

Review

Prostate stem cells and cancer

A.Y. Nikitin¹, A. Matoso¹ and P. Roy-Burman²

¹Department of Biomedical Sciences, Cornell University, Ithaca, New York and ²Department of Pathology and Biochemistry and Molecular Biology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

Summary. Properties shared by neoplastic and stem cells indicate a possibility that somatic stem cells or transit-amplifying cells that have reacquired stem cell properties, particularly the ability for self-renewal, represent favorable targets for malignant transformation. In this review we discuss significance of the stem cell model for understanding prostate cancer pathogenesis and describe relevant studies in animals. It is proposed that dissemination of rare cancer stem cells may lead to metastatic disease and that resistance of such cells to multiple drugs and androgen ablation make them responsible for failure of current treatments. Thus further understanding of the cancer stem cell biology is needed for development of efficient rationally designed therapy permitting better targeting and better treatment outcomes for patients with prostate neoplasms.

Key words: Adult stem cells, Androgen ablation, Carcinogenesis, Neuroendocrine differentiation, Mouse models, Prostate

Prostate cancer

Prostate cancer is the most common malignant neoplasm in men in the Western world. In the US, it is estimated that in 2007 there will be 218,890 newly diagnosed prostate cancers and 27,050 men will die from the disease. (Jemal et al., 2007). While the causes of prostate cancer are unknown, the best documented risk factors include age, ethnicity, and family history (Chodak, 2006).

Prostate carcinoma arises from the prostate epithelium. Normal prostatic epithelium is composed of three phenotypically distinct populations of cells. The most abundant population consists of secretory luminal cells, which express cytokeratin (CK8), CK18, prostate specific antigen (PSA), high level of androgen receptor (AR) and are dependent on androgen for survival. Basal

cells, the second most common cellular population, are separated from stroma by the basement membrane, express p63, CK5, CK14 and low level of AR (Bonkhoff and Remberger, 1993; Wang et al., 2001). Neuroendocrine (NE) cells are scarce and they express chromogranin A and synaptophysin, and lack AR and PSA (Bonkhoff et al., 1995). Although the function of NE cells is largely unknown, they might induce proliferation of adjacent cells modulated by paracrine secretion of neuropeptides (Bonkhoff et al., 1991). Additionally, there is a group of cells that have an intermediate phenotype expressing a mixture of basal and luminal markers (CK5, CK8, CK14, CK18 and PSA), and are believed to be the transit-amplifying cells (Bonkhoff et al., 1994; Bonkhoff and Remberger, 1996; Xue et al., 1998; Hudson et al., 2000). Whether basal cells are the progenitors of luminal cells or represent a terminally differentiated phenotype is a subject of controversy. Basal cells have been shown to survive in low androgen conditions and are thought to reconstitute the secretory cell compartment in response to androgen re-administration (Nagle et al., 1991; Peehl et al., 1994; De Marzo et al., 1998a,b; Robinson et al., 1998; Xue et al., 1998; Wang et al., 2001). However, this view has been challenged by an observation that grafting of p63-deficient prostate cells leads to formation of prostatic tissue consisting of neuroendocrine and luminal but not basal cells (Kurita et al., 2004; Signoretti et al., 2005). This supports the idea that basal and luminal cell populations are derived either entirely from independent lineages that are capable of self-renewing or maintained by a common p63-negative progenitor cell.

The great majority of prostate cancers manifests as adenocarcinoma, and usually proceeds through a series of defined stages, from prostatic intraepithelial neoplasia (PIN), to prostate cancer *in situ*, to invasive and metastatic cancer. Tumor cells in the adenocarcinoma are usually AR and PSA positive mimicking the phenotype of luminal epithelial cells. This disease is initially regarded as responding to androgen depletion because therapeutic hormonal castration induces massive apoptosis of malignant cells and clinically leads to tumor regression. However, after a remission period of up to

several years, cancer usually progresses despite the low levels of androgen (Chodak, 2006).

