Histol Histopathol (2007) 22: 219-226

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Pituitary tumor transforming gene: An important gene in normal cellular functions and tumorigenesis

C. Bradshaw and S.S. Kakar

James Graham Brown Cancer Center, Department of Medicine, University of Louisville, Louisville, USA

Summary. Pituitary tumor transforming gene (PTTG) is an oncogene which is found to be highly expressed in proliferating cells and in most of the tumors analyzed to date. Overexpression of PTTG induces cellular transformation and promotes tumor development in nude mice. PTTG is regulated by various growth factors including insulin and IGF-1. PTTG is a multifunctional and multidomain protein. Some of the functions of PTTG include inhibition of separation of sister chromatids, expression and secretion of angiogenic and metastatic factors. In this review we focus on expression of PTTG in normal and tumor tissues, define its biological function, its role in tumorigenesis, and its interaction with other proteins that may play important role in mediating tumorigenic function of PTTG.

Key words: PTTG, Securin, Sister chromatids separation, Tumorigenesis, Angiogenesis

Primary structure of PTTG

Pituitary tumor transforming gene (PTTG) in an oncogene which was initially cloned from rat pituitary tumor (Pei and Melmed, 1997). Subsequently, we and others cloned from human testis (Kakar and Jennes, 1999), fetal liver (Zhang et al., 1999), thymus (Dominguez et al., 1998) and ovarian tumor (Puri et al., 2001). Sequence analysis of the human PTTG cDNA revealed that it is composed of 656 nucleotides and encodes a protein of 202 amino acids that lacks homology with other known proteins (Fig. 1). Analysis of the protein sequence for PTTG revealed that the protein contains an N-terminal basic domain, and C-terminal acidic domain, a destruction box in the central region, a DNA binding domain, an anaphase-promoting complex (APC) binding site, and a transactivation domain (Fig. 2). The protein also has consensus motifs for cyclic AMP, and cyclic GMP-dependent protein kinase phosphorylation, casein kinase II phosphorylation, Akt phosphorylation and protein kinase C phosphorylation. It also contains proline-rich motifs (Fig. 2). These functional motifs represent potential SH3 docking regions and suggest that PTTG might be involved in SH3-mediated intracellular signaling. Three homologues of PTTG have been identified (PTTG 1, 2 and 3) (Fig. 3), of which only PTTG1 (PTTG) has been studied in detail to date (Chen et al., 2000).

Predominantly PTTG protein is localized in the cytoplasm, although some may be localized in the nucleus (Chien and Pei, 2000; Pei, 2000; Ramos-Morales et al., 2000). Nuclear translocation of PTTG can be facilitated, however, by either interaction with PTTG binding factor (PBF) or by activation of the mitogen-activating protein (MAP) kinase cascade (Chien and Pei, 2000; Ramos-Morales et al., 2000). The level of PTTG expression is increased in rapidly proliferating cells and is regulated in a cell cycle-dependent manner (Ramos-Morales et al., 2000; Yu et al., 2000). PTTG mRNA and protein expression are low at the G1/S interphase, gradually increasing during the S phase and peaking at the G2/M phase (Ramos-Morales et al., 2000). However, as the cells enter anaphase, PTTG is degraded and the daughter cells express very low amounts of PTTG. The degradation of PTTG most likely occurs via ubiquitin since PTTG contains a D-box, which is required for such proteolysis. The intracellular location of PTTG is predominantly nuclear at interphase with significant expression in the cytoplasm, but it is localized to the mitotic spindles during early mitosis (Yu et al., 2000). This duality in the intracellular localization of PTTG correlates with its activity as a human securin (Zou et al., 1999).

Biological functions of PTTG

PTTG possesses multiple biological functions that are crucial to the mitotic success of normal, non-cancerous cells. However, this significant cell cycle

Offprint requests to: Sham S. Kakar, Department of Medicine, James Graham Brown Cancer Center, 580 South Preston Street, Baxter II, Room 321 E, University of Louisville, Louisville, KY 40202, USA. e-mail: sskaka01@louisville.edu

protein, if expressed abnormally within a cell, has the potential to transform into an oncogene that can trigger tumorigenesis in a variety of tissues. By conducting research related many roles played by PTTG in cell processes, investigators have been able to better understand the dynamics of how this protein affects the biological stability of cells. PTTG's involvement in sister chromatids separation and in the secretion and expression of various growth factors such as bFGF, VEGF, and IL-8 is necessary in the regulation of a typical cell cycle and thereby contributes to the growth and longevity of the cell itself.

