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Original Article

Overexpression of Securin in Human Transitional Cell Carcinoma Specimens

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Abstract

Objective: Securin, the product of *PTTG* (pituitary tumor transforming gene), is overexpressed in several tumors, and plays important roles in cancer progression and invasion. In our previous report, securin expression was observed in transitional cell carcinoma (TCC; the most common pathological pattern of bladder cancer) cell lines, including BFTC905, T24, and TSGH8301. However, the existence of securin in human bladder cancer specimens has not been established.

Materials and Methods: Commercial bladder cancer tissue arrays (BL208 & BLC661) were used. Slides of paraffin-fixed human bladder tissues included all grades of TCC (18, 22 and 29 tissue samples for grades I, II, and III, respectively), 21 superficial and 41 invasive TCC specimens, and 11 normal urothelial tissue samples. The intensities of securin immunostaining were graded as background, mild, and strong (scored as 0, 1, and 2, respectively), and scores from the nucleus and cytosol were analyzed separately.

Results: We have demonstrated, for the first time, the expression of securin in bladder tissues. Securin was overexpressed in the cytosol and nucleus of all TCC samples compared to normal urothelium, but only cytosolic localization revealed statistical significance. There were no differences in securin immunoreactivity among the different grades of TCC. Significant overexpression of cytosolic but not nuclear securin was found in both superficial and invasive TCC samples compared to normal urothelium. No difference in immunoreactive staining for nuclear and cytosolic securin between superficial and invasive TCC was noted.

Conclusion: Significant enhanced expression of cytosolic securin protein was found in human bladder cancer specimens, suggesting that the level of tissue securin may be a potential biomarker for the diagnosis of bladder cancer. Studies that investigate the relationship between securin expression and the outcomes of bladder cancer patients could be conducted in the future. (*Tzu Chi Med J* 2010;22(4):171–176)

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1. Introduction

Urinary bladder cancer is among the nine most commonly diagnosed cancers in the world [1]. Pathologically, more than 90% of bladder cancers are transitional cell carcinoma (TCC), but small numbers of other histological types, such as squamous cell carcinoma and adenocarcinoma, have also been reported [2,3]. Histological findings revealed by microscope examination as well as clinical staging determined by imaging are the most common tools to evaluate the management and to predict the outcome of bladder cancer. The TNM system provides a detailed staging classification, but the invasive status divided into superficial and muscle-invading types is easier to use. There are some correlations between tumor grades and stages. Most well and moderately differentiated tumors are superficial and most poorly differentiated tumors are invasive into muscle [4].

Many proteins have been examined for potential roles as diagnostic or screening markers for bladder cancer detection [5]. However, the use of these molecular markers is usually not practical for extensive clinical application [6]. Survivin, for example, is an excellent urinary marker for bladder cancer detection [7–9]. Furthermore, survivin expression indicates a poor outcome and serves as a recurrent marker in bladder cancer patients [10–13]. However, our investigation (unpublished results) revealed some discrepant data between the *in vitro* cytotoxic effects and survivin expression after lestaurtinib treatment in TCC cells. Thus, a new marker for outcome monitoring of bladder cancer patients is needed.

Pituitary tumor-derived transforming gene (*PTTG*) was originally identified from rat pituitary tumor [14]. Subsequent studies demonstrated that human securin is identical to the product of *PTTG* [15]. Overexpression of securin has been observed in several human neoplasms other than pituitary tumors, e.g., carcinoma of the breast [16], esophagus [17], and liver [18]. In addition, high level expression of securin is associated with the invasiveness and aggressiveness of thyroid cancer [19]. Securin is also thought to be a proliferation marker and outcome indicator of invasive ductal breast cancer [20]. In our previous study, securin was easily detected by Western blot analysis in three TCC cell lines, i.e., BFTC905, T24, and TSGH8301 [21]. Inhibition of securin expression by TrkB blockade was followed by cytotoxicity [21]. As far as we know, there are no studies investigating the expression of securin in human bladder cancer tissues. In this study, we report the expression of securin in human bladder cancer specimens. We found significantly elevated cytosolic securin expression in all TCC samples regardless of grade or stage, or whether it is superficial or invasive.

