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CLINICAL STUDY

"Glia-like" cells of fibroblast morphology are present in cultures from injured human brain tissue

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ABSTRACT
OBJECTIVE: Astrocytes undergo morphological and molecular changes in response to numerous pathological conditions.
BACKROUND: Increased expression of glial fibrillary acidic protein (GFAP) has been reported as a characteristic feature of reactive astrocytes. However, GFAP-positive cells occur rarely in adult human brain cultures. These cultures are mostly composed of flat GFAP-negative "glia-like" cells, which remain poorly characterized in relation to reactive astrogliosis.
METHODS: We examined the cultures from macroscopically injured and normal brain tissue from patients with brain trauma, gliomas, or brain metastases. Immunofluorescence and immunohistochemical methods were used for reactive astrocytes detection.
RESULTS: The intensity of GFAP-positive staining was higher in reactive astrocytes in the brain tissue surrounding gliomas or metastases and lower in brain tissue damaged by traumatic injury. We did not observe any correlation between GFAP-positive reactive astrocytes in cultures and brain tissue. However, we found rapidly proliferating spindle-shaped cells in cultures prepared from injured brain tissue.
CONCLUSION: Present data demonstrate the unexplained phenomenon of disparate cell morphologies in cultures when prepared either from macroscopically normal or injured human brain tissue. While normal cultures are mainly comprised of flat cells, the cultures from severely damaged brain tissue may be entirely composed of spindle-shaped cells usually classified as fibroblasts. We suggest that this spindle-shaped cellular morphology is not specific for fibroblasts, but it rather can be interpreted as the most favorable shape for rapid cell proliferation under culture conditions. After brain trauma, unknown processes may be triggered, such as induced cell proliferation which can be revealed under culture condition. Accordingly, we conclude that spindle-shaped cells are activated precursors of glial cells *(Fig. 3, Ref. 15)*. Text in PDF www.elis.sk
KEY WORDS: reactive astrocytes, human glia, GFAP, vimentin, fibronectin.

Introduction

Astrocytes undergo morphological and molecular changes in response to numerous pathological situations such as trauma, ischemia, neurodegenerative disorders, or aging. In tissue, astrocytes surrounding the brain or spinal lesions are classified as reactive astrocytes and the condition as reactive astrogliosis (1–5). The first observation of reactive astrocytes was demonstrated in 1856 by Virchow, who described highly fibrillar scarring around necrotic spinal lesions (6). The astroglial fibrillary cytoplasmic structure is created mainly by GFAP, which was initially isolated from a glial scar of patients with multiple sclerosis (7, 8). Although the astroglial marker protein was first isolated from human CNS, most studies on reactive astrogliosis were performed on experimental animals rather than on human reactive astrocytes in culture. This may be attributable to the fact that adult human brain tissue cultures are of non-glial origin (9, 10). They are often classified as "glia-like" cells, remaining negatively stained for glial markers (9, 11, 12).

In this study, we examined the differences between the cultures from patients with brain trauma, i.e., with macroscopically injured otherwise normal brain tissue, compared to those from patients with gliomas or brain metastases. The comparative analysis was aimed at examining the presence and characteristics of reactive astrocytes in brain tissue and in cultures.

Materials and methods

Tissue cultures and brain specimens

Brain samples were kindly provided by the Department of Neurosurgery, Derer's Hospital, Bratislava. This study reports the results performed on brain biopsies collected between 1989 to 2000. Experiments with human brain biopsies were performed according to Slovak laws 272/1994, 76/2004 and approved by the Ethics Committee of UNB Bratislava. For tissue cultures, we chose 20 biopsies of brain tissue from adult patients undergo-

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ing neurosurgical interventions for gliomas or brain metastases. The samples were obtained from temporal or frontal lobes. We recultivated and examined 8 cultures from brain samples from donors with non-malignant diagnoses. Tissue cultures were prepared using an explat method. Samples were cut into small pieces and seeded into uncoated plastic dishes (25 cm^2). The culture medium consisted of MEM with glutamine, nonessential amino acids, and 10% fetal calf serum. Cultures were passaged using 0.2% EDTA and 0.25% trypsin. Simultaneously, the cells used for immunostaining were grown under the same conditions on uncoated glass coverslips.

