Pttg1/securin is required for the branching morphogenesis of the mammary gland and suppresses mammary tumorigenesis

Rashieda J. Hatcher[a,1], Jie Dong[b,c,1], Shuang Liu[a], Guangxing Bian[d], Alejandro Contreras[b], Tao Wang[b], Susan G. Hilsenbeck[b], Yi Li[b,c], and Pumin Zhang[a,d,e,2]

[a]Department of Molecular Physiology and Biophysics, [b]Lester and Sue Smith Breast Center, and [c]Department of Molecular and Cell Biology, Baylor College of Medicine, Houston, TX 77030; [d]State Key Laboratory of Proteomics, Beijing Proteomics Research Centre, Beijing Institute of Radiation Medicine, Beijing 100850, China; and [e]National Center for Protein Sciences Beijing, Beijing 102206, China

Edited* by Stephen J. Elledge, Harvard Medical School, Boston, MA, and approved December 9, 2013 (received for review September 25, 2013)

Pituitary tumor transforming gene 1 (Pttg1) **encodes the mammalian securin, which is an inhibitor of separase (a protease required for the separation of sister chromatids in mitosis and meiosis).** ***PTTG1*** **is overexpressed in a number of human cancers and has been suggested to be an oncogene. However, we found that, in** ***Pttg1***-**mutant females, the mammary epithelial cells showed increased proliferation and precocious branching morphogenesis. In accord with these phenotypic changes, progesterone receptor, cyclin D1, and** ***Mmp2*** **were up-regulated whereas p21 (*Cdkn1a*) was down-regulated. These molecular changes provide explanation for the observed developmental defects, and suggest that** ***Pttg1*** **is a tumor suppressor. Indeed, mice lacking** ***Pttg1*** **developed spontaneous mammary tumors. Furthermore, in human breast tumors, PTTG1 protein levels were down-regulated and the reduction was significantly correlated with the tumor grade.**

Originally identified as a gene overexpressed in rat pituitary tumors (1), *pituitary tumor transforming gene 1 (Pttg1)* was later found overexpressed in a large number of human malignancies, including those of the breast (2), uterus (3), lung (4), and thyroid (5). Its expression was also found to represent one of 17 genes that formed a molecular signature capable of predicting tumor metastasis (6, 7). Transgenic overexpression of human *PTTG1* in the mouse pituitary caused hyperplasia and adenoma (8). In addition, *Pttg1* deletion provides protection against *Rb* haploinsufficiency-induced pituitary tumorigenesis (9). These results suggest that *PTTG1* is an oncogene and plays a role in the proliferation of cells in the pituitary.

PTTG1 encodes a small protein with 202 residues. Previous studies suggest that PTTG1 directly or indirectly regulates transcription of a number of genes involved in tumorigenesis, including *Cdkn1a*, *Myc*, *Fgf2*, *Mmp2*, etc (10). However, these studies were cell culture- and overexpression-based. It is unclear whether PTTG1 regulates the expression of these genes in vivo, and, if it does, in what tissue context.

PTTG1 is also the mammalian securin (11). Securin is the inhibitor of separase, a protease required for the separation of sister chromatids in mitosis (11). To allow for the activation of separase, securin is degraded at the onset of metaphase to anaphase transition through ubiquitination by the anaphase promoting complex (APC)/cyclosome, an E3 ubiquitin ligase (12). In budding yeasts, loss of securin (*PDS1*) causes precocious sister chromatids separation and chromosome instability (13, 14). However, ablation of securin in mice did not result in apparent chromosome instability as seen in yeasts (15). Most likely, this is because in vertebrates separase is also inhibited by phosphorylation (16). Only when both inhibitory mechanisms are removed are defects in mitotic separation of sister chromatids seen (17, 18). Nonetheless, the function of securin as the inhibitor of separase fueled the speculation that the overexpression of *PTTG1* in tumors might be related to chromosome instability. Currently, however, there is no direct evidence to support such speculation.

Here, we report that *Pttg1* is required for proper morphogenesis of the mammary gland in mice. In the absence of *Pttg1*, the mammary gland epithelial cells display an altered gene expression profile. *Cdkn1a* and *Mmp2*, two of the potential transcriptional targets of PTTG1, were down-regulated in *Pttg1*-null mammary tissues, whereas cyclin D1 and progesterone receptor (PR) were up-regulated. These gene expression changes lead to increased proliferation in mammary epithelial cells, and defects in branching morphogenesis manifested as delayed migration of the primary branch and supernumeral secondary and tertiary branches. Consistent with these developmental defects, *Pttg1*-null female mice develop spontaneous mammary gland tumors. Further, we found a significant correlation between the levels of PTTG1 and the degree of malignancy in human breast tumors. These results indicate that *PTTG1* is a tumor suppressor in the mammary gland.

