Notch1-expressing Cells Are Indispensable for Prostatic Branching Morphogenesis during Development and Re-growth Following Castration and Androgen Replacement*

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Xi-De Wang‡**, Jianyong Shou‡§**, Peter Wong¶, Dorothy M. French†, and Wei-Qiang Gao||

From the Departments of ‡Molecular Oncology and ¶Pathology, Genentech, Inc., South San Francisco, California 94080

Notch1-expressing cells are indispensable for prostatic branching morphogenesis during development and re-growth following castration and androgen replacement. In rodents, the prostate is formed by budding of the urogenital sinus at late embryonic stages under the influence of androgen and epithelial cell interactions with stromal cells (1). At the cellular level, the glandular structure includes epithelium and stroma. The epithelium is composed of essentially two major types of cells, basal and luminal epithelial cells that can be in part distinguished by their expression of different markers. Whereas basal cells are positive for cytokeratin (CK)1, 5, CK14, and p63, luminal cells express CK8, CK18, and Nkx3.1 (2, 3). It has been well documented that androgen is required for prostatic morphogenesis and maintenance. Without androgen, the prostate cannot be generated during embryogenesis. Upon castration or androgen deprivation, significant apoptosis occurs in the mature epithelium, and the prostate shrinks in size (4). However, upon androgen replacement, the prostate re-grows to the original size. Defining the nature of the progenitor cells during prostatic development and re-growth will enhance our understanding of normal and pathologic processes in the prostate.

Recently, we have reported dynamic expression patterns of Notch1 during prostatic development and tumorigenesis (5). Immunostaining of prostates from a Notch1-GFP transgenic mouse line, in which expression of GFP is under control of the Notch1 promoter (6), revealed that Notch1-expressing cells localize within the basal cell compartment where progenitor cells are believed to reside. In addition, Notch1 expression is down-regulated in mature prostates but becomes up-regulated in certain primary and metastatic tumor cells in the TRAMP (transgenic adenocarcinoma of the mouse prostate) (7). These results suggest a possible role for Notch1 signaling during prostatic development and tumorigenesis.

Notch proteins are membrane-bound receptors. In mammals, four members including Notch 1, 2, 3, and 4 have been identified, and all of them share substantial homology with their original ortholog Notch in Drosophila (8). Notch expression is associated with progenitor cell types and is developmentally regulated in a variety of tissues/organs, including the brain (9), thymus (10), blood vessel (11), skin (12), eye (13), and ear (6, 14). Notch-mediated cell-cell interactions are well documented to be responsible for cell fate specification during embryogenesis (15). Aberrant activation or mutation of the Notch pathway not only leads to developmental disorders, including Alagille syndrome (16), but also neoplasia such as T-cell leukemia (17, 18) or mammary (19) and salivary adenocarcinomas (20). Of the four family members, Notch1 is by far the most extensively studied.

In the present experiments, we hypothesize that Notch1-expressing cells define the progenitor cells in the prostatic epithelium and, elimination of them would significantly impair prostatic branching morphogenesis and re-growth. To address...
this issue, we employed a transgenic approach to eliminate selectively the Notch1-expressing cells by targeted expression of a bacterial nitroreductase, which can convert 5-aziridinyl methyl benzimidazole into its active derivative. A prodrug consisting of a 5-aziridinyl methyl benzimidazole was introduced into the animal. The prodrug was dissolved in dimethyl sulfoxide at stock solution at 0.2 mg/ml. Phosphate-buffered saline (PBS) was used to dilute the stock solution to desired concentrations. For evaluation of apoptosis in various organs, prodrug at 50 mg/kg body weight was injected intraperitoneally daily for 5 days into 12-14-week-old mice. On the 6th day, the animals were euthanized with carbon dioxide, and various organs were harvested, and portions of these organs were fixed in 10% formalin for histology and eosin (H&E) staining or 4% paraformaldehyde for immunohistochemistry analyses.