Reactive astrocytes were identified in five samples of white matter surrounding the above-mentioned brain tumors and further samples from patients with brain traumatic contusions. The brain samples were fixed in 4% neutral formalin and embedded in paraffin for immunohistochemistry.

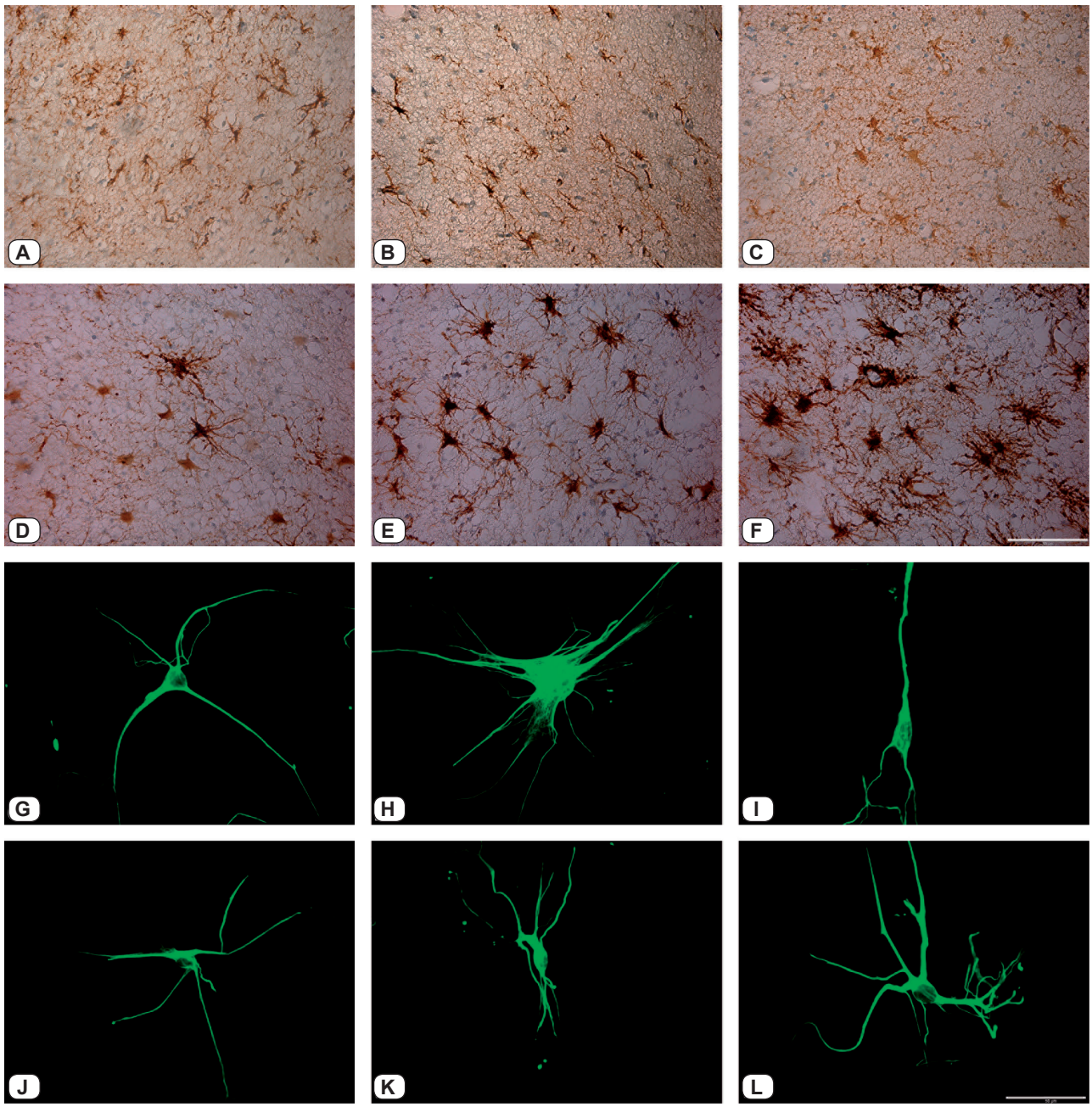

Fig. 1. Immunoperoxidase (A–F) and immunofluorescence staining for GFAP (G–L). Reactive and damaged astrocytes in the white matter positively stained for GFAP, bioptic samples from patients with brain contusion (A–C). Hypertrophic GFAP-positive astrocytes in the white matter surrounding glioma (D) and metastatic tumor (E, F). Morphological features of GFAP-positive astrocytes in cultures from brain tissue from patient with tumoral diagnoses.

Antibodies and immunofluorescence

For identifying astroglial cell types, we used antibodies against GFAP (clone GF-01, 1:100, Exbio, Prague, Czech Republic), and polyclonal sera to GFAP (1:100, Dako), antibodies to vimentin (clone V9, 1:100, Sigma), and polyclonal sera against fibronectin (1:100, Sigma). The secondary fluorescein- and rhodamine-conjugated antibodies were purchased from Sigma and Sevapharma, Prague.

Cells grown on glass uncoated coverslips were used for detecting GFAP, vimentin and fibronectin. Indirect immunofluorescence staining was performed on cells fixed in methanol-acetone solution (1:1) for 15 min and incubated at –15 °C for 1 h with primary antibodies and for 30 min with appropriate secondary antibodies diluted to the concentration of 1:50. Nuclei were stained with Hoechst 33258 fluorochrome (5 µg/ml in PBS, Sigma) for 1 min. Fluorescence microscopy was performed using an Olympus BX51 microscope (Hamburg, Germany). Formalin-fixed paraffin-embedded sections were stained for GFAP, incubated for 1 h with antibodies to GFAP and for 1h with peroxidase-conjugated anti-mouse secondary antibodies (DAKO). The diaminobenzidine reagent was used as chromogen. The sections were stained for 30 s with hematoxylin.

