Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 331 (2005) 86–92
BBRC
www.elsevier.com/locate/ybbrc

Regulation of the pituitary tumor transforming gene by insulin-like-growth factor-I and insulin differs between malignant and non-neoplastic astrocytes

Kathrin Chamaon [a], Elmar Kirches [a], Dimitrios Kanakis [b], Stefan Braeuninger [a], Knut Dietzmann [a], Christian Mawrin [a,*]

[a] Department of Neuropathology, University of Magdeburg, Leipziger Straße 44, 39120 Magdeburg, Germany
[b] Department of Pathology, Helios-Klinikum Berlin-Buch, Karower Straße 11, 13125 Berlin, Germany

Received 21 March 2005
Available online 30 March 2005

Abstract

The reasons for overexpression of the oncogene pituitary tumor transforming gene (PTTG) in tumors are still not fully understood. A possible influence of the insulin-like growth factor I (Igf-I) may be of interest, since enhanced Igf-I signalling was reported in various human tumors. We examined the influence of Igf-I and insulin on PTTG expression in human astrocytoma cells in comparison to proliferating non-neoplastic rat embryonal astrocytes. PTTG mRNA expression and protein levels were increased in malignant astrocytes treated with Igf-I or insulin, whereas in rat embryonic astrocytes PTTG expression and protein levels increased only when cells were exposed to Igf-I. Enhanced transcription did not occur after treatment with inhibitors of phosphoinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK), blocking the two basic signalling pathways of Igf-I and insulin. In addition to this transcriptional regulation, both kinases directly bind to PTTG, suggesting a second regulatory route by phosphorylation. However, the interaction of endogenous PTTG with MAPK and PI3K, as well as PTTG phosphorylation were independent from Igf-I or insulin. The latter results were also found in human testis, which contains high PTTG levels as well as in nonneoplastic astrocytes. This suggest, that PI3K and MAPK signalling is involved in PTTG regulation not only in malignant astrocytomas but also in non-tumorous cells.

Keywords: Pituitary tumor transforming gene; Igf-I; Insulin; Astrocytoma

Insulin-like growth factor-I (Igf-I) is involved in growth and differentiation of various cell types. Although liver is the primary source of circulating Igf-I, significant expression of this growth factor is seen in various tissues, including brain, where it is known to exert autocrine and paracrine functions [1]. Igf-I is overexpressed, along with its receptor during the progression of cancer. In diffusely infiltrating astrocytomas, Igf-I and the insulin-like growth factor receptor (Igf-IR) are present in all tumor grades. Moreover, the Igf-I content and pattern of expression correlate with the histopathologic grade [2]. The proportion of astrocytic tumor cells expressing Igf-I also correlates with the Ki-67 proliferation index. Treatment of astrocytoma cell cultures with Igf-IR antisense oligonucleotides induced apoptosis [3]. The tumorigenicity of a rat glioblastoma is lost by Igf-I antisense expression [4]. Igf-I is mitogenic for many glioma cell lines and is thought to function in some cell lines in an autocrine manner. The binding of Igf-I and insulin to its receptors was enhanced in glioblastomas compared to normal adult brain [5].

* Corresponding author. Fax: +49 391 671 3300.
E-mail address: christian.mawrin@medizin.uni-magdeburg.de (C. Mawrin).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.03.124

Until recently, the functions of insulin and insulin receptor (IR) in the central nervous system (CNS) have largely remained unclear. IR is abundantly expressed in several specific brain regions that govern fundamental behaviors such as food intake, reproduction, and high cognition. While the physiological functions of insulin and Igf-I are different, the IR and Igf-IR are structurally similar. Both are receptor tyrosine kinases and exist at the cell surface as $\alpha 2\beta 2$ tetramers. Ligand binding is thought to induce a conformational change, resulting in ATP binding and autophosphorylation. The two receptors become autophosphorylated at conserved tyrosine residues and activate similar signalling substrates. When studied in cell cultures, the two receptors are capable of producing similar cellular responses. Findings in genetically engineered mice indicate physiologically distinct but overlapping functions of Igf-I- and insulin systems [6].

Upregulation by growth factors may also play a role for the recently discovered oncogene pituitary tumor transforming gene (PTTG). In normal adult tissues, human PTTG mRNA is restricted to a small number of tissues, including testis, thymus, colon, and small intestine [7]. NIH-3T3 fibroblasts transfected with PTTG form tumors in nude mice, supporting the oncogenic potential of PTTG overexpression [8]. Although a role of PTTG in human tumorigenesis was mainly discussed in pituitary adenomas thus far, the protein is overexpressed in various tumors as well as in carcinoma cell lines. The cause for this upregulation is not well understood. It has been suggested that fibroblast growth factor (bFGF), epidermal growth factor (EGF), and tumor growth factor α (TGFα) are involved [9,10]. Here we show that PTTG is increased in human malignant astrocytomas compared with normal human brain tissue. Furthermore, we show that PTTG expression can be stimulated by Igf-I and insulin. In non-neoplastic embryonal astrocytes, PTTG was upregulated only by Igf-I.