2. Materials and methods

2.1. Surgical specimens from commercial tissue arrays

Slides of paraffin-fixed human bladder tissues including all grades/stages of TCC, and normal urothelial tissues from a total of 102 patients were purchased from Biomax, Inc. (BL208 & BLC661; Rockville, MD, USA). Sarcoma, squamous cell carcinoma and adenocarcinoma, and sections with absence of urothelial tissues were excluded from this study. Finally, examined tissues included 11 normal urothelial samples and 69 TCC cases. The case numbers of grades I, II and III TCC were 18, 22 and 29, respectively. Some neoplastic tissues were graded within a range (e.g., II–III), but for subsequent analysis, they were assigned to the higher grade (i.e., III). Some TCC tissues without definite staging ($T_xN_0M_0$ mentioned on the list) were also excluded from further analysis. Thus, the case numbers of superficial ($T_{is}N_0M_0$ and $T_1N_0M_0$) and invasive type ($\geq T_2N_xM_x$) were 21 and 41, respectively.

2.2. Immunohistochemical staining

The protocol and agents used for immunohistochemical staining have been previously described [22]. Initial steps included deparaffinization, rehydration, antigen retrieval, endogenous peroxidase quenching, and background blocking. Sections were incubated for 48 hours at 4°C with primary anti-securin monoclonal antibodies (1:200, ab3305; Abcam, Cambridge, MA, USA). Horseradish peroxidase-conjugated secondary antibodies were added for 30 minutes [NovoLink polymer; Novocastra, Leica Microsystems (UK) Ltd., Milton Keynes, Bucks, UK], and substrate 3,3′-diaminobenzidine (Dako Denmark A/S, Glostrup, Denmark) was added for 5 minutes. Strong, weak, and no immunoreactive staining of the nucleus or cytosol were scored as 2, 1, and 0, respectively [23]. The tissues were viewed under a microscope (×400), and the scores were confirmed by other staff members. A representative scored sample is demonstrated in Fig. 1.

2.3. Statistical analysis

The average score for each grade or stage was expressed as mean±standard error of the mean. The differences in the immunoreactive scores among each group were evaluated by nonparametric Mann-Whitney *t* test. In all cases, a significant difference was accepted at $p<0.05$.