One of the biological pathways through which PTTG has been shown to contribute to the oncogenic destiny of a cell is through its regulation of the secretion and/or expression of bFGF and VEGF. Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) play an integral part in angiogenesis, a process important in tumor growth. The relationship between PTTG and these two angiogenic growth factors was first examined in mouse fibroblast (NIH 3T3) cells. In one of the earliest investigations into PTTG's functions, a comparison between PTTG-transfected and control NIH 3T3 cells resulted in a distinct increase in bFGF mRNA and protein levels in the PTTG-transfected cells relative to the control (Zhang et al., 1999).

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  1 MATLIYVDKENGEPGTRVVAKDGLKLGSGPSIKALDGRSQVSTPRFGKTF  50
 51 DAPPALPKATRKALGTVNRATEKSVKTKGPLKQKQPSFSAKKMTEKTVKA 100
101 KSSVPASDDAYPEIEKFFPFNPLDFESFDLPEEHQIAHLPLSGVPLMILD 150
151 EERELEKLFQLGPPSPVKMPSPPWESNLLQSPSSILSTLDVELPPVCCDI 200
201 DI
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Fig. 1. Deduced amino acid sequence of the human pituitary transforming gene (PTTG). All amino acids are represented by single letter codes.

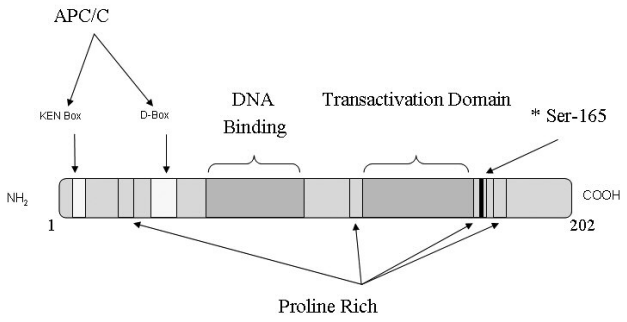

Fig. 2. Key functional domains of PTTG, including DNA binding region, transactivation region, anaphase promoting complex/cyclosome (APC/C) destruction site, and the critical SH3-interacting domains (praline-rich motifs).

Furthermore, cells transfected with mutant hPTTG failed to exhibit higher concentrations of bFGF mRNA. VEGF mRNA levels, in contrast, appeared to be relatively the same in the two cell groups. This data, nevertheless, provided the first evidence of the correlation between PTTG and bFGF and their subsequent capacity to promote angiogenesis and thus tumorigenesis in mouse fibroblast cells (Zhang et al., 1999). The combined ability of PTTG and bFGF to trigger angiogenesis was studied more in-depth in NIH 3T3 cells and ultimately yielded definitive results that PTTG does indeed cause angiogenesis through its interaction with bFGF. This conclusion was determined by examining the degree of angiogenesis induction in conditioned medium (CM) derived from NIH 3T3 cells overly expressing wild-type human PTTG (Ishikawa et al., 2001). As expected, the bFGF concentration of this medium was much higher than concentrations exhibited by the medium containing mutant hPTTG and the control medium. Moreover, by conducting a CAM (chorioallantoic membrane, i.e. the highly vascular membrane of bird and reptile eggs comparable to the mammalian placenta) assay of nine-day-old chick embryos, it was found that embryos treated with conditioned medium derived from the wild-type hPTTG induced the most angiogenesis, thus confirming PTTG's role in this process (Ishikawa et al., 2001). Although VEGF study was neglected in this experiment due to the difficulty of achieving accurate results *in vitro*, more recent experimentation with estrogen-regulated PTTG in rat cells has shown that increases in PTTG mRNA expression are associated with increased VEGF and bFGF levels *in vivo* (Heaney et al., 2002).

Since conclusive evidence of PTTG's upregulating effect on bFGF and VEGF growth factors in murine animals has been well established, the next logical step

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PTTG1   MATLIYVDKENGEPGTRVVAKDGLKLGSGPSIKALDGRSQVSTPRFGKTFDAPPALPKAT 60
PTTG3   MATLIYVDKENEEPGILVATKDGLKLGSGPSIKALDGRSQVSISCFGKTFDAPTSLPKAT 60
PTTG2   MATLIYVDKEIGEPGTRVAAKDVLKLESRPSIKALDGISQVLTPRFGKTYDAPSALPKAT 60
        ********** ***  *.:** *** * ******** ***  . ****:***.:*****