Methods

Immunohistochemistry. Paraffin sections of malignant astrocytomas and non-tumorous cortices (2–3 μm) were dewaxed, dried, and washed two times 5 min in xylol, rinsed in 100% ethanol, 75% ethanol, and aqua dest. Slices were boiled 20 min in target retrieval solution (DAKO), rinsed in aqua dest, and incubated with a monoclonal anti-securin (PTTG) antibody 1:100 (Novocastra) overnight at 4 °C. After washing, slices were incubated 30 min with a biotinylated secondary anti-mouse immunoglobulin (DAKO), and after repeated washing incubated for 30 min in streptavidin/horseradish peroxidase. Immunoreactivity was detected by staining with diaminobenzidine.

Immunofluorescensce. Cells of the glioma lines U87MG, U138MG, and LN405, and rat embryonal astrocytes were grown on slides, and the slides were fixed for 30 min in a solution containing 20 g paraformaldehyde in 150 ml saturated picric acid buffered in phosphate buffer, washed with PBS, and blocked in 10% goat serum. Further, slides were incubated with a monoclonal anti-securin antibody 1:100 (Novocastra) in PBS for 2 h, washed and incubated with an anti-mouse antibody, coupled to Alexa Fluor 488 (Molecular Probes). Images were recorded on a confocal laser scanning microscope.

RT-PCR. From cell cultures RNA was isolated with Trizol according to the manufacturer's instructions, and cDNA was synthesized with reverse transcriptase (Invitrogen) using 500 ng RNA per reaction. PTTG expression levels were analyzed by real-time-PCR on a LightCycler-System (Roche Molecular Biochemicals, Germany) using the DNA Master SYBR Green I kit (Roche) in at least three independent experiments. The sequences of the primers were as follows: 5′-GCT TTG GGA ACT GTC AAC AG-3′ (PTTG sense), 5′-ATC TGA GGC AGG AAC AGA GC-3′ (PTTG antisense), 5′-CCA GCA GAG AAT GGA AAG TC-3′ (β2-MG sense), and 5′-GAT GCT GCT TAC ATG TCT CG-3′ (β2-MG antisense). Quantification of each reaction product was performed by comparison of the results with a dilution series of a PTTG plasmid, generated by cloning of a PTTG fragment into a pPCR Script Amp SK (+) vector (Stratagene). Since varying sample sizes and RNA qualities may influence detectable copy numbers, all results for PTTG were normalized to the housekeeping gene β2-MG.

Cell culture. The two malignant human glioma cell lines U87MG and U138MG were purchased from the American Type Culture Collection (ATCC, Rockeville, USA) and grown to 80% confluency in Earl's minimal essential medium (EMEM, high glucose) from PAA laboratories (Linz, Austria), supplemented with 10% FCS (PAA). For the human glioma cell line LN405 (DSMZ, Braunschweig, Germany) and a primary culture of embryonal rat brain astrocytes, RPMI 1640 or DMEM (PAA) were used as grown media instead of EMEM. Non-confluent cells were serum-starved for 24 h and treated with 10 ng/ml Igf-I (Growth Peptide, Australia) or 200 nM (400 nM) insulin (Aventis, Germany) for 48 h prior to protein/RNA extraction. Protein kinase inhibitors wortmannin (50 nM and 20 μM), LY294002 (10 μM), and PD98059 (30 μM) were added 30 min before Igf-I/insulin treatment. Wortmannin and LY294002 were purchased from Sigma and PD98059 was from Alexis Biochemicals.

Immunoprecipitation. Cells from LN405, U138MG, and U87MG, and rat embryonal astrocytes were lysed in lysis buffer (0.75% v/v NP-40, 1 mM EDTA, 1% aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml leupeptin in PBS) for 30 min at 4 °C, sonificated, and centrifuged for 20 min at 14,000 rpm.

Human autoptical-derived testis tissue was homogenized in homogenization buffer (0.25 M sucrose, 50 mM Tris, pH 7.5, 1 mM DTT, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride), with an Ultra Thurrax homogenizer (IKA-Werke, Germany), sonificated, and centrifuged for 15 min at 14,000 rpm. Gamma bind Sepharose (Pharmacia) was washed two times in lysis buffer and 8 μl anti-securin (PTTG) antibody (Novocastra) in lysis buffer was added. After 2 h rotation antibody-Sepharose was centrifuged, washed in lysis buffer, and incubated with cell lysate or testis homogenate under gentle rotation for 4 h. After centrifugation for 7 min at 14,000 rpm, the pellet was washed with lysis buffer two times and was dissolved in loading buffer. All centrifugation steps were performed at 4 °C.

