Prostate Stem Cells and Prostate Cancer

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Understanding prostate stem cells (PSCs) may provide insight for the design of therapeutics for prostate cancer. We have developed a quantitative in vivo colony-forming assay and have demonstrated that the Sca-1 antigen is present on the surface of a prostate cell subpopulation that possesses multiple stem cell properties. Immunofluorescence analysis demonstrates that Sca-1 is expressed by both radial and luminal cells in the proximal region of the adult prostate, but is not expressed by either lineage in more distal regions. The proximal region has been suggested as the PSC niche based on BrdU label-retention analyses and the presence of distinct smooth-muscle cells that produce high levels of TGF-β. Sca-1 is also expressed by nearly all cells within fetal prostate epithelial cords, suggesting Sca-1 may be conserved on PSCs throughout development. Malignant epithelial cells from TRAMP mice, as well as normal prostate cells with lentiviral-mediated alteration of the PTEN/AKT signaling pathway, give rise to PIN lesions and prostate cancer in vivo. Alteration of PTEN/AKT signaling in Sca-1-unmarked PSCs also results in PIN lesions, suggesting that PSCs can serve as one target for prostate carcinogenesis.

Stem cells are defined by their capacity for self-renewal, multilineage differentiation, and replication quiescence. Pluripotent embryonic stem cells possess the most plasticity and can give rise to all tissues of an organism. Demonstration of stem cells in adult tissues was first realized in experiments in which lethally irradiated mice were rescued by transfer of bone marrow from donor mice. The existence of hematopoietic stem cells (HSCs) was later confirmed in limiting-dilution experiments demonstrating that small numbers of bone marrow cells could give rise to clonal colonies in the spleens of lethally irradiated hosts (for review, see Shizuru et al. 2005). Adult stem cells have since been identified in tissue systems from all three embryonic germ layers, including the lung, intestine, brain, breast, skin, and prostate. Debate still ensues over the existence of adult stem cells in some organs such as the pancreas, in which mature insulin-producing β islet cells rather than stem cells may divide to replenish the islet β-cell compartment (Dor et al. 2004).

Mutation of somatic stem cells may contribute to many types of human pathology such as cancer. Stem cells may be particularly vulnerable to malignant transformation because of their unique capacity for long-term self-renewal and multilineage differentiation. Many tumor types have been shown to be hierarchical with respect to differentiation status, where only a discrete subpopulation of “cancer stem cells” within the tumor are capable of initiating and sustaining tumor growth (for review, see Huntly and Gilliland 2005).

EXPERIMENTAL PROCEDURES

In vivo prostate regeneration. For development of the in vivo prostate colony-forming (CFU) assay, dissociated prostate cells were prepared from 6- to 10-week-old C57BL/6 and β-actin GFP transgenic mice as described previously and mixed in the ratios listed (Xin et al. 2003). β-Actin GFP transgenic mice were purchased from the Jackson Laboratory (C57BL/6-TgN[ACTbEGFP]1Osb). Each sample was resuspended in collagen, incubated overnight, and implanted under the kidney capsule of SCID mice as described previously (Xin et al. 2003).

For evaluation of the regenerative activity of Sca-1+ and Sca-1− prostate cells, cells were dissociated from β-actin GFP mice and were sorted into Sca-1+ and Sca-1− fractions using magnetic bead sorting, as described previously (Xin et al. 2005). Sca-1+ prostate cells were prepared from 6- to 10-week-old β-actin GFP and TRAMP mice. 1 x 105 cells from each source were mixed and implanted under the kidney capsule. Tumorigenic activity was measured by fluorescence microscopy and histological analysis.

