Inactivation of *Apc* in the Mouse Prostate Causes Prostate Carcinoma

Katia J. Bruxvoort,[1] Holli M. Charbonneau,[1] Troy A. Giambernardi,[1] James C. Goolsby,[2] Chao-Nan Qian,[3] Cassandra R. Zylstra,[1] Daniel R. Robinson,[1] Pradip Roy-Burman,[4] Aubie K. Shaw,[5] Bree D. Buckner-Berghuis,[2] Robert E. Sigler,[2] James H. Resau,[2] Ruth Sullivan,[6] Wade Bushman,[5] and Bart O. Williams[1]

[1]Laboratory of Cell Signaling and Carcinogenesis, [2]Laboratory of Analytical, Cellular, and Molecular Microscopy, and [3]Laboratory of Cancer Genetics, Van Andel Research Institute, Grand Rapids, Michigan; [4]University of Southern California, Los Angeles, California; [5]Division of Urology and McArdle Laboratory for Cancer Research; and [6]University of Wisconsin Comprehensive Cancer Center, University of Wisconsin, Madison, Wisconsin

Abstract

Alterations of the Wnt/β-catenin signaling pathway are positively associated with the development and progression of human cancer, including carcinoma of the prostate. To determine the role of activated Wnt/β-catenin signaling in mouse prostate carcinogenesis, we created a mouse prostate tumor model using probasin-Cre–mediated deletion of *Apc*. Prostate tumors induced by the deletion of *Apc* have elevated levels of β-catenin protein and are highly proliferative. Tumor formation is fully penetrant and follows a consistent pattern of progression. Hyperplasia is observed as early as 4.5 weeks of age, and adenocarcinoma is observed by 7 months. Continued tumor growth usually necessitated sacrifice between 12 and 15 months of age. Despite the high proliferation rate, we have not observed metastasis of these tumors to the lymph nodes or other organs. Surgical castration of 6-week-old mice inhibited tumor formation, and castration of mice with more advanced tumors resulted in the partial regression of specific prostate glands. However, significant areas of carcinoma remained 2 months postcastration, suggesting that tumors induced by Apc loss of function are capable of growth under conditions of androgen depletion. We conclude that the prostate-specific deletion of *Apc* and the increased expression of β-catenin associated with prostate carcinoma suggests a role for β-catenin in prostate cancer and offers an appropriate animal model to investigate the interaction of Wnt signaling with other genetic and epigenetic signals in prostate carcinogenesis. [Cancer Res 2007;67(6):2490–6]

Introduction

Prostate cancer is the second most common type of cancer and the third leading cause of cancer-related death in U.S. men. The American Cancer Society estimates that there will be about 234,000 new cases and more than 27,000 deaths attributed to prostate cancer in 2006 (1). Although localized prostate cancer is amenable to cure by either surgery or radiation therapy, many men present with nonlocalized disease or develop recurrent, metastatic disease after attempts at curative therapy. Androgen deprivation therapy has been a mainstay of palliative therapy for advanced disease, but most men will develop androgen-independent tumor progression (2, 3). Accordingly, there is an urgent need to create animal models to study the signaling events involved in prostate cancer progression and identify potential targets for therapeutic intervention.

The etiology of prostate cancer is multifactorial, and multiple signaling pathways have been identified in prostatic neoplasia. Loss-of-function mutations in *phosphatase and tensin homologue*, which encodes a lipid phosphatase that functionally inhibits the phosphatidylinositol 3-kinase/Akt pathway, and the homeobox gene *NKX3.1* are common in advanced prostate cancer (4, 5). The effect of mutation of these genes on prostate growth regulation has been studied by creating transgenic loss-of-function models and examining the effect of these mutations on differentiation, the appearance of dysplasia, and the development of prostate cancer (6). Further, creation of mice carrying both mutations revealed cooperative effects in the development of prostate cancer and progression to invasive disease (7). As many as 25% of advanced human prostate cancers exhibit activating alterations or dysregulation of β-catenin, suggesting that this is also a common feature of advanced prostate cancer (8).

β-catenin plays multiple roles in regulating cell growth and function, and the cytoplasmic levels and nuclear localization of β-catenin are normally tightly regulated. Of particular importance, β-catenin, together with adenomatous polyposis coli (APC), plays a critical role in the Wnt signaling pathway. Wnts are glycoproteins that initiate signals by binding to a protein complex containing both a member of the Frizzled family of seven transmembrane receptors and a molecule of the low-density lipoprotein receptor family (either LRP5 or LRP6; refs. 9–11). This leads to down-regulation of glycogen synthase kinase-3 (GSK-3) activity. Normally, GSK-3, in complex with APC and axin, phosphorylates β-catenin, marking it for ubiquitin-dependent degradation. Inhibition of either GSK-3 or APC leads to increased levels of β-catenin in the cytosol and nucleus, allowing β-catenin to interact physically with the Tcf/Lef class of DNA binding proteins and leading to transcription of Wnt target genes.

Alterations in the Wnt signaling pathway are highly associated with human prostate cancer. Specifically, alterations of the *APC* gene have been identified in both primary and metastatic prostate cancers, including both somatic alterations (12, 13) and promoter hypermethylation (14, 15). Moreover, activating oncogenic mutations in

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

K.J. Bruxvoort, H.M. Charbonneau, and T.A. Giambernardi contributed equally to this work.

Requests for reprints: Bart O. Williams, Laboratory of Cell Signaling and Carcinogenesis, Van Andel Research Institute, 333 Bostwick Avenue Northeast, Grand Rapids, MI 49503-2518. Phone: 616-234-5308; Fax: 616-234-5309; E-mail: Bart.Williams@vai.org.

doi:10.1158/0008-5472.CAN-06-3028

the *β-catenin* gene have been shown to occur in prostate cancer (16–18) with >20% of advanced prostate tumors, 77% of prostatic lymph node metastases, and 85% of prostatic skeletal metastases having elevated levels of β-catenin (8, 19). It is unclear why dysregulation of β-catenin occurs so commonly in advanced skeletal disease but possible explanations include β-catenin–induced promiscuity of the androgen receptor (AR; refs. 20–22) and tumor cell mimicry of β-catenin–mediated activities seen during normal bone development (23, 24).