Results

Abnormal Mammary Gland Branching Morphogenesis in *Pttg1*-Null Mice. In mice, mammary gland development and maturation starts at approximately 4 wk of age when puberty begins and continues through ~13 wk of age. We examined the mammary glands at different developmental stages via whole-mount staining. It was apparent that the loss of *Pttg1* caused defects in branch patterning and progression (Fig. 1). Compared with the WT controls, the mutant glands contained more terminal end buds (TEBs) at 4 and 7 wk (Fig. S1*A*), and showed a delay in progression at 7 wk

Significance

Pituitary tumor transforming gene 1 (Pttg1) **has been suggested as an oncogene. Here we show that it could act as a tumor suppressor, at least in mammary glands. Loss of** ***Pttg1*** **leads to spontaneous mammary gland tumorigenesis, whereas, in human breast tumors, there is a significant inverse correlation between the expression levels of PTTG1 and the tumor grade. This tumor suppressor function of Pttg1 is likely rooted in its role in the regulation of the proliferation of mammary gland epithelial cells and the morphogenesis of the ductal trees of the gland. We demonstrated that a number of genes related to proliferation and mammary gland branching morphogenesis were misregulated in the absence of Pttg1, which provides in vivo evidence that PTTG1 regulates gene expression.**

Author contributions: R.J.H., Y.L., and P.Z. designed research; R.J.H., J.D., S.L., G.B., and A.C. performed research; R.J.H., J.D., T.W., S.G.H., and P.Z. analyzed data; and R.J.H., Y.L., and P.Z. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]R.J.H. and J.D. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: pzhang@bcm.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1318124111/-/DCSupplemental.

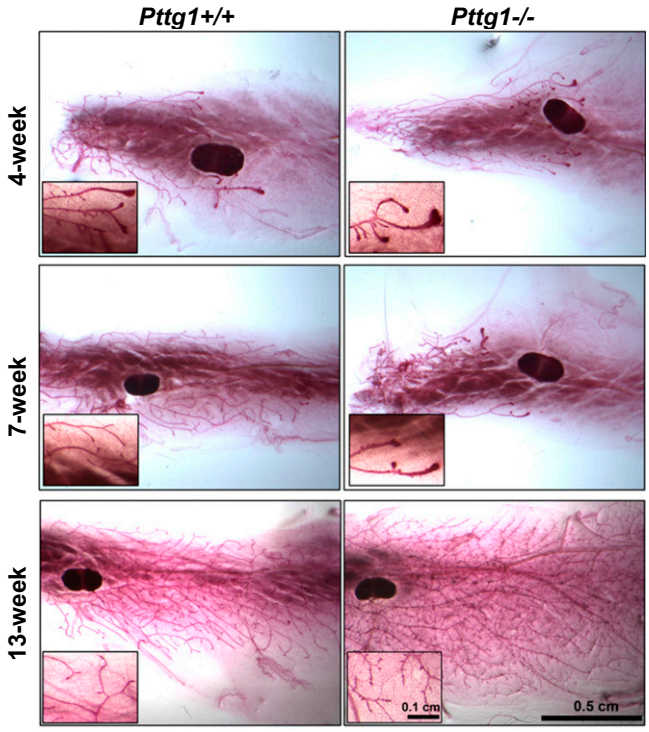


Fig. 1. Loss of *Pttg1* disrupts mammary gland morphogenesis. Whole-mount staining of mammary glands at different developmental stages.

as measured by the distance between the lymph node and the leading TEB (Fig. S1*B*). The delay is more or less recovered by 13 wk. However, strikingly, the mutants showed much more lateral side branching offshoots than WT. Counting the number of secondary (from mature branch) and tertiary (from secondary branch) branches for three primary branches per gland demonstrated that there was a significant increase in the lateral side branching in these mutant glands (Fig. S1 *C* and *D*).

The Abnormal Branching Morphogenesis in *Pttg1* Mutants Can Be Reproduced in Transplants. Given the potential function of *Pttg1* in the pituitary and other endocrine organs, the observed abnormal branching morphogenesis in *Pttg1* mutants might be a result of systemic reasons rather than defects within the mammary epithelial cells. To address this concern, we transplanted $Pttg1^{+/+}$ and $Pttg1^{-/-}$ mammary gland tissues into the cleared fat pads of WT recipient mice. The WT and the mutant mammary tissues were placed in the same mouse (WT on one side and the mutant on another) (19, 20). The transplants were allowed to develop uninterruptedly and were harvested for analysis at 5 and 8 wk after the procedure. Whole-mount staining clearly indicated the superenumerated secondary and tertiary branch pattern in the mutant glands, but not in the WT controls (Fig. 2*A*). Quantitative analysis (Fig. S2 *A* and *B*) demonstrated significant increases in bifurcation of the mutant glands. These results indicate that *Pttg1* is intrinsically required for the proper function of mammary epithelial cells.

We also allowed the transplant recipient animals to be pregnant and observed the transplanted glands. There was a delay in the expansion of alveolar structures in the mutants (Fig. S2*C*). It is unclear the reason for the delay but could be caused by changes in the expression of genes related to lactation.

***Pttg1* Is Expressed in Developing Mammary Gland.** Having established that *Pttg1* is required intrinsically in mammary gland development, we asked where and when *Pttg1* was expressed in the developing gland. Immunohistochemistry (IHC) staining of sections of mammary glands at the beginning (4 wk), middle (7 wk), and late stages (13 wk) of development showed that PTTG1 was highly detectable in the epithelial cells (Fig. S3 *A* and *B*). In the TEB at 4 wk, PTTG1 was expressed in cap and body cells (Fig. 3*A*), whereas, in the ductal region, it was evenly dispersed throughout the single-layer luminal epithelium (Fig. S3*A*). The same expression pattern maintained during mid and late puberty (Fig. S3*B*). At all stages examined, PTTG1 was present mainly in

