

The Emerging Role of Pituitary Tumor Transforming Gene in Tumorigenesis

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Pituitary tumor transforming gene (PTTG) is a newly discovered oncogene, and serves as a marker of malignancy grades in several forms of cancer, particularly endocrine malignancies such as pituitary adenomas. PTTG appears also to have a role in the genesis of some types of cancer. Also known as a human form of securin, PTTG is an anaphase inhibitor that prevents premature chromosome separation through inhibition of separase activity; hence, its degradation is required to start anaphase. Through this important function, PTTG participates in several key cellular events such as mitosis, cell cycle progression, DNA repair and apoptosis. The physiological importance of PTTG is indicated by the study of PTTG-null mice that have cell growth abnormalities in testis and pancreatic beta cells. Overexpression of PTTG has been observed in thyroid and colon cancers. In addition, 90% of pituitary adenomas overexpress PTTG, qualifying it as the best available marker for this disease. Although the exact mechanism is unknown, PTTG participates in the pathogenesis of various tumors, including pituitary tumors, by inducing aneuploidy and upregulating FGF-2, a potent mitogenic and angiogenic factor. Various growth factors, nuclear factors and hormones regulate PTTG expression in different tumor cells, which could be important to understand in order to obtain insight into the tumorigenic and tumor progression process. Here, we review the current knowledge of the biological and pathophysiological roles of PTTG.

Keywords: Aneuploidy; Angiogenesis; Cell cycle; Pituitary adenoma

Pituitary tumor transforming gene (PTTG) is a pituitary tumor-specific oncogene that was discovered a decade ago. It is expressed in pituitary tumors but not in normal pituitary cells and is currently the best available marker of pituitary adenomas. Available data increasingly implicate PTTG overexpression with malignancy grades in thyroid, colon and astroglial cancers. Furthermore, there is a rising body of evidence suggesting that PTTG, and its binding protein, participate in the genesis of some cancers.

Equal separation of sister chromatids is an essential step for chromosome segregation and is fundamental to the inheritance of genetic material. In somatic cells, sister chromatid separation occurs once in the cell cycle during a brief mitotic step called anaphase. Anaphase is triggered by a protease called separase, which is normally kept inactive by an associated inhibitor called securin.¹⁻³ When PTTG is ubiquitinated by the anaphase-promoting complex or cyclosome (APC/C),⁴⁻⁶ it is destroyed by the proteasome,

leading to separase activation, Scc1 cleavage, loss of chromosome cohesion and onset of anaphase.^{2,3} Loss of chromosome cohesion allows segregation of sister chromatids toward opposite spindle poles during anaphase. Under aberrant conditions, when sister chromatids are pulled to the same pole, aneuploid daughter cells are produced.

Aneuploidy, frequently noted in human cancers, is characterized by alterations in the number of chromosomes due to defects in a cell cycle surveillance mechanism, called the spindle checkpoint, resulting in chromosomal instability. PTTG, also known as human securin, is a mitotic checkpoint gene involved at the metaphase-anaphase interface. PTTG is abundantly expressed in several neoplasms. Its overexpression in human normal and cancer cells induces aneuploidy with micronuclei and multiple nuclei. PTTG also exhibits transforming activity, and its overexpression may lead to tumorigenesis. In this article we review the current developments in understanding the role of PTTG in tumorigenesis and the underlying biological mechanisms.

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Cloning, Characterization and Function of PTTG Indicate Its Tumorigenic Role

