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**RESEARCH ARTICLE**

**WT1 Expression Distinguishes Astrocytic Tumor Cells from Normal and Reactive Astrocytes**

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**Keywords**
astrocytoma; astrogliosis; neoplastic astrocytes; normal brain; WT1.

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**Abstract**

Particularly in small brain biopsies, it might be difficult to distinguish reactive astrogliosis from low-grade or infiltration zones of high-grade astrocytomas. So far no immunohistochemical marker allows a reliable distinction. Recently, the over-expression of Wilms’ tumor gene product WT1 was reported in astrocytic tumor cells. However, no sufficient data on WT1 expression in normal or reactive astrocytes are available. Therefore, we investigated WT1 expression in paraffin-embedded brain sections from 28 controls, 48 cases with astrogliosis of various etiologies and 219 astrocytomas [World Health Organization (WHO) grades I–IV] by immunohistochemistry. In normal brains and in astrogliosis, expression of WT1 was restricted to endothelial cells. In astrocytomas, WT1-positive tumor cells were found in pilocytic astrocytomas (66.7% of cases), diffuse astrocytomas (52.7%) WHO grade II (52.7%), anaplastic astrocytomas (83.4%) and glioblastomas (98.1%). Overall, the majority of all astrocytic neoplasms (84.5%) expressed WT1. Establishing a cut-off value of 0% immunoreactive tumor cells served to recognize neoplastic astrocytes with 100% specificity and 68% sensitivity and was associated with positive and negative predictive values of 1 and 0.68, respectively. Therefore, WT1 expression in astrocytes indicates a neoplastic origin and might represent an important diagnostic tool to differentiate reactive from neoplastic astrocytes by immunohistochemistry.

**INTRODUCTION**

In the central nervous system (CNS), morphological features of astrocytic neoplasms overlap with reactive astrogliosis occurring within or around brain lesions of various etiologies. Especially, the distinction between astrogliosis and low-grade astrocytomas or infiltration zones of high-grade astrocytomas may be difficult (9). In the normal human brain, astrocytes are associated with structural support, metabolic homeostasis and energy supply (14, 45, 49). They produce adhesion molecules, neurotrophic factors and extracellular matrix substances that are essential for structural integrity and repair (11). As a reaction to pathological changes they increase in number and size and secrete additional extracellular matrix components (1, 38) through inhibition of membrane-bound Na⁺/K⁺-ATPase (5) or activation of epidermal growth factor receptor (EGFR), a transmembrane receptor with tyrosine kinase activity (30). Reactive (non-neoplastic) astrocytes may become very large and may even display pleomorphic nuclei resembling neoplastic tumor cells (9). These morphologic features are especially observed in progressive multifocal leucoencephalopathy (PML) (13). Therefore, in small biopsy specimens, this may result in a diagnostic dilemma leading to inconclusive diagnosis (15) and might be the main reason for inter-observer variability (42). Especially, if both atypical astrocytes and mitotic figures are present in reactive lesions, an erroneous diagnosis of an astrocytic neoplasm might occur (56). So far, no tool is available to reliably prove the neoplastic nature of astrocytes in routine pathologic diagnosis. Upregulated expression of glial fibrillary acidic protein (GFAP) (11), vimentin (52) and N-cadherin (44) are the hallmarks of astrogliosis and may also be observed in distant areas of the lesion because of gap junction signaling (16). Under physiological conditions, the level of GFAP expression in the CNS is low. The increase of GFAP in reactive astrocytes makes GFAP an excellent immunohistochemical marker for labeling these cells (12). But its diagnostic usefulness in differentiating reactive and neoplastic astrocytes is very limited as GFAP is also commonly observed in astrocytomas (4, 55). Previous studies have examined EGFR controlling cellular functions in various gliomas (43), EGFR is not detected in normal astrocytes; however, reappearance occurs in reactive astrocytes under various pathophysiological conditions (21, 41). Other authors suggested that immunohistochemical presence of p53 protein might detect neoplastic transformation in glial tissue. However, it became evident that p53 is also expressed in a subset of reactive astrocytes (26, 54). The cell-surface receptor for hyaluronic acid, CD44 was considered to be significantly up-regulated in astrocytic neoplasms compared with normal brain (25). Later it became evident that normal brain and neoplastic tissues share the same splicing variant (35). In addition we were...
able to demonstrate in a previous study that CD44 up-regulation also occurs in reactive astrocytes at the border of infiltrative neoplasms of non-glial origin (57). Therefore, a reliable method to distinguish neoplastic from normal or reactive astrocytes suitable for routine diagnosis has not yet been established.