Immunohistochemistry and Immunofluorescence. Frozen sections were prepared from adult and fetal embryonic day (E)16–17 tissue and stained with rat monoclonal anti-Sca-1 (1:250, Covance, or rabbit monoclonal anti-Ck5 (1:1000, Covance). Sca-1 stained sections were subsequently incubated with a biotin-conjugated rabbit anti-rabbit antibody (1:200, DakoCytomation), and incubated with FITC-conjugated streptavidin (1:200, Jackson
IDENTIFICATION OF PROSTATE STEM CELLS

The concept of an adult prostatic stem cell (PSC) first emerged to explain the profound capacity of the organ for tissue regeneration. Androgen cycling experiments demonstrating that the rodent prostate can undergo more than 30 cycles of involution and regeneration revealed the presence of cells possessing self-renewal and multipotentiality (Isaacs 1987). Hoechst staining and FACS analysis of dissociated human prostate cells has further shown that stem-like cells more efficient at dye efflux, called side population cells, are present in the prostate (Bhatt et al. 2003). Extension of this operational definition of PSCs is essential to gain an in-depth understanding of PSC function. Recent efforts have therefore focused on the development of in vivo stem cell assays for isolating PSCs for functional analysis.

Some of the best assays for comparing stem cell enrichment strategies have been developed for the hematopoietic and central nervous systems. The spleen colony-forming assay (CFU-S) developed by Till and McCulloch in 1961 was used to first identify the antigenic profile of murine HSCs (Spangrude et al. 1988). In this assay, nodules appear within the intravascular transfer of bone marrow cells into lethally irradiated mice. The number of nodules can be counted in limit-dilution experiments to quantify the number of progenitor cells transferred (Wu et al. 1967). In the competitive repopulation (CRU) assay, HSC number is measured by coinjecting a decreasing number of fractionated cells with differentially marked unfractionated “helper” cells in order to count the number of recipients with donor-derived repopulation by the fractionated population (Harries et al. 1980). Neural stem cell (NSC) number can be assessed using an in vitro colony assay in which NSC colonies are enriched for neural stem cells. The prostate tissue fragment recombination procedure developed by Cunha and Lung (1978) is an excellent system for the study of prostate development and epithelial-mesenchymal interactions. This system uses tissue fragments dissected from rat or mouse urogenital sinus mesenchyme (UGSM) in combination with adult epithelial tissue to regenerate prostate-like tissues when implanted under the kidney capsule of immunodeficient mice. To define PSCs using the cell-fractionation approaches utilized for HSC and NSC enrichment, we modified this system to utilize dissociated cell populations of adult mouse epithelial cells in combination with E16 UGSM cells (Xin et al. 2003). Tissue regenerated using this method possesses a normal prostate marker profile and most closely resembles the anterior and ventral lobes of the murine prostate.

Previously, we modified the prostate regeneration system for use as a CFU assay to identify murine PSC markers. Analogous to the CFU-S and CRU assays used in studies of HSC enrichment, serial dilution experiments were performed using decreasing numbers of GFP+ prostate cells from β-actin GFP mice in combination with a constant number of cells from wild-type C57BL/6 mice. The lack of chimeric tubules containing both GFP+ and GFP- cells in prostate tissue regenerated from these mixed populations suggests that each tubule is clonal and derived from the outgrowth of a single cell (Fig. 2). Because each tubule appears to be clonal, GFP+ tubules can be counted as in the CFU-S assay to quantify the number of cells contributing to graft formation. This analysis suggests that, on average, about 1 in 2500 prostate cells possesses stem cell activity, which is significantly lower than previous in vitro reports of 1–4% for both human and rodent prostate cells (Hudson et al. 2000; Sawicki and Rothman 2002). This may be because in vitro and in vivo techniques measure different cell entities. For example, both stem cells and progenitors may grow in vitro, whereas whole tubule structures may only be regenerated from true stem cells in vivo. Alternatively, the seeding efficiency of PSCs may be lower in vivo than in vitro. Perhaps more stringent niche requirements are necessary for tubule growth in vivo than for colony growth in vitro.

Figure 1. Schematic representation of dissociated cell prostate regeneration system. Cells dissociated from adult murine prostate tissue are mixed with dissociated UGSM cells from E16 fetuses and implanted under the kidney capsule of immunodeficient mice for the regeneration of prostatic tissues.

<table>
<thead>
<tr>
<th>Fetal urogenital sinus</th>
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<tr>
<td>Adult urogenital system</td>
<td>Prostate</td>
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<tr>
<td>Enzymatic digestion</td>
<td>Remove UGSM</td>
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Dissection

Implantation

8-week incubation

Subcapsular

Prostate regeneration

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(Reynolds et al. 1992). This assay was used by Uchida et al. (2000) to demonstrate that the CD133+ CD24–/lo population of cells is enriched for neural stem cells.