PTTG was first identified by differential display polymerase chain reaction (PCR) between rat pituitary tumor cells (GH4) and normal pituitary tissue. It was observed that PTTG mRNA is exclusively expressed in pituitary tumor but not in normal pituitary tissue.⁷ Full-length PTTG mRNA, cloned from the GH4 cell line, is 974 base pairs (bp) with an open reading frame for 199 amino acids. The human PTTG family comprises at least three homologous proteins, of which the structure and function of only PTTG-1 have been studied in detail.^{8,9} The gene for human PTTG is located on chromosome 5 and encodes a protein of 202 amino acids (22 kDa) with no significant homology to other known proteins.¹⁰ PTTG is a multidomain protein consisting of a transactivation domain, a domain required for ubiquitin-mediated proteolysis and a DNA-binding domain (mapped from amino acids 60-118).¹¹ At the end of the metaphase of cell division, PTTG is rapidly degraded by an anaphase promoting complex that recognizes a conserved motif within its N-terminus called the destruction box, releasing the inhibition of separin, which in turn leads to the degradation of separin proteins.¹ Eventually PTTG is degraded by the ubiquitin pathway to ensure equal sister chromatid separation.¹²

Like any other oncogene, PTTG has normal physiological functions that were revealed by deleting the PTTG gene in mice. These PTTG-deficient mice exhibit a variety of cell growth abnormalities, including testicular and splenic hypoplasia, thymic hyperplasia and, diabetes mellitus secondary to defective beta-cell proliferation.¹³ However, the tumorigenic potential of PTTG was demonstrated by its ability to transform NIH-3T3 fibroblasts following PTTG overexpression. This transformation resulted in morphological changes and anchorage-independent growth of PTTG transfectants in soft agar.⁸ Furthermore, when nude mice were injected with PTTG-expressing NIH-3T3 cells, they developed large tumors at the injection sites, suggesting that PTTG is capable of cellular transformation without the requirement of a complimentary oncogene and that it is tumorigenic *in vivo*.⁷ These observations implicated PTTG in the genesis and progression of cancer. PTTG has dual roles in tumorigenesis. First, through its action as a key regulator of cell division, overexpression of PTTG initiates genetic instability. Second, as a transactivator of growth factors, high PTTG expression induces fibroblast growth factor (FGF)-2, vascular endothelial growth factor (VEGF) and other proangiogenic genes. Thus, PTTG appears to be both an initiator and a promoter of tumorigenesis, and one whose expression has been correlated with tumor progression and metastatic potential across a plethora of tumor types as discussed below.

PTTG and Pituitary Tumors

Pituitary adenomas are neuroendocrine tumors that produce endocrine and metabolic alterations and are the most common benign intracranial neoplasms. The genesis of pituitary

tumors is controversial and largely unclear. Several mechanisms, including intrinsic alterations in pituitary cells, hypothalamic dysregulation and locally produced growth factors, seem to underlie tumorigenesis. X-chromosomal inactivation analysis revealed that the majority of pituitary adenomas, as with most tumors, derive from the clonal expansion of a single pituitary cell. This transforming event provides selective growth advantage to the mutant cell, resulting in secondary mutations and/or alterations in the growth factors favorable to clonal expansion and tumor progression. Because of their clonal composition, most cases of pituitary tumor display excessive secretion of any given pituitary hormone which results in endocrine diseases such as Cushing's syndrome for adrenocorticotrophic hormone (ACTH), acromegaly for growth hormone, hyperprolactinemia for prolactin and thyrotoxicosis for thyroid-stimulating hormone (TSH).