The Wilms' tumor supressor gene encodes for a zinc-finger transcription factor (WT1) and is associated with genitourinary malformations due to reduced protein levels in human development (8). Allelic losses or mutations of the gene result in nephroblastoma, a common pediatric solid malignancy of the kidney (20). The WT1 protein is involved in proliferation, differentiation and apoptosis (22). Interaction with other transcription factors like EGR-1 and p53 shows that WT1 has oncogenic properties (31). Recent reports indicate that WT1 is expressed in glioblastoma cell lines (32) and in astrocytic tumor tissue (39) among other types of solid cancer. In addition, it has been shown that WT1 might be useful to differentiate between proliferating endothelial lesions and vascular malformations (28). In order to characterize the astrocytic WT1 expression pattern and its diagnostic usefulness, we investigated normal, reactive and neoplastic human CNS tissue specimens by immunohistochemistry.

**MATERIALS AND METHODS**

**Patient data**

In total, 295 cases from the tissue archives of the Institute of Brain Research (Neuropathology), University Hospital of Tuebingen were enrolled in this study, including normal brains (28 cases) and reactive CNS tissues (48 cases). The reactive CNS tissue samples were derived from different pathological conditions such as vascular malformations, brain metastasis, infiltrating meningiomas and ischemia (details on cases are shown in Table 1). Furthermore, astrocytic neoplasms (219 samples) consisting of World Health Organization (WHO) grade I pilocytic astrocytomas (n = 24), WHO grade II astrocytomas (n = 56), WHO grade III anaplastic astrocytomas (n = 54) as well as WHO grade IV glioblastomas (n = 105) were included in the present study. These patients underwent surgical treatment between 1995 and 2005 (epidemiologic details are shown in Table 2). Histological diagnosis and grading were performed according to the WHO classification system by at least two experienced neuropathologists (10).

**Immunohistochemistry**

After surgical removal, all specimens were fixed in 4% formalin (pH 7.4) and embedded in paraffin. Immunohistochemistry was performed using the established monoclonal WT1-antibody, clone 6F-H2 (dilution 1:50, Dako, Glostrup, Denmark). The antibody reacts with all isoforms of the full-length WT1 protein. In addition a polyclonal GFAP antibody (Z0334, dilution 1:1000, Dako) was used. Sections were deparaffinized, rehydrated and immunostained using the Benchmark immunohistochemistry system (Ventana Medical Systems, Strasbourg, France). The automated standard protocol is based on an indirect biotin-avidin system and consists of a cell conditioning pretreatment, use of I-View inhibitor (Ventana) for 4 minutes, application of the WT1 antibody for 20 minutes or GFAP-antibody for 6 minutes followed by an incubation of amplifier Systems A and B (Ventana), avidin-botin blocking for 4 minutes and one drop of I-View Biotin Ig (Ventana). For visualization the sections were incubated with one drop of I-View SA-HRP for 8 minutes and then with DAB/H2O2 for additional 8 minutes. The sections were finally incubated with a copper enhancer (Ventana) for 4 minutes and counterstained with hematoxylin. Wilms’ tumor tissue specimens were used as positive control, displaying a distinct nuclear staining pattern of tumor and endothelial cells. Negative control slides were processed in parallel with each batch of staining. To exclude unspecific binding of the monoclonal antibody, IgG isotype controls using the same IgG-concentrations as in the replaced primary antibody solutions were performed (IgG1, Dako).

