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**The emerging role of pituitary tumor transforming gene (PTTG)
in endocrine tumourigenesis.**

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Summary

It is now 10 years since PTTG was first cloned and isolated. Perhaps the major story of the intervening decade of work performed by numerous groups around the world is the sheer multifunctionality ascribed to this gene. PTTG has been implicated in mechanisms of gene transactivation, cell transformation, angiogenesis, metabolism, apoptosis, DNA repair, genetic instability and mitotic control, both in endocrine and non-endocrine settings. In the current review, we cast a critical eye over a decade of PTTG research within the field of endocrine neoplasia.

Introduction

Pituitary tumor transforming gene is a gene with two identities: *PTTG* and *securin*. This has encouraged an almost schizophrenic character to the gene, with those who refer to it as *PTTG* (or *PTTG1*, *hPTTG*, *hPTTG1*) generally confined to endocrine research and cell signalling, and those who call it *securin* generally engaged in cell cycle analysis. For the purposes of this review, we will cover both gene identities under the original nomenclature of *PTTG*.

PTTG was originally isolated from rat pituitary GH4 cells (Pei and Melmed, 1997), and shown to be transforming *in vitro* and tumourigenic *in vivo* (Zhang et al., 1999). Subsequently, the human homologue was identified (Dominguez et al., 1998), which had broadly the same attributes as its rat counterpart (Zhang et al., 1999). The same year, PTTG was identified as the human securin, a critical protein in late-stage mitosis (Zou et al., 1999). In the intervening period, a growing multifunctionality of the protein has emerged, with convincing evidence that PTTG plays a dual role in tumour development, being involved in both tumour initiation and subsequent progression towards more aggressive phenotypes. This review aims to summarise the important developments in PTTG biology and describe its emerging role in endocrine neoplasia, with particular focus upon tumour initiation, tumour progression and genetic instability.

The role of PTTG in tumour initiation

The multi-step hypothesis of tumourigenesis assumes that an initial transforming mutation provides the resulting cell with a gain of proliferative function allowing clonal expansion, and subsequent secondary mutations that favour tumour progression. The oncogenic mutations responsible for the initiation step may be facilitated by pre-existing hyperplasia.

Increased PTTG expression has been repeatedly demonstrated in hyperplastic disease states and benign adenomas of both pituitary and thyroid glands compared with corresponding normal tissue, suggesting PTTG may play a role in early development of pituitary and thyroid cancers (Boelaert et al., 2003, Heaney et al., 2001, McCabe et al., 2003). Additionally, PTTG overexpression has now been reported in multiple tumour types, including colon, breast and liver. However, the pioneering evidence of PTTG's role in tumour initiation came from examination of oestrogen induction of pituitary tumours via PTTG.

PTTG and Oestrogen

Oestrogen is mitogenic for lactotrophs and gonadotrophs and high doses of oestrogen induce rat lactotroph hyperplasia and adenoma formation (Heaney et al., 1999). Oestrogen has been shown to induce PTTG expression by acting upon an oestrogen-response element (ERE) in the PTTG promoter region (Heaney et al.,

1999). Further, in rats, pituitary expression of PTTG is induced early by oestrogen and precedes oestrogen-induced hyperplasia and adenoma formation (Heaney et al., 1999). These results indicate that oestrogen-induced PTTG expression is coincident with early pituitary lactotroph transformation and suggests a role for PTTG in the preliminary stages of tumour initiation.

Murine Models of PTTG Function

Elegant recent studies using PTTG transgenic mouse models provide support for the importance of PTTG in the initiation of endocrine tumours, again through the model of pituitary adenoma formation. Transgenic mice with the common alpha-subunit (α GSU) promoter driving PTTG expression were generated to determine the impact of early pituitary PTTG overexpression (Abbud et al., 2005). Targeted expression of PTTG resulted in focal PTTG expression in LH- and GH- producing cells ranging from hyperplasia to microadenoma development. To further understand the role of PTTG on pituitary tumour development, α GSU.PTTG mice were crossed with Rb^{+/-} mice, a well established mouse model for pituitary tumours that are heterozygous for the retinoblastoma (Rb) tumour suppressor gene (Donangelo et al., 2006, Jacks et al., 1992). The pituitary glands of the resulting bitransgenic α GSU.PTTGxRb^{+/-} mice exhibited marked hyperplasia and higher prevalence of pituitary tumours compared with singly transgenic α GSU.PTTG or Rb^{+/-} mice.

