Granular Cell Glioblastoma: A Malignant Granular Cell Neoplasm of Astrocytic Origin

MARIO KORNFELD, M.D.

Abstract. Granular cell tumors in the cerebral hemispheres are rare and of unknown origin. Two large aggressive cerebral neoplasms composed mainly of granular cells and also containing neoplastic astrocytes were studied histologically, ultrastructurally and immunohistochemically. In both instances, transitional forms between astrocytes and typical granular cells were demonstrated. In the second case which consisted of a central core of typical glioblastoma surrounded by a thick shell of granular cells, the latter spread in a fashion that is characteristic of intrinsic tumors of the central nervous system by forming abundant secondary structures of Scherer, particularly under the pia and around cortical blood vessels. All the evidence indicates that in these two cases granular cells were transformed neoplastic astrocytes. Paradoxically, many granular cells exhibited benign cytological features that belied their malignant nature.

Key Words: Astrocytes, transformed; Glioblastoma; Granular cell neoplasms; Granular cells; Immunocytochemistry.

INTRODUCTION

Granular cell tumors are relatively common neoplasms which arise in multiple sites particularly in the soft tissue of the head, neck, and upper extremities. Their histogenesis has been a subject of long-standing controversy. Striated muscle cells, peripheral nervous tissue, histiocytes, fibroblasts and undifferentiated mesenchymal cells have all been in turn considered as probable cells of origin. More recent studies have offered substantial evidence in favor of a Schwann cell origin (1). Tumors with identical morphologic features have on occasion been observed in the cranial cavity in the following sites: the sella turcica and its vicinity (2, 3), cranial nerves (4, 5), and within the cerebral hemisphere (6-12). Most of the intracranial examples have been found in the region of the sella. These, like the exceptional granular cell tumors arising from the intracranial portion of the fifth nerve, were characterized by slow noninfiltrating growth. Some information regarding the origin of the intracranial granular cell tumors in these two locations is available. Immunohistochemical studies implicate the astrocyte as the cell of origin in the former (2) and ultrastructural study suggests the Schwann cell origin for the latter (4). In contrast, the histogenesis of intrahemispheric granular cell tumors remains uncertain. In addition, the cerebral examples, at least those followed for adequate periods of time and with sufficient pathological data at hand, behaved in a malignant fashion.

This paper describes two large hemispheric tumors composed predominantly of granular cells and also showing unequivocal features of glioblastoma. The transitional features between the two components suggest that the granular cells originated from neoplastic astrocytes.

From the Department of Pathology, University of New Mexico School of Medicine, Albuquerque, New Mexico.

Correspondence to: Mario Kornfeld, M.D., Department of Pathology, University of New Mexico, School of Medicine, Albuquerque, NM 87131.

CASE REPORTS

Case 1

A 46-year-old man was admitted to the hospital for the evaluation of speech difficulties and occipital headaches of one month's duration. On examination, he was dysphasic, disoriented in time and his concentration span reduced. He had a right facial nerve paresis of central type and clumsiness and hyperreflexia of the right arm and decreased pinprick perception in the right hand. A computerized tomogram (CT) scan of the head showed a large contrast enhancing lesion surrounded by massive edema in the left frontoparietal region. At craniotomy, a firm, richly vascularized mass was found and partially resected. Subsequently the patient received radiation therapy consisting of 6,000 rads in 30 treatments over 45 days to the tumor. After a period of stabilization, his neurological status deteriorated and he died eight months after the onset of his first symptoms.

Case 2

This 49-year-old male developed tingling and numbness in the left arm and leg. Examination revealed a left quadrant hemianopsia, hyperreflexia, markedly impaired perception of all sensory modalities and asterognosis on the left side. Following studies (brain scan, right carotid angiogram and pneumoencephalogram) that suggested the presence of a right temporoparietal mass, an aspiration of the lesion through a right parietal burr hole was attempted but neither tissue nor fluid was obtained. The patient continued to deteriorate and died without the benefit of radiation therapy or chemotherapy, approximately two months after the onset of symptoms.

