Ultrastructural Characterization of Transitional Cells in Oligodendrogliomas

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Abstract. In oligodendroglioma tumors the expression of glial fibrillary acidic protein (GFAP) is found in cells with an astrocytic morphology representing preexistent or neoplastic astrocytes. In addition, a proportion of the GFAP-positive cells has the morphology of minigemistocytes (minigemistocytes or oligodendrocytes (gliofibrillary oligodendrocytes or GFOC). Both minigemistocytes and GFOC are considered as cells transitional between astrocytic and oligodendroglial lineage. Though minigemistocytes can readily be distinguished in routinely stained histological sections, GFAP immunostaining is obligatory for the identification of the GFOC. In the present study, the GFOC is characterized at the ultrastructural level using an immunogold-silver stain on semithin (1 μm) slides for identification of GFAP immunoreactivity and subsequent processing of the adjacent slide for immunoelectron microscopy. In analogy with the minigemistocytes, the glial filaments in the GFOC are arranged in parallel bundles. The finding of cells with ultrastructural features intermediate between those of GFOC and minigemistocytes suggests a close relationship and a possible interconvertibility between the two transitional cell types in oligodendrogliomas.

Key Words: Electron microscopy; Gliofibrillary oligodendrocyte; Glioma; Immunoelectron microscopy; Minigemistocyte; Oligodendrogloma.

INTRODUCTION

Although oligodendrogliomas consist predominantly of neoplastic oligodendrocytes, cells with obvious astrocytic differentiation have also been observed in these gliomas since the earliest microscopic investigations (1–8). Traditionally, cells with slender processes have been considered as interspersed preexisting astrocytes, while cells with coarse and irregular cytoplasm and eccentric nuclei have been interpreted as neoplastic astrocytic cells (9, 10). Various mixtures of neoplastic astrocytes and oligodendrocytes were found in mixed gliomas. The oligoastrocytomas are defined as glial tumors with separate areas (compact variant) or mixtures (diffuse variant) of two cell types (8). Whereas some authors refused to recognize mixed gliomas as a separate group (11, 12), others believed that basically all gliomas would be of a mixed character (13). The latter view was supported by the finding of mixed cell populations in experimentally induced gliomas (14, 15), although pure astrocytomas and pure oligodendrogliomas might be the result of experimental tumor induction as well (16).

Small-sized gemistocytic cells have been recognized in oligodendrogliarial tumors long before immunohistochemical techniques were available (7, 8, 17). These cells were considered as transitional cells that changed their oligodendroglial morphology into cells with an astrocytic appearance. It was speculated that tumor age, changes in vascularity and increase in intracranial pressure might be causal factors for this transformation (5). More recently, the application of immunohistochemistry led to the discovery of oligodendroglial cells which expressed glial fibrillary acidic protein (GFAP) (18–22) and the term “gliofibrillary oligodendrocyte” (GFOC) was introduced, while the minigemistocytes were called “minigemistocytes” (19). Most authors considered these GFAP-positive cells as true elements of oligodendroglial tumors (9, 18, 21–25), while some classified tumors with these cells as mixed gliomas (22). Oligodendrogliomas containing

<table>
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<th>Localization</th>
<th>Diagnosis</th>
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<td>Grade C</td>
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<td>Grade B</td>
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<tr>
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<td>Left fronto-parietal</td>
<td>Oligo-astrocytoma</td>
<td>Grade 2</td>
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<tr>
<td>7</td>
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<td>33</td>
<td>Left frontal</td>
<td>Oligo-astrocytoma</td>
<td>Grade 2</td>
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The oligodendrogliomas were graded according to the modified grading system of Smith (30), while the oligo-astrocytomas were graded according to the grading system derived from Kernohan (31).
GFAP-positive cells, i.e. GFOC or minigemistocytes, were termed transitional gliomas (22).

