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p63 in Prostate Biology and Pathology

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Abstract The identification of stem cells and differentiation programs regulating the development and maintenance of the normal prostate epithelium is essential for the identification of the cell type(s) and molecular alterations involved in the development and propagation of prostate cancer (CaP). The p53-homologue p63 is highly expressed in normal prostate basal cells and is a clinically useful biomarker for the diagnosis of CaP. Importantly, p63 has been shown to play a critical role in prostate development. Recent experimental evidence also suggests that this gene is essential for normal stem cell function in the prostate as well as other epithelial organs. Future studies aimed at better defining the role of p63 in the renewal of the adult prostate epithelium are likely to shed new light on the mechanisms involved in prostate carcinogenesis. J. Cell. Biochem. 103: 1354–1368, 2008. © 2007 Wiley-Liss, Inc.

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Prostate cancer (CaP) is the sixth most common cancer in the world, and together with lung and colon cancer it is the most frequent form of malignancy among men [Jemal et al., 2007]. In the last decades the overall mortality from CaP has been declining, due to significant advances in early detection and prevention. However, the long-term survival of patients with metastatic disease has not changed significantly. Great hope currently lies in the development of new therapeutic agents with higher specificity and decreased toxicity.

It has been proposed that tumors contain rare stem-like cells (i.e., cancer stem cells (CSC) or tumor initiating cells) that are characterized by unlimited self-renewing capacity, low proliferation rates, and ability to differentiate into proliferating tumor cells [Reya et al., 2001; Tan et al., 2006]. Current therapies are designed to treat the bulk of neoplastic cells and might not be effective in eradicating the stem-like cells responsible for initiating and maintaining tumor growth. Therefore, the CSC model has important clinical implications and its validation could lead to a major advance in both understanding the biology of CaP and developing new treatment strategies that selectively target the CSCs.

One major limitation in the identification of the cell type(s) that gives raise and maintains prostate carcinoma is that adult prostate stem cells have not been identified yet and the mechanisms regulating the renewal of the adult prostate epithelium both in physiologic and pathologic conditions remain largely unknown. Notably, the discovery of the p63 gene [Augustin et al., 1998; Osada et al., 1998; Yang et al., 1998] has recently provided new paradigms for understanding how the normal prostate epithelium develops and is maintained. It has been convincingly demonstrated that p63 is selectively expressed in the basal cells of both stratified and glandular epithelia, including the prostate and that its expression in required for normal prostate organogenesis [Signoretti et al., 2000; Kurita et al., 2004]. Importantly, our group has recently utilized the p63 knockout mouse as a tool to identify the stem cells in the developing prostate [Signoretti et al., 2005].

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In this review, we will outline the current knowledge about p63 function and its role in prostate organogenesis. Moreover, we will examine experimental approaches aimed at clarifying the function of the p63-positive basal cells in the homeostasis of the adult prostate epithelium. Finally, we will discuss the clinical relevance of p63 immunostaining as a diagnostic biomarker for CaP.

UNRAVELING p63 FUNCTION

The p63 Gene

The p63 gene was discovered almost a decade ago on the basis of its homology with the tumor suppressor gene (TSG) p53 [Augustin et al., 1998; Osada et al., 1998; Yang et al., 1998]. Together with p73, p63 belongs to the p53 family of genes and phylogenetic analysis indicates that it represents the most ancient member of the family [Moll et al., 2001; Little and Jochemsen, 2002; Melino et al., 2003; Moll and Slade, 2004; Deyoung and Ellisen, 2007]. p63 is located on chromosome 3, and contains 15 exons, covering a region of about 220 kb. It encodes for two main groups of transcripts (TAp63 and ΔNp63) that are generated by two independent promoters. The TAp63 transcripts contain the transactivating (TA) domain and derive from a promoter located upstream of exon 1, while a second promoter located within intron 3 encodes for the ΔNp63 transcripts, which lack the TA domain. All p63 proteins contain a DNA-binding domain, which has about 60% homology with the DNA-binding domain present in the p53 gene as well as an oligomerization domain with about 38% identity to the one in p53 (Fig. 1). The amino-terminal (N-terminal) TA domain, only contained in the TAp63 isoforms, has 22% homology with the p53 transactivating domain [Irwin and Kaelin, 2001]. Alternative splicing of the primary RNA transcripts at the carboxy-terminal (C-terminal) results in three variants (alpha, beta, gamma) for each isoform type, and further contributes to the diversity of the p63 protein products. Specifically, the alpha isoforms contain a sterile alpha motif (SAM) domain that mediates protein–protein interactions and is absent in the beta and gamma isoforms.

p63 isoforms are characterized by different levels and patterns of expression. It has been extensively shown that the ΔNp63alpha isoform is selectively expressed at high levels in the basal cells of stratified and glandular epithelia and that its expression decreases with cell differentiation [Yang et al., 1999a; Liefer et al., 2000; Signoretti et al., 2000; Pellegrini et al., 2001; DiRenzo et al., 2002; Nylander et al., 2002; Koga et al., 2003; Westfall et al., 2003]. In contrast, recent data demonstrate that TAp63 proteins are only detected in oocytes in meiotic arrest, where TAp63alpha is the most abundant isoform [Suh et al., 2006].