Results

Morphology and immunofluorescence staining of adult human brain cultures

Previously we described the morphological features of cultures prepared from brain biopsies from patients with non-tumoral diagnoses (12, 13). Briefly, at confluence, the primary cultures contained mainly flat "glia-like" cells (95 to 98%), microglial cells (2–5%), and astrocytes (0.1%). Areas of spindle-shaped cells were found in 8 of 70 cultures.

In this study, we recultured cryopreserved cultures with a higher number of spindle-shaped cells. After passage 5, only one culture (1/8) became homogeneous and entirely comprised of rapidly proliferating spindle-shaped cells arranged in parallel at confluence (Fig. 3A, B). The cells were stained positively only for vimentin and fibronectin (Fig. 3C, D). This culture was derived from severely injured hemorrhagic tissue from a patient with traumatic brain contusions.

To reveal the reactive astrocytes, we examined the cultures from 20 bioptic brain samples from patients with gliomas and brain metastases. The majority of brain samples were comprised of white matter surrounding brain tumors. Morphological features of brain cultures were observed on living cells using phase-microscopy. Cell morphologies in these cultures were similar to those derived from biopsies from patients with non-tumoral diagnoses described above. In all cultures, confluent cell layers formed over 3 to 6 weeks, composed mainly of flat cells (Fig. 2 A–C). In early passages, the cultures contained small zones or single spindle-shaped cells (Fig. 2C, E, F). Long thin process-bearing cells often overlayed the confluent layer (Fig. 2B). "Glia-like" cells were stained negatively for GFAP and positively for vimentin and fibronectin (Fig. 2D-F). Morphologic features of astrocytes were demonstrated by GFAP immunostaining. The three main cell morphology types, i.e., stellate, bipolar and large cytoplasmic cells, were found in all cultures (Fig. 1G-L). GFAP-positive cells which mimic reactive