At the ultrastructural level the minigemistocytes are readily recognized by their voluphtuous cell bodies, blunt cell processes and eccentric, flattened nuclei. In a previous study we showed that minigemistocytes contain characteristic dense bundles of GFAP-positive intermediate filaments in their cytoplasm which clearly separate this cell type from the classical gemistocytes (26). As yet, no ultrastructural description of the GFOC has been established, probably because this cell type is only recognized after GFAP immunohistochemistry (15, 27–29). The aim of the present study was to describe the ultrastructure of the gliofibrillary oligodendrocytes by using a gold-silver enhanced stain for detection of GFAP immunoreactivity on semithin plastic sections, and subsequently processing the adjacent ultrathin sections for immunoelectron microscopy. Comparison of the ultrastructural features of the minigemistocytes with those of the GFOC revealed a close relationship between these two cell types.

MATERIAL AND METHODS

Patient Material

Biopsy material of seven patients with oligodendroglial viz. mixed oligo-astrocytic tumors in which GFOC or minigemistocytes were found was selected for processing for immunoelectron microscopy in addition to routine histology. The clinical data of the seven patients are summarized in Table 1.

The oligodendrogliomas were graded according to the modified grading scheme of Smith (30). The mixed oligo-astrocytomas were graded following the grading scheme derived from Kernohan (31).

Histology and Immunohistochemistry

Freshly obtained surgical specimens were processed for paraffin embedding after fixation in phosphate buffered formalin while part of the material was kept apart for immunoelectron microscopy (see below). Sections of 5 μm were made and stained with hematoxylin and eosin. Consecutive sections of the same thickness were used for immunohistochemistry. The primary antibody included rabbit anti-GFAP antiserum (DAKO Corporation, Copenhagen, Denmark) diluted 1:60 in phosphate

buffered saline (PBS), pH 7.4. Endogenous peroxidase activity was blocked by treatment with 1% hydrogen peroxide in methanol. The two-step indirect immunoperoxidase technique was used on deparaffinized sections preincubated with 10% normal swine serum diluted in PBS. As the second step antibody, swine anti-rabbit immunoglobulin (Ig) antiseraum conjugated to horseradish peroxidase (DAKO) diluted 1:50 in PBS was used. The incubations were performed at 37°C in a humidified chamber for 30 minutes. Final visualization was achieved by incubation with 0.02% diaminobenzidine (DAB) in PBS and 0.075% H₂O₂ for 7 minutes in darkness. Control slides in which the primary antibody was replaced by PBS always were negative. The slides were counterstained with hematoxylin.

Neoplastic oligodendrocytes were recognized by their round or polygonal cytoplasmic borders, lack of cell processes, and round, centrally located nuclei. The tumor cells showed the typical honeycomb texture. The only distinction at the light microscopic level between the classic oligodendroglial cells and the gliofibrillary oligodendrocytes is the immunoreactivity for GFAP of the latter. Immunopositivity was seen as a small perinuclear rim. The transitional cells, or minigemistocytes, are readily recognized in the routine hematoxylin and cosin stain. The cytoplasm of these cells stains homogenously pink and gradually increases, leaving the nuclei in an eccentric position.

**Electron Microscopy**

Fresh tumor material was minced into 1 mm³ cubes and fixed immediately in 1% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.2) at 4°C. The specimens were fixed for 24 hours, transferred and stored in 0.1 mol/L phosphate buffer for 8 hours, and post-fixed in 1% OsO₄ in 0.1 mol/L phosphate buffer (pH 7.2) for 12 hours at 4°C. Subsequently, the specimens were rinsed in the same buffer, ethanol-dehydrated, and Epon-embedded for routine transmission electron microscopy. After ultrathin cutting, the sections were collected on mesh 100 copper grids and counterstained with uranyl acetate and lead citrate. Transmission micrographs were made on a Zeiss 902 transmission electron microscope at 80 kV.

