GRANULAR CELL TUMORS OF THE CENTRAL NERVOUS SYSTEM*†

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ABSTRACT

This paper describes two cases of granular cell tumor, the first such tumors believed to have arisen in the central nervous system with the exception of those in the neurohypophysis. The tumor in one case arose in the cervical spinal leptomeninges and in the other was located in a cerebral hemisphere. The light and electron microscopic characteristics were indistinguishable from granular cell tumors (myoblastomas) reported in other body sites. Ultrastructurally two cell types could be distinguished. The predominant form (Type II) was characterized by abundant cytoplasm filled with dense bodies, multivesicular bodies and vacuoles. A second smaller cell (Type I) contained few of the above organelles. It is considered likely that these cell types are evolutionary stages of a single kind of a cell in which Type I is transformed into Type II. Various cells, including Schwann cells, have been proposed as giving rise to granular cell tumors. The occurrence of these neoplasms in the central nervous system lessens the likelihood of a Schwannian source in all instances. An origin from Schwann cells of perivascular nerves cannot be excluded. The possibility remains that neoplasms designated as granular cell tumors may have more than one cell of origin.

INTRODUCTION

Granular cell tumors (myoblastomas) are found in a wide variety of cutaneous, oral and visceral sites. In the nervous system they have been reported in peripheral nerves (6, 7), and in the neurohypophysis where they occasionally become large enough to produce clinical symptoms (8, 12, 17, 19, 24, 25, 27, 39, 49). More often those in the posterior lobe or pituitary stalk are encountered incidentally at autopsy as microscopic nodules of large pale granular cells (8, 19, 26, 35). Two well-documented examples of metastatic intracerebral neo-

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plasms believed to have been granular cell myoblastomas have also been recorded (30, 41). In a third case the metastasis in the brain was not verified as a granular cell tumor histologically (32, case 8). One report (23) mentions the presence of cells thought to be those of a granular cell myoblastoma within a meningioma of the falx in a patient with multiple neurofibromatosis.

Two cases of granular cell tumors located in the central nervous system which we have recorded elsewhere in a brief communication (29) are described in greater detail here. One of the present tumors arose in the leptomeninges of the spinal cord and the other, probably also a primary tumor of the central nervous system, was situated in a cerebral hemisphere. Such tumors have not, to our knowledge, been previously reported to originate in the central nervous system except for those in the neurohypophysis.

CASE REPORTS

Case I

A 58-year-old male developed weakness of the left arm and leg one week before admission to the Columbia-Presbyterian Medical Center. Examination disclosed a left homonymous hemianopsia, left hemiparesis with hyperreflexia and extinction on the left side of the body. A RISA brain scan revealed a large uptake at 48 hours in the right parietal region. Right brachial and carotid angiograms showed a large, avascular, intra-axial, right retrosylvian mass. A thorough search demonstrated no primary or metastatic extracranial neoplasm. At operation a large, poorly vascular tumor was found in the right parieto-temporo-frontal region. It was of variable consistency, presented on the surfaces of the cortex and extended deeply into the white matter. A subtotal removal of the tumor was performed. Six months later the patient developed clinical evidence of recurrence of the tumor manifested by increasing hemiparesis, slurred speech, and repeated falling. A second operation again disclosed tumor in the parietal, temporal, and frontal lobes. It was believed to extend medially into the thalamus. Subtotal removal was again effected. Following the operation the patient received 3000 rads of radiocobalt therapy over a five week period without improvement in the clinical status. He died 17 months after the onset of symptoms without clinical evidence of tumor in the other organs. An autopsy was not performed.

Case II

A 73-year-old female had had numerous hospital admissions for upper gastrointestinal bleeding and cirrhosis of the liver with eventual hepatic coma. In 1966, three years before her death, she underwent a portacaval shunt. Her sixth and final admission in March, 1969, was for hepatic pre-coma thought to be due to a gastrointestinal hemorrhage. She lapsed into coma, developed numerous metabolic problems and died. She did not have neurological signs or symptoms referable to the cervical spinal cord. At autopsy the major findings were advanced portal cirrhosis and a non-metastasizing solitary hepatoma. The brain was normal except for the presence of Alzheimer type II astrocytes in the cerebral gray matter. A midline, firm, yellowish-white, round, unencapsulated mass measuring 6 mm x 5 mm x 3 mm, was found in the leptomeninges overlying the posterior columns of the first cervical spinal cord segment (Fig. 2, inset). The cord was not grossly compressed. No similar tumors were found elsewhere in the body.

