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Prostate cancer stem cells

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ABSTRACT

Prostate cancer is the most frequently diagnosed cancer in men. Despite recent advances in the detection of early prostate cancer there is little effective therapy for patients with locally advanced and/or metastatic disease. The majority of patients with advanced disease respond initially to androgen ablation therapy. However, most go on to develop androgen-independent tumours that inevitably are fatal. A similar response is seen to chemotherapeutic and radiotherapy treatments. As a result, metastatic prostate cancer remains an incurable disease by current treatment strategies. Recent reports of cancer stem cells have prompted questions regarding the involvement of normal stem/progenitor cells in prostate tumour biology, their potential contribution to the tumour itself and whether they are the cause of tumour initiation and progression. Although still controversial, the cancer stem cell is likely to be the most crucial target in the treatment of prostate cancer, and a thorough understanding of its biology, particularly of how the cancer stem cell differs from the normal stem cell, might allow it to be targeted selectively and eliminated, thus improving therapeutic outcome.

1. Introduction

Stem cells have an extensive capacity for self-renewal. This property enables their maintenance over the lifetime of the host, making them excellent candidates for the cells of origin of cancer. It was a remarkable series of transplant experiments, initiated in the 1960s, which proved that cancers are composed of a heterogeneous population of cells with marked differences in their potential to self-renew and reconstitute the tumour upon transplantation.[1–4] These early observations led the investigators to speculate that the entire population of a tumour's cells might arise from a small number of 'cancer stem cells'.[3] Two theories were proposed to explain these observations.[5] The stochastic theory predicts that every cell within a tumour is potentially tumour-initiating, but the probability that every cell will enter the cell cycle is low and is controlled stochastically. In contrast, the hierarchy theory proposes that the tumour is functionally heterogeneous and only a subpopulation of cells is capable of initiating tumour growth. Whilst the stochastic model predicts that the tumourigenic mechanisms are operative in all cells (and that studying the bulk population would identify the key properties of the tumour), the hierarchical model predicts that rare tumour-initiating cells are distinct from the non-tumour-initiating, bulk population of the tumour. This model also predicts that eradication of the bulk of the tumour may result in remission, but if the tumour-initiating cells (cancer stem cells) are not eliminated, the tumour will re-grow (Fig. 1). To differentiate between these two models it is necessary to define distinct populations of cells within tumours (based on cell surface phenotype), to purify them and to determine their function in vitro and in vivo. This was accomplished by Bonnet and Dick[6] who demonstrated that the $CD34^+$ cell fraction from patients with acute myeloid leukaemia (AML) contained all the leukaemia-initiating cells. Since this seminal paper, similar studies have demonstrated that sub-populations of tumour cells from breast[7] and brain[8] have cancer stem cell characteristics. These studies conclusively

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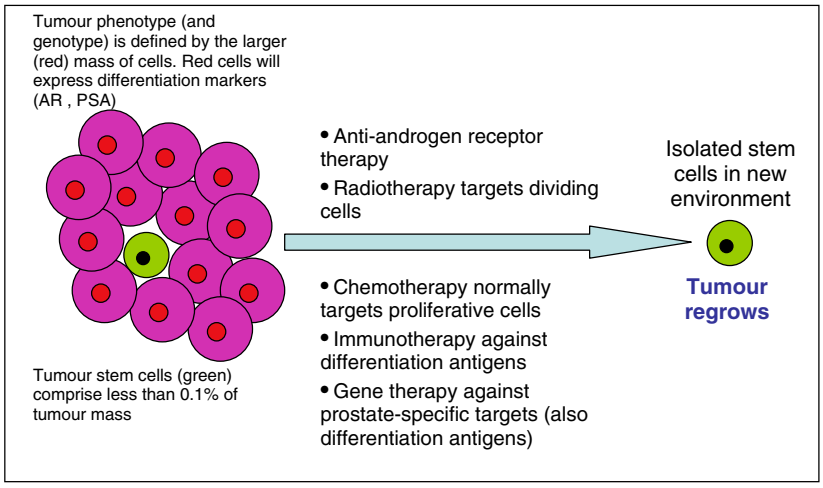

Fig. 1 – Hierarchical or stem cell model of cancer. Eradication of the bulk of the tumour will result in remission, but if the cancer stem cells are not eliminated the tumour will re-grow.

showed that the cancer or leukaemic clone is organised as a hierarchy in which only a rare subset of cancer cells possess the ability to initiate new tumour growth and recapitulate the original tumour heterogeneity.