**Immunoelectron Microscopy**

For postembedding immunoelectron microscopy, 1 mm³ tissue cubes were fixed in 0.1 mol/L phosphate buffer (pH 7.2) containing 1% acroleine and 0.4% glutaraldehyde at 4°C for 4 hours. Tissues were transferred and stored in a sucrose buffer of 1 mol/L sucrose in 0.1 mol/L phosphate buffer (pH 7.2) with 1% paraformaldehyde at 4°C until further processing for Lowicryl embedding as described previously (32). From Lowicryl-embedded material semithin (1 μm) and adjacent ultrathin sections were made with glass knives. The semithin sections were processed for gold-silver enhanced immunostaining for anti-GFAP as described below. The ultrathin sections were collected on carbon-coated Formvar-filmed mesh one hole copper grids. The immunological methods for visualization of rabbit anti-

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Fig. 2. Since the immunoreaction takes place on the cut surface while the tissue is within the Lowicryl, either the gold-silver stain or the cellular details are in focus in the photomicrographs. A) Semithin (1 μm) section of oligodendroglioma with GFAP and minigemistocytes. A GFOC (arrow) is tagged for ultrastructural investigation. (Patient 1, GFAP-immunostained gold-silver enhanced, counterstained with hematoxylin-azophloxin, x100). B) Ultrathin adjacent section demonstrating the same GFOC as tagged in Figure 2A (arrow). This cell contains large bundles of filaments and lacks significant cell processes. (Patient 1, immunoelectron microscopy stained for GFAP, x1,100). C) Detail of the cytoplasm of the GFOC from Figure 2A and B (frame). The filaments are arranged in parallel bundles.

The colloidal gold particles represent immunoreactivity with GFAP which are confined to the filaments. (Patient 1, immunoelectron microscopy stained for GFAP, x12,000).
GFAP were essentially as described previously (32). A 10 nm colloidal gold-labeled goat anti-rabbit antiserum (GAR-10, Aurion, Wageningen, The Netherlands) was used as the second step. Control sections were incubated with PBS, diluted in normal rabbit serum 1:60 in PBS, or the appropriate dilution of a similar monoclonal antibody nonreactive with glial tissue. Background staining was always negligible.

The semithin (1 μm) sections were incubated with anti-GFAP as the first step. Secondary incubation was done with Au-probe 1 M GAR-5 nm Au (Aurion). The immunogold-silver enhancement was done by magnification of the gold particles by precipitation of metallic silver (Aurion R Gient Developer and Enhancer, Aurion) in darkness at room temperature. The slides were counterstained with hematoxylin-a-azophloxin.

RESULTS

Patients and Histopathology

The patient group consisted of five males and two females. The mean age of the patients was 39 years with a range from 31 to 60 years. Most tumors had a frontal localization. The length of preoperative symptomatic period ranged from two years to one month.

In three tumors the cell density and the nuclear-cytoplasmic ratio was low, while vascular and endothelial proliferation, pleomorphism and necrosis were absent. Subsequently these tumors received Smith grade A. In the tumors of cases 3 and 5, an increased cell density and vascular and endothelial proliferation led to the attribution of grade B. Two gliomas had a considerable neoplastic astrocytic component and were subsequently diagnosed as oligo-astrocytomas (mixed gliomas). These tumors were graded according to the grading system derived from Kernohan (31). Both neoplasms received grade 2.

In the oligodendrogliomas, the GFOC and minigemistocytes were present in several areas. In Figure 1 an oligodendroglioma with transitional cells is shown stained with hematoxylin-a-azophloxin. In the mixed gliomas, the GFAP-positive oligodendrogial cells as well as the astrocytic cells were dispersed throughout the tumor.

Immunoelectron Microscopy

At the ultrastructural level, the oligodendroglial tumor cells were characterized by the absence of substantial numbers of filaments in the cytoplasm. Most of these cells had prominent mitochondria. The cells had round or oval nuclei. In the GFOC that were selected on semithin sections, the cytoplasm was filled with bundles of filaments in parallel arrangement (Figs. 2, 3). There were no other characteristics that separated these cells from the GFAP-negative neoplastic oligodendrocytes. The minigemisto-
cytes were readily recognized by their more voluminous cytoplasm without substantial cell processes and eccentric nuclei (Fig. 4). In the cytoplasm large intertwined bundles of filaments were seen (Fig. 4B, C). Regularly, cells with an intermediate phenotype between that of a GFOC and a minigemistocyte were seen (Fig. 3). These cells are characterized by a round or oval nucleus and cytoplasm densely filled with GFAP-positive bundles of filaments. In serial sections, it was confirmed that these cells indeed had a cytoplasmic volume between that of a GFOC and a minigemistocyte and, therefore, these cells did not represent superficially cut minigemistocytes.