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as a marker for the enrichment of hematopoietic, mammary, and lung stem cells (Spangrude et al. 1988; Welm et al. 2002; Kim et al. 2005). We previously used the dissociated prostate CFU assay to show that Sca-1 can also be used to enrich for murine prostate stem cells (Fig. 3) (Xin et al. 2005). Because each regenerated tubule is thought to be clonal in origin, the presence of both basal and luminal cells in each tubule suggests that Sca-1+ cells are at least bipotential. Using similar techniques, another group further demonstrated that the Sca-1 hi population contains more regenerative activity in vivo than either the Sca-1 lo or Sca-1 – population (Burger et al. 2005). Castration, which results in an enrichment for PSCs and progenitor cells, causes a concomitant increase in the percentage of Sca-1+ cells, and Sca-1 + cell fractions have been shown to contain increased percentages of replication-quiescent cells that localize to the proximal region of the murine prostate nearest to the urethra (Burger et al.

Short-term in vitro cell culture assays have been used to monitor PSCs and progenitor cells in multiple studies. Collins and colleagues (2001) demonstrated that CD44+ human prostate basal cells can be fractionated into integrin α2β1 high- and low-expressing populations based on differential adherence to type I collagen in vitro. Rapidly adherent cells possess 3.8-fold greater colony-forming efficiency (CFE) in vitro and can develop into phenotypically normal glands when grafted onto the flanks of athymic mice. Later studies by the same group found that subdivision of the α2β1 hi basal cell compartment using CD133, an NSC and HSC marker, results in an 11-fold increase in CFE in vitro (Richardson et al. 2004). CD133+ α2β1 hi cells can also give rise to glandular tissue in vivo, but the efficiency of tubule regeneration following this enrichment is unclear.

Stem cell antigen 1 (Sca-1), a glycosylphosphatidylinositol (GPI)-linked cell surface protein, has been used as a marker for the enrichment of hematopoietic, mammary, and lung stem cells (Spangrude et al. 1988; Welm et al. 2002; Kim et al. 2005). We previously used the dissociated prostate CFU assay to show that Sca-1 can also be used to enrich for murine prostate stem cells (Fig. 3) (Xin et al. 2005). Because each regenerated tubule is thought to be clonal in origin, the presence of both basal and luminal cells in each tubule suggests that Sca-1+ cells are at least bipotential. Using similar techniques, another group further demonstrated that the Sca-1 hi population contains more regenerative activity in vivo than either the Sca-1 lo or Sca-1 – population (Burger et al. 2005). Castration, which results in an enrichment for PSCs and progenitor cells, causes a concomitant increase in the percentage of Sca-1+ cells, and Sca-1+ cell fractions have been shown to contain increased percentages of replication-quiescent cells that localize to the proximal region of the murine prostate nearest to the urethra (Burger et al.

Figure 2. Development of in vivo CFU assay. Decreasing numbers of dissociated prostate cells from β-actin GFP mice are combined with a constant number (1 × 10⁵) of wild-type prostate cells and implanted under the kidney capsule for 8 weeks. Transillumination (TI) and fluorescent (GFP) images of regenerated tissues and tissue sections.

Figure 3. Cells dissociated from β-actin GFP mouse prostate tissue were separated into Sca-1+ and Sca-1– fractions using magnetic beads. 1 × 10⁵ cells from each fraction were combined with wild-type prostate cells and incubated under the kidney capsule. Transillumination (TI) and fluorescent (GFP) images of grafts and tissue sections are shown. Fluorescent signal was quantified by CCD camera (CCD). (Reprinted, with permission, from Xin et al. 2005 [© National Academy of Sciences].)
The careful balance of quiescence, self-renewal, and differentiation of somatic stem cells is maintained by specific interactions with supportive cells and extracellular materials that comprise their specialized microenvironment, or niche (Fuchs et al. 2004). Intra- and intercellular signaling of stem cells with neighboring cells in the niche is thought to occur through three main pathways: Wnt, Sonic hedgehog (Shh), and Notch. For example, inhibition of Wnt receptors by the trangenic expression of Dickkopf-1, a secreted antagonist of Wnt signaling, results in a complete loss of intestinal epithelial stem cell niches called crypts (Kuehnert et al. 2004). Ablation of Notch-1 in transgenic mice abrogates PSC function, resulting in the inhibition of cell growth, differentiation, and branching morphogenesis of the prostate in vitro as well as in vivo following androgen replacement after castration (Wang et al. 2004). Shh signaling is also an important component of fetal prostate development. Shh expression at epithelial evaginations of the urogenital sinus epithelium activates the transcription factor Gli-1 in the mesenchyme to promote early ductal morphogenesis. Shh expression then becomes strongest in advancing distal regions of budding tubules until expression stabilizes in the adult organ (Lamm et al. 2002; Freestone et al. 2003).