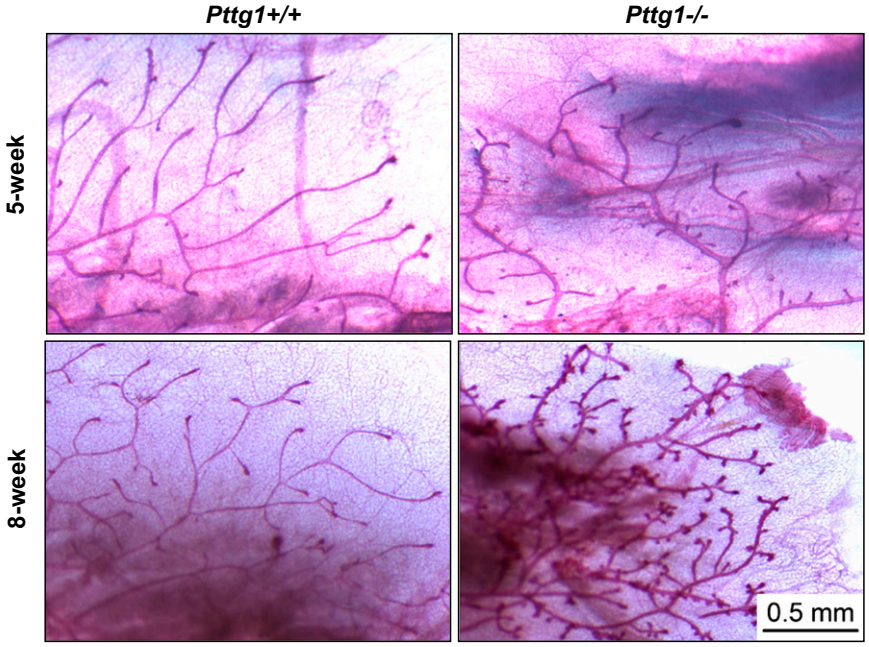


Fig. 2. *Pttg1* is intrinsically required for the mammary gland branching morphogenesis. Whole-mount staining of the transplanted mammary glands at 5 and 8 wk after the transplantation procedure.

the nucleus. As expected, the antibodies we used did not pick up any signal in *Pttg1*$^{-/-}$ mammary glands.

Given the fact that PTTG1 is present in the epithelium, we decided to determine if its absence affected the epithelial cell characters. We examined the expression of E-cadherin, keratin 8, and keratin 5, and found that the loss of *Pttg1* caused no changes in their expression (Fig. S4).

Mammary Epithelial Cells in *Pttg1* Mutants Show Increased Proliferation with Associated Up-Regulation of Cyclin D1 and Down-Regulation of p21. As *Pttg1* was implicated in the regulation of cell proliferation through modulating the expression of growth-related genes, we asked whether the loss of *Pttg1* was associated with a disruption in the control of proliferation of mammary epithelial cells. The mice at 4, 7, and 13 wk old were injected with BrdU, and, 2 h after the injection, the mammary glands were harvested and processed for IHC staining of BrdU (Fig. S5*A*). Quantitative analysis of BrdU incorporation at these different developmental stages indicated that there were significant increases in proliferation in the mutant TEBs at 4 and 7 wk (Fig. S5*B*). Although, at 4 and 7 wk, there were no significant increases in the proliferation of ductal epithelial cells in the mutants, there was a significant increase at 13 wk (Fig. S5*C*). At the same time, TUNEL assay did not detect any increase in the apoptosis rates in the mutants at all three ages examined (Fig. S6). Thus, the loss of *Pttg1* causes a net increase in the number of mammary epithelial cells, which underlies, at least in part, the defects in branching morphogenesis described earlier.

Next, we asked which proliferation-related genes were modulated by PTTG1. We did not observe any changes in the mutant mammary glands in the expression of *c-Myc*, *Fgf2*, or *cyclin D3* as previously reported (21, 22). Cyclin D1 (*Ccnd1*) is essential for the proliferation of mammary gland epithelial cells (23, 24). We therefore examined its expression in the mutants. IHC detected much stronger cyclin D1 expression in the mutant than in the WT controls at all three developmental stages (Fig. 3*A*). Quantitative PCR analysis demonstrated 10-fold (4 wk), 28-fold (7 wk), and threefold (13 wk) increases in *Ccnd1* mRNA levels in the mutant glands compared with the controls (Fig. S7*A*). At the same time, we found that p21 (*Cdkn1a*) expression was suppressed in the mutants (Fig. 3*B* and Fig. S7*B*). At 4 wk of age, the mutant glands showed much lower p21 IHC signals (Fig. 3*B*). By 7 and 13 wk, although the p21 protein could not be detected in WT and mutant mammary gland epithelial cells, the levels of p21 mRNA in mutant mammary epithelial cells were still much less than in WT control (Fig. S7*B*). Taken together, these results demonstrate that *Pttg1* negatively modulates the proliferation of mammary gland epithelial cells through regulating two important proliferation-related genes, *Ccnd1* and *Cdkn1a*.

