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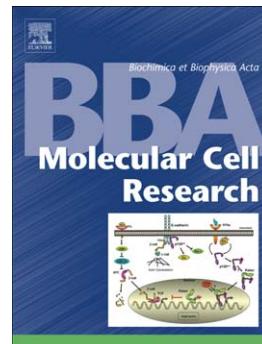
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The Molecular and Cellular Origin of Human Prostate Cancer

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Highlights

- Cancer stem cells (CSCs) provide a cellular origin for prostate cancer.
- The distinctive phenotype of CSCs overlaps that of normal prostate stem cells.
- Inflammation has a synergistic role in prostate tumourigenesis.
- Pre-malignancies support pre-tumour development in the prostate.
- Genetic and epigenetic aberrations create divergent molecular subtypes of cancer.

Abstract

Prostate cancer is the most commonly diagnosed male malignancy. Despite compelling epidemiology, there are no definitive aetiological clues linking development to frequency.

Pre-malignancies such as proliferative inflammatory atrophy (PIA) and prostatic intraepithelial neoplasia (PIN) yield insights into the initiating events of prostate cancer, as they supply a background “field” for further transformation. An inflammatory aetiology, linked to recurrent prostatitis, and heterologous signalling from reactive stroma and infiltrating immune cells may result in cytokine addiction of cancer cells, including a tumour-initiating population also known as cancer stem cells (CSCs). In prostate tumours, the background mutational rate is rarely exceeded, but genetic change via profound sporadic chromosomal rearrangements results in copy number variations and aberrant gene expression.

In cancer, dysfunctional differentiation is imposed upon the normal epithelial lineage, with disruption/disappearance of the basement membrane, loss of the contiguous basal cell layer and expansion of the luminal population. An initiating role for androgen receptor (AR) is attractive, due to the luminal phenotype of the tumours, but alternatively a pool of CSCs, which express little or no AR, has also been demonstrated. Indolent and aggressive tumours may also arise from different stem or progenitor cells.

Castrate resistant prostate cancer (CRPC) remains the inevitable final stage of disease following treatment. Time-limited effectiveness of second-generation anti-androgens, and the appearance of an AR- neuroendocrine phenotype imply that metastatic disease is reliant upon the plasticity of the CSC population, and indeed CSC gene expression profiles are most closely related to those identified in CRPCs.

Introduction

Prostate cancer is, apart from hereditary cases, a disease of old age with a peak incidence in men of ~70 years. (1) No evident epidemiology supports the high frequency of this malignancy, compared to chemical carcinogenesis such as smoking for lung cancers. (2) The heterogeneity of the gland itself has imposed many barriers in the search for an aetiological origin of prostate cancer. (3)

The human prostate is a glandular organ situated inferior to the bladder, **Figure 1A.** composed of epithelial acini arranged in a fibromuscular stromal network. The epithelia are highly organised into a contiguous basal layer containing 3 major cell sub-types; stem, transit amplifying (TA) and committed basal (CB) cells, which account for ~40% of total epithelial cell numbers, and a layer of columnar secretory luminal cells that make up the rest of the epithelium. **Figure 1B.** (3,4)

Figure 1. Human prostate anatomy and epithelial cell constitution

- A. *The prostate is located beneath the bladder and is composed of 3 distinct zones; the central zone (CZ) that contains the ductal tube from the seminal vesicle to where it meets the descending urethra, the peripheral zone (PZ) which is situated at the posterior of the gland and is the region from where the vast majority of PIN and cancer arises, and the transitional zone (TZ) that is directly below the bladder and surrounds the transitional urethra. BPH occurs in this region of the prostate.*
- B. *Diagrammatic representation of a normal prostatic acinus; the epithelial bilayer of basal and luminal cells, surrounded by fibromuscular stroma. The relative content of different epithelial cells in the normal prostate are summarised graphically; luminal (60%), basal (40%) with the stem cells constituting ~1% of total epithelia.*
- C. *Cellular composition of a cancerous acinus. Cancer is characterised by luminal hyperproliferation, loss of the basal layer, breakdown of basement membrane, immune cell infiltration and stromal reactivity. Cancer skews the epithelial cell percentages; the luminal cells make up >99% of tumours, basal CSCs are estimated to constitute <0.1% of tumour epithelial cells.*

It is likely that tissue stem cells source the prostatic epithelial lineage; asymmetric division produces a non-stem daughter TA cell that bulks the basal layer through rapid mitosis and differentiation into CB cells. **Figure 2A.** In humans, the end-stage basal cell then develops into a terminally differentiated luminal cell, and moves towards the acinar lumen. (5–7) In prostate cancer, and indeed in the disease's postulated pre-malignancies, there is disruption of the epithelial lineage, followed by a skewing of cell population numbers. (8) Prostate carcinomas present with a dominant luminal phenotype, disruption or absence of the basement membrane, (9,10) and contain reactive stroma that aid tumour growth through heterotypic signalling. **Figure 1C.** (11–15) In the search for a cell-of-origin that can establish the tumour, it is logical to suppose that the offender must be of a luminal phenotype. However a basal cell origin provides a less obvious but increasingly supported alternative in man. (16)

This review is centred upon discerning the origins of human prostate cancer and is divided into three parts. The first discusses the cellular origins of prostate cancer, the second; prostate cancer's inflammatory aetiology, and the third – the pre-malignancies of the disease alongside the specific molecular defects of the cancer.

Are all Tumour Cells Equal?

The prostate is composed of variable cell populations; within an organised epithelial lineage, a fibro-muscular stromal network, the endothelial vasculature and a variety of immune cells. **Figure 1B.** Most tumours represent a tissue established by a dysfunctional differentiation program based upon a similar cellular hierarchy. (17,18) Currently there are two hypotheses that account for the heterogeneity evident in cancers; malignancies that have probably arisen from multiple genetic and epigenetic changes in a single cell, and the existence of a cancerous progenitor cell which sources a dysregulated differentiation program to form the tumour.