2. Architecture of the prostate epithelium and its patterning during development

The prostate develops from the urogenital sinus in response to testosterone stimulation. The embryonic prostate initially consists of a multilayered epithelium surrounded by mesenchyma. In a process of ductal budding, which starts at 10 weeks of gestation, multiple epithelial outgrowths invade the surrounding mesenchyma. These epithelial buds form ducts that elongate and branch out from the urethra and terminate into acini. From the 20th week of gestation up to puberty, the immature prostatic acini and ducts are lined with multiple layers of immature cells with round nuclei and scant cytoplasm. Postnatal development includes a period of growth during the first year, quiescence during childhood, and further growth with the testosterone surge at puberty. During puberty, the immature multilayered epithelium differentiates into a two-layered epithelium consisting of peripheral flattened to cuboidal basal cells and inner secretory cylindrical epithelium[9] (Fig. 2).

Three main cell types are discernible within normal, mature prostatic epithelium: basal, secretory luminal and neuro-endocrine. The luminal or glandular cells constitute the exocrine compartment of the prostate, secreting prostate specific antigen (PSA) and prostatic acid phosphatase (PAP) into the glandular lumina. They are terminally differentiated, and represent the major cell type in normal and hyperplastic epithelium. They express high levels of the androgen receptor (AR)[10] and are dependent on androgens for their survival.[11] In contrast, basal cells are relatively undifferentiated and lack secretory activity. As their name suggests, basal cells rest on the basement membrane and morphologically they range from small flattened to cuboidal cells. They express low/undetectable levels of AR[12] and are independent of androgens for their survival.[11] Basal cells focally express the oestrogen receptor and proliferate under oestrogen therapy.[13]

Significant populations of neuro-endocrine cells also reside among the more abundant secretory epithelium in the normal prostate gland. These cells are found in the epithelium of the acini and in ducts of all parts of the gland. The major type of neuro-endocrine cell contains serotonin and thyroid-stimulating hormone. Neuro-endocrine cells are terminally differentiated, post-mitotic cell types that are androgen-insensitive.[14]

3. Experimental evidence for prostate epithelial stem cells in the adult gland

The existence of stem cells in the prostate is probably best illustrated by androgen cycling experiments in the rodent. The prostate is an androgen-dependent organ that undergoes involution after castration, but can completely regenerate if androgen levels are restored. As this cycle of involution–castration can be repeated many times, a population of long-lived, androgen-independent stem cells, responsible for the regeneration of the gland must exist.[15] These results led Isaacs and Coffey[16] to propose a stem cell model for prostate epithelia (Fig. 3), whereby androgen-independent stem cells give rise to a population of androgen-responsive (but independent) transit amplifying cells. These cells can respond to androgens and amplify the number of androgen-dependent, secretory luminal cells. This point is emphasised by the fact that it is possible to castrate adult male rats and allow an extended period (i.e. >3 years) before replacing androgen and still fully restore the gland.[16]

In the murine prostate, opinion is divided on the location and identity of stem cells. Each prostatic duct consists of a proximal region attached to the urethra, an intermediate region and a distal tip.[17] Proliferating cells are located at the tips of the ducts and can undergo significant growth when grafted, under the renal capsule, in combination with embryonic urogenital sinus mesenchyme.[18] Based on this finding, it was suggested that prostatic stem cells reside in the distal

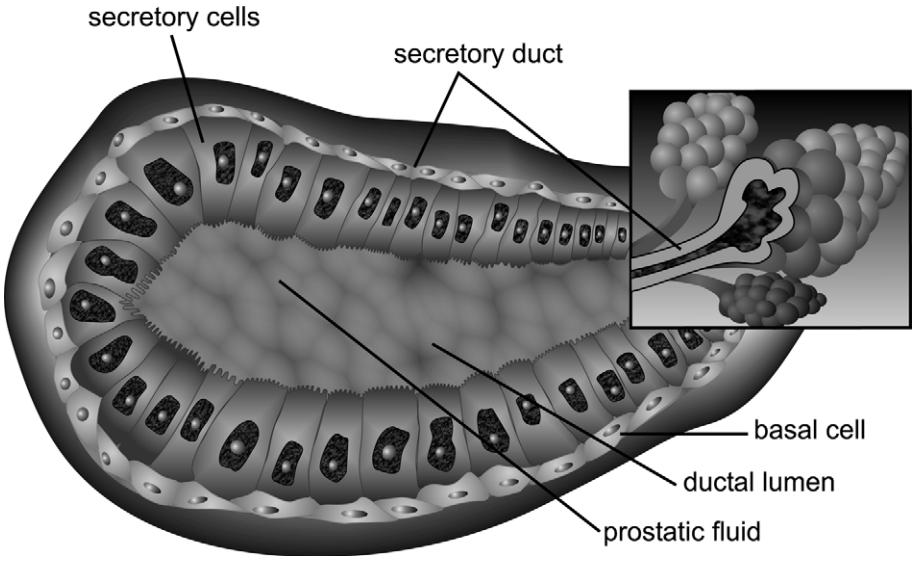

Fig. 2 – Organisation of the prostate gland. Cross-section of the ductal region with labels indicating cell types present in prostatic ducts, including luminal secretory cells and basal cells.