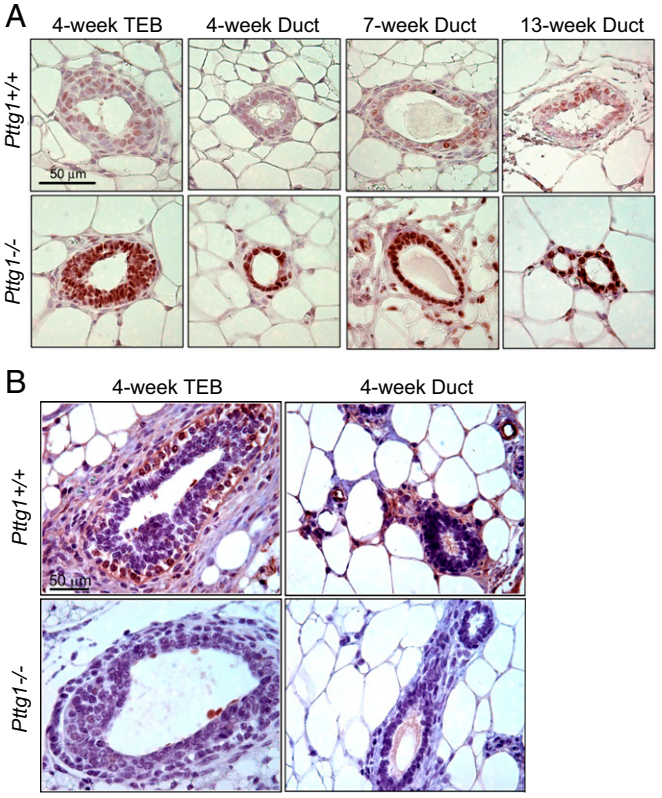


Fig. 3. *Pttg1* regulates cell cycle gene expression. (*A*) IHC staining of cyclin D1 in developing mammary glands. (*B*) IHC staining of the cycle inhibitor p21 in 4-wk mammary glands.

***Pttg1* Regulates the Expression of Genes Involved in the Branching Morphogenesis of Mammary Glands.** Early outgrowth of the mammary gland tree is primarily controlled by estrogen receptor (ER)-α (ERα) signaling (25). However, we did not detect any significant alterations in the expression of ERα in the mutants. Another critical regulator of mammary gland development is PR, especially later in puberty and during pregnancy and lactation. PR is necessary for the secondary and tertiary lateral side branching and lobuloalveolar budding during pregnancy (26, 27). Therefore, we decided to examine the status of PR expression in *Pttg1* mutant mice. At 4 wk of age, as expected, PR could be detected neither in WT control nor in mutant glands (Fig. S8*A*). However, at 7 and 13 wk, the mutant glands showed a much stronger PR expression than the control mice (Fig. 4*A*). We further analyzed the mRNA levels of PR. Again, the mutant glands showed much higher expression than the control, more than 10-fold at 7 wk and fourfold at 13 wk (Fig. S8*B*).

The normally controlled proliferation and invasion behavior of mammary gland epithelial cells relies on coordinated expression of a number of matrix metalloproteinases, including *Mmp2* and *Mmp3* (28, 29). Given that *Mmp2* was reported to be regulated by *Pttg1*, we analyzed the expression of *Mmp2* in *Pttg1* mutant animals. As shown in Fig. 4*B*, there were marked decreases in the levels of *Mmp2* mRNA in the mutants than in the WT controls at all developmental stages examined. Remarkably, genetic ablation of *Mmp2* led to a similar mammary gland branching defects as the loss of *Pttg1* (29). Together these findings suggest that *Pttg1* might regulate the branching morphogenesis through PR and/or Mmp2.

***Pttg1*-Null Female Mice Develop Spontaneous Mammary Gland Tumors.** The developmental defects, including the increased proliferation of epithelial cells observed in *Pttg1*-null mammary glands, suggest that *Pttg1* might have tumor suppressor function. In a cohort of 30 virgin female mice, we observed that three animals had spontaneous breast tumors, one of which appeared as early as 7 mo of age. Histological analysis indicated that these tumors were adenoma and epithelial in origin (Fig. 5*A*). These results suggest that *Pttg1* is a mammary gland tumor suppressor, albeit a weak one by itself. As the exogenous expression of *Ccnd1* in mammary glands is sufficient to drive tumorigenesis, the observation that the loss of *Pttg1* causes overproduction of *Ccnd1* is consistent with the tumor phenotype.

PTTG1 Expression Inversely Correlates with the Degree of Malignancy in Human Breast Tumors. The mammary gland tumor phenotype in *Pttg1*-null mice prompted us to examine the expression of PTTG1 in human breast tumors. To that end, we analyzed 10 normal breast tissues and tissue microarrays comprising 10 normal breast tissues and 239 breast tumor samples, with two cores for each sample. PTTG1 was visualized with IHC staining (Fig. 5*B*), and its expression levels were scored by two independent pathologists