Dissection and Culture of Prostate Tissue—The prostate whole mounts consisting of all lobes were dissected from postnatal mice and cut in two along the middle line, and each piece was placed individually onto cell culture inserts (8-μm pore size; BD Biosciences) in serum-free medium. The prostatic ductal organ culture was performed as described (29). Media was replaced with Dulbecco’s modified Eagle’s medium/F-12 with serum-free supplement (1-1884; Sigma), 2 mM glutamine, 5 mg/ml glucose, 100 units/ml penicillin, 100 mg/ml streptomycin, 1 × 10−8 M testosterone (Sigma), and 1:1000 epidermal growth factor (Clonetics). Unless otherwise noted, the medium was changed every other day; the prodrug concentration for treatment was 62.5 μM, and BrdUrd was always introduced into the medium 16 h prior to tissue fixation. Images of the cultures were taken under a Nikon TE300 inverted microscope using Comipx imaging systems with a cooled RGB CCD camera and analyzed with Photoshop 7.0. For BrdUrd pulse-labeling experiments, the prostate cultures (P9) were treated with 62.5 μM prodrug for 24 h first and then refed with medium containing BrdUrd. Two hours later, the cultures were rinsed in serum-free medium for 30 min and then either fixed or maintained for an additional 2 days before fixation with 4% paraformaldehyde.

Prostate Re-growth Following Hormone Replacement—Transgenic male mice as well as their wild type male littermates at the age of 12–14 weeks old were first castrated. Starting on day 9 after castration, all mice were injected with 50 mg/kg prodrug daily for 5 consecutive days. On day 14 after castration, some mice in each group were sacrificed, and prostates were harvested for evaluation. Other mice were implanted with testosterone pellets (15 mg/pellet/mouse, Innovative Research, Sarasota, FL). On day 17, i.e., 3 days after hormone replacement, mice were sacrificed, and their prostates were harvested. For this time point, the injections of BrdUrd at 50 mg/kg body weight were administered with 3 h intervals between. The weight of whole prostates harvested at each time point was measured with saline solutions completely absorbed by Kimwipes. Prostates were further analyzed by immunohistochemistry.

Histology, Immunohistochemistry, Image Acquisition, and Cell Counting—For general histologic analysis, tissues were collected and fixed in 10% neutral buffered formalin, then embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. For histochemistry, prostate whole mount tissues freshly dissected from mice or grown in culture were fixed with 4% paraformaldehyde in 0.1 M sodium phosphate, pH 7.4, at room temperature for 30–60 min. The tissues were rinsed with PBS and then either fixed in 2.5% glutaraldehyde in 0.1 M PBS at 4 °C. Cryosections of 5–20 μm thickness were cut using OCT as the embedding reagent. Immunostaining with anti-EGFP (chicken, 1:100; Chemicon, Temecula, CA), anti-cytokeratin 14 (rabbit, 1:10,000; Babco, Berkeley, CA), anti-cytokeratin 8 (sheep, 1:150; Pickell, Amsterdam, Netherlands), anti-caspase 3 (rabbit, 1:1000; R&D Systems, Minneapolis, MN), or anti-BrdUrd (mouse, 1:30; BD Biosciences, Palo Alto, CA) were detected with Texas Red- and/or fluorescein isothiocyanate-conjugated secondary antibodies. For BrdUrd and cytokeratin 14 double staining, sections were pre-treated with 2 N HCl for 40 min at room temperature before incubation with the primary antibodies. An in situ Cell Death Detection kit (TUNEL) (Roche Applied Science) was used to detect apoptosis. For double staining TUNEL and cytokeratin 14, TUNEL was performed after the immunostaining, including the washing of secondary antibody, was finished. Staining of whole mount tissue was performed similarly except that primary antibodies were incubated for 2 days at 4 °C with shaking. Slides were mounted in Fluoromount-G (Southern Biotechnology) supplemented with counter-staining dye 4,6-diamidino-2-phenylindole (DAPI) (Sigma). Immunostaining was observed under a fluorescence microscope. Unless otherwise noted, images were captured with Comipx imaging systems using a cooled RGB CCD camera and analyzed using Adobe Photoshop 7.0.