PTTG1   RKALGTVNRATEKSVKTKGPLKQKQPSFSAKKMTEKTVKAKSSVPASDDAYPEIEKFFPF 120
PTTG3   RKALGTVNRATEKSVKTNGPLKQKQPSFSAKKMTEKTVKAKNSVPASDDGYPEIEKLFPF 120
PTTG2   RKALGTVNRATEKSVKTNGPRKQKQPSFSAKKMTEKTVKTKSSVPASDDAYPEIEKFFPF 120
        *****************:** ******************:*.*******.******:***

PTTG1   NPLDFESFDLPEEHQIAHLPLSGVPLMILDEERELEKLFQLGPPSPVKMPSPPWESNLLQ 180
PTTG3   NPLGFESFDLPEEHQIAHLPLSEVPLMILDEERELEKLFQLGPPSPLKMPSPPWKSNLLQ 180
PTTG2   NLLDFESFDLPEERQIAHLPLSGVPLMILDEEGELEKLFQLGPPSPVKMPSPPWECNLFA 180
        * *.*********:******** ********* *************:*******:.**:

PTTG1   SPSSILSTLDVELPPVCCDIDI 202
PTTG3   SPLSILLTLDVELPPVCSDIDI 202
PTTG2   ------VSFKHSVDPGC----- 191
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Fig. 3. Amino acid sequence alignment of human PTTG1, PTTG2 and PTTG3. All amino acids are represented by single letter codes. PTTG2 contains point deletion at C-terminus resulting in frame shift and truncated protein. Amino acids absent in PTTG2 are indicated by dashes.

was to test the same hypotheses in the context of the human cell. In order to achieve a more in-depth look into the PTTG and VEGF relationship, experiments were conducted in fetal neuronal, breast cancer, and choriocarcinoma cells. Through *in vivo* and *in vitro* studies, a positive correlation between PTTG and VEGF was determined, with increased levels of PTTG expression coinciding with increased levels of VEGF as well as its receptor, KDR. Interestingly, promotion of VEGF secretion was independent of PTTG phosphorylation (McCabe et al., 2002). In a further effort to study the nature of the PTTG and VEGF relationship, research was conducted on human pituitary adenomas in which basal secretion of VEGF, among other things, was measured and compared to the degree of PTTG expression. Although all tumors exhibited some secretion of VEGF, there was unfortunately no distinct correlation between VEGF and PTTG concentrations, thus leading to the conclusion that angiogenesis in pituitary tumors is by a mechanism other than VEGF (Hunter et al., 2003). In addition to human pituitary cells, experimentation into PTTG and its effect on growth factors has been performed with human embryonic kidney cells (HEK293). This research, on top of studying the levels of VEGF and bFGF, also investigated PTTG's interaction with interleukin-8 (IL-8), another possible angiogenic contributor associated with tumorigenesis. By transfecting the HEK293 human embryonic cell line with PTTG cDNA and measuring the subsequent VEGF, bFGF, and IL-8 concentrations, it was evident that higher levels of these growth factors were present in PTTG-treated cells relative to controls. This positive effect was also examined under in vivo conditions, a procedure which yielded the same basic results. The augmenting effect of PTTG on VEGF, bFGF, and IL-8 therefore suggests that the relationship between PTTG and these growth factors may be the process by which the gene executes its oncogenic potential (Hamid et al., 2005).

Not only does PTTG interact with various angiogenic growth factors, but it is also involved in crucial components of the cell cycle, such as apoptosis and sister chromatids separation. Evidence of PTTG's involvement in the regulation of the cell cycle can be seen through its increased concentration in rapidly dividing cells and the phosphorylation of the protein during mitosis, the later suggesting that PTTG, on top of playing a role in the cell cycle, may be induced by regulatory mitotic pathways (Ramos-Morales et al., 2000). Since it is clear that PTTG levels within a cell possess influence over cell cycle progression, it is not surprising that research has indicated that overexpression of PTTG blocks the advancement of mitosis to anaphase. One study that utilized human cancer cell line H1299 revealed that cells transfected with medium or high levels of PTTG (along with green fluorescent protein) displayed an increased duration of prophase and metaphase (Yu et al., 2003). In a pursuit to identify the mechanism by which PTTG achieves this effect, PTTG interaction with mitosis checkpoint protein MAD2, a protein whose detachment from sister chromatids enables the start of anaphase, was examined. Unfortunately, PTTG did not appear to have any visible effect on MAD2's attachment to sister chromatids, therefore eliminating one possible mechanism for PTTG cell-cycle inhibition (Yu et al., 2003). The most likely impetus by which PTTG impedes cell-cycle progression is through its action as a human securin, a protein that inhibits the separase essential for sister chromatids separation until the completion of metaphase. It has been found that human securin is identical to the protein product of PTTG; this correlation suggests that overexpression of PTTG could possibly disrupt sister chromatids separation through its continued inhibition of separase into anaphase (Zou et al., 1999). It was later discovered that degradation of PTTG/securin is required for successful sister chromatids separation; through fzy and fzr's mediation, the anaphase promoting complex (APC) degrades securin and thus allows sister chromatids to equitably divide between daughter cells (Zur and Brandeis, 2001). It seems logical; therefore, that it would take more time to degrade overexpressed PTTG and the cell cycle would subsequently be delayed. In relation to PTTG's effect on sister chromatids separation is its recently discovered ability to aid the repair of damaged DNA. Regulated by DNA double-stranded breaks, PTTG binds to the Ku-70 protein, a subunit of the DNA-dependent protein kinase. Data also showed that DNA double-stranded breaks inhibit PTTG binding to Ku-70, thus augmenting the amount of PTTG available to be phosphorylated by DNA-protein kinase and suspend sister chromatids separation. This haltage of the cell cycle could consequently allow time for damaged DNA to be repaired (Romero et al., 2001). In addition to these roles, PTTG has also been shown to be an integral part of developing the human fetal brain through up-regulating fibroblast growth factor FGF-2, a molecule necessary for the maturation of the central nervous system (Boelaert et al., 2003).

