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Review

PTTG and cancer

T. Hamid and S.S. Kakar

Department of Medicine and James Brown Cancer Center, University of Louisville, Louisville, Kentucky, USA

Summary. Pituitary tumor transforming gene (pttg) is a recently isolated oncogene that is expressed in most of the tumors. Overexpression of pttg results in an increase in cell proliferation, induces cell transformation *in vitro*, and promotes tumor formation in nude mice. The gene encodes a protein of 202 amino acids with no significant homology with other known proteins. The protein is a multi domain consisting of a transactivation domain, domain required for ubiquitin-mediated proteolysis and a DNA binding domain. pttg protein is bestowed with a multitude of functions and seems to be involved in most of the important mechanisms of cell proliferation, differentiation and signaling. Given the number of processes that are involved in the manifestation of cancer, it thus becomes mandatory to study the role of this potent oncogene in relation to the processes of cell survival, death and functioning.

Key words: PTTG, Pituitary, tumor, Oncogene, Tumorigenesis

Introduction

Cancer is a broad term encompassing a plethora of conditions characterized by unscheduled and uncontrolled cellular proliferation. The causes of cancer are varied and include genetic predisposition, environmental influences, infectious agents and ageing. These events transform normal cells into cancerous by derailing a wide spectrum of regulatory and downstream effector pathways. Cancer is a common and widely publicized disease and inspite of ever increasing efforts to understand it as a process, its incidence in the population is rising. Cancer may affect any organ or tissue, but, while some cancers are common (for example, lung, breast, skin, gastrointestinal tract and prostrate) others are rare.

Pituitary tumor formation follows the general principles of tumorogenesis (Alexander et al., 1990; Shimon and Melmed, 1997). The current model hypothesizes that pituitary tumor originate from the uncontrolled proliferation of a single cell with a gain of proliferative function (initiation), secondary mutations and/or alteration of regulatory factors thereafter favor the clonal expansion and tumor progression (promotion).

Offprint requests to: Dr. Sham S. Kakar, Dept of Medicine, 570 S. Preston Street, Baxter Biomedical Building, Room 240 C, University of Louisville, Louisville, KY 40202, USA. Fax: 502-852-2356. e-mail: sskaka01@louisville.edu

PTTG: Cloning, expression and its molecular mechanism

Pituitary tumor transforming gene (pttg) is a recently characterized proto-oncogene that was originally cloned from a rat pituitary tumor by differential mRNA display polymerase chain reaction (PCR) (Pei and Melmed, 1997). Subsequently the human homologue of this transforming gene was cloned from human testis and liver (Kakar and Jennes, 1999) (Fig. 1). The two genes share a striking structural similarity. The nucleotide sequence of hpttg and rat pttg cDNA and their encoded proteins share 85% and 89% of their identity respectively. The normal tissue distribution of pttg expression is restricted to testis in case of rats (Pei and Melmed, 1997), however in humans the expression has been reported not only from testis but also from other tissues including thymus, colon, small intestine and weakly in brain and placenta. Interestingly, pttg is highly expressed in all tumors and tumor cell lines evaluated (Kakar and Jennes, 1999; Zhang et al., 1999).

Overexpression of hpttg in mouse fibroblast cells (NIH 3T3) results in increased cell proliferation, induction of cellular transformation *in vitro* and promotion of tumor formation in nude mice (Kakar and Jennes, 1999). In addition to its role in cellular transformation, pttg overexpression also induces basic fibroblast growth factor (bFGF) production both at mRNA and protein levels (Zhang et al., 1999; Kakar et al., unpublished results). Increased bFGF expression has been reported in several human tumors in which it is considered to be stimulating growth factor important for angiogenesis (Ray and Melmed, 1997). The primary structure of pttg gene has been evaluated. The gene spans over 10 Kb and consists of five exons and four introns and is localized to human chromosome 5q35.1

(Kakar, 1999). The Southern blot analysis of human genome and chromosomal mapping of hpttg eventually led to the identification of two additional members of this pttg gene family (Chen et al., 2000). Based on the similarity in the sequences of these genes, Chen et al. (2000) proposed that hpttg be named as hpttg1 and the two other members of the family be called hpttg2 and hpttg3 respectively. The existence of the new homologues was confirmed by localization of hpttg2 to chromosome 4p15.1 and hpttg3 to chromosome 8q13.1 (Chen et al., 2000). Northern blot analysis for expression of these new genes indicated that hpttg2 is expressed in most of the normal and tumor tissues, whereas, the expression of hpttg3 is limited to cancer tissues and cancer cell lines (Prezant et al., 1999; Chen et al., 2000). Taken together these data indicate a tissue-specific expression of these gene and differential roles they might play in normal cellular function and tumorigenesis.