Epithelial Development

Due to the high degree of similarity between p63 and p53, it was first thought that the two genes would also have homologous functions. However, significantly different biophysical properties in the DNA sequences result in distinct roles in epithelial biology [Klein et al., 2001; Ho et al., 2006]. Indeed, in contrast to p53, p63 has been shown to play a role in the regulation of epithelial cell development and differentiation [Koster and Roop, 2004; Barbieri and Pietenpol, 2006], as well as cell proliferation, apoptosis and senescence [Osada et al., 1998; Yang et al., 1998; Katoh et al., 2000; Guo and Mills, 2007]. There is also substantial evidence that p63 is required for maintaining the proliferative potential of stem cells in the thymus epithelium and the epidermis [Senoo et al., 2007].

Significant understanding of p63 function has been achieved with the analysis of two independently generated p63 deficient (−/−) mouse models. p63 −/− mice are born with severe defects in craniofacial, limb, and epithelial development. More specifically, these mice show complete absence of several epithelial organs such as the epidermis, salivary, lachrymal, and mammary glands, as well as severe defects in the development of the urogenital system [Mills et al., 1999; Yang et al., 1999a]. Importantly, mice lacking p63 die soon after birth for maternal neglect and dehydration, due to the absence of an epidermal barrier. Both craniofacial abnormalities (including cleft lip and palate and lack of teeth) and limb agenesis are caused by an abnormal communication between the defective ectoderm and the underlying mesenchyma. Indeed, the interaction between a specialized epithelial structure called the apical ectodermal ridge (AER) and the mesenchyme is known to be required for normal limb formation [Niswander, 1997]. Similarly,

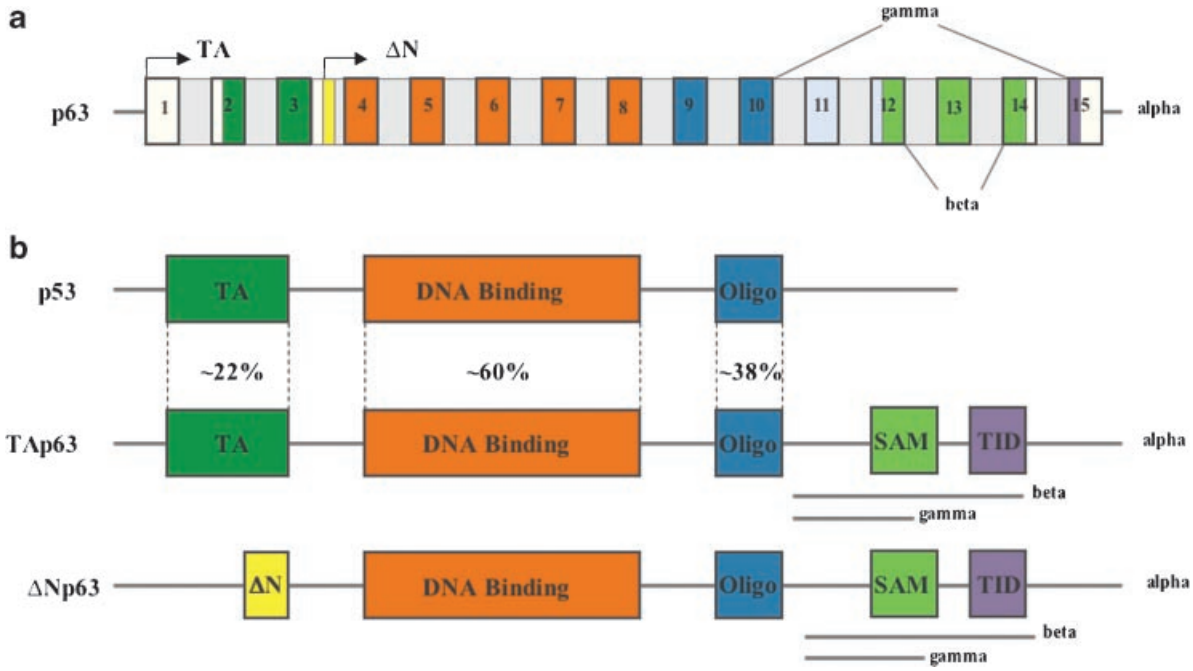

Fig. 1. Schematic of the p63 gene and protein isoforms. **a**: Numbered boxes indicate exons, gray shading indicates introns, cream shading indicates untranslated sequences. Two alternative promoters generate the TA and ΔNp63 transcripts. C-terminal alternative splicing events give rise to three isoforms: alpha, beta, and gamma. **b**: The transactivation domain (TA), ΔN specific domain, DNA binding domain, oligomerization domain, sterile alpha motif (SAM), and transactivation inhibitory domain (TID) are highlighted in different colors. The level of homology between p53 and p63 protein domains is also shown.

normal development of craniofacial structures depends on a normally functioning ectoderm [Francis-West et al., 1998].