In order to study PTTG's effect on apoptosis, relationship between PTTG and the most widely known apoptotic agent, p53, has been conducted. Experimentation has provided conclusive evidence that PTTG has the ability to regulate apoptosis in the presence or absence of p53. One example of this action sans p53 can be seen in the human lung cancer cell line H1299, a cell line which does not express p53 and died due to the overexpression of PTTG (Yu et al., 2003). A p53-expressing cell line such as the human choriocarcinoma JEG-3 yielded similar results when PTTG was overexpressed (Yu et al., 2003). A previous study involving MCF-7 breast cancer cells and MG-63 osteosarcoma cells provided the groundwork for further examination into the PTTG-p53 relationship. In the first cell line, which was p53-positive, PTTG was shown to up-regulate and transports p53 to the nucleus and increase expression of p53 enhanced PTTG-triggered apoptosis; consistent with these results, inactivation of

p53 noticeably minimized PTTG's apoptotic effect. However, despite the absence of p53, PTTG was still able to cause apoptosis in the latter cell line directly following its suspension of the cell cycle (Yu et al., 2000). In addition to these findings, more recent studies involving the MCF-7 cell line as well as a human embryonic kidney cell line (HEK293) have shown that overexpression of PTTG regulates the expression of p53 indirectly, with this mechanism being mediated by c-myc expression. Furthermore, PTTG overexpression indirectly augments the activity of the Bax gene, a target of p53 that triggers apoptosis in HEK293 cell line (Hamid and Kakar, 2004). Additional insight into the PTTG-p53 interaction has revealed that PTTG inhibits the binding of p53 to DNA, preventing its transcriptional and apoptotic actions (Fig. 4). Similarly, PTTG was found to aid in the production of new human lung cancer cells that compensated for those killed by p53-transfection, thus providing clues to PTTG's tumorigenic effects (Bernal et al., 2002).

Distribution and expression of PTTG

Pituitary tumor transforming gene (PTTG) is a novel oncogene that is present in multiple tumorigenic cell lines and tissues as well as their normal counterparts. Its distribution and expression within the cell varies with the cell cycle and includes location in the nucleus and the cytoplasm. As research into this enigmatic gene continues, it is no surprise that its involvement in promoting cancer is becoming more and more important.

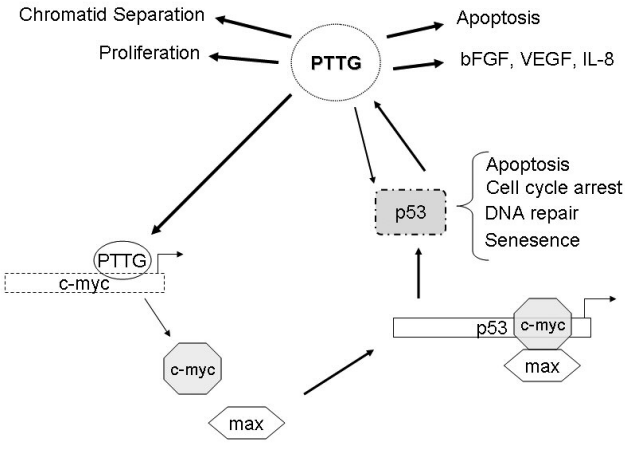

Fig. 4. Interrelationship between PTTG and p53. Increased in expression of PTTG regulates the expression and secretion of bFGF, VEGF, and IL-8; inhibits sister chromatids separation and cell proliferation. In addition it interacts with p53 protein and regulates cell apoptosis. Furthermore, increase in expression of PTTG results in activation of transcription of c-myc gene. C-myc binds to its partner max at C-terminus which as a complex (c-myc/max) binds to p53 promoter and regulates its expression. Increase in p53 expression regulates cell apoptosis, cell cycle arrest, DNA repair and cell senescence. Increase in p53 also down-regulates the expression of PTTG.