The protein sequence of pttg consists of 202 amino acids and has a calculated molecular mass of 22,024 dalton and an isolelectric point of 6.55. The protein can be divided into two domains, an amino-terminal domain with isoelectric point of 11.2 and a carboxy-terminal domain with isoelectric point of 3.8. The protein has consensuses motifs for cyclic AMP, and cyclic GMP dependent protein kinase phosphorylation, casein kinase II phosphorylation and protein kinase C phosphorylation. The pttg protein contains a proline-rich region, which in the human sequence includes three P-X-X-P motifs as opposed to a single P-X-X-P motif in rat pttg protein (Fig. 2). These functional motifs represent potential SH3 docking regions, suggesting that pttg might be involved in SH3-mediated intracellular signaling.

pttg protein is predominantly localized to the cytoplasm with a partial nuclear localization. However, nuclear translocation of pttg can be facilitated either by interaction with pttg binding factor (Chein and Pei, 2000) or by activation of mitogen-activated protein kinase cascade (Pei, 2000). It is although not clear how pttg is targeted to nucleus since it does not have a nuclear localization signal. It is speculated that its small size might be responsible for its entry into the nucleus. The amino acid sequence of pttg does not show absolute homology with any of the known protein sequences but does bear a close similarity with some DNA binding proteins, including a known transcription factor: mouse stromelysin factor (Dominguez et al., 1998). The similarity along with its partial nuclear localization led to the evaluation of its role as a transcriptional activator, however, our recent studies indicate that pttg also acts to repress its own transcription (Flynn and Kakar, unpublished results). Yeast two-hybrid screening studies in testicular germ cells have identified ribosomal protein S10 and a novel human homologue of the bacterial heat shock protein DnaJ; HSJ2 as binding partners to pttg. The fact that pttg mRNA is expressed highly in a stage specific manner in spermatocytes and spermitids during rat spermatogenic cycle (Pei, 1998) suggests that pttg may be involved in regulating male gem cell differentiation. The expression levels of pttg are also found to be modulated in a cell cycle-dependent manner in rapidly proliferating cells, with the levels peaking at mitosis (Ramos-Morales et al., 2000) suggesting that pttg might also play important role in cell proliferation.

The involvement and importance of pttg in cell proliferation is further supported by the recent findings that implicate its role in sister chromatid separation during cell division (Zou et al., 1999). pttg by virtue of its function as human securin ensures that there is no premature separation of sister chromatids. By the end of metaphase pttg is degraded by an anaphase promoting complex (APC) that recognizes a conserved motif localized within its N-terminus called the destruction box (D-box) releasing inhibition of separin, which in turn lead to the degradation of separin proteins that glue the sister chromatids together (Fig. 3). Premature

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  1   MATLIYVDKE NGEPGTRVVA KDGLKLGSGP SIKALDGRSQ VSTPRFGKTF DAPPALPKAT
 61   RKALGTVNRA TEKSVKTKGP LKQKQPSFSA KKMTEKTVKA KSSVPASDDA YPEIEKFFPF
121   NPLDFESFDL PEEHQIAHLP LSGVPLMILD EERELEKLFQ LGPPSPVKMP SPPWESNLLQ
181   SPSSILSTLD VELPPVCCDI DI
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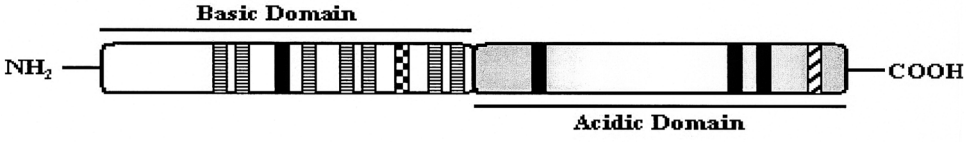
Fig. 1. Deduced amino acid sequence of the pttg protein. The sequence is available from EMBL/GenBank under accession number AF074242.