PSCs are traditionally thought to reside in the basal cell layer of the prostate, where they divide to give rise to highly proliferative transit-amplifying cells (TACs) that in turn differentiate to produce neuroendocrine cells and terminal, secretory luminal cells (Bosnich and Remmberger 1996). Several lines of evidence support this theory. The prostate regenerates survi-vability of basal cells and loss of terminal, androgen-dependent cells following androgen deprivation suggests that the self-renewal and proliferative activities that confer tissue regeneration following androgen replacement are most likely possessed by basal cells (English et al. 1987). Cell kinetic studies demonstrating that 70% of proliferating human epithelial cells localize to the basal compartment suggest this compartment may house both the stem and transit-amplifying elements (Bosnich et al. 1994). Human basal cells also uniformly express bcl-2, a protein frequently found up-regulated in many types of stem cells, which is known to protect cells from apoptosis (Verhagen et al. 1992). The discovery of subsets of human cells with basal/luminal and basal/neuroendocrine lineages (Verhagen et al. 1992). The most striking evidence, however, supporting the concept that PSCs reside in the basal cell compartment is the discovery that mice with a homozygous knockout of p63, a prostate basal cell marker, are born without limbs or epithelial organs such as the mammary and prostate glands (Mills et al. 1999; Yang et al. 1999; Signoretti et al. 2000).

An intriguing modification of this model was recently proposed by Tsujimura et al. (2002). Using BrdU labeling to identify slow-cycling cells, this group found that approximately 25% of both basal and luminal cells in the proximal region of the murine prostate nearest to the urethra retain the label following repeated rounds of androgen cycling. However, the majority of label-retaining cells in the intermediate and distal regions of the prostatic ducts were of the basal phenotype (Tsujimura et al. 2002). These data suggest that although slow-cycling stem-like cells are not confined to the basal cell lineage in the proximal region, only basal cells function like stem cells in the distal regions of prostatic tubules. This group also found that rapidly cycling TACs were predominantly localized in the distal and intermediate ductal regions where the fastest cycling cells displayed a luminal phenotype. This is consistent with prior findings that actively proliferating cells are located at the tips of prostatic ducts (Yagimura et al. 1986).

Murine prostate stem cell populations identified by functional analysis in in vivo regeneration assays also appear to localize to the proximal region of the murine prostate. Both groups identifying Sca-1 as a murine PSC marker demonstrated that the proximal region contains at least a 3- to 4-fold higher percentage of Sca-1+ cells than more distal regions of the prostate (Burger et al. 2005; Xin et al. 2005). In an attempt to resolve some of the debate regarding the location of PSCs, we investigated the relationship of basal, luminal, and Sca-1+ cells in the proximal region. Immunofluorescent analysis of serial sections of murine prostate tissue demonstrates that Sca-1+ cells colocalize with both basal (CK5) and luminal (CK8) cell markers in the proximal region of prostatic ducts (Fig. 4). The basement membrane and/or some of the stromal cells in the region may also stain positive for Sca-1. However, neither basal, luminal, nor stromal cells in the distal tips of prostatic tubules express the Sca-1 antigen.