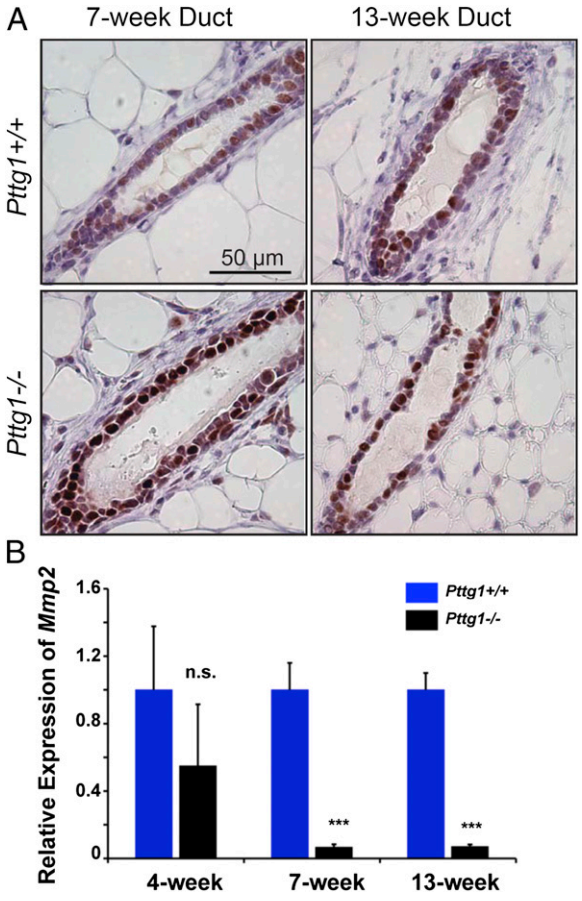


Fig. 4. *Pttg1* regulates the expression of genes involved in mammary gland morphogenesis. (*A*) IHC staining of PR in 7- and 13-wk mammary glands. (*B*) Quantitative RT-PCR analysis of *Mmp2* expression in developing mammary glands. Student *t* tests were performed to determine statistical significance. n.s., no significance (***$P < 0.001$).

on a scale from 0 to 4. After quality control and resolution of discrepancies, values from duplicate cores were averaged to yield a single value for each sample. All 10 normal samples were scored at 4, whereas the tumor sample scores varied (Fig. 5*C*). The expression levels in tumors were significantly lower than the normal ducts (Wilcoxon rank-sum test, $P < 0.001$; Fig. 5*D*). We also found that lower PTTG1 levels of expression were associated with higher grade (Fig. 5*E*) of the tumors ($P = 0.0017$ by Wilcoxon rank-sum test).

Discussion

Faithful transmission of genetic materials either from generation to generation or from a parent cell to daughter cells is critical. Elaborate mechanisms have evolved that ensure equal chromosome segregation in mitosis and meiosis, one of which is the spindle assembly checkpoint (SAC). SAC blocks the separation of sister chromatids by inhibiting separase, the protease which catalyzes the dissolution of chromatid cohesion (30). In vertebrates, there are two mechanisms to inhibit separase, phosphorylation and binding by securin (16). We have shown previously that these two mechanisms are redundant in most somatic cells (17), but, in mice, postmigration germ cells rely entirely on phosphorylation to inhibit separase (18). Mammalian securin, *PTTG1*, has been associated with cancer before it is known as securin. *PTTG1* was found overexpressed in many types of tumors. However, these analyses were primarily mRNA-based, and whether or not PTTG1 protein is also overexpressed in cancer cells is not clear. Further, the exact function of *PTTG1* (if any) in tumorigenesis remained elusive.

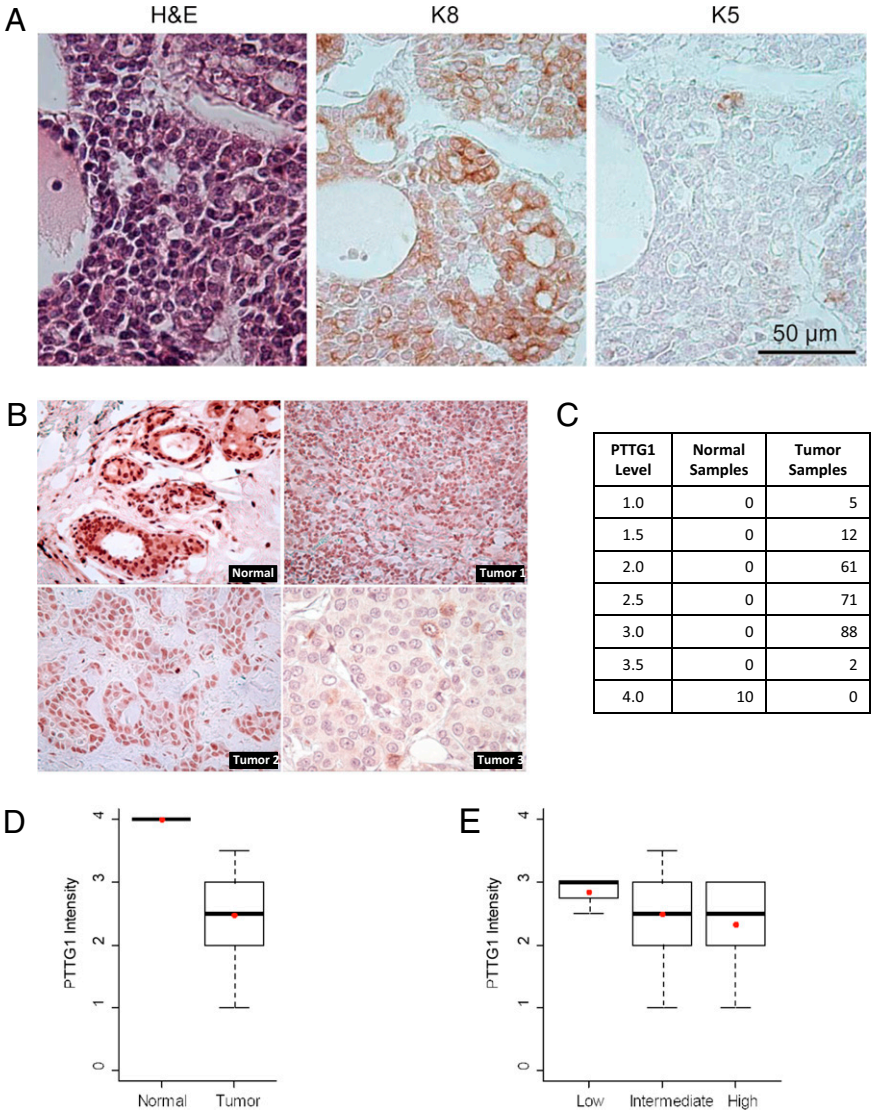
Our analyses demonstrate that *PTTG1* is a tumor suppressor, at least in the mammary gland. The mammary gland is a unique specialized epidermal appendage that originates during embryogenesis, but is not fully formed until puberty and not functional until lactation (31). The timed release of hormones in response to puberty and pregnancy is critical to the development and function of the mammary gland (32–36). During puberty (approximately between 4 and 9 wk after birth in the mouse) and under the influence of ovarian estrogen, the TEBs guide the branching network advancing across the fat pad. TEBs are complex structures consisting of multicell layers and have high rates of proliferation and apoptosis, reflecting extensive tissue remodeling. It is the expanding and forwarding movement of TEBs that drives the gland's invasion of the fat pad and leaves behind ducts. These ducts consist of an inner luminal epithelium and an outer myoepithelium. Following puberty, these ducts can undergo side branching under the influence of progesterone, and this ductal tree can further expand and form alveoli under the influence of additional reproductive hormones such as prolactin.