▤ : Protein kinase C phosphorylation site; ▨ : Casein kinase phosphorylation site.
▩ : cAMP and cGMP dependent protein kinase phosphorylation sites;
■ : Proline rich region;

Fig. 2. Schematic representation of pttg protein. The different motifs present in the protein are depicted.

activity of APC is under the inhibition of kinetochore. The securin function of pttg has been confirmed by the observations that overexpression of mutant pttg leads to disruption of sister chromatid separation (Zou et al., 1999). Interestingly, securin degradation has also been reported to be dispensable for chromatid separation and successful execution of either mitosis or cytokinesis (Zur and Brandeis, 2001).

Over expression of pttg inhibits mitosis and delays cell division since the cells require more time to degrade overexpressed pttg, that acts as securin, before anaphase (Yu et al., 2000). Loss of yeast Pds1p or Drosophilla securin pimples is lethal (Funabiki et al., 1996). However, mice lacking pttg are viable and fertile but do exhibit distinct cellular and physiological phenotypes including splenic and testicular hypoplasia, thymic hyperplasia, thrombocytopenia, aberrant cell cycle progression and premature centromere division (Wang et al., 2001). These findings implicate the involvement of more than one mechanism(s) to regulate mammalian sister chromatid separation.

Cellular characteristics of pttg have also been addressed (Yu et al., 2000). pttg mRNA and protein expression are low at G_1/S interphase, gradually increasing during the S phase and as reported earlier (Ramos-Morales et al., 2000) peaking at G_2/M phase. However as the cells enter anaphase, pttg is degraded and daughter cells express little pttg. The degradation of pttg occurs via ubiquitin since pttg contains a D-box that is required for such proteolysis (Zou et al., 1999). Intracellular pttg localization is predominantly nuclear at interphase with significant expression in the cytoplasm, but localized to mitotic spindles during early mitosis (Zou et al., 1999). This dual intracellular localization of pttg correlates with its activity as a human securin.

Analysis of the protein sequence for pttg revealed that the protein contains an amino-terminal basic domain and a carboxy-terminal acidic domain. In many eukaryotic transcription factors, the presence of an acidic region correlates with the transactivation domain (Ptashne and Gann, 1988). Transient transfection of fusion constructs containing GAL4 DNA-binding domain and C-terminal region of pttg indicated that pttg could indeed act as a transactivating protein (Dominguez et al., 1988, Flynn and Kakar, unpublished data). The downstream targets of pttg gene function have also been examined. Among these candidate targets, the most important ones include c-myc oncogene and basic fibroblast growth factor (bFGF). Further point mutations within the SH3 binding domains within the C-terminal region of pttg led to a decrease in bFGF levels (Zhang et al., 1999).

The role of pttg in cell proliferation and differentiation implicate its role in cell signaling pathways. Many of these pathways converge on the mitogen-activated protein kinase (MAPK) cascade. Of many of the roles of this system is the regulation of gene expression within nucleus. The presence of a consensus