MATERIALS AND METHODS

Tissue obtained by biopsy in Case 1 was divided into two parts, one part was fixed in 10% buffered formalin, embedded in paraffin and the sections stained with hematoxylin and eosin (H&E), Masson trichrome stain, periodic acid Schiff (PAS) with and without digestion with diastase, phosphotungstic acid hematoxylin (PTAH), Gomori reticulin stain, PAS–luxol fast blue (LFB) and van Gieson stain. Immunostaining with antibodies to glial fibrillary acidic protein (GFAP) (DAKO) and protein S-100 (DAKO) was also performed on paraffin sections using the peroxidase-anti-peroxidase (PAP) technique. Frozen sections of material fixed in formalin were used for staining with Sudan black and oil red O. The other part of the biopsy specimen was used for electron microscopy, minced fragments of tumor were immediately immersed in 2.5% buffered glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated and embedded in epoxy resin. Semi-thin (1 μm) sections were stained with azure II–methylene blue and the ultrathin sections with uranyl acetate and lead citrate. In Case 2, the brain was removed at autopsy, placed in 10% buffered formalin and cut after fixation. For light microscopy, the paraffin and frozen sections were treated as in Case 1. Blocks for electron microscopy were washed in phosphate buffer, osmicated, and processed as indicated for Case 1.

RESULTS

Case 1—Light Microscopy

This richly vascularized, densely cellular neoplasm was composed predominantly of diffuse sheaths of round or oval cells 10 to 50 μm in diameter. Neoplastic cells had abundant cytoplasm that was packed with fine granules (Fig. 1). The granules were eosinophilic, argentophilic, and strongly PAS-positive before and after exposure to diastase (Fig. 2A); granules were bright red with the trichrome stain, tan with van Gieson stain and light blue with PTAH. The nuclei were irregularly rounded or oval,

often centrally located and of moderate size. Chromatin was finely stippled and a prominent nucleolus visible in some nuclei. In many cells, the nucleus was small, elongated, hyperchromatic, frequently crenated and located directly under the cell membrane. An occasional granular cell was in mitosis or had a large irregular nucleus with dusky chromatin. Scattered among granular cells were cells with hyperchromatic, pleomorphic, sometimes bizarre nuclei and eosinophilic cytoplasm that was devoid of granules (Figs. 2B, 3). Their irregularly shaped bodies usually had multiple processes. However, some cells of this type were round and without processes. The PTAH stain demonstrated numerous fibrils in the majority of the cells with processes. In contrast, the round nongranular neoplastic cells were usually not fibrillar. Multinucleated cells of this type were also noted and mitotic figures were much more common than in the granular cells. The nongranular cells occurred singly or in small clusters. Immunostaining with antibodies to GFAP revealed a number of strongly positive cells as well as many nonreacting cells. Cells of intermediate reactivity as well as cells in which the reaction product was limited to the peripheral cytoplasm were also present. While the majority of cells expressing GFAP antigen possessed multiple processes which were well-defined by the reaction product, most of the rounded cells did not show any reactivity (Fig. 4). There were, however, a number of round cells that were strongly or moderately positive. Among the latter were the cells with heavily granular cytoplasm (Fig. 4 inset). Immunostaining with antibodies to S-100 protein closely followed the pattern obtained with antibodies to GFAP. The numerous small blood vessels often had a hypertrophic endothelial lining. The lumens of several larger arteries were obstructed by fresh or organized thrombi, and in their walls there were occasional foci of fibrinoid necrosis. Several small areas of necrosis and fibrosis were scattered throughout the neoplasm. Reticular fibers were present in the fibrotic zones and in the immediate vicinity of blood vessels but none were found around or among the tumor cells of any type.

