The prostate is a glandular organ comprising three distinct cell types (basal, luminal and neuroendocrine) embedded in a fibro-muscular stroma (see Figure 1). Prostate basal cells form a layer along the basement membrane of each duct, and luminal cells form a layer above the basal cells.

Neuroendocrine cells are a minor population scattered throughout the basal layer. 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 about 60 per cent of the cell mass in the normal prostate, but in prostatic cancer they account for about 99 per cent of the cell mass. These luminal cells express high levels of the androgen receptor and are dependent on androgens for their survival (1). In contrast, basal cells are relatively undifferentiated, lack secretory activity and express low/undetectable levels of androgen receptors and are independent of androgens for their survival (2).

Prostate cancer remains a clinically and biologically difficult problem for translational researchers; with improved methods for the selection, propagation and phenotypic analysis of the critical tumour-initiating cells, real progress towards rational therapies can now be made.

Prostate cancer is the most frequently diagnosed cancer in men and is second only to lung cancer as the leading cause of male cancer-related mortality. For organ-confined disease, initial treatment is prostatectomy or radiation, which is usually curative. However, approximately 20 per cent of patients are not cured by such treatments and their cancer recurs – sometimes with long latencies – and some patients are diagnosed only after the cancer has spread. Despite recent advances in the detection of early prostate cancer, there remains 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, due to the androgen-dependent nature of the vast majority of prostate cancer cells. However, with very high frequency, androgen-independent cancers emerge and subsequently widespread metastases occur. Despite the frequency of prostate cancer, we know little about the target cells for oncogenic change and, in particular, the tumour initiating cell – both within the prostate and at the preferential sites of metastatic disease in bone.

Cancer Stem Cells

Cancer stem cells (CSCs) are a sub-population of cancer cells that possess characteristics associated with normal stem cells (SCs), such as self-renewal and the ability to differentiate into multiple cell types. CSCs are tumorigenic (tumour-forming), in contrast to the bulk of cancer cells, which are thought to be non-tumorigenic. CSCs persist in tumours as a distinct population, and cause relapse and metastasis by giving rise to new tumours as they have been shown to be resistant to radio- and chemotherapy (3). The CSC hypothesis infers that if the CSC were eliminated, the tumour would simply
regress due to differentiation and cell death. Normal SCs appear to be the ideal candidates for cancer transformation because cells with long lifespans are more likely to accumulate the appropriate number and variety of genetic lesions necessary to acquire full tumorigenic capacity. In addition, normal SCs, which are already indefinitely self-renewing, need a few number of mutations to be transformed into malignant cells.

CSC Markers

The identification of CSCs by the use of specific phenotypic markers represents an essential tool for their isolation, further biological characterisation and the development of new therapeutic strategies that selectively target them. The first identification of CSCs as CD34+CD38− leukaemia-initiating cells in acute myeloid leukaemia (AML) was demonstrated by John Dick and colleagues, who characterised these rare cells by surface markers overlapping with normal hematopoietic stem cells but distinct from the bulk of the tumour (4). More recent studies have demonstrated that a minor population of tumour-initiating cells resides within solid tumours, including brain, breast and colon tumours (5-6). These tumour-initiating cells were identified by their unique surface markers overlapping with normal hematopoietic stem cells but distinct from the bulk of the tumour. The defining surface markers for the tumour-initiating population are thought to overlap with stem cell markers in the respective normal tissues. In solid tumours the CSCs were also in many cases phenotypically similar to the resident normal tissue SCs. CD44+CD24−ESA+ CSCs were identified in breast carcinoma (8), while CD133+ CSCs have been described in cancers from a variety of tissues including brain, prostate, pancreas, colon, lung, liver and ovary (9). Other CSC markers that have been utilised include the membrane ATP-binding cassette (ABC) drug transporters, and the detoxifying enzyme aldehyde dehydrogenase 1 (ALDH1), which has been involved in drug resistance (8,9).

Prostate Cancer Stem Cells

As the stem cell is likely to be the cell in the prostate with the most opportunity to acquire and accumulate mutations, predisposing to tumour development, it seemed most likely that it would share antigen expression with its tumour-initiating counterparts. Accordingly, Collins et al used the CD133+/α2β1 integrin+/CD44 phenotype to select for cells with high clonogenicity and an invasive capacity (10). These cells represent approximately one per cent of the cells in the normal prostate but are rare in prostate cancer biopsies (see Figure 1). The cancer-derived cells also had a longer lifespan and a higher cell output capacity than their normal equivalents. The cells were basal in phenotype and showed some evidence of the genomic instability required for an adaptable cancer stem cell.

Prostate CSC Markers

To generate new markers for the prostate CSC population, Maitland’s laboratory has recently carried out whole-genome expression analysis using RNA from purified cell populations (11). A unique, cancer-specific phenotype was detected, but only when tumours containing a detectable Gleason pattern 4 were scored as cancers. The gene signature provided clear evidence of different gene sets expressed in the CSCs, their amplifying progeny and the normal equivalents. In addition to a cancer-specific signature, there was also a set of differentiation-specific genes – some of which were already well established in the prostate such as c-met, prostate stem-cell antigen and microseminoprotein B – which could be segregated from the tumour signature. The prostate CSCs also share the expression of a number of cell-surface antigens with their normal counterparts; for example, over-expression of beta 1 integrin, CD44 and CD133 were all
independently confirmed by the expression analysis. One of a new series of markers for prostate CSC identified by the Maitland group was a unique cell surface target that is over-expressed in prostate CSCs and transit-amplifying (TA) cells, but is absent from basal cells (see Figure 2). Knock-out of this target leads to inhibition of colony forming capacity and proliferation of tumorigenic prostate cells in vitro, and inhibition of tumour formation in vivo.