Western blot. Proteins were denatured in loading buffer (2 min 100 °C). Protein concentration was measured by Bradford assay, with BSA as standard. Proteins were separated by SDS–polyacrylamide gel electrophoresis on 12% acrylamide gels. Gels were electrophoretically transferred to nitrocellulose membranes. Membranes were incubated in 5% nonfat milk in TBS with 0.1% Tween, followed by incubation with antibodies to PTTG 1:500, (Usbio), PI3K 1:1000 (MBL), MAPK 1:500 (Calbiochem), anti-phosphoserine 1:100 (Biomeda), P-Akt 1:1000 (Cell Signaling) or β-actin 1:10,000 (Sigma–Aldrich) overnight at 4 °C. After washing in TBS with 0.1% Tween, blots were incubated with appropriate secondary antibodies conjugated to horseradish peroxidase for 2 h at room temperature. After further washes, immunoreactivity was visualized by ECL chemiluminescence detection system (Amersham).

Results

PTTG is overexpressed in malignant astrocytomas

PTTG was strongly detectable in distinct tumor cells of malignant astrocytomas by immunohistochemistry, but was not present in normal brain (Figs. 1A and B). Controls of the malignant astrocytoma sections incubated without primary antibody showed no specific staining. PTTG mRNA content of 26 malignant astrocytomas (16 glioblastoma multiforme [GBM] WHO grade 4, nine anaplastic astrocytomas WHO grade 3, and one recurrent GBM) was analyzed by real-time-PCR. Strong expression was found in all malignant astrocytomas but mRNA was nearly undetectable in normal brain ($p < 0.01$, Fig. 1C). Although the normal brain tissue was autoptic material, it was judged to be an appropriate control, since the expression levels of the reference gene in the real-time-PCR did not differ significantly between brain and malignant astrocytoma tissue.

PTTG is expressed in malignant astrocytoma cell lines and rat embryonal astrocytes

Immunofluorescence stainings demonstrated PTTG expression in the astrocytoma cell lines U87MG (a), U138MG (b), and LN405 (c), and in rat embryonic astrocytes (d), as shown in Fig. 2. Interestingly, there were different expression patterns, including cells with or without nuclear staining for endogenous PTTG. Such different localization was also documented for GFP-PTTG constructs expressed in different cell lines by Mu et al. [11]. Negative controls of cells stained without primary antibody were immunonegative.

PTTG protein is upregulated after Igf-I and insulin stimulation in LN405 cells

We sought to find out whether Igf-I or insulin influences PTTG protein level. A non-confluent serum-starved LN405 cell culture was treated with 10 ng/ml Igf-I or 200 nM insulin. PTTG expression was enhanced in both,

