Human von Willebrand factor (factor VIII-related antigen) in glial neoplastic cells of brain gliomas

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In order to determine whether the glial neoplastic cells within the brain gliomas show human von Willebrand factor (factor VIII-related antigen [FVIII-rA]) expression, and the possible distribution of FVIII-rA positive glial cells within the neoplasm, morphological examination and computerised morphometric analysis were conducted in 35 brain neoplasms, divided into 2 groups: glioblastoma multiforme (15 cases) and astrocytoma II (20 cases). Only in glioblastomas was the expression of FVIII-rA within the glial neoplastic cells found to be significantly higher than in background. The groups of FVIII-rA positive glial cells were usually found near the vascular glomeruloid structures. On the basis of the examination, the following conclusions were drawn: at least a part of the glial neoplastic cells within the glioblastoma multiforme may present expression of FVIII-rA; lack of FVIII-rA expression in the glial cells of astrocytomas II suggests that the activity of FVIII-rA within these cells is connected with the malignancy of gliomas; the role of FVIII-rA positive glial cells remains unknown, but the tendency to location near the vascular glomeruloid structures of glioblastomas might suggest their potential functional connection with endothelial cells.

key words: von Willebrand factor, FVIII-rA, glial cells, gliomas

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Glial fibrillary acidic protein (GFAP), as the most specific marker of astrocytes, provides the best histological diagnosis of astrocytic tumors [16]. Its evident expression in mature and reactive, as well as neoplastic, astrocytes is generally accepted. The other common markers of astroglia there are: vimentin, S-100 protein, glutamine synthetase [16]. Human von Willebrand factor (factor VIII-related antigen [FVIII-rA]) is a plasma protein engaged in haemostasis - it regulates platelet adhesion to the endothelium of the injured vessel wall and stabilises factor VIII [3]. The FVIII-rA is produced by the endothelium, platelets and megakaryocytes [4, 17]. The FVIII-rA, besides fibronectin, laminin, alpha-actin, type IV collagen and vascular endothelial growth factor, is designed for the evaluation of neovascularisation and endothelial proliferation, also within the neoplasms [2, 11, 13, 16]. In former reports there was no information on the expression of FVIII-rA in astroglia, either reactive or neoplastic [7, 12]. Recently it has been suggested that astrocytes, similarly to endothelial cells, may also express endothelin-1 [14] or endothelial specific protein [1], or secrete ADAM-17 (TACE) [6]. Furthermore, sporadic papers postulate that astrocytes might act as brain endothelial cells [5].

We decided to determine whether the glial neoplastic cells within the brain gliomas show FVIII-rA expression and the possible distribution of FVIII-rA positive glial cells within the neoplasm.

MATERIAL AND METHODS

Morphological examination and computerised morphometric analysis were conducted in 35 brain neoplasms, divided into 2 groups: glioblastoma multiforme — 15 cases (group I) and astrocytoma II — 20 cases.
(group II). The tumours were taken during routine neurosurgical procedure. The age of the patients ranged from 23 to 71 years. The histological specimens were fixed in formalin for 18–22 hours. All the specimens were 2 micrometres thick and were stained and impregnated under the same conditions. The paraffin-embedded specimens were stained with haematoxylin and eosin. The sections were also immunostained for GFAP and FVIII-rA. An image-computerised analysis was conducted by means of morphological material scanning in light microscope (Axioskop) and a two-tube colour TV camera. The illumination source was a 100 W halogen bulb. The Plan-Neofluar objective 100 × (1.3 oil) was used for scanning and measurements. Each scanned image comprised 0.0048 mm² for magnification at 100 ×. Ten to fifteen images were scanned from each neoplasm. The images were analysed with the KONTRON KS-100 v.2.0 imaging system (license No. 0100176). For densitometric analysis, the images were defined on grey (brightness) scale. A grey value of 0 represented black (maximal density), a grey value of 255 represented white (minimal density). The mean (MEAND) GFAP and FVIII-rA density within the neoplastic cells was analysed. The densitometric parameters of GFAP were referred to background while FVIII-rA to background and to endothelial expression of FVIII-rA (control measurements). The distribution of densitometric values in scanned cells and in the background along the x coordinate was also examined by means of the function ‘PROFILE’. The PROFILE curve consists of three true colour components: red, green, blue (RGB). Each colour component is also described by means of corresponding numerical value (0–255), measured for every pixel between the first and the last point of the PROFILE. The PROFILE procedure allows us to estimate the density of the measured area to be performed (the more elevated and separated colour components indicated a lesser density of the area being measured).

Statistical differences between numerical values of the grey scale were determined by means of Wilcoxon, Kruskal-Wallis and U Mann-Whitney tests. The level of significance was taken as p < 0.01 and α = 0.05.

RESULTS

Among the examined neoplasms, only in glioblastomas was the expression of FVIII-rA within the glial neoplastic cells found to be significantly higher than in background (Fig. 1 — the lower the bar, the greater the expression; p < 0.05). In astrocytomas II the expression was observed as well, much less evidently however, and was only slightly higher in comparison with unspecific background reaction. Conversely, the expression of GFAP within the glioblastomas was lower than in astrocytomas II (Fig. 2). Also, in giant cells of glioblastomas, GFAP expression appeared to be less evident, while FVIII-rA positive reaction was observed in part of them. The groups of FVIII-rA positive glial cells were usually found near the vascular glomeruloid structures (Fig. 3).

