COMMENTARY

PTTG – a new pituitary tumour transforming gene

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Abstract

The pathogenesis of sporadic pituitary tumours remains elusive. Recently, a new candidate gene has been described which is able to induce pituitary cell transformation, and the expression of which appears to be strongly correlated with pituitary tumorigenesis. The so-called pituitary tumour transforming gene (PTTG) encodes a 23 kDa, 202 amino acid protein, and is located on chromosome 5q33, a locus previously associated with recurrent lung cancer and acute myelogenous leukaemias.

Although the precise function of PTTG protein is unknown, *in vitro* experiments have demonstrated that it is capable of inducing fibroblast growth factor (FGF) expression. Mutation of the two proline-rich domains of the PTTG protein has also been shown to abolish subsequent FGF induction. Furthermore, in patients with pituitary adenomas, serum FGF concentrations fall post-operatively after successful excision of the tumour.

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Introduction

Following characterisation of the multiple endocrine neoplasia 1 (MEN 1) gene and acknowledgement of its undoubted importance in the aetiology of hereditary endocrine cancers, it comes with much disappointment that MEN 1 does not live up to its potential in being a significant aetiological factor in sporadic pituitary tumours. Data increasingly suggest that the MEN 1 gene fails to play a pivotal role in sporadic pituitary tumorigenesis (Zhuang *et al.* 1997, Prezant *et al.* 1998). Recently, a potent pituitary tumour transforming gene termed PTTG has been identified, which appears to be expressed in the majority of sporadic pituitary tumours. The capacity of PTTG to induce cellular transformation in the absence of other oncogenes is unique. Furthermore, transplanted PTTG-transformed cells are capable of tumour growth in nude mice. This commentary describes the current understanding of the role of the PTTG in pituitary tumorigenesis.

Isolation and characterisation of PTTG

PTTG was first isolated from rat growth hormone (GH)-secreting pituitary cell lines by differential mRNA display (Pei & Melmed 1997). Two pituitary tumour-specific mRNAs were identified using this approach, one of which was the insulin-induced growth response protein, and the

other a differentially expressed, 396 bp mRNA showing no homology to current Genbank entries. Use of this mRNA fragment to probe a rat pituitary tumour cDNA library enabled the isolation and characterisation of a full length (976 bp) PTTG cDNA clone. The gene was found to be composed of 5 exons which encoded a 199 amino acid, 25 kDa protein (Pei & Melmed 1997). The full length PTTG cDNA sequence appeared to bear some homology to several partial transcripts of unknown function expressed during mouse embryonic development and also expressed in ovarian cancer, although subsequent similarity searches performed on the PTTG protein revealed no homology with known protein sequences. In addition, no common functional motifs have been recognised in the PTTG product.

Northern and reverse transcription-PCR analysis of rat PTTG expression has revealed two potential functional mRNA isoforms of the gene, resulting from alternative splicing, use of alternative promoters or polyadenylation sites. Such variants are tissue specific in their expression – a truncated PTTG mRNA isoform of approximately 1 kb is expressed in adult rat testis (both germ and supporting Sertoli and Leydig) cell lines and in embryonic liver at relatively low levels, whereas a 1.3 kb isoform is strongly expressed in pituitary tumour cells (see Table 1). In adult rat tissues, only the testis contains significant PTTG; other organs appear to be devoid of detectable PTTG mRNA. Interestingly, this pattern of PTTG mRNA expression is similar to that of the proto-oncogenes *c-mos* and *c-abl* and

Table 1 Expression of full length and truncated PTTG transcripts in embryonic and adult rats

	PTTG location	Transcript	Expression
Cell lines	Leukaemia	Full length (ca. 1.3 kb)	High
	Lung carcinoma		
	Lymphoma		
	Melanoma		
	HeLa		
Adult rat	Testis	Short (ca. 1 kb)	Low
Embryo	Liver	Short	Low
Tumours	Pituitary	Full length	High

the homeobox gene, PEM, although it remains to be determined whether this co-localisation of expression is of functional significance.

Due to the lack of sequence homology of PTTG with other proteins, and the absence of clear functional domains, there are few clues as to the mechanism by which PTTG is able to effect cellular transformation. A number of experiments have been performed both *in vitro* and *in vivo*, however, which serve to highlight the potential significance of PTTG expression in pituitary tumour cells. NIH 3T3 fibroblast lines have been stably transfected with the entire coding sequence of PTTG and clones that express high levels of PTTG protein assessed for changes in growth characteristics. Interestingly, growth rates in transfected cells were reduced to 50–75% of untransfected levels. Given the pedestrian growth rate of human and rat pituitary adenomas, the suppression of cell proliferation by the PTTG product is to be expected if the gene does indeed play a key role in pituitary tumorigenesis.

In addition to measuring growth velocity of PTTG-transfected 3T3 fibroblasts, evidence of cell transformation has also been sought. Transfectants expressing PTTG were found to exhibit colony formation and anchorage independent growth in soft agar, features not apparent in non-transfected cells, indicating cellular transformation (Pei & Melmed 1997). To determine whether PTTG might induce tumour formation in adult animals, PTTG-transfected fibroblasts were injected subcutaneously into athymic nude mice. All such mice developed tumours within three weeks, compared with no such changes in controls, confirming that PTTG is a potent transforming gene *in vivo* (Pei & Melmed 1997).

Human PTTG

Using the rat cDNA as a probe, molecular characterisation of the human PTTG gene from a human fetal liver cDNA library has recently been possible (Zhang *et al.* 1998). Through the use of fluorescent *in situ* hybridisation (FISH), human PTTG has been localised to the chromo-

somal location 5q33, interestingly a genomic region previously associated with a number of recurrent neoplastic abnormalities of the lung (Hosoe *et al.* 1994, Wu *et al.* 1995, Hosoe 1996) and with acute myelogenous leukaemias (Wlodarska *et al.* 1996, Abe *et al.* 1997). The 202 amino acid protein sequence of human PTTG contains both proline- and basic amino acid-rich domains, features important for signal transduction.