In two mixed oligo-astrocytomas GFOC and minigemistocytes and cells with an intermediate phenotype were seen in some areas. Furthermore, large parts of these tumors consisted of GFAP-negative oligodendrocytes intermixed with (neoplastic) astrocytes (Fig. 5). The astrocytic cells had cell processes of variable lengths filled with an abundance of glial filaments (Fig. 5B, C).

**DISCUSSION**

To date, ultrastructural studies have not been able to identify unequivocal lineage-specific differences between neoplastic cells of putative oligodendroglial or astroglial lineage. A variety of ultrastructural features was claimed to be preferentially present in oligodendroglialomas, but none of these seemed to be specific for oligodendroglial tumor cells (12, 27–29, 33–45). In spite of the incidental observation of the entire cytoplasmic occupation by proteoglycans in neoplastic oligodendrocytes (34), glial filaments were not found as a prominent ultrastructural feature of neoplastic oligodendrocytes in other studies (33, 46). Ebhardt et al (44) occasionally found microtubuli and cytoskeletons in typical oligodendroglial cells, but these structures are a common finding in astrocytic cells.

Kamitani et al (27, 28) also reported the presence of “glial” filaments in neoplastic oligodendrocytes, and therefore supposed that these cells belong to the astrocytic lineage. The absence of GFAP in a proportion of oligodendroglial cells was attributed to the relative scarcity of filaments (27). The presence of abundant glycogen particles and perivascular end-feet was interpreted as additional evidence for the astrocytic nature of some oligodendroglial tumor cells (27). Now, the discrepancies with regard to the finding of filaments in neoplastic oligodendrocytes by different authors can easily be explained by our observation that a subset of (light microscopical) oligodendroglial cells contains dense bundles of filaments.

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Fig. 4. A) Semithin (1 μm) section of oligodendroglialoma with GFOC and minigemistocytes. A typical minigemistocyte (arrow) is seen adjacent to a typical (GFAP-negative) oligodendroglial cell (asterisk). The latter has a clear cytoplasm. In the upper left corner an interspersed astrocytic cell with prominent cell processes is strongly immunoreactive for GFAP. (Patient 1, GFAP-immunostained gold-silver enhanced, hematoxylin-azophloxin counterstained, ×100). B) Ultrathin adjacent section. The cytoplasm of the minigemistocyte (arrow) is filled with dense bundles of filaments, in contrast to the cytoplasm of the oligodendroglial cell (asterisk) which shows an electron lucent cytoplasm. In the latter cell, mitochondria represent the most prominent cell organelles. (Patient 1, immunoelectron microscopy stained for GFAP, ×1,160). C) Detail of GFAP-positive cell from Figure 4A and B (frame). The immunogold particles are associated with the intertwined bundles of filaments. (Patient 1, immunoelectron microscopy stained for GFAP, ×12,000).
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(29, 33, 42, 43). Since these glioblastomatous oligodendrocytes (GFOC) are defined both with light microscopical and immunohistochemical criteria (i.e. reactivity with anti-GFAP antibody), immunoelectron microscopy was required to identify GFOC at the ultrastructural level. Using adjacent semithin GFAP immunostained sections of the corresponding tumors, we showed that GFAP-positive tumor cells with the light microscopical morphology of GFOC were identical to the cells examined with the transmission electron microscope.