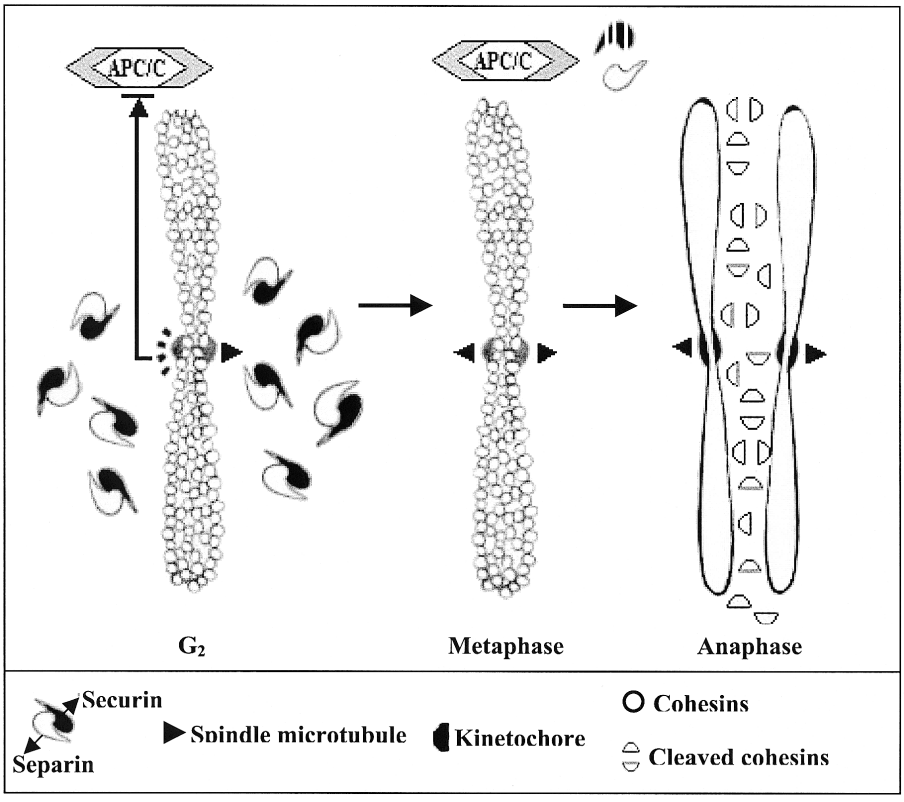


Fig. 3. Schematic representation of sister chromatid separation as it occurs in yeast.

MAP kinase phosphorylation site (Pro-X-Ser/Thr-Pro) within the transactivation domain of pttg led to the findings that transactivation function of pttg is enhanced by the activation of MAP kinase cascade (Pei, 2000), further pttg was also demonstrated to be phosphorylated at Ser162 *in vitro* and that pttg interacts with MEK1 through SH3-binding motif located between the amino acids 51 and 54 at the N-terminus of pttg (Pei, 2000). Taken together these findings indicate that a growth factor stimulated MAP kinase plays an important role in modulating pttg function.

Among other genes that are influenced by pttg expression include the *c-myc* oncogene. Overexpression of *c-myc* stimulates the cell cycle progression, leads to transformation, blocks differentiation and induces apoptosis (Henriksson and Luscher, 1996). pttg has been shown to bind to c-myc promoter near the transcription initiation site in a protein complex containing the upstream stimulatory factor (USF-1). The pttg DNA-binding site was mapped to a region between amino acids 61 and 118 (Pei, 2001). These results indicate that pttg has a DNA binding domain and that this domain is important for its transactivating properties, since mutant-containing deletion of this domain is unable to bind DNA and also inhibits the transcriptional activation by wild type pttg (Pei, 2000). The amino acid sequence in this region is rich in basic amino acids containing 12 lysine and arginines and does not contain any common DNA binding motifs. pttg DNA binding domain may thus represent a new motif for DNA-binding proteins.

Another protein that interacts with hpttg is KU-70, the DNA binding component of DNA-dependent protein kinase that is a DNA-activated nuclear serine/threonine protein kinase, that consists of a regulatory subunit, the hetrodimeric Ku protein (composed of a 70 Kda and 80 kda subunit). These proteins are involved in repairing ionizing radiation-induced damage and in V(D)J recombination (Featherstone and Jackson, 1999). Hpttg interacts both *in vivo* and *in vitro* with Ku heterodimer and is a substrate for DNA-PKcs (Romero et al., 2001). These observations suggest that hpttg might connect the DNA damage-response pathway with sister chromatid separation pathways.

Since over expressed pttg leads to the disruption of normal cell cycle, the role of pttg in cell survival and apoptosis has also been investigated. The results indicate that overexpression of pttg leads to an increased p53 expression along with its translocation to the nucleus (Yu et al., 2000; Hamid and Kakar, unpublished results). Interestingly, pttg overexpression also induces apoptosis in p53-negative cells indicating that pttg-induced apoptosis can either be p53 dependent or independent.