Experimental mice models with deleted or reduced PTTG expression demonstrated supportive reciprocal findings. Using the Rb/PTTG transgenic mouse model, PTTG deletion (Rb^{+/-}.PTTG^{-/-}) resulted in decreased organ size and decreased cell proliferation in the pretumorous pituitary gland compared with parental mice (Chesnokova et al., 2005). Further, PTTG deletion was shown to protect Rb^{+/-} mice from developing pituitary tumours (Chesnokova et al., 2005). Subsequent *in vitro* studies showed that PTTG repression was associated with p21 upregulation and reduced Rb phosphorylation, suggesting that the delay in tumour formation in Rb^{+/-}.PTTG^{-/-} mice might arise as a consequence of pituitary p21 overexpression and Rb hypo-phosphorylation leading to inhibited proliferation (Chesnokova et al., 2005).

Much of the available data describes the role of PTTG in pituitary tumourigenesis. However, other evidence suggests that PTTG also plays an important role in the initiation of other endocrine-related tumours such as thyroid and breast. Thyroid cancer is a particularly well described model, with increased PTTG expression in thyroid hyperplasia and adenomas, as well as carcinomas, suggesting early involvement of PTTG in thyroid tumour development (Boelaert et al., 2003, Heaney et al., 2001). *In vitro*, PTTG overexpression induces proliferation in human follicular thyroid cancer FTC133 cells (Kim et al., 2006a). Further, overexpression of PTTG in rat FRTL5 and primary human thyroid follicular cells caused *in vitro* transformation and produced a dedifferentiated phenotype (Heaney et al., 2001).

The TR β PV transgenic mouse model expresses a dominant-negative mutation of thyroid hormone receptor β (Kaneshige, 2000). These mice spontaneously develop follicular thyroid cancers with distant metastases, and exhibit elevated PTTG expression (Ying, 2003), similar to human thyroid cancers. However, when these mice are crossed with PTTG^{-/-} mice, thyroid glands are significantly smaller and show decreased thyroid cell proliferation compared with parent TR β PV mice (Kim et al., 2007a). Evaluation of the Rb-E2F pathway in these mice demonstrated a decrease in the level of phosphorylated Rb and an increase in p21 expression (Kim et al., 2007a). These findings are consistent with Rb^{+/-}.PTTG^{-/-} mice data in pituitary glands (Chesnokova et al., 2005). However, in contrast, there was no difference in the

rate of FTC occurrence between TR β PV and TR β PV/PTTG $^{-/-}$ mice, which indicates that PTTG removal does not prevent initiation of FTC (Kim et al., 2007a). Therefore, in this model it appears PTTG has a more prominent role in tumour growth and progression rather than initiation. One caveat, however, is the background of the TR β PV mutation, which is unlikely to be generally apparent in human FTC, and may elicit altered pathways of tumour initiation. Further studies are merited in this important and intriguing model.

The role of PTTG in tumour progression

Following the mutational events which elicit the initial transformation of a normal to a neoplastic cell and clonal expansion, further genetic changes are required for the acquisition of novel properties that allow continued growth and progression towards malignancy. In addition to its role in tumour initiation PTTG appears to play an important role in tumour growth and progression.