Some of the first research efforts to characterize PTTG included investigation into its degree of expression in various types of rat tissues, both fetal and adult. Differing lengths of the PTTG transcript were observed in various rat cell lines, tissues, and tumors, with the completeness of the transcript proportional to the degree of PTTG expression; full length transcripts were apparent in rat leukemia, lung cancer, lymphoma, and melanoma cell lines and in pituitary tumors whereas shorter versions were present in adult rat testis and embryonic liver. The disparity in transcript lengths could be due to splicing of the gene that confers a specific function upon it depending on the tissue location (Pei, 1998). Eventually, the transition was made from analyzing PTTG levels in rats to researching those in humans. In fetal tissues, PTTG mRNA was strongly detected in the liver (Kakar and Jennes, 1999). As for normal adult tissues, intense expression was only apparent originally in the testis (Pei and Melmed, 1997) but was later found in high levels in the thymus and in low levels in the colon, small intestine, brain, placenta, and pancreas (Zhang et al., 1999). The first evidence of PTTG's role in cancer was acquired through the discovery of its overexpression in a plethora of malignant tumor tissues. In the testing of human malignant tumor cells, PTTG was abundant in all cell lines studied; moreover, the gene was highly present in many human pituitary tumors (Zhang et al., 1999). Since pituitary tumors were the first cancerous growths in which PTTG was found to be abnormally expressed, further research was conducted with these tumors in order to obtain more information about PTTG's role in cancer. Using *in situ* hybridization, it was found that PTTG was expressed at low levels in normal human pituitary tissue whereas increased expression was observed in tumor tissues, specifically hormone-secreting ones. Furthermore, PTTG mRNA levels in benign pituitary tumors were lower than in malignant ones, thus suggesting that PTTG may play a role in metastasis. This novel research was the first to discover a human transforming gene that was consistently overexpressed in almost all tumorigenic pituitary tissues (Saez et al., 1999; Zhang et al., 1999).

The strong indication of early research that PTTG possesses an important role in cell transformation and tumorigenesis spawned many other investigations into the gene's prevalence in other tissues besides those of the pituitary. Not surprisingly, PTTG was found to be expressed at high levels in a plethora of cancers, including breast, thyroid, esophageal, and colorectal. Very recent research has provided conclusive evidence into PTTG's role in breast cancer, where abundant cytoplasmic and nuclear expression was observed in all 90 breast tumors studied; although PTTG was also present in some normal breast tissue samples, it was only localized to the cytoplasm, suggesting the importance of the genes transferal to the nucleus in performing its oncogenic function (Ogbagabriel et al., 2005). The gene

is highly prevalent in thyroid tumors, as well, particularly follicular neoplasms (Heaney et al., 2001). A similar relationship was observed in esophageal cancer, in which PTTG expression was higher the more invasive the cancer (Shibata et al., 2002). As for colorectal cancer, PTTG was overexpressed in all 48 carcinomas studied, thus further contributing to the notion that high amounts of PTTG may be an indicator of potential tumorigenesis (Heaney et al., 2000). In addition, increased amounts of the gene have been witnessed in human tumors of the liver, kidney, endometrium, uterus, and ovary (Kakar and Jennes, 1999; Kakar and Malik, 2006; Kakar at al., 2001; Puri et al., 2001) (Fig. 5). Studies have also shown that PTTG is prevalent in human T-lymphoma cell lines and hematopoietic malignancies (Dominguez et al., 1998). More recently, PTTG has been indicated in astrocytoma (Tfelt-Hansen et al., 2004).