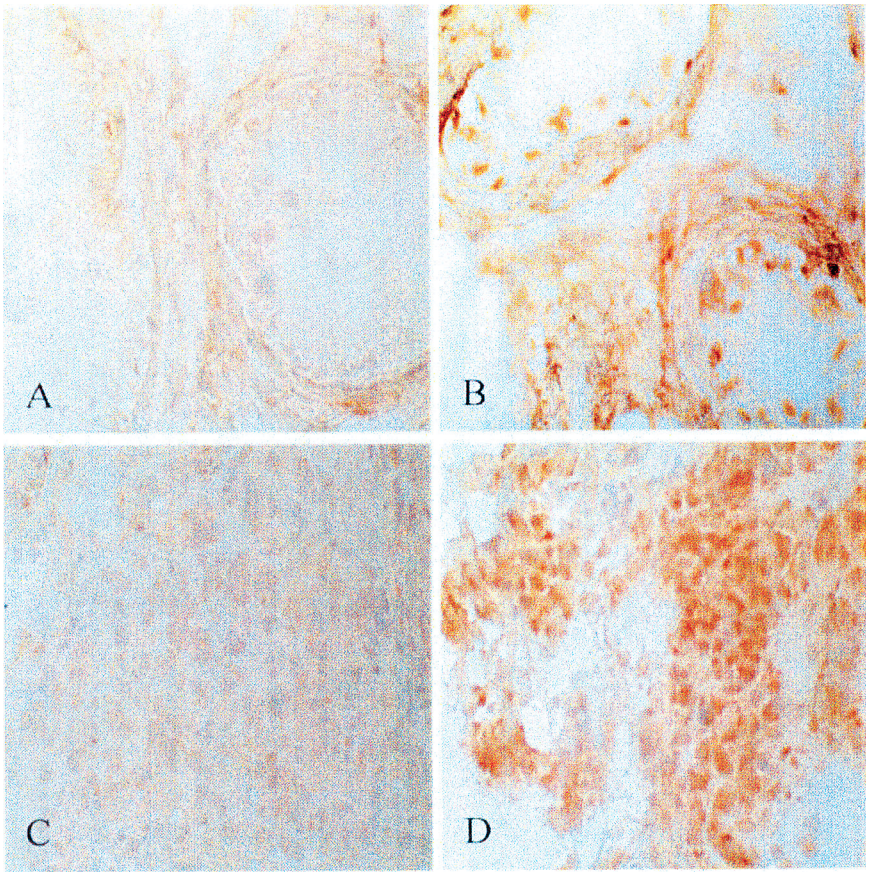


Fig. 4. Immunohistochemical analysis of the human testis and testicular tumor. **A, B.** Normal testis. **C, D.** Testicular tumor. **A, C.** Preimmune serum. **B, D.** polyclonal antibody against pttg diluted 1:1500. The levels of expression of pttg protein was higher in the tumor than that observed in normal tissue. (Reproduced, with permission from Kakar et al., Characterization of Polyclonal Antibody to Human Pituitary Tumor Transforming Gene 1 (PTTG1) Protein, J. Histochem. Cytochem. 49(12): 1537-1545, 2001).