**Table 1.** Overview of gliosis and normal brain samples.

<table>
<thead>
<tr>
<th>CNS tissue samples with reactive astrogliosis</th>
<th>No. of cases (n = 76)</th>
<th>Male/female</th>
<th>Mean age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavernous hemangioma</td>
<td>12</td>
<td>5/7</td>
<td>37</td>
</tr>
<tr>
<td>Arteriovenous-Malformation</td>
<td>2</td>
<td>0/2</td>
<td>42</td>
</tr>
<tr>
<td>Carcinoma, metastatic</td>
<td>15</td>
<td>10/5</td>
<td>67.6</td>
</tr>
<tr>
<td>Meningioma, brain-invasive</td>
<td>6</td>
<td>4/2</td>
<td>60.0</td>
</tr>
<tr>
<td>Melanoma</td>
<td>7</td>
<td>3/4</td>
<td>62.1</td>
</tr>
<tr>
<td>Ischemia</td>
<td>4</td>
<td>1/3</td>
<td>65.7</td>
</tr>
<tr>
<td>Progressive multifocal leuencephalopathy</td>
<td>2</td>
<td>2/0</td>
<td>55.0</td>
</tr>
<tr>
<td>Normal human brain</td>
<td>28</td>
<td>15/13</td>
<td>55.4</td>
</tr>
</tbody>
</table>

**Table 2.** Overview of tumor samples.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>WHO grade</th>
<th>No. of cases (n = 219)</th>
<th>Male/female</th>
<th>Mean age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilocytic astrocytoma</td>
<td>I</td>
<td>24</td>
<td>14/10</td>
<td>21.8</td>
</tr>
<tr>
<td>Diffuse astrocytoma</td>
<td>II</td>
<td>36</td>
<td>24/12</td>
<td>43.9</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>III</td>
<td>54</td>
<td>36/18</td>
<td>56.1</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>IV</td>
<td>105</td>
<td>63/42</td>
<td>58.3</td>
</tr>
</tbody>
</table>
Evaluation and statistics

Only samples showing WT1 expression in endothelial cells serving as internal control were scored (28). Tumor cells were considered positive, when cytoplasmic staining of cells matched at least the intensity observed in endothelial cells (47). The scores of WT1+ tumor cells of the whole neoplastic specimen available was estimated independently by three raters (J.S., M.M., R.B.) using a semiquantitative score including 0 (no staining), 1 (singular positive cells <1%), 2 (2–19%), 3 (20–50%) and 4 (>50%) allowing fast and easy scoring of the tissue. Only if two or more scores were identical for the same case, this score was taken into account. Ordinal logistic regression was performed for each comparison followed by a likelihood ratio chi-squared test. To test inter-observer’ variability of the three experienced neuropathologists in the WT1 assessment, a weighted kappa statistic was calculated. A summary kappa was calculated both for each pairwise combination and for all raters as a group. Kappa values of 0–0.20 have been defined as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial and 0.81–1.0 as almost perfect (27). JMP 7.0 was used for statistical analysis (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.0001).

RESULTS

The applied WT1 scoring method is an easy and reproducible method

Inter-observer variability for all cases examined was at most one score level. The agreement about the WT1 scores between the three raters taken as a group was substantial (κ = 0.683). Furthermore, all individual pairwise comparisons showed substantial agreement ranging for κ = 0.613 (SD = 0.033) to κ = 0.758 (SD = 0.029). Agreement across categories was almost perfect (κ = 0.9560) for WT1 score 0 (no WT1 expression), which is the most relevant factor for the diagnosis of positive and negative cases.

WT1 expression in normal brain is restricted to endothelial cells

In all 28 normal human brains, expression of WT1 was limited to endothelial cells of small blood vessels, both in gray and in white matter (internal control). No astrocytes, oligodendroglia, ependymal or neuronal cells showed WT1 immunoreactivity (score 0; Figure 1A,B). Weak cytoplasmic staining of the epithelial choroid plexus lining was considered nonspecific as it did not match endothelial staining of capillaries and small vessels of CNS and leptomeninges (not shown). Regions examined included samples from all four cerebral lobes, cerebellum, diencephalon and brainstem. In Wilms’ tumor (positive control), a strong nuclear expression of WT1 was observed in tumor cells and in endothelial cells (Figure 1C).