Recent work from several groups has shed light on the relative contribution of TAp63 and ΔNp63 isoforms to epithelial development. Developmental defects of p63−/− mice share similarities with those observed in a ΔNp63-deficient zebrafish model, whose phenotype is characterized by fin truncations and lack of epidermal morphogenesis [Bakkers et al., 2002; Lee and Kimelman, 2002]. These results seem to highlight the importance of ΔNp63 isoforms in regulating epithelial development. On the other hand, in vivo genetic complementation of p63−/− mice by TAp63alpha and/or ΔNp63alpha recently suggested that the two isoforms play cooperative roles in epidermal formation [Candi et al., 2006]. More recently, however, mice lacking exons 2 and 3 encoding the TA-specific N-terminal of the p63 gene were generated and showed normal epithelial morphogenesis [Suh et al., 2006]. Thus, these findings unequivocally demonstrated that TAp63 is dispensable for the development of epithelial organs, which is regulated by ΔNp63. Importantly, however, a critical role for TAp63 in the protection of the female germ line was unravelled [Suh et al., 2006]. Specifically, this recent study established that TAp63alpha is required for a process of DNA damage-induced oocyte death, which is independent of p53.

Heterozygous germ line mutations of the human p63 gene cause rare autosomal dominant developmental disorders [van Bokhoven and McKeon, 2002]. These disorders are characterized by a variety of phenotypes that partially resemble those of the p63 deficient mice, and include ectrodactyly–ectodermal dysplasia–clefting (EEC) [Celli et al., 1999], ankyloblepharon–ectodermal dysplasia–clefting (AEC) [McGrath et al., 2001], acro-dermato-ungual-lacrimal-tooth (ADULT) [Rinne et al., 2006b], Rapp-Hodgkin [Rinne et al., 2006a], and limb-mammary (LMS) [van Bokhoven et al., 1999] syndromes. In addition, p63 mutations can also cause two non-syndromic disorders: split-hand/split-foot malformations (SHFM) [Ianakiev et al., 2000] and non-syndromic cleft lip [Leoyklang et al., 2006]. Interestingly, in line with the defects of the p63 knock-out mice, abnormalities commonly observed in these

patients include dry hypopigmented skin, absence or reduced number of sweat glands, mammary gland hypoplasia, abnormal salivary and lachrymal glands, and genitourinary malformations.

As opposed to the Li-Fraumeni syndrome, in which germline heterozygous mutations of the p53 gene cause carcinogenesis, patients with germline p63 mutations are not more prone to developing tumors. Nevertheless, the involvement of p63 in cancer remains controversial.

Carcinogenensis

The controversy concerning the role of p63 in neoplastic transformation is mainly related to the fact that susceptibility to cancer has been shown to be substantially different in the two existent p63-deficient mouse models [Flores et al., 2005; Mills, 2006; Flores, 2007; Koster et al., 2007]. The p63 heterozygous mice constructed by Yang et al. are prone to spontaneous tumors [Flores et al., 2005], while the ones generated by Mills and co-workers are not. In spite of the fact that p63 mutations have rarely been detected in human malignancies [Osada et al., 1998; Ikawa et al., 1999; Sunahara et al., 1999; Hibi et al., 2000], other studies seem to support the hypothesis that p63 can function as a TSG. For instance, TAp63 overexpression causes the activation of p53 responsive genes, leading to cell cycle arrest and apoptosis [Jost et al., 1997; Yang et al., 1998]. Additional experiments have shown that p63 and p73 act together with p53 or in an obligatory parallel pathway to induce apoptosis subsequent to DNA damage [Flores et al., 2002]. Recent in vitro studies performed on squamous cell carcinoma (SCC) cell lines have demonstrated that disruption of p63 causes upregulation of genes associated with a higher potential to metastasize and invade [Barbieri et al., 2006]. Finally, analyses of bladder and breast cancers have reported a marked reduction in the expression of p63 in invasive tumors [Urist et al., 2002; Wang et al., 2002]. On the other hand, there is evidence that ΔNp63 actually promotes cancer development and thus functions as an oncogene. More specifically, SCCs of the lung and head and neck are characterized by high-level amplifications of the p63 locus as well as high levels of expression of the ΔNp63alpha isoform [Crook et al., 2000; Hibi et al., 2000; Yamaguchi et al., 2000; Senoo et al., 2001; Tonon et al., 2005]. ΔNp63alpha overexpression leads to enhanced cell growth in soft agar, and increased tumor size in mice [Hibi et al., 2000]. The molecular mechanisms through which ΔNp63 promotes tumor growth are currently under investigation. It has been shown that ΔNp63 inhibits GSK3beta and induces intranuclear accumulation of beta-catenin with activation of beta-catenin-dependent transcription [Patturajan et al., 2002]. Moreover, work from Rocco et al. demonstrates that ΔNp63alpha promotes survival in squamous epithelial malignancy by repressing a p73-dependent proapoptotic transcriptional program [Rocco et al., 2006]. Additionally, ΔNp63alpha acts as a survival factor and controls a pathway for p73-dependent cisplatin sensitivity in a subset of human breast cancers [Leong et al., 2007].

In summary, the role of p63 in tumorigenesis is clearly complex and needs further clarification. Current data suggest that TA and ΔN isoforms might play opposite roles in this process. The generation of animal models with altered expression of specific p63 isoforms will be critical for dissecting the function of the different p63 proteins during tumor development and progression.