Clonal Evolution or Stochastic Model

In this model, a definitive transformational event first affects an oncogenic or tumour suppressing gene, and a subsequent mitosis produces another cell that has equal tumour propagating potential. Heterogeneity is generated by further step-wise mutation in the progeny of the founder cell. Sequential accumulation of other genetic lesions in this field creates variant clones that can be traced to the founder event. (19–21) **Figure 2C.** This has been modelled through transformation of cells by introduction or removal of genes recurrently identified to be altered in human cancer across multiple cell types. Around 140 so called tumour suppressor and oncogenes have now been identified to drive cancers across different tissue types. (22)

Further evidence for a sequential model comes from the observation of field cancerisation (FC). As first described in oral cancers,(23) FC explains the spatial proximity of multi-focal primary tumours, where an area of tissue represents a "field" that has a predisposition to carcinogenesis. Each individual cell in the field thus has an enhanced propensity to undergo transformational change and to establish sub-fields in which further mutagenesis can occur.(24) There is increasing evidence for FC in prostate cancer. (reviewed in 25) For example, by determination of methylation ratios of APC and RAR β 2 across adjacent benign and malignant tissue, an underlying epigenetic field could be detected. (26) More recently, deep-sequencing of three prostate

cancers identified a surprisingly high number of somatic mutations in “normal” epithelia, which in the prostate, where a low background mutation rate has been postulated, is totally unexpected. The authors attributed this elevated base-level of mutation to a background field effect that sourced the multi-focal distribution of the tumours that they were investigating. (27) Another experimental approach to FC, is the ex vivo sequential mutagenesis of normal human prostate epithelial cells. Goldstein & Witte *et al.* introduced the driver genes ERG and AKT, by lentivirus, into basal cells that were then able to constitute lesions which resembled prostatic intraepithelial neoplasia (PIN). Further addition of androgen receptor (AR) caused formation of adenocarcinomas that, in some cases, lost the basal layer and had concomitant expansion of an AR⁺ luminal population. This observation supports the premise that histological character of the cancer upon presentation is not congruent with the state of the malignancy at its origin. (28)

Cancer Stem Cell Model

The cancer stem cell (CSC) hypothesis states that not all of the cells in the tumour are able to reconstitute the tumour mass after dissociation. There exists a distinct and, in most cases, minor sub-population of cells that is responsible for tumour initiation, renewal and relapse post-treatment, and mirror normal proliferative tissue dependence on a somatic stem cell population. This CSC pool lies at the base of an aberrant differentiation lineage, which results in the cellular variance of prostate and many other major human tumours. (29–32) **Figure 2D.**

However the stochastic and CSC models are definitely not mutually exclusive. **Figure 2D.** It is entirely plausible that the CSCs can establish early pre-tumour development and also allow for relapse, whereas in late-stage or aggressive disease it is more likely that a CSC-derived dominant clone with an enhanced proliferative and invasive capability will drive the cancer. The CSC pool can also maintain genetic heterogeneity that will derive successful clones. (33)

The CSC hypothesis is compatible with an intermediate stage in carcinogenesis; pre-tumour development, where all the driver oncogenic mutations and genetic aberrations accumulate in the stem or progenitor cell, prior to actual tumourigenesis and the phenotypic emergence of visible cancer. Necessarily, this would occur over a long period of time. Adult stem cells are the only cell type in a tissue that aren't, under normal conditions, depleted through either differentiation, development or cell death. The slow turnover and relative quiescence of the stem cell also explains the slow progression and late presentation of prostate cancer. (18,34) A recent publication from Tomasetti & Vogelstein (35) commented on the positive correlation between number of stem cell divisions and the incidence of cancer in tissues. However, misappropriation of the term “bad luck”, underestimation of environmental factors, the use of data from only the United States and absence of major cancer types including; breast, stomach, cervix and prostate, in analysis has attracted subsequent criticism. (36–42) Carcinogenesis is a far too convoluted process to attribute random mutation in stem cell divisions as the major and unavoidable risk factor in disease development. The high frequency of cancer incidence among the population and the number of mutations seen in solid tumours, without the acquisition of a mutator phenotype through defective mismatch repair or genomic instability, is not explicable in a stochastic multi-hit model using a baseline mutation rate of 10^{-7} - 10^{-9} (34,43–45) and is therefore suggestive of a Darwinian selection through micro-evolutionary processes. (34,46,47) Selection of mutations through conferred proliferative or survival phenotypic advantages, in the setting of field cancerisation, would create a niche microenvironment where successive mutations are accrued in a single cell. In a recent and thought provoking study, Ling *et al.* analysed ~300 samples from a single hepatocellular carcinoma.(48) They found unexpected genetic diversity amongst clonal populations, with mathematical modelling suggesting a Non-Darwinian mode of selection in the solid tumour, and an extremely high mutational load. Relation of genetic heterogeneity to the definitive functional non-equivalence of cells, especially on the drift of low frequency passive mutations, alongside an inability to predict exact selection pressures within the tumour mass itself limits analysis. A further study conducted by Williams *et al.*, using mathematical modelling on the evolution of cancer genome sequences, identified presence of driving mutations in the “first” cancer cell and that the noise of passenger mutations creates observed intratumoral heterogeneity whilst separate lineages evolve in a neutral manner and accumulate passive driver events that can, later, confer treatment resistance.(49) Thus supporting a dominant role of pre-tumour development over subsequent clonal evolution within cancers.

Figure 2. The Human Prostatic epithelial hierarchy, stem cell division and Stochastic/CSC models of cancer.

- A. *Simplified prostate epithelial lineage hierarchy. From a self-renewing stem cell, luminal cells are formed by the step-wise differentiation of the cell through transit amplifying and committed basal states.*
- B. *Outcomes of stem cell division. 95% of division occurs asymmetrically, maintaining the stem cell pool and the epithelial cell lineage. The remaining 5% of divisions are symmetrical, leading to either the expansion or the extinction of the stem cell population.*
- C. *Stochastic model of tumour heterogeneity. In C₁, mutation x1 transforms a TA cell that then produces a field of more differentiated CB cells. The second mutation is incurred in a CB cell that can only propagate this in the CB and luminal cell populations. The mutations are assumed to confer an ability of self-renewal so that the cells aren't lost by progression through the epithelial cell lineage, thus every cancer cell has roughly equal tumourigenic potential in this model of clonal evolution from a field of cancerisation. C₂ – A schematic of a tumour representing the epithelial lineage of the C₁ stochastic model.*
- D. *Cancer Stem Cell model. The postulate for the hypothesis; is that only the CSC has the potential to generate a tumour. Mutation x1 in the SC creates a tumour lineage derived from this stem cell. Mutation x2 represents a merging of the CSC and stochastic models, as the two are not mutually exclusive. Here, after symmetrical division of the CSC in niche succession, a secondary mutation occurs in the CSC, resulting in a variant tumour hierarchy, that is genetically/epigenetically different from the first CSC lineage. D₂ – A schematic of a tumour representing the lineage of the D₁ CSC model.*