Previous studies have examined the effect of expressing activated β-catenin based on the mouse mammary tumor virus promoter in transgenic animals (25, 26). The transgenic males exhibited prostatic hyperplasia and squamous metaplasia but no tumor formation. Absence of tumor progression could reflect a level of β-catenin expression that was insufficient to induce malignant transformation but could also signify that increased β-catenin expression alone was not sufficient to induce tumor formation. To resolve this issue and to provide a robust and tractable mouse model of activated β-catenin signaling with which to study the role of β-catenin in prostate cancer, we created a conditional *Apc* mutant mouse.

Mice heterozygous for an inactive allele of *Apc* develop intestinal tumors similar to patients with familial adenomatous polyposis (FAP); however, no prostate-related pathology has been reported [e.g., benign prostatic hyperplasia (BPH), carcinoma, and prostatitis; ref. 27]. Homozygous mutant embryos are not viable past early stages of gestation (28–30). The *Apc* conditional mutant characterized here was created using probasin-driven Cre expression to achieve excision of the floxed *Apc* alleles in the luminal prostate epithelium. We show that inactivation of *Apc* leads to the rapid nuclear localization of β-catenin and a coordinated series of cellular changes leading to the emergence of androgen depletion–independent prostate cancer.

Materials and Methods

Overview of mouse crosses. The production of *PB-Cre4* transgenic mice and floxed *Apc* mice was described previously (31, 32). All animals were used in protocols that were reviewed and approved by the Van Andel Research Institute (VARI) Institutional Animal Care and Use Committee. *PB-Cre4* heterozygous male mice (*PB-Cre*$^+$; maintained on a mixed background of approximately equal parts of C57Bl/6J and 129Sv/J) were crossed with female mice homozygous for a floxed allele of *Apc* (*Apc*$^{580S/580S}$; abbreviated *Apc*$^{flox/flox}$) to generate *PB-Cre*$^+$;*Apc*$^{flox/+}$ mice. Because of previously observed levels of Cre activity in the female germline of *PB-Cre* mice, we restricted our next round of matings to crosses of male *PB-Cre*$^+$;*Apc*$^{flox/+}$ mice with *Apc*$^{flox/flox}$ females. These crosses generated litters in which approximately one fourth of the offspring were the *PB-Cre*$^+$;*Apc*$^{flox/flox}$ genotype. Males of *PB-Cre*$^+$;*Apc*$^{flox/flox}$ genotype were viable and fertile before 6 months of age and were therefore used in matings with *Apc*$^{flox/flox}$ females to generate litters, in which half of the offspring were the *PB-Cre*$^+$;*Apc*$^{flox/flox}$ genotype. Cohorts of animals from these crosses were the main source for our studies, with the males lacking the *Cre* transgene being used as controls.

Genotype analysis. DNA was prepared from tail biopsies using an AutoGenprep 960 automated DNA isolation system. PCR-based strategies were then used to genotype these mice (32, 33).

Laser capture microdissection and PCR verification. To confirm that *Apc* exon 14 had been deleted in the neoplastic prostate epithelium, laser capture microdissection (LCM) and PCR amplification were done as described (33). Three samples were analyzed: (*a*) laser-captured prostate neoplastic epithelium from a 3-month-old *PB-Cre*$^+$;*Apc*$^{flox/flox}$ mouse; (*b*) tail DNA from the same mouse; and (*c*) tail DNA from a *PB-Cre*$^-$;*Apc*$^{flox/flox}$ mouse.

Histology and immunohistochemistry. For β-catenin, Ki67, AR, and p63 immunohistochemistry, prostate tissues were fixed in 10% neutral buffered formalin for 24 h, embedded in paraffin, and sectioned at 5-μm thickness. H&E was used to stain specific sections. Immunohistochemical staining for β-catenin, Ki67, and AR was optimized using the Discovery XT System (Ventana, Tuscon, AZ) with the following antibodies: anti–β-catenin mouse monoclonal antibody (1:100; BD Biosciences PharMingen, San Jose, CA), rat anti-mouse Ki67 monoclonal antibody (TEC-3; 1:50; DAKO, Glostrup, Denmark), and AR (1:50; Cell Signaling, Danvers, MA). To detect p63 proteins, deparaffinized slides were subjected to antigen retrieval, hydrogen peroxide treatment, and incubation with anti-p63 mouse monoclonal antibody (1:500; BD Biosciences PharMingen). For immunohistochemical staining of APC, prostate tissues were fixed in 4% paraformaldehyde for 2 h, embedded in ornithine carbamyl transferase freezing compound (Tissue-Tek, Torrance, CA), and cut 5 μm thick. After

