Pituitary tumor-transforming gene in endocrine and other neoplasms: a review and update

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Abstract

Pituitary tumor-transforming gene (PTTG) was only recently discovered. Its overexpression occurs in a wide variety of endocrine and non-endocrine tumors, including ones of pituitary, thyroid, ovary, breast, prostate, lung, esophagus, colon, and the central nervous system. It affects tumor invasiveness and recurrence in several systems, functions as a securin during cell cycle progression, and inhibits premature sister chromatid separation. PTTG is involved in multiple cellular pathways, including proliferation, DNA repair, transformation, angiogenesis induction, invasion, and the induction of genetic instability. In thyroid carcinomas, PTTG expression is a marker of invasiveness. PTTG is overexpressed in most pituitary adenomas, where it appears to correlate with recurrence and angiogenesis. Increasing evidence also points to the role of PTTG in endocrine organ development. For example, PTTG knockout mice show defective pancreatic β-cell proliferation. Herein, we review the current knowledge regarding PTTG-mediated pathways based on evidence from in vivo and in vitro studies as well as knockout mice models. We also summarize the issue of PTTG expression and its correlation with clinicopathologic parameters in patients with neoplasms, particularly of endocrine organs. In addition, we discuss in vitro and in vivo therapeutic models targeting PTTG overexpression.

Endocrine-Related Cancer (2008) 15 721–743

Introduction

The pituitary tumor-transforming gene (PTTG) was first isolated from GH4 rat pituitary tumor cells (Pei & Melmed 1997). Subsequent studies demonstrated its function as a securin, mediating sister chromatid separation during mitosis (Zou et al. 1999). The PTTG family includes PTTG1, PTTG2, and PTTG3. In this review, we focus specifically on PTTG1, the most abundant and widely studied form of the substance, and will refer to PTTG1 as PTTG. Subcutaneous injection of PTTG-transfected cells into nude mice has been shown to induce tumors formation. Overexpression of PTTG reportedly occurs in several neoplasms, including pituitary tumors, as well as carcinomas of lung, breast, esophagus, colon, rectum, and ovary. In colon and thyroid cancers, its expression is associated with tumor invasiveness and aggressive behavior (Heaney et al. 2000, Boelaert et al. 2003a). PTTG is implicated in several normal cellular processes, including DNA damage repair, apoptosis, and angiogenesis. It also interacts with a number of factors both in vivo and in vitro. These include p53. PTTG also possesses transactivating activity and induces upregulation of several other genes, specifically basic fibroblast growth factor (bFGF) and c-myc (Zhang et al. 1999b, Pei 2001, Hamid & Kakar 2004, Kim et al. 2006a, Tfelt-Hansen et al. 2006). In neoplasms, PTTG is thought to induce aneuploidy and genetic instability. Recent evidence suggests that PTTG is implicated in stem cell proliferation as well as in cardiac hypertrophy, thus expanding the broad spectrum of processes which it regulates. PTTG knockout mouse models have been developed to investigate the role of PTTG in tumor initiation and
progression particularly in the pituitary and thyroid gland. Recently, Vlotides et al. (2007) reviewed PTTG action within the context of endocrine cell function. In this review, we provide an update of the current knowledge regarding the role of PTTG in physiologic and pathologic processes as well as its significance as a prognostic indicator in human neoplasia. In vivo models of PTTG-targeted therapies are also discussed.

PTTG: structure and function

PTTG1, PTTG2, and PTTG3

In humans, PTTG1 exhibits 91 and 89% amino acid sequence homology with PTTG2 and PTTG3 respectively (Prezant et al. 1999). By RT-PCR, low levels of PTTG2 have been detected in normal pituitary, brain, placenta, small intestine, colon, liver, spleen, thymus, prostate, testis and ovary, as well as pituitary adenomas, the primary fibroblast cell line LL-24, and in five cancer cell lines (Prezant et al. 1999, Chen et al. 2000). By contrast, PTTG3 mRNA is extremely low or absent in these tissues (Chen et al. 2000). A variant form of PTTG with no transactivating function or NIH3T3 cell-transforming ability has also been detected (Wang & Melmed 2000b); C-terminus deletion reinstates both functions, suggesting that variant PTTG forms may compete with other forms and that their balance may determine function (Wang & Melmed 2000b). The physiologic role and the significance of PTTG2 and PTTG3 remain unclear.

PTTG tissue expression

Early studies established that in normal tissue, PTTG levels are seen in testis predominantly, with lower or absent expression levels in other tissues (Pei & Melmed 1997, Pei 1998). Early studies established that in normal tissue, PTTG levels are seen in testis predominantly, with lower or absent expression levels in other tissues (Pei & Melmed 1997, Pei 1998). By RT-PCR, low levels of PTTG2 have been detected in normal pituitary, brain, placenta, small intestine, colon, liver, spleen, thymus, prostate, testis and ovary, as well as pituitary adenomas, the primary fibroblast cell line LL-24, and in five cancer cell lines (Prezant et al. 1999, Chen et al. 2000). By contrast, PTTG3 mRNA is extremely low or absent in these tissues (Chen et al. 2000). A variant form of PTTG with no transactivating function or NIH3T3 cell-transforming ability has also been detected (Wang & Melmed 2000b); C-terminus deletion reinstates both functions, suggesting that variant PTTG forms may compete with other forms and that their balance may determine function (Wang & Melmed 2000b). The physiologic role and the significance of PTTG2 and PTTG3 remain unclear.

Subcellular localization

Subcellular PTTG localization, particularly the significance of cytoplasmic versus nuclear expression, remains controversial. Nuclear PTTG is thought to function as a securin, inhibitor of premature sister chromatid separation as well as a potential transcriptional activator, whereas the role of cytoplasmic PTTG remains unclear (Zhang et al. 1999b, Zhou et al. 2005, Kim et al. 2006a, Tfelt-Hansen et al. 2006). While differential PTTG localization may be due to the variations in cell lines and tumor types examined, cell cycle-dependent expression of PTTG may also account for the reported differences (Fujimoto et al. 1999, Zou et al. 1999, Hunter et al. 2003, Akino et al. 2005). A summary of reported PTTG expression patterns is found in Tables 1 and 2. Translocation of PTTG from cytoplasm to nucleus may be mediated by PTTG-binding factor (PBF), which contains a nuclear localization signal (Chien & Pei 2000). Another mechanism proposed to be involved in PTTG translocation involves the mitogen-activated protein kinase (MAPK) pathway. The expression of a constitutively active form of MAPKK (MEK1) facilitated PTTG translocation to the nucleus in Cos-7 cells (Pei 2000). Recent studies demonstrated that pituitary PTTG is a secretory protein in pituitary tumor cells (Minematsu et al. 2007). Recently, Wierinckx et al. (2007) found only nuclear PTTG expression to be predictive of aggressive pituitary tumor behavior. Thus, PTTG subcellular localization appears to be a significant factor in determination of its tumorigenic role.

Src homology (SH-3)-binding domain (SH-3BD)

The human PTTG C-terminus contains two proline-rich motifs that form an SH-3BD, and enables PTTG to interact with SH-3-containing proteins (Dominguez et al. 1998, Boelaert et al. 2004). Several studies have demonstrated the significance of SH-3BD in PTTG functions, including its transforming ability, proliferative effects, transactivation activity, induced sodium iodide symporter (NIS) expression, and as an inducer of hormone secretion in pituitary cells (Pei 1998, 2000, Heaney et al. 2001, Wang et al. 2001, Saez et al. 2002, Boelaert et al. 2007). The SH-3BD of PTTG may also be of importance for its angiogenic effects (Zhang et al. 1999b, Ishikawa et al. 2001, McCabe et al. 2002, Saez et al. 2002, Boelaert et al. 2004, Kim et al. 2006a,b,c), thus providing a potential target in the treatment of tumors overexpressing PTTG. The isolation of SH-3-containing proteins that interact with PTTG would illuminate on the mechanisms of PTTG SH-3BD function.
The phosphorylation of PTTG may facilitate its translocation to the nucleus (Pei 2000) and has been observed in a cell cycle-dependent manner (Ramos-Morales et al. 2000, Romero et al. 2001). Phosphoinositol-3-kinase (PI3K) and MAPK both physically interact with PTTG, thus suggesting that PTTG phosphorylation occurs via these two cascades (Wang & Melmed 2000a, Chamaon et al. 2005). PTTG phosphorylation by DNA-dependent protein kinase (DNA-PK) subunit (Ku70) has been demonstrated, but its significance is unclear (Romero et al. 2001).