Transcriptional regulation of the PTTG gene has only been examined to date in the rat promoter region (Pei 1998). Transfection-deletion analysis has identified the major site of upstream regulation of PTTG to be a 115 bp region some 500 bp from the transcriptional start site. DNaseI footprinting assays revealed interaction of nuclear proteins from testicular cell lines within this regulatory sequence, including interactions at two consensus binding sites for the general transcription factor, Sp1. Deletion of the 115 bp regulatory sequence resulted in abolition of promoter function, although, interestingly, mutation of the two Sp1 sites failed to significantly impair promoter action, indicating that the Sp1 binding sites are not vital elements of the enhancer sequence of PTTG. At least another three nuclear proteins have been shown to be able to interact with the PTTG upstream regulatory region in a cell-specific manner, although the nature of such proteins is at present unknown.

Possible mechanisms of PTTG-induced cellular transformation

Currently, the mechanism by which PTTG is able to cause cell transformation remains unclear (see Fig. 1). However, preliminary data (Zhang *et al.* 1998) suggest that PTTG may act through the fibroblast growth factor (FGF) family. FGF2 is important in pituitary regulation and has mitogenic, angiogenic, and hormone regulatory functions (Gospodarowicz *et al.* 1987, Rifkin & Moscatelli 1989). Abnormal expression of FGF2 (Silverlight *et al.* 1990) and its receptor isoforms (Abbass *et al.* 1997) have been reported previously in pituitary adenomas. Furthermore, following successful surgical removal of anterior pituitary tumours, patients show reduced serum FGF concentrations compared with pre-operative levels (Zhang *et al.* 1998). Also, PTTG has been shown to signal FGF transcription *in vitro* (Zhang *et al.* 1998), providing a possible mechanism for PTTG in inducing cell transformation. Additional evidence that PTTG acts through FGF is provided by the observation that injection of stably transfected 3T3 cells carrying PTTG sequences with mutated proline-rich domains not only abolishes the transforming ability of the gene in mice, but also fails to cause increased expression of FGF that is observed in animals harbouring the intact, full length PTTG.

Whatever the precise function of PTTG *in vivo*, its cellular localisation appears, from *in situ* evidence, to

Figure 1 Possible mechanisms of induction and promotion of sporadic pituitary tumours. As a potent transforming gene, PTTG is able to induce cellular transformation of differentiated stem cells, most likely through the family of FGFs.

be cytoplasmic. The observation that PTTG has been identified in lung carcinoma, melanoma, leukaemia and lymphoma immortalised and malignant cell lines (Pei 1998), would suggest a general role for the full length PTTG in tumorigenesis *per se*, rather than exclusively in the pituitary. In the adult rat, PTTG mRNA is exclusively expressed in the testis, suggesting that this shorter, alternatively spliced isoform may perform some other function in the testis. Indeed, the testis relatively frequently gives rise to transcripts that differ from their somatic counterparts due to differential processing or use of alternative promoters (Wolgemuth *et al.* 1986, 1987, Featherstone *et al.* 1988, Propst *et al.* 1988, Garrett *et al.* 1989, He *et al.* 1989, Rosner *et al.* 1990, Wolgemuth *et al.* 1991, Watrin & Wolgemuth 1993).

Clinical relevance of PTTG in human pituitary adenomas

Preliminary data relating PTTG expression in human pituitary adenomas shows that, of 46 secreting and non-secreting anterior pituitary tumours analysed, all expressed PTTG. Furthermore, 83% of tumours expressed PTTG at a level at least 50% greater than that observed in normal pituitaries. Although only small numbers of tumours have been examined, in subsets of GH- and prolactin-secreting tumours, a correlation between levels of PTTG expression and tumour invasiveness has been noted (Zhang *et al.* 1998).

The future for PTTG

The future for research into the role of PTTG in pituitary tumorigenesis is exciting. Clearly, PTTG is a potent transforming gene which is expressed in rat pituitary cell lines and human pituitary adenomas. PTTG is a tumour promoter gene that has the ability to act alone, and does not require the presence of a complimentary oncogene, as

is generally necessary (Land *et al.* 1983, Ruley 1983, Schwab *et al.* 1985), to induce cell transformation. Little is currently known of what regulates PTTG transcription, thus mechanisms underlying the activation of PTTG expression remain wholly speculative. Preliminary data suggest that PTTG may function through the family of FGFs, although the steps involved in this activation are not understood. PTTG has been shown to inhibit cell proliferation in a manner akin to cell transformation due to transforming growth factor β , although the rapid growth of subcutaneously grown PTTG-transfected fibroblast tumours in athymic nude mice implies that once transformed, cell proliferation may be accelerated.

The observation that the transforming ability of PTTG is dependent upon proline-rich domains may be of significance in the future in terms of therapeutic manoeuvres in the treatment of pituitary tumours. If further studies confirm a relationship between PTTG expression and tumour invasiveness, there is a potential role for PTTG expression to be used as a prognostic marker in surgically excised pituitary tumours, allowing selection of subgroups of tumours for early radiotherapy, for example, in tumours with high levels of expression of PTTG. It is becoming evident that PTTG is not a pituitary-specific transforming gene, given the observation of other, non-pituitary, cell lines showing increased expression of the gene (Pei 1998). Expression of PTTG may thus be of aetiological significance in a range of other tumour types. This may be expected, considering its genomic location at chromosome 5q33, a site previously identified as being associated with a number of malignant conditions.

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