Reactive astrocytes lack WT1 expression

In all 48 brain specimens comprising reactive astrogliosis because of the various pathological conditions (Table 1), an increased number of astrocytes showed gemistocytic morphology and marked expression of GFAP (Figure 2). Reactive CNS tissue

Figure 1. WT1 immunoreactivity in normal brain and Wilms’ tumor. In normal brain specimens expression of WT1 is restricted to endothelial cells, both in gray (A) and in white matter (B). No WT1 expression is found in glial cells (A, B). In Wilms’ tumor serving as positive control the vast majority of tumor cells and endothelial cells showed strong nuclear expression of WT1 (C) (scale bar: 100 µm).
around cavernomas (Figure 2A) and arteriovenous malformations displayed strong GFAP expression in reactive astrocytes close to the pathologic vessels (Figure 2B). The same areas remained negative except for endothelial vessels on consecutive sections stained with WT1 antibody (Figure 2C). Gliomastocytic astrocytes around infarct lesions (Figure 2D) showed prominent GFAP immunostaining (Figure 2E) compared with the surrounding neuropil. These all showed a lack of WT1 expression (Figure 2F). Non-primary brain tumors as invasive meningiomas, melanomas and carcinoma metastases (Figure 2G) showed reactive gliotic changes at the tumor border with strong GFAP expression, too, (Figure 2H) but remained negative for WT1 (Figure 2I). Expression of WT1 was always detectable in endothelial cells of CNS and tumors (internal control) but was never observed in astrocytes including those with gemistocytic morphology (score 0). All seven melanomas used in our study exhibited at least focally some cytoplasmic (7/7) or nuclear (2/7) WT1 staining of tumor cells (not shown). Two cases of PML with presence of JC-virus DNA confirmed by molecular genetic methods did not express WT1 including their pleomorphic astrocytes.

Expression of WT1 in astrocytomas differs significantly from normal brain and reactive gliosis

Among all 219 glioma samples, cytoplasmic WT1 immunoreactivity occurred in endothelial cells, with 185 tumors (84.5%) showing astrocytic WT1 expression as well. The distribution of WT1-positive tumor cells varied between different WHO grades (Table 3). Score variability in astrocytic tumors is illustrated in different glioblastomas (Figure 3). Numbers of WT1-positive tumor samples were higher in pilocytic astrocytomas (16/24) than in WHO grade II astrocytomas (19/36). There was a significant increase of WT1-positive tumors in anaplastic astrocytomas (45/54) with almost all glioblastomas showing WT1-positive tumor cells (103/105). Likelihood ratio chi-squared test showed that the distribution scores of astrocytic tumors combined versus reactive CNS were significantly different ($P < 0.0001$). Comparison of WT1 expression in astrogliosis versus astrocytomas also showed statistically significant differences (Table 4). Detailed $P$-values in comparison of normal brain, gliosis and astrocytomas also were summarized in Table 4. Similar analysis of gliomas and astrocytomas (WHO grades I–IV, respectively) also differed significantly ($P < 0.0001$, each). Similarly, comparison of gliosis and individual subtypes of astrocytomas (WHO grades I–IV, respectively) revealed significant results ($P \leq 0.0001$, each). The frequency distribution of different WT1 scores observed in normal brain, gliosis and astrocytomas WHO grades I–IV is illustrated in Figure 4. Using score 0 as a cut-off value, neoplastic astrocytes are recognized with 100% specificity and 68% sensitivity and are associated with positive and negative predictive values of 1 and 0.68, respectively. Using score 1 (<1% immunoreactive cells) instead for the cut-off, neoplastic astrocytes are recognized with 100% specificity and 52% sensitivity and are associated with positive and negative predictive values of 1 and 0.52, respectively.