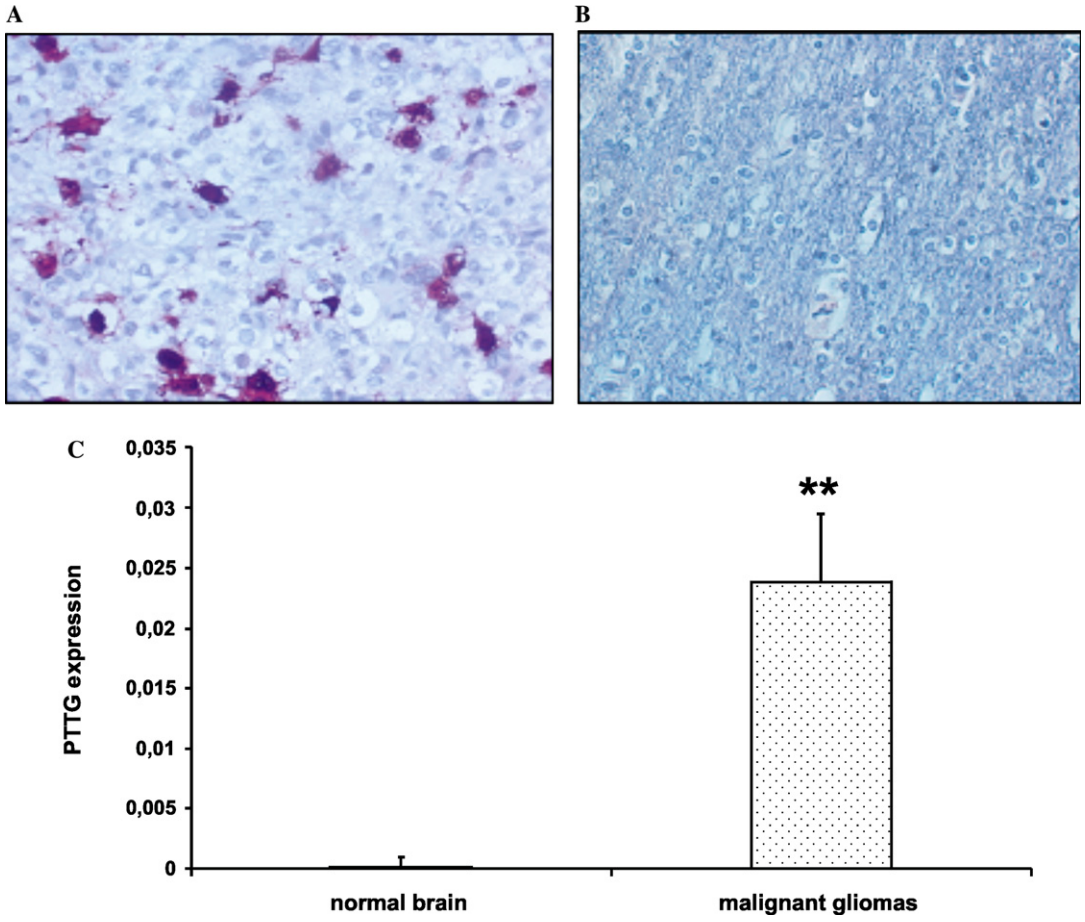

Fig. 1. Paraffin section of a glioblastoma multiforme biopsy shows strong PTTG staining in distinct astrocytic tumor cells (A). Section from normal human brain shows no PTTG immunoreactivity (B). Analysis of PTTG mRNA amount of 26 malignant astrocytomas (16 glioblastoma multiforme WHO grade 4, nine anaplastic astrocytomas WHO grade 3, and one recurrent glioblastoma multiforme) in comparison to normal brain tissue by real-time-PCR (C). PTTG expression (y-axis) represents the β2-MG-normalized gene copy number of PTTG. The difference of PTTG expression between tumor and normal brain was significant ($p < 0.01$) using a t test.

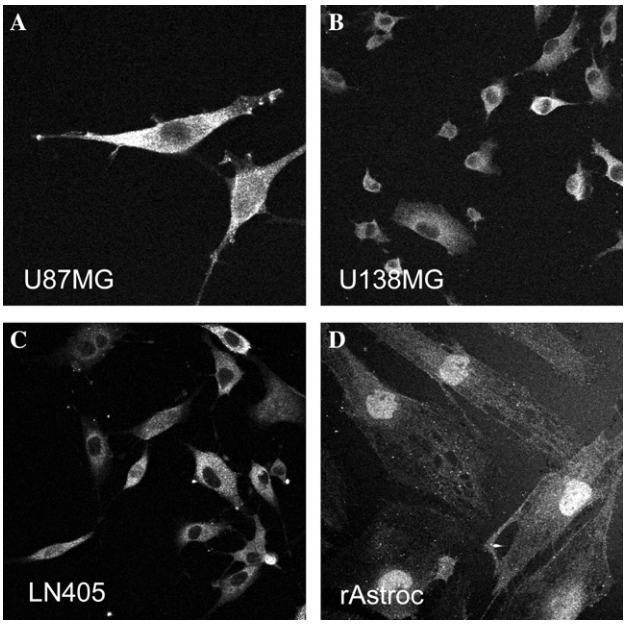

Fig. 2. Immunocytochemical detection of PTTG with a monoclonal anti-PTTG antibody and secondary Alexa-488 coupled antibody in three human glioma cell lines and rat embryonal astrocytes, grown in medium with 10% FCS. Immunofluorescence microscopy revealed a high PTTG expression for U87MG (A), U138MG (B), LN405 (C), and non-neoplastic embryonic rat astrocytes (rAstroc, D).

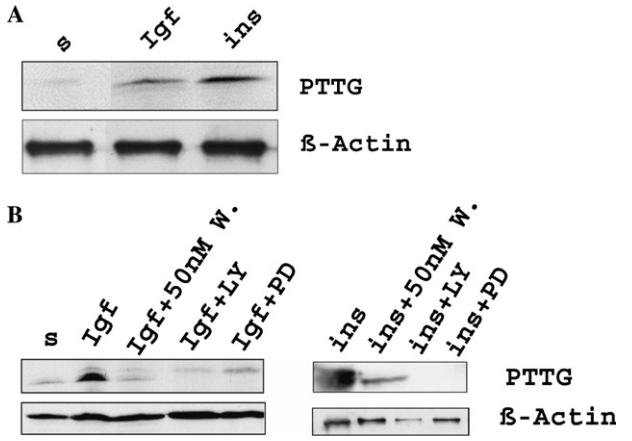

Fig. 3. (A) Enhanced PTTG expression after treatment of non-confluent serum-starved (s) LN405 cells with Igf-I (10 ng/ml) and insulin (200 nM, ins). Proteins detected by Western blots (50 μg protein per lane) were developed with a rabbit PTTG-antibody, which detected a protein band of expected size of around 22 kDa. The β-actin immunoreactivity shows equal protein loading. (B) PTTG expression of serum-starved LN405 cells (s) after Igf-I (10 ng/ml) stimulation and inhibition of Igf-I treated cells with PI3K inhibitor wortmannin (50 nM) and LY294002 (10 μM) or MAPK inhibition adding PD98059 (30 μM). Additionally shown PTTG expression of serum-starved LN405 cells after insulin treatment (200 nM, ins) and PI3K inhibition of insulin stimulated cells with wortmannin (50 nM), LY294002 (10 μM) or MAPK inhibition with PD98059 (30 μM). PTTG protein on Western blots was detected by rabbit-PTTG antibody and equal protein loading was confirmed by β-actin detection of stripped blots.