Cell counting was performed on the digital images captured from the slides. DAPI staining was used to obtain the total number of cells in a given area. Cytokeratin 14-expressing cells were counted as basal cells, and the rest of cells in the epithelium were luminal cells. At least five randomly selected regions such as epithelial ducts from different sections were counted for each group, and a two-way, unpaired f test was used for statistical analyses where necessary.
RESULTS

Establishment of Notch1-NTR-IRES-EGFP Transgenic Mouse Line—To eliminate selectively Notch1-expressing cells, we expressed the bacterial nitroreductase gene (NTR) (28, 30) under the control of the Notch1 promoter. This promoter had been used for generation of Notch1-GFP transgenic mice and had been shown to be able to drive GFP expression faithfully as demonstrated by Notch1 in situ hybridization and GFP labeling in parallel E14 sections (6). Cells that express nitroreductase can be selectively induced to undergo apoptosis in the presence of the prodrug CB1954, i.e., 5-aziridinyl-2,4-dinitrobenzamide (22). We also inserted the IRES-EGFP cassette following the NTR gene to facilitate visualization of Notch1-expressing cells that are undetectable due to lack of suitable anti-Notch1 antibodies and/or relatively low expression levels of Notch1 protein in the prostate. A schematic map of the construct is shown in Fig. 1A. Routine genotyping of mice was performed using the EGFP sequence as an amplicon in PCR (Fig. 1B). The results of using EGFP or NTR sequences for PCR genotyping are 100% concordant (data not shown). Of the 228 mice genotyped, 110 (48.2%) were positive for EGFP. We further verified the expression of both NTR and EGFP genes in transgenic animals. As shown in Fig. 1C, NTR mRNA was detected in the prostates of transgenic mice but not in those of wild type mice by RT-PCR. To verify the expression of the transgene EGFP, we dissected prostates from male neonates (P2–P5) and examined a piece of the prostate tissue mounted onto slides under a fluorescence microscope. Green fluorescence could be selectively detected in the epithelial region of the freshly dissected, live prostate tissue but not in the stromal area of the transgenic mice tissue or in wild type tissue (data not shown), as demonstrated in the Notch1-GFP line (5). Green fluorescence became undetectable following fixation. To verify the EGFP expression pattern at the cellular level, we used an anti-EGFP antibody to enhance the signal (see “Experimental Procedures”). As shown in Fig. 1D, all the epithelial cells in the epithelial bud but not stromal cells of P3 prostate were labeled by the EGFP antibody, and at this stage, they all expressed CK14 (Fig. 1D). At stage P10, when luminal cell differentiation is prominent (31), EGFP expression became restricted to cells in the basal cell compartment, which are CK14-positive (Fig. 1, D3 and D4). This EGFP expression pattern is consistent with the Notch1 expression pattern, which has been determined to be associated with CK14-positive cells (5).

Specific Cell Targeting in Notch1-NTR-IRES-EGFP Transgenic Mice Upon Prodrug Treatment—To determine whether the expression of bacterial nitroreductase could cause destruction of cells by treatment with prodrug, we injected the prodrug into adult transgenic and wild type animals. Following 5 days of daily injections, individual apoptotic cells, as revealed by the condensation and degradation of the nuclei, were evident in the epithelial lining of the prostatic epithelium in the transgenic mice (Fig. 2B, arrow) but not in the corresponding regions of wild type mice (Fig. 2A). To verify that apoptosis occurred in the targeted compartment, i.e. the basal cell compartment, we performed double staining of TUNEL and the basal cell marker cytokeratin 14. As shown in Fig. 2D, the majority of apoptosis occurred in CK14-positive cells. Cell counts from randomly selected areas revealed that ~11% of basal cells were TUNEL-positive. In sharp contrast, wild type mice treated with the prodrug had no apparent cytotoxicity (Fig. 2C), and the prostates from these mice were histologically normal (Fig. 2, A and
Fig. 2. Selective targeting of cells in the basal layer in the prostate of transgenic mice treated with prodrug. H&E staining (A and B) and TUNEL/CK14 double labeling (C1 and D1) of wild type (WT) and transgenic (TG) mice treated with prodrug. Note that while there is obvious cell death in the basal cell compartment of the transgenic prostate epithelium (B and D1), the wild type tissue shows normal histology (A and C1). C2 and D2 show DAPI counterstaining for the slides shown in C1 and D1, respectively. Scale bar, 30 μm.
Notch1-expressing Cells in Prostate Development and Re-growth