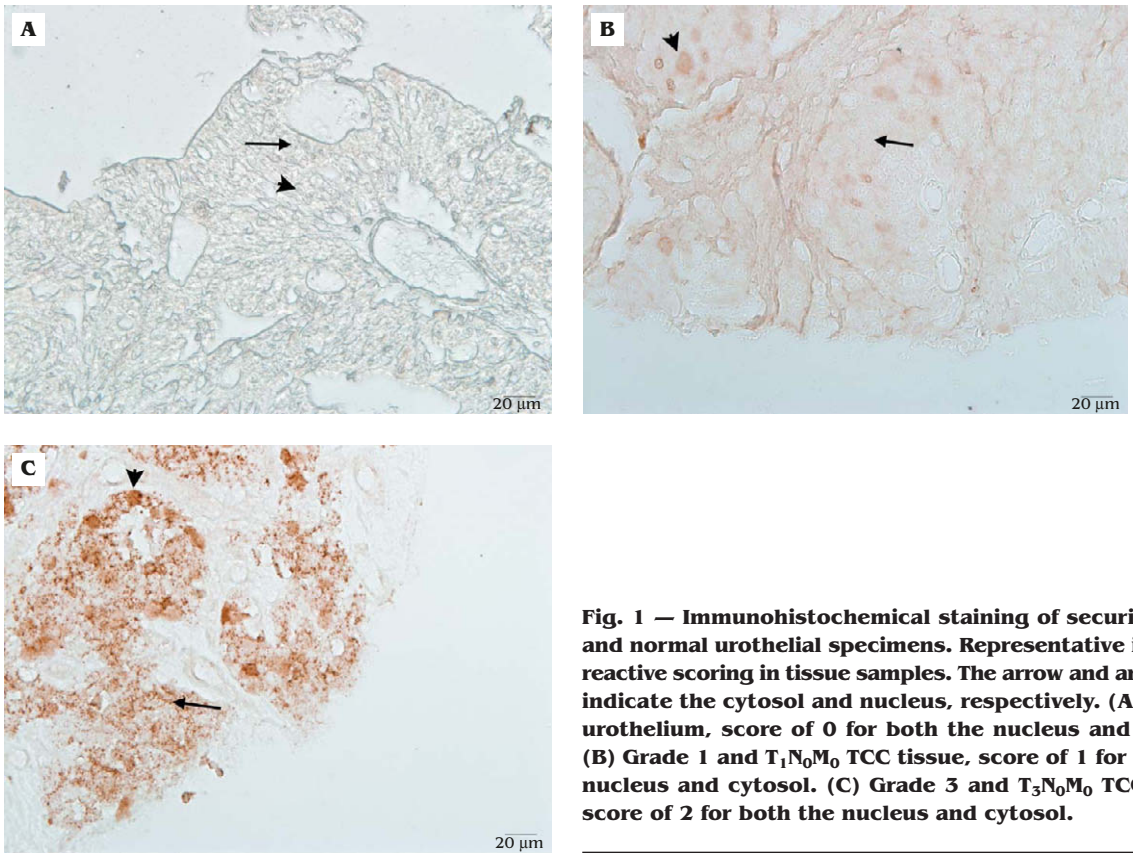

Fig. 1 — Immunohistochemical staining of securin in TCC and normal urothelial specimens. Representative immunoreactive scoring in tissue samples. The arrow and arrowhead indicate the cytosol and nucleus, respectively. (A) Normal urothelium, score of 0 for both the nucleus and cytosol. (B) Grade 1 and $T_1N_0M_0$ TCC tissue, score of 1 for both the nucleus and cytosol. (C) Grade 3 and $T_3N_0M_0$ TCC tissue, score of 2 for both the nucleus and cytosol.

3. Results

3.1. *Securin is overexpressed in the nucleus and cytosol of TCC specimens*

The difference in immunoreactive scores for cytosolic securin between normal urothelium and all TCC samples reached statistical significance (0.45±0.16 *vs.* 1.04±0.06 for normal urothelium *vs.* TCC, respectively; $p=0.006$; Fig. 2). Although nuclear securin in TCC tissues was also overexpressed compared to normal urothelium (0.73±0.30 *vs.* 1.23±0.08, normal urothelium *vs.* TCC, respectively), the difference did not reach statistical significance ($p=0.09$).

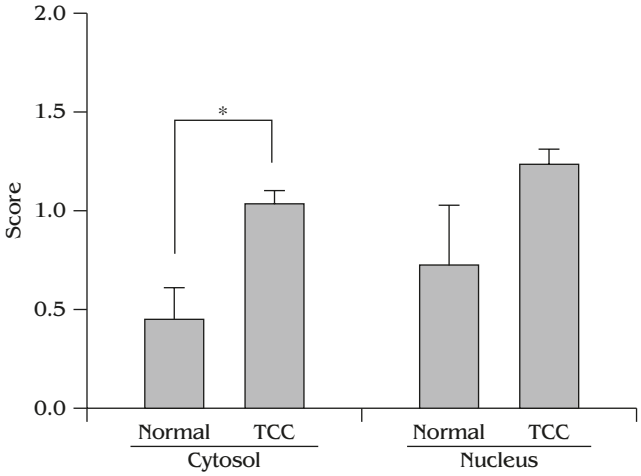

Fig. 2 — Securin immunostaining scores for all TCC samples. The average cytosolic and nuclear securin scores for TCC and normal urothelial tissue samples. *$p<0.05$.