PTTG and Angiogenesis

Angiogenesis is a key rate-limiting step in tumour progression and critical for metastatic spread (Folkman, 1990, Hanahan and Weinberg, 2000). The concept of 'angiogenic switch' has been proposed to describe a discrete and critical rate limiting event in tumour growth, and represents the transition from the pre-vascular to the vascular phase in early tumour progression (Bergers and Benjamin, 2003, Hanahan and Folkman, 1996). Many dormant tumours, including incidental pituitary adenomas and thyroid microcarcinomas, are discovered during autopsies in individuals who have died of causes other than cancer (Black and Welch, 1993), thus supporting the notion that only a small subset of dormant tumours enters the second vascular phase, following the 'angiogenic switch', in which exponential growth ensues.

PTTG upregulates FGF-2 (Zhang et al., 1999) and VEGF (McCabe et al., 2002) expression, and has been shown to induce angiogenesis (Ishikawa et al., 2001). The conditioned medium from NIH3T3 cells stably overexpressing PTTG induced proliferation, migration and tube formation of human umbilical vein endothelial cells *in vitro* and chick chorioallantoic membrane spoke-wheel-like appearances *in vivo* (Ishikawa et al., 2001). Thyroid cancers in TR β PV.PTTG $^{-/-}$ transgenic mice, where PTTG was eliminated, had significantly reduced vascular invasion and vessel density and were associated with reduced metastases. Further, *in vitro* motility assays using primary cultures of thyroid cells from these mice demonstrated rescued cell motility independent of angiogenesis and proliferation (Kim et al., 2007a). Thyroid tumours induced in mice by stable expression of PTTG showed increased MMP-2 expression and activity. MMP-2 specific antibody treatment led to decreased cell migration and invasion *in vitro* (Malik and Kakar, 2006). These findings lend further support to PTTG's role in tumour invasion and metastasis.

Using microarray analysis PTTG over-expression in primary thyroid cells was shown to enhance expression of multiple angiogenic stimulators and repress expression of angiogenic inhibitors (Kim et al., 2006b), analogous to the angiogenic switch. The precise role played by each of these angiogenic factors awaits further investigation. Of the multiple genes shown to be differentially expressed, it is likely that a small number of angiogenic genes are critical to PTTG's direct promotion of angiogenesis, whereas others may be involved downstream of these key genes.

Growth Factor Induction

Self-sufficiency of growth factors is believed be another crucial property acquired

during tumour progression. Autocrine and paracrine growth promoting mechanisms would allow tumours to become insensitive to external control. A recent study demonstrated KDR expression in human thyroid epithelial cells and significantly elevated expression in differentiated thyroid cancers (Kim et al., 2006a). Receptor functionality was confirmed by demonstrating VEGF activation of the KDR-dependent downstream MAPK pathway. Using a KDR-specific inhibitor, VEGF was shown to promote thyroid cell proliferation via KDR activation. Furthermore, up-regulation of the transcriptional factor Inhibitor of DNA-binding 3 (ID3) was demonstrated to be critical to this autocrine mechanism. These findings suggest that secreted VEGF may stimulate thyroid cell proliferation via an autocrine or paracrine mechanism involving a KDR-dependent MAPK-ID3 downstream pathway. Importantly, PTTG was shown to up-regulate both KDR expression and VEGF secretion, and to stimulate KDR-dependent cell proliferation (Kim et al., 2006a). Therefore, high PTTG expression, apparent in thyroid cancers, may promote a VEGF-KDR-ID3 autocrine/paracrine mitogenic pathway, a potentially important mechanism in thyroid tumorigenesis.