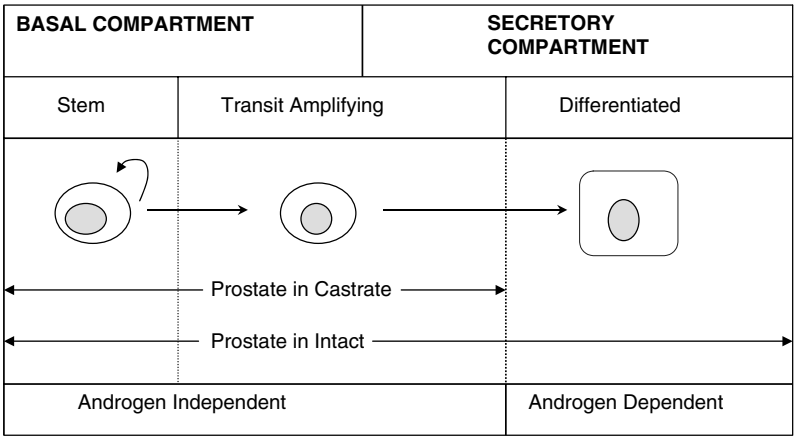

Fig. 3 – Stem cell model for the organisation of the prostate epithelium. Stem cells in the basal cell layer give rise to a population of transit amplifying (TA) cells, which in turn differentiate into the functional, secretory luminal cells. The survival of the secretory luminal cells is dependent upon androgens.

region.[18] However, Tsujimura and colleagues[19] demonstrated that the proximal region is enriched in a subpopulation of epithelial cells that are cycling slowly, possess a high in vitro proliferative potential and can reconstitute highly branched glandular ductal structures in collagen gels. They proposed that prostatic epithelial stem cells are concentrated in the proximal region of the ducts and give rise to the proliferating transit-amplifying cells that migrate distally. Both these studies indicate that a stem cell hierarchy exists in the prostate, as luminal and basal cell layers can be regenerated from proximal and distal tissue. More recent work has concentrated on identifying stem cells using cell surface markers, such as Sca-1.[20] These authors demonstrated that $Sca1^{+}$ enriches for a prostate-regenerating cell population that is concentrated in the proximal region of the mouse prostatic duct. However, they also reported sporadic Sca-1 expression in the distal region of ducts and regenerating activity could also be attributed to $Sca\text{-}1^{-}$ cells.[20] Further functional studies are needed to definitively identify the stem cell in murine prostate. These should include self-renewal of enriched populations' in vitro and serial transplantation of homogeneous cell populations in an immune-deficient host.

Although there is no single definition for a stem cell, there is general agreement that such a cell should exhibit clonogenicity and, more importantly, the ability to regenerate the different cell types that constitute the tissue in which it exists. Thus, transplanted cells should be capable of self-renewal and producing progeny that differentiate into a fully functional epithelium. Basal cells directly isolated from human prostatic tissue, on the basis of rapid adherence to type I collagen, are clonogenic[21,22] and can reconstitute prostatic-like glands in immunocompromised mice with morphological and immuno-histochemical evidence of prostate-specific differentiation.[21] Unlike the previous studies in murine prostate, our group observed that in the human prostate, $\alpha_2\beta_1^{hi}$-selected cells (the rapidly adherent population) were randomly

distributed throughout acinar and ductal regions.[21] In order to augment our initial stem cell marker, integrin $\alpha_2\beta_1^{hi}$, we recently identified CD133, which is also expressed by primitive haematopoietic,[23] endothelial,[24] neurones and glial[25] stem cells as a further stem cell marker for prostate epithelia.[26] CD133 cells are restricted to the $\alpha_2\beta_1^{hi}$ population and are located in the basal layer of the prostate, often at the base of a budding region or branching point (Fig. 4). $\alpha_2\beta_1^{hi}$/CD133$^+$ cells exhibit two important attributes of epithelial stem cells: they possess a high in vitro proliferative potential and can reconstitute prostatic-like acini in immune-compromised male nude mice with concomitant expression of differentiation markers, such as AR, PAP and K18.[26]

Of relevance to the determination of lineage(s) is the finding that cells that are morphologically and phenotypically intermediate between basal and luminal cells have been identified within the normal prostatic epithelium. For example, electron microscopic studies of the human prostate have identified foci of cells with morphological features typical of basal and secretory cells.[27] Brandes[28] noted similar transitional forms of basal cells with similarities to luminal cells following experimental castration and androgen administration.