Expression patterns of WT1 in neoplastic astrocytes

In the majority of high-grade astrocytomas, the tumor cells showed a strong cytoplasmic staining including delicate cell processes. With a few exception in the glioblastomas, astrocytomas were devoid of nuclear staining. A considerable up-regulation of WT1 immunoreactivity was observed in areas of pseudopalisading around necrosis (Figure 5A). Except for a thin internally located endothelial layer of WT1-positive cells, proliferating vessels were immunonegative for WT1 (Figure 5B). Infrequently, astrocytes in close vicinity to microvessel proliferation also showed increased WT1 staining compared with the more distant tumor areas (Figure 5B). In most instances, cells with exceptionally anaplastic morphology (large, multinucleated tumor cells) also displayed strong immunoreactivity for WT1 (Figure 5C). In anaplastic astrocytomas, there was usually a diffuse spread of WT1-positive tumor cells intermingled with WT1-negative tumor cells (Figure 5D), but

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal brain</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gliosis</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse astrocytoma</td>
<td>17</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>9</td>
<td>13</td>
<td>15</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>2</td>
<td>8</td>
<td>29</td>
<td>37</td>
<td>29</td>
</tr>
</tbody>
</table>

Table 3. Distribution scores of WT1.

Table 4. Statistical results. n.s. = not significant, values after Bonferroni-Holm correction.

<table>
<thead>
<tr>
<th>Level A</th>
<th>Level B</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliosis</td>
<td>Normal brain</td>
<td>(n.s.) all values $= 0$</td>
</tr>
<tr>
<td>Gliosis</td>
<td>WHO I</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>Gliosis</td>
<td>WHO II</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>Gliosis</td>
<td>WHO III</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>Gliosis</td>
<td>WHO IV</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>Normal brain</td>
<td>WHO I</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>Normal brain</td>
<td>WHO II</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>Normal brain</td>
<td>WHO III</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>Normal brain</td>
<td>WHO IV</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>WHO I</td>
<td>WHO II</td>
<td>0.3631 (n.s.)</td>
</tr>
<tr>
<td>WHO I</td>
<td>WHO III</td>
<td>0.04 (n.s.)</td>
</tr>
<tr>
<td>WHO I</td>
<td>WHO IV</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>WHO II</td>
<td>WHO I</td>
<td>0.001$^*$</td>
</tr>
<tr>
<td>WHO II</td>
<td>WHO III</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>WHO II</td>
<td>WHO IV</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>WHO III</td>
<td>WHO I</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>WHO III</td>
<td>WHO III</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>WHO I-IV</td>
<td>Normal brain</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>WHO I-IV</td>
<td>Gliosis</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
</tbody>
</table>

* $P \leq 0.05$, ** $P \leq 0.01$. 

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in some cases, clustering of WT1-positive cells was present resulting in WT1-positive areas next to WT1-negative areas. Similar WT1 immunoreactivity of tumor cells was present in astrocytomas WHO grade II with exception of gemistocytic astrocytomas which exhibited moderate to strong WT1 expression in most cases (Figure 5E). Further, most pilocytic astrocytomas displayed WT1+ tumor cells including the delicate piloid processes (Figure 5F).

**DISCUSSION**

Distinction between reactive astrogliosis and an astrocytic neoplasm is often difficult, especially in cases when diffuse low-grade astrocytoma or the infiltration zone of a high-grade astrocytoma is considered (9). So far, no single immunohistochemical marker can clearly distinguish these cases. In recent years, attempts to discriminate between reactive and neoplastic astrocytes have employed various molecular genetic methods, including in situ hybridization to detect chromosomal aberrations absent in non-neoplastic tissue (50). However, these methods are elaborate, expensive and cannot replace the usefulness of immunostaining.

There is a known period of transient WT1 up-regulation in ependymal cells of brain and spinal cord during embryogenesis, suggesting involvement in differentiation of glial cells (3, 46). There is no evidence of WT1 reactivation in normal brain or reactive astrogliosis so far (39). Expression of WT1 has previously been reported in astrocytomas, although the number of tumors examined was rather low (32, 39). In the present study, we were able to confirm that WT1 is indeed expressed in astrocytic neoplasms. The consistent expression of WT1 in blood vessels of the brain even in cases lacking WT1 expression in tumor cells serves as a good internal control for immunohistochemistry and has been noted previously in mesotheliomas (24). WT1 expression in the vessels may be surprising at first glance; however, recent studies were able to demonstrate that WT1 transcriptionally regulates vascular endothelial growth factor through interaction of its zinc-finger DNA binding domain and thus might not only be present in normal endothelium but also associated with angiogenesis in neoplasia (17, 18).