At Pro-Cure Therapeutics, we are developing a humanised monoclonal antibody (mAb) therapeutic that targets PTT256, one of our proprietary targets. PTT256 is a cell surface single pass transmembrane protein that is up-regulated in prostate cancer stem cells. This is a novel and attractive therapeutic target for prostate cancer as:

- It is up-regulated in cancer stem cells relative to benign cells and expressed in TA cells
- It is not significantly expressed in other tissues
- We have shown that inhibition of this target prevents tumour initiation in vivo
- PTT256 is localised on the cell surface in primary human prostate cancer cells: CSCs and TA cells

Table 1: Prostate CSC resistance to conventional cancer therapy

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Primary Resistance</th>
<th>Secondary Resistance</th>
</tr>
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<tbody>
<tr>
<td>Surgery</td>
<td>Residual tumour</td>
<td>Genomic instability</td>
</tr>
<tr>
<td>Androgen therapy</td>
<td>CSCs AR negative</td>
<td>Genomic instability</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>CSCs divide slowly</td>
<td>Expression of known radio-resistance</td>
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<tr>
<td></td>
<td></td>
<td>genes elevated in CSCs</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>CSCs divide slowly</td>
<td>Expression of drug resistance genes</td>
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<tr>
<td></td>
<td></td>
<td>including ATP-Binding Cassette (ABC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>transporters in stem cells</td>
</tr>
<tr>
<td>Immunotherapy/vaccine</td>
<td>Normally directed against</td>
<td>Genomic instability</td>
</tr>
<tr>
<td></td>
<td>common and differentiated cell</td>
<td></td>
</tr>
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<td></td>
<td>antigens not expressed in CSCs</td>
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<tr>
<td>Gene therapy</td>
<td>CSCs do not express common</td>
<td>Genomic instability</td>
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<td></td>
<td>differentiated cell targeting</td>
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</tr>
<tr>
<td></td>
<td>molecules</td>
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</tbody>
</table>

CSCs and Therapy Resistance in Prostate Cancer

An important consequence of the well-documented CSC resistance to radiation and chemotherapy is that these therapies often serve to enrich the resistant CSC sub-population and in addition have little effect on the ability of the remaining CSCs to regrow tumours (12). Thus, CSC enrichment may be the basis for the relative inability of most single modality cancer treatment strategies to control long-term cancer growth.

The gene expression patterns of CSCs and amplifying cells in the prostate can also provide some clues as to mechanisms of therapy resistance. In other cellular systems, the residual disease is frequently of a primitive phenotype, distinct from the more differentiated cells treated in the original disease (13). In prostate cancer, the androgen receptor (AR)-negative, basal phenotype of the CSCs provides a ready explanation for minimal residual disease after castration therapies (see Table 1).

How to Eliminate CSCs in the Prostate

Therapeutic strategies to repair or eliminate mutated stem cells are at their earliest stages, and the dangers associated with targeting ‘stemness’ per se (for example, the Wnt, Notch and hedgehog pathways) remain to be assessed in the clinic (14). In determining the phenotypic differences between normal and malignant stem cells in the prostate, there is a phenotypic signature that can differentiate not only cancer from benign cells, but also stem from TA cells ((11), see Figure 1). The cancer element is more tractable, and overlaps the stem and TA compartments; destruction of stem and TA cells would provide a more lasting therapy than just elimination of the more differentiated progeny cells as is used in current clinical practice. By selectively targeting CSCs, it would be possible to treat patients with aggressive, non-resectable tumours, as well as to prevent the tumour from metastasising. Thus, to target the stem-cell compartment for the elimination of CSCs will require new strategies and assay systems that are quite distinct from those used to derive the anti-proliferative therapies that are the currently favoured targets of the pharmaceutical industry (see Figure 3, page 24).

A promotion from the stem to the amplifying compartment would include an increase in growth rate, and would make the stem cells susceptible to conventional killing. Induction of differentiation from...
the AR-negative stem and TA compartments would
also render the stem cells susceptible to multiple anti-
androgen therapies. Ultimately, therapies directed at
all cellular compartments in the prostate are likely
to be the most successful, by exposing the stem-cell
compartment and treating it with a CSC drug (such as
anti-PTT256) after destruction of cells that express the
AR-positive luminal phenotype.

Conclusion

Prostate cancer remains a clinically and biologically
difficult problem for translational researchers. By
invoking a CSC mechanism, many of the confounding
features of this common tumour can be explained,
particularly with respect to therapy resistance. With
improved methods for the selection, propagation and
phenotypic analysis of the critical tumour-initiating
cells, real progress towards rational therapies can now
be made. A key feature of the stem-cell phenotype is
the separable nature of differentiation from malignancy
– the targeting of which should complement the
current androgen- and proliferation-based approaches
to therapeutic intervention.

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