MATERIALS AND METHODS

For light microscopy tissues from both cases were fixed in concentrated formaldehyde diluted 1:10 (4% formaldehyde) and were embedded in paraffin or utilized for frozen sections. Paraffin-embedded sections were stained by the following methods: hematoxylin
and eosin, periodic acid-Schiff (PAS), Masson trichrome, phosphotungstic acid hematoxylin (PTAH), mucicarmine, the Laidlaw and Gomori silver techniques for reticulin, and the Nissl stain for myelin sheaths. Frozen sections were stained with Sudan Black B and Oil Red O.

For electron microscopy the biopsy specimen from Case I was fixed in 6.25% glutaraldehyde adjusted to pH 7.4 with 0.1 M phosphate buffer for 2 hours. Blocks of tissue were post fixed in cold 2% osmium tetroxide buffered to pH 7.4 with veronal acetate and after dehydration with cold graded acetone solutions were embedded in Durcupan (Fluka, A.G., Buchs, S.G., Switzerland). Thin sections were cut with glass or diamond knives, mounted on uncoated grids, and stained with uranyl acetate and lead citrate. The tissues from Case II had been in 4% formaldehyde for 4 weeks before processing. Blocks were removed from the tumor and washed repeatedly in phosphate buffer, post fixed in 2% osmium tetroxide, after which they were processed in a manner similar to Case I. Electron micrographs were taken with a Siemens Elmiskop I and an RCA EMU-3F electron microscope.

RESULTS

Gross and Light Microscopic Observations. Case I. The formalin-fixed samples of tumor, which had been removed piece-meal, were moderately firm and rubbery, pale grey or yellowish-white and presented a homogeneous smooth surface on section. There was no line of separation between the abnormal tissue and the parenchyma with which it merged. The histologic findings in the two biopsy specimens were similar and need not be described separately. The tumor was composed of sheets of large-bodied, generally closely-approximated, round or oval cells lacking any characteristic arrangement (Fig. 1). Most were of the order of 25–35 µ in maximum diameter, with a range of about 12–43 µ. The rounded contours of the cells were altered in some instances by flattening, or by shallow indentation at the side where they were molded against their neighbors, or by blunting of one end. Very rarely a thin process appeared to emerge from the cell body to end or pass out of the plane of section after a short course. The most characteristic feature was the presence in the cytoplasm of a great abundance of small, discrete, lightly eosinophilic granules. The granules were moderately PAS-positive. They were pale red in the Masson trichrome stain, light blue with PTAH, and mucicarmine-negative. In frozen sections they did not stain with Oil Red O or Sudan Black B. They seemed to vary comparatively little in size and were often uniformly distributed throughout the voluminous cell body. In many instances, however, particularly in the larger cells, the cytoplasm was quite pale and agranular, because of a paucity or virtual absence of granules (Fig. 1). The agranular area was often limited to the central part of the cell but sometimes occurred diffusely throughout the perikaryon. The tumor cell nuclei were small relative to the size of the cell body and more often than not they were eccentrically placed. As a rule they were round or oval and contained fine chromatin particles and a prominent, but small, nucleolus. However, in many cells the nucleus was shrunken and dark or irregularly shaped. Much of this nuclear pyknosis may have been an artefact, perhaps occurring during fixation. Cells undergoing mitosis were occasionally seen in the second biopsy specimen. Small blood vessels, usually of capillary size coursed through the tumor but the cells showed no apparent orientation about them.
Fig. 1. Case I. Dense cellular tumor composed of cells with small dark nuclei and abundant granular cytoplasm. Note several cells with clear cytoplasm centrally (arrows). Hematoxylin and eosin stain, × 505.