Niche Independence as a Driver Mutation

Normal somatic and CSCs *in vivo* are maintained by, and indeed maintain, a protective niche within tissues. In light of this, it is likely that a key driver mutation class in cancer will be to confer niche independence or the ability to generate a new niche to a CSC. The best evidence for a CSC niche comes from glioblastoma, in which CSCs are maintained by a hypoxic micro-environment, (50) yet can also trigger angiogenesis through a CSC fraction that acts as an endothelial progenitor. (51,52) In the prostate, this encapsulating micro-environment of cellular contacts and a potent cocktail of growth factors is most likely situated on the basement membrane, due to high stem cell expression of collagen-binding $\alpha_2\beta_1$. Here, the stem cell is exposed to heterotypic interaction with the underlying stromal compartment. (15,53,54) Extrinsic factors supplied by this niche, along with intracellular molecules contained within progenitors themselves, will affect key developmental decisions such as symmetric or asymmetric division. **Figure 2B.** (55,56) The niche for prostate epithelial stem cells requires further research, as this may establish a direct difference that can be attributed to a malignant stem cell phenotype and maintenance. (57) Interestingly, niche similarity or mimicry may explain the bone tropism displayed by metastatic prostate cancer. (58)

How do Driver Mutations occur?

Accumulation of driver mutations by fixation and passenger mutations by drift can be modelled in selective sweeps of niche succession by an individual stem cell. Niche succession is thought to be a cyclic process that occurs once every 8.2 years (median) in the colon, and presumably takes place at much slower rates in low turnover tissues such as the prostate. (34,59) Advantageous mutations, at least in initial pre-tumour development would confer an enhanced ability of the stem cell to remain niche-bound and may also promote expansive symmetric division. Stem cell biology is also significantly different from that of a differentiated cell, with cell fate structured around different pathways. On current evidence, “successful” CSCs manage to maintain their stemness pathways such as Notch, Wnt and Hedgehog intact or in a hyper-activated state, as molecular inhibitors targeting these networks abrogate CSC effectivity. (60) There is also a strong possibility that mutations deleterious to cells of a more differentiated phenotype can be tolerated, or may in fact remain silent/have reduced penetrance. This is observed in pediatric tumours, in the tumour progenitor population. (22,61) A situation can therefore exist in which somatic stem cells, from birth, act as a potential neoplastic field for accumulative genetic change over an individual's life-time. In prostate, the initiation events most likely occur during the two waves of prostatic development and expansion; neonatally and at puberty. Such neonatal expansion is driven (in AR⁻ cells) by growth factors supplied through the stromal micro-environment. (3)

Direct transformation of somatic stem cells presents a compelling case for establishment of a founder event in the stem cell, rather than a near-progenitor as observed in colorectal crypt cells. Here, *only* transformation of a stem cell allowed formation of an adenocarcinoma, whereas malignant initiating TA cells had limited self-renewal and thus could only form abortive cancers. (29) The colon constitutes an excellent model for studying direct transformation as so much is known of crypt cells, niche succession and mitotic turnover. (62) Transformation of a stem cell in the colon was the most plausible explanation for initiation of colorectal cancer, as mutations in more differentiated cells would have no time to accumulate due to the rapid turnover of cells in the intestine. Transformation of a non-stem cell would require mutation alongside an additional co-incident event such as micro-environmental change or an inflammatory stimulus, which is improbable yet possible. (63) In the prostate, an organ which has a much slower cell turnover, the chances of non-stem originated cancer will be increased in accord with this reasoning, yet a stem cell event would still remain the most logical origin for tumour initiation.

The Evidence for Cancer Stem Cells

CSCs were postulated as a cell-pool long before the ability to fractionate individual cellular populations. (17) When technology was able to isolate living cells with specific cell surface phenotypes, the first direct evidence of a clonogenic cell fraction in cancers came from acute myeloid leukaemia (64). Here leukaemic progenitor cells were selected using the immunophenotype of haematopoietic stem cells (HSCs); CD34⁺CD38⁻, and were able to not only reconstitute the leukaemic blast hierarchy but also to possess extensive self-renewal capabilities. CSCs have now been derived in many cancers using commonly conserved immunophenotypic markers. (30) **Table 1**

Table 1 –immunophenotypes of Human Cancer Stem Cells

Isaacs and Coffey were first to postulate the existence of an aberrant stem cell population in the prostate. (78) This prediction was realised when Collins *et al.* enriched a tumour initiating cell-type from human prostate cancers. (32) These basal stem cells, classified through expression of canonical markers including p63, CK5 and CK14, and a lack of AR, prostate specific antigen (PSA) and PAP luminal identifiers, (77, reviewed in 16) can be fractionated from radical prostatectomy biopsy cores by selection of a CD44⁺ $\alpha_2\beta_1^{hi}$ CD133⁺ phenotype. The fact that these markers also isolate stem cells of the non-malignant prostate adds further weight to a CSC origin of prostate cancer. (53,80) This cell class constitutes fewer than 0.1% of tumour cells and has markedly enhanced invasive potential and self-renewal over normal tissue stem cells. (32) We have also demonstrated that hallmarks of canonical chromoplexy such as the TMPRSS2-ERG fusion (81,82) and PTEN deletion (Butler *et al.* manuscript in preparation) exist in this cell-pool, although further work to fully characterise the extent of genomic aberration is required. Classical stem cell markers such as Bmi-1 and nanog have also been identified in prostate epithelial and cancer stem cells. (83–85) Recently, basal cells have demonstrated a significantly enhanced organoid generating ability from single cells over those of a luminal phenotype, further evidencing a basal cell of origin. (86)

Surface marker classification is by no means a definitive determination of the CSC cadre of the prostate. Inter-CSC variance has been known to exist for decades. (87) If we view the tumour like its tissue counterpart, (88) aberrant acini will have separate stem cells and thus CSC niches. In addition, the “cancer” phenotype can be reached by multiple genetic and epigenetic routes, which will be superimposed on a more consistent stem-like phenotype. Both intra- and inter-niche heterogeneity will contribute to the plethora of sub-clonal properties of CSCs in constitution of the disease itself, (89–93) metastases, (94,95) dormancy (96) and therapy resistance. (30,97) One truly interesting prospect is that indolent and aggressive cancers may have non-convergent roots in distinct CSC sub-types, a topic of further intrigue if it is found that Gleason grades are divergent rather than progressive.

