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Immunohistochemical Expression of PTTG in Brain Tumors

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Abstract. *Background: Pituitary tumor-transforming gene (PTTG1) has been implicated in several oncogenic processes. The aim of this study was to determine PTTG expression in brain tumors. Materials and Methods: We investigated 88 benign and malignant brain tumors. PTTG immunoexpression was evaluated using a scale of 0 to 3. PTTG immunoexpression was nuclear and cytoplasmic in most tumors, except for medulloblastomas and hemangiopericytomas. Expression was highest in medulloblastomas. Higher grade gliomas including glioblastoma multiforme (GBM) IV and astrocytoma III had the highest level of PTTG expression, whereas low-grade gliomas had the lowest levels of PTTG expression. Hemangiopericytomas had the lowest levels of PTTG immunoreactivity, with meningiomas and schwannomas exhibiting similarly low PTTG levels. Nuclear PTTG immunoreactivity was higher than cytoplasmic in higher-grade tumors. Conclusion: Our results indicate that PTTG immunoexpression is higher in aggressive brain tumors including medulloblastomas, GBM IV, and astrocytoma III, whereas in more benign tumors, PTTG immunoexpression is lower.*

Pituitary tumor-transforming gene (PTTG), first isolated from GH4 rat pituitary tumor cells, is a cell-cycle protein that prevents premature sister chromatid separation during cell-cycle progression (1, 2). PTTG is abundantly expressed in all tumors studied to date (3-10). Injection of PTTG-transfected cells into athymic nude mice induces neoplastic transformation (1). PTTG is implicated in several tumorigenic mechanisms, including genetic instability, DNA repair mechanisms, angiogenesis and p53-dependent apoptosis (11). Several studies have shown PTTG to be a valuable prognostic indicator with respect to patient tumor recurrence, invasiveness, metastatic potential and patient survival (6, 12). With respect to patients with glioma, one study has shown PTTG expression to be correlated with poor clinical outcome and shorter survival times, highlighting the need for further investigation of PTTG expression in brain tumors (6). Malignant gliomas account for up to 70% of newly-diagnosed malignant primary brain tumors (13). Many are associated with a poor prognosis, with median survival of only 12 to 15 months seen in patients with glioblastoma. A deeper understanding of the mechanisms underlying tumorigenesis will aid in the development of new treatments and the identification of clinically useful biomarkers. The immunohistochemical expression of PTTG in other intra- and extra-axial tumours of the brain has not been explored to date, and the aim of our study was to investigate this pattern.

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Materials and Methods

Eighty-eight brain tumors were selected from the archive of the Department of Laboratory Medicine, St. Michael's Hospital, Toronto, Canada. All tumors were surgically-obtained from patients with brain neoplasms and were classified according to the 2007 WHO Classification of Tumors of the Nervous System (14). Tumor types and respective WHO grades included 13 glioblastomas (GBM) IV, 5 astrocytomas (III), 4 anaplastic oligodendrogliomas (III), 8 oligoastrocytomas (III), 9 pilocytic astrocytomas (I), 10 myxopapillary ependymomas (I), eight schwannomas, and 23 meningiomas (I). All specimens had been fixed in 10% neutral buffered formalin, routinely processed, paraffin-embedded, cut at 4-6 μm and stained with hematoxylin and eosin (H&E).

Sections were then microwaved in 0.1 mm sodium citrate buffer (pH 6.0), incubated with goat anti-serum and exposed to the streptavidin-biotin peroxidase complex. Diaminobenzidine served as the chromogen. Mouse testicular tissue served as the positive control for PTTG immunoreactivity. Replacement of the primary antiserum with PBS served as the negative control. Immunostains for MIB-1 (Immunotech, Westbrook, ME, USA) were performed to detect Ki-67 labeling. For immunostaining of PTTG, a mouse monoclonal antibody (1:75; Abcam, Cambridge, UK) was used. Routine de-paraffinization, rehydration, and blockade of endogenous peroxidase activity were carried out. Routine staining for diagnostic purposes was carried out using glial fibrillary acidic protein (GFAP), S100, vimentin, cytokeratin and epithelial membrane antigen

(EMA), and Ki-67. Both the intensity and percentage of positive cells were studied blindly and independently by two observers who assessed ten high-power fields per specimen. Nuclear and cytoplasmic PTTG immunostaining intensity was assessed using a 0-3 scale (0=none, 1=slight, 2=mild, 3=strong staining). The percentage of PTTG-immunopositive cells was independently determined by each observer by counting positive and total cells in 10 high-power fields and calculating an average of the 10 fields.

Statistical analysis. Student *t*-test was employed to find significant differences between brain neoplasms subtype in regard to PTTG expression.