In our study, we examined WT1 immunoreactivity in normal brain, reactive CNS tissue with astrogliosis resulting from various underlying pathologies (eg, vascular malformations, brain metastasis, ischemia, PML) and astrocytic tumors of different WHO grades. We were able to show that normal and reactive astrocytes do not display any visible WT1 immunoreactivity. Our

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**Figure 2.** WT1 immunoreactivity in reactive astrogliosis. Thin pathological vessels of a cavernoma [A, hematoxylin and eosin (H&E)] are surrounded by reactive astrocytes with eosinophilic cytoplasm (inset). Reactive astrocytes are strongly positive for glial fibrillary acidic protein (GFAP, B), whereas WT1 expression remains restricted to endothelial cells of capillaries and cavernomatous vessels (C). Residual infarction aged 4 months shows glial scar formation with reactive astrocytes (D, H&E), which express GFAP (E). In the same region WT1 immunoreactivity also remains restricted to endothelial cells (F). Astrogliosis because of a brain metastasis of an adenocarcinoma (G, H&E). Reactive astrocytes express GFAP (H) but not WT1 (I). Note WT1 expression in tumor vessels and in brain capillaries (scale bars: A–I, 200 μm; insets, 50 μm).

**Figure 3.** Illustration of WT1 immunoreactivity scores. Representative photomicrographs illustrating WT1 immunoreactivity scores in glioblastomas. **A.** WT1 expression is limited to endothelial cells of capillaries (score 0). **B.** A few WT1-positive cell processes and single (<1%) WT1+ tumor cells (arrows) are present (score 1). Glioblastomas showing WT1 expression in 1–19% (C, score 2), in 20 to 50% (D, score 3) or in more than 50% of tumor cells (E, score 4). Isotype control demonstrating specificity of the primary WT1 antibody (F) (scale bar: 100 μm).
Figure 4. Distribution of different WT1 scores in neoplastic glial cells. Overall illustration of the frequency of different WT1 scores in astrocytomas grades I–IV WHO. Note absence of WT1 expression in normal brain and astrogliosis (all cases score 0, not shown). In astrocytomas of each WHO grade, all WT1 scores are present in variable frequency, respectively.

Figure 5. WT1 immunoreactivity in astrocytomas. Immunostaining for WT1 in astrocytic neoplasms. A. Glioblastoma (grade IV WHO) with areas of pseudopalisading necrosis (score 4); B. Glioblastoma with WT1 enhancement around pathological tumor vessels (score 3); C. Another glioblastoma with WT1 expression predominating in pleomorphic tumor cells (score 3); D. Anaplastic astrocytoma, grade III WHO (score 3); E. Gemistocytic astrocytoma grade II WHO (score 2); and F. Pilocytic astrocytoma grade I WHO (score 3) show cytoplasmic WT1 in varying number of positive cells (scale bars: A–F, 100 μm).
observations on ischemic CNS lesions correlate with previous observations of Wagner and colleagues, describing WT1 up-regulation in kidney and heart but not in brain of rats kept under hypoxic conditions (48).