### Table 1 Pituitary tumor-transforming gene (PTTG) nuclear and cytoplasmic localization in cell lines

<table>
<thead>
<tr>
<th>Study</th>
<th>Cell line</th>
<th>Cytoplasmic</th>
<th>Nuclear</th>
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</thead>
<tbody>
<tr>
<td>A. Predominantly cytoplasmic PTTG localization</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Kim et al. (2007c)</td>
<td>HCT166</td>
<td>+ + +</td>
<td>+</td>
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<tr>
<td>Akino et al. (2005)</td>
<td>Hepatocytes</td>
<td>+ + +</td>
<td>-</td>
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<tr>
<td>Mu et al. (2003)</td>
<td>HeLa</td>
<td>+ + +</td>
<td>+</td>
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<tr>
<td></td>
<td>Cos-7</td>
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<td></td>
<td>DU145</td>
<td>+ + +</td>
<td>+</td>
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<tr>
<td>Yu et al. (2000b)</td>
<td>JEG-3</td>
<td>+ + +</td>
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<td></td>
<td>3T3</td>
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<td>GH3</td>
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<td>AtT20</td>
<td>+ + +</td>
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<td></td>
<td>SKOV3</td>
<td>+ + +</td>
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<td></td>
<td>MCF-7</td>
<td>+ + +</td>
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<tr>
<td></td>
<td>COS-7</td>
<td>+ + +</td>
<td>+ +</td>
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<tr>
<td>B. Predominantly nuclear PTTG localization</td>
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<tr>
<td>Dominguez et al. (1998)</td>
<td>Jurkat cells</td>
<td>+</td>
<td>+ + +</td>
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<tr>
<td>C. Variable nuclear and cytoplasmic PTTG localization</td>
<td></td>
<td>Variable*</td>
<td>Variable*</td>
</tr>
<tr>
<td>Chamaon et al. (2005)</td>
<td>U87MG</td>
<td>Variable*</td>
<td>Variable*</td>
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<td></td>
<td>U138MG</td>
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<td></td>
<td>LN405</td>
<td>Variable*</td>
<td>Variable*</td>
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<tr>
<td>Mu et al. (2003)</td>
<td>A459</td>
<td>Diffuse</td>
<td>Diffuse</td>
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<td></td>
<td>DLD-1</td>
<td>Diffuse</td>
<td>Diffuse</td>
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<td></td>
<td>NIH3T3</td>
<td>Diffuse</td>
<td>Diffuse</td>
</tr>
</tbody>
</table>

++, predominant expression; +++, considerable expression; +, little expression; −, no expression; * variable, variable nuclear and cytoplasmic patterns.

### Table 2 Pituitary tumor-transforming gene (PTTG) localization in normal or neoplastic tissue

<table>
<thead>
<tr>
<th>Study</th>
<th>Tissue</th>
<th>Cytoplasmic</th>
<th>Nuclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Predominantly cytoplasmic PTTG localization</td>
<td>Pituitary adenomas</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>Minematsu et al. (2006)</td>
<td></td>
<td></td>
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<tr>
<td>Boelaert et al. (2003a,b)</td>
<td>Fetal brain</td>
<td>+</td>
<td>-</td>
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<td></td>
<td>Adult brain</td>
<td>+ + +</td>
<td>-</td>
</tr>
<tr>
<td>Boelaert et al. (2003b)</td>
<td>Pituitary adenomas</td>
<td>+ + +</td>
<td>-</td>
</tr>
<tr>
<td>Saez et al. (1999)</td>
<td>Pituitary adenomas</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>B. Predominantly nuclear PTTG localization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filippella et al. (2006)</td>
<td>Pituitary adenomas</td>
<td>−</td>
<td>+ + +</td>
</tr>
<tr>
<td>Genkai et al. (2006)</td>
<td>Gliomas</td>
<td>−</td>
<td>+ + +</td>
</tr>
<tr>
<td>Uccella et al. (2005)</td>
<td>Pituitary adenomas</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>Saez et al. (1999)</td>
<td>Breast adenocarcinomas</td>
<td>+ +</td>
<td>+ +</td>
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<tr>
<td></td>
<td>Lung adenocarcinomas</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Saez et al. (2002)</td>
<td>Hodgkin’s lymphoma**</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>C. Variable PTTG localization</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wierinckx et al. (2007)</td>
<td>PRL pituitary tumors***</td>
<td>Variable***</td>
<td>Variable***</td>
</tr>
<tr>
<td>Winnepenningckx et al. (2006)</td>
<td>Melanomas</td>
<td>Variable*</td>
<td>Variable*</td>
</tr>
</tbody>
</table>

++, predominant expression; +++, considerable expression; +, little expression; −, no expression; variable*, variable nuclear and cytoplasmic patterns; **, Reed–Sternberg cells; variable***, only cytoplasmic in non-invasive and invasive tumors, but both cytoplasmic and nuclear in aggressive–invasive ones.

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One study has shown that the expression of constitutively phosphorylated PTTG results in increased colony-forming ability and cell proliferation (Boelaert et al. 2004). It will be important to distinguish between the phosphorylated and non-phosphorylated PTTG expressions in future studies, to shed light on the role of phosphorylation in modulating PTTG function.

PTTG interacting factors

Sp1

Interaction of PTTG with Sp1, an important transcription factor involved in the regulation of several genes associated with cellular growth and differentiation (Clem et al. 2003) is well documented (Pei 1998, Kakar 1999, Wang & Melmed 2000a, Clem et al. 2003). It was recently proposed that the PTTG Sp1-binding region may play a role in the development of cardiac hypertrophy in transforming growth factor-β-inducible early gene knockout male mice (Rajamannan et al. 2007). The Sp1-binding site has been shown to be important in the regulation of PTTG transcriptional activity (Pei 1998, Kakar 1999, Wang & Melmed 2000a, Clem et al. 2003). Additionally, PTTG and Sp1 were found to cooperatively mediate G1/S-phase transition (Tong et al. 2007). Recently, Chesnokova et al. (2007) showed that PTTG binds to the p21 promoter region via Sp1, thereby suppressing p21 activity. Given the important function of transcription factors in neoplastic mechanism, including apoptosis, angiogenesis, and metastasis (Xie et al. 2006), characterization of their interaction with PTTG requires further investigation. Recent studies of Sp1 inhibition have shown promising anti-angiogenic and anti-fibrotic effects in prostate cancer as well as in kidney and lung fibrosis respectively (Chae et al. 2006, Yuan et al. 2008). Thus, the effects of Sp1 inhibition in neoplasms overexpressing PTTG should be investigated and may provide a potential therapeutic target in these neoplasms.

Other interacting factors

Physical interaction and binding of PTTG with several factors have been demonstrated. These include: S10, a ribosomal protein; HSJ2 (heat shock protein J2), a human homolog of DnaJ; Ku70, the regulatory subunit of DNA-PK; and c-myc, an important mediator of cell proliferation (Pei 1999, 2001, Romero et al. 2001, Hamid & Kakar 2004). S10 is constitutively expressed and associates with ribosomes, whereas HSJ2 is a novel homolog of human DnaJ, which mediates dissociation of several protein complexes (Pei 1999). It is possible that S10 and DnaJ regulate ribosomal association and dissociation of PTTG by directly interacting with its C-terminus (Pei 1999). Interaction of PTTG with Ku70 has been demonstrated in vitro and in vivo, PTTG forming a complex with Ku70 in intact HL-60 cells without affecting Ku70–Ku80 heterodimer formation (Romero et al. 2001). Interestingly, DNA double-stranded (dsDNA) breaks inhibited PTTG/Ku70 interaction, thus suggesting that increased DNA damage frees sequestered Ku70, which can then enhance DNA association with DNA-PK (Romero et al. 2001). The proposed hypothesis is that PTTG functions as a regulator of DNA-PK. Finally, the c-myc oncogene directly interacts with PTTG and is upregulated by it (Pei 2001, Hamid & Kakar 2004). Thus, PTTG, via interaction with a plethora of factors, is implicated in a host of cellular and neoplastic functions including oncogenesis and DNA damage repair. Further examination of the nature of such interactions would aid our understanding of the precise role of PTTG in cellular physiologic as well as neoplastic processes.

PTTG regulatory factors

Estrogen

Estrogen regulation of PTTG expression has been shown to occur (Heaney et al. 1999, 2002, Yin et al. 2001). In a study by Yin et al. (2001) in two out of the four rat strains examined, estrogen increased pituitary PTTG mRNA levels in parallel with estrogen induced pituitary tumor development. In a dose- and time-dependent manner, lactotrope tumors induced by estrogen in Fischer rats, showed PTTG expression within 24 h after estrogen treatment, reaching maximal levels after 48 h (Heaney et al. 1999). In mice, estrogen induced PTTG transcription in growth hormone (GH)/prolactin (PRL)-secreting GH3 cells and pituitary PTTG mRNA levels both increased concomitantly with the proestrus serum estradiol surge (Heaney et al. 2002). Estrogen regulation of PTTG expression may actually be cell specific. For example, in U87 glioma cells, 17β-estradiol (E2) does not affect PTTG mRNA levels (Tfelt-Hansen et al. 2004). Furthermore, PTTG is induced in oophorectomized rats treated with estrogen in a strain-dependent manner similar to the ability of estradiol to induce pituitary tumors in these rats, thus suggesting that PTTG expression may mediate E2-induced pituitary tumorigenesis (Pei 1998). Since most pituitary tumors express high levels of estrogen receptors as well as PTTG, anti-estrogen management of pituitary tumors may prove useful in suppressing PTTG expression and subsequently
suppress aggressive tumor behavior (Heaney et al. 2002, Wierinckx et al. 2007).

**Insulin**

An effect of insulin and insulin-like growth factor (IGF-I) on PTTG expression has been demonstrated in several cell lines (Chamaon et al. 2005, Thompson & Kakar 2005). For example, two insulin-responsive sequences have been found in the PTTG promoter region, thus implicating insulin and IGF-I regulation in PTTG expression (Kakar 1999). Treatment of human glioma cell lines (LN405 and U87MG) with IGF-I or insulin resulted in PTTG protein expression via PI3K and MAPK (Chamaon et al. 2005). In addition, in MCF-7 breast cancer cells, insulin and IGF-I treatment each stimulated PTTG mRNA and/or protein synthesis in a dose- and time-dependent way, peak PTTG mRNA levels being seen 48 h after insulin administration (Thompson & Kakar 2005).

