Dynamics of Notch Expression during Murine Prostate Development and Tumorigenesis

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ABSTRACT

Notch signaling has been widely demonstrated to be responsible for cell fate determination during normal development and implicated in human T-cell leukemia and mouse mammary carcinomas. Here we show that Notch signaling may be involved in prostatic development and cancer growth. In situ hybridization and reverse transcription-PCR analyses revealed that Notch1 was expressed in prostate epithelial cells during normal development and in prostate cancer cells. Characterization of Notch1-green fluorescent protein transgenic mice, in which the expression of reporter green fluorescent protein is under the control of the Notch1 promoter, indicated that Notch1-expressing cells were associated with the basal epithelial cell population in the prostate. Examination of the transgenic adenocarcinoma of the mouse prostate showed that expression of Notch1 was elevated in malignant prostatic epithelial cells of primary and metastatic tumors. Expression of Notch ligands, however, was low or undetectable in cultured prostate cancer cells or in malignant prostatic epithelial cells in transgenic adenocarcinoma of the mouse prostate. Furthermore, overexpression of a constitutively active form of Notch1 inhibited the proliferation of various prostate cancer cells, including DU145, LNCaP, and PC3 cells. Taken together, our data indicate for the first time that Notch signaling may play a role in murine prostatic development and tumorigenesis.

INTRODUCTION

The majority of prostate tumors are adenocarcinomas, which arise from overproliferation of epithelial cells (1, 2). There are two major types of epithelial cells within the prostatic epithelium: luminal and basal cells. These cells can be distinguished according to their location, morphology, function, and expression of specific cytokeratins (3). It is generally believed that the luminal cells are terminally differentiated cells that are androgen dependent and produce secretory proteins. The basal cells are located between the luminal cells and the underlying basement membrane. A subpopulation of the basal cells has progenitor cell features (4, 5) and may have the capacity to give rise to luminal cells during normal prostatic development or regeneration following castration and androgen replacement therapy (6). Whereas the luminal cells express cytokeratin 8 and 18, the basal cells produce cytokeratin 5 and 14 (3). Prostatic tumorigenesis is attributable to dysregulation of cell proliferation and differentiation of either basal or luminal epithelial cells.

The mechanisms controlling proliferation, differentiation, and tumorigenesis of prostatic epithelial cells are still largely unknown. Cell-cell interactions (1) and specific genes (2) are shown to play an important role in these processes. In an attempt to identify additional genes that might be involved during prostatic development and carcinogenesis, we have focused our attention on the Notch signaling molecules. The Notch family of receptors and ligands are good candidates because they have been shown to be expressed in numerous types of epithelial cells where they mediate a variety of cell interactions specifying cell fate during embryogenesis (7). In addition, alterations in Notch signaling have been linked to human T-cell leukemia (8, 9) and mouse mammary carcinomas (10).

Originally identified as a Drosophila mutation displaying abnormal differentiation of neural versus epidermal cell lineages (11), the Notch receptors are generally associated with nonterminally differentiated, often proliferating cell populations. Activation of Notch signaling influences the ability of these cells to acquire or maintain a particular cell fate (7). To date, four vertebrate Notch genes have been identified: Notch1/TAN-1, Notch2, Notch3, and Notch4/int-3. Each of these Notch genes is widely expressed throughout development and has both overlapping and unique expression patterns relative to each other (12, 13). The proteins encoded by these genes appear to bind nonpreferentially to each of the identified Notch ligands, the DSL2 family of ligands. Similar to the Notch receptors, DSL ligands are transmembrane proteins originally identified in Drosophila as Delta and Serrate. Six vertebrate DSL family genes have been identified, including the Serrate-like homologues Jagged1 and Jagged2 (14, 15), and the Delta-like homologues Delta, Delta2, Delta3, and Dll4 (16–18).

In the present study, we first examined the expressing patterns of Notch1 receptor during rodent prostatic development and in various human prostate cancer cells, using RT-PCR and in situ hybridization techniques. We also characterized Notch1-GFP transgenic mice in which the reporter gene GFP is driven by the Notch1 promoter (19). We found that Notch1 expression was spatially and temporally regulated during prostatic development. In addition, we determined Notch1 expression patterns in cultured prostate cancer cells and in TRAMP (20). We observed that Notch1 was expressed in various cancer cells and that its expression was elevated in malignant and metastatic prostatic epithelial cells, whereas Notch1 ligand expression was undetectable or low. We then performed experiments to determine whether activation of Notch signaling would influence the growth of prostate cancer cells by expression of constitutively active Notch1 (21). Activation of Notch1 signaling resulted in a growth inhibition of prostate cancer cells. Thus, the present experiments suggest that Notch1 signaling is involved in murine prostatic development and tumorigenesis.