Igf-I and insulin stimulated cells, compared with serum-starved cells (Fig. 3A). We were unable to detect PTTG in other astrocytoma cell lines by Western blotting, probably due to low protein levels. In these cell lines, the protein was clearly detectable only in immunoprecipitates.

Increase of PTTG protein after Igf-I stimulation is inhibited by PI3K and MAPK inhibitors

To find out which pathway of Igf-I und insulin signalling mediates the increased PTTG expression, we blocked either the PI3K pathway using Wortmannin and LY294002 or the MAPK pathway with PD98059. Application of PI3K- and MAPK-inhibitors blocked the effect (Fig. 3B). With a polyclonal anti-PTTG antibody two bands for PTTG protein were detectable what may indicate different phosphorylation states [12].

PTTG mRNA is upregulated after Igf-I and insulin stimulation in malignant astrocytoma cells

We performed real-time-PCR from all three astrocytoma cell lines to examine PTTG mRNA levels after growth hormone stimulation and kinase inhibitor treatment. Cells were grown in serum-free medium and serum-free medium with Igf-I or insulin. As shown in Fig. 4A, Igf-I substantially increased PTTG expression in U87MG cells. No specific mRNA was detectable after PI3K inhibition, whereas MAPK inhibitors abolished the effects of Igf-1. Comparable results were obtained for the other two cell lines, although PI3K inhibitors did not block PTTG expression completely (data not shown).

In all cell lines, PTTG induction by insulin treatment was less pronounced as with Igf-I and was abolished by PI3K inhibitors (as shown for U87MG in Fig. 4B). Insulin-mediated enhancement of PTTG was also abolished by MAPK inhibition in U138MG and LN405 cells (data not shown), whereas no effect of PD98059 was seen in U87MG cells (Fig. 4B).

Interestingly, substantial upregulation of PTTG in proliferating rat embryonal astrocytes was seen only after Igf-I (Fig. 4C), but not after treatment with insulin, even in higher concentrations (400 nM) (not shown).

Since general toxicity of PI3K- or MAPK-inhibitors may lead to unspecific suppression of PTTG transcription, which would hamper the dissection of both pathways as transducers of insulin or Igf-I effects, a control experiment was performed. The unstimulated endogenous PTTG-mRNA levels were measured in U87MG cells and in embryonal rat astrocytes, treated only with Wortmannin or PD98059 or the corresponding concentration of the solvent DMSO. No significant differences were observed between inhibitor and pure solvent (data not shown).