Gross aneuploidy is reported in sporadic pituitary adenomas at a prevalence of approximately 50%. Genetic instability, or chromosomal alteration, is a classical mechanism of tumor formation, generating mutations in or overexpression of oncogenes and tumor suppressor genes. Sporadic and low-frequency mutations of oncogenes and tumor suppressor genes are observed in pituitary adenomas,^{14,15} but give no specific information about tumor development. Activating mutations of the oncogene, *Gsp*, in 40% of cases of growth hormone-producing adenomas has served as the best marker for pituitary adenoma, thus far.¹⁵ Cloning of PTTG was crucial in revealing that approximately 90% of pituitary adenomas overexpress this gene compared with normal pituitary tissue, which expresses very little PTTG. Several reports show that, although PTTG was upregulated in all histological subtypes, its expression is highest in ACTH-secreting and nonfunctioning pituitary tumors.¹⁶⁻¹⁸ One study reported higher expression levels of PTTG, PTTG binding factor, and FGF-2 and its receptor FGFR1 mRNAs in pituitary adenomas as compared to normal tissue in 121 *ex vivo* pituitary tumors. In addition, FGFR1 expression was higher in hormone-secreting pituitary tumors that invade bone (the sphenoid bone) than in those that do not.¹⁹ These data suggest a causative association between the PTTG signaling pathway and FGF-2 in pituitary adenomas. In the clinical setting, where the measurement of conventional markers of aggressive tumors (e.g., expression of proliferating cell nuclear antigen (PCNA), Ki67 or bromodeoxyuridine labeling) have failed in cases of pituitary adenomas,²⁰⁻²³ PTTG and possibly FGF-2 and FGFR1 are currently the best available markers of pituitary tumors. The high sensitivity of PTTG as a marker of pituitary adenomas may be due to the fact that this protein participates in the transition of normal cells to benign tumors as in the case of pituitary adenomas, whereas PCNA and Ki67 overexpression is generally associated with the malignant transition of the tumors. In this regard, it would be interesting to determine whether the conventional treatments of pituitary tumors are accompanied by downregulation of PTTG expression, which could then be applied to monitor treatment efficacy.

Abundant expression of PTTG in all adenoma subtypes indicates its importance in the genesis of pituitary tumors. This was shown elegantly in a mouse model where targeted overexpression of PTTG in pituitary tissue resulted in focal pituitary hyperplasia and adenoma formation.²⁴ The cellular mechanism involves specific downregulation of a cyclin-dependent kinase inhibitor, p21, whose normal expression restrains tumor formation. In addition, mice bearing a single allele of the tumor suppressor gene, retinoblastoma (Rb), develop pituitary tumors with almost complete penetrance.^{25,26} Deleting the PTTG gene in Rb^{+/-} mice has been shown to exert a protective effect on the high levels of penetrance observed in Rb^{+/-} PTTG^{-/-} mice.²⁷ These studies suggest that the genesis of pituitary adenoma involves downregulation of Rb and p21 expression due to PTTG overexpression, since Rb stimulates p21 promoter activity.

PTTG is also known to promote tumor progression by stimulating expression and secretion of basic fibroblast growth factor (bFGF),^{8,18} a potent mitogenic and angiogenic factor.^{28,29} Overexpression of PTTG in transfected cells stimulates the expression of bFGF, and a point mutation of the putative SH3-binding site within the C-terminal region of PTTG abrogates an increase in bFGF expression.³⁰ In 2001, Pei³⁰ demonstrated that PTTG transactivates bFGF transcription by interacting with PTTG-binding factor, which suggests that transcriptional activation is one of the important activities of PTTG. The PTTG transactivation domain has been mapped to a region between amino acids 119 and 164, which includes sequences that are acidic and proline-rich, characteristic of the transactivation domains of many other transcription factors. Transcriptional regulation of FGF2 expression by PTTG appears to require PTTG-binding factor to initiate PTTG's entry into the nucleus.

In the pituitary, members of the FGF family regulate pituitary growth and the transcription and secretion of prolactin.³¹⁻³³ FGF-4 is also found in pituitary tumors,³⁴ where its expression is higher than in normal cells and is detectable in large prolactinomas. In experimental prolactin-secreting tumors, FGF-4 correlates with proliferation.³³ Finally, clinical studies³⁵ reveal that PTTG-binding factor, FGF-2 and VEGF are elevated in pituitary tumors and mostly correlate with PTTG levels. These results corroborate experimental reports of the role of PTTG in angiogenesis and mitogenesis of pituitary adenomas. However, one group showed lack of correlation between PTTG overexpression and VEGF production in somatotrophic tumors, suggesting a more complex cause and effect relationship between PTTG overexpression and the pathogenesis of pituitary adenoma.³⁶ Despite this complex relationship, it is safe to suggest that paracrine activation of growth factor expression and secretion accounts for the induction of pituitary tumor formation by PTTG¹⁸ and thus, qualifies it as a potential target for drug development for the treatment of pituitary adenoma. PTTG expression has also been correlated with progression of malignancy in other tissues.

