Granular cell astrocytoma. A case report with immunohistochemical and ultrastructural characterization

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Abstract
Granular cell astrocytoma (GCA) is an uncommon type of granular cell tumours (GCTs) in the central nervous system. Granular cells in these tumours are of enigmatic origin. We report a case of cerebral GCA in a 59-year-old man who suffered from diabetes and Addison-Biermer disease. The tumour was localized in the left parietal lobe. Microscopically, the tumour was almost entirely composed of large, polygonal cells with round to oval, granular eosinophilic, PAS-positive cytoplasm. The nuclei were located centrally or eccentrically and sometimes exhibited nucleolar vacuoles. The tumour cells were arranged in nests surrounded by blood vessels and connective tissue. Immunohistochemically, the granular tumour cells were reactive for GFAP and vimentin. They were intensively stained for ubiquitin and some of them were reactive for CD68. Moreover, a lot of stromal cells expressed CD68 reactivity. Ultrastructurally, most tumour cells were round or oval with only a few or without filaments. Their cytoplasm was filled with electron-dense granular material limited by a single membrane and autophagic vacuoles. Another type of tumour cells, present in a significantly lower number, revealed abundant cytoplasm with numerous intermediate filaments, swollen rough endoplasmic reticulum, mitochondria and a few clusters of granular material. Cells with numerous condensed electron-dense, bizarrely-shaped mitochondria and few filaments were occasionally observed. Among granular cells, macrophages with vacuoles and/or lamellar structures were visible. In our case, both immunohistochemical and ultrastructural analysis supported astroglial origin of the granular cell tumour.

Key words: granular cell astrocytoma, immunohistochemistry, ultrastructure.

Introduction
Granular cell tumour was first mentioned by Weber [33] and described by Abrikossoff, who referred to it as “granular cell myoblastoma” of the tongue [1]. The term “granular cell tumour” (GCT) was first introduced by Lack et al. in 1980 [15] and up to now about 200 cases of GCT have been described. GCTs are uncommon lesions, which may occur in humans and domestic animals at any age in al-
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Granular cells in GCTs are of enigmatic origin. They were initially believed to be of myogenic origin and then of histiocytic and subsequently of neuronal origin [7,22,28].

Granular cell astrocytoma (GCA) is a rare histopathological variant of astrocytoma exhibiting a prominent population of typical granular cells. These tumour cells contain numerous eosinophilic granules and show cytoplasmic immunoreactivity to glial fibrillary acidic protein (GFAP) [3,6,9,12,13,18,23,25,31].

We report a case of cerebral GCT in a man who suffered from diabetes and Addison-Biermer disease. The astroglial origin of this rare entity was confirmed by immunohistochemical and ultrastructural examinations.

Case report

A 59-year-old man was admitted to our hospital with a one-week history of progressive headache and visual disturbances. His medical history was significant for long-standing (20 years) and well-controlled insulin-treated diabetes mellitus type 2 and Addison-Biermer disease recognized 6 years before admission. Physical examinations revealed pale skin and neurological testing revealed left-sided homonymous hemianopsia. The motor strength was intact, and no sensory deficits were detected. The Babinski sign was absent. Laboratory examination showed a slight decrease of white cell count: 3.6 K/μl (4.1 to 10.9). Mild anaemia was noted with red blood cell count of 3.89 M/μl (4.2 to 5.70), haemoglobin was 11.7 g/dl (12.6 to 17.2) and haematocrit was 33.6% (38.0 to 49.0). Sedimentation rate, coagulation profile, liver tests and the results of the renal function test were insignificant. The results of the chest X-ray and abdominal computed tomography (CT) were normal. Gastric endoscopic examination revealed diffuse atrophic gastritis. Biopsies taken at the time of endoscopic examination evidenced H. pylori infection.