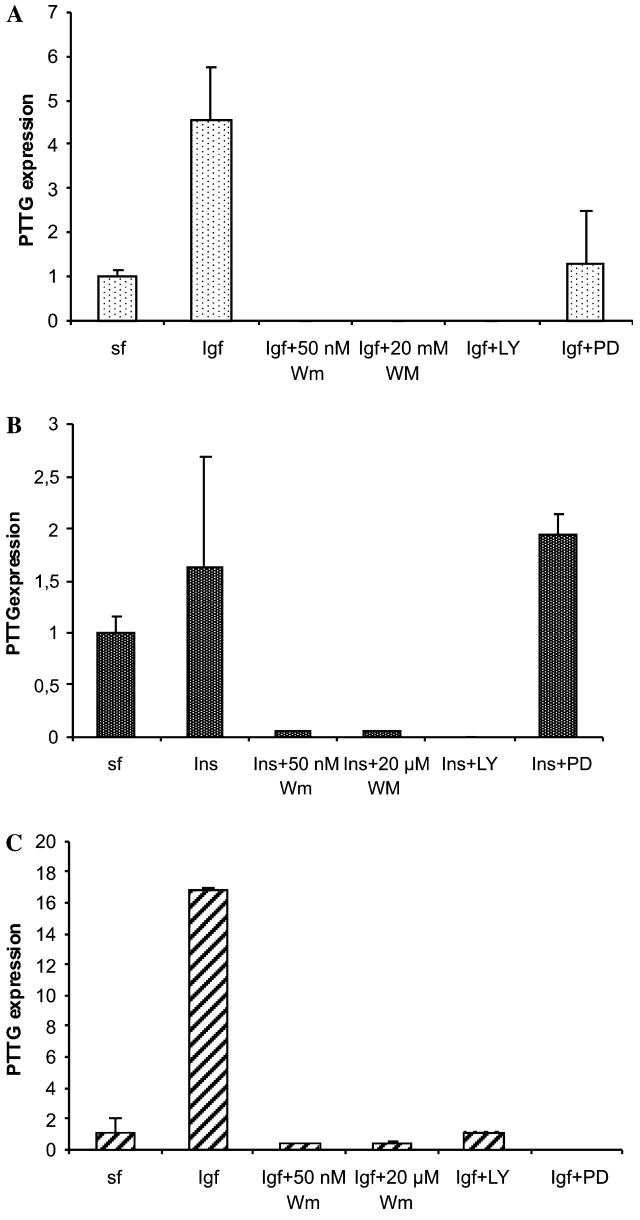

Fig. 4. PTTG transcript levels in the astrocytoma cell line U87MG (A,B) and rat embryonic astrocytes (C) determined by real-time-PCR. PTTG copy number was normalized to β2-MG as a reference gene and expressed as fold increase compared to serum-free (sf) cells. Cells were cultured in serum-free medium, serum-free medium with Igf-I (10 ng/ml) (A,C) or with 200 nM insulin (ins) (B). Additionally, Igf-I/insulin stimulated cells were grown with either wortmannin (Wm; 50 nM and 20 µM), LY294002 (10 µM) or PD98059 (30 µM). Data represent results of at least three independent experiments.

Endogenous PTTG is (serine)phosphorylated in astrocytoma cell lines and in non-malignant cells

Besides increased PTTG expression in tumors, phosphorylation may also be involved in the downstream function of this oncoprotein. Therefore, we performed immunoprecipitation studies using different cell lines and adult testis tissue. PTTG itself was found in all immunoprecipitates from cell lines LN405, U87MG, U138MG, rat embryonal astrocytes, and testis. Using a phosphoserine-specific antibody, (serine)phosphorylated PTTG was detectable not only in insulin stimulated cells, but also in serum-starved cells (Fig. 5A). Since the PTTG antibody detected only a single band comigrating with the one detected by the phosphoserine antibody, PTTG seems to be generally phosphorylated in the investigated samples. These results further suggest that PTTG phosphorylation is important for its function, independent from stimulation with the two specific growth factors used in this study. Previously, it was shown that cdc2 [12] and MAPK [13] phosphorylate PTTG. Whether PI3K is also involved in this process requires further investigations.

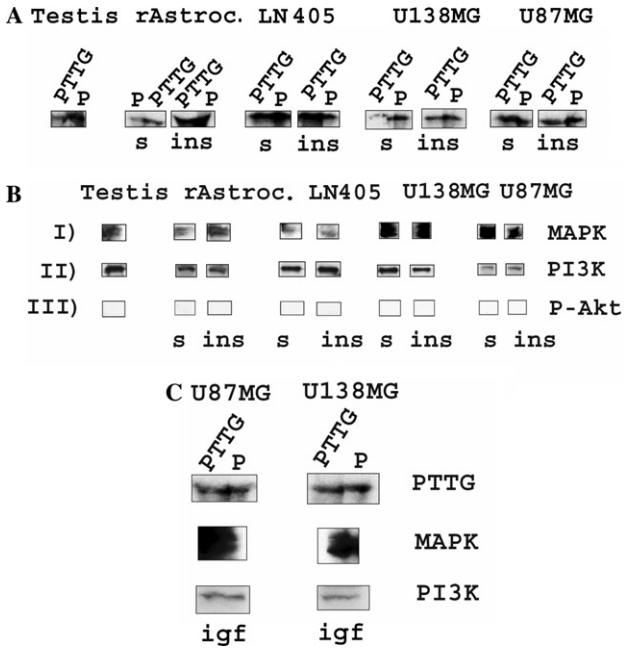

Fig. 5. Immunoprecipitations (IP) from various tissue lysates with a PTTG antibody and subsequent detection of PTTG, phosphoserine (P), MAPK, PI3K, and pAkt by immunoblotting (IB): (A) immunoprecipitation of PTTG with a PTTG-antibody from human adult testis tissue, rat embryonal astrocytes (rAstroc), and astrocytoma cell lines LN405, U138MG, and U87MG and after serum-starvation (s) or insulin (ins) treatment. Western blot lanes loaded with PTTG immunoprecipitates were cut into half. One-half of each lane was developed with PTTG antibody from rabbit (PTTG) and the other half with an anti-phosphoserine antibody (P). (B) Detection of MAPK (ERK1, ERK2) (I) and PI3K (II) in PTTG immunoprecipitates of human adult testis tissue, rat embryonal astrocytes, and astrocytoma cell lines LN405, U87MG, and U138MG after serum-starvation (s) or insulin (ins). No immunoreactivity for P-Akt (III) in PTTG-immunoprecipitates was found. (C) Detection of a serine-phosphorylated band comigrating with PTTG in PTTG immunoprecipitates from U87MG and U138MG astrocytoma cells after stimulation with 10 ng/ml Igf-I.