3.2. *Securin is overexpressed in the nucleus and cytosol of each grade of TCC*

Securin immunostaining in all grades of TCC (1.11±0.11, 0.91±0.09, and 1.10±0.10 for grades I, II, and III TCC samples, respectively) showed statistically higher scores in the cytosol than that of normal urothelium ($p=0.01$, 0.04, and 0.009; grades I, II, and III TCC *vs.* normal urothelium; Fig. 3). However, there was no difference in nuclear securin expression between normal urothelium and each grade of TCC. In addition, securin expression among each grade of TCC, both in the nucleus and cytosol, did not reach statistical significance.

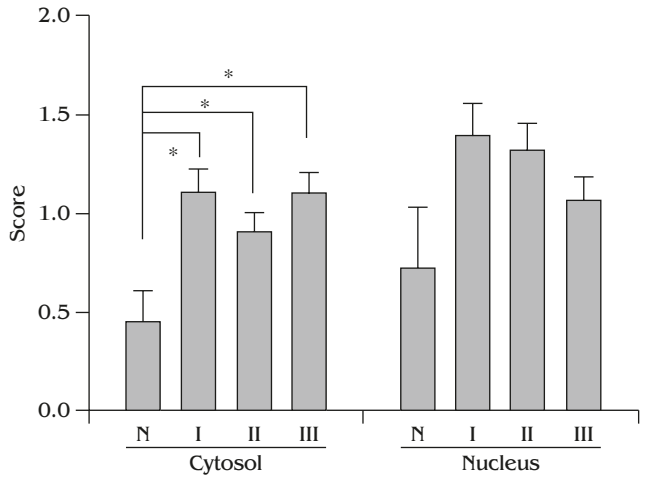

Fig. 3 — Securin scores for all grades of TCC. The average cytosolic and nuclear securin scoring differences between each grade of TCC (grades I, II, and III) tissues and normal urothelium (N). *$p<0.05$.

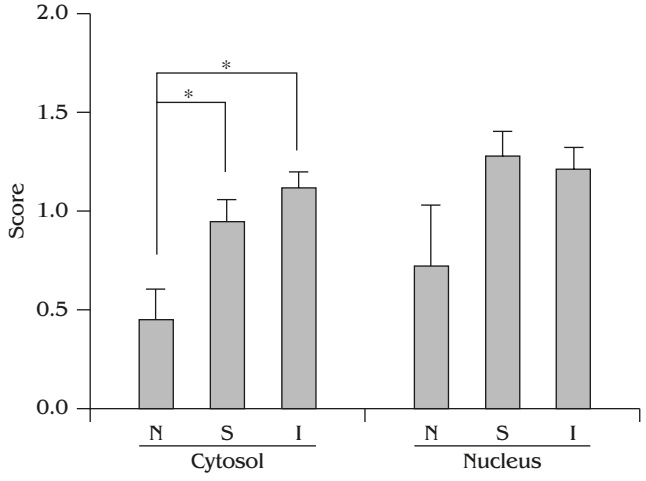

Fig. 4 — Securin scores for both superficial and invasive TCC samples. The average cytosolic and nuclear scoring differences for securin expression between superficial (S)/ invasive (I) TCC tissues and normal urothelium (N). *$p<0.05$.

3.3. Securin is overexpressed in the nucleus and cytosol of superficial and invasive TCC

Cytosolic securin was overexpressed in the superficial and invasive TCC samples (0.95±0.11 and 1.12± 0.08, respectively) compared to normal urothelium ($p=0.04$ and 0.004, respectively; Fig. 4). However, there was no difference in nuclear securin expression between normal urothelium and superficial or invasive TCC samples. In addition, no difference in nuclear and cytosolic securin expression between superficial and invasive TCC was found.