The magnetic resonance imaging (MRI) scan of the head revealed a tumour (15 × 25 × 15 mm) localized in the left parietal lobe (Figs. 1, 2). The tumour was shown as a well-defined, bordered, homogeneous mass, surrounded by oedematous brain tissue and compressing the posterior horn of the right lateral ventricle. Right parietal, parasagittal craniotomy was performed. A well bordered, brown-yellowish, hard pathological tissue was found 15 mm under the surface of the oedematous cerebral cortex. The tumour was completely removed within its borders.

Methods

The tumour tissue for morphological examination was fixed in 10% buffered formalin and paraffin embedded. The specimens were stained with H&E, PAS, PAS-dimedon, Gomori’s method and immunohistochemically with the following antibodies: glial fibrillary acid protein (GFAP, DAKO, 1:500), vimentin (DAKO, clone PG-M1, 1:100), CD34 (Novocastra, 1:30), CD68 (DAKO, clone PG-M1, 1:100), CD34 (Novocastra,
1:300), epithelial membrane antigen (EMA, clone-GP 1,4, Novocastra, 1:300), Ki-67 (clone MIB-1, DAKO, 1:100), cytokeratin (CK, clone AE1/AE3, DAKO, 1:50) S-100 protein (DAKO, 1:400), ubiquitin (DAKO, 1:200), and anti-neuronal nuclei (NeuN, Chemicon, clone-A60, 1:100).

For electron microscopic evaluation, a small fragment of the tumour was taken from paraffin blocks. After deparaffinizing and washing in water, the material was postfixed in 2.5% glutaraldehyde, 2% osmium tetroxide and routinely processed to Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, then examined in a transmission electron microscope (Opton DPS 109).

Results

Microscopically, the tumour was almost entirely composed of round, oval or large polygonal cells with granular eosinophilic cytoplasm. The cells were arranged in a nest-like form (Figs. 3, 4). The blood vessels and reticular fibres compartmentalized the tumour cells (Fig. 5). The plump cytoplasm of tumour cells was eosinophilic with numerous PAS-positive granules often surrounded by a light “halo” (Fig. 6).
The nuclei of tumour cells were round to oval as well as bacilliform or lobular in shape (Fig. 7). They were located centrally or eccentrically and sometimes exhibited nuclear pseudovacuoles.

Immunohistochemically, the granular tumour cells were reactive for GFAP, vimentin and EMA (Figs. 8-10), whereas S-100 protein expressed weak positivity (Fig. 11). The majority of tumour cells were intensively stained for ubiquitin (Fig. 12). Cytokeratin, NeuN and SY immunostains were negative. Fibroconnective stromal cells and some of the granular cells were positively stained for CD68 (Figs. 13, 14). Labeling index of Ki-67 was low in tumour cells, while it was increased in stromal cells (Fig. 15).

At the ultrastructural level, a majority of tumour cells were round or oval and filled with electron-dense granular material limited by a single membrane (Fig. 16). Some of the vacuole-containing areas of the cytoplasm with mitochondria resembled autophagic vacuoles. Processes with bundles of intermediate filaments or collagen fibres could be seen between granular cells. In the cytoplasm of tumour cells usually sparse bundles of filaments were visible (Fig. 17). Sometimes, the filaments encircled the nucleus (Fig. 18). The nuclei of tumour cells exhibited more or less condensed chromatin and prominent nucleoli (Fig. 19). The cytoplasm of cells was often swollen with decreased electron density. Less numerous tumour cells revealed abundant cytoplasm with numerous intermediate filaments, swollen rough endoplasmic reticulum, mitochondria and a few clusters of granular material (Fig. 20). Sometimes, a few cells contained...
Fig. 10. Diffuse immunoreactivity for EMA, ×200

Fig. 11. Weakly expressed S-100 protein in the tumour, ×100

Fig. 12. Tumour cells intensively positive for ubiquitin, ×20

Fig. 13. Strong immunoreexpression of CD 68 in stromal cells, ×200

Fig. 14. Various immunolabelling for CD68 in large granular tumour cells, ×400

Fig. 15. Ki 67 labelling index low in tumour cells and higher in stromal cells, ×100
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numerous condensed, electron-dense mitochondria with bizarre, altered shape, markedly dilated granular endoplasmic reticulum and non-numerous filaments (Fig. 21). We also observed processes filled with numerous filaments and vacuoles with electron-dense material. Macrophages with a large number of vacuoles and lamellar structures were visible among granular cells. Small blood vessels usually demonstrated normal morphology of endothelial cells although numerous collagen fibres were seen around vessels.