Electron Microscopy

Ultrastructurally, four types of cells could be distinguished in the tumor. Of these, two types were closely related and undoubtedly represented the ultrastructural correlate of granular cells as observed with the light microscope. They had round, large cell bodies and a smooth plasmalemma which was either closely apposed to the membrane of similar neighboring cells or facing an irregular intercellular space. Also, both types of cells had a voluminous cytoplasm with numerous membrane-bound bodies with a densely granular content. In the first type of cell, the granular bodies were round or oval, of relatively uniform size with uniformly granular or sometimes microvesicular content (Fig. 5A). The cytoplasm among the granular bodies had numerous cisternae of granular endoplasmic reticulum, mitochondria, vesicles or a Golgi apparatus. Occasionally, a delicate irregular network of 8–9 nm filaments was seen. A rare cell had thick bundles or skeins of such filaments in its cytoplasm (Fig. 5B); the single nucleus was irregularly rounded, and the chromatin uniformly distributed. The second type of cell was characterized by more numerous and more pleomorphic granular bodies (Fig. 6A) with diameters varying from 0.4 to 4.5 μm; the larger ones often had scalloped contours apparently due to the fusion of individual round or oval granular bodies. Their contents were nonhomogeneous and consisted only in part of fine granules and vesicles; irregular dense bodies and large rounded vesicles were also present. Very little remaining cytoplasm was discernible among the granular bodies that occupied most of the perikaryon. The nuclei of this cell type showed abundant heterochromatin and were often highly irregular in shape due to
multiple infoldings. Although usually neither the type I nor the type II cell had any cytoplasmic extensions, a rare cell of either type (more commonly type I) had a few short stout processes which were free of granular bodies and contained an irregular network of 9 nm filaments (Fig. 6B). A third cell type was a rare cell with numerous bundles of 8–9 nm filaments which enclosed many elongated, membrane-bound structures with densely granular contents (Fig. 7). A few cells were identified in which only a portion of the perikaryon was filled with bundles of filaments and elongated granular structures while the remainder showed the features of a type I cell. The fourth cell type was a cell with usual characteristics of a neoplastic astrocyte.

Case 2—Gross Findings

In the brain there was herniation of the right uncus and parahippocampal gyrus through the incisura tentorii. The posterior portion of the right cingulate gyrus was herniated under the falx. In coronal sections, a deeply situated tumor measuring 6 cm at its widest was found in the right parietal lobe. It also involved the central portions of the right posterior frontal lobe and extended into the posterior portion of the corpus callosum. The tumor, which grossly appeared well demarcated, consisted of gray, soft tissue with disseminated creamy foci and multiple zones of hemorrhage.

Light Microscopy

The highly cellular neoplastic tissue was composed of aggregates of cells of different sizes and shapes with single or multiple, hyperchromatic, highly pleomorphic nuclei showing variable numbers of mitotic figures (Fig. 8A). There were many spindle-shaped as well as multipolar-cells, the processes of which blended into a delicate network of eosinophilic fibers. Except for pseudopalisade formations around frequent areas of necrosis, no specific architectural arrangement of tumor cells was noted. Vascular changes consisted of capillary proliferation, endothelial hyperplasia and thrombosis of larger blood vessels. At what grossly appeared to be the margin of the tumor, the histological features abruptly changed and the pleomorphic tumor was continuous with a mass of closely packed large cells with voluminous granular cytoplasm. These cells were, with minor exceptions, morphologically identical with the granular cells in Case 1 and showed the same tinctorial characteristics (Fig. 8B). One of the differences was the size since many cells reached a diameter of 75–80 μm. Another difference was the greater frequency of granular cells with atypical nuclei even though the majority of granular cells had small, rather uniform nuclei. Also, in the deepest layers of the shell many granular cells were stellate or polygonal in shape rather than round. Many such cells were only partially granulated; the granules were usually located peripherally in the perikaryon and in the proximal parts of the processes (Fig. 9). Occasional neoplastic astrocytes were also present. The densely packed granular cells formed a 1–3.5 cm thick shell around the major part of the pleomorphic mass. Where this shell was defective as in the corpus callosum the pleomorphic cells were present in decreasing numbers in the neighboring paren-

---

Fig. 1. Case 1. The neoplasm consists of a homogeneous population of closely packed, round cells with fine cytoplasmic granules. H&E. × 650.