Fig. 2. Case II. Tumor cells similar to those in Fig. 1 showing prominent cytoplasmic granules. Hematoxylin and eosin stain, × 800. Inset: Granular cell tumor (arrows) on dorsal surface of first cervical spinal cord (SC) segment.
There were several small fields of necrosis infiltrated by sparse polymorphonuclear leukocytes and containing rare, recently thrombosed, small vessels. Inconspicuous wisps of collagen fibers, best recognized by their blue color in the Masson stain, could often be distinguished between masses of the large cells, along with occasional elongated cells with narrow fusiform nuclei thought to be fibrocytes. Reticulin fibers appeared to be limited to the walls of the blood vessels. Connective tissue fibers and cells were more prominent at the edges of some of the larger lobules of the tumor, chiefly where the growth abutted upon the pial surface, but there was no encapsulation. The presence of remnants of cerebral tissue at the edges of the biopsy samples made it possible to recognize that active invasion of both cortex and white matter was in progress. Here the large tumor cells became progressively more widely separated from one another in the degenerating parenchyma and were interspersed with hypertrophied astrocytes which were identified by their large nuclei, agranular, homogeneous, eosinophilic bodies and multiple processes. In the infiltrated cortex scattered nerve cells could also be seen persisting among the tumor elements.

Case II. The tumor on the surface of the spinal cord consisted of a compact, sharply-edged mass of large cells forming a single nodule in the subarachnoid space (Fig. 2, inset). It was not encapsulated although a tenuous layer of narrow cells, probably those of arachnoid trabeculae, could be seen on its free surface in a few places. The flattened base of the mass rested on the pia overlying the posterior columns. Beneath this the external glial membrane had become slightly thickened due to an increase in glial fibers and astrocytes. A few narrow columns of tumor cells continuous with those in the body of the tumor had invaded the pia and glial tissue in this area. The neural parenchyma underlying the mass was minimally compressed but was otherwise normal. Some small spinal nerve roots traversed the subarachnoid space but none was present near the tumor. The tumor cells (Fig. 2) had the same morphological characteristics as those in Case I. They were compactly arranged throughout. Their abundant granules were uniformly dispersed through the cell body but in some cells the cytoplasm was very pale due to a relative lack or absence of granules. The walls of the capillaries permeating the tumor were rich in collagen and reticulin fibers and occasionally strands of them extended outward for a short distance among the masses of adjacent tumor cells. However, no network of connective tissue fibers could be seen throughout the growth with the Mallory or Laidlaw methods. Large zones of necrosis were not present but several focal aggregations of macrophage-like cells with clear bodies were encountered toward the center. They were filled with Sudan Black B and Oil Red O-positive material. Whether these cells were derived from degenerating tumor cells or some other source was not determined.

Electron Microscopic Observations. The characteristic granular cells seen in Case I by light microscopy were readily identified electron microscopically by their abundant cytoplasm replete with dense bodies, multivesicular bodies and vacuoles of various sizes and shapes (Figs. 3, 4). We have designated these as the Type II cell because, as indicated later, we believe they are derived from a
Fig. 3. Case I. Electron micrograph demonstrating several adjacent granular tumor cells (Type II cells). Note nucleus (N), numerous dense bodies and vacuoles in cytoplasm. At bottom of micrograph cells are separated from collagen (C) by basement membrane (arrow). A portion of an endothelial cell is included (E). × 5,500.
smaller less frequently seen cell. The dense bodies in the Type II cells were
round, ovoid or irregular in shape and surrounded by a single membrane (Fig.
5). They had fine granules within a homogeneously electron dense background
but occasionally also included small vacuoles (Fig. 5). Multivesicular bodies
had a limiting membrane enclosing many closely packed circular or ovoid
vesicles (Fig. 6). Vacuoles (Fig. 7) were characterized by an outer membrane
surrounding an electron lucent center which often contained scattered, small,
circular vesicles indistinguishable from the vesicles of the multivesicular bodies.
Other less well defined densities and structures which occasionally resembled
shrunken mitochondria were also seen in vacuoles. In some Type II cells the
central portion of the cytoplasm was composed primarily of vacuoles, while the
dense bodies and multivesicular bodies were located at the periphery (Fig. 4).
Fig. 5. Case I. Type II tumor cell containing dense bodies (DB); note small vacuoles in a dense body near upper border at right. At lower right is a separate large vacuole (V). Note basement membrane (arrows) separating cell from collagen (C), \( \times 24,750 \).