MATERIALS AND METHODS

In Situ Hybridization. [35]P]UTP-labeled sense and antisense riboprobes were generated from PCR products cloned into transcription vectors (22). The murine Notch1 cDNA, cloned in pBlueScript, (Stratagene, La Jolla, CA) spanned nucleotides 3773–4639 of the mouse Notch1 sequence (GenBank accession no. Z11886). The murine Jagged1 cDNA, also cloned in pBlue-Script, corresponded to the nucleotide sequence 702–2425 of the rat Jagged1 sequence (GenBank accession no. L38483). Formalin-fixed, paraffin-embedded, dissected mouse prostatic tissue (postnatal days 3 and 16, and adult) was sectioned and deparaffinized. The in situ hybridization was performed as described previously (19). Tissue pretreatments consisted of a proteinase-K digestion (4 μg/ml) for 30 min at 37°C, a 0.5× SSC (1× SSC: 0.15 m NaCl, 0.15 m sodium citrate) wash for 10 min at room temperature, and dehydration

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2 The abbreviations used are: DSL, Delta/Serrate/jagged; RT-PCR, reverse transcription-PCR; GFP, green fluorescent protein; TRAMP, transgenic adenocarcinoma of the mouse prostate; PIN, prostatic intraepithelial neoplasia.

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prior to application of the prehybridization solution. Slides were processed for autoradiography and exposed for 4 weeks before development. Sense control probes revealed no hybridization above background (not shown).

**TaQMan RT-PCR Gene Expression Analysis.** TaQMan real-time quantitative RT-PCR analysis was performed with the 5′-exonuclease assay using fluorescent nonextendible oligonucleotide probes (TaQMan PCR detector 7700; Perkin-Elmer Applied Biosystems) as described (23, 24). Prostatic tissue was dissected from rats at various developmental stages (postnatal day 1, postnatal day 10, and adult) in lysis buffer and homogenized through a QiAshredder column (Qiagen, Valencia, CA). Total RNA was immediately isolated using RNaseasy columns (Qiagen). We used specific probe and primers for rat *Notch1* (probe, 5′-CCTGGCCTTTGAGCTTCTACATCCTGTTG-3′; forward primer, 5′-AAGTCATGTCAAGGACCAC-3′; reverse primer, 5′-CAGCGTAAGCAGCTATGAT-3′). The specific probe and primers for the control housekeeping gene, rat *gapdh*, were the same as reported previously (25). Expression levels of genes of interest were normalized to *gapdh*. Initial RT-PCR amplifications were also examined by agarose gel electrophoresis to ensure that bands were visible only at the expected molecular weights.

For TaQMan analysis on the human prostate cancer cells and PrE cells, total RNAs were isolated using RNAzol (Tel-Test, Friendswood, TX) from cultured DU145, LNCaP, PC3, and PrE cells (Clonetics), respectively, treated with DNase I (Roche Molecular Biochemicals) for 30 min at room temperature, cleaned up on RNeasy columns (Qiagen), and then reverse transcribed by Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). cDNAs were subjected to TaQMan PCR analysis in the same as described above. The sequences for the specific primers and probes are listed as follows:

Human *Notch1*: probe, 5′-CCGCTCTGAGCAGGACA-3′; forward primer, 5′-CAGTGGTGGCCGGCC-3′; reverse primer, 5′-GTTTGATGTGTTCGGACCAT-3′.

Human *Jagged1*: probe, 5′-CCTGGCCAGCGAAAATCGAAA-3′; forward primer, 5′-CAACCGCATGTCGTCG-3′; reverse primer, 5′-CGCTCCACCAAGAATTGAT-3′.

Human *Human Dll4*: probe, 5′-TGACACCTCCTCTTGAACCAAACCGCA-3′; forward primer, 5′-CCTGTATGAAAGGAGAGATT-3′; reverse primer, 5′-CCGAGTGAAGAATAAAGAAGATT-3′.