**Other regulatory factors**


**Cell cycle regulation and PTTG**

By acting as a securin protein and inhibiting premature sister chromatid separation, PTTG plays an important role in mitosis. Cohesin binds sister chromatids during metaphase and is degraded by separin to allow sister chromatid separation at anaphase. It has been shown that PTTG blocks sister chromatid separation during metaphase by binding to separin and preventing cohesin degradation (Zou et al. 1999). The fact that PTTG−/− mice are viable indicates that PTTG is not required for sister chromatid separation, thus suggesting that there are other redundant mechanisms involved in this process (Yin et al. 2001). Nonetheless, the PTTG−/− mouse embryo fibroblasts showed aneuploidy and aberrant chromosome morphology, including many binucleate and multinucleate cells (Wang et al. 2001). The cells also demonstrated slower growth rate, shortened G1 and prolonged G2/M-phases, as well as premature centromere division (Mei et al. 2001, Wang et al. 2001). Although PTTG expression appears to be cell cycle dependent and has been shown to be abrogated immediately after mitosis (Zou et al. 1999, Yu et al. 2000b), being degraded by an anaphase promoting complex that targets PTTG (Zou et al. 1999), PTTG has recently been shown to mediate G1- to S-phase transition as well. The latter involves interaction with Sp1 (Tong et al. 2007). Whether the reported inconsistencies in the expression of PTTG in pituitary adenomas are due to its cell cycle-dependent regulation should be taken into consideration in future studies.

**P53-dependent and P53-independent apoptosis**

PTTG overexpression induced p53 upregulation and translocation to the nucleus, as well as apoptosis in MCF-7 breast cancer cells that express wild-type p53 (Yu et al. 2000a). PTTG also induced P53-independent apoptosis in MG-63 osteosarcoma cells deficient in p53, wherein p53 expression did not affect PTTG-induced apoptosis rate (Yu et al. 2000a). In insulin-secreting insulinoma MIN6 cells, PTTG transfection induced apoptosis, which increased overtime (Yu et al. 2006). Similarly, PTTG transfection of HEK293, a human embryonic kidney cell line expressing wild-type p53, induced elevated p53 protein expression and apoptosis, whereas transfection of PC-3 cells expressing a mutant p53 form did not (Hamid & Kakar 2004). The analysis of p53 promoter showed increased activity in MCF-7, PC-3, and HEK293 cells, implicating transcriptional activation of p53 by PTTG (Hamid & Kakar 2004). Additionally, PTTG transfection induced enhanced transcription and expression of the Bax gene, a pro-apoptotic factor, in a dose-dependent manner in MCF-7 and HEK293 cells. By contrast, there was no change in Bax expression in PC-3 cells that lacked a functional p53, suggesting that PTTG modulation of the pro-apoptotic factor occurs through p53. By contrast, inhibition of apoptosis by PTTG has also been shown. PTTG knockdown using short interfering RNA (siRNA) enhanced apoptosis in UV-irradiated HeLa cells, whereas PTTG overexpression suppressed UV-induced apoptosis in these cells (Lai et al. 2006). Similarly, transfection of SH-J1 hepatoma cells with siRNA against PTTG activated p53 and induced apoptosis (Cho-Rok et al. 2006). In HCT116 colorectal cancer cells, PTTG depletion resulted in p53-dependent cytotoxicity (Cho-Rok et al. 2006). Bernal et al. (2002) similarly found that PTTG transfection of H1299 cells inhibited p53-dependent apoptosis in a dose-dependent way. P53 apoptotic effects were further enhanced in PTTG−/− cells (Bernal et al. 2002). These results suggest that PTTG may exert its oncogenic effects at least in part by affecting p53-dependent pathways (Bernal et al. 2002) (Table 3).
PTTG and cell proliferation

The effect of PTTG on cell proliferation is unclear, as studies have reported contradictory findings. Whereas, in its functional capacity as a securin, PTTG overexpression is expected to reduce cell proliferation by arrest of mitosis and inhibition of sister chromatid separation, as a cell-transforming oncogene one would expect a pro-proliferative action. Several PTTG transfection/deletion as well as correlation studies support the latter notion (Heaney et al. 2002, Wang et al. 2003, Yao et al. 2003, Akino et al. 2005, Chesnokova et al. 2005, Tsai et al. 2005, Cho-Rok et al. 2006, Filippella et al. 2006, Genkai et al. 2006, Vlotides et al. 2006, Kim et al. 2007a, Rubinek et al. 2007).

In clinical studies, a correlation has been demonstrated between PTTG expression and cell proliferation (Tsai et al. 2005, Filippella et al. 2006). For example, its expression correlated with proliferation marker immunoreactivity, proliferating cell nuclear antigen (PCNA) or Ki-67, in pituitary adenomas (Filippella et al. 2006) and in leiomyomas (Tsai et al. 2005). Further support for the pro-proliferative role of PTTG was seen in PTTG−/− mice, which exhibited a reduction in β-cell islet mass and a decrease in cell proliferation (Wang et al. 2003). Similarly, in mouse models of pituitary tumorigenesis (Chesnokova et al. 2005) and follicular thyroid cancer (Kim et al. 2007a), PTTG ablation lead to decreased pituitary and thyroid cell proliferation respectively. PTTG expression was associated with proliferation of pituitary cells, hepatocytes, the U87 glioma cell line, and cultured leiomyoma cells (Heaney et al. 2002, Yao et al. 2003, Akino et al. 2005, Tsai et al. 2005, Cho-Rok et al. 2006, Vlotides et al. 2006). Several PTTG deletion studies using knockout or siRNA techniques have also confirmed the stimulatory role of PTTG in proliferation of bone marrow stem cells, as well as of glioma, sarcoma, hepatoma, and corticotrope cells (Chesnokova et al. 2005, Cho-Rok et al. 2006, Genkai et al. 2006, Rubinek et al. 2007).

By contrast, the anti-proliferative function of PTTG has also been demonstrated in animal as well as in cell
transfection studies (Pei & Melmed 1997, Mu et al. 2003, Yu et al. 2006). In contrast to one report of PTTG-induced cell proliferation in FTC133 follicular carcinoma cells (Kim et al. 2006b), other studies showed no correlation between PTTG expression and thyroid cancer cell proliferation (Saez et al. 2002, Boelaert et al. 2003a). Although PTTG expression was correlated with cell proliferation in the normal pituitary, no correlation was found in a series of 101 pituitary adenomas (Minematsu et al. 2006). PTTG suppression of cell proliferation may occur through induction of apoptosis and delay of mitosis (Akino et al. 2005).

Hence, it is currently unclear whether PTTG promotes or suppresses cellular proliferation. Dose dependency and/or phosphorylation status of PTTG may be additional factors affecting the stimulatory/inhibitory role of PTTG upon cell proliferation (Boelaert et al. 2003b, 2004). As neoplasia is intimately associated with cell proliferation, further studies to illuminate on the role of PTTG in proliferation will be important.

PTTG, FGF, and vascular endothelial growth factor (VEGF)

At least in part by induction of bFGF and VEGF angiogenic factors PTTG is involved in angiogenesis (McCabe et al. 2002, Kim et al. 2006a,b,c, 2007a, Minematsu et al. 2006). Transactivation of FGF-2 by PTTG has been well documented (Zhang et al. 1999b, Kim et al. 2006a, Tfelt-Hansen et al. 2006). In a mouse model of follicular thyroid cancer, PTTG deficiency resulted in reduced FGF-2, FGF receptor (FGF-R1), and VEGF levels (Kim et al. 2007a). Thus, an autocrine mechanism involving PTTG and bFGF may exist (Heaney et al. 1999, Tsai et al. 2005). Both PTTG and bFGF expressions were increased in acute leukemia, a positive correlation being noted between their expression levels (Cong et al. 2005). Concordant PTTG and FGF-2 expression patterns were detected in developing fetal brain cortex (Zou et al. 1999). PTTG-dependent bFGF and/or VEGF expression was observed in several cell lines (Ishikawa et al. 2001, McCabe et al. 2002, Hunter et al. 2003, Tfelt-Hansen et al. 2003, Chen et al. 2004b). It was found that PTTG promoted human umbilical vein endothelial cell proliferation and tube formation, as well as in vitro vessel growth (Ishikawa et al. 2001). In pituitary adenomas, a significant positive correlation was found between VEGF and PTTG mRNA expressions, as well as between PTTG and KDR (kinase insert domain receptor; VEGF receptor) mRNA (McCabe et al. 2002, Minematsu et al. 2006). Also, VEGF and PTTG showed co-localization (Minematsu et al. 2006). PTTG upregulation of inhibitor of DNA-binding-3 (ID3), an important mediator of VEGF induced angiogenesis and downregulation of thrombospondin-1 (TSP-1), an angiogenic inhibitor, has been demonstrated in FTC133 human follicular cancer cells (Kim et al. 2006b). Thus, PTTG appears to play an essential role in tumor angiogenesis and may provide a therapeutic target in neoplasms with high vascular density.

DNA repair and damage

PTTG binds to Ku70, the regulatory subunit of DNA-PK, which is involved in repair of double-stranded DNA breaks. The PTTG binding of Ku70 is inhibited by dsDNA breaks, with resultant freeing and activation of Ku70 and DNA repair mechanisms (Romero et al. 2001). Etoposide-induced dsDNA damage was elevated in PTTG-transfected HCT116 cells and human fibroblasts and associated with lower Ku70 function, thus suggesting that PTTG suppresses Ku70 activity (Kim et al. 2007a). Similarly, PTTG-deficient bone marrow stem cells (Rubinek et al. 2007), as well as pituitary gland cells (Chesnokova et al. 2007) showed upregulation of genes involved in DNA damage and repair pathways, supporting a role for PTTG in suppression of these pathways. PTTG inhibition and/or proteosomal degradation as a result of DNA damage has also been demonstrated (Romero et al. 2001, Zhou et al. 2003, Kim et al. 2007a). Additionally, a recent study by Bernal et al. (2008), demonstrated that loss of PTTG in cell culture lead to hindered cell proliferation after genotoxic stress (Bernal et al. 2008), providing a potential rationale for PTTG overexpression in many malignancies. These findings support a pivotal role for PTTG inhibition of DNA damage repair pathways necessitating more research on this aspect of PTTG action in malignancies.