Fig. 3. Specific targeting of Notch1-expressing cells in transgenic prostate tissue. A, caspase 3 and EGFP double staining of P3 transgenic prostate. B, caspase 3 and EGFP double staining of P8 transgenic prostate. The prostates dissected from P3 and P8 pups were treated with 20 μM prodrug for 2 days. Note that caspase 3 (Csp3) staining was colocalized with EGFP-positive cells in the basal layer of P8 prostate (arrow in B) and undifferentiated epithelium of P3 prostate (A) as well as the distal duct tip of P8 prostate (arrowhead in B). Str, stroma; Epi, epithelium. Scale bar for A1–A3, 50 μm; for B1–B3, 60 μm.

Ablation of Notch1-expressing Cells Impairs Prostatic Re-growth in Castrated Mice Following Testosterone Replacement—An important feature of the prostate is that androgen is required for the maintenance of prostatic structure and function. Traumatic damage to the testis or castration causes prostatic involution and a decrease in its secretion function. However, subsequent administration of exogenous testosterone induces re-growth, differentiation, and resumption of the structure and function to its original size or level (33). Based on these findings, it is assumed that there are progenitor cells residing in the epithelium, which retain the capacity to repopulate the lost epithelial cells. However, the nature of these progenitor cells is uncharacterized. We hypothesized that Notch1-expressing cells define this progenitor population that plays an important role in the prostatic re-growth process.

To test this hypothesis, we followed the prostatic re-growth process of transgenic mice that had been castrated and treated with prodrug, and we compared it with that of wild type mice undergoing the same procedure. In agreement with the literature (33), 14 days after castration, wild type mice showed dramatic shrinkage of the prostate as compared with uncstrated normal mice of the same age. Transgenic mice also showed prostatic shrinkage following castration. Grossly, the wet weight of prostate in both groups was similar, averaging ~34 mg. TUNEL assay and immunostaining with anti-active caspase 3 in both wild type and transgenic prostate revealed prominent apoptosis in the luminal cell compartment and showed only small numbers of apoptotic cells in the basal cell compartment and the stroma (data not shown). The ratio of luminal cells and basal cells at this time point was about 1:1 (data not shown). Consistent with the results shown in Fig. 2, apoptosis in the basal cell compartment of transgenic tissue was elevated as compared with that in wild type tissue (data not shown). Following hormone replacement, transgenic mice
exhibited impaired prostate re-growth. On day 17, i.e. 3 days after replacement, the mean prostate weight of transgenic mice (55.9 ± 3.3 mg, n = 3) was significantly lower than that of the control group (91.6 ± 6.1 mg, n = 3). Immunostaining for CK14 in prostatic sections showed that while in wild type tissue, basal cells were separated by 1–2 luminal cells (Fig. 8, A1 and A2), the basal cells in the transgenic tissue frequently remained contiguous or much more reminiscent of the histology before hormone replacement (Fig. 8, A3 and A4). The ratio of luminal cells to basal cells in transgenic prostate epithelium (1.8 ± 0.2:1, n = 8) was >2-fold less than that in wild type tissue (4.3 ± 0.7:1, n = 8) (Fig. 8A5). BrdUrd incorporation was also compared at this time point as an index for the number of progenitor cells that were undergoing proliferation. As shown in Fig. 8B3, remarkably lower incorporation of BrdUrd was detected in the transgenic prostate epithelium than in the wild type tissue (Fig. 8B1). In contrast to the high percentage of cells (25.9 ± 5.1%, n = 8) incorporating BrdUrd in the wild type tissue, only 11.5 ± 3.3% (n = 8) of cells in the transgenic tissue were proliferating (Fig. 8B5). Based on their laminar position and morphology, the majority of cells that were BrdUrd positive did not appear to be luminal cells, in agreement with the