Fig. 6. Case I. Higher magnification of multivesicular body containing many vesicles of variable size (arrows), \( \times 39,100 \).

Fig. 7. Case I. Higher magnification of membrane-bound vacuoles (V) containing a few vesicles and an unidentified osmiophilic structure, \( \times 40,000 \).
The cytoplasm of Type II cells also contained fine filaments of approximately 100 Å thickness usually located at the periphery of the cell along the plasma membrane or near the nucleus (Fig. 8). They were also scattered between cytoplasmic organelles and were infrequently clumped in tight bundles in the central portion of the cell. Processes containing similar filaments, approximately 100 Å in width, with a few mitochondria and vacuoles were observed between Type II cell bodies and probably arose from them as suggested in Fig. 9. Such processes were only occasionally present and were usually short. Many of the large rounded Type II cells apparently had no processes (Fig. 4). From the surface of some Type II cells, however, there did project small villi (Fig. 8). Endoplasmic reticulum and Golgi apparatus were rarely seen in the bodies of these cells. Mitochondria, which were moderately electron-dense, were present in moderate numbers.

A second, much less numerous cell (designated as the Type I cell) was also found within the neoplasm (Figs. 10 and 11). Its nucleus, though slightly larger, was similar to that of the more common Type II cell but its less voluminous cytoplasm contained many mitochondria, dilated cisterns of smooth endoplasmic reticulum, and a few membrane-bound vacuoles. Dense bodies and multivesicular bodies were rarely seen. Type I cells gave rise to long processes containing closely packed parallel filaments measuring approximately 100 Å, a few mitochondria and some glycogen granules (Fig. 12 and Fig. 12, inset). The processes were most commonly seen abutting on a basement membrane which separated it from the large connective tissue space (Fig. 12). Both cell types were located in compartments separated from the abundant connective tissue spaces by basement membrane (Fig. 3). The relationship of the Type I and Type II cells to the basement membrane generally differed. The Type I cells and their elongated processes frequently were completely covered on one surface by basement membrane (Figs. 10, 11, and 12). Elsewhere Type I cells gave rise to thin filament-containing processes which were interposed between Type II cells and the basement membrane. Only rarely were the Type II cells in contact with the basement membrane at some part of their surfaces (Fig. 5). Basement membrane was never seen completely surrounding Type I or Type II cells.

The neoplasm contained a moderate number of capillaries within the connective tissue spaces. Immediately outside of the endothelium was a basement membrane or several layers of basement membrane beyond which there were abundant collagen fibers (Fig. 13). Another basement membrane separated the connective tissue from the tumor cells. No long-spacing collagen fibers were seen.

Electron microscopy of Case II revealed poorly preserved tissue since only formalin-fixed autopsy material was available, but the morphologic pattern of the granular cells and their cytoplasmic content was generally similar to that of the Type II cell seen in Case I. A great number of collagen fibrils was scattered throughout.

Neither axons nor myelin were observed in either neoplasm.
Fig. 8. Case I. Type II tumor cell showing cytoplasmic filaments (arrows) along plasma membrane and adjacent to nucleus (N). Note villous process (crossed arrow) arising from cell. × 22,000.

Fig. 9. Case I. Filamentous processes (P) between adjacent Type II tumor cells. Process appears to be arising from cytoplasm of cell on right (arrow). × 15,500.
Fig. 10. Case I. Electron micrograph of Type I tumor cell adjacent to basement membrane (arrow). Note dilated endoplasmic reticulum. Nucleolus (Nu), nucleus (N). × 9,900.

Fig. 11. Case I. Type I tumor cell with processes adjacent to basement membrane (arrows). Nucleus (N), collagen (C). × 10,500.
Fig. 12. Case I. Electron micrograph showing elongated process (white arrow) of Type 1 tumor cell with basement membrane (black arrows) on surface facing collagen (C). Small capillary lumen (L). Outside of portion of endothelial cell are multiple basement membranes (crossed black arrow). × 16,000. Inset: Higher magnification of process arising from Type 1 cell containing filaments (arrow) and glycogen granules (crossed arrow). × 53,500.
Fig. 13. Case I. Small capillary in tumor has a red cell in its lumen (L). Note multiple basement membranes (arrow) interspersed between collagen fibers. At bottom left is a portion of cytoplasm of Type I tumor cell. × 7,500.