Fig. 2. Case 1. A. Cytoplasmic granules react strongly with PAS. PAS. × 300. B. Admixture of two cell populations: granular cells and pleomorphic cells with features characteristic of astrocytes, two are multinucleated (arrow). H&E. × 475.
chyema. Granular cells behaved similarly. They infiltrated the adjacent white matter. Consequently, the shell of densely packed granular cells did not have a sharp peripheral border. However, at interfaces between the white and gray matter, e.g. around the putamen and claustrum or under the cerebral cortex denser aggregates of neoplastic granular cells were present. A few granular cells were noted in many places in the cerebral cortex of the convexity and in the medial aspects of the cerebral hemispheres particularly in the parietal lobe where abundant, compact aggregates formed under the pia mater (Fig. 10). In some of the foci the granular cells exhibited marked nuclear pleomorphism. Compact perivascular aggregates of granular cells were also frequent in the cortex. Immunostaining for GFAP revealed numerous strongly GFAP-positive tumor cells in the central pleomorphic mass. The granular cells whether in the shell surrounding the tumor or distal from it were GFAP-negative. In the shell of granular cells, especially its deeper layers, there were strongly GFAP-positive stellate cells as well as round cells showing different degrees of affinity for GFAP antibody. While the reaction product outlined two or three short processes in a few of the GFAP-positive round cells, most had none. Again, the results of immunostaining with antibody to the S-100 protein closely resembled those with antibody to GFAP.

Electron Microscopy

The tissue sampled for electron microscopy from the immediate vicinity of the central mass was relatively well preserved for material obtained at autopsy and fixed in formalin. The sample consisted of closely aggregated round cells, all with the ultrastructural characteristics of type II cells as described in Case 1 (Fig. 11).

DISCUSSION

Each of the two malignant hemispheric tumors presented in this study consisted of two morphologically distinct populations of cells: malignant astrocytes and granular cells. The light microscopic features, tinctorial affinity and electron microscopic characteristics of granular cells were indistinguishable from those observed by others in granular cell tumors located in the intracranial compartment, or in various soft tissues. Because of the dual cell population, these two neoplasms could be interpreted as mixed tumors. Accordingly, one could envision the first case as a result of simultaneous focal neoplastic transformation of astrocytes and a non-glial element giving rise to granular cells. Alternatively, one could postulate an induction of neoplasm in astrocytes trapped within the confines of a granular cell tumor. A reverse sequence of events, namely an induction of a granular cell tumor by a glioblastoma might be considered in Case 2. The occasional occurrence in the central nervous system (CNS) of mixed tumors of which one component is glial (13–18), lends some support to this hypothesis. However, in each case, several lines of evidence suggest that the granular cells had developed from neoplastic astrocytes, rather than rep-

Fig. 3. Case 1. In this area, pleomorphic cells (arrowheads) with voluminous eosinophilic cytoplasm and large, sometimes multiple nuclei with prominent nucleoli, are scattered among granular cells. H&E. ×740.