Sca-1 has been used to enrich for both fetal and adult murine HSCs and therefore may be conserved on HSCs during development (Ding et al. 2005). To determine whether Sca-1 is also present on both fetal and adult PSCs, we harvested urogenital sinus tissue from E16 fetuses. At E16–17, solid epithelial chords begin to bud into the fetal mesenchyme where they later canalize in a proximal to distal manner (Hayward et al. 1996). Immunofluorescent analysis indicates that Sca-1 is expressed by nearly all cells within developing epithelial chords (Fig. 5). Sca-1 expression also appears to overlap with CK5 and CK8 expression at this developmental stage. These data suggest that Sca-1 expression may be conserved on the surface of fetal and adult prostate stem and progenitor cells throughout prostate development.

Many hypotheses can be proposed to explain the fact that subsets of Sca-1+ cells possess basal and/or luminal markers. It is possible that true PSCs are not confined to the basal cell lineage. This would explain the label retention and Sca-1 expression of both basal and luminal cells in the proximal region of the prostate. Recent work by Kurita et al. (2004) supports this model. Using tissue reconstitute strategies, this group demonstrated that lu-
minal and neuroendocrine cells, but not basal cells, can develop from urogenital sinus tissue of p63–/– fetuses. Although the ductal morphology in regenerated tissue was not completely normal, this indicates that luminal cells may arise by pathways other than basal cell differentiation.

An alternative hypothesis is that PSCs may lie specifically in the basal cell layer of the proximal murine prostate. On the basis of their work, Tsujimura et al. (2002) proposed a model in which prostate stem cells in the proximal region give rise to early TACs that migrate distally where they contribute to the periodic replenishment of mature secretory cells along the tubule axis. In this model, perhaps Sca-1+ basal stem cells in the proximal region differentiate to give rise to early TACs possessing a more luminal-like morphology that retain the Sca-1 marker (Tsujimura et al. 2002). This would explain findings that the Sca-1+ prostate cell subset contains both quiescent and actively dividing cells, and the fact that Sca-1 is expressed on a larger population (15%) of prostate cells than could be true stem cells (Xin et al. 2005). Furthermore, the small numbers of Sca-1+ cells in more distal regions previously identified by fluorescence-activated cell sorting (FACS) analysis may constitute the micro-niches of slow-cycling stem-like cells and early TACs identified by Tsujimura et al. (2002). Differentiation of the progeny of TACs into terminal luminal cells may then accompany loss of the Sca-1 marker.

The proximal region of the murine prostate near the urethral/prostatic boundary is an excellent candidate for the stem niche. The tubules in this region appear to form concave pocket structures highly analogous to the structures of other niches such as the intestinal crypt. Additionally, the proximal region contains a thick band of smooth muscle cells that produce high levels of the mitogen transforming growth factor-β (TGF-β), whereas stromal cells in more proliferative distal regions resemble fibroblasts and produce lower levels of the mitogen (Nemeth and Lee 1996; Nemeth et al. 1997). TGF-β has been implicated in maintaining the quiescence of HSCs (Cashman et al. 1990; Tumbar et al. 2004). Absence of TGF-β or its receptor has been shown to induce prostate epithelial cell proliferation and to diminish normal levels of apoptosis in the proximal region of transgenic mice (Kundu et al. 2000). Furthermore, recent studies by Salm et al. (2005) have demonstrated that the proximal-distal gradient of active TGF-β signaling is reversed following androgen ablation, resulting in apoptosis of distal tip cells.

Figure 4. Analysis of cytokeratin expression profile of Sca-1+ prostate cells. Longitudinal sections were prepared from 8-week-old wild-type prostate tissue. Immunofluorescent analysis for Sca-1 (green, column 1 and 3), CK5 (red, column 2 and 4), and CK8 (green, columns 2 and 4) was performed and sections were counterstained with DAPI (blue) for visualization of cell nuclei. Fluorescent images were taken at three anatomical locations: the distal tips of tubules of the anterior lobe (Distal), the proximal region of anterior lobe tubes near the urethra (Proximal), and the prostatic/urethral epithelium (PU).

Figure 5. Sca-1 expression during fetal prostate branching morphogenesis. Immunofluorescent staining for Sca-1 in E16 epithelial chord is shown in left panel. CK5 (red) and CK8 (green) staining in the same region is shown in middle panel. Left panel shows immunostaining for CK5 (red) and SMA (green). Nuclei were counterstained with DAPI (blue).
and a sensitization of proximal cells to growth stimula-
tion by cytokines that induce prostate regeneration fol-
lowing androgen replacement. This group suggests a
model in which high levels of active TGF-β signaling in
the proximal region maintain stem cell quiescence in in-
tact animals, whereas low levels of signaling in more dis-
tal regions allow the proliferation and differentiation of
TACs (Salm et al. 2005).