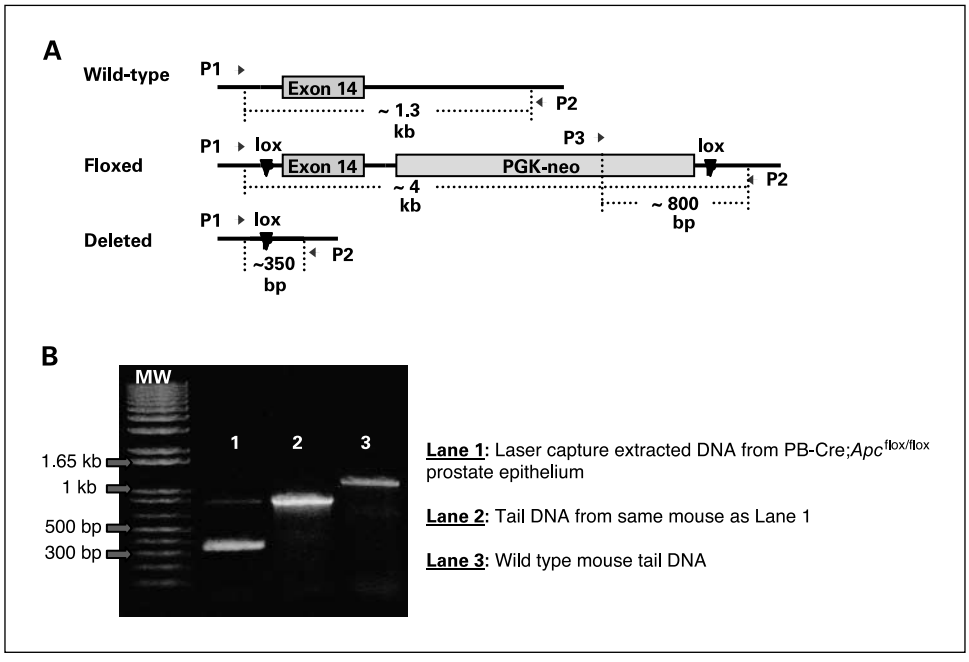

Figure 1. Elimination of *Apc* by Cre-lox recombination. *A,* schematic diagram of the PCR strategy used to confirm loss of *Apc* in the prostatic epithelium. Three primers (*P1, P2*, and *P3*) were designed to amplify exon 14 of the *Apc* gene. *B,* PCR analysis of DNA from prostatic epithelial cells of a 3-month-old *PB-Cre*$^+$;*Apc*$^{flox/flox}$ mouse isolated by laser capture microdissection confirmed the deletion of exon 14 of the *Apc* gene.

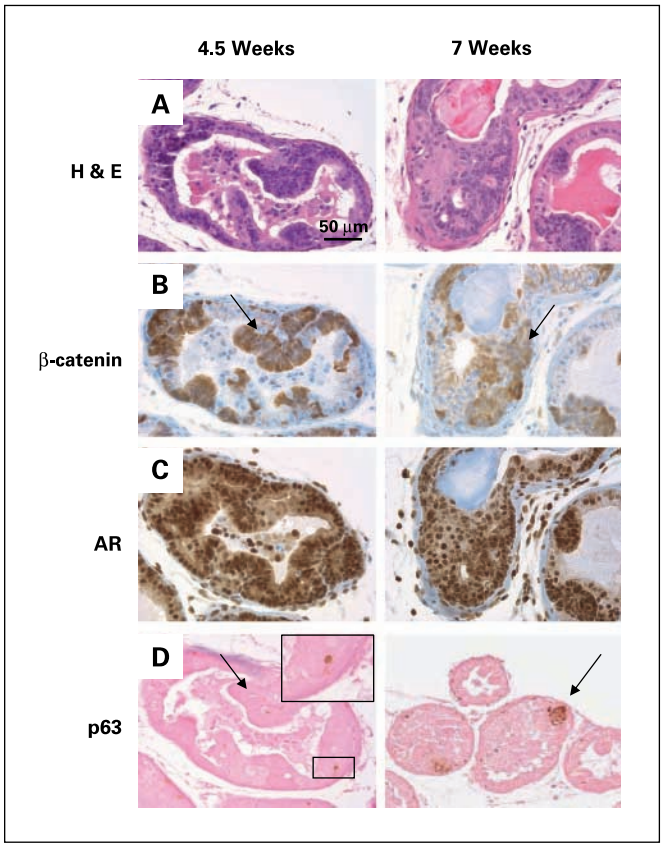

Figure 2. Regions of hyperplasia evident in 4.5- and 7-wk-old *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice. *A,* H&E of 4.5-wk-old (*left*) and 7-wk-old (*right*) *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate tissue. *B,* β-catenin immunohistochemistry of sections consecutive to those shown in (*A*). Note the regions of stabilized β-catenin (*right* and *left, arrows*). *C,* AR immunohistochemistry of sections from the same region shown in (*A* and *B*). *D,* p63 immunohistochemistry at 4.5 wks (*left*) and 7 wks (*right*) In (*D*), note the presence of the solitary p63-positive basal cell characteristic of staining seen in control tissues (*left, inset*) and the cluster of p63-positive cells in the hyperplastic region (*right, arrow*).

postfixation in 4% paraformaldehyde and quenching of endogenous peroxidases, sections were blocked in 10% goat serum and incubated for 1 h with rabbit anti-mouse APC polyclonal antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA). Visualization of p63 and APC proteins was accomplished with the Vectastain Elite avidin-biotin complex method kit (POD; Vector Laboratories, Burlingame, CA), 3,3′-diaminobenzidine, and hematoxylin counterstaining.

Surgical castration. Surgical castration was used to model androgen deprivation. A single longitudinal incision was made in the skin and body cavity rostral to the preputial gland. The epididymal fat pad, vas deferens, and testis were exteriorized, the testis was removed at the vas deferens by cauterization, and the epididymal fat pad was returned to the body cavity. The procedure was repeated on the contralateral side. The incision site in the body wall was sutured and the skin was wound clipped. Wound clips were removed 10 days following surgery. Postcastration mice showed no signs of infection and were sacrificed at designated time points.

Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay. Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) for determination and quantification of cell death/apoptosis was done according to the instructions for the *In situ* Cell Death Detection kit (POD; Roche, Basel, Switzerland).

Results

Several previously published studies have shown the penetrance and effectiveness of PB-Cre–mediated recombination (34, 35). To confirm the specificity and effectiveness of Cre-mediated deletion of Apc in *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ males, we examined the loss of Apc in prostate tumors via LCM followed by specific PCR to detect deletion of the floxed sequences. PCR analysis of 3-month-old *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice confirmed the deletion of exon 14 of the *Apc* gene (ApcΔ14) in the prostatic epithelial cells (Fig. 1). Deletion of this exon has been shown previously to inactivate *Apc* gene function in several tissues (31, 36).

To identify early phenotypic alterations in *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice, we sacrificed cohorts of animals for analysis at 4.5 weeks [mutant, $n = 7$; wild-type (WT), $n = 4$] and 7.0 weeks (mutant, $n = 7$; WT, $n = 6$). A histologic analysis of prostates from these animals showed that features consistent with prostatic epithelial hyperplasia and prostatic intraepithelial neoplasia (PIN) were evident as early as 4.5 to 7 weeks (Fig. 2*A*). Because inactivation of Apc results in increased levels of cytoplasmic β-catenin in numerous cell types, we carried out immunohistochemical analysis for β-catenin expression. Prostate tissue from *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice displayed increased staining for β-catenin specifically in regions of hyperplasia (Fig. 2*B*). However, immunohistochemical staining of consecutive sections for AR presented no difference between normal and hyperplastic regions at 4.5 and 7 weeks (Fig. 2*C*). At these time points, we also examined the expression of the basal cell marker, p63 (37, 38). In WT tissues, p63^{+} cells were found in the basal layer as expected. In hyperplastic regions of the *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate, we observed occasional pockets of p63^{+} cells that were not restricted to the basal layer; however, not all hyperplastic regions contained p63^{+} cells (Fig. 2*D*).