Fig. 4. Case 1. Cells with cytoplasm which binds GFAP antibody diffusely vary in shape from stellate to oval and round. They occur singly or in small groups (open arrows). Two GFAP-positive giant cells are seen left of center. Granular cells in this field do not express any GFAP antigen and are all unstained. GFAP. ×195. Inset. Group of cells with strongly GFAP-positive granules. GFAP. ×475.
Fig. 5. Case 1. A. This type I cell has multiple granular, lysosome-like bodies which vary in size, but are structurally uniform, in its cytoplasm. Its nucleus contains dispersed chromatin. Heterochromatin is present in the nucleus of a type II cell, a small portion of which is seen on the right. ×14,250. B. The cytoplasm of this granular cell contains a large skein (thick arrow) and two smaller bundles (thin arrows) of intermediate filaments. ×16,625.
Fig. 6. Case 1. A. This round type II cell without processes has a perikaryon filled with pleomorphic granular bodies. Its nucleus is multilobed and has condensed chromatin. ×6,293. B. A stout process of a type II cell contains randomly distributed intermediate filaments and sparse granules resembling glycogen. ×6,293.
resenting a second component of separate origin. The first tumor consisted predominantly of granular cells enclosing foci of typical malignant astrocytes. The immunohistochemical staining with antibodies to GFAP and S-100 protein clearly separated the two types, the granular cells being negative, the malignant astrocytes positive with the two antibodies. However, there were also some round, GFAP-positive cells without granules as well as GFAP-positive granular cells. By electron microscopy, most cells could be clearly identified as either granular cells or neoplastic astrocytes, but there were also two additional cell types with intermediate features: 1) multipolar cells with the ultrastructural features of astrocytes, but also containing numerous granular bodies; 2) cells with the ultrastructural features of granular cells but possessing processes. The second tumor was composed of the same two components as the first one but the two were separated: the glioblastoma formed a central core and the granular cells formed a shell around it. However, at the contact zone transitional forms among the cell types were present. With the PAS stain these transitional forms were represented by granular cells with multiple processes. Often such cells were only partially granulated and the central part of the perikaryon remained free of granules. In addition, the granular cells in this tumor spread in a fashion that is characteristic of intrinsic CNS tumors, by forming abundant secondary structures of Scherer at interfaces between the white and gray matter, perivascularly and under the pia mater. In each case, therefore, light microscopy, immunohistochemistry, and electron microscopy demonstrated cells with features that were intermediate between typical granular cells and neoplastic astrocytes. In the second case, the granular cells also exhibited an infiltrative behavior typical of neoplastic glia. On the basis of these observations, one might postulate a transformation beginning with increasing numbers of granular bodies in neoplastic astrocytes, followed by a further increase in
**Fig. 8.** Case 2. A. Central portion of the lesion: pleomorphic glial neoplasm with occasional multinucleate giant cells, foci of necrosis (lower right) and mural hyperplasia of blood vessels (lower left). H&E. ×320. B. At the periphery of the central mass, densely packed round cells with granular cytoplasm and no processes form a thick shell. H&E. ×500.
granular bodies, a reduction in filaments, and cell processes, and finally formation of granular cells first of type I and then type II as previously suggested by Markesbery et al (6) (Table 1). The loss of GFAP antigenicity seems to parallel the disappearance of cytoplasmic filaments, but on rare occasions (probably an early stage in the transformation) even the granules were GFAP-positive. Therefore, it appears justified to conclude that the two tumors were not mixed neoplasms but were glioblastomas with transformations of astrocytes into granular cells. The term granular cell glioblastoma seems to be an appropriate label for such and similar tumors. One might speculate that the previously recorded cerebral examples of granular cell tumors also belong to this category. Evidence for that speculative conclusion is strongest in the case described by Pasquier et al (10); their case was very similar to Case 1 of this study because it consisted of intermixed malignant astrocytes and granular cells. There were transitional forms between these two cell types and some granular cells were GFAP-positive. The hemispheric granular cell tumor investigated by Lechevalier et al (12) also had a substantial astrocytic component but this apparently lacked anaplastic features. Case 2 of Sakurama et al (11) studied in biopsy only was very similar to our Case 2 in that the central part was a glial neoplasm and granular cells formed its periphery. While the glial part was described as a partly fibrillary and partly “gemistocytic” astrocytoma, the aggressive behavior of the tumor suggests that an anaplastic component was probably present at the time of biopsy. The concept is further supported by a recent immunohistochemical study of granular cell tumors from various sites (19) which included the material from the malignant cerebral tumor reported previously by Ule et al (7) and found GFAP-positive, immature granular cells at the periphery of that neoplasm. In the remaining granular cell tumors

of the brain, which lacked strong evidence of an astrocytic derivation (6, 11), other features such as infiltrative growth, vascular abnormalities, necrosis and clinical course were certainly compatible with a diagnosis of glioblastoma.