THE ROLE OF p63 IN THE PROSTATE

The Prostate Epithelium

The prostate is an exocrine gland of the male reproductive system that develops by epithelial budding of the intermediate region of the primitive urogenital sinus (UGS), a hindgut derivative of endodermal origin [Sugimura et al., 1986; Timms et al., 1994; Roy-Burman et al., 2004]. Several similarities and differences in the gross and microscopic anatomy of human and murine prostate have been described. For instance, the human prostate is subdivided in four different glandular regions, or zones (peripheral, central, transition, and anterior), while the mouse prostate is divided into four distinct lobes (anterior, ventral, lateral, and dorsal) surrounded by a mesothelial-lined capsule that separates the lobes from each other [McNeal, 1981; Cunha et al., 1987]. As opposed to the human prostate, the murine gland is characterized by the presence of very modest fibromuscolar stroma surrounding the ducts [Marker et al., 2003]. Despite the differences, there is general agreement that the mouse prostate represents a relevant and invaluable model for studying prostate development and

carcinogenesis in humans [Roy-Burman et al., 2004].

Both human and murine prostate glands are composed of an outer basal cell layer, which lies on a basement membrane and an inner secretory (luminal) cell layer lining the lumina. Additionally, neuroendocrine cells represent a minor cell population that is located between secretory cells, without extending to the lumen. The expression of molecular markers and hormonal regulation allow a clear differentiation among basal, secretory and neuroendocrine cells. Secretory cells are characterized by the expression of androgen receptor (AR), prostate specific antigen (PSA), and low molecular weight cytokeratins (LMWCK) 8 and 18 [Ware, 1994]. In contrast, the basal cell compartment is androgen independent and expresses high molecular weight cytokeratins (HMWCK) 5 and 14 [Ware, 1994]. Neuroendocrine cells are rich in serotonin-containing granules and also contain a variety of peptide hormones, including somatostatin, calcitonin, and bombesin. Similarly to basal cells, neuroendocrine cells do not express AR and PSA.

The way the prostate epithelium develops has long been debated and the hierarchical relationship between basal and secretory cells has been highly controversial. Several lines of evidence suggest that secretory cells derive from progenitor basal cells [Bonkhoff and Remberger, 1996; De Marzo et al., 1998b]. For example, cells that are phenotypically intermediate between basal and secretory cells have been observed both in vitro and in vivo [Jones and Harper, 1992; Verhagen et al., 1992; Peehl et al., 1994; Bonkhoff and Remberger, 1996; Robinson et al., 1998; Xue et al., 1998; De Marzo et al., 1998a; Hudson et al., 2001; Wang et al., 2001]. By immunohistochemical analysis, Wang et al. showed that both human and mouse UGS epithelia are highly enriched for cells that express both prostate secretory and basal cell markers [Wang et al., 2001]. On the basis of these results, they hypothesized that these cells with intermediate phenotypic characteristics represent prostate epithelial stem cells. Interestingly, cells with analogous molecular features can also be sporadically detected in the mature prostate epithelium and other investigators have postulated that such cells correspond to basal stem cells in the process of developing into secretory cells (i.e., transient amplifying cell population). In line with the hypothesis that stem cells reside in the basal cell layer, other sets of experiments have demonstrated that in both human and rodent prostate epithelia, basal cells display a higher proliferation rate than secretory cells [Evans and Chandler, 1987a; Bonkhoff et al., 1994b]. Specifically, the proliferation capacity of the basal cell compartment has been shown to exceed that of the secretory cell layer by a factor of 7 [Bonkhoff et al., 1994b]. As androgen withdrawal causes massive apoptosis of prostate secretory cells [Kyprianou and Isaacs, 1988; Waltregny et al., 2001], the reconstitution of the prostate epithelium after castration and subsequent androgen administration is thought to be sustained by basal stem cells, which are capable of surviving in low androgen environments [Bonkhoff et al., 1994a; Bonkhoff, 1996; Bonkhoff and Remberger, 1996]. Interestingly, basal cells are characterized by the expression of the antiapoptotic bcl-2 protein, which is frequently upregulated in several stem cell populations [Verhagen et al., 1992]. In spite of this experimental evidence, the role of basal cells as stem cells has not been accepted unanimously [English et al., 1987; Evans and Chandler, 1987b]. In contrast to this hypothesis, several kinetic studies of both rodent and human prostate clearly demonstrate that secretory cells have the ability to proliferate in vivo [English et al., 1987; Evans and Chandler, 1987a,b]. Other experiments have shown that administration of androgens after castration induces a proliferative response especially in the luminal compartment [English et al., 1987; Evans and Chandler, 1987b]. Furthermore, a pulse-chase labelling approach was recently used to identify the slow cycling cells (putative stem cells) in the prostate epithelium after castration and androgen-induced regeneration [Tsujimura et al., 2002]. This study demonstrated that the label retaining cell population resides within the proximal prostate ducts and, remarkably, consists of both basal and secretory cells. Overall, these latter observations are consistent with the concept that both the basal and the luminal compartments have self-renewal capacities and are not hierarchically related.