Endogenous PTTG protein interacts with PI3K and MAPK in astrocytoma and non-tumorous cells

Pei [13] showed the interaction of His-tagged PTTG, transfected in COS cells, and a HA-tagged MAPK

(MEK1). Here we show a direct interaction of endogenous PTTG with endogenous MAPK in tumor cell lines and non-tumorous cells. MAPK immunoreactivity was found in PTTG-immunoprecipitates of human adult testis, rat embryonal astrocytes, and all astrocytoma cell lines (Fig. 5B). The association of PTTG and MAPK is independent of insulin stimulation, since the interaction is also found in serum-starved, Igf-I/insulin untreated cells. Fig. 5C shows PTTG immunoprecipitations of U87MG and U138MG cell extracts after Igf-I stimulation with the same results. The interaction of PTTG with MAPK generally is independent of the transformed state of the cells, since it was also found in human testis and embryonal rat astrocytes. Although PI3K can be found in PTTG immunoprecipitates of all astrocytoma cell lines, non-tumor human testis, and rat embryonal astrocytes, p-Akt does not co-immunoprecipitate with PTTG (Fig. 5B), suggesting that this downstream element of PI3K signalling does not target the PTTG protein directly.

Discussion

In this study, we presented data concerning the expression of the oncogene PTTG in proliferating tumorous and non-tumorous astrocytic cells. We showed that PTTG mRNA is overexpressed in high grade astrocytomas compared to normal brain tissue, which is in line with recent findings [10]. However, the mechanisms of PTTG upregulation in tumors are largely unknown.

Many tumor genes are induced by both, Igf-I and insulin [14]. Moreover, the Igf-I content and pattern of expression correlate with the histopathologic grade in astrocytomas [2]. A possible regulation of PTTG by insulin is suggested by the presence of two insulin-responsive sequences in the promoter: AP-1 motif (tgaG/Ctca) and GAPH IRE-A-like motif (CCCGCCTC), which mediate stimulatory effects on gene transcription [15].

The activation of both, insulin-like growth factor I receptor (Igf-IR) and insulin receptor (IR), induces two main signalling cascades: (1) one involving SH2-containing proteins (Grb2, Nck, Crk, and SHP2) and MAPK, and (2) one involving PI3K and Akt. Here we demonstrate the upregulation of PTTG by these two signalling pathways in malignant astrocytoma cells. However, we show that even in unstimulated tumor cells and in non-tumorous cells, PTTG is already phosphorylated and interacts with PI3K and MAPK. It suggests that a second regulatory route from the kinases directly to the PTTG protein is basically present in many cell types. However, the reinforcement of growth factor signalling to the PTTG promoter by as yet unknown mechanisms may be an essential aspect in tumors. Different roles for Igf-I and insulin in tumor- and non-tumorous proliferating cells may exist, as we did not find a stimulating effect of insulin on PTTG expression in rat embryonal astrocytes. Results of some studies showed that the overall mitogenic activity seems to be greater for the Igf-IR than for the IR [16]. There are further hints for different effects of Igf-1 and insulin on proliferating cells. It was shown, for instance, that the widely expressed oncogene Crk-II is rapidly phosphorylated by Igf-I treatment in both, 293 cells and NIH-3T3 fibroblasts, whereas phosphorylation of Crk-II by insulin treatment occurred only at high insulin concentrations [17]. Although it had been shown that PTTG is phosphorylated by MAPK [13], we did not find a hint for aberrant PTTG phosphorylation.

A probably more important regulation mechanism may be enhanced transcription. In pituitary adenomas, high levels of PTTG expression were connected with high expression of bFGF and NIH-3T3 cells treated with bFGF showed a 2.4-fold induction of PTTG mRNA [9]. Important is also the observation that PTTG upregulates bFGF expression [18], suggesting the existence of an autocrine/paracrine loop in PTTG regulation at least in pituitary adenomas. The existence of a similar autocrine/paracrine loop for PTTG and Igf-I/insulin cannot be refused and must be examined. In malignant astrocytomas, a possible relationship between other growth promoting factors and PTTG may be considered. In line with this, Tfelt-Hansen et al. [10] recently showed an upregulation of PTTG after EGF and TGFα stimulation of the U87MG cell line. These candidates bind to tyrosine kinase receptors, which are often overexpressed by gene amplification or enhanced transcription in astrocytomas.

Taken together, our experiments show that the Igf-I/insulin-pathways generally regulate PTTG transcription. Additionally, we found differences in the Igf-I and insulin effects between astrocytic tumor cells and non-tumorous rat embryonal astrocytes, since in the latter insulin does not enhance PTTG expression even at higher concentrations. Additionally, phosphorylation of PTTG and its interaction with PI3K and MAPK are not an exclusive feature of tumor cells, suggesting a second putative route for growth factor signalling, directed towards the PTTG protein itself. However, the exact mechanisms for the Igf-I/insulin-mediated PTTG mRNA upregulation in human malignant astrocytoma cells remain to be elucidated.

Acknowledgments

This work was supported by a start-up grant from the Otto-von-Guericke University. For technical assistance we thank Mrs. Schellhase and Ms. Fuchs from the Department of Neuropathology of the Otto-von—Guericke University Magdeburg.