We then sacrificed cohorts of *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ animals ($n = 14$) and WT littermates ($n = 13$) at 7 months for detailed histologic analysis. Gross analysis of the prostate at necropsy revealed enlargement of all prostate lobes in the *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ males. We dissected prostate tissue, fixed it in 10% neutral buffered

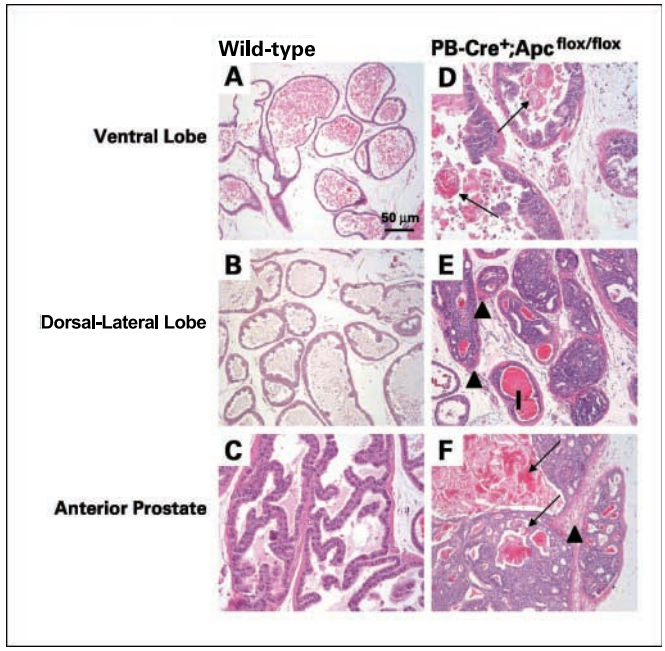

Figure 3. Prostate adenocarcinoma development in 7-mo-old *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice. *A* to *C,* ventral (*A*), dorsal-lateral (*B*), and anterior (*C*) lobe sections from WT mouse prostate. *D* to *F,* ventral (*D*), dorsal-lateral (*E*), and anterior (*F*) lobe sections from the prostate of a *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mouse. *Arrows,* areas of keratinization; *arrowheads,* areas of inflammation.

formalin, and prepared H&E-stained samples from paraffin-embedded sections for histologic analysis (Fig. 3). All lobes displayed neoplasia with hyperplasia multiple foci of squamous metaplasia and keratinization, and a prominent stromal reaction, including edema, inflammatory reaction, and stromal hyperplasia. The anterior prostate generally exhibited the most severe phenotype followed by the dorsal-lateral prostate and the ventral prostate. Small foci of tumor invasion into the stroma were identified (data not shown), but the invasive phenotype was not a prominent or common feature. These changes were fully penetrant, with these characteristics seen in all 14 *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ males. None of these changes were observed in an equivalent number of age-matched WT littermates.

Immunohistochemical analysis of prostate tissue at 7 months of age confirmed that regions of *Apc* deletion corresponded with both increased levels of β-catenin and regions of cellular proliferation (Fig. 4*A* and *B*). Prostate tissue from 7-month-old WT mice displayed β-catenin localization only at the membrane (Fig. 4*A, left*), consistent with the presence of β-catenin in adherens junctions. In addition, WT prostate tissue showed high levels of staining for Apc (Fig. 4*B, left*) but little to no staining for Ki67 (Fig. 4*C, left*). p63 staining was restricted to the basal layers of prostate tissues from control mice (Fig. 4*E, left*). In contrast, *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate tumors showed increased levels of β-catenin staining with localization in the cytoplasm and nucleus (Fig. 4*A, right*), decreased levels of Apc staining (Fig. 4*B, right*), and a high level of staining for the proliferation marker Ki67 (Fig. 4*C, right*). Levels of AR expression were generally maintained in hyperplastic tissue at 7 months of age (Fig. 4*D, right*); however, a decrease in AR staining intensity was noted in some regions of squamous metaplasia (data not shown). Areas of enhanced p63 staining could be detected within tumors derived from the *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice, with immunoreactivity particularly evident in regions of squamous metaplasia (Fig. 4*E, right*; data not shown).

An analysis of *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice between 12 and 15 months of age (n = 19) further showed the growth and diffuse involvement of adenocarcinoma. Tumor-reactive lymphadenopathy of the lumbar lymph nodes was observed in one third of these mice (39), and *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice over 12 months of age consistently developed hepatomas compared with 30% of controls (n = 10). However, PCR analysis of DNA extracted from these tissues did not reveal evidence for Cre-mediated deletion of the *Apc* allele in these samples (data not shown). Thus, we have not found evidence for metastasis of prostate tumors to lymph nodes or other organs. Additionally, five *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice at this age developed unidentified tumors in the scrotal region, not obviously associated with the testes. Tumors were 1 to 1.5 cm in diameter, cystic, and histologically consistent with epithelial origin (data not shown).