PROSTATE STEM CELLS IN PROSTATE
CANCER
Most human cancers are considered clonal diseases in
which one cell that has accumulated sufficient transform-
ing mutations initiates unstrained cancerous growth. De
Marzo et al. (1998) have suggested that most human
prostate cancers are derived from the accumulation of
oncogenic events in non-terminally differentiated secre-
tory luminal cells that still maintain some proliferative
activity. In this model, mutational events disrupting cell
cycle control may result in the production of secretory
cells that do not undergo terminal differentiation, thereby
causing the development of high-grade prostatic intraep-
thelial neoplasia (HGPIN). This is supported by findings
that basal cells are not readily identifiable in most human
prostate cancers, and that the bulk of cancer cells express
luminal-cell-specific markers such as cytokeratin 8
(CK8), cytokeratin 18 (CK18), and prostate-specific anti-
gen (PSA) (Okada et al. 1992).

This model is difficult to reconcile, however, with the
identification of prostate cancer cells coexpressing both
basal and luminal cell markers that may represent less-
differentiated cells preceding secretory cell development
in the traditional lineage hierarchy. Verhagen et al. (1992)
suggested that the presence of intermediate cells indicates
that prostate cancers are more likely to arise from
intermediate, transit-amplifying basal cells that nor-
mally express both basal and luminal cell markers. Find-
ing that PSCA, a putative marker of normal late-interme-
diate prostate cells, is also up-regulated in most stages of
prostate cancer further supports this model (Reiter et al.
1998; Tran et al. 2002).

One prevailing theory for tumor initiation in many tis-
sues implicates somatic stem cells as the predominant tar-
gets for transformation. Since stem cells persist in organ-
isms for longer than differentiated cells, they theoretically
have more opportunity to accumulate the mutations nec-
cessary for transformation (Reya et al. 2001). More muta-
tional events would also be required to confer stem cell
properties such as self-renewal in more differentiated
cells. Additionally, tumorigenesis and normal organogen-
esis are similar. The cells comprising most tumors are
heterogeneous with respect to cellular morphology, dif-
ferration status, and function, indicating that cancer-
initiating clones must have some degree of multipotential-
ity that is characteristic of somatic stem cells (Reya et al.
2001). Similarly, most prostate tumors contain cells re-
sembling three of the four known cell types, such as
CK5/CK18+ luminal cells, intermediate cells expressing
CK5 and CK8, and scattered neuroendocrine cells ex-
pressing markers such as serotonin (Okada et al. 1992;

The overexpression of genes typically associated with
stem cells in prostate cancers further suggests that PSCs
may be targets for prostate tumor initiation. The anti-
apoptotic protein bcl-2 frequently present in stem cells
and prostate basal cells is up-regulated in hormone-re-
fractory prostate cancer (HRPC) (McDonnell et al. 1992;
Colombo et al. 1993). Telomerase, a reverse transcrip-
tase that promotes cell immortality by preventing telo-
mere shortening, is another typical stem cell gene com-
monly expressed in prostate cancers (Sommerfeld et al.
1996). Furthermore, both telomerase and bcl-2 are pre-
sent in HGPIN, which is a generally accepted precursor
lesion of prostate cancer.