Genetic instability

The association between PTTG overexpression and increased aneuploidy as well as genetic instability in tumors and cell lines is well established (Mu et al. 2003, Yu et al. 2003, Kim et al. 2005, 2007a). In colorectal cancers patients, such instability correlates with higher tumor grade, indicating that PTTG and genetic instability may be linked in these tumors (Kim et al. 2007a). PTTG expression also correlates with aneuploidy in melanomas (Winneppeninckx et al. 2006) and in follicular as well as papillary thyroid carcinomas (Kim et al. 2005). In vitro transfection of colorectal (Kim et al. 2007a) and follicular thyroid
carcinoma cells (Kim et al. 2005) as well as of HeLa and A549 cells (Mu et al. 2003) has been shown to induce both genetic instability and aneuploidy. Indeed, cell transfection with a non-degradable PTTG mutant was shown to result in incomplete cytokinesis and lack of chromosomal segregation in 51 out of 55 cultured cells (Yu et al. 2003). Interestingly, aneuploidy was also evident in the pituitary gland cells of PTTG-deficient mice (Chesnokova et al. 2007). Thus, aberrant PTTG expression is associated with aneuploidy, which may significantly contribute to its tumorigenic potential (Yu et al. 2003).

**Invasion**

Several studies have shown a correlation between tumor PTTG expression and invasiveness. This is true of endocrinologically functioning pituitary adenomas as well as colorectal and breast carcinomas (Zhang et al. 1999a, Heaney et al. 2000, Solbach et al. 2004). In the TRβPV/PV (thyroid receptor beta) model of follicular thyroid carcinoma, PTTG deficiency attenuates tumor vascular invasion and the occurrence of lung metastasis (Kim et al. 2007a). Recently, PTTG regulation of matrix metalloproteinase 2 (MMP-2) has been shown, which may account for decreased tumor invasiveness in the PTTG-deficient TRβPV/PV mice (Malik & Kakar 2006). In vivo, PTTG overexpressing HEK293 cells injected into nude mice were seen to induce increased MMP-2 expression and tumor formation. PTTG also facilitated endothelial tube formation and growth in 3D Matrigel matrix, effects mediated through MMP-2 expression (Malik & Kakar 2006). Finally, PTTG depletion by siRNA reduces tumor invasiveness in several glioma cell lines (Genkai et al. 2006). Thus, PTTG may stimulate mediators of tumor invasiveness, contributing to enhanced invasive potential of tumors. Accordingly, PTTG targeting to reduce or abrogate tumor invasiveness warrants further investigation.

**Transgenic models**

Several models of PTTG deficiency have been developed to study the role of PTTG in tumorigenesis and in other pathophysiologic processes, including diabetes (Wang et al. 2001, 2003, Abbud et al. 2005, Chesnokova et al. 2005, Donangelo et al. 2006, Fraenkel et al. 2006, Kim et al. 2007a). Although PTTG knockout (PTTG−/−) mice initially appeared viable and fertile (Wang et al. 2001), female subfertility, testicular and splenic hypoplasia, thymic hyperplasia, and thrombocytopenia have been noted in these mice (Wang et al. 2001). The use of PTTG knockout and transgenic mouse models has confirmed the tumor-inducing capacity of PTTG in the pituitary. Interestingly, PTTG deficiency in Rb+/− mice susceptible to pituitary tumor formation protects against pituitary tumorigenesis (Chesnokova et al. 2005), reducing tumor formation at 17 months from 86% in Rb+/−PTTG+/+ mice to 30% in Rb+/−PTTG−/− mice (Chesnokova et al. 2005). By contrast, pituitary-specific PTTG overexpression in Rb+/− mice is associated with pituitary hyperplasia and an increased incidence of adenoma development (Donangelo et al. 2006). Similarly, pituitary-specific PTTG overexpression in a wild-type background caused plurihormonal hyperplasia and induced microadenoma formation (Abbud et al. 2005). The TRβPV/PV mouse model of follicular thyroid carcinoma-producing distant metastasis has also been crossbred with PTTG−/− mice to create TRβPV/PV PTTG−/− mice. TRβPV/PV expresses a dominant negative mutation in the TRβ, which results in thyroid hormone resistance (Kim et al. 2007a,b). TRβPV/PV PTTG−/− mice had smaller thyroid glands compared with the PTTG+/+ mice and exhibited decreased thyrocyte proliferation as well as FTC aggressiveness reflected in slower tumor progression and increased survival (Kim et al. 2007a). In PTTG−/− male mice, PTTG deficiency affects pancreatic β-cell proliferation and induces diabetes (Wang et al. 2003, Fraenkel et al. 2006), often associated with weight loss, increased food uptake, and polyuria as well as decreased islet mass and β-cell proliferation (Wang et al. 2003). Thus, animal models have provided solid evidence of tumor-inducing capacity of PTTG, particularly in endocrine organs.

**PTTG in stem cells**

That PTTG−/− mice develop organ-specific hypoplasia suggests a role for PTTG in progenitor cell function (Wang et al. 2001). Indeed, the PTTG is transcribed in the one-cell stage of mouse development (Yao et al. 2003), the transcript showing a 39% increase in the zygote as compared with the oocyte. Furthermore, gene profiling of immature and mature oocytes showed differential expression of PTTG3 during oocyte meiosis (Yao et al. 2003). Thus, PTTG may play a role in zygote-stage gene activation (Yao et al. 2003). In a recent study, PTTG was shown to be essential for maintenance and proliferation of bone marrow stem cells (Rubinek et al. 2007). Gene profiling of proliferating and differentiated neural stem cells has shown PTTG to be one of the genes upregulated in proliferating neural progenitor cultures.
as compared with differentiated neural cells (Karsten et al. 2003). Similarly, PTTG is seen to be upregulated following treatment of human mesenchymal stem cells with bone morphogenic protein, the upregulation being associated with stem cell proliferation (Akino et al. 2003). These findings cumulatively suggest that PTTG expression in stem and progenitor cells is associated with proliferative potential.

PTTG in tumors
Endocrine tumors
Pituitary

PTTG expression in pituitary tumors has been demonstrated in several studies. In a study of 12 pituitary adenomas (six non-functioning, four GH, one PRL, and one adrenocorticotropic hormone (ACTH)), northern blot analysis showed elevated expression of PTTG mRNA as compared with its low or undetectable expression in non-tumoral pituitary (Saez et al. 1999). In addition, immunostaining of 36 pituitary adenomas showed cytoplasmic PTTG immunoreactivity in all but four pituitary adenomas (one PRL, one ACTH, and two non-functioning); nuclear staining was observed in only a few cells (Saez et al. 1999).

Several studies have investigated the potential predictive value of PTTG. A recent study of 25 PRL-secreting pituitary tumors showed that although PTTG expression did not per se predict aggressive behavior, nuclear PTTG staining was one of the histological features (numerous mitoses, high Ki-67 index, and nuclear labeling of PTTG and P53) distinguishing aggressive–invasive PRL tumors from those behaving less aggressively (Wierinckx et al. 2007). Interestingly, a comparative RT-PCR study of 54 pituitary adenomas (13 GH, 10 PRL, 1 ACTH, and 30 non-functioning) showed no correlation between tumor stage and PTTG mRNA levels in non-functioning adenomas, whereas in hormone-secreting tumors significantly higher PTTG expression was seen in invasive tumors (Zhang et al. 1999a). With respect to tumor recurrence, examination of 45 pituitary tumors (14 PRL, 8 GH, 6 ACTH, 11 luteinizing hormone (LH)/follicle-stimulating hormone (FSH), and 6 non-functioning) demonstrated nuclear PTTG immunoreactivity (using a monoclonal antibody) in 89% of the tumors (Filippella et al. 2006); its expression showed a strong correlation with Ki-67 immunopositivity, and was higher in recurrent tumors. The cut-off value separating recurring and non-recurring tumors was 3.3% for PTTG immunopositivity (60% sensitivity and 76% specificity). There was no significant correlation between PTTG immunopositivity and tumor size or radiologic grade, patient age, and gender or treatment (Filippella et al. 2006). In 27 of the 45 patients for which 1-year follow-up was available, a cut-off of 2.9% for both PTTG and Ki-67 positivity predicted recurrence versus non-recurrence, Ki-67 being the superior predictor (Filippella et al. 2006). These findings indicate the need for thorough investigation of the predictive value of PTTG as a marker of aggressive pituitary tumor behavior.

Other studies have investigated a potential correlation between PTTG expression and pituitary tumor subtype. PTTG mRNA levels in 40 pituitary tumors (12 GH, 5 PRL, 5 ACTH, and 18 non-functioning) showed elevated expression in GH-secreting adenomas (2.7-fold) compared with non-functioning adenomas (Hunter et al. 2003), suggesting cell type-dependent expression of PTTG. In another study, although it was higher in GH adenomas as compared with PRL and ACTH adenomas, this difference was not statistically significant (Wang et al. 2003). Additionally, Zhang et al. (1999a) found a significant difference in PTTG association with tumor stage between non-functioning and hormone-secreting pituitary neoplasms. This distinction suggests differences in the molecular mechanisms underlying PTTG-mediated tumor initiation and/or progression (Zhang et al. 1999a). It is of note however, that Minematsu et al. (2006), examining 101 pituitary adenomas (29 GH, 12 PRL, 6 ACTH, 1 FSH, 3 thyroid-stimulating hormone (TSH), and 50 non-functioning) revealed elevated PTTG mRNA levels in most pituitary adenoma subtypes; differences between them were not statistically significant. One study investigated anterior pituitary hormone levels in relation to PTTG levels in primary tumor cultures in vitro (Hunter et al. 2003). The results showed that only GH levels were significantly correlated with PTTG expression (Hunter et al. 2003). Presently, PTTG association with pituitary tumor subtype remains unclear.