Prostate cancer can also progress towards NE differentiation. The spectrum of NE differentiation can vary from presence of scattered or focal NE cells to carcinoid tumors and small cells prostate carcinoma (SCPC). Carcinomas with increased NE phenotype have been closely correlated with tumor progression and androgen depletion-resistance (Bonkhoff et al., 1991, 1995; Hoosein et al., 1993; Culig et al., 1994; Weinstein et al., 1996; Hobisch et al., 1998; Jongsma et al., 2000; di Sant'Agnese, 2001; Ito et al., 2001; Huang et al., 2005; Vashchenko and Abrahamsson, 2005). However, some other studies have led to findings that differed from such correlation (Cohen et al., 1991; Ahlegren et al., 2000; Bostwick et al., 2002), and, thus, the role of focal NE differentiation in prostate cancer remains controversial. However, it is not disputed that pure small cells tumors have indeed an aggressive course (Cohen et al., 1991; di Sant'Agnese, 1992). Importantly, these cases are sometimes mistakenly diagnosed as poorly differentiated adenocarcinomas and found to progress rapidly to metastatic disease without raising PSA or responding to androgen ablation therapy (Yashi et al., 2006). Although SCPC occurs in 1-2% of all prostate carcinomas (Clegg et al., 2003), its ominous course makes it responsible for up to 10% of all prostate cancer related deaths.

Many investigators increasingly favor a possibility that initiation of carcinogenesis occurs in a multi-potent stem cell that can give rise to neuroendocrine and secretory cell lineages. The similarities and differences between cells that give rise to the normal epithelium and those that form neoplasm are largely unknown and subject of extensive research.

Cancer stem cells

The hypothesis that tissue stem cells may be the cellular origin of cancer was proposed 150 years ago by pathologists Cohnheim and Durante (Sell, 2004). However, only recent advances in stem cell biology have permitted the isolation of these cells from malignant neoplasms of the hematopoietic system (Lapidot et al., 1994; Bonnet and Dick, 1997), brain (Singh et al., 2004), breast (Al-Hajj et al., 2003), and colon (O'Brien et al., 2007; Ricci-Vitiani et al., 2007).

Stem cells are important for maintenance of different somatic tissues by being able to replenish the cell population as necessary. They are defined by the presence of several distinct properties: long-term self-renewal, the capability to develop into multiple lineages, the potential to proliferate extensively, the ability to develop multidrug resistance and sustain telomerase expression. Their most defining property is the potential to generate more stem cells and, at the same time, progenies that also differentiate (Betschinger and Knoblich, 2004; Clevers, 2005; Morrison and Kimble, 2006). This can be accomplished by asymmetric cell

division, whereby each stem cell divides to yield one daughter with stem cell fate and one daughter that differentiates. To have control of their own numbers, stem cells can also use symmetric division, by which both daughter cells have the same fate to either stay as stem cells or differentiate (Morrison and Kimble, 2006). These dividing cells with capacity to differentiate will then constitute the progenitor cells of a given tissue.

Adult mammalian tissues are composed primarily of differentiated cells, but also have populations of cells that are between the stem cells and its terminally differentiated progeny. These cells are usually called transit-amplifying cells, and their main function is to proliferate and increase the number of differentiated cells produced by each stem cell division. In most tissues, the only long-lived cells are the stem cells (Potten and Loeffler, 1990; Sell, 2004). Like normal tissues, tumors consist of heterogeneous populations of cells, and only a small portion of them might have the capacity of self-renewal. These are the so called "cancer stem cells" (Pierce, 1967).

Over the last 30 to 40 years, evidence supporting the cancer stem cell hypothesis has gained impetus, and an expanding body of literature has addressed the similarities in the biology of stem cells and cancer initiating cells (Reya et al., 2001; Taipale and Beachy, 2001; Reya and Clevers, 2005). Signaling pathways that regulate normal stem cell self-renewal and maintenance, such as *wnt*, *notch*, *oct4*, and *sonic hedgehog*, have been found to be altered in cancer (Monk and Holding, 2001; Beachy et al., 2004; Weng and Aster, 2004; Reya and Clevers, 2005). A delicate balance between signals that favor quiescence and those that allow cell cycle progression seems to be essential for maintenance of the tissue homeostasis and normal regulation of their cellular population. Quiescence of stem cells has been shown to be maintained by p16 (Janzen et al., 2006), p18 (Yuan et al., 2004), p21 (Cheng et al., 2000b; Kippin et al., 2005), p27 (Cheng et al., 2000a), TGF β (Salm et al., 2005) and pRb (Hatzfeld et al., 1991). An increased stem or progenitor cell potential has been found in dermal (Topley et al., 1999), neural (Durand et al., 1998) or otic (Lowenheim et al., 1999) tissues of *p21^{-/-}* or *p27^{-/-}* mice. In hematopoietic and neural stem cells, the Bmi1 protein is critical for stem cell self-renewal (Park et al., 2003; Molofsky et al., 2005) at least in part due to its regulation through the Ink4a locus (Jacobs et al., 1999; Bruggeman et al., 2005). The *ink4* locus encodes two tumor suppressor proteins: p16^{ink4a} and p14^{Arf}, for which Rb and p53 are respective downstream targets. Therefore, it is not surprising that two of the pathways most frequently altered in human cancer, p53 and Rb, are also implicated in the regulation of stem cell self-renewal and quiescence. However, whether p53 and Rb alterations, individually or in combination, may preferentially transform stem cells and/or their early progeny and if such transformation may determine aggressiveness of the neoplasia, are important issues that remain to be investigated.