To determine whether prostate tumors arising in *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice were androgen depletion independent, we induced androgen deprivation by surgical castration. *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice castrated at 6 weeks and sacrificed 32 weeks later showed regions of hyperplasia, squamous metaplasia, and some areas of elevated, cytoplasmic β-catenin, but not carcinoma (Fig. 5, *middle*). Prostate tissue from castrated WT mice of the same age displayed minimal immunostaining for β-catenin along the cell membranes (Fig. 5, *left*), consistent with the normal phenotype (Fig. 5*A*). In contrast, prostates from *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice that were castrated after tumors were grossly developed and sacrificed 2 months postcastration still maintained significant regions of adenocarcinoma in all lobes (Fig. 5, *right*). β-Catenin staining was elevated and localized in the cytoplasm of many tumor cells (Fig. 5*B, right*), and Ki67 staining was likewise elevated in tumorous regions (Fig. 5*C, right*). Castration did not seem to affect the intensity of AR staining (Fig. 5*D*) or the presence of p63^{+} cells within the tumor tissue (Fig. 5*E*). TUNEL staining for cell death/apoptosis conducted on prostates from mice castrated at similar ages and sacrificed 4 days later showed apoptosis in some tumor cells, but most tumor cells were negative for TUNEL staining (Fig. 6*A, right*). In contrast, TUNEL staining of prostates from WT mice castrated and sacrificed at a similar age verified regression of all WT lobes, with the ventral lobe being the most apoptotic (Fig. 6*A, left*). Staining of adjacent sections showed that β-catenin was still stabilized in the cytoplasm of apoptotic tumor cells (Fig. 6*B*) and Ki67 staining was elevated (Fig. 6*C*) compared with the castrated WT controls.

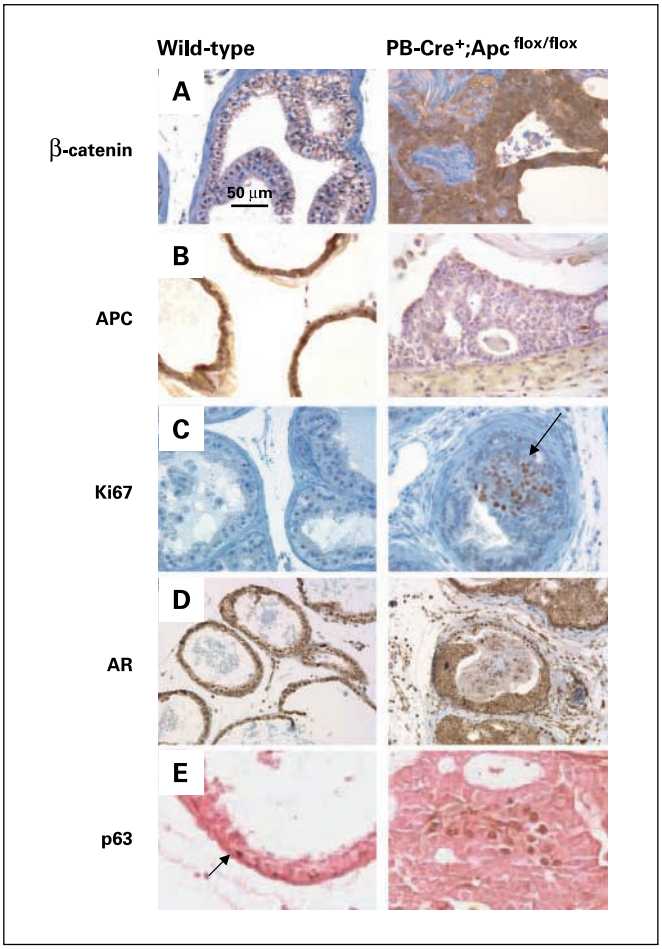

Figure 4. Cytoplasmic levels of β-catenin are increased, but levels of *APC* are decreased, in prostates of 7-mo-old *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice. *A, left,* β-catenin immunohistochemistry of WT prostate, showing the localization of β-catenin at the cell membrane consistent with its function in adherens junctions; *right,* β-catenin immunohistochemistry of *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate, showing widespread stabilization of β-catenin. *B,* Apc immunohistochemistry of WT prostate (*left*) and *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate (*right*). Note the lack of Apc immunoreactivity in the hyperplastic tissue. *C,* Ki67 immunohistochemistry of WT prostate (*left*) and *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate (*right*). Note pocket of Ki67 cells (arrow). *D,* AR immunohistochemistry of WT prostate (*left*) and *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate (*right*). *E, left,* p63 immunohistochemistry of WT prostate. Note basal cells positive for p63 (*arrow*). *Right,* p63 immunohistochemistry of *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate. Some tumors contain areas with pockets of cells stably expressing p63. Higher magnification *E*, *right*.

Discussion

In this study, we investigated the role of Wnt/β-catenin signaling in prostate cancer, using Cre-mediated recombination methods to create a conditional knockout of the *Apc* gene in prostate epithelial cells. We show that loss of Apc leads to the development of prostatic adenocarcinoma, positively correlated with increased cytoplasmic and nuclear levels of β-catenin.

These results are consistent with previous findings that aberrations in Wnt/β-catenin signaling leading to increased levels of β-catenin in the cytoplasm and nucleus are associated with tumor formation. The best-known example is human colon cancer, where >85% of colorectal cancers contain inactivating mutations in Apc, all of which have been associated with β-catenin stabilization in the cytoplasm (40). Mutations in β-catenin itself, which inhibit its phosphorylation by GSK-3, have also been found in colorectal cancer and other tumor types (41). The relevance of this model to human prostate cancer is supported by the observation that as many as 25% of advanced human prostate cancers are associated with activating alterations or dysregulation of β-catenin (19).

The mouse model of prostate cancer described here unequivocally evolved from a tissue-specific deletion of *Apc*. By 4.5 weeks of age, *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice harbored prostatic epithelial hyperplasia and morphologically distinct stages of PIN. By 7 months, adenocarcinoma displaying increased immunoreactivity for β-catenin and Ki67 was observed in all prostate lobes. By 15 months, all *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice had carcinoma and died or were sacrificed due to poor health, with no metastasis noted. The tumors exhibited multiple foci of squamous metaplasia. Squamous metaplasia is a feature atypical in human prostate cancer; however, squamous metaplasia has been described in prostate cancer as well as in high-grade PIN and BPH (42) and is a prominent feature of the estrogenized prostate (43). The appearance of squamous metaplasia in this model is likely to be a consequence of activated β-catenin signaling, as squamous metaplasia was also noted in previous mouse mammary tumor virus–based models of activated β-catenin expression (25, 26). Recent studies showing a functional connection between β-catenin signaling and estrogen receptor activity suggest a possible mechanism for this effect (44).