As animal and in vitro studies have shown correlations between expression and function of PTTG and that of other factors including bFGF and VEGF, several studies have investigated such potential correlations in pituitary tumors. Using semi-quantitative RT-PCR analysis, PTTG and bFGF expressions were examined in 41 human pituitary adenomas (8 GH, 7 PRL, 1 ACTH, 1 TSH, 15 non-functioning, and 8 plurihormonal); significant correlation was found between PTTG and bFGF expressions ($R = 0.84$; Heaney et al. 1999). By contrast, although elevated PTTG, FGF, and FGF-R1 levels were elevated in a cohort of 111 pituitary adenomas, no correlation was
reported between PTTG and FGF or FGF-R1 expression (McCabe et al. 2003). Nonetheless, FGF-R1 expression was significantly higher in invasive adenomas (McCabe et al. 2003). In addition, PBF mRNA was elevated in all pituitary tumor subtypes. A significant correlation was observed between PBF and PTTG expressions, but no significant correlation was seen between PBF or PTTG mRNA expression and patient age, sex and estrogen status, and recurrence or invasion (McCabe et al. 2003). Examination of 103 (out of 111) pituitary adenomas with suitable RNA (15 GH, 4 PRL, 4 ACTH, 3 TSH, and 85 non-functioning) showed a significant positive correlation between PTTG and VEGF mRNA levels, as well as PTTG and KDR expression levels (McCabe et al. 2002). Similarly, in another study, many tumor cells demonstrated PTTG co-localization with VEGF (Minematsu et al. 2006). The analysis of CD34 immunopositivity and blood vessel distribution in tumors showed a high correlation between PTTG expression and microvascular density in GH-secreting pituitary adenomas (Minematsu et al. 2006). The authors suggested that PTTG may be involved in angiogenic activity in adenomas, and in cell proliferation in the normal pituitary (Minematsu et al. 2006). Although these findings support the hypothesis that PTTG promotes angiogenesis through VEGF, Hunter et al. (2003) did not find any correlation between PTTG levels in primary tumor cell cultures and in vitro VEGF secretion. Interestingly, they found VEGF secretion to be correlated with secretion of S100 protein, a marker of folliculostellate cells, suggesting that these cells are the source of VEGF (Hunter et al. 2003). That these results are inconsistent with the findings of other studies showing a correlation between PTTG, VEGF, and KDR expressions may be due to the in vitro setting. The relationship between PTTG and VEGF expressions in pituitary adenomas should be addressed in future investigations.

Mouse models of pituitary tumors have contributed to our understanding of PTTG action (Fig. 1; Heaney et al. 1999, Abbud et al. 2005, Chesnokova et al. 2005, Donangelo et al. 2006). In the Rb+/− mouse model, PTTG deficiency was protective for pituitary tumor development, delaying and reducing their incidence, through inhibition of cellular proliferation (Chesnokova et al. 2005). In a recent study, Chesnokova et al. (2007) demonstrated that PTTG deficiency in mice is associated with pituitary cell senescence, thereby restraining development of pituitary tumors. In keeping with the pro-tumorigenic role of PTTG in the pituitary, pretumorous pituitary of Rb+/− PTTG+/+ mice had elevated PTTG levels when compared with 2- to 4-month-old wild-type (WT) mice (Chesnokova et al. 2005).

PTTG-targeted overexpression in gonadotropes and thyrotropes, using the α-GSU (glycoprotein subunit) promoter, was associated with focal hyperplasia and

Figure 1 Pituitary PTTG1 content correlates with gland plasticity and tumor formation potential. On the left side of the inverted triangle are listed mouse models with descending pituitary PTTG1 content, with or without the combination with tumorigenic Rb+/−. Horizontal bars represent observed effects of the different genotypes on pituitary trophic status, which correlates with pituitary tumorigenic potential (arrow) (Reproduced with permission from Donangelo & Melmed 2005. Copyright 2007, The Endocrine Society).
increased neoplasia (Abbud et al. 2005). The hyperplastic and neoplastic LH and TSH cells overexpressed PTTG. Surprisingly, using double immunostaining, GH cells also expressed PTTG (Abbud et al. 2005). Similarly, pituitary-targeted PTTG overexpression in the Rb+/− also using the α-GSU promoter, resulted in focal pituitary hyperplasia. The incidence of anterior pituitary tumor development was increased in α-GSU.PTTGxRb+/− mice when compared with Rb+/− animals, there being no changes in the cumulative incidence of pituitary tumors when intermediate lobe tumors were also considered (Donangelo et al. 2006). These findings clearly support the role of PTTG in pituitary tumorigenesis (Fig. 1).

Recent characterization of PTTG-deficient mice showed that PTTG−/− mice pituitary glands exhibited senescence, associated with elevated p53, p21, and Bcl-2 expressions as well as slow cell cycle progression (Chesnokova et al. 2007). However, this senescence was not associated with telomere shortening, a feature of premature aging (Chesnokova et al. 2007).

The expression of PTTG in Fischer 344 rat pituitaries and human pituitary adenomas in vivo was shown to be estrogen dependent (Heaney et al. 1999). The levels of PTTG protein were suppressed by an average of 64% following anti-estrogen treatment of primary pituitary tumor cell cultures from four out of the eight invasive pituitary macroadenomas (Heaney et al. 2002). In three of the four tumors, reduced PTTG levels were associated with decreased PCNA expression (47%); reduced bFGF and Bcl-2 expressions were seen in two of the four tumors (Heaney et al. 2002).

One study showed low hPTTG2 expression in normal pituitary but moderately elevated levels in some adenomas, suggesting that, in addition to PTTG1, PTTG2 plays a role in pituitary tumor development and progression (Prezant et al. 1999). PCR analysis of DNA from 25 pituitary tumors (2 GH, 5 PRL, 2 ACTH, 2 TSH, and 14 non-functioning) failed to show any insertions/deletions or specific mutations. Thus, aberrant PTTG overexpression in pituitary adenomas may have other causes, including epigenetic factors such as hypomethylation (Kanakis et al. 2003).

**Thyroid**

Elevated PTTG levels in differentiated thyroid carcinomas have been documented by many studies (Heaney et al. 2001, Boelaert et al. 2003a,b, Kim et al. 2005, 2006b, Stratford et al. 2005). Its mRNA expression has been found to be increased in thyroid lesions (9 follicular adenomas, 2 follicular carcinomas, 8 papillary carcinomas, 15 thyroid hyperplasias, and 3 cases of Hashimoto’s disease), the highest expression being seen in a subset of follicular adenomas, follicular carcinomas, and thyroid hyperplasias (Heaney et al. 2001). RT-PCR analysis of 66 lesions (25 multinodular goiter, 14 Graves’ disease, 8 follicular, and 19 papillary cancers) showed that PTTG mRNA was 9.5-fold elevated in all 27 thyroid cancers as compared with normal thyroid tissue; no significant differences were noted between the two subtypes (Boelaert et al. 2003a). The expression of PTTG protein paralleled the mRNA findings as evidenced by western blot analysis. Intriguingly, PCNA expression was not significantly elevated in thyroid neoplasms, suggesting that PTTG expression and proliferation are likely unrelated in thyroid cancer (Boelaert et al. 2003a). Although there was no significant association between PTTG mRNA expression and the occurrence of metastasis, PTTG expression was significantly elevated in recurrent carcinomas. An independent association between PTTG mRNA and early tumor recurrence was confirmed by multiple regression analysis, suggesting that PTTG may be used as a prognostic marker in differentiated thyroid cancers (Boelaert et al. 2003a).

Furthermore, PBF and PTTG expressions correlated significantly in a cohort of 24 thyroid carcinomas (17 papillary and 7 follicular; Stratford et al. 2005). The expression of PBF was 3.3-fold increased over that of normal thyroid tissue. No evidence of mutations or sequence alterations was found in the coding region of PBF in the 24 thyroid carcinomas studied (Stratford et al. 2005). PBF immunohistochemical expression was evident both within nuclei and cytoplasm of normal thyroid glands and in thyroid carcinomas, great variability being seen between different samples (Stratford et al. 2005). The causal relationship between PBF and PTTG expressions was confirmed by the transfection of primary follicular thyroid cancer cultures with wild-type and SH-3 mutant PTTG, the former resulting in significant PBF induction. By contrast, SH-3 mutant PTTG transfectants did not induce significant PBF expression (Stratford et al. 2005). Stable PBF transfection of NIH3T3 cells resulted in colony formation (Stratford et al. 2005). In vivo, PBF overexpression stimulated tumor formation in nude mice (Stratford et al. 2005).