In contrast to normal and reactive astrocytes, neoplastic astrocytes frequently express WT1. In our series of 219 astrocytomas WT1+ tumor cells were found in pilocytic astrocytomas (66.7% of cases examined), WHO grade II astrocytomas (52.8%), WHO grade III astrocytomas (83.4%) and WHO grade IV glioblastomas (98.1%). Overall, the majority of all astrocytic tumors (84.5%) expressed WT1 at least focally. Thus, WT1 is significantly up-regulated in astrocytomas of any WHO grade when compared with normal brain or astrogliosis. Using score 0 (lack of WT1-positive tumor cells) as a cut-off, astrocytic neoplasms can be diagnosed with 100% specificity and 68% sensitivity. In cases where WT1 expression is present on astrocytic cells, the diagnosis clearly points to an astrocytic neoplasm as there is an excellent positive predictive value of 1. In cases lacking WT1 immunostaining differentiation from reactive gliosis remains difficult with a negative predictive value of 0.68. We have employed WT1 in our routine diagnostic panel for glial neoplasms and reactive gliosis for several months as well during the review process and have not experienced a single false positive case so far. However, we are very well aware that there will be always an exception that proves the rule and expect someone to report this. It is important to remember that WT1 is known to be expressed not only in endothelial cells but also in fibroblasts (37). The presence of WT1 in brain tissue, therefore, should not prompt a straightforward diagnosis of an astrocytoma. Rather to rule out the presence of a glial scar on the one side and mesenchymal or desmoplastic reaction on the other side, the pathologist should apply appropriate additional stainings when in doubt. Moreover, WT1 should not be regarded as an astrocytoma-specific marker as its expression in other glial neoplasms (ependymomas, oligodendrogliomas, etc.) has been demonstrated recently (19). Our findings in astrocytic tumors are in accordance with a previous report by Oji et al who found expression of WT1 protein in 18/18 high-grade gliomas and in 5/6 low-grade gliomas examined (39). We have limited the current immunohistochemical study to astrocytic neoplasms as low-grade astrocytic neoplasms are most prone to misdiagnoses compared with other glial neoplasms. In the past months, WT1 staining helped to clarify several challenging cases in our institute. Some of these cases are illustrated in Figure 6.
It is also noteworthy that the WT1 antibody can be applied to frozen tissue as well.

It has previously been suggested by some authors that cytoplasmic staining represents cross-reactivity with an epitope unrelated to WT1 (24). However, recent studies were able to demonstrate presence of WT1 in the cytoplasm via western blot (36) and reverse transcriptase-polymerase chain reaction (32). In addition, immunohistochemical staining of WT1 in the cytoplasm of several types of cancer has been demonstrated (36). This suggests that strong over-expression leads to an inactive state of the WT1 transcription activity, and after phosphorylation, it shuttles back into the nucleus, where it is able to bind DNA (37). In addition, recent identification of an immunogenic T-cell binding epitope within WT1 suggests a specific tumor-antigen directed immunological approach in therapy (6).

Tumor-restricted antigens that are not expressed in normal and/or reactive astrocytes include the intracellular vimentin-binding 300-kDa intermediate filament associated protein (29). Its expression in radial glia of the embryonic CNS is terminated post-natally and re-expression occurs only during tumorigenesis as demonstrated in seven high-grade gliomas and cultured cells (53). To date, however, no information on expression in low-grade astrocytomas or in reactive astrocytes has been provided. Integrin alpha3, a 140-kDa protein is reported to be present in malignant gliomas but does not show antibody reaction within the normal brain (23). As a cell membrane adhesion molecule, its expression might be a potential target for immunotherapy, yet its role in reactive astrocytes still needs to be elucidated. One of the best evaluated proteins is the mutated isoform of the EGFRvIII. Considered as a marker for malignancy it is expressed in approximately half of all gliomas examined (2, 51); however, immunohistochemical results on paraffin-embedded specimens remain poor (7). Another interesting marker is the tumor-specific murine antibody 8H9 directed against a 58-kDa surface antigen expressed among neuroectodermal, mesenchymal and epithelial tumors, which has been reported in 15/17 frozen glioblastoma and 6/8 frozen astrocytoma samples and does not react with normal human brain (33). Recombinant immunotoxins raised against this epitope are currently tested against various types of cancer (40). To date, no information for 8H9 on paraffin-embedded specimens exists. The common acute lymphoblastic antigen (cALLA, CD10), also known as neutral endopeptidase was suggested not only as a marker of malignant progression but also considered lacking expression in reactive astrocytes in frozen tissue sections (34). Although showing promising results, routine use of the previously mentioned antibodies is limited because the staining of paraffin-embedded specimens is the method of choice in diagnostic pathology.

In conclusion, WT1 immunohistochemistry is a valid and promising diagnostic tool to distinguish neoplastic astrocytes from normal or reactive astrocytes in routinely paraffin-embedded tissue. We have also shown that our scoring method shows substantial agreement and therefore is a valid and reproducible tool for quick assessment of WT1 expression.

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REFERENCES