Another phenomenon supporting the stem cell model of
tumorigenesis is the frequent dysregulation in cancer
cells of pathways typically involved in the self-renewal
and homeostasis of normal stem cells. Loss of the adeno-
matus polyposis coli (APC) gene, a scaffold protein that
binds β-catenin in the Wnt signaling pathway, is found in
at least 60% of sporadic colorectal cancers and is the
principal cause of familial adenomatous polyposis
(Powell et al. 1992; Kinzler and Vogelstein 1996). Muta-
tion and loss of heterozygosity of the APC gene in human
prostate tumor samples have been reported by several
groups (Brewwater et al. 1994; Phillips et al. 1994; Watan-
abe et al. 1996). Disruptions in Wnt signaling may also
contribute to the progression of prostate cancer to andro-
gen independence. A higher percentage of hormone-re-
fractory (38%) than hormone-sensitive (23%) prostate
 cancers present abnormal β-catenin staining (de la Taille
et al. 2003). This may be explained by recent observa-
tions that nuclear β-catenin can act as an activator of the
androgen receptor (AR). Overexpression of β-catenin in
prostate cancer cell lines results in enhanced androgen-
stimulated transcription by the AR and reduces the antag-
omic capacity of bicalutamide against AR signaling
(Truica et al. 2000; Chester et al. 2002). Wnt signaling
changes may therefore represent a cause of oncogenic al-
teration of AR signaling that is commonly found in the
progression to HRPC.

The Notch and Shh pathways critical in PSC function
during development also play key roles in prostate tu-
morigenesis. Immunohistochemical analysis has shown
that expression of the Notch1 ligand Jagged1 is higher in
metastases than in benign prostate tissue or localized
prostate cancer (Santagata et al. 2004). Karhadkar et al.
(2004) demonstrated that Shh expression is 200- to 400-
fold increased in prostate cancer cell lines such as PC3,
DU145, and LnCAP relative to normal epithelial cells. This
group further found that stable expression of the
transcription factor Gli in normal prostate epithelial cells
results in the formation of aggressive tumors in vivo
(Karhadkar et al. 2004). Prostate tumors are also depen-
dent on sustained Shh signaling for vitality (Steeca et al.
has been achieved by the administration of anti-hedgehog
antibodies, small inhibitory RNAs (siRNAs) against Gli1,
and cyclopamine that antagonizes smoothened, a receptor
for hedgehog proteins (Karhadkar et al. 2004; Sanchez et
al. 2004).
Dysregulation of signaling in neighboring cells in the stem cell niche, rather than PSC themselves, may also contribute to cancer initiation. For example, conditional inactivation of the TGF-β type II receptor in the fibroblasts of transgenic mice results in an increase in fibroblast number as well as epithelial proliferation and the formation of PIN lesions (Bhowmick et al. 2004). Since the production of TGF-β by stromal cells in the putative PSC niche is thought to function in maintaining the quiescent state of PSCs, disruption of this signaling in stromal cells by inactivation of the TGF-β receptor may cause sufficient oncogenic insult to induce transformation of PSCs and initiate tumorigenesis.

In most types of tumors, only small subpopulations of cancer cells possess the stem-like capacity to initiate and sustain tumor growth. These cells, called “cancer stem cells,” may represent the cancerous progeny of the initial transformed stem cell clone of origin of the tumor and therefore support the stem cell theory of tumor initiation. Cancer stem cells were identified in human acute myeloid leukemia (AML) by Bonnet and Dick (1997), who demonstrated that the CD34+CD38− cell fraction representing only 0.1–1% of the AML cell population contained all of the tumor-initiating capacity when transplanted into NOD/SCID mice. The discovery of cancer stem cells in solid tumors such as the brain and mammary gland suggest cancer stem cells may also exist in prostate tumors (Al-Hajj et al. 2003; Singh et al. 2004). Since normal PSCs are androgen-independent, the presence of prostate cancer stem cells in primary tumors may explain the rise of androgen-independent cancer following androgen ablation therapies. Small numbers of preexisting androgen-independent cancer stem cells residing within primary tumors may become selected during androgen withdrawal and expand to produce new tumors composed entirely of androgen-independent cells. This model also explains the overexpression of stem and basal cell genes such as bcl-2 in HRPC, and provides a mechanism for which luminal, transit-amplifying neuroendocrine cells are all produced within clonally derived tumors.

It is equally possible that differentiated cells initiate prostate tumorigenesis and then evolve to display the PSC property of androgen independence when placed under selective pressure during androgen ablation. It is also possible that initial oncogenic events take place in PSCs that do not themselves become transformed, but require additional mutations in their progeny to lead to tumor initiation. Recent efforts to investigate tumor initiation have therefore taken a reductionist approach to directly assess the tumorigenic potential of different somatic cell populations. For example, Passegue et al. (2004) inactivated junB, a transcriptional regulator of myelopoiesis, in HSCs and later progenitor cells to demonstrate that only HSCs could give rise to myeloproliferative disorders similar to human chronic myelogenous leukemia (CML) in transgenic mice.