The loss of *Pttg1* results in a number of defects in mammary gland development, and the defects are mammary gland-intrinsic, as the mutant glands transplanted into WT recipients displayed similar developmental defects. Morphologically, migrating through the fat pad by mutant TEBs was delayed and there were increases in the secondary and tertiary braches. At the molecular level, we observed increased proliferation of the mammary gland epithelia and altered gene expression profiles in the mutants. The decrease in *Cdkn1a* (p21) expression and the increase in *Ccnd1* (cyclin D1) expression caused by the lack of *Pttg1* provide an explanation for the increased proliferation, whereas the delay in passing through fat pad by the mutant glands and the supernumeral secondary and tertiary branches could be accounted for by the decrease in *Mmp2* expression, as these morphological defects are much like those in *Mmp2*-deficient mice (29).

The developmental defects in *Pttg1* mutant mammary glands are unlikely a result of loss of the securin function as an inhibitor of separase. We have shown previously in cells and in mice that securin and separase phosphorylation were redundant functionally (17). It is also very unlikely that mitotic errors (if any) caused by the loss of *Pttg1* could account for the profound changes in gene expression. In fact, we could not detect any mitotic defects in *Pttg1*-null mammary epithelial cells, nor in the spontaneously developed tumors. In agreement, none of the mouse strains carrying SAC-disrupting mutations or mutations in other mitotic regulators (both of which were more severe than the loss of securin in terms of misregulation of mitoses) was shown to cause mammary gland defects or mammary gland tumors (37–40). Thus, it is most likely the nonmitotic function of PTTG1/Securin that is important for mammary gland development.

It has long been suggested that PTTG1 functions in regulating transcription. In vitro chromatin immunoprecipitation experiments suggested the involvement of PTTG1 in global transcription regulation, probably through interaction with SP1 (10, 22). Our analyses indicate that the transcription-regulating function of PTTG1 is important for mammary gland development in mice, although the exact mechanism remains to be determined. It is possible that PTTG1 directly or indirectly inhibits the function of ER in the mammary gland because its absence results in the up-regulation of two ER targets, PR and cyclin D1.

Our analyses in mice indicate *Pttg1* is a tumor suppressor in the mammary gland, which is supported by our human data demonstrating a general reduction in PTTG1 levels in breast tumors and a significant correlation between the degree of reduction and tumor grade. These results are in contrast to the notion that *PTTG1* is an oncogene. This discrepancy might be a result of the assay used (mRNA vs. protein), the sample size



PTTG1 Level	Normal Samples	Tumor Samples
1.0	0	5
1.5	0	12
2.0	0	61
2.5	0	71
3.0	0	88
3.5	0	2
4.0	10	0

Fig. 5. *Pttg1* is a breast tumor suppressor. (*A*) Analyses of breast tumors developed in *Pttg1*-null female mice through histology (H&E stain) or keratin 8 (K8) or keratin 5 (K5) IHC staining. (*B*) IHC staining of PTTG1 in normal human breast tissue and in breast tumors. Representatives of different strength of staining/expression level were presented. Tumor 1 was scored as 3, tumor 2 as 2, and tumor 4 as 1. (*C*) Summary of the staining scores of all samples. (*D*) Comparison of PTTG1 levels in normal breast tissue and breast tumors. (*E*) PTTG1 expression levels correlated with tumor grades ($P = 0.0017$ by Wilcoxon rank-sum test).

(249 samples were analyzed here), or molecular/cellular context. For example, the fact that *Pttg1* is required for the expression of *Mmp2* qualifies *Pttg1* as an oncogene because of its potential in promoting metastasis. This fact might also underlie its inclusion in the metastasis signature of breast tumors (6, 7).

Materials and Methods

Mouse Colony. *Pttg1*-KO mice were reported previously (15). The mice were backcrossed to C57/BL6 more than 15 times before they were crossed five times (F5) to FVB/NJ. All experimental data were obtained with F5 animals.