PTTG and Other Cancers

Thyroid cancer

PTTG is expressed at high levels in a variety of human primary tumors in addition to pituitary adenomas and tumor cell lines (table 1). Thyroid cancer is the most common endocrine malignancy and, like pituitary tumors, is found more frequently in women. This form of cancer carries a fairly good prognosis, with a 10-year survival rate for differentiated thyroid carcinoma of approximately 90%.³⁷ The prognosis of patients with thyroid carcinoma is affected by factors related to the patient, the disease and the treatment. Disease- and patient-related positive factors are 1) a small and histologically well-differentiated primary tumor, 2) tumors limited to the thyroid gland, and 3) young age.³⁸ Currently, there is no good molecular marker for the prognosis of the disease. A study investigating the expression of PTTG in thyroid neoplasias found PTTG mRNA to be upregulated in 50% (4/8) papillary carcinomas, 100% (2/2) minimally invasive follicular carcinomas, 77.8% (7/9) follicular adenomas, and 66.7% (10/15) thyroid hyperplasias.³⁹ Another study also showed bFGF and its receptor to be upregulated in thyroid neoplasias.⁴⁰ The causal link between PTTG and bFGF was established in FRTL5 (Fisher rat thyroid cell line 5) thyroid cells, in which overexpression of PTTG upregulated bFGF secretion. The most convincing data regarding the oncogenic potential of PTTG in thyroid cells were obtained by overexpressing PTTG in FRTL5 cells and in primary human thyroid cells *in vitro*, which resulted in transformation and produced a dedifferentiated, neoplastic phenotype. This observation was supported by a separate group reporting upregulation of PTTG and bFGF in differentiated thyroid cancers.⁴¹ In addition, expression of PTTG-binding protein mRNA was found to be higher in differentiated thyroid carcinomas than in normal thyroid and was independently associated with tumor recurrence.⁴² To summarize, the data suggest that PTTG, PTTG-binding protein, and bFGF are potential prognostic markers for thyroid malignancies, and the use of these markers may in the future guide the therapeutic regimen offered to the patients. However, the roles played by PTTG, PTTG-binding protein and bFGF in the genesis of thyroid cancer are uncertain at present.

Colon cancer

Colon cancer is one of the most common and most deadly forms of cancer in the western world. An investigation of PTTG expression in samples of colorectal polyps and tumors showed significant upregulation of PTTG mRNA in 68 consecutive unselected patients.⁴³ Overexpression of PTTG also correlated with a higher tumor grade (stage C and D) than the lower grade (stage A and B) carcinomas of the Duke classification. Furthermore, highly vascular colorectal carcinomas and adenomas express higher levels of PTTG mRNA than less-vascular samples. Finally, Heaney et al⁴³ demonstrated that colorectal tumors with lymph node invasion had higher PTTG expression than carcinomas without lymph node invasion. Therefore, the detection of high PTTG expression in surgically excised colorectal tumor tissue

Table 1. Current data on PTTG expression in various malignancies.

	Cell-line/primary culture	Patient samples
Pituitary cancer	+7†	+17,18
Thyroid cancer		+41,59
Esophageal squamous cancer		+53,60
Uterine Leioma cells		+61
Lung cancer	+47	+62
Lymphoid cancer	+63	+64
Colon cancer		+43
Gastric carcinoma		+65
Testis cancer	+52	
Breast cancer		+66
Astrocytoma cancer	+45,46	+45,46

+: indicates available data on PTTG expression *in vitro* (cell lines or primary culture) or *in vivo* (from patient samples).

† The numbers refer to the cited references.

may be adjuvant information to the histology grade and useful for appropriate selection of post-surgical treatment. The mechanism behind the PTTG mRNA upregulation in malignant colonic tissue is currently unknown, but several factors are now known to regulate PTTG expression in a variety of cell systems. Overall, it is currently premature to state that disrupting PTTG actions could serve as a therapeutic approach for the treatment of colon cancer.