<table>
<thead>
<tr>
<th></th>
<th>GBM</th>
<th>Astrocytomas II</th>
<th>Background</th>
<th>Endothelial.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII-rA</td>
<td>mean = 247.2, SD ± 5.4</td>
<td>mean = 179.3, SD ± 10.4</td>
<td>mean = 231.4, SD ± 15.2</td>
<td>mean = 157.8, SD ± 20.1</td>
</tr>
<tr>
<td>GFAP</td>
<td>mean = 177.7, SD ± 15.1</td>
<td>mean = 145.4, SD ± 17.5</td>
<td>mean = 194.0, SD ± 12.9</td>
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</table>

**Figure 1.** FVIII-rA and GFAP expression within the glial neoplastic cells of glioblastoma multiforme (GBM) and astrocytomas II (Astroc. II).
FVIII-rA expression within the glial cells was always less evident and more homogeneous than in the endothelial cells of vascular walls. In the latter, the immunoreaction was usually granular, which was particularly well visible in densitometric PROFILE (Fig. 4). In the glial cells, FVIII-rA expression was never granular.

**DISCUSSION**

It is assumed that both reactive and neoplastic glia cells do not reveal positive FVIII-rA reaction [9, 10, 12]. Our observations, supported by computerised morphometric analysis, suggest that the glial neoplastic cells might present FVIII-rA expression. This concerns the glial cells of glioblastoma multiforme, however it was not observed in astrocytic cells of astrocytoma II. It seems to us that FVIII-rA positive reaction in glial neoplastic cells, including some giant cells of glioblastoma multiforme, might result from their poor differentiation. In mature astrocytes, forming tumors of II degree, there was no evidence of FVIII-rA expression. Within the cells demonstrating evident GFAP reaction, the expression of FVIII-rA was lower.

The principal question is whether the cells which we regarded as FVIII-rA positive glial cells are really of glial origin. These cells demonstrated co-expression of GFAP and FVIII-rA and abounded with cytoplasm, with tendency to dendritic processes formation. Certainly, the expression of FVIII-rA within the glial cells was not as evident as in the endothelium. Moreover, the glial expression of FVIII-rA is homogeneous, while in the endothelium it is granular. It should be stressed that only areas with many co-expressed cells were taken into consideration. Single cells, suspected to be an accidental find, were not analysed.

The next question is what would be the role of FVIII-rA positive glial cells in glioblastoma multiforme. The FVIII-rA plays a pivotal role in both haemostasis and pathological intravascular thrombosis, and especially contributes to both platelet adhesion/aggregation and blood coagulation through its multiple adhesive functions for the platelet membrane receptors [17]. At in vivo rheological situations, where platelets are flowing with high speed in the bloodstream, the only reaction that can initiate mural thrombogenesis is the interaction of FVIII-rA with platelet glycoprotein 1b-alpha [17]. Recently, it has been proved that there are many proteins synthesised by both endothelial and glial cells, for instance tissue factor pathway inhibitor, endothelin-1 — which induces vasoconstriction, vascular endothelial growth factor [1, 14, 15]. It seems that extensive necrotic areas, characteristic for glioblastomas, may not only result from discrepancies between ineffective development of neovascularisation and neoplasm growing. Maybe in glioblastoma multiforme the glial neoplastic cells, demonstrating FVIII-rA expression, might aid the endothelial cells in their pro-coagulative activity and also result in the formation of neoplastic necrosis.

The above-mentioned new concepts on glial cell function and our suggestion seem to indicate that the biological role of FVIII-rA and the connection between the glia and endothelium in glioblastomas is more complex than was previously thought [3, 8].

**CONCLUSIONS**

1. At least a part of glial neoplastic cells within the glioblastoma multiforme may present expression of FVIII-rA.

2. Lack of FVIII-rA expression in glial cells of astrocytomas II suggests that the activity of FVIII-rA within these cells is connected with the malignancy of gliomas.

3. The role of FVIII-rA positive glial cells remains unknown, but the tendency to location near the vascular glomeruloid structures of glioblastomas might suggest their potential functional connection with endothelial cells.
Fig. 4. Distribution of densitometric values along x coordinate (densitometric PROFILE) of GFAP and FVIII-rA expression within the glial neoplastic cells of glioblastoma multiforme.

<table>
<thead>
<tr>
<th>Figure</th>
<th>R</th>
<th>G</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>mean = 238.4, SD ± 6.9</td>
<td>mean = 148.4, SD ± 29.2</td>
<td>mean = 144.2 SD ± 18.4</td>
</tr>
<tr>
<td>4b</td>
<td>mean = 247.2, SD ± 5.4</td>
<td>mean = 195.3, SD ± 11.2</td>
<td>mean = 163.3 SD ± 9.7</td>
</tr>
<tr>
<td>4c</td>
<td>mean = 232.5, SD ± 6.9</td>
<td>mean = 183.9, SD ± 10.6</td>
<td>mean = 144.3 SD ± 9.4</td>
</tr>
<tr>
<td>4d</td>
<td>mean = 243.3, SD ± 5.6</td>
<td>mean = 192.5, SD ± 9.9</td>
<td>mean = 154.2 SD ± 12.1</td>
</tr>
<tr>
<td>4e</td>
<td>mean = 231.4, SD ± 15.2</td>
<td>mean = 157.8, SD ± 20.1</td>
<td>mean = 146.7 SD ± 17.5</td>
</tr>
<tr>
<td>4f</td>
<td>mean = 254.2, SD ± 6.7</td>
<td>mean = 232.4, SD ± 12.5</td>
<td>mean = 203.3 SD ± 10.9</td>
</tr>
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</table>

*R — red, G — green, B — blue

**Differences between densitometric values shown on fig. d, e, and c, f statistically significant (p < 0.01)
REFERENCES