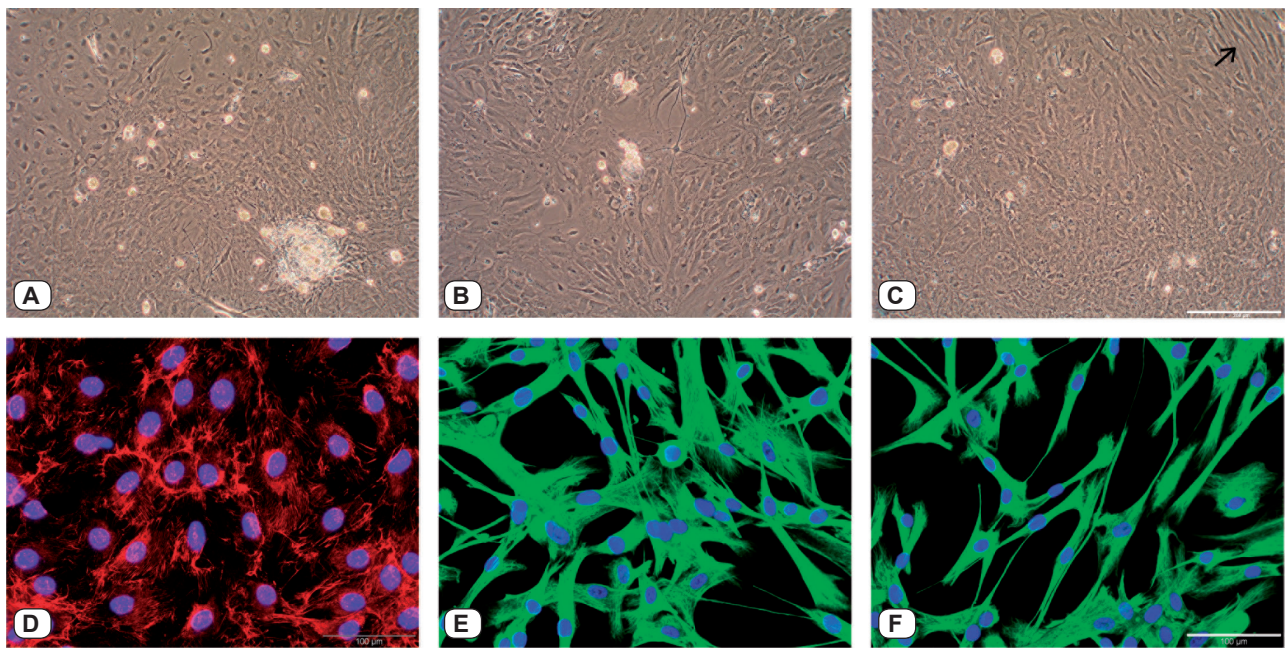

Fig. 2. Cultures derived from bioptic brain samples from patients with tumoral diagnoses. Morphology of living cells, phase-contrast microscopy (A–C), small area of spindle-shaped cells, arrow. Indirect immunofluorescence, "glia-like" cells positively stained for fibronectin (D) and vimentin (E, F).

astrocytes were not observed, other than several cells sharing a disputable morphology similar to reactive astrocytes in the brain tissue (Fig. 1L). They occurred extremely rarely and could be attributed to the persistence of reactive astrocytes under culture condition.

Immunohistochemistry of human brain tissue

To identify reactive astrocytes, we performed indirect immunoperoxidase staining using marker antibodies to GFAP. The staining was performed on brain tissue surrounding brain metastases or gliomas, and on injured brain tissue caused by brain trauma. Staining showed intensely defined reactive hypertrophic astrocytes for GFAP in the brain samples obtained from patients with brain tumors (Fig. 1D-F). In injured brain tissue, the GFAP-positive staining was less defined due to lower hypertrophy of astrocytes (Fig. 1A-C). These findings are consistent with individual patient diagnoses.