Cytokeratin expression serves as a valuable marker for epithelial phenotypes. Analysis of cytokeratins in normal, hyperplastic and malignant prostate[29–33] as well as primary[34] and organ cultures[35] has identified cell phenotypes intermediate between basal and luminal cells. PSA-expressing cells have even been identified within the basal layer.[36] Bonkoff and colleagues also reported simultaneous expression of neuro-endocrine markers with PSA and neuro-endocrine cells expressing basal-specific cytokeratins.[36] These observations demonstrate that basal and luminal cells are linked in a hierarchical pathway, but to resolve the issue of lineage it will be necessary to track the progeny and differentiation of a marked or isolated stem cell – either as a clonal regeneration assay (regenerating a culture from a single cell) or using a transfected marker.

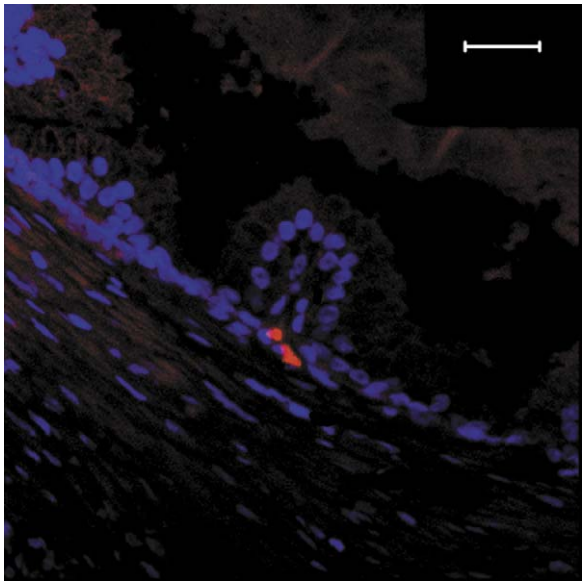

Fig. 4 – A rare sub-set of basal cells express CD133$^+$. A paraffin section of prostatic acini labelled with the nuclear stain DAPI (blue) and anti-CD133 directly conjugated to PE (red).

4. Cellular origin of cancer stem cells

The cellular origins of prostate cancer are still controversial. It has been suggested that prostate cancers arise from the terminally differentiated luminal cells[37–39] because the bulk population of tumour cells, in the most common form of prostate cancer, express luminal cell-specific markers (cytokeratins 8, 18 AR, PSA and PAP), but lack expression of basal cell markers, such as p63. Other studies suggest that the disease is derived from intermediate progenitors that have acquired the ability to self-renew.[40] Xin and colleagues[20] recently reported that introduction of constitutively active AKT in Sca-1-enriched murine prostate epithelial cells resulted in the initiation of prostate tumourigenesis. Moreover, Sca-1 sorted cells, enriched for cells with prostate-regenerating activity, showed evidence of basal and luminal lineage. Liu and colleagues[41] also observed that most primary tumours consist of luminal cells, whereas the majority of metastases contain basal cells.

The stem cell model proposes that mutations occur in stem cells only. According to this model, heterogeneity results from the variable ability of these primitive cancer stem cells to differentiate (depending on the influence of specific transformation or progression-related genes). Thus, the observation that the majority of prostate cancer cells express markers specific for the secretory cell compartment reflects the ability of the cancer stem cell to give rise to progeny that are characteristic of the tissue from which they were derived.

While the number of mutations necessary to induce neoplastic change is debatable, it is generally accepted that cancer development occupies a significant time-span in humans. In many tissues the restricted progenitors and differentiated cells tend to have a short life-span. In contrast to the stem cells, which persist throughout life in these tissues, there is little opportunity for mutations to accumulate in the progenitor/differentiated cells. The critical signalling pathways that regulate stem cell maintenance are associated with carcinogenesis. For example, bcl-2 and telomerase (normally restricted to the basal cells in normal prostate) are overexpressed in prostate cancer.[42,43] Many other intracellular signalling pathways, such as Notch, Sonic hedgehog and Wnt which regulate stem cell self-renewal, are also associated with oncogenesis [reviewed in 44]. As cancer stem cells must self-renew, it is logical to suppose that they are derived from either self-renewing normal stem cells or from progenitors that have acquired the ability to self-renew (Fig. 5). In leukaemia there is now strong evidence in support of stem cells as targets for mutation.[6,45] The evidence is not so definitive for solid tumours. This is partly due to the paucity of cell surface markers available to enable cell sorting of normal tissue stem cells. Recently, Singh and colleagues[8] demonstrated that CD133, which is a marker for neural stem cells, is present in a high proportion of brain tumours and that this marker could be used to isolate the tumour-initiating population.