PTTG is associated with angiogenic pathways in thyroid carcinomas. A microarray study identified ID3 and TSP-1, a pro- and anti-angiogenic genes respectively, as two genes differentially regulated following PTTG transfection of primary thyroid cells (Kim et al. 2006b). Subsequent characterization of 34 thyroid carcinomas (8 follicular and 26 papillary) showed that similar to in vitro microarray results, PTTG and ID3
mRNA levels were significantly correlated in thyroid carcinomas; follicular thyroid cancers showed significantly higher ID3 mRNA levels being higher in follicular than papillary carcinomas (Kim et al. 2006b). Although TSP-1 levels in normal and thyroid carcinoma tissues were not significantly different, the results being highly variable, there was a significant inverse correlation between PTTG and TSP-1 mRNA levels. Correlation of clinicopathologic parameters and gene expression showed ID3 expression to be significantly higher in patients over 45 years of age; an independent association between ID3 and both patient age and tumor type was also demonstrated (Kim et al. 2006b). TSP-1 expression was independently associated with early tumor recurrence, TSP-1 expression in recurrences being lower than normal thyroids (Kim et al. 2006b). Taken together, these results suggest that PTTG mediates angiogenesis by regulating angiogenic promoters and inhibitors (Kim et al. 2006b). The same group also found a significant correlation between VEGF and ID3 expression in 38 thyroid carcinomas (9 follicular and 29 papillary; Kim et al. 2006b). In vitro treatment of FTC133 cells with VEGF elevated ID3 mRNA and protein expressions (Kim et al. 2006b). Elevated expression of KDR mRNA in these thyroid tumors was highly correlated with increased PTTG mRNA expression. KDR mRNA expression was also associated with early recurrence as well as lymph node metastasis (Kim et al. 2006b). The findings of these studies suggest that PTTG may play a major role in angiogenesis through its involvement in both pro- and anti-angiogenic pathways.

Another tumorigenic mechanism associated with elevated PTTG levels in thyroid neoplasms is DNA ploidy status. A correlation between PTTG expression and DNA ploidy status has been demonstrated in differentiated thyroid carcinomas (Kim et al. 2005). Fluorescent inter-simple sequence repeat PCR analysis of 19 thyroid carcinomas (10 follicular and 9 papillary) showed 6.7–72.7% increased genomic instability as compared with normal thyroid samples, a result significantly correlated with PTTG mRNA expression. Instability was greater in follicular than in papillary thyroid carcinomas (Kim et al. 2005). In vitro transfection of FTC133 thyroid follicular cells with PTTG resulted in a dose-dependent increase in genetic instability (Kim et al. 2005). Thus, PTTG overexpression in thyroid carcinomas may promote tumor development by inducing genetic instability.

Recently developed mouse models have been used to investigate the role of PTTG in thyroid tumorigenesis. The TRβPV/PV mouse develops metastasizing follicular thyroid carcinoma (Kim et al. 2007a,b,c). PTTG-deficient, TRβPV/PV mice developed smaller thyroids compared with the PTTG+/+ mice, the basis being inhibited thyroid cell proliferation (Kim et al. 2007a). These same mice had longer survival times, and the follicular thyroid carcinomas they developed were significantly less aggressive, having lower rates of vascular invasion and of lung metastasis; nonetheless, no difference in the overall incidence of follicular thyroid carcinoma development was noted (Kim et al. 2007a). These results indicate a role for PTTG in thyroid carcinoma progression rather than initiation (Kim et al. 2007a).

Cell transfection studies have also examined the role of PTTG in thyroid carcinomas (Kim et al. 2006b, Boelaert et al. 2007). Transient PBF and PTTG transfection of primary human thyroid cells resulted in NIS mRNA inhibition by 95 and 86% respectively (Boelaert et al. 2007). SH-3 mutant PTTG also suppressed NIS mRNA by 81%, somewhat less than intact PTTG. Iodide uptake was also reduced (59% WT PTTG, 34% SH-3 mutant PTTG, and 68% PBF). PTTG or PBF and reporter construct co-transfection in rat thyroid FRTL-5 and human thyroid cells showed repression of NIS promoter activity via the human upstream enhancer element. Mutations in various promoter regions demonstrated specifically that a complex paired box gene 8 (PAX8) upstream-stimulating factor (USF1)-response element was essential for NIS suppression by PTTG and PBF. Further analysis showed that the USF1-response element was particularly important for PTTG inhibition of NIS, whereas PAX8, which was required for PBF action, was not essential for this effect (Boelaert et al. 2007). Thus, PTTG appears to reduce thyroid carcinoma sensitivity to radioiodine treatment by downregulating NIS, affecting the prognosis of patients with differentiated thyroid carcinomas (Boelaert et al. 2007).

Studies of the role of PTTG in thyroid carcinomas have proven promising by suggesting PTTG as a potential marker of aggressive tumor behavior. PTTG functional studies in thyroid have shown its involvement in both promotion and inhibition of angiogenic pathways, as well as induction of genetic instability. Extensive studies to further characterize the role of PTTG in thyroid tumorigenesis are warranted.

Testis/ovary

Using RT-PCR, high PTTG expression was demonstrated in ovarian tumors, including germ cell tumors, seminomatous and non-seminomatous germ cell tumors of the testis, including gonadal epithelial, sex
cord, and stromal tumors (Puri et al. 2001). High levels of PTTG mRNA are present in all three major testicular cell types (Leydig, Sertoli, and germ (GC2) cells; Pei 1998). PTTG knockout mice have lower testicular weights by 45–55%, hypoplasia being more pronounced in sexually mature mice (Wang et al. 2001). This suggests that PTTG may be implicated in G1- and S-phases regulation during spermatogenesis (Wang et al. 2001). The expression of PTTG was stage specific in spermatocytes and spermatids (Pei 1999). Although northern blot analysis of developing rat testis detected low PTTG levels by day 14, PTTG mRNA levels increased significantly, reaching adult levels by day 28. Additionally, two PTTG interacting proteins, the ribosomal subunit protein S10 protein and a HSJ2, novel human homolog of DnaJ, a bacterial heat shock protein, were identified in testicular cells. It was proposed that PTTG interaction with S10 mediates its ribosomal targeting and that HSJ2 may facilitate PTTG dissociation from ribosomes. The significance of such interaction, however, remains unclear (Pei 1999).

In rat H500 Leydig tumor cells, high Ca2+ rapidly induced PTTG expression in a dose-dependent manner via transcriptional regulation and de novo PTTG mRNA synthesis (Tfelt-Hansen et al. 2003). Ca2+-induced PTTG stimulation was shown to be regulated by the calcium-sensing receptor (CaR), a G-protein-coupled receptor (Tfelt-Hansen et al. 2003). CaR is found to be expressed in many cancer cells (Li et al. 2005), but PTTG upregulation is specific to H500 Leydig tumor cells. Thus, whether PTTG as a potential marker in testicular cancers merits more studies.

Pancreatic islets

Evidence of the importance of PTTG in pancreatic β-cell proliferation emerged from PTTG−/− animals that presented with sexually dimorphic diabetes (Wang et al. 2003, Fraenkel et al. 2006, Yu et al. 2006). Examination of PTTG−/− pancreatic β-cell neogenesis showed defective β-cell proliferation in association with normal differentiation of pancreatic ducts (Yu et al. 2006). PTTG−/− male mice reportedly develop diabetes, weight loss, increased food uptake, polyuria, and hyperglycemia (Wang et al. 2003). Glucose tolerance tests elicited no response in PTTG−/− males, confirming glucose metabolism defects (Wang et al. 2003). Although insulin sensitivity was not altered, reduced β-cell mass, decreased β-cell proliferation, and low insulin content were noted (Wang et al. 2003). The protective effect of estrogen was evidenced by oophorectomy-induced fasting hyperglycemia in PTTG−/− mice (Wang et al. 2003). In addition to pancreatic β-cell expansion being hindered as the mice aged, insulin secretion was significantly lower in PTTG−/− males compared with male controls (13 vs 53 pg per islet per h; Wang et al. 2003). Further evidence for the development of sex hormone-dependent diabetes emerged from the study of gonadectomized PTTG−/− male mice treated with estrogen (Wang et al. 2003). Interestingly, manipulation of sex steroid presence affected diabetes development in PTTG−/− male mice. Gonadectomy of PTTG−/− males and their subsequent treatment with estradiol prevented hyperglycemia (Fraenkel et al. 2006). The onset of diabetes was delayed due to gonadectomy in PTTG−/− male mice, and was further prevented when gonadectomy was combined with estradiol treatment. These findings indicate that the development of diabetes in PTTG-deficient mice is sexually dimorphic and can be reversed by sex steroid treatment. Reversal is thought to take place subsequent to changes in peripheral insulin sensitivity as shown by insulin tolerance testing (Fraenkel et al. 2006). The mechanisms underlying sexually dimorphic diabetes development in PTTG-deficient mice are not understood. In one cell culture study, the measurement of intracellular insulin in enhanced green fluorescent protein-coupled PTTG (PTTG-EGFP)-expressing MIN6 insulinoma cells showed similar levels to non-transfected cells, indicating that PTTG does not influence insulin production or secretion (Yu et al. 2006). PTTG function as a securin was confirmed in these cells; thus, defective insulin secretion was attributed to defective β-cell proliferation rather than to a defect in cell differentiation or insulin production/secretion (Yu et al. 2006).

Non-endocrine tumors

Brain tumors

The expression of PTTG has been demonstrated in fetal as well as in adult brain (Boelaert et al. 2003b, Pemberton et al. 2007). In the ventricular zone of the developing ferret cortex, PTTG was expressed during E11.5–E13.5, peaked in its expression at E15.5, and was mainly found in mitotically active compared with differentiated tissue (Tarabykin et al. 2000). The levels of PTTG began to decrease at E18.5 and were detectable in P2, but were no longer expressed in adult brains (Tarabykin et al. 2000). In human fetal brain, PTTG mRNA and protein expressions were detectable from 7 weeks of gestation, significantly lower PTTG expression being seen in the first and second trimesters as compared with the adult cerebral
PTTG doses stimulates NT-2 cell proliferation (150% cell proliferation. Whereas transfection with lower the dose-dependent association of PTTG levels and 2007). These contradictory results can be explained by stimulation of cell proliferation (Pemberton et al. et al. 2003b). By contrast, FGF-2 expression patterns parallel to those of PTTG. In vitro, PTTG expression upregulates FGF-2 in fetal neuronal NT-2 cells. It is of note that on SH-3BD mutant form of PTTG is unable to induce elevations in FGF-2 (Boelaert et al. 2003b). The functional significance of these variations in PTTG expression in fetal and adult brains is unclear and calls for future studies.

