membranes. In other cell types, glycogen occasionally has been observed engulfed by autophagic vacuoles (23). However, the presence of a single unit membrane delimiting the chordoma cell vacuoles shows that these structures are not of the autophagic variety. If, in chordoma cells, the two classes of glycogen—vacuolar and cytoplasmic—are identical, then the mode of entry of the cytoplasmic glycogen into the vacuoles is unknown.

In addition to cytoplasmic vacuoles, intranuclear vacuoles also are present in chordoma cells. In the case described by Friedmann et al. (15), some nuclei contained "patchy" inclusions of unknown nature. We have also observed several of these structures, and they apparently correspond to the class of nuclear inclusions termed "sphaeridions" by Büttner and Horstmann (4), which are widely distributed in many species of normal and pathologic cells (3). In the chordoma studied by Spjut and Luse (29), a different type of intranuclear inclusion was present; this was described as an area of cytoplasm trapped within the nucleus. In our material similar configurations were common. It was clearly evident, however, that such inclusions were in direct continuity with the cytoplasm and that they in fact represented cross-sections of villiform cytoplasmic extensions. In addition to these pseudo-inclusions, several examples of true intranuclear inclusions were observed in the present study. These usually appeared as watery vacuoles bounded by a single membrane. Like the cytoplasmic vacuoles, the cytogenesis of the nuclear vacuoles is unclear.

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REFERENCES

Figs. 1-5 are light micrographs. Figs. 1 and 2 are of paraffin-embedded material, while Figs. 3-5 are of half-micron-thick sections of epoxy-embedded tissue.

Fig. 1. An area of tumor showing strands of vacuolated physaliferous cells and small stellate cells separated by large areas of intercellular matrix. H & E, X 120.

Fig. 2. Typical tumor cells embedded in a dense, collagenous matrix. H & E, X 120.

Fig. 3. This section shows physaliferous cells displaying varying degrees of vacuolization. Some are of the “signet-ring” type, with the nucleus and cytoplasm displaced to the cell periphery by a large vacuole (arrows). The stellate cells are smaller and have a denser cytoplasm. The cells are stained various shades of blue, while the matrix is stained pink. Toluidine blue, X 400.

Fig. 4. A higher power micrograph showing numerous intracellular vacuoles, some of which contain flocculent material (V1). The difference in staining between the clear vacuoles (V2) and the intercellular matrix is obvious. Note the small vacuoles in the stellate cell at the upper right. Toluidine blue, X 1350.

Fig. 5. Two types of nuclear inclusions are present in this bilobed nucleus. One type is a transversely sectioned process of cytoplasm, thus constituting a pseudoinclusion (P1). In this connection, note the deep clefts in the nuclear membrane of the larger lobe. Two true intranuclear inclusions (INI) are demarcated by a halo of low density. The darkly stained nuclear structures are nucleoli. The adjoining cell contains a large vacuole (V) filled with flocculent material. The dense structures (*) in the cytoplasm are mitochondrial endoplasmic reticulum complexes. Toluidine blue, X 1880.

Figs. 6-16 are electron micrographs of thin sections stained serially with uranyl acetate and lead citrate.

Fig. 6. Survey micrograph of chordoma cells. A physaliferous cell containing a huge vacuole (V) is at the upper left. The other cells are of the stellate type. They are characterized by a highly filamentous cytoplasm and by a relative paucity of vacuoles. At the right is a series of cell processes, each of which is enwrapped by other cells. This cellular interdigitation is common among all types of chordoma cells. Clusters of glycogen are distributed throughout the cytoplasm, being especially prominent in the cell process at the upper right. X 7,500.

Fig. 7. A cytoplasmic process surrounded by portions of two other cells and bound to them by small desmosomes (D). The process contains numerous filaments (F), which appear as dots or granules in transverse section. The surrounding cells have abundant smooth endoplasmic reticulum (SER). X 24,000.

Fig. 8. A nucleus demonstrating two types of nuclear inclusions. The large central vacuole appears to be within the nucleus but actually is contained in a cytoplasmic extension, here seen in cross-section. A narrow rim of cytoplasm that includes a mitochondrion (arrow) envelopes the vacuole. The cytoplasmic process is demarcated by nuclear envelope (E) with associated condensed chromatin. A similar process (*), also in transverse-section but without vacuoles, is seen at the upper left. A true intranuclear inclusion (NB) of the nuclear body type is present at the lower right of the nucleus. NU, nucleolus. X 11,000.

Fig. 9. A nucleus displaying many pseudoinclusions, some of which contain cytoplasmic vacuoles. X 21,000.

Fig. 10. A group of true intranuclear inclusions. These usually are clear vacuoles with a single limiting membrane. Unlike the pseudoinclusions, no chromatin is condensed at their periphery. A nucleolus is at the lower right. X 21,000.

Fig. 11. A typical mitochondrial endoplasmic reticulum complex. The regular alternation of organelles is well demonstrated. The mitochondria are attenuated in their central regions and bulbous at their ends. Elements of smooth endoplasmic reticulum and Golgi complex are present in the cytoplasm. Small clusters of glycogen are scattered through the cytoplasm, and a deposit of this substance is present in a vacuole (arrow). A portion of the nucleus is at the left. X 59,000.

Fig. 12. Distended endoplasmic reticulum cisternae with associated mitochondria. Note that ribosomes are present on the cisternal membranes primarily where mitochondria are closely apposed to the membrane. X 50,000.

Fig. 13. A mitochondrial endoplasmic reticulum complex in a region rich in smooth endoplasmic reticulum. Note the transition of rough into smooth endoplasmic reticulum at the periphery of the complex (arrow). This area is shown at higher magnification in the next micrograph. X 33,000.

Fig. 14. An enlarged portion of Fig. 13, showing the direct continuity of rough endoplasmic reticulum and smooth endoplasmic reticulum. X 48,000.

Fig. 15. A Golgi complex displaying the fenestrated appearance typical of this organelle in chordoma cells. A cytoplasmic vacuole is present at the top of the micrograph. X 44,000.

Fig. 16. A grazing section of a fenestrated Golgi cistern. Several vesicles appear to be in continuity with the Golgi membrane (arrow). X 55,000.
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