PTTG and Iodide Uptake

The sodium-iodide symporter, NIS, is responsible for the active and avid uptake of iodine by the thyroid gland. Use of radioiodine therapy for well-differentiated thyroid tumours relies upon this selective uptake for effective ablation of the thyroid gland and reduced toxicity for other tissues. However, numerous studies have shown that thyroid cancers, especially in more aggressive and advanced cases, are associated with reduced or absent NIS function (Boelaert et al., 2007, Smanik et al., 1997, Tanaka et al., 2000). Two studies have demonstrated NIS expression to be suppressed by overexpression of PTTG (Boelaert et al., 2007, Heaney et al., 2001). Recently, immunohistochemical analysis of thyroid tumours demonstrated a significant association between PTTG expression, persistent disease and reduced NIS function (Saez et al., 2006). More detailed mechanistic examination of the interaction between PTTG and NIS showed that PTTG – as well as its binding factor PBF – repressed NIS expression and function by specific inhibition of NIS promoter activity (Boelaert et al., 2007). A complex PAX8-USF1 response element was shown to be critical both to the basal promoter activity and to PTTG repression of NIS. It is conceivable that reduced NIS function secondary to PTTG overexpression may be responsible for less than optimal ablative radioiodine treatment and the observed higher recurrence rate. For differentiated thyroid cancers, then, PTTG overexpression has implications at three critical levels of disease progression: initiation, promotion and treatment (see **Figure 1**).

PTTG and p53

The ability to evade apoptosis is a critical property acquired by many cancers. DNA damage activates p53, triggering either cell-cycle arrest and DNA damage repair response or programmed cell death (apoptosis) (Levine, 1997, Vousden, 2000). One major study has shown PTTG to interact with p53 (Bernal et al., 2002). PTTG blocked the binding of p53 to DNA and thus inhibited its transcriptional activity and its ability to induce apoptosis (Bernal et al., 2002). In addition, a potentiation of the apoptotic and transactivating functions of p53 was demonstrated in PTTG-deficient HCT116 cells, highlighting the critical physiological relevance of this interaction. However, the exact nature of the functional interaction between PTTG and p53 is a complex one.

p53 has been reported to repress PTTG expression (Zhou et al., 2003), a finding which has been explained by p53 binding to the PTTG promoter (Kho et al., 2004). Further, it has been demonstrated that p53 is necessary for the repression of PTTG in response to DNA damage (Chiu et al., 2006).

The functional interaction between PTTG and p53 therefore appears to pivot upon the presence or absence of DNA damage. In its absence, PTTG binds p53 and interferes with transcriptional and apoptotic pathways. If a cell detects significant DNA damage, p53 represses PTTG expression, presumably overcoming its repression of apoptosis and gene transactivation. The paradox then arises that if PTTG itself induces DNA damage (Kim et al., 2005, Kim et al., 2007b), its levels should be constitutively inhibited by p53. However, most tumour types demonstrate prolonged PTTG over-expression. Further complexities arise in the PTTG/p53 story. siRNA repression of PTTG has been shown to induce p53 activation (Cho-Rok et al., 2006), resulting in apoptosis, and PTTG repression in response to arsenic-induced DNA damage is independent of p53 status in colorectal HCT116 cells (Chao et al., 2006). In breast cancer MCF-7 cells, PTTG overexpression caused p53 stimulation and apoptosis (Yu et al., 2000), a finding confirmed in separate studies in MCF-7 and HEK293 cells (Hamid and Kakar, 2004). In addition, overexpression of p53 along with PTTG augmented apoptosis, whereas expression of the human papillomavirus E6 protein, which downregulates p53, inhibited apoptosis in MCF-7 cells (Yu et al., 2000). Finally, one recent investigation has failed to confirm protein binding of PTTG and p53 *in vitro* (Sanchez-Puig et al., 2005). It is thus apparent that the PTTG-p53 relationship is incompletely understood and further studies are required to elucidate their complex and intriguing interactions in the presence and absence of DNA damage.

PTTG & genetic instability

The ‘mutator phenotype’, proposed by Loeb et al in 1974 (Loeb and Springate, 1974), suggested that cancer cells acquire a degree of ‘genetic instability’ early on as a result of mutations in genes involved in the maintenance of DNA integrity during replication.

Thyroid cancers have been demonstrated to exhibit both chromosomal and intra-chromosomal instability. Allelic deletions are an indication of chromosomal instability and, in several studies of tumour loss-of-heterozygosity (LOH) patterns, specific chromosomal regions such as 3p, 2p, 2q and 11q appear more susceptible to allelic loss in thyroid tumours (Marsh et al., 1997, Kubo et al., 1991, Ward et al., 1998, Zedenius et al., 1995) compared with normal thyroid tissue. Aneuploidy is a common feature of thyroid follicular adenomas (29%) and carcinomas (56%), as well as of many human thyroid carcinoma cell lines (Joensuu et al., 1986, Joensuu and Klemi, 1988). Further, development of chromosomal instability has been suggested to underlie the progression to the more aggressive phenotype of anaplastic thyroid cancer.