Although the extensiveness of PTTG expression in various tissues is an important quality to measure, its distribution and concentration within the cell itself can also provide crucial insight into its function. Some of the first research conducted into this area revealed that PTTG is primarily expressed in the cytoplasm of the cell with minimal amounts present in the nucleus. For instance, this distribution is the case for pituitary adenoma cells (Saez et al., 1999; Zhang et al., 1999) as well as hematopoietic neoplasms (Dominguez et al., 1998). Interestingly, studies show that PTTG expression is cell-cycle dependent, thus suggesting PTTG significance in the cell cycle. This reliance of PTTG on the cell cycle is typical of a securin protein, and later studies have confirmed that this is indeed PTTG's function within the cell (Zou et al., 1999). By monitoring PTTG levels during mitosis of human placental JEG-3 cells, one study was able to measure the amount of PTTG present in a cell at a specific point in its lifespan. The level of PTTG mRNA was low at the beginning of mitosis in the G_1 phase, increased during S phase, and exhibited its highest amount at the G_2/metaphase border. As the cell cycle returned to the G_1 phase, PTTG levels decreased. The subcellular localization of the gene also varied as mitosis progressed. During interphase, PTTG was observed at a high extent in the nucleus with some present in the cytoplasm; in contrast, once mitosis was initiated, PTTG associated with the mitotic spindles in the cytoplasm and was eventually degraded before anaphase (Yu et al., 2000). Although this later data indicating that PTTG exists predominantly in the nucleus during one point of the cell cycle contradicted earlier research suggesting the gene's primary location to be cytoplasmic, further examination into the matter enabled the discrepancy to be eradicated. Additional research revealed that PTTG's interaction with its binding factor (PBF) facilitates the gene's transferal to the nucleus and also enables it to activate bFGF. Therefore, there appears to be a link between PTTG localization to the nucleus and its subsequent oncogenic activities (Chien and Pei, 2000).

Tumorigenic function of PTTG

Due to the presence of PTTG in a plethora of cancerous cell lines and tissues, its involvement in tumorigenesis, although still enigmatic, has been solidly confirmed. Therefore, the direction of research has logically transitioned to investigating the mechanism through which PTTG executes its oncogenic functions.

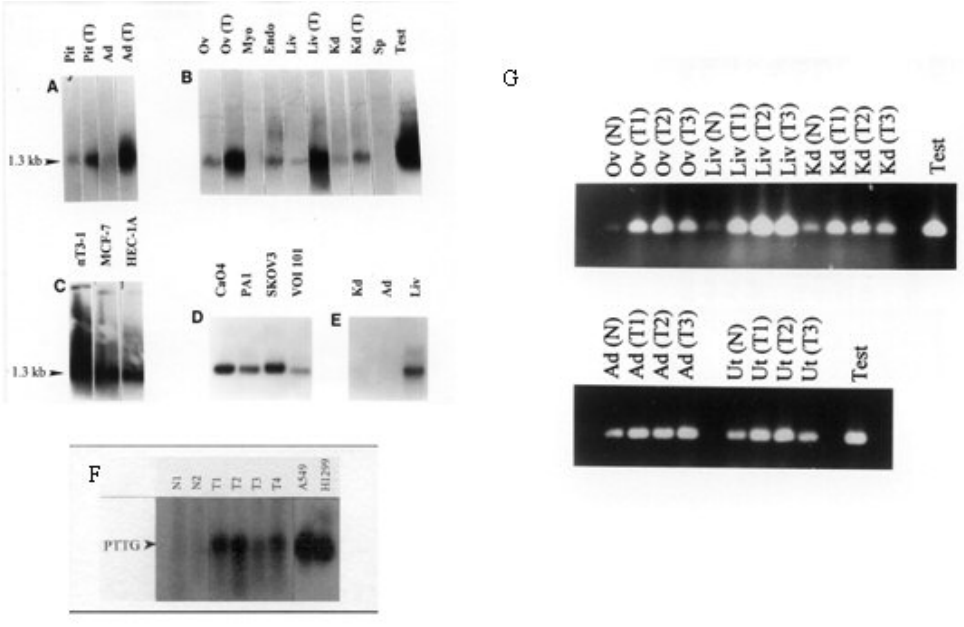

Fig. 5. Expression of PTTG in various tumors and normal tissues. Expression of PTTG in various tumors and normal tissues analyzed by Northern blot analysis (A-F), and RT/PCR (G). Pit: pituitary; Ad: adrenal; Ov: ovary; Myo: myometrium; Endo: endometrium; Kd: kidney; Sp: spleen; Test: testis; αT3-1: mouse pituitary gonadotrope cell line; MCF-7: human breast tumor cell line; HEC-1A: endometrium tumor cell line; Ca04, PA1, SKOV3 and VOI 101: ovarian tumor cell lines. Panel E: human fetal tissues. Panel F: lung tumor and normal tissues; T: tumor and N: normal. Reproduced from Kakar and Jennes (1999); Kakar and Malik (2006) with permission.