PTTG expression is higher in neoplastic than in normal astrocytes, whereas no differences in PBF expression are noted (Tfelt-Hansen et al. 2004). Immunohistochemical staining of 44 glioma specimens (9 astrocytomas, 9 anaplastic astrocytomas, and 26 glioblastomas) showed nuclear PTTG expression, to be significantly higher in glioblastomas than in the lower grade astrocytomas (Genkai et al. 2006). In that PTTG immunoexpression showed a significant correlation with patient survival (Genkai et al. 2006), it was proposed to be a prognostic marker in such patients (Genkai et al. 2006). In yet another study, astrocytomas were found to show strong PTTG immunoreactivity, whereas normal brain tissue was immunonegative (Chamaon et al. 2005). Not surprisingly, PTTG mRNA expression was also strong in malignant astrocytic tumors (glioblastoma multiforme IV and anaplastic astrocytoma; Chamaon et al. 2005). Various nuclear and cytoplasmic PTTG localization patterns were noted in astrocytoma cell lines U87MG, U138MG, and LN405 as well as in rat embryonic astrocytes (Chamaon et al. 2005). The tumorigenic mechanisms associated with PTTG overexpression in brain neoplasms remain unclear.

Employing the siRNA technique, PTTG depletion in T98G; ON12, and U251 glioma cell lines resulted in a decrease in cell proliferation and invasion as assessed by cell counts and Matrigel invasion assay (Genkai et al. 2006). By contrast, in fetal neuronal NT-2 cells, PTTG repression by means of siRNA method resulted in increased Rad21 and separase levels as well as stimulation of cell proliferation (Pemberton et al. 2007). These contradictory results can be explained by the dose-dependent association of PTTG levels and cell proliferation. Whereas transfection with lower PTTG doses stimulates NT-2 cell proliferation (150% compared with a vector-transfected control), higher doses inhibit cell proliferation (86% compared with control; Boelaert et al. 2003b). An SH-3BD mutant form abrogated the proliferative effect of low-dose PTTG transfection (Boelaert et al. 2003b). The intimate role of PTTG in brain cell proliferation was further supported in a study of murine PTTG−/− model, where overexpression of separase and Rad21, involved in sister chromatid separation and cohesion respectively, was seen (Pemberton et al. 2007). Microarray analysis of the brain in these mice showed upregulation of several mitosis-associated proteins, including cyclin C and sestrin 2 (Pemberton et al. 2007). These results suggest that coordinated expression of PTTG and other mediators of mitosis, including separase and Rad21n, is required for the normal development of murine brain (Pemberton et al. 2007).

PTTG is tenfold downregulated in Wallerian degeneration slow (Wld(S)) mutant mice, which feature blocked Wallerian degeneration of injured axons and synapses, by the expression of a chimeric gene (Nmnat-1/truncated-Ube4b; Gillingwater et al. 2006). In vitro transfection of HEK293 and neuronal NSC34 cells with Wld(S)-EGFP construct also stimulates PTTG1 expression, suggesting that PTTG reduction plays a role in the neuroprotective effects of Wld(S) gene expression (Gillingwater et al. 2006).

Breast
PTTG expression in breast cancers has been documented (Saëz et al. 1999, Solbach et al. 2004, Ogbagabriel et al. 2005). High PTTG expression as determined by RT-PCR was demonstrated in both in situ and infiltrating ductal carcinoma (Puri et al. 2001). Lo et al. (2007) hypothesizing that genes associated with mitotic checkpoint may be a linked to breast cancer, investigated single nucleotide polymorphisms (SNPs) in these genes including PTTG. Their results indicated that PTTG SNPs were associated with increased risk of breast cancer development in women, suggesting that the loss of chromosomal integrity and aneuploidy may underlie breast tumorigenesis. However, the precise mechanism by which PTTG SNPs contribute to elevated incidence of breast cancer is unclear and requires further research.

PTTG was shown to be one of the four marker genes detected in circulating tumor cells in the blood of Taiwanese women with breast cancer (n=92). Detection of these genes, including PTTG, correlated with tumor size, histologic grade, the presence of lymph node metastasis, and tumor node metastasis (TNM)

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stage (Chen et al. 2006). In one cohort of 90 breast tumors, cytoplasmic and nuclear PTTG expressions were seen as compared with low cytoplasmic expression in normal breast tissue. PTTG expression was maximal in invasive ductal carcinoma, metastatic breast carcinoma, and breast carcinoma cell cultures. PTTG mRNA also correlated with invasion in breast tumors (Ogbagabriel et al. 2005). The analysis of PTTG mRNA in 72 breast carcinoma samples demonstrated a correlation between its expression and lymph node involvement as well as recurrence over a 5-year period of follow-up (Solbach et al. 2004). Evidently, PTTG overexpression in breast cancers is associated with tumor invasion and metastasis (Solbach et al. 2004, Ogbagabriel et al. 2005, Chen et al. 2006), but the contribution of PTTG to these aspects of neoplasia remains unclear.

In breast cancer MCF-7 cells, insulin and IGF-I stimulate PTTG mRNA and protein expressions in a time- and dose-dependent manner (Thompson & Kakar 2005). The PI3K pathway was implicated in PTTG induction by insulin and IGF-I in these cells, since PI3K inhibitors completely abrogated PTTG induction. It appears that MAPK was partially involved, as MAPK inhibitors partly reduced PTTG expression. Thus, the PI3K cascade seems to be the dominant pathway by which insulin and IGF-I exert their PTTG-inducing effect in breast carcinoma cells (Thompson & Kakar 2005).

Although PTTG upregulation has been demonstrated in breast carcinoma, PTTG was one of the genes downregulated after knockout of BRCA, a gene implicated in increased breast cancer risk (Bae et al. 2005). Microarray analysis showed downregulation of PTTG following BRCA siRNA knockout in prostate DU-145 and MCF-7 breast carcinoma cell lines (Bae et al. 2005). These findings echo the role of BRCA in regulation of several mitotic spindle checkpoint proteins. It appears that BRCA positively regulates PTTG levels, but the precise mechanisms of interaction between BRCA and PTTG remain unknown (Bae et al. 2005).

**Prostate**

In a study of 41 prostate carcinoma specimens, 34 (83%) were found to express PTTG, a relatively high figure when compared with 36% of non-tumorous prostate (Zhu et al. 2006). Transfection of PTTG into LNCaP prostate cancer cell line induced both cellular proliferation in vitro and tumor formation in nude mice (Zhu et al. 2006). PTTG knockout using antisense PTTG leads to suppressed cell proliferation (Zhu et al. 2006). Interestingly, PTTG was among the genes downregulated following genotoxic stress (irradiation) in C4-2 prostate carcinoma cells (Crosby et al. 2007). In addition, the transcription factor E2F4 co-immunoprecipitated with PTTG promoter regions, indicating that following irradiation and DNA damage, E2F4 downregulates PTTG expression in order to minimize the proliferation of DNA-damaged cells (Crosby et al. 2007). These studies implicate PTTG in proliferation of prostate carcinoma cells.

**Uterus**

The analysis of 23 leiomyomas and paired normal myometrial tissue samples demonstrated that PTTG mRNA and protein expressions were significantly higher in leiomyomas the results being independent of the menstrual cycles (Tsai et al. 2005). Immunohistochemical staining and western blotting showed higher PCNA expression in leiomyomas and a significant correlation between it and PTTG expression. In addition, bFGF induced elevated PTTG expression in a dose-dependent way within 48 h after initial treatment in cultured leiomyoma cells (Tsai et al. 2005). PTTG expression was associated with increased cell proliferation, as evidenced by increased expression of cell cycle regulators, cyclins A and B1, as well as by increased 3H-thymidine incorporation. PTTG transfection of leiomyoma cells in culture resulted in increased bFGF and VEGF mRNA expressions. In turn, bFGF induced significant PTTG expression, thus indicating the existence of a positive feedback loop between bFGF and PTTG in leiomyoma cells. The failure of estrogen and progesterone treatments of cultured leiomyoma to affect PTTG expression may partly explain the lack of efficacy of anti-estrogen and anti-progesterone treatments in eradication of tumor (Tsai et al. 2005).

**Other cancers**

High level of PTTG mRNA expression has also been documented in leukemia, lung carcinoma, lymphoma, and melanoma (McCabe & Gittoes 1999). High levels of PTTG mRNA and protein were also demonstrated by northern blot and immunohistochemistry in 78 non-small cell lung carcinomas (NSCLC) including large cell undifferentiated carcinoma as well as moderately differentiated squamous cell carcinoma, levels being very low or undetectable in normal lung tissue (Honda et al. 2003). PTTG immunohistochemical staining of 136 primary small cell lung carcinomas (SCLC) and 91 primary NSCLC samples showed that 98% of NSCLC tumors and 64% of SCLC tumors exhibited PTTG expression. SCLC patients in whom the tumors had no or low-level PTTG immunopositivity had a shorter survival time (265 vs 379 days) compared with those with higher PTTG levels (Rehfeld et al. 2006). By contrast, in NSCLC patients, a reverse correlation was observed; strong PTTG expression was associated with a shorter survival time (306 vs 463 days; Rehfeld et al. 2006). PTTG expression also correlated with more aggressive NSCLC tumors, lymph node, and distant metastases being more frequent (Rehfeld et al. 2006). The lung tumor cell lines A549 (lung carcinoma) and H1299 (NSCLC) also showed high levels of PTTG (Solbach et al. 2005, Kakar & Malik 2006).