The identification of the p63 gene and its restricted expression in the basal compartment of various epithelial organs [Yang et al., 1998, 1999a; Mills et al., 1999], has allowed some progress in the understanding of the role of

basal cells in these tissues. Work from our group has shown that within the normal prostate epithelium, p63 is selectively expressed in the basal cell layer and consistently absent in the secretory and neuroendocrine cells [Signoretti et al., 2000]. Double immunostaining for p63 and high molecular weight cytokeratins demonstrated co-localization of the two antigens in the majority of basal cells. However, a small subset of p63-positive and HMWCK-negative cells was also observed, suggesting that the prostate basal cell compartment is phenotypically and functionally heterogeneous and contains cells in different stages of differentiation. In accordance with what has been observed in other epithelial tissues (e.g., epidermis, breast, and urothelium), ΔNp63alpha is the main isoform expressed by basal cells of the prostate [Signoretti et al., 2000].

Prostate Organogenesis

The central role played by the p63 gene in regulating the formation of the prostate gland during embryogenesis was highlighted by histological analysis of the urethral region of p63−/− newborn mice [Signoretti et al., 2000]. Interestingly, the epithelial lining of the urethra showed major developmental abnormalities, including lack of stratification and complete absence of basal cells. Most importantly, prostate buds, normally located within the ventral and dorsolateral regions of the periurethral mesenchyma, were not detected. The agenesis of the early prostate suggests that p63 is required for the formation of prostate stem cells that reside in the basal layer of the UGS epithelium. However, since p63−/− mice die immediately after birth and the prostate gland undergoes significant post-natal development, the possibility that p63 deficiency simply causes a developmental delay in the prostate cannot be ruled out by this analysis. To address such a possibility, both our group and others utilized renal grafting of the UGS, a procedure that has been extensively employed to study urogenital development [Kurita et al., 2004; Signoretti et al., 2005]. In these experiments, the UGS was isolated from p63−/− male mice, transplanted under the kidney capsule of recipient wild-type (WT) mice, and analyzed a few months after transplantation. Remarkably, the p63−/− grafts formed glands lined by cells with histological and molecular characteristics of prostate secretory cells with complete absence of basal cells. Intriguingly, the grafts also contained an abundant population of mucinous cells, which focally presented unequivocal features of intestinal differentiation. Importantly, cells expressing prostate cell markers (e.g., Nkx3.1) were consistently negative for expression of intestinal cell markers (e.g., Cdx-2). It is worth noting that both prostatic and intestinal epithelia originate from the embryonic endoderm. However, in contrast to the prostate, the intestine does not express p63 nor do p63−/− mice present defects in the development of this organ. Thus, our findings support the hypothesis that the lack of p63 in the UGS endoderm prevents its full commitment to the prostate cell lineage and allows it to differentiate towards the intestinal cell lineage, which does not require p63 for normal development (Fig. 2).

As mentioned above, p63 deficiency prevents the development of basal cells, while secretory cell formation is not significantly affected. Surprisingly, such results prove that p63 is dispensable for the differentiation of the secretory cell compartment. Nevertheless, a clear understanding of the hierarchical relationship between basal and secretory cells during normal prostate development cannot be achieved by the simple analysis of the prostate defects of p63−/− mice. To gain insight on prostate cell lineages and test the hypothesis that p63-positive basal cells represent the progenitors of secretory cells during normal prostate organogenesis, the developmental defects of p63−/− embryos were abrogated by the injection of normal embryonic stem (ES) cells into p63−/− blastocysts [Signoretti et al., 2005]. The blastocyst complementation system provided key information on the way the prostate epithelium develops. First, we were able to demonstrate that p63 functions in a cell autonomous manner to regulate the development of epithelial tissues, including the prostate. Most importantly, our results indicated that the early prostate (prostate buds) consist exclusively of p63-positive cells that subsequently differentiate into basal and secretory cells of the mature prostate epithelium and thus function as progenitor/stem cells.

Prostate Homeostasis

The existence of stem cells has been demonstrated in various adult tissues including brain, bone marrow, peripheral blood, blood vessels,

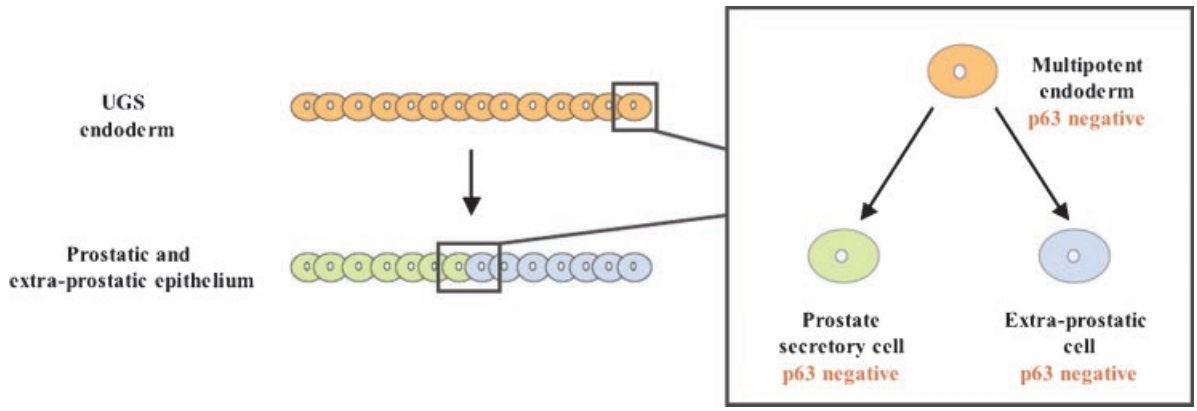

Fig. 2. Model of endodermal differentiation in p63−/− mice. In absence of p63, the UGS endoderm maintains the ability to differentiate towards prostate secretory cells and extra-prostatic (i.e., intestinal) epithelium.