Histology. ***Mammary gland whole mount.*** No. 4 and no. 9 breast glands were excised, spread on glass slides, and fixed in Carnoy fixative (EtOH:$CHCl_3$:glacial acetic acid, 6:3:1) for 4 h at room temperature. The fixed tissues were washed in 70% (vol/vol) EtOH and gradually rehydrated to distilled H_2O before the overnight incubation in carmine alum stain (1 g carmine and 2.5 g aluminum potassium sulfate in 500 mL H_2O, boiled for 20 min). The stained tissues were washed in increasing concentrations of EtOH and cleared in xylene overnight. ***H&E staining.*** No. 4 and no. 9 mammary glands and tumor samples were fixed in 4% (wt/vol) PFA for 2 h, dehydrated, and embedded in paraffin according to standard procedures. The tissues were sectioned at 3 μm, and the sections were dewaxed in xylene (3×) and rehydrated. H&E staining was performed following standard procedures. Stained sections were mounted with Permount (Fisher Scientific) and imaged.

IHC. The tissue sections were processed the same as for H&E staining. After rehydration, the sections were placed in a pressure cooker with 0.1 M citric buffer (pH 6.0) and incubated for 20 min at subboiling temperatures for antigen retrieval. Immunostaining was performed by using the MOM (Mouse-on-Mouse) immunodetection kit (Vector Labs) according to manufacturer instructions. The sections were incubated with primary antibodies overnight at 4 °C and with secondary antibodies for 1 h at room temperature after extensive washing to remove the primary antibodies. The secondary antibodies were then visualized with NovaRed peroxidase staining (Vector Labs). Primary antibodies used were PR (rabbit; sc-7208; Santa Cruz), PTTG (mouse; sc-22772; Santa Cruz), α-tubulin (mouse; no. 625902; Biolegend), ERα (rabbit; sc-542; Santa Cruz), and p21 (mouse; sc-6246; Santa Cruz). Secondary antibodies used were HRP-conjugated goat anti-mouse (no. 1706516; Bio-Rad) or goat anti-rabbit (no. 1706515; Bio-Rad) immunoglobins.

BrdU incorporation and apoptosis analyses. Mice 4, 7, and 13 wk old were intraperitoneally injected with BrdU (dissolved in PBS solution at 1 mg/mL) at a dose of 25 μg/g body weight. At 2 h after the injection, the no. 4 and no. 9 mammary glands were harvested, processed, and sectioned. The sections were dewaxed and rehydrated for the detection of BrdU. We used BrdU antibody (B-5002;

Sigma) for the IHC detection and followed the protocol recommended by the antibody manufacturer. Apoptotic cells were detected with the DeadEnd Fluorometric TUNEL System (Promega) and counted. BrdU- or TUNEL-positive cells were scored in more than five fields per section, at least 1,000 cells were counted for each section, and at least three sections were analyzed per mouse.

Quantification of bifurcation, transplantation procedures, and quantitative RT-PCR analysis are detailed in *SI Materials and Methods*.

ACKNOWLEDGMENTS. This study was supported by National Institute of Health (NIH) Grants CA116097 (to P.Z.), CA122623 (to P.Z.), and CA124820 (to Y.L.); National High Technology Research and Development Program of China 863 Program Grant 2012AA020206; and National Natural Science Foundation of China Grant 81171920. The pathological and statistic analysis of human breast tumor tissues were performed in the Dan L. Duncan Cancer Center at Baylor College of Medicine with support from NIH Grant CA125123.