4. Discussion

Although securin is expressed at a low level or is even absent in most normal tissues [14], its mRNA has been observed in some normal human cells, e.g., the testis, small intestine, and fetal liver [24]. Securin binds separase to inactivate the latter's proteolytic activity during metaphase to prevent sister chromatid separation [25]. During the metaphase–anaphase transition, securin is degraded by ubiquitin ligase [26]. In addition, DNA double-strand breaks prevent the association of securin and Ku-70, the regulatory subunit of the DNA-dependent protein kinase, resulting in DNA repair by Ku-70 activation [27]. Thus, it is not surprising that securin expression is found in these proliferative normal tissues and functions as a regulator of the cell cycle. Urothelium is also one type of proliferative epithelial cell. No investigations examining the securin expression in urinary bladder tissue have been reported. In this study, 6 of 11 normal cytosolic and 7 of 11 normal nuclear urothelial tissues did not show immunostaining for securin. But strong nuclear immunostaining for securin was found in 4 of 11 normal urothelium samples. Therefore, securin may also play some role in the cell cycle and DNA repair of normal urothelium. The signaling of securin in urothelium proliferation remains to be investigated.

Both subcellular localization of securin in the nucleus and/or cytoplasm in various cell lines and tissue types was reported and summarized by Salehi et al [28]. Translocation of securin from the cytoplasm to the nucleus is mediated by some molecules such as PBF (PTTG-binding factor) [29], and mitogen-activated protein kinase cascades [30]. Nuclear expression of securin functions as the inhibitor of premature sister chromatid separation, while cytoplasmic expression is thought to be a transcriptional factor [15,31,32]. The significance of securin localization remains unclear in cancer biology. Thus, we analyzed the subcellular localization of securin expression in human bladder cancer tissues.

In the present study, abundant securin expression was found in both the cytosol and nucleus of all grades and all TCC tissues compared to normal urothelium. Although we did not observe a significant difference for nuclear securin expression between normal and cancerous urothelium, which might be due to the limited number of samples used, especially of normal urothelium, there was a trend toward elevated expression in bladder cancer specimens. These results are similar to those for adenocarcinoma of the breast and lung [33,34]. However, predominant nuclear localization was found in patients with glioma [35], while predominant cytoplasmic expression was found in patients with pituitary adenoma [36]. Although the roles of its expression in the nucleus or cytosol of

TCC should be further investigated, our data indicate that securin undoubtedly plays important roles in the tumorigenesis of TCC. Due to the distinct difference in its expression between TCC and normal urothelium, securin might have the potential to become a new biomarker for TCC screening.

Recent evidence has demonstrated the correlation between securin and the invasive abilities of some cancers. A high recurrence rate has been noted in breast cancer patients with overexpression of securin (37). High securin expression in colorectal and esophageal cancer has been demonstrated to correlate with lymph node metastasis (38,39). Follicular thyroid carcinoma grown in *PTTG*$^{-/-}$ mice exhibited a significant reduction in vascular invasion and less development of lung metastasis when these mice progressively aged (40). Knock-down securin by siRNA also inhibited the invasion of glioma cells *in vitro* (34). Thus, securin indeed plays important roles in neoplasmic invasiveness. Both cytosolic and nuclear securin overexpression in superficial and invasive TCC tissue was found in this study, but only cytosolic localization revealed statistical significance. Therefore, the precise role of securin in the invasive ability of TCC remains unclear.

Only 69 human TCC tissues and 11 normal urothelial samples were examined in our study. The existence of a correlation between securin expression and the severity of TCC, if present, cannot be completely confirmed, and larger numbers of samples should be collected and evaluated. In addition, the immunohistochemical staining score is not a perfect method for quantification of the target protein. In future investigations, securin detected by Western blot or enzyme-linked immunosorbent assays in fresh TCC samples will be needed to determine the relationship between clinical outcome and expression levels of securin.