Cytoplasmic granules similar to or identical with those observed in granular cell tumors have also been noted as a minor change in well-differentiated astrocytoma. The earliest observation of this kind is that of Zülch (20) who, however, thought that the granular cells were probably macrophages. Hossmann and Wechsler (21) established by electron microscopy not only the fine structure of the granules but
also the fact that they developed within the tumor cells. In a case of ganglioglioma, Rubinstein and Herman (22) also found granules in the glial component and stressed their almost invariable association with cytoplasmic filaments. In a fibrillary astrocytoma, Barnard and Scott (23) found that the eosinophilic granules often showed a positive reaction when stained for GFAP. Large globoid cells with eosinophilic granules in the cytoplasm repeatedly observed in the brain with edema (24, 25) and radiation (26, 27) are also very similar to the constituents of granular cell tumors. In the past, such granulated globoid cells were considered of microglial or oligodendroglial origin, but recent studies of pion irradiated brains using immunohistochemical techniques disclosed the presence of GFAP antigen in many of them (28). Thus, there is evidence that the astrocyte whether neoplastic and of any degree of malignancy, or reactive might be transformed into a granular cell. The exact circumstances that might trigger the transformation of the malignant astrocyte into a granular cell are not clear. In benign astrocytes the granules tend to develop in the vicinity of cysts (23, 29). In the two cases reported here, no cysts were present. Although necroses were frequent there was no spatial relationship between them and granular cells. Granular cells are often found in nonneoplastic tissue in irradiated brain and neither of the two cases reported here had radiation therapy before tissue diagnosis. Since it is associated with the development of granular cells, edema also should be considered as a potential trigger of granular change in glioblastomas, since edema is a common accompaniment of that neoplasm. While edema indeed might be important, additional factors or special circumstances would have to be invoked since edema is common and granular cell transformation is rare in glioblastoma.

Some insight into the genesis of the granules in granular cell tumors of soft tissues was obtained by the immunohistochemical studies that have identified peripheral myelin proteins within granules (1). In our material the fact that granules displayed GFAP-reactivity and neoplastic cells were packed with filaments and large numbers of lysosome-like bodies, suggest that in these tumors of the CNS the primary abnormality might be in the glial filament. The granules in soft tissue tumors and those
TABLE 1
Presumed Stages in Development of Granular Cells

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFAP Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple granular bodies (GrB) among 9 nm filaments in perikaryon and processes; GFAP+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Further increase in GrB, rounding of perikaryon, reduction of filaments, persistence of processes, sometimes GFAP+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerous GrB, round perikaryon, sparse filaments, loss of processes, usually GFAP-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masses of pleomorphic GrB and dense bodies, no filaments, no processes, GFAP-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GFAP+ = positive for glial fibrillary acidic protein; GFAP- = negative for glial fibrillary acidic protein.

in granular tumors of the CNS are morphologically identical; they have the appearance of autophagic vacuoles. However, the lysosomal system in one instance seems to be stimulated by a membrane component of the Schwann cell and by an astrocytic cytoskeletal element in the other.

Secondary changes can substantially alter the morphological characteristics of many tumors including the tumors of the CNS. The accumulation of lipid is a recognized phenomenon in some meningiomas, Schwannomas, and hemangioblastomas. Similarly, heavy lipidization of neoplastic glia can alter the usual features of malignant gliomas (30). In pleomorphic xanthoastrocytoma, large amounts of lipid may obscure the glial nature of the neoplasm (31). Cartilage-like foci in some gliomas might result from the secretion of basement membrane-like materials by neoplastic astrocytes (32). The development of granular cells in glioblastomas can also be placed into this category of secondary change. It is of practical importance that not only the identity of the tumor might be obscured but also that the usual cytological features of malignancy are not expressed by many granular cells. For these reasons, large areas of the granular cell glioblastoma might be morphologically indistinguishable from the biologically different granular tumors of theellar region or those developing in the cranial nerves that not only differ in biological behavior but probably in histogenesis as well.

REFERENCES


(Received 26 August 1985/Accepted 31 December 1985) MS85-60