skeletal muscle, skin, breast, lung, and liver [Till and Mc, 1961; Beltrami et al., 2003; Merkle et al., 2004; Oliver et al., 2004; Tumbar et al., 2004; Urbich and Dimmeler, 2004; Collins et al., 2005; Kim et al., 2005; Herrera et al., 2006; Stingl et al., 2006].

We have shown that the p63-positive cell population of the developing prostate contains progenitor/stem cells able to give rise to both basal and secretory cells. This observation suggests that p63-positive basal cells of the fully developed prostate include stem cells responsible for maintaining and repairing the adult prostate epithelium (i.e., adult stem cells). As an alternative, however, it is possible that differentiated basal and secretory cells of the adult prostate are maintained by a process of self-renewal. There is indeed evidence that differentiated cells of epithelial organs can be self-sustaining. For instance, a recent lineage tracing within the pancreatic epithelium surprisingly demonstrated that pre-existing beta-cells, rather than multipotent stem cells, are the major source of new beta-cells during adult life [Dor et al., 2004].

In line with studies performed in the hematopoietic system, several investigators have attempted to isolate adult prostate cells with stem/progenitor cell characteristics by using cell surface markers known to be expressed by stem cells of other organs [Richardson et al., 2004; Burger et al., 2005; Xin et al., 2005]. Stem cell antigen 1 (Sca-1) is a marker commonly used for enrichment of stem and progenitor cells from various murine tissues [Holmes and Stanford, 2007]. Recently, Sca-1 expression was successfully utilized to enrich for murine prostate cells with regenerating activity in tissue recombination assays [Burger et al., 2005]. Interestingly, Sca-1-positive cells were most abundant within the proximal prostate ducts that had been previously shown to harbor a slow cycling putative stem cell population. CD133 or prominin-1 is a transmembrane glycoprotein originally found on neuroepithelial stem cells in mice [Weigmann et al., 1997]. In humans, CD133 antigen expression is restricted to undifferentiated cells that include endothelial progenitor cells [Peichev et al., 2000], hematopoietic stem cells [Yin et al., 1997], fetal brain stem cells [Uchida et al., 2000], embryonic epithelium [Weigmann et al., 1997; Corbeil et al., 2000], and myogenic cells [Torrente et al., 2004]. In the prostate, CD133 was recently shown to be expressed in a subpopulation of cells localized in the basal cell compartment. Importantly, the CD133 cells, which co-expressed high integrin $\alpha2\beta1$ levels, displayed high clonigenic activity in vitro, and were capable of differentiating into prostate epithelium in vivo [Richardson et al., 2004]. Finally, CD44 is another surface molecule expressed in the basal cell compartment of the prostate. In vitro analysis of the CD44-positive basal cell populations in the presence of dihydrotestosterone (DHT) and Matrigel showed increased production of PSA, suggesting differentiation of basal cells into secretory cells [Liu et al., 1997]. As a whole, the observations that subpopulations of cells expressing stem cell markers (i.e., CD133, $\alpha2\beta1$, and CD44) and displaying stem-like properties are localized within the basal cell layer suggest that the hierarchy between p63-positive basal cells and secretory cells identified during development is maintained throughout adulthood.

As illustrated above, most of the current knowledge on adult prostate stem cells is based on in vitro and in vivo assays performed on putative stem cell populations purified/enriched on the basis of surface cell marker expression. While this approach is powerful in identifying cell subsets that have stem abilities and are able to reconstitute prostate glands when removed from their natural environment, it does not provide significant information on how the prostate epithelium in situ is renewed either in physiological conditions or after cell injury. This issue can only be addressed by tracing the fate of a specific cell type and its progeny within the adult prostate. State-of-the-art mouse engineering technology allows to genetically label a given cell type and to follow its progeny within the tissue of interest. The Cre-LoxP system, which has been successfully utilized for this purpose [Rajewsky et al., 1996; Rossant and McMahon, 1999], is based on the development of genetically engineered mouse models with selective expression of the Cre recombinase in the cell type of interest [Indra et al., 1999]. Cre expression determines the permanent activation of a reporter gene (e.g., ß-galactosidase, GFP) in all the cells expressing Cre as well as in their progeny. To date, there are no lineage tracing studies that investigate the origin of the differentiated prostate secretory cells. Thus, genetic tracing of the p63-positive basal cell population in the adult prostate has the potential to unequivocally determine whether basal cells represent the adult prostate stem cells during normal homeostasis and upon tissue damage.