DISCUSSION

Granular cell myoblastoma was originally described in 1926 by Abrikossoff (1). The histogenesis of this tumor remains controversial. Among the various types of cells from which it has been thought to arise are striated muscle fibers (1, 9, 22, 33), fibroblasts (10, 36), histiocytes (5, 50), undifferentiated mesenchymal cells (3) and cells of peripheral nerves, particularly Schwann cells (4, 6, 7, 13, 14). Multiple cells of origin have also been suggested (18, 42). Many terms have been used to designate the tumor (31). While most authors consider the granular cell myoblastoma a neoplasm, others have suggested that it represents a degenerative change (48, 51), a metabolic disorder (18, 42), or a lipid storage disorder (5).

The growth characteristics of granular cell tumors in sites outside of the central nervous system have been variable. While most reports have emphasized their benign nature, several authors have described malignant forms (2, 15, 20, 22, 28, 47). The histochemical and ultrastructural features of a metastasizing malignant granular cell tumor studied by Al-Sarraf et al (2) were similar to those of the benign granular cell tumors. Although the two tumors in the present report had a similar structure their clinical courses and growth characteristics were quite different. The tumor in Case I was within neural
parenchyma. It infiltrated the white matter and cortex of the right cerebral hemisphere, recurred relatively rapidly and ultimately caused the patient's death. The absence of clinical evidence of a primary tumor outside the nervous system before the onset of neurological signs, or during the 17 month period following the appearance of such signs is in favor of a primary origin in the brain but metastasis cannot be completely excluded. The patient's clinical course following radiation therapy suggested that the tumor was not radiosensitive. The clinical symptoms and signs and the results of the brain scan and cerebral angiography were indistinguishable from those associated with many other types of infiltrating cerebral neoplasms. In Case II the tumor was sharply circumscribed and did not significantly distort or compress the underlying spinal cord. It arose in, and remained confined to, the leptomeninges (subarachnoid space) except for minimal extension into the pia and external glial membrane. No granular cell tumor was found elsewhere post mortem. The tumor was clinically "silent," though one may speculate that it might have produced a filling defect had myelography been performed.

The light microscopic characteristics of the cells in these two cases with their voluminous cytoplasm containing abundant PAS-positive granules were similar to those previously described in granular cell tumors in other body sites. In the neurohypophysis tumors which were probably also granular cell tumors have been variously described as granular cell myoblastomas (8, 12, 19, 39, 49), choristomas (35, 38, 46) or pituiyctomas (25). Recently, Popovitch et al. (38) compared the ultrastructural and histochemical characteristics of two neurohypophyseal choristomas with a laryngeal granular cell myoblastoma. They found that the cytoplasmic granules were identical; the tumor cells of each were surrounded by basement membrane and histochemically they were similar. The authors proposed that choristomas and granular cell myoblastomas be "regarded as members of a family of 'granular cell tumors,' probably derived from Schwann cells."

We believe that the Type I and Type II cells which we have described electron microscopically may be variants of the same cell and that the Type I cell is the precursor. The Type II cell contained fine filaments which appeared to be displaced toward the periphery of the cell and toward the nucleus by the numerous cytoplasmic organelles which are presumed also to distend the cell. The much less common Type I tumor cells contained ordinary cytoplasmic organelles and only an occasional vacuole or dense body. It is possible that as dense bodies, multivesicular bodies and vacuoles develop within Type I cells they enlarge and become rounded and the processes containing filaments are "withdrawn" or shortened. This, together with an impaired capacity to form basement membrane, may result in a transformation of Type I cells into Type II cells. This concept would be in keeping with the observations of Sobel et al (45). In a study of seven granular cell myoblastomas they described "early" and "mature" tumor cells in which the former contained numerous fine fibrils, large Golgi apparatus, many mitochondria, well developed endoplasmic reticulum and few granules. The "mature" cells contained many more small and large
granules and fewer other cytoplasmic organelles and fibrils. Perhaps the Type I cells in our study correspond to the "early" tumor cells described in their report.