Novel areas of research include PTTG effects on the genetic instability of the cell, and this study has required its role in sister chromatids separation to undergo more intense scrutiny. In addition, examination of PTTG relationship with p53, apoptosis, angiogenesis, and cell proliferation may also provide clues as to the impetus by which the gene achieves cell transformation.

Genetic instability has recently been identified as a hallmark of tumorigenesis in colorectal and oral squamous cell carcinomas, as well as others (Stoler et al., 1999; Viswanathan et al., 2003). Encompassing such genetic aberrations as aneuploidy, intrachromosomal insertions and deletions, and point mutations, genetic instability can be defined as any deviation from the normal functioning of the genetic machinery that is cell division and/or gene arrangement. Genetic instability was first linked to cancer by Duesberg et al. (1998), in which aneuploidy was hypothesized to be the source of instability within the genome. As expected, experiments with aneuploid cells from Chinese hamster embryos indicated that the greater the degree of aneuploidy, the more unstable the karyotype, thus leading to cancer. Aneuploidy, also termed chromosomal instability (CIN), is one of the most researched areas of genetic instability and suggests that proteins involved in chromosomal segregation during mitosis may be likely candidates for oncogenic agents. Once abnormal chromosome number, and thus genetic instability, was shown to be a cause of cell transformation (Li et al., 1996), subsequent research sought to identify the catalysts behind this atypical segregation process, one of which is PTTG. Since PTTG plays a significant role in sister chromatids separation by acting as a securin, it is no surprise that its overexpression could inhibit proper degradation and consequently result in the failure of chromosomes to evenly divide between daughter cells. The first indication of PTTG's connection with aneuploidy was provided by Yu et al. (2000), in which experiments revealed that PTTG-transfected osteosarcoma cells exhibited an increased occurrence of aneuploidy as opposed to control cells. The same group went on to confirm their results using human H1299 lung cancer cells; cells overexpressing PTTG underwent abnormal chromosome segregation, sometimes resulting in a macronucleus (Yu et al., 2003). The effects of PTTG overexpression on genetic instability were also studied in thyroid and colorectal cancer cells. In order to more easily analyze the data, a genomic instability index (GI) was utilized, a process which involves conducting FISSR-PCR on the tested cells and dividing the number of aberrant bands by the total number of bands. This testing revealed that the GI for PTTG-transfected thyroid cells was consistently higher than that of vector-only-transfected cells. Moreover, when thyroid cells were transfected with different doses of PTTG, the GI index increased as the dosage amount increased (Kim et al., 2005). The promising results yielded by this experiment have prompted the pursuit for confirmation in other cancer cell lines, such as colorectal and ovarian cancers.

Another possible mechanism by which PTTG induces tumorigenesis is through its regulation of various growth factors, such as bFGF, VEGF, and IL-8. These specific molecules play integral roles in angiogenesis, a process necessary to maintain the survival of tumors. Of course, the first evidence of PTTG upregulating effect on the above growth factors was found in murine animals, with the results eventually being confirmed in humans. Zhang et al. (1999) were the first to delve into this area of research utilizing the mouse fibroblast cell line NIH 3T3. After injecting human PTTG-transfected NIH 3T3 cells into nude mice and observing solid tumor growth, Zhang et al. (1999) hypothesized the tumorigenic mechanism to due to PTTG upregulation of angiogenic growth factors. Their hypothesis was strengthened when ELISA revealed that conditioned medium derived from PTTG-transfected cells exhibited increased levels of bFGF mRNA. Later research went a step further by actually measuring the extent of new vessel formation in PTTG-transfected chick embryos. Through a CAM (chorioallantoic membrane) assay, it was evident that the number of induced vessels was greater in medium derived from PTTG-containing cells than in controls (Ishikawa et al., 2001). Further investigation with choriocarcinoma, neuronal, breast cancer, and human embryonic cells affirmed the upregulating influence of PTTG on various growth factors. These cells, when PTTG overexpression was introduced, were capable of inducing tumorigenesis, and, as expected, exhibited elevated levels of bFGF and VEGF (McCabe et al., 2002; Hamid et al., 2005). Similarly, increased amounts of another growth factor, interleukin-8, were found in the human embryonic cells transfected with PTTG (Hamid et al., 2005). The consistency of this data, therefore, strongly suggests that PTTG executes its tumorigenic function through the upregulation of angiogenic growth factors such as bFGF, VEGF, and IL-8.