p63 AND PROSTATE CANCER

Prostate Cancer Diagnosis

The human prostate is prone to pathology, especially with advancing age. Around the fourth decade, men start developing a benign proliferation of the epithelium and stroma of the prostate called nodular hyperplasia [De Marzo et al., 2003a,b]. Epithelial hyperplasia is not considered to be a precancerous lesion, unless cellular atypia is observed. The latter is called dysplasia, also known as prostate intraepithelial neoplasia (PIN). PIN usually occurs spontaneously and its prevalence increases with age. PIN lesions are divided in two subgroups, low and high grade [Iczkowski, 2006; Humphrey, 2007]. High grade PIN is considered to be a precancerous lesion, and its increase in grade is characterized by the progressive loss of the basal cell layer. The invasion of the basal membrane with infiltration of the stroma represents the hallmark of progression of high grade PIN to actual cancer. The pathological diagnosis of CaP is based on the presence of major and minor microscopic criteria. Major criteria include the absence of the basal cells, infiltrative glandular growth pattern and nuclear atypia. Minor criteria include, among others, mitotic figures, adjacent high grade PIN and nuclear hyperchromasia [Humphrey, 2007].

Immunohistochemical assays for the identification of basal cells represent a valuable tool for the differential diagnosis of benign versus malignant prostatic lesions, when morphology alone is not sufficient for a definitive diagnosis [Varma and Jasani, 2005; Egevad et al., 2006]. In the past decades, basal cell-specific antibodies recognizing HMWCK (e.g., 34ßE12 and cytokeratin 5/6) have been utilized for this purpose [Totten et al., 1953; Brawer et al., 1985; Kahane et al., 1995; Wojno and Epstein, 1995]. The absence of staining for basal cell-specific cytokeratins is almost definitively diagnostic for cancer, excluding rare exceptions (e.g., atypical adenomatous hyperplasia and postatrophic hyperplasia) [Bostwick and Brawer, 1987; Gaudin and Epstein, 1995; Googe et al., 1997; Amin et al., 1999; Yang et al., 1999b; Oliai et al., 2002; Herawi et al., 2005]. Unfortunately, formalin fixation has been shown to affect HMWCK immunoreactivity [Varma et al., 1999]. Indeed, the immunoreactivity of these antibodies diminishes as the time of fixation increases, leading to false negative results.

As previously mentioned, we recently demonstrated that p63 is selectively expressed in the basal cell compartment of normal prostate glands [Yang et al., 1999a; Signoretti et al., 2000]. Importantly, we also showed that p63 is not expressed in the vast majority of prostate carcinomas. Specifically, 126 of 130 (97%) prostate tumors were completely negative for p63 expression, while in the remaining four cases, only a small percentage ($<2\%$) of p63-positive cells were detected. Thus, our study was the first to highlight the potential relevance of p63 as a diagnostic biomarker for CaP in the clinical setting [Signoretti et al., 2000]. Several groups have subsequently replicated

our findings and confirmed that p63 is strongly and diffusely expressed in normal and hyperplastic prostate glands, patchily expressed in proliferative atrophy and high-grade PIN, and almost always absent in prostate adenocarcinoma [Parsons et al., 2001; Oliai et al., 2002; Shah et al., 2002]. To date, the specificity and sensitivity of p63 expression versus other basal cell markers has been investigated in a few studies, which have led to the conclusion that p63 immunohistochemistry offers an advantage compared to HMWCK in the diagnostic evaluation of tissue specimens obtained by transurethral resection of the prostate (TURP) [Shah et al., 2002; Weinstein et al., 2002]. In the attempt to increase the sensitivity of the assay, the combination of HMWCK and p63 staining has been also evaluated. The use of such antibody cocktail allows the identification of both the cytoplasmic (cytokeratins) and nuclear (p63) components of basal cells. However, these analyses have not generated entirely consistent results. While one study showed an increased sensitivity in the detection of the basal cells [Zhou et al., 2003b], results from a second analysis indicated that the advantage of using a basal cell marker cocktail compared to the use of p63 staining alone, was not significant [Shah et al., 2004].

It must be noted that the use of basal cell markers for the diagnosis of CaP is based on the absence of immunostaining and can thus lead to false negative results. As a consequence, a significant advance in the field was achieved through the identification of alpha-methylacyl coenzyme A racemase (AMACR) as a gene selectively expressed in prostate adenocarcinoma [Xu et al., 2000]. AMACR, which plays a role in ß-oxidation of branched chain fatty acids, was identified by high throughput microarray screening of prostate carcinoma [Xu et al., 2000]. This marker was first reported by Jiang and co-workers to be selectively expressed in CaP cells, with rare expression in normal prostate glands [Jiang et al., 2001]. Several studies have evaluated immunohistochemical expression of AMACR as a molecular marker of CaP [Jiang et al., 2001, 2002; Beach et al., 2002; Rubin et al., 2002; Yang et al., 2002; Magi-Galluzzi et al., 2003; Zhou et al., 2003a; Wu et al., 2004; Hameed et al., 2005]. The sensitivity of the assay is high but varies among studies, ranging from 68 to 100%. Importantly, it has been shown that the sensitivity of AMACR immunostain depends on the type of antibody used, fixation protocols and tissue processing methods [Magi-Galluzzi et al., 2003].