The development of squamous epithelia requires the basal cell marker p63. Mice lacking p63 undergo embryonic agenesis of epithelial organs (37). Additionally, p63 is expressed in prostate basal cells, but not in neuroendocrine or secretory cells, and was

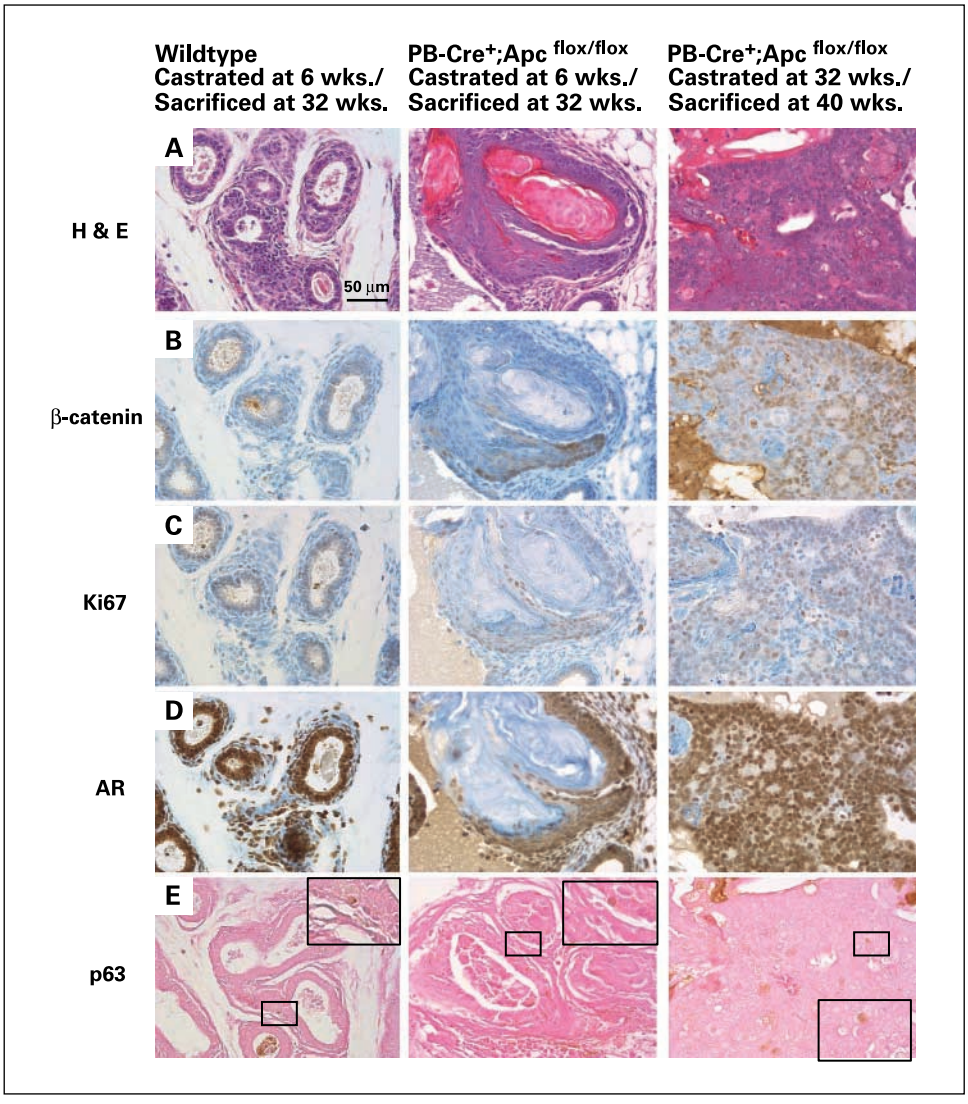

Figure 5. Androgen is necessary for tumor formation but is not necessary for tumor maintenance. *Left,* prostate tissue from WT mice castrated at 6 wks and sacrificed at 32 wks; *middle,* prostate tissue from *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice castrated at 6 wks and sacrificed 32 wks; *right,* prostate tissue from *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice castrated at 32 wks and sacrificed at 40 wks. *A,* H&E staining. *B,* β-catenin staining. *C,* Ki67 staining. *D,* AR staining. *E,* p63 staining. The regions from which higher power views in (*E*) are taken are indicated by the small boxes.

found to be absent in human prostate adenocarcinomas, which usually express markers for secretory cells (38). Importantly, the same laboratory has suggested that basal cells may include prostate stem cells. Our results showed that some clusters of *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ tumor cells expressed p63, especially in regions of squamous metaplasia, indicating possible variation in the cells of tumor origin. The majority of tumor cells did not express p63 but did express AR, a putative marker of transit-amplifying or differentiated secretory cells. These findings suggest that hyperplasia and tumor development in this model involves proliferation and transformation of cells with progenitor or transit-amplifying cell phenotypes and may explain the androgen-independent phenotype of established tumors.

PB-Cre^{+};*Apc*$^{flox/flox}$ mice display prostate cancer under conditions of androgen depletion if androgen ablation occurred once tumors were established. We found that some *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice castrated at 6 weeks of age developed hyperplasia, but not carcinoma, as examined 6 months postcastration. However, mice that were castrated after advanced tumors had developed still had prostatic adenocarcinoma 2 months postcastration. Taken together, these results suggest that androgen may be necessary for tumor formation in *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice but not for tumor maintenance and progression.

Prostate cells normally require androgens, primarily dihydrotestosterone, to grow and survive. Androgens both stimulate proliferation and inhibit apoptosis through binding to the AR and initiating transcription of androgen-responsive genes. Various mechanisms have been identified by which androgen-independent prostate cancer develops (45), including aberrant activation of AR by non-androgen steroids and androgen antagonists. Although selection for gain-of-function mutations in the ligand-binding domain of the AR could result in AR promiscuity, alterations in coregulatory molecules could also be responsible. Interestingly, β-catenin has been reported to be an AR transcriptional coactivator in transient transfections using reporter genes (20, 21, 46). High-level expression of AR has also been found to suppress activation of transcription by Tcfs, suggesting that AR and Tcf transcription factors compete for β-catenin binding (22). Alternatively, androgen depletion–independent growth in *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice could be occurring by growth factor activation of AR or via an alternative pathway that bypasses the AR. However, we found that AR levels were maintained in WT and *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ prostate tissue, and castration did not affect AR expression. Future studies in this model will further explore the role of β-catenin in androgen-independent growth.