Human *Delta*: probe, 5′-TCTGCGTGACGCAGAAGCC-3′; forward primer, 5′-TGTGTTGACGAAACTAATGCGAG-3′; reverse primer, 5′-GTGATAAGTGGCGGAGGC-3′.

**RESULTS**

**Temporal and Spatial Expression Patterns of Notch1 during Prostatic Development.** To determine whether *Notch1* is expressed in the prostate and whether its expression is developmentally regulated, we performed TaQMan real-time, quantitative RT-PCR analysis using RNA extracted from whole-mount prostate organs prepared from rats at different developmental stages. As shown in Fig. 1A, *Notch1* was expressed at high levels in the developing prostate at postnatal day 1 (P1) to postnatal day 10 (P10), and its expression was down-regulated in the adult.

To uncover the cellular expression pattern, i.e., whether *Notch1* is expressed by epithelial cells or stromal cells in the prostate, we carried out *in situ* hybridization on tissue sections prepared from developing mouse prostates, using a specific radiolabeled antisense probe. Although the sense probe did not show any labeling (data not shown), *Notch1* signal was seen selectively in epithelial, but not in stromal cells at both postnatal day 3 (P3) and postnatal day 16 (P16; Fig. 1B).

**Notch1 Expression Is Associated with Basal Epithelial Cells in Prostate.** Although radioactive *in situ* hybridization showed highly restricted *Notch1* expression patterns in the epithelium, it did not provide the cellular resolution to distinguish which epithelial cell type specifically expressed *Notch1*. To precisely determine the cellular expression of *Notch1* in the prostatic epithelium, we analyzed protastatic sections from mice containing a transgene of GFP under the control of the *Notch1* promoter (19). In the *Notch1*-GFP transgenic mice, *Notch1*-expressing cells fluoresce green. Previous side-by-side comparison of sections of E14 whole embryos showed that the distribution of *Notch1* signal in *Notch1*-GFP transgenic mice was similar to that observed in E14 embryos by *in situ* hybridization with *Notch1* mRNA (19).

At postnatal day 3, there was robust GFP signal in the epithelium surrounding the lumen of the budded ductal epithelial units. At this stage, all of the cells in the epithelium were labeled (Fig. 2A), and they all showed a typical barrel-shaped morphology, extending their cytoplasm from the base to the lumen. By postnatal day 12, although the majority of the cells in the epithelium were still GFP-positive (Fig. 2B), some had lost *Notch1* expression. In the adult, GFP labeling became much weaker, and only a small fraction of cells located in the deep, basal layer of the epithelium were GFP-positive (Fig. 2C), indicating a down-regulation of *Notch1* expression. These GFP-positive cells in the adult prostate displayed a small, round cell body with thin processes instead of a columnar or barrel-like shape. No fluorescence was observed in wild-type mouse prostates (data not shown).

The GFP-positive cells in the *Notch1*-GFP transgenic mice ap-
shown that TRAMP mice may serve as a useful model of prostate cancer (20, 26). We were interested in whether there is any change in the levels of Notch1 expression in the TRAMP mouse prostate, in particular, in malignant cells during prostatic tumorigenesis. In situ hybridization of the tissues prepared from TRAMP mice revealed that although normal mature prostate expressed undetectable or very low levels of Notch1 (Fig. 4, A and B), malignant cells, including some areas of PIN (data not shown), moderately differentiated adenocarcinoma cells (Fig. 4, C and D) and some areas of poorly differentiated adenocarcinoma cells (data not shown) expressed high levels of Notch1. Similarly, the prostate tumor cells that had metastasized to the lymph node also expressed markedly higher levels of Notch1 compared with the neighboring normal tissue (Fig. 4, E and F).

Because Notch1 expression is associated with basal cell population during prostatic development, the finding that Notch expression is up-regulated in the malignant cells in the TRAMP prostate raises a question whether these malignant cells possess basal cell futures. We therefore carried out immunohistochemistry using anticytokeratin 14 antibody, a basal cell marker, in prostatic tissue prepared from the TRAMP and wild-type mice. We found that the majority of the cells were cytokeratin 14-negative in the PIN (data not shown) and in the well- to moderately differentiated TRAMP carcinomas (Fig. 4H), although a few cytokeratin 14-positive cells were observed. There was no apparent increase in the number of cytokeratin 14-positive cells in the PIN and adenocarcinomas of the TRAMP mice compared with the normal prostate in wild-type mice (Fig. 4, G and H). In fact, in moderately and poorly differentiated carcinomas of the TRAMP, virtually all cells were cytokeratin 14-negative (data not shown). These results suggest that in the malignant cells of TRAMP, Notch1 expression is uncoupled from cytokeratin 14 expression.