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References

1. Murta-Nascimento C, Schmitz-Drager BJ, Zeegers MP, et al. Epidemiology of urinary bladder cancer: from tumor development to patient's death. *World J Urol* 2007;25:285–95.
2. Fleshner NE, Herr HW, Stewart AK, Murphy GP, Mettlin C, Menck HR. The National Cancer Data Base report on bladder carcinoma. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer* 1996; 78:1505–13.
3. Tavora F, Epstein JI. Bladder cancer, pathological classification and staging. *BJU Int* 2008;102:1216–20.
4. Ramos Soler D, Ferrer Lozano J, Navarro Fos S, Llombart-Bosch A. Multiple analysis of morphologic factors with prognostic value in transitional cell papillary carcinoma of the bladder. Retrospective study of 571 cases. *Actas Urol Esp* 1999;23:119–26.
5. Quek ML, Sanderson K, Daneshmand S, Stein JP. New molecular markers for bladder cancer detection. *Curr Opin Urol* 2004;14:259–64.
6. Goebell PJ, Groshen SL, Schmitz-Drager BJ. Guidelines for development of diagnostic markers in bladder cancer. *World J Urol* 2008;26:5–11.
7. Shariat SF, Ashfaq R, Karakiewicz PI, Saeedi O, Sagalowsky AI, Lotan Y. Survivin expression is associated with bladder cancer presence, stage, progression, and mortality. *Cancer* 2007;109:1106–13.
8. Smith SD, Wheeler MA, Plescia J, Colberg JW, Weiss RM, Altieri DC. Urine detection of survivin and diagnosis of bladder cancer. *JAMA* 2001;285:324–8.
9. Kenney DM, Geschwindt RD, Kary MR, Linic JM, Sardesai NY, Li ZQ. Detection of newly diagnosed bladder cancer, bladder cancer recurrence and bladder cancer in patients with hematuria using quantitative rt-PCR of urinary survivin. *Tumour Biol* 2007;28:57–62.
10. Shariat SF, Karakiewicz PI, Godoy G, et al. Survivin as a prognostic marker for urothelial carcinoma of the bladder: a multicenter external validation study. *Clin Cancer Res* 2009;15:7012–9.
11. Ku JH, Kwak C, Lee HS, Park HK, Lee E, Lee SE. Expression of survivin, a novel inhibitor of apoptosis, in superficial transitional cell carcinoma of the bladder. *J Urol* 2004; 171:631–5.
12. Schultz IJ, Kiemeney LA, Karthaus HF, et al. Survivin mRNA copy number in bladder washings predicts tumor recurrence in patients with superficial urothelial cell carcinomas. *Clin Chem* 2004;50:1425–8.
13. Shariat SF, Casella R, Khoddami SM, et al. Urine detection of survivin is a sensitive marker for the noninvasive diagnosis of bladder cancer. *J Urol* 2004;171:626–30.
14. Pei L, Melmed S. Isolation and characterization of a pituitary tumor-transforming gene (PTTG). *Mol Endocrinol* 1997; 11:433–41.
15. Zou H, McGarry TJ, Bernal T, Kirschner MW. Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. *Science* 1999;285: 418–22.
16. Ogbagabriel S, Fernando M, Waldman FM, Bose S, Heaney AP. Securin is overexpressed in breast cancer. *Mod Pathol* 2005;18:985–90.
17. Shibata Y, Haruki N, Kuwabara Y, et al. Expression of PTTG (pituitary tumor transforming gene) in esophageal cancer. *Jpn J Clin Onco* 2002;32:233–7.
18. Fujii T, Nomoto S, Koshikawa K, et al. Overexpression of pituitary tumor transforming gene 1 in HCC is associated with angiogenesis and poor prognosis. *Hepatology* 2006; 43:1267–75.
19. Boelaert K, McCabe CJ, Tannahill LA, et al. Pituitary tumor transforming gene and fibroblast growth factor-2 expression: potential prognostic indicators in differentiated thyroid cancer. *J Clin Endocrinol Metab* 2003;88:2341–7.
20. Talvinen K, Tuikkala J, Nevalainen O, et al. Proliferation marker securin identifies favourable outcome in invasive ductal breast cancer. *Br J Cancer* 2008;99:335–40.
21. Huang YH, Lai PC, Wu CC, Cheng CC, Chiu TH. TrkB antibody elicits cytotoxicity and suppresses migration/invasion