**Fig. 4.** Correlation between the level of cell death and the level of Notch1 expression in non-prostate organs. A–D shown are H&E staining indicating strong apoptosis in the thymus of transgenics (B1 and B2, low and high magnification, respectively) compared with wild type tissue (A1 and A2, low and high magnification, respectively) and undetectable cell death in transgenic (D) and wild type kidneys (C). E, the level of Notch1 expression in a panel of normal adult mouse tissues was evaluated using Taqman real time quantitative RT-PCR with mouse RNA purchased from Clontech. The value has been normalized to glyceraldehyde-3-phosphate dehydrogenase in the same samples. Scale bars in A1 and B1, 200 μm; in A2 and B2, 25 μm; in C and D, 100 μm.
concept that luminal cells are terminally differentiated and non-dividing cells. In addition, the intensity of BrdUrd staining in transgenic prostate was much weaker than that in wild type (Fig. 8, B1 and B3).

**Notch1 Expression Is Elevated Following Castration and High in PrEC Cells**—Based on the above findings, we predicted that Notch1 expression pattern would match those of other basal cell markers that define the compartment of progenitor cells. In a separate study in which we followed prostate gene expression profiles of normal adult mice during the course of castration and hormonal replacement processes by using the microarray technique (Fig. 9A), we found indeed that the expression pattern of Notch1 highly correlated with those of p63 and cytokeratin 14, two basal cell markers (see review Ref. 1). During the entire castration and hormonal replacement course, there was a peak elevation at 14 days following castration and a resumption to nearly normal levels after 3 days of hormone replacement. In contrast, expression levels of Nkx3.1, a luminal cell marker, showed an opposite pattern (Fig. 9A).

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**Fig. 5. Blockade of branching morphogenesis of cultured P4 transgenic prostates in the presence of prodrug.** A, representative images of cultured prostate at different time points from triplicate experiments. Although either the wild type (WT) tissue maintained in the presence of prodrug (left column) or the mock-treated transgenic (TG) prostate tissues (right column) shows normal branching morphogenesis, transgenic tissue treated with prodrug showed a completely blocked branching morphogenesis (middle column). The final concentration of prodrug was 62.5 μM, and prodrug was added on day 0 (d0) when the prostates were freshly dissected and plated in culture dishes. The cultures were refed with medium containing prodrug on day 3 (d3). Experiments with higher concentrations of the prodrug (125, 250 μM) yielded similar results without showing toxicity in prodrug-treated wild type tissue (not shown). B, epithelial staining of whole mount prostate after prodrug treatment using a combination of CK8 and CK14 antibodies. Note that while wild type tissue showed normal branching morphogenesis, the epithelial budding in transgenic tissue was blocked. Scale bar, 500 μm.
**DISCUSSION**

By taking advantage of the nitroreductase activity, we have successfully established a transgenic mouse line in which Notch1-expressing cells can be selectively ablated. By using this strategy we found that Notch1-expressing cells may define the progenitor cells in the prostatic epithelium. These cells are necessary for the epithelial branching morphogenesis during prostatic development and are important contributors for prostatic re-growth following castration and androgen replacement.