PTTG is the human securin (Zou et al., 1999), a key mitotic checkpoint gene involved at the metaphase-anaphase transition, and sequesters the active protease separase, preventing the premature separation of sister chromatids. PTTG has been shown to be involved in both UV and chemotoxic DNA damage response pathways in mammalian cells (Zhou et al., 2003, Romero et al., 2004). Theoretically, the increased expression of PTTG apparent in many cancers would suppress PTTG-dependent DNA damage responses, resulting in the rapid accumulation of mutations. More

recently, PTTG's interaction with separase, and separase's subsequent stabilisation, was proposed as a novel double-stranded DNA (dsDNA) break repair mechanism, acting through the cleavage of cohesin (Nagao et al., 2004). PTTG has been shown to interact with other DNA repair genes, including p53 and Ku70 (Romero et al., 2001, Bernal et al., 2002). Together these observations suggest that PTTG may play a critical role in the development of genetic instability in endocrine tumours.

The first direct evidence of its role in the development of genetic instability came from the demonstration that PTTG over-expression induces aneuploidy, arising from chromatid mis-segregation (Yu et al., 2003). Subsequently, a strong relationship between PTTG expression and the degree of genetic instability was observed in thyroid cancers, and PTTG over-expression was shown to induce genetic instability in a dose dependent manner in FTC133 thyroid *in vitro* (Kim et al., 2005).

Ku70 is a critical protein which acts to trigger the non-homologous end-joining (NHEJ) repair pathway, the predominant mammalian route for repairing dsDNA breaks (Collis et al., 2005). PTTG over-expression in HCT116 cells was shown to inhibit etoposide-induced dsDNA damage repair activity, and to inhibit Ku70 DNA-binding function (Kim et al., 2007b). Based on these data it was hypothesised that PTTG forms an inhibitory complex with Ku70. Previously, PTTG was shown to dissociate and release free and active Ku70 in response to DNA damage (Romero et al., 2001). However, against a background of aberrantly high PTTG expression, PTTG sequestration of Ku70 would inhibit the NHEJ repair pathway, and this may therefore represent a critical mechanism in the development of genetic instability in thyroid and other cancers.

Summary

In the 10 years since its discovery, there has been a significant progress in our understanding of PTTG's role in tumourigenesis. Studies have demonstrated PTTG to be involved in inducing multiple cancerous traits in endocrine tumours including invasion, metastasis, dedifferentiation, evasion of apoptosis and angiogenesis, which are critical for tumour progression. Recent studies using transgenic mouse models of both PTTG overexpression and inactivation support a causal role for PTTG in the development of endocrine tumours. The wealth of data to date suggest therefore that PTTG may play a dual role in endocrine tumour development, being involved in both tumour initiation and subsequent progression towards more aggressive phenotypes. Emerging work continues to dissect these elaborate and critical mechanisms. Central to future endeavours will be understanding the precise nature of PTTG's interaction with p53, dissecting common mechanisms of induction in endocrine and endocrine-related tumour types, and uniting PTTG research within the cell cycle and endocrine communities.

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Figure Legend

The multiple implications of PTTG overexpression in differentiated thyroid cancers. PTTG induces genetic instability and chromosomal abnormalities as early initiation events in thyroid cells. PTTG's interaction with its binding factor PBF and p53 may modulate these processes. Overexpression of PTTG results in altered regulation of growth factors such as FGF-2, VEGF and ID3, which have been associated with increased angiogenesis. In terms of radioiodine treatment of differentiated thyroid tumours, high PTTG expression represses NIS promoter activity and iodine uptake, reducing the efficacy of treatment.