Gleason Grading of Prostate Cancer

In prostate cancer there are currently no definitive markers that can differentiate indolent and malignant varieties of tumour better than the classic histopathological Gleason grading system (98) which distinguishes malignancy on comparative tissue architecture. **Figure 3A.** (10,99) However, it is still not confirmed whether Gleason grade of tumour tissue is transitional; increasing over time as disease progresses, or clonal; with

divergent cells of origin for Gleason 3, 4 and 5 patterns. Both transitional and clonal models have supporting evidence and may not necessarily be mutually exclusive. Temporally repeated biopsy of the disease in the same prostate is theoretically the best way to conduct this research. However this strategy will be restricted by significant needle biopsy sampling errors, prostate tissue heterogeneity, and the frequently multifocal nature of primary prostate cancer. (99–101)

In support of a transitional model; Gleason grade correlates positively with age at detection i.e. older men are diagnosed with more aggressive cancers. (101) This could however be countered by the fact that higher Gleason grade cells may be relatively undetectable earlier in the natural history of disease and may initiate growth at a later point. Laser capture micro-dissection (LCM) of adjacent G3 and G4 tissues, followed by genome sequencing of the lesions identified sharing of common breakpoints and early genetic events such as chromothripsis and TMPRSS2-ERG fusions, between the two tissue grades. Even in samples that, at first, seemed genetically disparate, it was shown that a small population of the Gleason 3 cells had “stemmed” the Gleason 4 lineage through sufficient sequencing depth, providing molecular evidence of a transition. **Figure 3C.** (102) Equally, there could still be a common progenitor clone for each lesion.

Figure 3. Gleason grading and progression hypotheses

- A. *Gleason patterns adapted from the 2010 ISUP consensus on pathology. Cumulative Gleason grades are given for the 2 dominant patterns in cancerous tissue, meaning a score of 2-10 can be awarded.*
- B. *Clonal model of Gleason progression. Here Gleason 3 and 4 tissues have distinct progenitors that stem respective indolent and aggressive cancers.*
- C. *Transitional model of Gleason progression. A Gleason 3 cell accumulates further epigenetic/mutational changes required for the cell to progress and form a more advanced Gleason pattern.*

Indication of separate clonal events propagating Gleason 3 and 4 tissues is seen in both population and expression studies. PSA testing has permitted detection of prostate cancer earlier in the individual tumours' natural history, than before implementation of the biomarker. Thus earlier detection of cancers should reduce the absolute numbers of patients diagnosed with high Gleason cancer, as lower grade cancers haven't had time to progress to the more malignant phenotype. However a large population study found that, at diagnosis, the incidence of >G7 cancers in the pre and post-PSA testing eras had remained at the same level. (101) The presence of Gleason pattern 4 in tumours significantly reduces progression free survival. Gleason 3+3 cancers only rarely progress to lethal disease (103,104) yet the presence of a tertiary component of Gleason 4 is predictive of biochemical relapse of the cancer, (105) suggesting that a cell detached from the Gleason 3 tissue is responsible for malignant progression. **Figure 3B.**

Gleason 4 and 5 cancers are reported to be indistinguishable in their expression signatures, possibly due to heterogeneity, whereas there is a clear separation of Gleason 3 and 4 transcriptomes. (106) An 86 gene signature was able to divide 81% of cancers on this molecular correlate and identified genes such as monoamine oxidase A that are upregulated in Gleason 4 pattern lesions over those of Gleason 3 status. We also showed that Gleason 6 (3+3) cancers were distinguishable in their expression profiles from Gleason 7 cancers. Interestingly when these Gleason 6 cancers were included in total analysis, the distinction of malignancy (cancer-benign) and differentiation state seen with >G7 cancers was lost. (81) However such robust expression signatures for separate Gleason grades weren't identified in a similar micro-array based investigation, one possible explanation of this disparity would be a dominant luminal cell expression signature masking the expression differences observed by our study. (107)

While doubt still remains about the nature of prostate cancer progression and lineage involvement from a cell of origin in the disease, more research into the molecular evidence of progression is required. Studies that account for disease heterogeneity by cellular fractionation, LCM or deep sequencing will hold the true discovery potential in future prostate cancer research. The more benign nature of lower Gleason grade

tumours (108) is now reflected in a histopathological consensus classification which still bears Donald Gleason's name. (109)

Quiescence and Stem Cell Initiation of Cancers

CSCs don't necessarily have to gain an enhanced proliferative potential to fuel a "successful" cancer. Maintenance of a quiescent state is advantageous, as it removes the threats of chemotherapy and the "fixing" of deleterious mutations from radiotherapy, as seen in therapy-resistant normal adult HSCs. (110)

The loss or breaking of adult stem cell dormancy transitions stem cells to an "activated" state of increased mitotic potential. (111) Loss of a differentiated cellular hierarchy through bleeding (112) or chemotherapy (113) primes dormant HSCs into an activated state, allowing for re-constitution of the haematopoietic lineage. These cells then return to dormancy in their niche. (114) Somatic stem cell niche maintenance seems to be extremely important in the retention of a quiescent state. (113,115) Whether this model can translate effectively to the epithelial stem cells of the prostate is still to be determined.

CSC-initiated relapse involves an extended period of quiescence; a dormant subpopulation of CSC may direct this observed pause before relapse of metastatic disease. (30,116) Possible treatment scenarios for this population include; maintenance of stem cell dormancy so that they can't direct regrowth, or priming the cells into a cycling mitotic/more differentiated state then following up with conventional chemotherapeutic killing. (96,113) Differentiation therapy is an intriguing possibility that has been discussed previously in prostate cancer (43), however the main challenge in establishing this treatment is to ensure that the induced development is selective for CSCs, while leaving normal adult stem cell quiescence uninterrupted. Outcome in non-targeted treatments of this sort would be deleterious to gland integrity.