Discussion

Granular cell tumour (GCT) is a rare, usually benign tumour of heterogeneous origin. This lesion is most frequently encountered in the tongue, but can occur in various visceral and cutaneous sites. On histological examination, this tumour consists of typical large, rounded or polygonal cells containing an abundance of eosinophilic, PAS-positive cytoplasmic granules. On ultrastructural examination, osmiophilic granules of varied size could be observed in the cytoplasm of tumour cells.

The histogenesis of GCA has remained controversial. Most recently the tumour has been thought to be of Schwann cell origin on the basis of morphological and immunohistochemical studies [8,32]. Some authors now consider GCT as a hamartomatous lesion and others as a tumour of uncertain histogenesis.

It has been documented that tumour cells of intracranial and intraspinal GCTs exhibited distinct immunohistochemical and ultrastructural features that were sometimes site-dependent [4,17-19,25,31]. Thus, granular cells in meningioma were immunoregulated for epithelial membrane antigen (EMA). Ultrastructurally, desmosomes confirmed the diagnosis of meningioma [14,19]. Granular cell tumours, occurring in cranial nerves, were positive for S-100 protein...
protein, being among others a marker of Schwann cells [5,11]. Granular cells in medulloblastoma co-expressed neuronal immunohistochemical markers, synaptophysin and NeuN [24]. Granular cells in tumours of the pituitary stalk, hypophysis, sellar or suprasellar region and oligodendroglioma exhibited similar immunoreactivity and ultrastructural features [7,22,27,35]. Genetic alterations specific to GCA could not be found, except for a high frequency of allelic loss on 9p and 10q [6].

The present case was diagnosed as granular cell astrocytoma as the astrocytic origin of the predominant granular cell population was supported by both immunohistochemical and ultrastructural studies. The tumour demonstrated the morphological picture of granular cell tumour. It was characterized by nests of plump cells with eosinophilic granular cytoplasm, positive for PAS stain and immunoreactive for GFAP, vimentin, ubiquitin and also positive for EMA, similarly to all previously reported cases of GCA.

The Ki-67 labelling index in granular cells was low (<2). Granular cells were surrounded by blood vessels and stromal cells. Tumour necrosis was not present, but focal endothelial proliferation was observed.

The cases of GCT reported in the literature were mostly benign, regarding their histopathology and biological behaviour. It has been suggested that granular cell astrocytoma might be of any grade according to the WHO grading system. However, some GCA are biologically more aggressive than the same grade astrocytoma lacking granular cell features [13,24,30].

Ultrastructurally, the majority of tumour cells exhibited typical morphology of granular cells. Their cytoplasm was filled with numerous vacuoles with granular, electron-dense material and sometimes

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**Fig. 19.** Granular cell with nucleus exhibiting slightly condensed nuclear chromatin and prominent nucleoli (Nu). Orig. magn. ×4400

**Fig. 20.** Tumour cells with abundant cytoplasm (TC) and typical granular tumour cells (GCT). Orig. magn. ×4400

**Fig. 21.** Fragment of tumour cell cytoplasm showing intermediate filaments (IF), markedly diluted granular endoplasmic reticulum (RER) and mitochondria (M). Orig. magn. ×7000
with autophagosomes. Intermediate filaments corresponding with GFAP-immunoreactive glial filaments were observed in the cytoplasm of tumour cells.

References