Cell ablation is an important technique that has been elegantly used in cell lineage and cell-cell interaction studies in the developing nervous system. Initially, cell ablation was achieved via laser beam, cytotoxin injection, or mechanical surgery in the invertebrate nervous system. These early studies clearly demonstrated how the presence or absence of a given cell affects cell fate and growth patterns of adjacent cells (36–38). More recently, cell death genes such as *ICE* and *ced-3* have been employed under the control of specific promoters to effectively and specifically kill target cells but not adjacent cells (39). To achieve cell ablation at specific time points, sophisticated systems such as herpes simplex virus 1-thymidine kinase (40, 41), diphtheria toxin, and its receptor approaches (42, 43) have been designed such that cell ablation can happen only when the substrates or ligands are administered. However, these systems exhibited certain levels of non-specificity or could potentially induce secondary effects. The approach we used in the present experiments is a combination of the bacterial nitroreductase and a specific Notch1 promoter. With this approach, we are not only able to target a cytotoxin to a specific cell population but also able to eliminate cells at various developmental time points by using an enzymatic activity that is exogenous to the host. Interestingly, this tissue specificity and the ability to control timing of administration have resulted in consideration of the prodrug strategy for gene therapy (44, 45).
In addition, we demonstrated that specific organs such as prostate can be dissected and placed in culture in the presence of the prodrug to study the role of Notch1-expressing cells during prostatic development. This model may also facilitate studies on other Notch1-expressing organs such as the thymus and developing nervous system.

The present experiments reinforce the idea that Notch-expressing cells are important for cell growth and differentiation. First, at an early developmental stage, such as P3, Notch1 expression is associated with all prostatic epithelial cells that are proliferating or act as progenitor cells. Elimination of the Notch1-expressing cells at this stage completely blocks the epithelial branching morphogenesis. Second, at later stages when cell differentiation becomes prominent (e.g. P10), Notch1 expression is localized to basal cell compartment where prostatic progenitor cells are believed to reside. Our data showed that when Notch1-expressing cells are destroyed at this later stage, the number of cells in the basal layer decreased, and differentiation of new luminal epithelial cells was greatly inhibited as indicated by the BrdUrd pulse labeling experiments. Third, Notch1 expression is down-regulated in mature prostates, and this corresponds to the decreased proliferation in the epithelium at this stage.

Although previous studies have documented the prostatic re-growth process, the progenitor cells within the epithelium responsible for the re-growth are uncharacterized. During prostate regression following castration, massive apoptosis occurred in the luminal layer of the epithelium, resulting in a dramatic decrease in the ratio of luminal cells to the basal cells from ~10:1 to about 1:1 at the time point of 2 weeks after hormone deprivation (33). Following hormone replacement, robust cell proliferation happens in the epithelium, and the ratio of luminal cells to basal cells resumes. Our work that prodrug treatment significantly affected the prostate re-growth suggests that Notch1 expression may define the progenitor cells that undergo proliferation in this process. This model is supported by the correlation of Notch1 gene expression patterns and the dynamics of the basal cell population in the entire process following hormone ablation and replacement. Our microarray experiments showed that castration leads to an increased level of Notch1 in the prostate, and androgen replacement reversed the change. The pattern of Notch1 expression matched very well with other known markers of progenitor cells, i.e. p63 and CK14. In addition, the data that PrEC cells, which represent the proliferating basal cells of mature prostate epithelium, expressed much higher levels of Notch1 than prostatic samples that are mainly composed of luminal cells, provided additional support for the notion that Notch1 expression

Fig. 7. Ablation of Notch1-expressing cells in P9 transgenic prostates inhibits proliferation and subsequent differentiation of new luminal cells. P9 prostates were treated with prodrug for 1 day, pulse-labeled with BrdUrd for 2 h, and then either fixed immediately or allowed to grow for 2 additional days in the absence of BrdUrd. Tissues of both wild type (WT) and transgenic mice (TG) at different stages of experiments were collected for analyses including CK8/CK14 double staining after prodrug treatment (A and B), BrdUrd and CK14 double staining either immediately (C and D) or 2 days (E and F) after BrdUrd pulse labeling. Scale bar, 20 μm.
is associated with the progenitor cells that contribute to the re-growth process.