Asymmetric division facilitates the self-renewal of the stem cell population. It has been hypothesised that this process also confers mutational protection on the stem cell pool. The immortal strand hypothesis was proposed by Cairns in 1975 (117) – as the mutational accumulation in epithelia is lower than expected background rates, stem cells may non-randomly retain their template chromosomes or strands so that mutations incurred in the replication process are passed on to their non-stem daughter. (118,119) This makes the assumption that stem cells must minimise recombinatory DNA repair and limit mitotic sister chromatid exchange to keep the immortal strand "pure" and, counterintuitively, HSCs have been found to have deficient DNA repair. (120) Selective strand segregation has been observed in embryonic fibroblasts, (121) mouse intestinal stem cells (122) and skeletal muscle stem cells. (123) More recent evidence however has argued against the hypothesis. HSCs (124) and mouse intestinal stem cells (125) have been shown to randomly separate their template chromosomes through temporal dilution of variant DNA-labels and a recent meta-analysis of sequencing data has also provided an argument against existence of an immortal strand. (126) By modelling expected mutational accumulation in healthy stem cells using the assumption of an immortal strand system, it was shown that cancers of the head and neck, blood and colon had a greater than hypothesised mutational load – suggesting random chromosomal segregation into daughter cells. The limitations of comparing modelled normal stem cells and cancer sequencing data must be noted, as this doesn't account for particular mutations in the cancer that could support a mutator phenotype. Equally, a hallmark of CSCs in pre-tumour development may be the disruption of regulatory pathways that govern strand segregation, proposed to be centred upon p53, (127) to create a setting for cumulative mutations in the tumour progenitors.

Mouse Models of Prostate Cancer

The favoured animal model for human prostate disease is the mouse. Studies using mice have permitted the application of xenograft experimentation and genetic engineering. (reviewed in 10,126) Xenografting of cell lines and primary human cells has yielded significant information on the molecular mechanisms of prostate cancer yet also presents new challenges, due to the heterologous mouse tissue environment and the lack of an endogenous immune system in the mice. (10) In terms of CSC dynamics, the niche in mouse tissue will not recapitulate that of the cell in culture or indeed in the human prostate. Hypothetically the xenografting of tumour cells may select for a variant dominant CSC that supports tumour initiation in the mouse. The stromal compartment of the prostate is extremely important in the development of cancer, and addition of human stromal cell lines into xenografting studies enhances tumourigenicity of the cancer cells. (129,130)

Current evidence can support both a basal (131,132) and luminal (133,134) CSC model of disease in the mouse prostate (135) via multipotent, bipotent (136) and unipotent (137) stem cells that also differ in symmetry of their division to produce cells with inequivalent aggressiveness.(138) However human cancers provide more proof for a basal CSC (32,83,139–141) despite some evidence for luminal progenitors. (142)

Although studies using transgenic mice have shed light on many human diseases, there are many anatomical differences between the mouse and human prostates. Firstly, the mouse prostate doesn't spontaneously develop neoplasia and instead atrophies with increasing age,(143) a situation in stark contrast to benign prostatic hyperplasia (BPH) and cancer development in the human organ. Basic anatomy is also divergent; the mouse prostate is composed of three lobes around the urethra whereas the human prostate is alobular and envelopes the upper portion of the urethra. (144) Glandular structure between the two species also differs. Human acini are composed of two distinct epithelial layers compared to the single mixed layer of epithelial cells found in the mouse prostate. (3) As prostate cancer doesn't naturally develop in the mouse, any parallels drawn between the two species have to be carefully considered. The act of forcing a non-naturally occurring scenario on a system will inherently introduce artefacts that are open to misinterpretation.

Epigenetic Aetiology of Cancer

Over recent years it has been observed that the epigenome of cancer cells is perturbed to the same extent as their genomes. (145) Epigenetics, the marking of DNA (by repressive methylation) or histones (primarily through activating acetylation and both activating and repressive methylations), allows for plastic and flexible control of gene expression. (146) This is entirely in keeping with current hypotheses of gene regulation in adult stem cells, which have to simultaneously remain in a dedifferentiated state yet retain the potential to resolve gene-sets and enter distinct lineages of differentiation. (55,147,148) Meta-regulation of the epigenetic control of gene expression can become dysregulated in tumours by over-expression, mutation or loss of chromatin modifying enzymes, a common occurrence in prostate cancers. (107,149,150)

Early stem cell histone marks have been shown to predict gene downregulation, followed by stabilising DNA methylation in many cancers (151–153) including that of the prostate. (154) Initial bivalent histone modifications at tumour suppressing loci can thus predict the epigenetic silencing of the gene in malignancy. Epigenetic plasticity is also employed in epithelial to mesenchymal transition (EMT) – a metastatic signature dominant in the CSCs of the prostate. (79,81) Tumour cells thus can use epigenetics to retain more plastic and adaptable de-differentiated states, without relying on inflexible and fixed mutations to drive carcinogenesis. These epigenetic mechanisms also have the propensity to silence mutant alleles until a time when they become tolerable in the cell i.e. when quality control pathways have been dysregulated. Oncogene activation has been previously reported through loss of promoter DNA methylation (155) and repressive histone marks. (156) Indeed promoter hypomethylation in prostate cancer has been reported to facilitate aberrant expression of Wnt5a, production of which correlates with disease malignancy (157) and the utilisation of the Wnt pathway by circulating tumour cells (CTCs) in castrate resistant disease. (158)

Heterogeneity of Gene Expression and Random Monoallelic Expression in Prostate Cancer

Prostate cancer presumably develops due to genetic and epigenetic insults accumulated by a single cell, which then proceeds to generate an extensively heterogeneous tumour. Accounting for cellular variance in disease and understanding the mechanisms of tumour cell diversification is critical to all investigations of prostate cancer development. Most discussions of tumour heterogeneity centre upon genetic aberration and mutational differences that separate distinct sub-clones. However there is also the factor of expression heterogeneity. In the situation of common heterozygous mutation, is selective expression of the mutant transcript over that of the wild-type allele critical for tumour development? This impacts upon cancer therapies, as it can generate unimaginable variance within a tumour, through production of stochastic intra-clonal heterogeneity. (159,160) In turn, generation of vast heterogeneity significantly raises the potential of the selection of one cell by favourable adaptation to a micro-environmental change, or in response to a new treatment regimen. Thus there are two primary variables to take account of when assessing expressional variance amongst cells;

The first is the level of mRNA transcript – in tumours; excessive oncogenic mRNA or loss of transcription at a tumour suppressing locus will inevitably affect cellular contributions to the cancer. Recent single-cell studies have indicated that normal cellular mRNA expression varies greatly, even in clonal cells (161) with the same also holding true in cancer cells. (162–165)