Gliomas

Astrocytomas are the most common and most deadly primary tumors found in the brain and carry a particularly poor prognosis, especially high-grade astrocytomas or gliomas. The activity and regulation of a number of mitogenic signaling pathways are aberrant in astrocytomas and are thought to play a crucial role in their development. The cascade of events leading to the formation and progression of astrocytomas from low- to high-grade is well characterized.⁴⁴ Due to the emerging promalignant/oncogenic roles of PTTG, the positive correlation between PTTG expression and the degree of pituitary tumor invasiveness, and its high expression in most cancers including those of the thyroid and colon, we and others sought to study the expression pattern and regulation of PTTG in glioblastoma.^{45,46} These two studies demonstrated an upregulation of PTTG mRNA in astrocytomas as compared to normal astrocytes. In one study, the PTTG-binding protein was also found to be upregulated. In addition, knocking down PTTG expression by RNA silencing resulted in the inhibition of proliferation of an astrocytoma cell line.⁴⁶ These studies again suggest that

PTTG is a very promising marker of malignancy. In the future, more conclusive studies will show whether PTTG is causative in the genesis of these malignancies, which would make PTTG a potential drug target.

Although, overexpression of PTTG has been implicated in the genesis and/or pathogenesis of various cancers, there is an exception. Overexpression of human PTTG in HeLa and A549 lung cancer cells inhibited cell growth and stimulated expression of the p21 gene,⁴⁷ which is in contrast to the effects observed in pituitary adenomas. Therefore, PTTG overexpression may not always be associated with cancer cell growth, and we should be careful to determine the effect of its overexpression in other types of cancers in the future. In addition, how PTTG switches from normal physiologic function to behaving as an oncogene, as well as the importance of PTTG in the genesis of tumors other than pituitary adenoma, need to be determined.

In summary, there is a growing body of data demonstrating the utility of PTTG as a possible marker of thyroid carcinomas, colon adenocarcinoma, and gliomas, in addition to being a well-established marker of pituitary adenomas. Although PTTG was first discovered in pituitary adenomas, its importance in malignant transition appears to be more generalized than previously thought. PTTG expression in various cancer tissues is listed in table 1, and its regulation is discussed below.

Regulation of PTTG Expression in Malignant Cells and Tissues

Understanding aberrant activation of PTTG expression may provide insight into the function of the protein in various cancers. Characterization of the human PTTG promoter revealed that it consists of two transcription start sites, 37 bp and 317 bp, upstream of the translational start site.⁴⁸ The existence of these two sites suggests that the expression of PTTG is differentially regulated in tumor and normal tissues. The regulation of PTTG expression has been investigated in a number of malignant tissues. For example, PTTG is upregulated by estrogen and bFGF and downregulated by a peroxisome proliferator-activated receptor- γ agonist, rosiglitazone, in pituitary adenomas.^{49,50} Astrocytic tumors, where PTTG expression positively correlates with the malignancy grades of astrocytomas, are upregulated by tumorigenic cytokines, such as epidermal growth factor, transforming growth factor- α , hepatocyte growth factor and insulin-like growth factor-1.^{45,46} In H-500 Leydig cell tumors, a humoral hypercalcemia of malignancy model, PTTG is upregulated by extracellular Ca^{2+} acting via the calcium-sensing receptor.^{51,52} Therefore, in pituitary adenomas, gliomas and Leydig cell tumors, PTTG expression shows a pattern of upregulation by tumor-promoting factors specific to these tumors. In human esophageal squamous cell carcinoma, overexpression of PTTG has been correlated with increased expression of the wnt signaling protein, β -catenin/T-cell factor (TCF), which plays an important role in differentiation, morphogenesis and tumorigenesis.^{53,54} This report also showed that in human esophageal squamous carcinoma cells, the overexpression of PTTG probably results from possessing a putative TCF4-binding element in the PTTG gene.⁵³ Interestingly, in both astrocytomas and MCF-7 breast tumor cells, upregulation of PTTG expression by insulin and insulin-like growth factor-1 involves a

pro-survival PI3 kinase pathway, suggesting that mechanisms of tumor cell survival lead to overexpression of PTTG. At present, however, it remains unclear whether induction of PTTG by insulin and insulin-like growth factor-1 leads to tumorigenesis. The regulation of PTTG by various factors summarized in table 2 may help to understand the possible mediatory roles played by this protein in the genesis and/or progression of various cancers.