The ultrastructural features observed in the neoplasm in Case I are comparable to those described previously in granular cell tumors outside of the central nervous system (2, 3, 9, 13, 16, 18, 31, 43, 45). In our material the cytoplasmic granules of the more common Type II tumor cells seen by light microscopy appeared to be composed of the dense bodies and multivesicular bodies observed electron microscopically. The cells with central pale agranular cytoplasm seen by light microscopy were readily identified in electron micrographs. In their centers were large numbers of vacuoles peripheral to which were many dense bodies. The dense bodies, with their finely granular or homogeneously electron dense center surrounded by a single membrane, have the appearance of lysosomes but the demonstration of hydrolytic enzyme activity in them would be needed to support this view. The vesicle-containing organelles, referred to as multivesicular bodies, have also been considered to be lysosomes (21) and have been observed previously in granular cell tumors in other sites (3, 13, 18, 31, 43, 45). The heterogeneous vacuoles containing a few vesicles and fragments of other organelles most likely represent autophagic vacuoles as described by de Duve (11). Such heterogeneous vacuoles have been seen in other ultrastructural studies of granular cell tumors and interpreted as residual bodies (16), phagosomes (31), and autophagic vacuoles (44). Although tissue was not available for histochemical study of the components of the cytoplasm in our cases, some of the granules in tumor cells studied by others (16, 44, 45) have been shown to exhibit acid phosphatase activity, generally accepted as a lysosomal marker. It could be supposed that there is a common lysosomal origin for the dense bodies, the multivesicular bodies and the vacuoles of the tumor cells. Transformation of dense bodies and multivesicular bodies into vacuoles might ensue as the result of an autophagic process. These observations lead us to the view that the cells which have pale centers as seen by light microscopy are Type II cells in a more advanced state of degeneration.

The cell of origin of the tumors in this study is uncertain. The support for a Schwann cell origin for granular cell tumors is based upon the occurrence of the tumors in peripheral nerves (6, 7), the presence of axons in a few of the reported cases (13, 16, 45), the existence of basement membranes about tumor cells (13, 16, 38, 45), and the appearance in degenerating Schwann cells of cytoplasmic vacuoles like those in the tumor cells (16). We did not observe myelin or axons in the tumors in our cases and in Case II the tumor was remote from any nerve roots. Basement membranes were present in many parts of the tumor of Case I generally separating the abundant collagen fibers from the compartments of tumor cells. Between most of the tumor cells, however, there was a conspicuous absence of basement membrane. This is in contrast to schwannomas in which basement membrane is present between many tumor cells and between their processes. In our experience, however, as schwannoma cells become "foamy" in type they may lose their basement membrane (Duffy,
P. E. and Defendini, R., Unpublished observations). Multiple folded basement membranes were occasionally seen intermeshed with collagen between the small capillaries and tumor cell compartments in Case I. Nathaniel and Pease (34) described multiple basement membranes forming around reactive Schwann cells during nerve regeneration. Abundant collagen was observed ultrastructurally between the granular cells of Case II, but due to poor tissue preservation no definitive statement regarding the relationship of basement membrane to cells can be made in this case. The demonstration of granular cell tumors in the central nervous system in the present cases and their occurrence within the neurohypophysis, make it less likely that all granular cell tumors are derived from Schwann cells. Their development from Schwann cells of small peri vascular nerves which have been observed in the leptomeninges and possibly in central neural tissue (37, 40), cannot be completely excluded.

Significant in the present work is the demonstration that primary granular cell tumors do occur in the central nervous system outside of the neurohypophysis, that the granules seen by light microscopy can be equated electron microscopically with dense and multivesicular bodies, and that cells with clear centers by light microscopy have undergone a further vacuolar change, perhaps due to autophagia of dense and multivesicular bodies. It is also possible, in view of the ultrastructure described, to suggest that the tumor is composed of a single type of cell in various stages of development or degeneration.

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