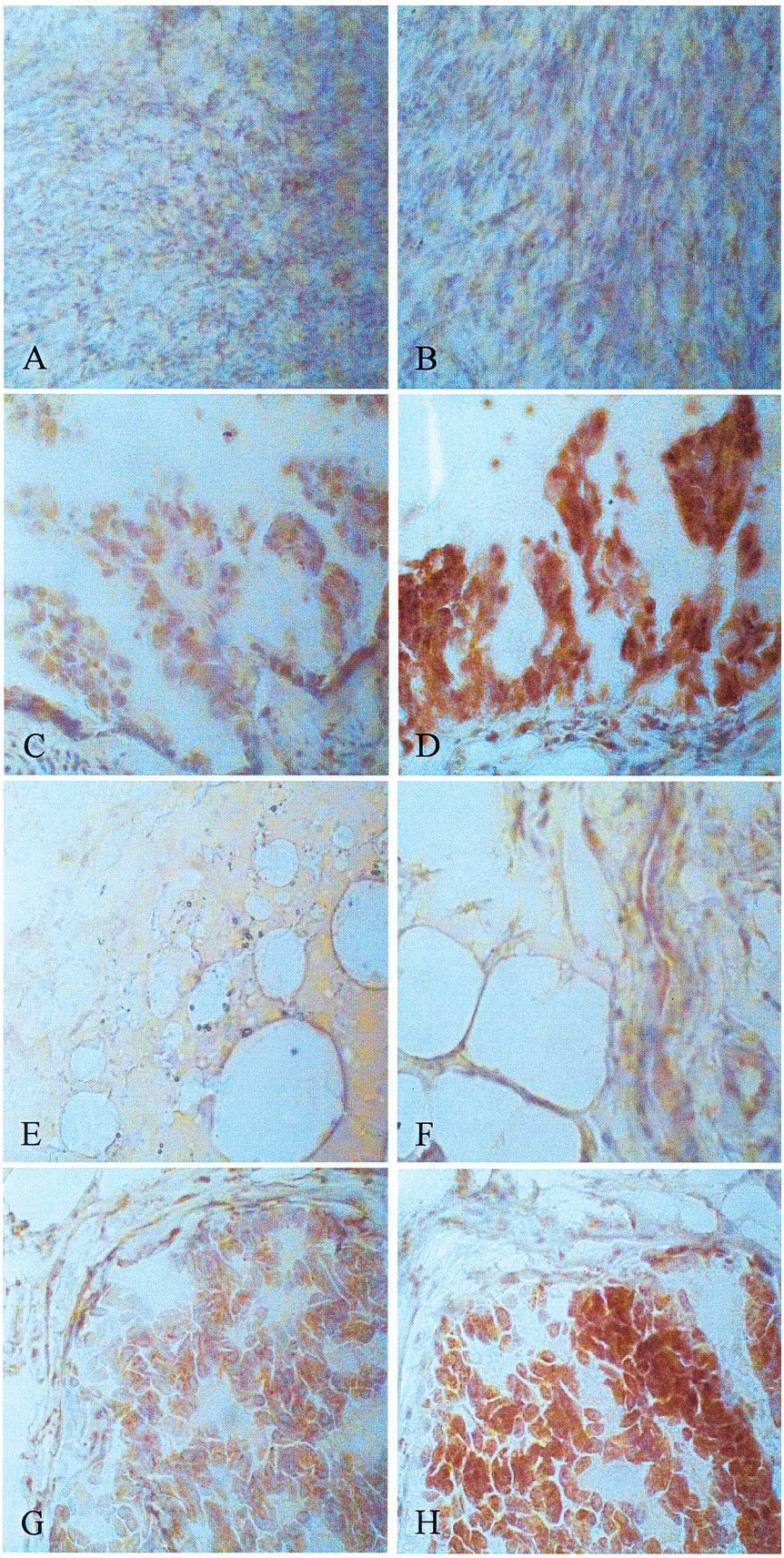


Fig. 5. Immunohistochemical analysis of the human ovary, ovarian tumor, breast and breast tumor. **A, B.** Normal ovary. **C, D.** Ovarian tumor. **E, F.** Normal breast. **G, H.** Breast tumor. **A, C, E, G.** Preimmune serum. **B, D, F, G.** polyclonal antibody against pttg diluted 1:1500. The levels of expression of pttg protein were higher in tumors than those observed in normal tissues. (Reproduced, with permission from Kakar et al., Characterization of Polyclonal Antibody to Human Pituitary Tumor Transforming Gene 1 (PTTG1) Protein, J. Histochem. Cytochem. 49(12):1537-1545, 2001).

However, p53-negative cells exhibit signs of aneuoploidy such as enhancement of micronuclei, macronuclei and chromosomal bridges (Yu et al., 2000). These data indicate that the exact mechanism of p53 activation by pttg is not understood but do suggest that aneuoploidy may be one of the mechanisms for pttg-induced tumorogenesis.

In recent times, it has been debated whether the expression of pttg might serve as a prognostic molecular marker in various cancers. This hypothesis is based on the findings that the primary structure of pttg gene remains unaltered (Puri et al., 2001) and does not reveal any mutations when analyzed in various ovarian tumors, suggesting that its overexpression in human tumors is not related to the accumulation of its altered gene product.

High levels of pttg protein have recently been reported in patients with colorectal and thyroid tumors (Heaney et al., 1999, 2000; Heaney and Melmed, 2000), with the levels correlating positively with cell cycle, tumor metastasis and invasion. Based on these findings and the importance of pttg protein in various cancers, Kakar et al. (2001) developed and characterized a polyclonal antibody against hpttg1 protein by immunization of rabbits with purified hpttg protein. They further demonstrated by immunocytochemical studies that the antibody is specific and is able to localize pttg1 protein in both normal and malignant tissues and proliferating cells (Kakar et al., 2001) (Figs. 4, 5). The specific nature of this antibody suggests that it can be used in the identification of proteins specifically interacting with pttg gene product during the process of tumorogenesis and could also suffice clinical purposes as a diagnostic tool to detect the levels of pttg protein expression in tumor tissues.