In addition to the alterations of the pathways involved in quiescence, oncogenic transformation of cells could also be triggered by reactivation of pathways involved in normal embryonic development when rapid division of pluripotent cells give rise to the different tissues and cell lineages. Accordingly, differentiated cells can be reprogrammed to an embryonic like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem cells. Furthermore, the addition of certain transcription factors into differentiated fibroblasts grown under stem cell culture conditions has induced their transformation into pluripotent cells with embryonic characteristics (Takahashi and Yamanaka, 2006). Intriguingly, one of the transcription factors involved in this transformation is the potent oncoprotein MYC, which has been found to be overexpressed in many human cancers, including prostate cancer (Dalla-Favera et al., 1982; Pompetti et al., 1996; Nesbit et al., 1999).

The altered balance between senescence and self-renewal signals often comes from external signals such as secreted factors, cell-cell interactions, integrins and the extracellular matrix (Watt and Hogan, 2000; Song et al., 2002; Zhang et al., 2003). Adult or somatic stem cells generally have little to no function without the niche. The niche must have both anatomic and functional dimensions enabling stem cells to self-renew. Evidence of the importance of the microenvironment is provided by the observation that embryonal carcinoma cells injected subcutaneously in mice formed teratocarcinomas, whereas the same cells injected into blastocyst gave rise to normal chimeric mice (Mintz and Illmensee, 1975). Additionally, it has been shown that genetic manipulation of stromal cells can result in tumor formation, and thus the original cause of tumor may be the neighboring cells. For example, experiments performed with prostate tissue have shown that rendering stromal fibroblasts unresponsive to TGF β leads to intraepithelial neoplasia (Bhowmick et al., 2004). Therefore, the cancer stem cell hypothesis should be extended to include the microenvironment as a key player in the control of oncogenic signals from the cells as well as the generation of carcinogenic conditions.

Prostate stem cells and carcinogenesis

A stem cell model for prostate organization proposes that a pluripotent stem cell is the progenitor of differentiated basal, secretory luminal and NE cells (Isaacs and Coffey, 1989; Signoretti et al., 2005). Support for this hypothesis comes from animal studies of prostate response to androgens. Androgen deprivation leads to rapid involution of the gland, but when androgens are restored, the gland regenerates completely. As this cycle of involution of the gland can be repeated many times, populations of stem cells that are able to survive in a low androgen environment are thought to reconstitute the organ (Isaacs et al., 1987). Presence of stem cells in the adult human prostate is also

supported by the observation that a small number of cells within the prostate possesses strong proliferative capacity and can form glandular-like structures in reconstituted systems (Hudson et al., 2000).

Although the overall organization of the rodent prostate differs from that of the human gland (Roy-Burman et al., 2004), it provides a unique opportunity to study many important features of the prostate, including the localization and properties of stem cells. Each mouse prostatic duct consists of a proximal region attached to the urethra, an intermediate region, and a distal tip. Proliferating cells are located at the distal tip (Sugimura et al., 1986a,b; Cunha et al., 1987). These rapidly proliferating cells are likely to be the transit-amplifying cells. Cells with stem cell-like properties, such as low cycling rate and high *ex vivo* proliferative potential, were later identified to be in the proximal region of the prostatic duct (Figure 1 and Ref. Tsujimura et al., 2002). Correspondingly, cell suspensions derived from the proximal region have been shown to form significantly more prostatic tissue than those obtained from the distal regions in an *in vivo* transplantation model. Furthermore, these cells were shown to share the expression of such specific antigens as stem cell antigen-1 (Sca-1), α 6 integrin and Bcl-2 with stem cells of other organs (Burger et al., 2005), and to survive prolonged androgen deprivation (Goto et al., 2006).