Expression Levels of Notch ligands Are Low or Undetectable in Prostate Cancer Cells. Given the fact that Notch1 is expressed in prostate cancer cells, we were interested in whether these cells also express Notch ligands. We therefore performed TaqMan RT-PCR analysis using specific primers and probes for Notch ligands with cultured DU145, LNCaP, and PC3 cells and human prostatic epithelial cells (PrE cells; Clonetics). At the time we carried out these experiments, only four cloned sequences of human Notch ligands were available from GenBank, including Jagged1, Jagged2, Delta, and Dll4 (16–18). As shown in Fig. 5A, only Jagged1 was expressed in the PrE cells, and it was expressed at low levels in LNCaP cells. Jagged2 was expressed at low levels in LNCaP cells, whereas Delta and Dll4 expression was negligible in all four types of cells examined.

To further characterize the expression profile of Jagged1, we performed in situ hybridization with a specific antisense probe on prostatic sections prepared from the TRAMP and wild-type mice. A strong signal was detected in the endothelial cells of blood vessels in normal and malignant prostate tissues; however, no signal was seen in the malignant epithelial cells in TRAMP tumors (Fig. 5B) or in normal prostatic epithelium in wild-type mice. Activation of Notch Signaling Leads to Inhibition in Proliferation of Prostate Cancer Cells. Because Notch1 is expressed in prostate cancer cells and Notch ligand expression is relatively low, we wondered whether activation of Notch signaling would influence the growth of prostate cancer cells. We transfected various prostate cancer cells with a constitutively active form of Notch1, mN1-IC, the intracellular domain of mouse Notch1 (21). Overexpression of the constitutively active form of Notch1 inhibited DNA synthesis in all three prostate cancer cells examined, including DU145, LNCaP, and PC3 cells. As shown in Fig. 6A, there was a statistically significant reduction in the proliferation rates in all three prostate cancer cells compared with those transfected with a control vector (P < 0.005).

Previous studies have revealed that DNA-binding protein suppressor of hairless [Su(H)] in Drosophila (29) and its homologue CBF1

![Expression patterns of Notch1 during prostatic development. A. TaqMan RT-PCR analysis of Notch1 expression in whole-mount prostatic tissue at different developmental stages: postnatal day 1 (P1), postnatal day 10 (P10), and adult. Two independent RNA samples were prepared from rat ventral prostate and examined. Experiments were performed in duplicate and the data were normalized to gapdh. B, bar chart shows the expression levels of Notch1 mRNA in different stages of prostatic development.](link)

**Fig. 1. Expression patterns of Notch1 during prostatic development.** A: TaqMan RT-PCR analysis of Notch1 expression in whole-mount prostatic tissue at different developmental stages: postnatal day 1 (P1), postnatal day 10 (P10), and adult. Two independent RNA samples were prepared from rat ventral prostate and examined. Experiments were performed in duplicate and the data were normalized to gapdh. B, bar chart shows the expression levels of Notch1 mRNA in different stages of prostatic development.
RBPJ/KLF2 in mammals (30) are downstream mediators of Notch signaling. It has been shown that activation of Notch transactivates the CBF1 pathway and subsequently leads to inhibition of neurogenesis (31) and muscle cell differentiation (14, 32, 33). To explore whether activation of the Notch pathway by mN1-IC in prostate cancer cells also transactivates the CBF1 pathway, we performed a reporter assay using a luciferase reporter construct (33). As shown in Fig. 6B, cotransfection of the CBF1-luciferase construct and the mN1-IC resulted in a significant (P < 0.001) increase in luciferase activity in cultured PC3 (a 300-fold increase) and LNCaP (a 1900-fold increase) cells compared with that in the cultures cotransfected with the CBF1-luciferase construct and a control vector. These results indicate that transactivation of the CBF1 pathway is a downstream event following Notch activation in prostate cancer cells.