Direct comparison of different prostate cell populations has so far been hindered mostly by the absence of cell surface markers for cell separation, and lack of in vivo tumorigenesis assays to assess tumor initiation and progression. To develop an in vivo tumorigenesis assay, we tested whether cells with known oncogenic potential could initiate tumorigenesis in the dissociated cell prostate regeneration system. Dissociated prostate cells from TRAMP mice expressing the viral SV40 small t and large T antigens (Greenberg et al. 1995) were mixed with prostate cells from β-actin GFP mice and UGSM and implanted in the subcapsular renal space (Fig. 6A). Eight weeks later, grafts were harvested, and sectioned tissue was examined for cancerous lesions. Figure 6B shows the presence of GFP+ PIN lesions and GFP+ normal tissue (panel 3). PIN lesions also stained positive for the SV40 antigen as expected (panel 2), indicating that PIN lesions were derived from TRAMP cells.
This regeneration assay can also be used to assess the impact of introducing oncogenic stimuli in normal murine prostatic epithelial cells. Infection of adult murine prostate cells with a lentivirus producing a constitutively active form of AKT, a serine/threonine kinase in the PTEN/AKT signaling pathway that is often disregulated in human prostate cancer, results in the growth of low-androgen-high-gleason tumors, as well as some areas of carcinoma in older grafts. Infection of normal prostate cells by lentivirus containing siRNA to knock down PTEN expression also results in the formation of PIN lesions and prostate cancer in vivo (Xin et al. 2005).

Since Sca-1 has been identified as a putative murine prostate stem/progenitor cell marker, some insight about the tumor-initiating capacity of primitive prostate cells can be gained by investigating the oncogenic potential of these cells. We have demonstrated that murine prostate Sca-1+ stem/progenitor cells expressing activated AKT can give rise to PIN lesions following an 8-week incubation in vivo (Xin et al. 2005). Interestingly, tumor tissue derived from normal prostate cells infected with lentivirus containing AKT contains high (75%) percent identification of murine and human prostate cancer stem cells. Gleevec, a targeted anticancer therapy, is a Bcr-Abl kinase inhibitor that induces remission in over 90% of newly diagnosed patients with CML (Kantarjian et al. 2002). However, the majority of cells eliminated by Gleevec are differentiated leukemic cells. Multiple studies have identified residual CML stem/progenitor cells in patients following Gleevec exposure, indicating that these cells may be preferentially resistant to the drug (Graham et al. 2002; Bhatia et al. 2003; Chu et al. 2005; Michor et al. 2005).

A similar problem may exist in current treatments for metastatic prostate cancer. Although surgical resection is an effective treatment for clinically localized prostate cancer, androgen ablation therapies to eradicate androgen-dependent metastatic prostate cancer cells are only effective for a short period of time before HRPC's appear in the absence of androgen (Klein et al. 1997). Since primitive PSCs and TACs survive but do not proliferate in the absence of androgen, it is plausible that small numbers of androgen-independent metastatic prostate cancer stem cells survive androgen ablation therapy. Theoretically, such prostate cancer stem cells should remain quiescent, or give rise to androgen-dependent progeny that die rapidly due to their androgen dependency. However, mutations conferring androgen independence may arise in prostate cancer stem cells or their progeny, enabling more differentiated prostate cancer cells to survive in androgen-depleted environments. Revival of androgen receptor signaling via amplification, mutation, and/or cross-talk with other signaling pathways has been documented in patients with HRPC and in prostate cancer cell lines (Visakorpi et al. 1995; Tilley et al. 1996; Hobsch et al. 1998).

The existence of many cell types and complex intercellular interactions within most tumors make them a challenging target for therapeutic design. The increasing evidence for the role of adult stem cells in tumor initiation suggests that most cancers are developmental disorders in which pathological stem cell function results in aberrant tissue development. Therapies aimed at treating epithelial cancer as a chronic developmental disorder may therefore prove to be as efficacious as those aimed at proliferative events.

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