of transitional cell carcinoma cells. *Int J Oncol* 2010;37: 943–9.
22. Lai PC, Chiu TH, Huang YT. Overexpression of BDNF and TrkB in human bladder cancer specimens. *Oncol Rep* 2010;24:1265–70.
23. Tsuruta H, Kishimoto H, Sasaki T, et al. Hyperplasia and carcinomas in Pten-deficient mice and reduced PTEN protein in human bladder cancer patients. *Cancer Res* 2006; 66:8389–96.
24. Zhang X, Horwitz GA, Prezant TR, et al. Structure, expression, and function of human pituitary tumor-transforming gene (PTTG). *Mol Endocrinol* 1999;13:156–66.
25. Alexandru G, Uhlmann F, Mechtler K, Poupart MA, Nasmyth K. Phosphorylation of the cohesin subunit Scc1 by Polo/ Cdc5 kinase regulates sister chromatid separation in yeast. *Cell* 2001;105:459–72.
26. Zur A, Brandeis M. Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis. *EMBO J* 2001;20:792–801.
27. Romero F, Multon MC, Ramos-Morales F, et al. Human securin, hPTTG, is associated with Ku heterodimer, the regulatory subunit of the DNA-dependent protein kinase. *Nucleic Acids Res* 2001;29:1300–7.
28. Salehi F, Kovacs K, Scheithauer BW, Lloyd RV, Cusimano M. Pituitary tumor-transforming gene in endocrine and other neoplasms: a review and update. *Endocr Relat Cancer* 2008;15:721–43.
29. Chien W, Pei L. A novel binding factor facilitates nuclear translocation and transcriptional activation function of the pituitary tumor-transforming gene product. *J Biol Chem* 2000;275:19422–7.
30. Pei L. Activation of mitogen-activated protein kinase cascade regulates pituitary tumor-transforming gene transactivation function. *J Biol Chem* 2000;275:31191–8.
31. Zhou C, Liu S, Zhou X, et al. Overexpression of human pituitary tumor transforming gene (hPTTG), is regulated by beta-catenin/TCF pathway in human esophageal squamous cell carcinoma. *Int J Cancer* 2005;113:891–8.
32. Kim DS, Franklyn JA, Stratford AL, et al. Pituitary tumor-transforming gene regulates multiple downstream angiogenic genes in thyroid cancer. *J Clin Endocrinol Metab* 2006;91:1119–28.
33. Wierinckx A, Auger C, Devauchelle P, et al. A diagnostic marker set for invasion, proliferation, and aggressiveness of prolactin pituitary tumors. *Endocr Relat Cancer* 2007; 14:887–900.
34. Saez C, Japon MA, Ramos-Morales F, et al. hPTTG is overexpressed in pituitary adenomas and other primary epithelial neoplasias. *Oncogene* 1999;18:5473–6.
35. Genkai N, Homma J, Sano M, Tanaka R, Yamanaka R. Increased expression of pituitary tumor-transforming gene (PTTG)-1 is correlated with poor prognosis in glioma patients. *Oncol Rep* 2006;15:1569–74.
36. McCabe CJ, Khaira JS, Boelaert K, et al. Expression of pituitary tumour transforming gene (PTTG) and fibroblast growth factor-2 (FGF-2) in human pituitary adenomas: relationships to clinical tumour behaviour. *Clin Endocrinol* 2003;58:141–50.
37. Solbach C, Roller M, Fellbaum C, Nicoletti M, Kaufmann M. PTTG mRNA expression in primary breast cancer: a prognostic marker for lymph node invasion and tumor recurrence. *Breast* 2004;13:80–1.
38. Heaney AP, Singson R, McCabe CJ, Nelson V, Nakashima M, Melmed S. Expression of pituitary-tumour transforming gene in colorectal tumours. *Lancet* 2000;355:716–9.
39. Yan S, Zhou C, Lou X, et al. PTTG overexpression promotes lymph node metastasis in human esophageal squamous cell carcinoma. *Cancer Res* 2009;69:3283–90.
40. Kim CS, Ying H, Willingham MC, Cheng SY. The pituitary tumor-transforming gene promotes angiogenesis in a mouse model of follicular thyroid cancer. *Carcinogenesis* 2007; 28:932–9.