DISCUSSION

We determined that expression of Notch1 is temporally and spatially regulated in the rodent prostate during normal development. Notch1 expression correlates well with the basal-luminal cell segregation in immature prostates (3) and is restricted to the basal prostatic epithelial cells in the adult. The rodent prostate is formed by budding from the urogenital sinus just before birth. Before postnatal day 5, all of the cells in the epithelium are proliferative and possess progenitor cell features. Our experiments demonstrate that at this early developmental stage, Notch1 is expressed in all prostatic epithelial cells. Beginning with postnatal day 5, some cells in the epithelium undergo terminal differentiation and become luminal cells (34), whereas others remain proliferative and stay in the basal layer. In more mature prostate, although the luminal cells are cytokeratin 14-negative but cytokeratin 18-positive, the basal cells are cytokeratin 14-positive but cytokeratin 18-negative (3). Our study showed that at the intermediate and later stages (from postnatal day 5 to adult), the Notch1-expressing cells are cytokeratin 14-positive cells, indicating that notch expression is associated with the basal cell population. Further support for this result comes from our RT-PCR analysis of the basal cell-derived PrE cells, which express Notch1 (data not shown). The PrE cells obtained from Clonetics represent essentially basal cells because they were established via selection of proliferative and surviving human prostatic epithelial cells in the initial cultures (35) and expressed cytokeratin 14. On the other hand, our Notch1 expression data were obtained by examining Notch1 mRNA expression using in situ hybridization and quantitative RT-PCR, or by monitoring Notch1 promoter-directed GFP transgene expression. Expression of Notch1 protein in the prostate remains to be determined.

The expression pattern of Notch1 during prostatic development appears to be similar to that during neurogenesis. In the nervous system, Notch1 usually is expressed in neural progenitor cells, whereas terminally differentiated neurons are Notch-negative (7). It is generally accepted that Notch signaling is involved in cell differentiation events resulting in the segregation of developmentally equiv-
lent precursors into distinct cell types, through either lateral inhibition/specification or inductive signaling (for a review, see Ref. 36). The present data suggest that early in development, Notch1 signaling might be involved in cell fate determination, in particular, the acquisition of the luminal cell versus basal cell identity in the prostatic epithelium.

An important finding of this study is that Notch1 expression is up-regulated in the malignant cells, including moderately differentiated adenocarcinoma (C and D) and metastasized lymph node (arrows in E and F versus the adjacent normal lymphatic tissue, indicated by arrowheads). G and H, cytokeratin 14 immunohistochemical staining in normal prostate of wild-type (G) and in a well-differentiated adenocarcinoma of a TRAMP mouse (H). Red arrows indicate cytokeratin 14-positive cells. The majority of cells are cytokeratin 14-negative in the TRAMP adenocarcinoma. Bar, 100 µm for A and B, 200 µm for C–F, 50 µm for G and H.

Fig. 4. Notch1 and cytokeratin 14 expression in the TRAMP prostate. A-D, Notch1 in situ hybridization signals in normal prostatic tissue from an adult wild-type mouse (A and B) and from a moderately differentiated adenocarcinoma from a 5-month-old TRAMP mouse (C and D). E and F, Notch1 in situ hybridization signals in metastatic prostatic epithelial cells in the lymph node of a 5-month-old TRAMP mouse. Dual H&E brightfield (A, C, and E) and darkfield (B, D, and F) images were taken from the same visual field. Note that although the expression levels are undetectable in normal prostatic tissue in wild-type mice (A and B), Notch1 expression is up-regulated in the malignant cells, including moderately differentiated adenocarcinoma (C and D) and metastasized lymph node (arrows in E and F versus the adjacent normal lymphatic tissue, indicated by arrowheads). G and H, cytokeratin 14 immunohistochemical staining in normal prostate of wild-type (G) and in a well-differentiated adenocarcinoma of a TRAMP mouse (H). Red arrows indicate cytokeratin 14-positive cells. The majority of cells are cytokeratin 14-negative in the TRAMP adenocarcinoma. Bar, 100 µm for A and B, 200 µm for C–F, 50 µm for G and H.