1. Pei L, Melmed S (1997) Isolation and characterization of a pituitary tumor-transforming gene (PTTG). *Mol Endocrinol* 11(4):433–441.
2. Puri R, Tousson A, Chen L, Kakar SS (2001) Molecular cloning of pituitary tumor transforming gene 1 from ovarian tumors and its expression in tumors. *Cancer Lett* 163(1):131–139.
3. Kakar SS (1999) Molecular cloning, genomic organization, and identification of the promoter for the human pituitary tumor transforming gene (PTTG). *Gene* 240(2): 317–324.
4. Zhang X, et al. (1999) Structure, expression, and function of human pituitary tumor-transforming gene (PTTG). *Mol Endocrinol* 13(1):156–166.
5. Boelaert K, et al. (2003) Pituitary tumor transforming gene and fibroblast growth factor-2 expression: potential prognostic indicators in differentiated thyroid cancer. *J Clin Endocrinol Metab* 88(5):2341–2347.
6. Ramaswamy S, Ross KN, Lander ES, Golub TR (2003) A molecular signature of metastasis in primary solid tumors. *Nat Genet* 33(1):49–54.
7. Hunter K, Welch DR, Liu ET (2003) Genetic background is an important determinant of metastatic potential. *Nat Genet* 34(1):23–24.
8. Abbud RA, et al. (2005) Early multipotential pituitary focal hyperplasia in the alpha-subunit of glycoprotein hormone-driven pituitary tumor-transforming gene transgenic mice. *Mol Endocrinol* 19(5):1383–1391.
9. Chesnokova V, Kovacs K, Castro AV, Zonis S, Melmed S (2005) Pituitary hypoplasia in Pttg−/− mice is protective for Rb+/− pituitary tumorigenesis. *Mol Endocrinol* 19(9): 2371–2379.
10. Tong Y, Eigler T (2009) Transcriptional targets for pituitary tumor-transforming gene-1. *J Mol Endocrinol* 43(5):179–185.
11. Zou H, McGarry TJ, Bernal T, Kirschner MW (1999) Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. *Science* 285(5426):418–422.
12. Uhlmann F (2003) Chromosome cohesion and separation: From men and molecules. *Curr Biol* 13(3):R104–R114.
13. Yamamoto A, Guacci V, Koshland D (1996) Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). *J Cell Biol* 133(1):99–110.
14. Yamamoto A, Guacci V, Koshland D (1996) Pds1p is required for faithful execution of anaphase in the yeast, Saccharomyces cerevisiae. *J Cell Biol* 133(1):85–97.
15. Mei J, Huang X, Zhang P (2001) Securin is not required for cellular viability, but is required for normal growth of mouse embryonic fibroblasts. *Curr Biol* 11(15): 1197–1201.
16. Stemmann O, Zou H, Gerber SA, Gygi SP, Kirschner MW (2001) Dual inhibition of sister chromatid separation at metaphase. *Cell* 107(6):715–726.
17. Huang X, Hatcher R, York JP, Zhang P (2005) Securin and separase phosphorylation act redundantly to maintain sister chromatid cohesion in mammalian cells. *Mol Biol Cell* 16(10):4725–4732.
18. Huang X, et al. (2008) Inhibitory phosphorylation of separase is essential for genome stability and viability of murine embryonic germ cells. *PLoS Biol* 6(1):e15.
19. Medina D (2010) Of mice and women: A short history of mouse mammary cancer research with an emphasis on the paradigms inspired by the transplantation method. *Cold Spring Harb Perspect Biol* 2(10):a004523.
20. Cardiff RD, Kenney N (2011) A compendium of the mouse mammary tumor biologist: From the initial observations in the house mouse to the development of genetically engineered mice. *Cold Spring Harb Perspect Biol* 3(6).
21. Tong Y, Falk J (2009) Genome-wide analysis for protein-DNA interaction: ChIP-chip. *Methods Mol Biol* 590:235–251.
22. Tong Y, Tan Y, Zhou C, Melmed S (2007) Pituitary tumor transforming gene interacts with Sp1 to modulate G1/S cell phase transition. *Oncogene* 26(38):5596–5605.
23. Fantl V, Stamp G, Andrews A, Rosewell I, Dickson C (1995) Mice lacking cyclin D1 are small and show defects in eye and mammary gland development. *Genes Dev* 9(19): 2364–2372.
24. Sicinski P, et al. (1995) Cyclin D1 provides a link between development and oncogenesis in the retina and breast. *Cell* 82(4):621–630.
25. Bocchinfuso WP, Korach KS (1997) Mammary gland development and tumorigenesis in estrogen receptor knockout mice. *J Mammary Gland Biol Neoplasia* 2(4):323–334.
26. Lydon JP, et al. (1995) Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev* 9(18):2266–2278.
27. Brisken C, et al. (1998) A paracrine role for the epithelial progesterone receptor in mammary gland development. *Proc Natl Acad Sci USA* 95(9):5076–5081.
28. Fata JE, Werb Z, Bissell MJ (2004) Regulation of mammary gland branching morphogenesis by the extracellular matrix and its remodeling enzymes. *Breast Cancer Res* 6(1):1–11.
29. Wiseman BS, et al. (2003) Site-specific inductive and inhibitory activities of MMP-2 and MMP-3 orchestrate mammary gland branching morphogenesis. *J Cell Biol* 162(6): 1123–1133.
30. Hauf S, Waizenegger IC, Peters JM (2001) Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. *Science* 293(5533):1320–1323.
31. Bissell MJ, Polyak K, Rosen JM (2011) *The Mammary Gland as an Experimental Model*. Cold Spring Harbor Perspectives in Biology (Cold Spring Harbor Laboratory Press, Plainview, NY).
32. Brisken C (2002) Hormonal control of alveolar development and its implications for breast carcinogenesis. *J Mammary Gland Biol Neoplasia* 7(1):39–48.
33. Brisken C, et al. (1999) Prolactin controls mammary gland development via direct and indirect mechanisms. *Dev Biol* 210(1):96–106.
34. Mallepell S, Krust A, Chambon P, Brisken C (2006) Paracrine signaling through the epithelial estrogen receptor alpha is required for proliferation and morphogenesis in the mammary gland. *Proc Natl Acad Sci USA* 103(7):2196–2201.
35. Oakes SR, Rogers RL, Naylor MJ, Ormandy CJ (2008) Prolactin regulation of mammary gland development. *J Mammary Gland Biol Neoplasia* 13(1):13–28.
36. Soyal S, et al. (2002) Progesterone's role in mammary gland development and tumorigenesis as disclosed by experimental mouse genetics. *Breast Cancer Res* 4(5): 191–196.
37. Babu JR, et al. (2003) Rae1 is an essential mitotic checkpoint regulator that cooperates with Bub3 to prevent chromosome missegregation. *J Cell Biol* 160(3):341–353.
38. Baker DJ, et al. (2004) BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat Genet* 36(7):744–749.
39. Li M, Fang X, Wei Z, York JP, Zhang P (2009) Loss of spindle assembly checkpoint-mediated inhibition of Cdc20 promotes tumorigenesis in mice. *J Cell Biol* 185(6): 983–994.
40. Dai W, et al. (2004) Slippage of mitotic arrest and enhanced tumor development in mice with BubR1 haploinsufficiency. *Cancer Res* 64(2):440–445.