The second factor is that different cells will have variant allelic preferences amongst gene sets. The phenomenon of random monoallelic expression (RME) is distinct from that established by aberrant promoter methylation, or locus deletion detected in cancers. An allele has a stochastic activation upon development from a stem cell, meaning that the gene can be either; biallelically, maternal monoallelically, paternal monoallelically expressed or isn't expressed at all. The allelic "choice" is mitotically stable in the non-stem daughter upon asymmetric division and adhered to throughout mitosis and differentiation. **Figure 4B.** (166,167) Fixed monoallelic expression in TA cells, and more differentiated progeny, will thus populate the tissue, creating an ever shifting cellular expressional mosaic based on initial allelic "choice" established from the CSC. **Figure 4C.** Indeed mosaic expression has been shown for key driver genes amongst single cells in glioblastoma. (164) RME was initially observed in cell culture (168,169) and hypotheses that it exists as an artefact of this artificial environment have now been dispelled by multiple *in vivo* studies. (166,170–172)

The regulation of allelic choice, in the majority of RME genes (166) is not controlled by correlative methylation of promoter CpG islands (82,173,174) as originally thought. Strand-specific methylation could account for switching in active alleles at mitosis, however recent higher resolution promoter analysis implies that RME is defined by an asymmetric chromatin signature in which the gene body histones of the active allele are trimethylated at H3K36 and the silenced allele are trimethylated at H3K27. (170) This fingerprint has been shown to be predictive of RME status and is conserved in orthologous mouse genes (175) suggesting that regulation of allelic expression is hardwired in our DNA, due to the evolutionary distance between mice and humans. Interestingly many of the genes found included in the RME chromatin structure were also bivalent in ESCs. (170) Bivalent chromatin is formed by dual deposition of respective activating and repressive modified histones H3K4me3 and H3K27me3 at promoter regions, allowing for rapid activation of genes upon stem cell differentiation. (176) Resolution of the somatic stem cell bivalent marks into those seen in RME is an important hypothesis. (170,177) Bivalent genes, like RME gene-sets, are lineage specific and are involved in cancers of various tissues (152,153,178–182) including the prostate. (154) **Figure 4A.**

Figure 4. Epigenetics and Tissue Patterns of Random Monoallelic Expression

- A. *Histone and DNA methylations involved in the maintenance of bivalent and randomly monoallelically expressed genes. Green represents transcriptionally activating marks while the red denotes marks involved in gene repression. The stars indicate that the methyltransferase/demethylase has been shown to be dysregulated in prostate cancer.*
- B. *Generation of RME from bivalent stem cell genes. The "choice" of allelic activation is made at each allele independently, allowing production of variant TA populations. The number of genes affected by RME and the stochastic resolution of allelic expression upon development allows for heterogeneity of expression to develop more extensively across tissues.*
- C. *Tissue mosaicism of allelic expression. The stability of RME patterns throughout mitosis and differentiation reasons that tissues would form mosaics, based upon allelic expression that originates from the SC niche and spans both basal and luminal epithelial layers.*

Current evidence of RME affecting cancer initiation is sparse, as most studies have focused on development. The first reports of tumourigenic RME occurred in chronic lymphocytic leukaemia patients that had haploinsufficiency of DAPK1, (183) where the RME status of the gene was confirmed in peripheral mononuclear blood cells.(169) TMPRSS2-ERG, the commonest genetic abnormality in prostate cancer, (149,184) is regulated by RME pre-fusion. Upon recombination with the ERG gene, the fused allele becomes dominant and is selectively monoallelically expressed in the CSC. Suppression of the unfused TMPRSS2 allele is later relaxed in development, with randomisation of allelic expression outside of the stem cell compartment. This perturbation of RME in cancer, generates epigenetic plasticity of the propagating cell and results in expression heterogeneity. (82)

RME in cancer has the potential to both silence tumour suppressing loci and to allow selective expression of oncogenic alleles. Conversely, it is probable that tumourigenic alleles are silenced in this manner during pre-tumour development and later re-activated. This can lead to situations of variable penetrance and may account for some instances of haploinsufficiency. For a greater insight, single-cell RNA-sequencing datasets in prostate cancer must be generated to distinguish any recurrent allelic alterations. Currently one study of this nature has been conducted in prostate CTCs (158) and future investigations will hopefully encompass all situations of disease states.

Inflammation of the Prostate – A Route to Cancer

Evidence of an inflammatory aetiology for prostate cancer has been proposed for many years. (reviewed in 166,167) Development of inflammation due to infection (prostatitis itself is the most common prostatic disorder) (186) stimulates the infiltration of immune cells. Secretion of chemo- and cytokines in this environment has the propensity to transform the stem cell population. Our lab has previously identified an inflammatory gene signature specific to prostate CSCs that includes active NF- κ B, IL-6 and IFNGR1. (81) Elevated serum levels of the IL-6 cytokine are common among prostate cancer patients with late-stage disease and can be correlated with poor prognosis. (187–189) Induced IL-6 secretion by CSCs will therefore establish an autocrine signalling loop, as the CSCs constitutively express IL-6 receptors. Activation of a downstream survival pathway reliant upon phospho-STAT3 evidently presents a selective advantage for the stem cell. (190) A comparable positive feedback loop incorporating NF- κ B, IL-6 and STAT3 induced transformation in a breast epithelial cell line and simultaneously enriched for a CSC phenotype. (191) Similarly, STAT3 activation acts as a bypass pathway of androgen dependence, during chemical castration in prostate cancer (192) and in the maintenance of self-renewal pathways of glioblastoma CSCs. (193) Development of STAT3 phosphorylation (190,192,194) and DNA-binding inhibitors both mark promising alternative therapies for prostate cancer. (195)

Whilst persistent infection triggers an exogenous supply of IL-6 from invading lymphocytes and/or macrophages to produce an initial inflammatory stimulus, IL-6 addition to the niche promotes an adaptive change in the stem cell which is then manifested into a positive feedback signal. Activation of a stem cell, by what is termed cytokine addiction, in this environment will grant a selective advantage in the new pro-inflammatory nature of the niche. The raised systemic IL-6 concentration from non-tumour cells, including stroma, in advanced prostate cancer patients may also provide an additional factor for successful expansion/survival of metastatic CSC clones.(187) Other CSCs have been observed to create feed forward favourable selection pressures; skin cancer progenitors establish a VEGF autocrine loop to support extrinsic vasculature and intrinsic self-renewal pathways.(196) This ultimately demonstrates the potential of prostate CSCs to modulate the niche micro-environment in response to inflammation. Given the convergence of many inflammatory signalling pathways via NF- κ B, IL-6 may represent only one of many routes to stem cell transformation. (81,197,198)