The origin of the "prostate cancer stem cell" remains unclear. Since multiple mutations are necessary for a cell to become fully cancerous, it is unlikely that all the mutations could occur during the short life span of progenitors and secretory cells. Using a prostate-specific gene deletion approach (Wu et al., 2001), a study of prostate epithelium-specific inactivation of *Pten* alleles (Wang et al., 2006) led to the observation that *Pten* loss is associated with increased basal cell density and concomitant expansion of a prostate stem/progenitor-like subpopulation as evidenced by the progressive increase of Sca-1- and Bcl-2-positive cells. Such altered stem/progenitor cell proliferation may lead to increased transit-amplifying cell population, from where prostate cancer could potentially originate. However, the exact mechanism by which PTEN controls prostate stem cell behavior remains to be defined.

To test directly the role of p53 and Rb in prostate carcinogenesis, we have also conditionally inactivated these genes in the mouse prostate epithelium (Zhou et al., 2006). Inactivation of both p53 and Rb were necessary to produce prostate cancer. Tumors are phenotypically diverse containing large number of cells that co-express the NE marker synaptophysin and epithelial luminal cell markers CK8 and AR but not basal cell marker CK5. These tumors progress to extensive metastasis in the liver, lung and lymph nodes, and despite their expression of markers of luminal differentiation, they became androgen-depletion independent very early after castration. Importantly, the mixed phenotype of these cancer cells suggest their possible origin from bi-potent stem cell (Fig. 2). This

possibility, as well as an alternative, although less likely, scenario of the acquisition of excessive phenotypical plasticity during carcinogenesis, is currently being investigated.

The pattern of gene expression associated with p53 and Rb deficient prostate cancer is very similar to that observed in aggressive human prostate carcinoma. Notably, of the 18 genes known to have expression

alterations in aggressive human prostate cancer, 16 are similarly affected in our mouse model. Among those, Nkx3.1, myosin heavy chain 11, and Kai1 are down regulated and Bmi1, Ezh2 and Pim1 are up regulated. The polycomb group Ezh2 gene, which is particularly reported to be involved in progression to metastatic prostate cancer in humans (Varambally et al., 2002), has been reported to be associated with the stem cell fate

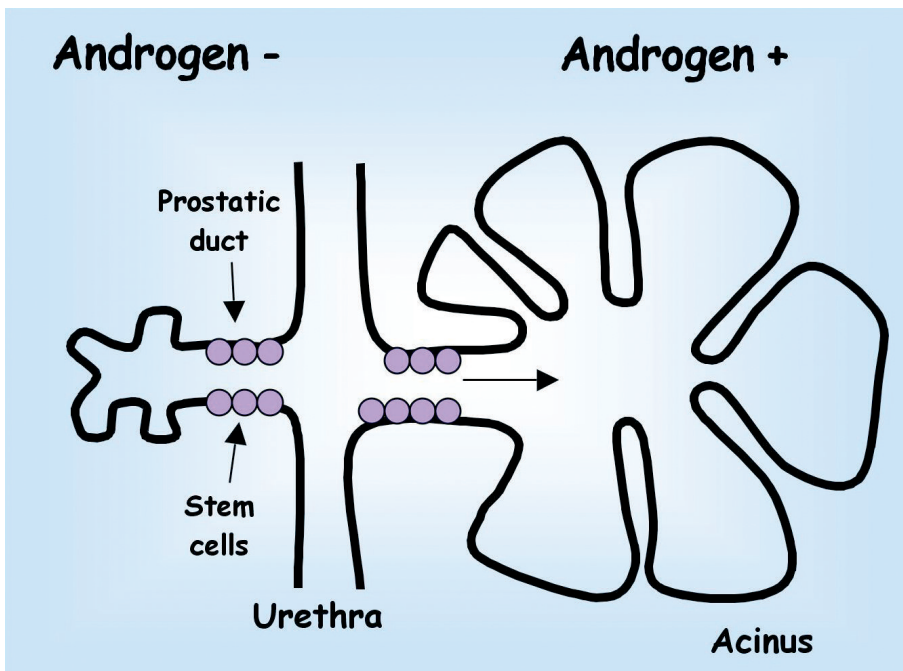


Fig. 1. Stem cell model of prostate organization. Prostate stem cells are independent of androgen for survival and present in the proximal region of prostatic duct. In the presence of androgen, stem cells give rise to transit-amplifying cells that have progressively diminished proliferative capacity and form terminally differentiated basal, luminal and neuroendocrine cells populating the acinus.