Future directions

pttg is a multifunctional gene. Its overexpression leads to an increase in cell proliferation, promotion of tumor formation in nude mice, inhibition of sister chromatid separation and secretion of angiogenic factors (Fig. 6). Based on the results and the studies being carried on pttg it appears that it has far more important

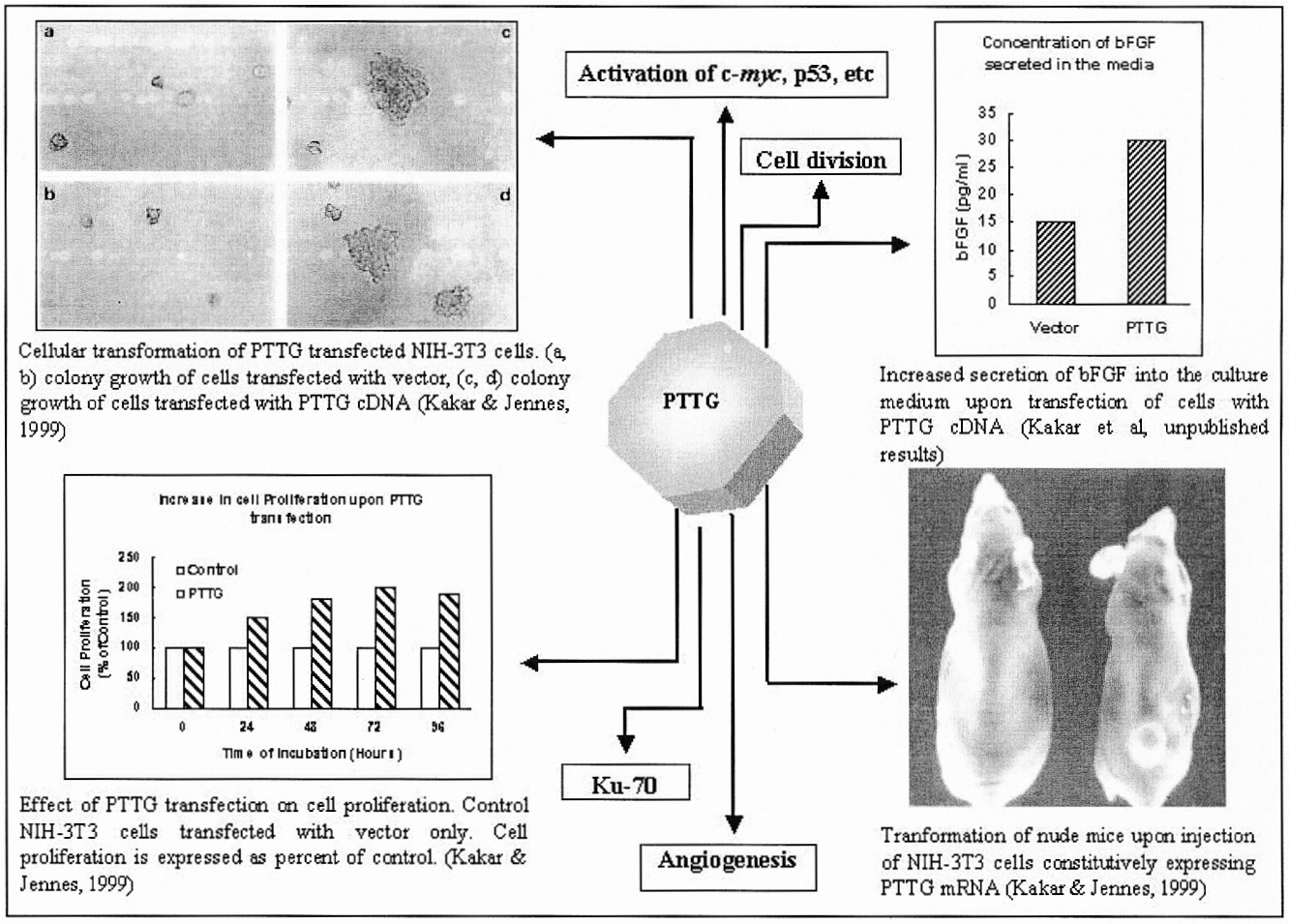


Fig. 6. Some of the important functions ascribed to pttg.

role to play in cancers, then it appears. The function of the other members of the pttg family is yet to be demonstrated and that should provide the researchers with a further insight in the mechanism of action of this oncogene and also to understand the language of its cross talk with other proteins and genes. These studies would help in elucidation of the molecular mechanism of pttg action in human tumorogenesis.

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