References

[1] D.N. Ishii, G.W. Glazner, S.F. Pu, Role of insulin-like growth factors in peripheral nerve regeneration, Pharmacol. Ther. 62 (1994) 125–144.

[2] H. Hirano, M.B. Lopes, E.R. Laws Jr., T. Asakura, M. Goto, J.E. Carpenter, L.R. Karns, S.R. VandenBerg, Insulin-like growth factor-1 content and pattern of expression correlates with histopathologic grade in diffusely infiltrating astrocytomas, Neuro-oncology 1 (1999) 109–119.

[3] D.W. Andrews, M. Resnicoff, A.E. Flanders, L. Kenyon, M. Curtis, G. Merli, R. Baserga, G. Iliakis, R.D. Aiken, Results of a pilot study involving the use of an antisense oligodeoxynucleotide directed against the insulin-like growth factor type I receptor in malignant astrocytomas, J. Clin. Oncol. 19 (2001) 2189–2200.

[4] J. Trojan, B.K. Blossey, T.R. Johnson, S.D. Rudin, M. Tykocinski, J. Ilan, J. Ilan, Loss of tumorigenicity of rat glioblastoma directed by episome-based antisense cDNA transcription of insulin-like growth factor I, Proc. Natl. Acad. Sci. USA 89 (1992) 4874–4878.

[5] R.P. Glick, R. Gettleman, K. Patel, R. Lakshman, J.C. Tsibris, Insulin and insulin-like growth factor I in brain tumors: binding and in vitro effects, Neurosurgery 24 (1989) 791–797.

[6] J.J. Kim, D. Accili, Signalling through IGF-I and insulin receptors: where is the specificity?, Growth Horm. IGF. Res. 12 (2002) 84–90.

[7] A.L. Clem, T. Hamid, S.S. Kakar, Characterization of the role of Sp1 and NF-Y in differential regulation of PTTG/securin expression in tumor cells, Gene 322 (2003) 113–121.

[8] W. Chien, L. Pei, A novel binding factor facilitates nuclear translocation and transcriptional activation function of the pituitary tumor-transforming gene product, J. Biol. Chem. 275 (2000) 19422–19427.

[9] A.P. Heaney, G.A. Horwitz, Z. Wang, R. Singson, S. Melmed, Early involvement of estrogen-induced pituitary tumor transforming gene and fibroblast growth factor expression in prolactinoma pathogenesis, Nat. Med. 5 (1999) 1317–1321.

[10] J. Tfelt-Hansen, S. Yano, S. Bandyopadhyay, R. Carroll, E.M. Brown, N. Chattopadhyay, Expression of pituitary tumor transforming gene (PTTG) and its binding protein in human astrocytes and astrocytoma cells: function and regulation of PTTG in U87 astrocytoma cells, Endocrinology 145 (2004) 4222–4231.

[11] Y.M. Mu, K. Oba, T. Yanase, T. Ito, K. Ashida, K. Goto, H. Morinaga, S. Ikuyama, R. Takayanagi, H. Nawata, Human pituitary tumor transforming gene (hPTTG) inhibits human lung cancer A549 cell growth through activation of p21(WAF1/CIP1), Endocr. J. 50 (2003) 771–781.

[12] F. Ramos-Morales, A. Dominguez, F. Romero, R. Luna, M.C. Multon, J.A. Pintor-Toro, M. Tortolero, Cell cycle regulated expression and phosphorylation of hpttg proto-oncogene product, Oncogene 19 (2000) 403–409.

[13] L. Pei, Activation of mitogen-activated protein kinase cascade regulates pituitary tumor-transforming gene transactivation function, J. Biol. Chem. 275 (2000) 31191–31198.

[14] E. Zelzer, Y. Levy, C. Kahana, B.Z. Shilo, M. Rubinstein, B. Cohen, Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT, EMBO J. 17 (1998) 5085–5094.

[15] R.M. O'brien, R.S. Streeper, J.E. Ayala, B.T. Stadelmaier, L.A. Hornbuckle, Insulin-regulated gene expression, Biochem. Soc. Trans. 29 (2001) 552–558.

[16] V.A. Blakesley, A. Scrimgeour, D. Esposito, D. Le Roith, Signaling via the insulin-like growth factor-I receptor: does it differ from insulin receptor signaling?, Cytokine Growth Factor Rev. 7 (1996) 153–159.

[17] D. Beitner-Johnson, D. LeRoith, Insulin-like growth factor-I stimulates tyrosine phosphorylation of endogenous c-Crk, J. Biol. Chem. 270 (1995) 5187–5190.

[18] X. Zhang, G.A. Horwitz, A.P. Heaney, M. Nakashima, T.R. Prezant, M.D. Bronstein, S. Melmed, Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas, J. Clin. Endocrinol. Metab. 84 (1999) 761–767.