Although PTTG's angiogenic mechanism has been well-established, the gene may also trigger tumorigenesis through its regulation of cell proliferation. Overexpression of PTTG has been shown to disrupt the normal capabilities of the cell, and cell proliferation is not exempt from this effect. The PTTG influence on cell proliferation has been quite ambiguous, with some results in rats indicating that it inhibits the process whereas others, specifically involving human placental JEG-3 cells, suggest that it enhances it (Pei and Melmed, 1997; Yu et al., 2000). Despite these contradictions, PTTG, depending on the degree of expression, has been adequately shown to induce cell proliferation, therefore making this interaction a likely candidate for one of the gene's tumorigenic functions. Indeed, overexpression of human PTTG was found to trigger cell proliferation and transformation in NIH 3T3 cells and tumor formation in nude mice (Kakar and Jennes, 1999). The later research of Pei (2001) concentrated on PTTG's effect on downstream target genes known to cause cell proliferation, including c-myc and MEK1 (mitogen-

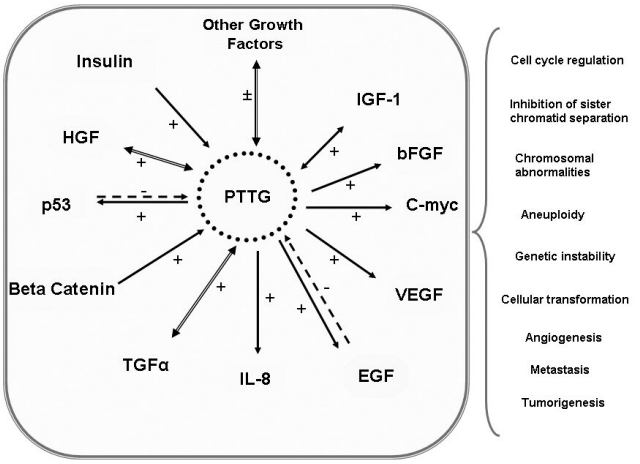

Fig. 6. Regulation of PTTG by various growth factors and its predicted cellular functions. Growth factors that regulate the expression of PTTG, and genes and cellular functions that are regulated by overexpression of PTTG are shown.

activated protein kinase). It was shown that PTTG-transfected cells induced *c-myc* and subsequent cell proliferation, thereby indicating the PTTG-*c-myc* pathway as a possible mechanism for tumorigenesis. In addition, c-myc has also been recently shown to mediate the interaction between PTTG and p53. Since a c-myc binding site on the p53 promoter has been discovered (Kirch et al., 1999), it was not surprising when PTTG induction of the gene affected the degree of p53-mediated apoptosis (Hamid and Kakar, 2004). The importance of PTTG in producing cell proliferation was additionally studied in PTTG-null male mice, in which proliferation of pancreatic beta cells was drastically minimized due to the absence of the gene (Wang et al., 2003). This result is reasonable when PTTG's role as a human securin, and thus as a vital player in sister chromatids separation, is taken into account.

Additional pathways through which PTTG could perform its tumorigenic function may also be found in its relationship with apoptotic agent p53 as well as the Ku-70 heterodimer. Although in many cases PTTG has been shown to upregulate p53 and thereby induce apoptosis (Yu et al., 2000, 2003; Hamid and Kakar, 2004), there is also evidence that PTTG possesses the ability to inhibit cell suicide, thus possibly contributing to cell accumulation and tumor formation. For example, studies conducted by Bernal et al. (2002) with human lung cancer cell line H1299 revealed that PTTG is able to inhibit binding of p53 to DNA and its apoptotic effects. Moreover, the gene performed a compensatory function in that it triggered accumulation of new cells to replace those killed by p53. These results, therefore, suggest that PTTG may enact its oncogenic potential through inhibiting and nullifying the effects of p53. Other than indirectly affecting DNA activity through p53, PTTG has also been shown to interact with the Ku-70 heterodimer and DNA-protein kinase, an enzyme important in repairing DNA double-stranded breaks. Normally, it seems that when DNA-protein kinases are activated, PTTG will dissociate from Ku-70 and become phosphorylated, eventually resulting in a blockage of the cell cycle that would provide time for DNA repair (Romero et al., 2001). However, if abnormal PTTG expression interferes with this process, the cell cycle may continue and perpetuate daughter cells containing harmful mutations that could possibly contribute to cancer formation.

Unfortunately, the precise mechanism through which PTTG executes its tumorigenic function is still unclear, but research is continuing to reveal that the gene possesses a plethora of functions that could serve some oncogenic purpose. The possible mechanisms and pathways by which PTTG induces its tumorigenic effects are summarized in Fig. 6.

Acknowledgements. The authors like to thank Mr. Mohammed Tariq Malik for technical help. This work was supported by a grant NCI CA82511 (SSK) and summer fellowship from KBRIN (CB).

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Accepted August 24, 2006