In these transgenic mice, tumors are usually formed in the prostate when the mice are 12 weeks or older. In 24–30-week-old TRAMP mice, metastatic tumors can be found in other tissues, such as the lymph node (20, 26). Whereas in the adult wild-type mouse prostate, Notch1 expression is either undetectable (by in situ hybridization) or low (by a more sensitive method, TaqMan RT-PCR), its expression is evident in malignant or metastatic prostatic cells. Our finding that Notch1 is up-regulated in malignant prostatic epithelial cells is in agreement with a previous study in which increased expression of Notch1 was reported in cervical carcinomas, colon adenocarcinoma, and lung squamous carcinoma (37).
The finding that expression of Notch1 is increased in TRAMP tumors is intriguing because theoretically the probasin promoter is a cell-specific promoter targeting genes to luminal, but not basal, prostatic epithelial cells. The observation that Notch1-expressing TRAMP tumors are cytokeratin 14-negative suggests that Notch1 expression becomes uncoupled from basal cells in the TRAMP. At present we do not know the mechanism for this uncoupling phenomenon. One possibility is that, usually, luminal cells are a postmitotic, terminally differentiated population, but when they become malignant, they are proliferative. The TRAMP luminal tumor cells are probably undergoing a dedifferentiation process. They may behave as progenitor cells, with which Notch1 expression is usually associated.

The function of Notch signaling in tumorigenesis can be either oncogenic or antiproliferative; the function is context dependent. A truncated form of human Notch1 has been shown to be capable of inducing T-cell leukemia. When bone marrow infected with a recombinant retrovirus expressing a truncated form of Notch1 was transplanted, clonal T-cell leukemia was formed in ~50% of the mice (9). Similarly, truncations caused by the insertion of a mouse mammary tumor virus (MMTV) within the Notch4 gene can transform mammary epithelial cells, leading to mammary carcinomas (10). On the other hand, our experiments show that activation of Notch signaling can inhibit the growth of prostate cancer cells. Consistent with our findings, a recent study in small cell lung cancer cells also demonstrated a growth-arresting effect of Notch signaling (38). There may obviously be tissue-specific differences in responsiveness. For example, estrogen enhances the proliferation of breast cancer cells (39), but inhibits prostate cancer cell growth (40). In addition, gene expression profiling on Affymetrix gene chips showed an opposite trend of expression of certain genes in prostate versus breast tumor tissues.3 Furthermore, up-regulation of genes that are antiproliferative in prostate tumors is not unprecedented. For example, bone morphogenetic proteins that belong to transforming growth factor-β family have been shown to be expressed at high levels in prostate tumors (41). However, bone morphogenetic protein-2 inhibits LNCaP cell growth in the presence of androgen (42). Another example is increased expression of the cell death receptors tumor necrosis factor receptor-1 and -2 in prostatic carcinomas (43). It has been proposed that there may be a feedback mechanism for the growth of tumor cells during tumorigenesis (43).

It is conceivable that activation of Notch may push cells into terminal differentiation to become mitotically inactive. Supportive evidence for this type of function comes from studies on multipotent neural crest stem cells during development of peripheral nervous system and on differentiation of subpopulation of T cells. Recently, Morrison et al. (44) demonstrated clearly that activation of Notch signaling does not maintain neural stem cells in an uncommitted state, but rather acts instructively to promote the progenitor cells to differentiate into glial cells. Even transient Notch activation can initiate an irreversible switch from neurogenesis to gliogenesis. Thus, activation of Notch signaling does not necessarily always lead to a negative

\[3\text{ J. Shou, R. Soriano, P.M. Williams, and W-Q. Gao, unpublished observations.}\]
regulation, but rather acts as a positive differentiation factor. Similarly, during T-cell differentiation, Notch acts at two distinct checkpoints to promote differentiation of the αβ, but not γδ T-cell lineage and later to push cells into the CD4−CD8+ lineage rather than the CD4+CD8− lineage (45, 46). Therefore, activation of Notch signaling can force proliferative progenitor cells to exit the cell cycle to adapt a specific cell fate, which they would not normally do. It is possible that this is also the case in the prostate.

In summary, the present study demonstrates for the first time that Notch1 is expressed in developing prostatic epithelial cells and in human prostate cancer cells. There is up-regulation of Notch1 receptor in the malignant and metastatic prostate epithelial cells in TRAMP mice, whereas the expression of Notch ligands is low or undetectable. These results suggest that in the prostate tumor cells, Notch signaling is probably not physiologically activated or that its activation is minimal. Activation of Notch signaling by expression of a constitutively active form of can result in inhibition of the proliferation of prostate cancer cells. Further understanding the contribution of Notch signaling to prostate tumorigenesis could help us to identify additional therapeutic targets for the treatment of human prostate cancer.

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