Stromal cells also contribute towards carcinogenesis and become reactive even during pre-malignancy (PIN). (129) Heterotypic signalling between the stroma and the epithelium is perturbed in disease (reviewed in 15) and the normal stromal cell phenotypic constitution alters; smooth muscle is replaced by cancer associated fibroblasts that are activated to myofibroblasts – a transition regularly observed at sites of wound healing and tissue remodelling. (199) Reactive stroma can promote the tumourigenesis of prostate epithelial cells in mice and in culture, highlighting the importance of reconstituting a favourable micro-environment in “successful” cancers. (11,200) Altered extracellular matrix ligand expression, such as higher levels of collagen 1, is also seen in disease and may aid constitution of the CSC niche. (53,129) TGF- β overexpression has been shown *in vitro* to induce reactive stroma, a molecular event that also begins in high-grade PIN tissues. (129,201) Stromal production of chemokines can produce synergistic effects alongside TGF- β signalling to enhance tumourigenic epithelial growth. (202) The same chemokines have also been linked to prostate cancer invasion, metastasis and immune cell infiltration of the primary tumour. (203)

Elevated intra-tumoural presence of immune cells can, counterintuitively, aid progression of the cancer. (204) Indeed, T cell infiltrates are often quiescent or “exhausted”, as shown by the expression of canonical markers such as PD-1 and its cognate ligand. (205) These clusters also have high numbers of regulatory T cells

suggesting a negative effect is compounded upon wider T cell responses against the cancer. (205,206) The presence of tumour associated macrophages is linked to a heightened risk of biochemical recurrence, (207) whereas increased levels of natural killer cells are associated with lower chances of disease progression. (208) B cell numbers are also elevated in tumours, as identified by matched tissue analysis from prostatectomy samples. (209) The presence of these lymphocytes can activate stem-like transcription of Bmi-1 in cancer cells via non-canonical NF- κ B signalling, to contribute towards self-renewal processes. (210) Tissue damage in the prostate, followed by the influx of immune cells, produces a reactive milieu of cytokines and growth factors, a cocktail that could potentially fuel the transformation of the epithelial stem cell niche as identified through inflammatory expression signatures in this rare cell population. (81,190)

Aetiological Inducers of Prostate Cancer: Inflammation may also be linked to Dietary, Chemical and Infectious agents

In an intriguing example of how environmental factors affect prostate tumourigenesis is the increased incidence of cancer in Japanese migrants to Hawaii, from extremely low to the level of the indigenous population. (211) The mere act of crossing the Pacific Ocean is certainly not attributable to this rise in incidence; a change in a habit, diet (212) or an endemic infection is much more likely. However, this data can also be interpreted as a racial predisposition of prostate cancer which is more intimately linked to environmental factors than previously thought.

Epidemiological studies have connected an increase in prostate cancer incidence with the ingestion of red meat and animal fat; (213,214) a possible mechanism of carcinogenesis postulated to be through formation of heterocyclic amines (HCAs). (215) Dietary supplementation of one such HCA; 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in rats induced formation of intestinal, breast and prostate carcinomas. (216) PhIP triggered an increased mutational frequency in the prostate and was found to have lobe-specific effects in the rodents which progressed through stages of epithelial atrophy, PIN and, finally, into cancer. (185,217,218) Recent research has found that dietary PhIP synergises with bacterial prostatitis and the IL-6 cytokine in promoting prostate carcinogenesis. (219,220)

Do Infectious Agents Induce Prostate Cancer?

Infection of the prostate by pathogenic organisms also produces an inflammatory response. Most of these agents are sexually transmitted, including; *Neisseria gonorrhoeae*,(221) *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Mycoplasma genitalium*,(222) *Treponema pallidum* (syphilis), *Ureaplasma urealyticum*, Herpes Simplex Virus types 1 and 2, Human Herpes Virus 8, Human Papillomaviruses (HPV) (223) and Cytomegalovirus. (224) Recent meta-analysis of results accrued on sexually transmitted infections (STI) influencing prostate cancer concluded that men infected with gonorrhoea have a 20% increased risk of developing malignancy and a past history of any STI correlates with heightened incidence of cancer later in life. (225)

Propionibacterium acnes, a bacterium primarily associated with acne, (226) is also recurrently found in prostate tissues with histological evidence of inflammation. (227,228) *In vitro* and *in vivo* studies utilising *P.acnes* have uncovered striking results. Co-culture of the bacterium with the RPWE-1 prostate epithelial cell line produced a complex immunological response with secretion of cytokines, including IL-6, and concomitant activation of NF- κ B and STAT3 (229) - a scenario resembling the inflammatory phenotype of prostate CSCs. (81,190) A mouse prostate model of *P.acnes* infection showed stimulation of epithelial neoplasia accompanied by inflammation and molecular changes including downregulation of NKX3.1 and AR. (230)

HPV, more frequently associated with cervical carcinoma, has become increasingly linked to prostate cancer aetiology. Infection with the virus correlates with high Gleason grade and lower survival rates. (231,232) Conversely, other studies have found absence of HPV sequences in prostate tissues (233,234) suggesting that the presence of the virus may just be correlative, yet further studies are required to assess the involvement of the pathogen in disease.

Epstein-Barr virus (EBV), previously implicated in both haematological and epithelial malignancies, has also been detected in prostate tissues.(235) It is thought that EBV may enhance HPV 18-mediated prostate oncogenesis.(236) Human BK polyomavirus has also been repeatedly identified in prostate cancer and its proposed precursor lesions. (237,238) BK T antigen is a protein that can nullify canonical p53 and Rb tumour

suppressor proteins, facilitating a non-mutagenic route of simultaneously abrogating gene function early in disease. (238) However, seropositivity to BK virus in prostate cancer patients has been linked to a reduced risk of biochemical recurrence, suggesting the virus is a positive prognostic factor although larger sample sizes are required to elucidate the role of the virus in disease development. (239)

With the depth of next-generation sequencing technologies, it is now possible to detect low abundance DNA of bacterial and viral pathogens in whole prostate tissue, (240) although targeted PCRs have allowed previous, if limited, detection of novel-prostate inhabiting species.(241–243) It is entirely probable that an unknown species may play a pivotal role in prostate cancer yet their inability to be successfully cultured from patient tissue has abrogated identification of the pathogen.

Further Evidence for an Inflammatory Aetiology of Prostate Cancer

Other studies that suggest an inflammatory aetiology for prostate cancer, through infectious agents or a compromised immune system, are the findings that; immunosuppression in transplant patients increases the risk of subsequent prostate cancer development, (244) the frequent use of non-steroidal anti-inflammatories slightly lowers incidence of prostate cancer, (185,245–247) and that inflammation of surrounding benign prostate tissue is positively associated with higher Gleason grade cancers. (248) All could indicate either a divergent inflammatory initiating event or a persistent progression stimulus.