It should be pointed out that although our study links Notch1 expression with progenitor cells, it does not differentiate the so-called stem cell and transit-amplifying cell populations in the prostate (46, 47). Recent work suggests that there are possibly two types of proliferative cells in the prostatic epithelium: the strict-sense stem cells that can give rise to all cell types in the epithelium and so-called intermediate or transit-amplifying cells that give rise to specific types of cells (reviewed in Ref. 48). The idea that there are probably stem cells in the prostate has been mainly based on the observation that the prostate has the capacity of self-renewal as it can undergo regression and re-growth process caused by castration and hormonal replacement for as many as 30 cycles (4, 49). However, the nature of this population in vivo still remains elusive. On one hand, the stem cell population is generally believed to localize in the basal cell layer (48). On the other hand, recent studies suggest that, in addition to expression of basal cell markers cytokeratin 5, 14, and p63, these cells may also express luminal cell markers cytokeratin 8 and 18, as well as other markers such as GSTπ, α,β integrin (50), or prostate stem cell antigen (25, 51). Moreover, it has been proposed that prostate stem cells are not equally distributed in different regions of the prostate. The proximal region of prostate ducts is reported to contain higher density of cells that resemble stem cells.

**Fig. 8. Impaired prostatic re-growth in adult transgenic mice treated with prodrug.** The re-growth of the prostate was measured qualitatively by the separation of basal epithelial cells (A1–A4) and quantitatively by the ratio of luminal and basal cells in the epithelium (A5) as well as the incorporation of BrdUrd (B1–B5) in transgenic (TG) and wild type (WT) tissue, respectively. Scale bar for A1–4 in A4, 20 μm; for B1–4 in B4, 40 μm.
cancer and developmental abnormalities (for reviews see Refs. 55 and 56). Our previous work showed that Notch1 expression is up-regulated in certain types of prostatic malignant cells (5), but whether Notch signaling is directly involved in prostatic tumorigenesis remains to be determined. Several mouse models for prostate cancer have been established (57), such as the transgenic adenocarcinoma of the mouse prostate (7), PTEN/ Nkx3.1, or conditional mutant mouse (58, 59), and these reported more recently, including prostate-specific PTEN knock-out model (60) and Myc-driven transgenic model (61). For further determination on how Notch1-expressing cells play a role during prostate tumorigenesis, these prostate cancer animal models can be crossed with our transgenic line to generate a compound model, and the prodrug can be injected into the compound model either before tumor formation to determine the importance of progenitor cells during tumor initiation or during tumor growth to determine the consequence of elimination of Notch1-expressing cells in tumor progression. Grafting of transgenic/knockout prostate tissue into the renal capsule of wild type animals may also be considered for use to minimize the complication of wide expression of Notch1. Future studies in this direction would advance our understanding on the role of Notch1-expressing cells in prostatic tumorigenesis.

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Fig. 9. High expression level of Notch1 in proliferating prostatic epithelial cells. A, Notch1 expression profile data obtained from a separate study using microarray to understand the process following castration and hormone replacement in mice. N, normal adult mice. C3, 3 days after castration. C14, 14 days after castration. C14 + T3, 3 days after hormone replacement performed on the 14th day after castration. The intensity of fluorescence on microarray from 5 samples in each group was used to calculate the relative expression of each gene. The data have been normalized to the levels in normal controls for comparison. B, Taqman real time RT-PCR is performed for comparison of Notch1 mRNA level in human samples. BPH-1, an immortalized cell line derived from benign prostate hyperplasia. Note that expression of Notch1 in mice tightly correlates with basal cell markers p63 and CK14 but not luminal cell marker Nkx3.1 in the process following castration and hormonal replacement (A) and that PrEC human progenitor epithelial cells express a much higher level of Notch1 than more differentiated prostate samples (B).
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Xi-De Wang, Jianyong Shou, Peter Wong, Dorothy M. French and Wei-Qiang Gao

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