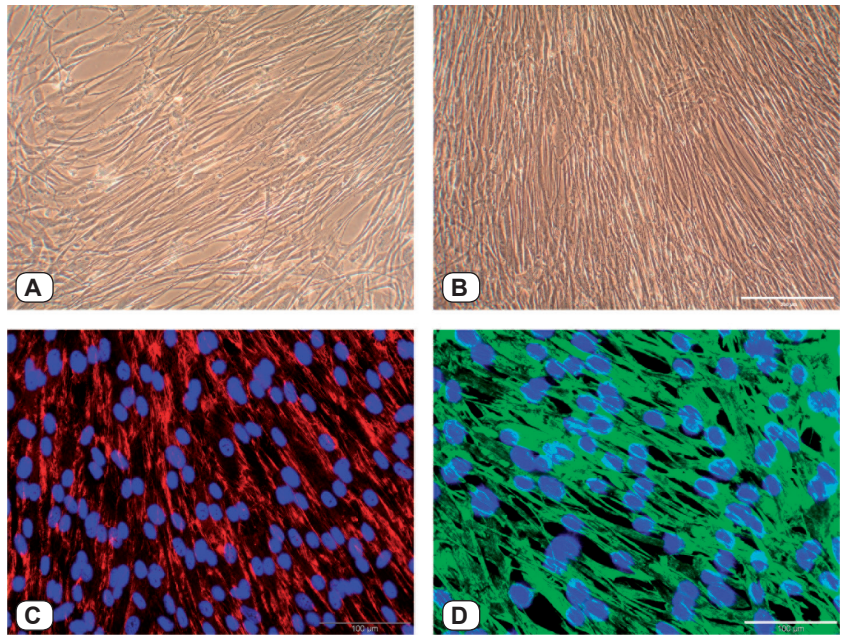

Figure 3. Morphology and immunostaining of spindle-shaped cells in cultures derived from traumatic injured brain tissue. Living cells, phase-contrast microscopy (A–B). Indirect immunofluorescence, cells positively stained for fibronectin (C) and vimentin (D).

Discussion

In this study, we tried to define the properties and fate of reactive human astrocytes under culture conditions. Brain cultures were prepared from patients with gliomas or brain metastases, mainly from white matter surrounding these tumors. We expected to find reactive astrocytes that were specific in morphology and immunostaining. However, we did not observe morphologically distinct GFAP-positive reactive astrocytes.

Previously we reported that adult human brain cultures are mostly composed of flat GFAP-negative "glia-like" cells. This was confirmed on cultures derived from 70 patients with non-tumoral diagnoses (12). In all cultures, we observed only a low percentage of stellate, bipolar or large plasmatic GFAP-positive astrocytes (0.1%). However, much more abundant areas of spindle-shaped cells growing in parallel arrays appeared in 8 of 70 cultures. In this study, we recultured these cryopreserved cultures. After passage 5, only one culture (1/8) was entirely comprised of rapidly proliferating spindle-shaped cells. This culture was prepared from macroscopically injured brain tissue due to brain contusion. Cells of a similar shape have been found in 1 of 73 cultures derived from an unspecified part of human brain and were suspected to be fibroblasts (14). We conclude that spindle-cell morphology is not specific for fibroblasts but can rather be interpreted as the most favorable shape for rapid cell proliferation under culture conditions. This may be confirmed by our previous observation, where cultures from injured brain tissue were mostly composed of spindle-shaped cells while the morphology of those derived from tissue adjacent to apparently normal brain tissue was flat. Both cultures, initially GFAP-negative, became GFAP-positive under prolonged subcultivation (15).

Immunohistochemical stains showed GFAP-positive hypertrophic astrocytes in the brain samples obtained from patients with brain tumors. In injured brain tissue, GFAP-positive staining was of lower intensity while being accompanied by various morphologically damaged astrocytes. We concluded that astroglial hypertrophy is associated with a longer period of brain noxa. On the other hand, a rapidly increasing brain pressure after trauma may trigger unknown processes such as induced cell proliferation. This may be revealed under culture conditions where rapidly proliferating spindle-shaped cells, often considered as fibroblasts, occur. We concluded that these cells are activated precursor glial cells.

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