A striking similarity was found between GFOC and minigemistocytes in that both neoplastic cell types contain large bundles of GFAP-positive intermediate filaments in their cytoplasm. The presence of cells with ultrastructural features intermediate between GFOC and minigemistocytes indicates that the two cell types are closely related. In a retrospective analysis of the distribution of GFOC, minigemistocytes and large classic gemistocytes in oligodendroglomas, with special reference to prognostic implications of these cells, GFOC were seen in combination with classic gemistocytes only if minigemistocytes were present (47). Therefore, a transformation of GFOC into classic gemistocytes via the minigemistocytic phenotype was suggested. The total number of GFAP-positive cells increased with the degree of malignancy of the tumor, and the presence of classic gemistocytes was correlated with significantly shorter survival rates, whereas the presence of transitional cell types viz. GFOC and minigemistocytes did not influence the survival times (47). These clinicopathologic observations are supportive for the hypothesis of close kinship between the GFOC and the minigemistocytes. Furthermore, an eventual transformation of a minigemistocyte into a classic gemistocyte cannot be excluded. The association between classic gemistocytes and short survival might be explained by the appearance of classic gemistocytes in a later developmental phase of the neoplasm.

No morphological counterpart for the gemistocytic cell has been described during normal development of humans or animals. In developing brain tissue, oligo- and astroglial cells are believed to derive from a common precursor cell. In tissue cultures of developing glial cells derived from the optic nerve of the fetal rat and the human fetus, it was found that a common glial precursor cell may transform into either an astrocyte or an oligodendrocyte depending on the presence of fetal calf serum in the culture medium (48, 49). The dual character of developing glial cells was illustrated by the finding of astrocytic features of glycogen particles and glial filaments

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Fig. 5. A) Semithin (1 μm) section of a mixed oligo-astrocytoma. The oligodendroglial cells are free of immunostain. The interspersed glial processes, however, react strongly for anti-GFAP. A cell with the morphological features of an astrocyte with intense GFAP-positivity is framed. (Patient 6, GFAP-immunostained gold-silver enhanced, hematoxylin and eosin counterstained, ×100). B) Ultrathin adjacent section. A neoplastic astrocitic cell and neighboring oligodendroglial cell are seen (from frame, Fig. 5A). While the cytoplasm of the oligodendroglial cell lacks filaments, the cell processes of the astrocytic cell are densely filled with bundles of filaments. (Patient 6, immunoelectron microscopy stained for GFAP, ×1,100). C) Detail of GFAP-positive astrocytic cell from Figure 5A and B (from frame, Fig. 5B). The filaments are densely labeled by the gold particles. (Patient 6, immunoelectron microscopy stained for GFAP, ×12,000).
in cells with the light microscopical morphology of oligodendrocytes in the human spinal cord and in cell cultures of the mouse brain (50–53). In cell cultures derived from adult human white matter obtained at autopsy, a large increment of the GFAP-positive cell population was found along with a significant number of cells expressing both the oligodendroglial surface marker galactocerebroside (GC) as well as GFAP (54). Because none of these cells incorporated radiolabeled thymidine, a direct metamorphosis of an oligodendrocytic cell into a cell with phenotypical properties of an astrocyte was suggested. The transformation was promoted by the addition of di-butyryl cyclic AMP, which provides additional evidence for the interconvertibility of developing astrocytes and oligodendrocytes (54). These in vitro studies illustrate the potential for developing glial cells to express astrocytic and oligodendroglial markers simultaneously.

Recently, the glial precursor surface marker A2B5 was selectively found in oligodendroglomas, but not in astrocytomas (55, 56). Since the oligodendroglial lineage of astrocytic tumor cells in some mixed oligo-astrocytomas was suggested by immunostainability for A2B5 (55), A2B5 cannot be used as a specific oligodendroglial tumor cell marker (10, 55, 57). Nevertheless, the immunohistochemical studies provide some circumstantial evidence for conversion potency of oligodendroglial into astrocytic lineage under neoplastic circumstances. The results of the present study suggest that in transitional tumors oligodendrocytes form a separate lineage together with the GFOC and minigemistocytes. In keeping with data from the literature, this lineage might be a reflection of a conversion of neoplastic oligodendroglial to astrocytic lineage.

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