In summary, the immunohistochemical assays currently utilized to facilitate CaP diagnosis either document the absence of the basal cell compartment (HMWCK and p63), or specifically stain the neoplastic epithelium (AMACR) [Varma and Jasani, 2005; Egevad et al., 2006]. Very recently, the combination of such markers has also been successfully introduced in the clinical setting [Molinie et al., 2004; Sanderson et al., 2004; Hameed et al., 2005]. Importantly, the p63/AMACR antibody cocktail presents increased sensitivity compared to the use of a single marker and is highly recommended for the evaluation of small, diagnostically difficult foci in prostate needle biopsy tissue [Luo et al., 2002; Molinie et al., 2004; Zhou et al., 2004; Hameed et al., 2005].

Cell of Origin of Prostate Cancer

Cancer develops through the progressive acquisition of multiple genetic and epigenetic changes. Recent work in the hematopoietic system suggests that the alterations required for cancer development might vary according to the differentiation stage of the target cell [Daley, 2004; Jamieson et al., 2004]. As a consequence, the identification of the cell(s) of origin of CaP is essential for a full understanding of the molecular events underlying prostate carcinogenesis and the potential development of more effective therapeutic strategies.

Since the vast majority of CaPs display a secretory phenotype, it has been generally assumed that prostate carcinoma originates from the malignant transformation of secretory cells [De Marzo et al., 1998b]. However, a subset of CaPs, especially in androgen-independent stage, contain a cell population co-expressing both secretory and basal cells markers, suggesting that they might originate from the putative basal stem cell population [Jones and Harper, 1992; Verhagen et al., 1992; Peehl et al., 1994; Bonkhoff and Remberger, 1996; Robinson et al., 1998; Xue et al., 1998; De Marzo et al., 1998a; Hudson et al., 2001; Wang et al., 2001].

The CSC model predicts that only a small subset of neoplastic cells with stem-like capacities promote the maintenance and development of the tumor. Efforts aimed at isolating putative prostate CSCs from cultured CaP cell, xenografts, and patient tumors are currently

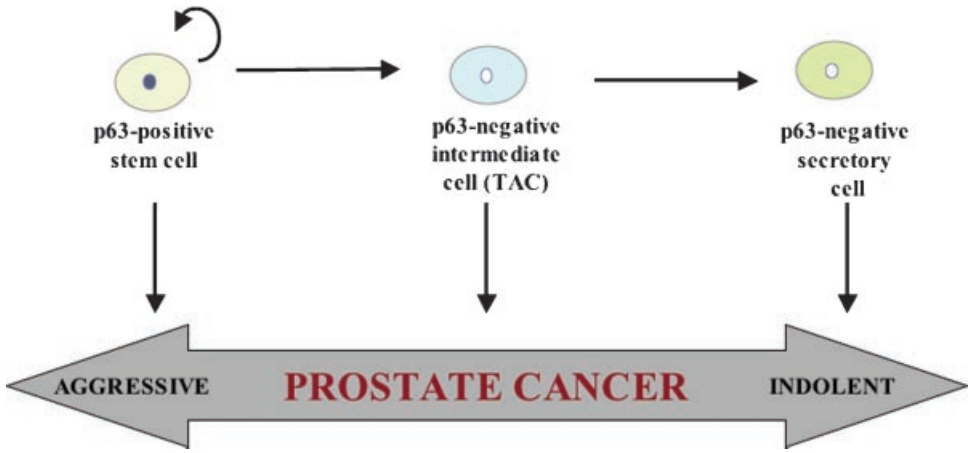

Fig. 3. Hypothetical model of prostate cancer origin. Prostate carcinoma can originate from multipotent basal stem cells, intermediate cells, or differentiated secretory cells. The differentiation stage of the target cell correlates with the clinical behavior of the tumor.

underway [Tang et al., 2007]. In hematologic malignancies, CSCs have been shown to derive from normal hematopoietic stem cells characterized by oncogene activation [Daley, 2004]. Alternatively, however, there is evidence that genetic/epigenetic changes occurring in committed progenitor cells can cause them to become neoplastic through dedifferentiation and acquisition of stem cell characteristics (i.e., self-renewal) [Jamieson et al., 2004]. In line with these recent findings, it is possible to speculate that different subsets of CaP may originate from cells that are in different stages of differentiation (Fig. 3). Intriguingly, the most lethal form of CaP, that is, androgen independent CaP, might emerge from androgen independent, p63-positive basal stem cells. It must be noted, however, that the validation of this hypothetical model of CaP origin cannot be achieved without the definitive identification of adult prostate stem cells and their differentiation lineages.

CONCLUDING REMARKS

The discovery of the p63 gene and its critical function in genitourinary epithelia has recently lead to important progress not only in the diagnosis of prostate carcinoma but also in the understanding of the basic mechanisms that regulate prostate organogenesis. Future studies aimed at assessing the role of p63 in the maintenance of adult prostate stem cells are required. Importantly, the identification of the cell type(s) that maintain the homeostasis of the adult prostate epithelium is likely to open novel research avenues that will significantly accelerate our understanding of the mechanisms underlying CaP development and progression.

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