Many cancers require accumulated genetic changes or "multiple hits." This is well shown by colorectal cancer, which may require multiple genetic events to develop. Patients with FAP, which is caused by a heterozygous germ-line mutation in APC, develop hundreds to thousands of benign colorectal polyps. Loss of the remaining functional allele is required for polyp formation. Subsequent mutations in other genes, such as *K-ras* and *p53*, result in tumor progression and metastasis (47, 48). A similar multiplicity of genetic and epigenetic changes are likely to play a role in the genesis and progression of prostate cancer. A variety of transgenic mouse models are being created to examine the effects of genetic disruption in specific pathways, and cross-breeding experiments are revealing synergies in tumorigenesis and metastasis. The *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice provide a needed model of Wnt/β-catenin dysregulation to be included in these studies. In addition, the distinctive features of p63^{+} cell

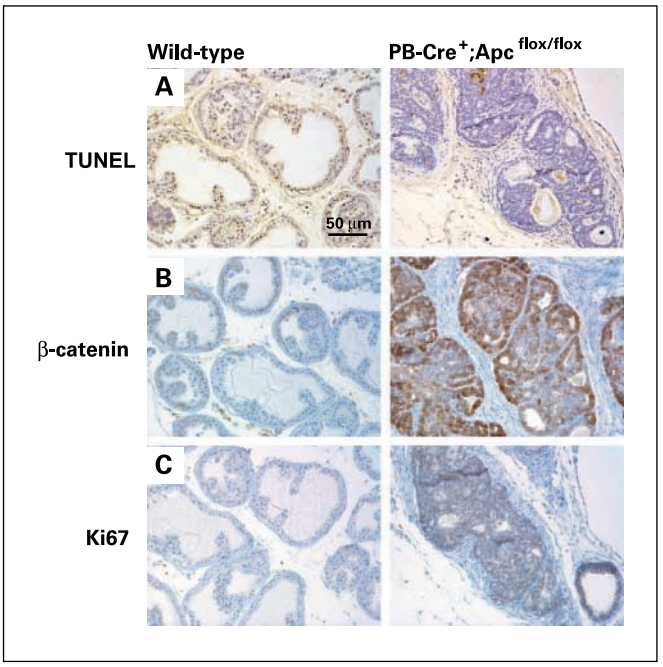

Figure 6. Androgen ablation does not lead to apoptosis of all tumor cells in *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mice. *A* to *C, left,* prostate tissue from a WT adult mouse castrated and sacrificed 4 d later; *right,* prostate tissue from a *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ mouse castrated and sacrificed 4 d later. TUNEL staining for cell death/apoptosis (*A*). Note the extensive staining in the WT tissue (*left*) compared with the *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ tumor (*right*). β-catenin immunohistochemistry (*B*). Ki67 immunohistochemistry (*C*). Both β-catenin and Ki67 staining are elevated in the *PB-Cre*$^{+}$;*Apc*$^{flox/flox}$ samples.

hyperplasia, epithelial metaplasia, and robust growth after androgen depletion make this a particularly interesting model. Whereas some tumor cells expressed p63 and we observed pockets of p63^{+} cells, most tumor cells did not express p63 but did express AR, a marker of transit-amplifying and differentiated secretory cells. There is controversy on the issue of whether basal cells are progenitors of the secretory luminal cells or they represent a terminally differentiated phenotype. Potentially, our model could also be explored to derive insight into this important problem as it pertains to tumorigenesis. Finally, the observations presented here suggest that dysregulation of Wnt/β-catenin signaling in human prostate cancer may be an important mechanism affecting epithelial differentiation, tumor grade, and responsiveness to androgen deprivation therapy.

Acknowledgments

Received 8/15/2006; revised 12/15/2006; accepted 1/10/2007.