To address the cellular origins of prostate cancer, we have looked for cells within prostate tumours that expressed the prostate epithelial stem cell markers $\alpha_2\beta_1^{hi}$/CD133$^+$, in a series

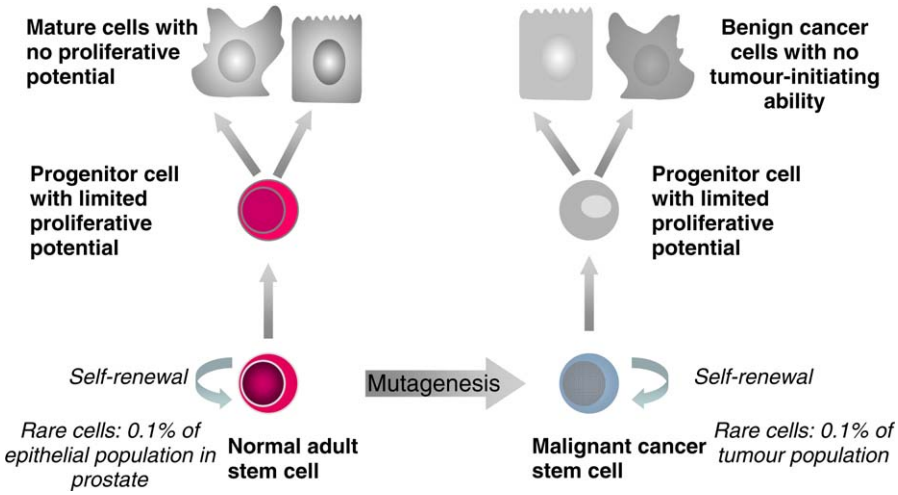

Fig. 5 – Stem cell model of normal tissue renewal and prostate cancer. The malignant stem cell arises from transformation of the normal stem cell. The histology of the tumour reflects the stage of differentiation arrest.

of biopsies from patients with primary and metastatic prostate cancer. Approximately 0.1% of tumour cells expressed this phenotype ($n > 40$). Similar to our previous findings with normal prostate epithelial stem cells, the tumour stem cells have a basal phenotype, proliferate extensively in vitro, unlike the more differentiated tumour phenotypes. Tumour-derived $\alpha_2\beta_1^{hi}/CD133^+$ cells also differentiate in vitro to an AR^+/PAP^+ phenotype after treatment with androgen (data not shown).

Ultimately, it will be necessary to determine whether prostate cancer cells that express $\alpha_2\beta_1^{hi}/CD133^+$ are tumour-initiating in immune-deficient hosts and can recapitulate the original tumour heterogeneity in vivo.

5. Conclusion and future directions

If we view prostate cancer as a differentiating system in which the initiating cell represents the most primitive (stem) cell, this provides a new framework for viewing the cellular and molecular mechanisms that underlie the heterogeneity seen in prostate cancer. The goal of existing therapies has been to eradicate the bulk of cells within a tumour. However, most patients go on to develop androgen-independent disease, which remains incurable by current treatment strategies. The major unanswered question remains as to the origin of this resistance: is it that existing therapies fail to kill cancer stem cells effectively? Normal stem cells from various tissues appear to be more resistant to chemotherapeutic reagents than do mature cell types[46] and characteristically express drug-resistance proteins, such as MDRI and ABC transporters.[47,48] If this were true of cancer stem cells, therapies that are more specifically directed at the cancer stem cell might result in more durable responses for primary as well as metastatic disease.

The identification of cancer stem cells in AML,[6] breast cancer[7] and brain cancer[8] raises the question as to the extent to which these cells have a different gene expression profile from non-tumour-initiating cancer cells. To date, DNA and tissue microarrays of tumours have failed to account for cellular heterogeneity and differences in the proliferative potential of the different populations within tumours. By directing expression analysis to enriched populations of tumourigenic cancer cells, the identification of novel diagnostic therapeutic targets should be more effective.

Conflict of interest statement

None declared.

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