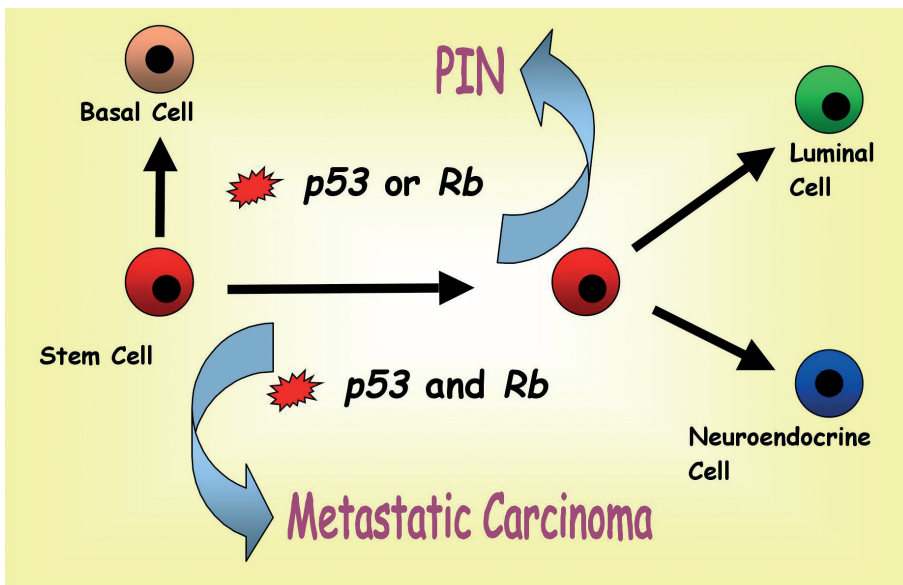


Fig. 2. Proposed mechanism of carcinogenesis associated with p53 and Rb deficiency. Inactivation of either p53 or Rb in the prostate epithelium leads to intraepithelial neoplasia (PIN) in cells of luminal differentiation (green), while inactivation of both p53 and Rb is necessary for malignant transformation of the stem cell (red) common for luminal and neuroendocrine (blue) lineages leading to invasive and metastatic cancer with luminal and neuroendocrine differentiation (blue). An earlier committed or independent lineage might give rise to basal cells (orange).

(Valk-Lingbeek et al., 2004; Saramaki et al., 2006).

The cancer stem cell hypothesis supports that most cancer cells lack the ability to form a new tumor and only dissemination of rare cancer stem cells can lead to metastatic disease. These cells must bear a complete set of mutations responsible for the carcinogenic properties (Reya et al., 2001), and may differ from their differentiated progeny by epigenetic controls (Feinberg et al., 2006). According to this concept, tumor mainly consists of cells that underwent genetic and epigenetic changes incompatible with the cancer stem or progenitor cell properties and do not contribute to the maintenance of neoplasia. Such cells will be responsible for the initial response to androgen deprivation therapy in prostate cancer, where most differentiated cells are AR positive. However, since this therapy is not designed to target androgen depletion-independent cancer stem cells, the disease is likely to relapse later as recurrent cancer (Collins and Maitland, 2006).

Conclusions

The prostate cancer stem cell model is being increasingly supported by the accumulation of experimental evidence. Although it is currently only at early stage of development, with time this model is likely to become crucial for better understanding of the critical steps in prostate carcinogenesis as well as for assessing successes and failures of androgen ablation therapy. It is projected that a parallel understanding of adult stem cell biology would be required to provide essential clues for the scrutiny of mechanisms that govern prostate cancer stem cells and, then to exploit those for therapeutic interventions to ultimately cure prostate cancer.

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