Results

PTTG immunoexpression intensity. Astrocytoma III (2±0.7) and glioblastoma IV (1.9±0.5) had the highest PTTG immunoexpression (Table I). The lowest PTTG immunoexpression was found in pilocytic astrocytoma and ependymoma (both 0.9±0.3). Pairwise comparison of different glioma tumor types showed that astrocytoma III and GBM IV had significantly higher PTTG expression than oligoastrocytoma III, oligodendroglioma III, pilocytic astrocytoma I, ependymoma I, and schwannoma ($p<0.0005$). Meningiomas and schwannomas exhibited low PTTG immunostaining (0.9± 0.3 and 1.5±1.2 respectively) (Table I).

Cellular localization of PTTG. In GBM IV, astrocytoma III and oligoastrocytoma III, nuclear expression of PTTG was more pronounced than its cytoplasmic expression. Nuclear and cytoplasmic PTTG expression was not significantly different in oligodendrogliomas, pilocytic astrocytomas, ependymomas, schwannomas and meningiomas.

Nuclear expression of PTTG and tumor aggressiveness. GBM IV and astrocytoma III tumors (1.9±0.5 and 2±0.7 respectively) showed the highest nuclear PTTG intensity, whereas oligoastrocytoma III (1.3±1.7), oligodendroglioma III (1±1), pilocytic astrocytoma I (0.9±0.3) and ependymoma I (0.9±0.3) exhibited significantly lower nuclear expression of PTTG ($p<0.0001$). Among extra-axial tumors, the highest PTTG nuclear expression was seen in schwannoma (1.5±1.2), followed by meningioma (0.9±0.3).

Table I. *Immunohistochemical expression of pituitary tumor-transforming gene (PTTG) in brain tumors examined, arranged by nuclear expression.*

Tumor type	Number (n)	Expression	
		Nuclear	Cytoplasmic
Intra-axial tumors			
Pilocytic astrocytoma I	9	0.9±0.3	0.9±0.3
Ependymoma I	10	0.9±0.3	0.9±0.3
Oligodendroglioma III	4	1±0	1±0
Oligoastrocytoma III	8	1.3±0.7	0.6±0.7
GBM IV	13	1.9±0.5	1.2±0.6
Astrocytoma III	5	2±0.7	1±0.7
Extra-axial tumors			
Meningioma I	23	0.9±0.3	1±0.3
Schwanomma I	8	1.5±1.2	1.4±1
Total	88	1.3±0.8	1.03±0.5

GBM: Glioblastoma multiforme.

Discussion

Nuclear PTTG expression is higher in malignant tumors than in benign tumors. Cellular localization of PTTG is important for determination of its role in pathways associated with tumor development and progression. The limited number of studies investigating expression of PTTG in brain tissue and neoplasms present contradictory results. Whereas Genkai *et al*. found PTTG to be solely expressed in tumor cell nuclei, Chamaon *et al*., reported nuclear, as well as cytoplasmic PTTG expression (15). Others have not reported PTTG cellular localization in their investigations (15-17). Given the contradictory findings reported in various cell lines and neoplasms, it is possible that PTTG undergoes regulated translocation between the cell nucleus and the cytoplasm.

Within gliomas, nuclear expression of PTTG was significantly higher than its cytoplasmic immunostaining in GBM IV, astrocytoma III, and oligoastrocytoma III ($p<0.05$). There may be several reasons for our observed increase in the nuclear/cytoplasmic ratio of PTTG expression. Firstly, nuclear PTTG may specifically be involved in promoting transcriptional activity of other factors involved in oncogenesis, including the fibroblast growth factor (FGF) family and c-myc. In gliomas, expression of FGF2 and its receptors is often found to be elevated (18, 19). Interestingly, elevated PTTG levels parallel those of FGF2 in the developing cerebral cortex, as well as in NT-2 neurons (20). Furthermore, PTTG stimulates FGF2 expression (20). The association of PTTG with bFGF expression and regulation is supported by several lines of evidence. These include a) PTTG and bFGF levels are correlated in acute leukemia (21), b) significant correlation exists between PTTG and bFGF expression in pituitary adenomas (22), and c) the fact that in cultured leiomyoma cells, bFGF and PTTG are involved in a positive autocrine feedback loop thought to promote tumorigenesis (9). Recently, it was shown that glioblastoma cell growth is inhibited by disruption of FGF pathway signaling (23). Thus, nuclear PTTG expression in glioma, particularly in astrocytic tumors, may elicit elevated bFGF and FGF2 expression and contribute to the tumorigenic process.