Grant support: Michigan Economic Development Corporation and the Michigan Technology Tri-Corridor (Michigan Animals Models Consortium grant 085P1000815). Van Andel Research Institute and a Young Investigator Award through the Department of Defense Prostate Cancer Research Program grant DAMD17-03-1-0031 (B.O. Williams). NIH grant NIH RO1CA59705 (P. Roy-Burman). T.A. Giambernardi was an American Cancer Society (Cathy Tartikoff-Fifer) postdoctoral fellow during this study.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Tetsuo Noda for the *Apc-flox* embryonic stem (ES) cells and Pam Swiatek and the VARI Mouse Germline Modification Core for generating mice from these ES cells; David Nadziejka and Rick Hay for critically reading the manuscript; and Bryn Eagleson, Sylvia Marinelli, and the VARI vivarium staff for expert animal husbandry.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106–30.
2. Sharifi N, Gulley JL, Dahut WL. Androgen deprivation therapy for prostate cancer. JAMA 2005;294:238–44.
3. Daskivich TJ, Oh WK. Recent progress in hormonal therapy for advanced prostate cancer. Curr Opin Urol 2006;16:173–8.
4. Dong JT. Prevalent mutations in prostate cancer. J Cell Biochem 2006;97:433–47.
5. Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. Genes Dev 2000;14:2410–34.
6. Abate-Shen C, Shen MM. Mouse models of prostate carcinogenesis. Trends Genet 2002;18:S1–5.
7. Abate-Shen C, Banach-Petrosky WA, Sun X, et al. Nkx3.1; Pten mutant mice develop invasive prostate adenocarcinoma and lymph node metastases. Cancer Res 2003;63:3886–90.
8. Yardy GW, Brewster SF. Wnt signalling and prostate cancer. Prostate Cancer Prostatic Dis 2005;8:119–26.
9. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20:781–810.
10. Polakis P. Wnt signaling and cancer. Genes Dev 2000; 14:1837–51.
11. Seto ES, Bellen HJ. The ins and outs of Wingless signaling. Trends Cell Biol 2004;14:45–53.
12. Brewster SF, Browne S, Brown KW. Somatic allelic loss at the DCC APC, nm23-1 and p53 tumor suppressor gene loci in human prostatic carcinoma. J Urol 1994;151: 1073–7.
13. Phillips SM, Morton DG, Lee SJ, Wallace DM, Neoptolemos JP. Loss of heterozygosity of the retinoblastoma and adenomatous polyposis susceptibility gene loci and in chromosomes 10p, 10q, and 16q in human prostate cancer. Br J Urol 1994;73:390–5.
14. Jeronimo C, Henrique R, Hoque MO, et al. A quantitative promoter methylation profile of prostate cancer. Clin Cancer Res 2004;10:8472–8.
15. Yegnasubramanian S, Kowalski J, Gonzalgo ML, et al. Hypermethylation of CpG islands in primary and metastatic human prostate cancer. Cancer Res 2004;64:1975–86.
16. Chesire DR, Ewing CM, Sauvageot J, Bova GS, Isaacs WB. Detection and analysis of β-catenin mutations in prostate cancer. Prostate 2000;45:323–34.
17. Voeller HJ, Truica CI, Gelmann EP. β-Catenin mutations in human prostate cancer. Cancer Res 1998; 58:2520–3.
18. Gerstein AV, Almeida TA, Zhao G, et al. APC/CTNNB1 (β-catenin) pathway alterations in human prostate cancers. Genes Chromosomes Cancer 2002;34:9–16.
19. Chen G, Shukeir N, Potti A, et al. Up-regulation of Wnt-1 and β-catenin production in patients with advanced metastatic prostate carcinoma: potential pathogenetic and prognostic implications. Cancer 2004;101:1345–56.
20. Truica CI, Byers S, Gelmann EP. β-Catenin affects androgen receptor transcriptional activity and ligand specificity. Cancer Res 2000;60:4709–13.
21. Willert K, Jones KA. Wnt signaling: is the party in the nucleus? Genes Dev 2006;20:1394–404.
22. Masiello D, Chen SY, Xu Y, et al. Recruitment of β-catenin by wild-type or mutant androgen receptors correlates with ligand-stimulated growth of prostate cancer cells. Mol Endocrinol 2004;18:2388–401.
23. Glass DA II, Karsenty G. Molecular bases of the regulation of bone remodeling by the canonical Wnt signaling pathway. Curr Top Dev Biol 2006;73:43–84.
24. Holmen SL, Zylstra CR, Mukherjee A, et al. Essential role of β-catenin in postnatal bone acquisition. J Biol Chem 2005;280:21162–8.
25. Bierie B, Nozawa M, Renou JP, et al. Activation of β-catenin in prostate epithelium induces hyperplasias and squamous transdifferentiation. Oncogene 2003;22: 3875–87.
26. Gounari F, Signoretti S, Bronson R, et al. Stabilization of β-catenin induces lesions reminiscent of prostatic intraepithelial neoplasia, but terminal squamous transdifferentiation of other secretory epithelia. Oncogene 2002;21:4099–107.
27. Shoemaker AR, Gould KA, Luongo C, Moser AR, Dove WF. Studies of neoplasia in the Min mouse. Biochim Biophys Acta 1997;1332:F25–48.
28. Luongo C, Moser AR, Gledhill S, Dove WF. Loss of Apc^{+} in intestinal adenomas from Min mice. Cancer Res 1994;54:5947–52.
29. Moser AR, Shoemaker AR, Connelly CS, et al. Homozygosity for the Min allele of Apc results in disruption of mouse development prior to gastrulation. Dev Dyn 1995;203:422–33.
30. Oshima M, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M. Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene. Proc Natl Acad Sci U S A 1995;92:4482–6.
31. Shibata H, Toyama K, Shioya H, et al. Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. Science 1997;278:120–3.
32. Wu X, Wu J, Huang J, et al. Generation of a prostate epithelial cell-specific Cre transgenic mouse model for tissue-specific gene ablation. Mech Dev 2001;101:61–9.
33. Qian CN, Knol J, Igarashi P, et al. Cystic renal neoplasia following conditional inactivation of apc in mouse renal tubular epithelium. J Biol Chem 2005;280: 3938–45.
34. Powell WC, Cardiff RD, Cohen MB, Miller GJ, Roy-Burman P. Mouse strains for prostate tumorigenesis based on genes altered in human prostate cancer. Curr Drug Targets 2003;4:263–79.
35. Wang S, Gao J, Lei Q, et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 2003;4:209–21.
36. Hasegawa S, Sato T, Akazawa H, et al. Apoptosis in neural crest cells by functional loss of APC tumor suppressor gene. Proc Natl Acad Sci U S A 2002;99: 297–302.
37. Yang A, Schweitzer R, Sun D, et al. p63 is essential for regenerative proliferation in limb, craniofacial, and epithelial development. Nature 1999;398:714–8.
38. Signoretti S, Waltregny D, Dilks J, et al. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 2000;157:1769–75.
39. Qian CN, Berghuis B, Tsarfaty G, et al. Preparing the "soil": the primary tumor induces vasculature reorganization in the sentinel lymph node before the arrival of metastatic cancer cells. Cancer Res 2006;66: 10365–76.
40. Powell SM, Petersen GM, Krush AJ, et al. Molecular diagnosis of familial adenomatous polyposis. N Engl J Med 1993;329:1982–7.
41. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and β-catenin signalling: diseases and therapies. Nat Rev Genet 2004;5:691–701.
42. Melissari M, Lopez Beltran A, Mazzucchelli R, Froio E, Bostwick DG, Montironi R. High grade prostatic intraepithelial neoplasia with squamous differentiation. J Clin Pathol 2006;59:437–9.
43. Risbridger GP, Wang H, Frydenberg M, Cunha G. The metaplastic effects of estrogen on mouse prostate epithelium: proliferation of cells with basal cell phenotype. Endocrinology 2001;142:2443–50.
44. Hou X, Tan Y, Li M, Dey SK, Das SK. Canonical Wnt signaling is critical to estrogen-mediated uterine growth. Mol Endocrinol 2004;18:3035–49.
45. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001;1:34–45.
46. Terry S, Yang X, Chen MW, Vacherot F, Buttyan R. Multifaceted interaction between the androgen and Wnt signaling pathways and the implication for prostate cancer. J Cell Biochem 2006;99:402–10.
47. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
48. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159–70.