In a cohort of 89 patients with squamous cell carcinomas of the head and neck (HNSCC), PTTG mRNA was significantly elevated when compared with very low or undetectable levels in normal adult mucosa of the upper respiratory and digestive tract (Solbach et al. 2005). Furthermore, the levels correlated with tumor stage. In addition, both lymph node stages, the most significant prognostic indicator in HNSCC, as well as tumor recurrence correlated significantly with PTTG levels. Thus, PTTG may serve as a prognostic marker in HNSCC (Solbach et al. 2005).

Microarray analysis of esophageal carcinomas identified PTTG1 as one of the four potential tumor markers, 2.4-fold higher expression levels being found in a series of 20 primary esophageal carcinomas as compared with non-tumorous tissue (Sato et al. 2006). As demonstrated by immunohistochemical staining of 69 primary esophageal squamous cell carcinoma (ESCC) samples, in ESCC, PTTG co-localized with β-catenin (Zhou et al. 2005). The latter accumulated within the cytoplasm (as opposed to its usual location at the cell membrane) in most cases, 70% of which also showed PTTG overexpression. Thus, there was a significant correlation between PTTG and β-catenin expressions (Zhou et al. 2005). When compared with paired normal samples, 67% of the 27 ESCCs expressed higher PTTG mRNA levels (Zhou et al. 2005). Several human cancer cell lines such as human ESCC cell lines KYSE180, KYSE410, KYSE510, and other cancer cell lines showed strong PTTG mRNA expression. PTTG promoter activity was assessed by a reporter construct and was found to be higher in these cell lines, confirming high transcriptional activity of the PTTG (Zhou et al. 2005). T cell factor (TCF)-4-binding element was found to play an essential role in transcriptional regulation of PTTG by the β-catenin/TCF pathway (Zhou et al. 2005). In 37 of the 48 ESCCs, PTTG mRNA was found to be highly overexpressed when compared with paired normal samples. Examination of clinicopathologic parameters showed PTTG mRNA expression to be significantly higher in tumors of high pathological stage IV disease, as compared with ones of stages 0–III (Shibata et al. 2002). PTTG expression also correlated with lower survival times, as illustrated by Kaplan–Meier curves (8.5 vs 14 months), but it was not an independent prognostic indicator on multivariate analysis (Shibata et al. 2002). Nonetheless, univariate analysis showed PTTG expression to be a prognostic indicator of survival (Shibata et al. 2002).

In a series of 68 colorectal carcinomas, PTTG expression was elevated in 71% (Heaney et al. 2000). It was also increased in 85% of 20 colonic polyps (Heaney et al. 2000). High microvascular density and lymph node involvement significantly correlated with higher PTTG expression (Heaney et al. 2000). Examination of 22 colorectal carcinoma specimens showed PTTG mRNA and protein expressions to be higher than in matched normal tissues (Kim et al. 2007c). PTTG expression also correlated with tumor stage. In vitro examination of PTTG overexpression in the HCT166 colorectal cell line found that PTTG induced genetic instability in a dose-dependent manner. In the 22 resected colorectal cancer specimens, such instability also correlated with higher tumor grade, suggesting that PTTG expression and genetic instability are linked in the pathogenesis of colorectal cancer (Kim et al. 2007c). In a series of 75 primary gastric adenocarcinomas, PTTG immunohistochemical expression was observed in 65% of cases, a significant increase being seen in metastatic lymph node deposits as compared with primary tumors; the observation implicates PTTG in the development of lymph node metastasis (Wen et al. 2004).

A study of 62 hepatocellular carcinomas showed increased PTTG expression compared with nontumorous liver, there being a significant correlation between PTTG expression, α-fetoprotein increase and microvascular density (Fujii et al. 2006). PTTG expression was also an independent prognostic marker
of disease-free survival (Fujii et al. 2006). In yet another series of 147 hepatocellular carcinomas, PTTG expression was found in 61% but showed no correlation with survival (Su et al. 2006). PTTG expression was, however, correlated with increased α-fetoprotein levels and higher tumor stage (Su et al. 2006).

In a series of 29 nodular and 29 superficial spreading melanomas, PTTG correlated with aneuploidy as well as the nodular type (Winnepenningx et al. 2006). The expression of PTTG mRNA in multiple myeloma (N=33) has been shown to be significantly higher than in normal controls (Wang et al. 2006). Assessment of 28 patients with leukemia, lymphoma, or myelodysplastic disease showed that 70% of patients overexpressed PTTG compared with normal control samples, thus implicating the role of PTTG in hematopoietic disease (Dominguez et al. 1998). Immunohistochemical analysis of 72 B cell and 37 T cell lymphomas, as well as 41 Hodgkin’s lymphomas showed that 70% of B- and T-cell lymphomas and 73% of Hodgkin’s lymphomas showed elevated PTTG expression. Among B-cell tumors, plasma cell tumors, follicular lymphomas, and diffuse large cell lymphomas showed the highest expression levels, but T cell lymphomas generally did show intense immunoreactivity. Northern blot analysis of 35 of these lymphomas confirmed the immunohistochemical findings and demonstrated alteration of PTTG at transcriptional levels. These results suggest that PTTG is involved in lymphoma initiation and/or progression (Saez et al. 2002).

In summary, PTTG is promising as a prognostic marker in a variety of neoplasm examined. In lung and hepatocellular carcinomas as well as in ESCC, PTTG expression levels are predictive of survival time. Additionally, PTTG levels are directly correlated with tumor stage in HNSCC, ESCC, and colorectal carcinomas. Higher PTTG levels in colorectal carcinomas are also associated with metastasis. Further studies are needed to better characterize and standardize the predictive role of PTTG as a marker of aggressive tumor behavior.

PTTG and treatment

Several in vitro and in vivo models have demonstrated the therapeutic potential of PTTG (Horwitz et al. 2003, Chen et al. 2004a, Tfelt-Hansen et al. 2004, Solbach et al. 2005, Cho-Rok et al. 2006, Genkai et al. 2006, Kakar & Malik 2006). Disruption of PTTG pathways in PRL- and GH-secreting pituitary GH3 cells using a C-terminus truncated form of PTTG suppressed PRL promoter activity as well as secretion (Horwitz et al. 2003). In addition, s.c. injection of GH3 cells transfected with truncated PTTG demonstrated the formation of smaller pituitary tumors in rats (Horwitz et al. 2003). These results suggest PTTG may be a therapeutic target in the treatment of PRL-secreting pituitary adenomas and control of their hypersecretion (Horwitz et al. 2003). Yet another study showed PTTG depletion by siRNA in U87 glioma cells, resulting in inhibition of cell proliferation associated with reduced BrdU incorporation (Tfelt-Hansen et al. 2004). Related studies showed similar results. For example: a) siRNA knockdown of PTTG in FTC133 follicular thyroid carcinomas cells suppressed ID3 levels and increased TSP-1 levels, a result in keeping with PTTG stimulation of angiogenic factors (Kim et al. 2006c), b) PTTG antisense transfection of the SK-OV-3 ovarian carcinoma cell line both reduced bFGF expression by 53% and suppressed colony formation (Chen et al. 2004a), c) PTTG knockout using siRNA in the lung cancer cell line H1299 resulted in reduced colony formation as assayed by the soft agar colony formation assay (Kakar & Malik 2006), d) nude mice injected with PTTG siRNA-transfected H1299 cells inhibited tumor development and growth without adverse effects (Kakar & Malik 2006), and e) the expressions of Ki-67, bFGF, and CD34 were significantly reduced in PTTG siRNA-transfected tumors (Kakar & Malik 2006).

Transfection of HeLa-S3 human cervical cancer cells with antisense PTTG mRNA decreased cell viability and reduced the mitotic index. Alterations in cell phenotype included aberrations in sister chromatid separation, DNA bridges, and apoptosis (Solbach et al. 2005). Owing to its anti-tumor effects, PTTG antisense has been proposed as a potential therapeutic target (Solbach et al. 2005). Similarly, simply depleting PTTG in T98G, ON12, and U251 glioma cell lines using the siRNA technique resulted in decreased cell proliferation and reduced invasion as studied by cell counts and the Matrigel invasion assay (Genkai et al. 2006). Antisense injection of mice was unassociated with side effects (Genkai et al. 2006). Adenoviral vector encoding siRNA to silence the PTTG resulted in PTTG depletion and in the SH-J1 hepatoma cell lines, induced apoptosis. In HCT116 colorectal cancer cells, PTTG silencing also showed dose-dependent cytotoxicity. In vivo, growth inhibition of Huh-7 hepatoma cell lines and SH-J1 tumor xenografts in nude mice was achieved by PTTG silencing via siRNA. The results suggest the therapeutic potential of PTTG silencing in the treatment of hepatic tumors as well as other neoplasms expressing high levels of PTTG (Cho-Rok et al. 2006).
Conclusion

PTTG is involved in a wide array of physiologic and oncogenic functions. Molecular and clinical studies implicate PTTG in cell cycle regulation, DNA repair, genetic instability, transactivating function, angiogenesis, and tumor invasion. PTTG-deficient mouse models have contributed to our understanding of PTTG function as an oncogene. In many neoplasms, PTTG expression correlates with invasiveness and tumor stage, thus suggesting that PTTG may be a prognostic biomarker. Indeed, PTTG downregulation in vitro and in vivo shows promise as a therapeutic tool. Further investigations of the PTTG pathway and its role as a biomarker and therapeutic target are warranted.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

Authors are indebted to the Jarislowsky Foundation and the Lloyd Carr–Harris Foundation for their generous support.

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