PTTG and Tumor Suppressor Genes

The p53 tumor suppressor gene is thought to be central in protecting against the development of cancer. The p53 protein functions as a transcription factor, regulating downstream genes involved in cell cycle arrest, DNA repair and programmed cell death. DNA damage is a potent stimulus for activation of p53 protein in repairing DNA damage and induces cellular apoptosis if the damage is too severe. Although available reports show an intimate relationship between PTTG and p53, its exact nature remains unclear.⁵⁵⁻⁵⁸ For example, PTTG overexpression, as occurs in many cancers, has been shown to induce cellular apoptosis by both p53-dependent and p53-independent mechanisms. The apoptotic effect is p53-dependent in MCF-7 breast cancer cells and p53-independent in MG-63 human osteosarcoma cells. In MCF-7 breast cancer cells, PTTG overexpression upregulates p53 expression and nuclear translocation with consequent apoptosis by activating a pro-apoptotic gene, bax. Upregulation of p53 by PTTG is indirect and is mediated through an oncogene, c-myc. These observations suggest that p53 is downstream of PTTG and that PTTG overexpression has a protective role against tumorigenesis by inducing cellular apoptosis.

Although overexpression of PTTG activates the p53 gene, PTTG itself is a downstream target of p53, suggesting an

Table 2. Regulation of PTTG expression by various growth factors and hormones.

Growth factors/hormones	Cells	Effects on PTTG expression
Estrogen	Pituitary adenoma ^{50*}	+
bFGF	Pituitary adenoma ⁵⁰	+
PPAR- γ agonists	Pituitary adenoma ⁴⁹	-
EGF/TGF α	Astrocytoma ⁴⁶	+
HGF	Astrocytoma ⁴⁶	+
Insulin, IGF-1	Astrocytoma ⁴⁵ , MCF-7 breast cancer cells	+
Ca^{2+} via the CaR	H-500 Leydig cell tumor ⁵²	+

* The numbers indicate references.

+, upregulation. -, downregulation.

bFGF, basic fibroblast growth factor; CaR, calcium-sensing receptor; EGF, epidermal growth factor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; PPAR- γ , peroxisome proliferator-activated receptor-gamma; PTTG, pituitary tumor transforming gene; TGF, transforming growth factor