Nuclear PTTG may also play a role in increasing transcriptional activation of c-myelocytomatosis oncogene

cellular homologc (c-Myc), as well as of several angiogenic factors such as vascular endothelial growth factor (VEGF), inhibitor of DNA binding-3 (ID3) and thrombospondin (TSP-1) (11). Given the fact that angiogenesis is important to progression in glioma and reckons in histological grading of malignancy, increased nuclear expression of PTTG is an expected feature of high-grade gliomas. It is of note that since PTTG is involved in cell cycle regulation by inhibition of premature sister chromatid separation, its elevation in malignant gliomas may simply be a reflection of the greater proliferative activity of these highly proliferative tumors. Lastly, elevated nuclear PTTG expression in malignant gliomas may be mediated by as yet unidentified mechanisms.

Glioma. Among glial tumors, GBM IV, astrocytoma III, anaplastic oligoastrocytoma (III) and anaplastic oligodendroglioma (III) exhibited the highest level of PTTG expression, whereas pilocytic astrocytomas and ependymomas were found to have the lowest PTTG immunoexpression. This observation may be related to the low proliferation rate of these tumors, since PTTG is implicated in tumor cell proliferation and aggressive behavior. Low PTTG expression may also be a reflection of the differences in the molecular pathways involved in their tumorigenesis and progression when compared to those of the high-grade infiltrative tumors studied which exhibited greater nuclear PTTG expression. For example, as shown in one study of gene expression, pilocytic astrocytomas (WHO grade I) and diffuse astrocytomas of low grade (WHO II) both differ from GBM (WHO IV) by the expression of five genes [fibronectin, osteopontin, human cartilage glycoprotein-39 (YKL-40), keratoepithelin and fibromodulin] which are involved in invasion and angiogenesis (24). *PTTG* appears to be yet another gene differentially expressed in these tumors. It is of note that low-grade infiltrative gliomas, whether astrocytic or oligodendroglial, usually progress to high-grade in terms of histology and clinical characteristics (14). It is reasonable to propose that the elevated PTTG levels seen in higher-grade gliomas undergoing transition from lower-grade are necessarily-acquired over time.

Schwannoma. Immunoexpression of PTTG was significantly higher in schwannoma than in pilocytic astrocytoma, anaplastic oligodendroglioma, ependymoma, and meningioma. This elevated PTTG expression level, as compared to other low-grade CNS tumor types, was notable. Schwannomas are benign and grow slowly. Very little is known of their molecular pathways. They are tumors of peripheral nerve and are causally related to loss of function of the neurofibromatosis-2 (*NF2*) gene, which encodes for the protein merlin (14). Our findings of relatively high PTTG expression in schwannomas contribute to the body of knowledge on this entity.

Meningioma. Albeit as a low level when compared to most malignant tumors, PTTG immunoexpression was present in meningiomas. As a rule, these common CNS tumors are mostly benign; fewer than 5% are WHO grade III (14). Meningiomas occur more frequently in females and their frequency is increased during pregnancy (14). Additionally, over 30% of meningiomas express estrogen receptors (14). Estrogen is known to mediate PTTG up-regulation in prolactin (PRL)-secreting pituitary adenomas (25). As such, it may be that PTTG in meningiomas interacts with estrogen pathways and contributes to tumorigenesis. Another oncogenic mechanism, increased genetic instability, is highly correlated with tumor grade in meningiomas (14). It is proposed that aberrant chromosomal segregation underlies the instability. In most other tumors, including carcinomas of thyroid, breast and colon, PTTG expression is highly correlated with aneuploidy and genetic instability (11). Therefore, the role of PTTG expression in aneuploidy, as seen in meningiomas of varying grades, requires further investigation. PTTG quantification and its correlation with aneuploidy in meningiomas may prove useful in meningioma classification and grading.

The present study found evidence of a correlation between immunohistochemical expression of PTTG and WHO tumor grade in a spectrum of CNS tumors. Of particular interest was the finding of increased PTTG staining in astrocytomas of WHO grades III and IV, as compared to lower grade gliomas of WHO grade I (pilocytic astrocytoma, myxopapillary ependymoma), as well as anaplastic oligodendroglioma. Further investigation into the precise molecular mechanisms that lead to PTTG overexpression in malignant tumors is warranted. The function of PTTG has been associated with several tumorigenic mechanisms, including cell proliferation, invasion, apoptosis, DNA repair mechanisms, and angiogenesis. Determining the precise role of PTTG in brain tumor development and growth will require elucidation of the active pathways in the various brain tumor subtypes. Mouse models of PTTG deficiency have advanced our understanding of pituitary tumor development and progression. Similar models need to be studied or developed to determine the role of *PTTG* in brain tumor biology. In glioma cells in culture, knockdown of *PTTG* using the small inhibitory RNA (siRNA) technique results in reduced cell proliferation (6). Thus *PTTG* may be a promising target in the treatment of tumors demonstrating elevated PTTG expression. More research is required to elucidate the role played by *PTTG* in oncogenic pathways.

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