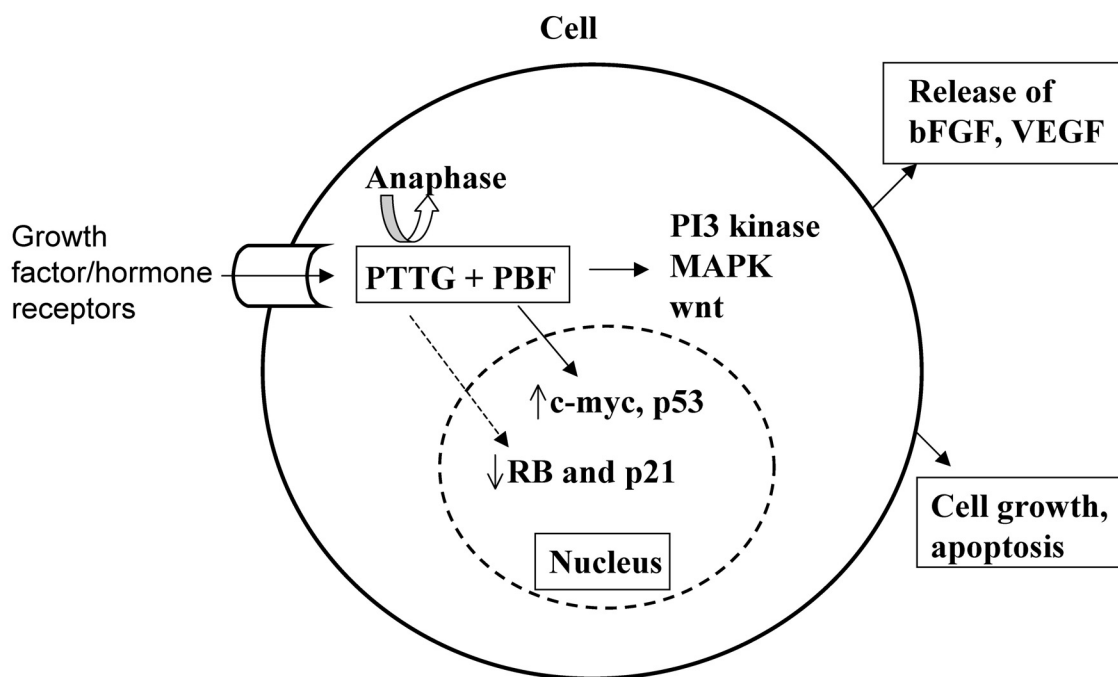


Figure 1. Schematic illustration of the regulation of PTTG expression, its activation and downstream signaling pathways in pituitary tumor cells. PTTG functions by activating MAP and PI kinase pathways, upregulating p53 and downregulating Rb and p21 expressions. Activation of PTTG has been shown to stimulate two pro-angiogenic factors, VEGF and bFGF. Overall effect is increased cellular growth and angiogenesis.

important feedback mechanism between these two molecules.⁵⁶ In a different study,⁵⁸ induction of DNA damage by the chemotherapeutic drugs, doxorubicin and bleomycin, suppressed PTTG expression in the p53-expressing human colorectal cancer cell line, HCT111, and U2OS osteosarcoma cells, but not in p53-deficient cells, demonstrating that p53 is upstream of the PTTG gene and downregulates its expression in order to facilitate DNA repair.

Finally, another report demonstrated that overexpression of PTTG in human A549 lung cancer cells inhibits cellular growth through upregulation of p21, a cell cycle protein that is downstream of p53.⁴⁷ However, no change in p53 mRNA was observed in A549 cells, indicating that PTTG regulates human lung cancer cell function by modulating the p21 pathway that bypasses the p53 protein. Heterogeneity in the regulation of cancer cell biology could only explain a varying relationship between PTTG and the tumor suppressor gene, p53. Whether disrupting the feedback relationship between PTTG and p53 could serve as a therapeutic strategy for cancer cells, needs to be investigated. A schematic illustration of how PTTG regulates cellular signaling and the resultant cellular response has been provided in figure 1 using a pituitary tumor cell as the model.

Conclusion

Since the cloning of PTTG in 1997 from pituitary tumor cells, our understanding of its structure, function and clinical importance has made impressive progress. A great body of data has enabled us to understand the molecular and cellular

regulation of PTTG, and its biological functions. Increasing evidence regarding the roles of PTTG in various cancers, including pituitary adenoma, is making it a candidate marker of malignancy and a potential chemotherapeutic target. Data on PTTG mRNA expression in thyroid malignancies and colorectal cancer show high expression of PTTG in samples from patients with high-grade malignancies. Furthermore, the colorectal cancers that had lymph node invasion also had upregulation of PTTG, making PTTG upregulation a possible tool in the decision to use adjuvant therapy. In thyroid carcinomas, PTTG, PTTG-binding protein and bFGF are very promising prognostic markers for thyroid malignancies, and the use of these markers may, in the future, guide the therapeutic regimen offered to patients. In pituitary neoplasms and thyroid cancer, emerging evidence suggests that PTTG and its binding protein are involved in the malignant transformation of cells. Future studies will reveal whether PTTG is a drug target in these malignancies.

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