Current evidence (249) suggests that inflammation and infection play a role in prostate carcinogenesis. Future studies, especially those involved in sequencing of large patient cohorts, should focus on identifying recurrently present infectious species with a further aim to distinguish molecular mechanisms of pathogenicity.

Prostate Cancer Pre-malignancies

Current carcinogenesis models argue strongly for the existence of an “activated” pre-malignant state, as previously discussed. The prostate is an organ in which pre-neoplastic disorders, including proliferative inflammatory atrophy (PIA) and PIN are relatively common. Tumours develop almost exclusively in the peripheral zone of the organ mimicking the zonal selectivity of hypothesised pre-malignancies; PIA and PIN. (185) **Figure 5.**

Damage to the tissue by an initiating inflammatory insult leads to PIA; a focal regenerative hyper-proliferative response from the epithelia. PIA has been shown to merge with regions of PIN and cancerous tissues – providing further evidence for both a field effect of development and a step-wise disorder progression of carcinogenesis. (250,251) Most studies have shown that these atrophic regions do not harbour genetic defects (107) yet anti-apoptotic Bcl-2 and protective GSTP1 (251) are both upregulated whilst known prostate cancer tumour suppressor genes; PTEN, NKX3.1 (252) and CDKN1B (253) are downregulated. This may represent a field of epigenetic activation prior to carcinogenesis.(254,255) These downregulated genes are also targets of heterozygous deletion in adenocarcinoma. (185) A recent study utilising an extensive amount of biopsies found a link between prostatitis and PIA yet not a transition between PIA and low grade PIN, (256) thus additional molecular and histological proof is required in support of this theory.

Figure 5. Phenotypic, micro-environmental and molecular changes incurred through the pre-malignant states of prostate cancer. Processes describe ongoing changes during progression and molecular changes are characteristic of each individual stage and transitional change through pre-malignancy into cancer.

There is probably stronger evidence for PIN as the major pre-cancerous lesion of the prostate.(257) This is characterised by enhanced luminal cell hyperplasia, and is biochemically, genetically and phenotypically similar to prostate cancer yet lacks any disruption of basement membrane.(10,107) The relative mitotic rates of the cells in the epithelial bilayer are reversed in PIN and the secretory cell population expands, foreshadowing the aberrant differentiation program of prostate cancer.(258) Numerous studies have reported coincidence of cancerous and PIN tissue, again supporting the FC theory. (259,260) The expressional profiles of PIN and prostate cancer have greater congruity between them than observed at any other defined stage in disease progression.(107) Additionally Tomlins *et al.* highlighted a reliance on ETS transcription factor driven gene-

expression in PIN. A more recent study has identified that, in some cases, high-grade PIN (hgPIN) lesions are retrograde carcinomas that have invaded normal glands. By establishing clonality through utilisation of unique TMPRSS2-ERG breakpoints and tracking cancer lesions with adjacent hgPIN, sub-clonality of PTEN loss was then used to mark molecular progression in the cancer. The identification of PTEN deletion in the “hgPIN” showed that the histopathologically identified neoplasia was in fact cancerous.(261) This suggests that other studies stating adjacent PIN has cancer-initiating defects, including the TMPRSS2-ERG fusion, (262) SPOP mutation (263) and NKX3.1 loss, (258) may have in fact been sequencing cancer cells that, by pathology, mimic PIN. The conversion rate of hgPIN into prostate cancer has also been interrogated. One study repeated biopsy 1 year following diagnosis of neoplasia and found that only ~13% of cases had “progressed” into cancer. This approach does have limitations, including the inaccuracy of core biopsies that may have missed cancer nodes upon PIN diagnosis and also the relatively short time frame between subsequent biopsies.(264) Whilst correlation of numerous molecular details is attractive the fact however remains that, currently, we have no absolute evidence of the progression of PIN into prostate cancer.

Prostate Cancer; Incidence in Autopsies

For more than 80 years, detection of prostate cancer at random autopsy has also yielded valuable information about the disease.(265,266) Striking observations from these studies, in which patient mortality wasn't attributable to cancer, include; (i) the number of “latent” cancers found and (ii) prevalence of cancer directly proportional to the age of the subject. Detection of prostate cancer in men in their twenties has also been reported.(259) Globally, prostate cancer prevalence is roughly equivalent with variation in racial population incidence and mortality rates.(267) The extremely high discovery rate of cancers that had not progressed to a clinically detectable stage, suggests that prostate tumours may pass through a 15-20 year latency period, as implied by PSA testing of men in their early forties.(268,269) Whether this is universal for progression or is a feature unique to indolent prostate cancer remains unknown.(267,270) However if there is an extended time of latency in cancer, as suggested by modelling in the colon (34), then this pause could be attributable to the CSC hypothesis.

Benign Prostatic Hyperplasia (BPH)

Normal post-natal prostatic growth occurs during puberty, due to a rise in endogenous testosterone. The organ can also undergo abnormal enlargement later in life, for example due to BPH. The latter is a chronic disease that exclusively affects the transitional zone of the organ, and causes constriction of the descending urethra – resulting in urinary retention and discomfort.(271) However, this hyper-proliferative disorder doesn't result in cancer. Our array data comparing states of cancer and BPH found that they are transcriptomically disparate (81) whilst also being phenotypically distinct in the fact that BPH can show hyperproliferation of the basal epithelial layer whereas cancer involves pronounced expansion of luminal cells.(272) In a recent publication detailing telomere dynamics through BPH, accounting for telomerase (TERT) activity, we have also shown the possible existence of divergent progenitors; one for the basal cells and the other for the luminal epithelia. (273)

Telomeres and Telomerase; a clue for Aberrant Differentiation in Cancer

Telomeres act as a buffering system to the end-replication problem in mammalian cells. Chromosome ends are reduced by 100-200bp after every cell division, thus telomeres shorten with age. Reductions in telomere length cause genomic instability and can result in chromosomal rearrangements.(274) Reactivation of telomerase in the presence of shortened telomeres, where tumour suppressor gene function has been compromised, is sufficient to generate multiple genetic rearrangements with the propensity to drive bone trophic metastases in a mouse model.(275) Telomere shortening is commonly seen in prostate cancer (276–279) alongside a restoration of, or indeed, an increase in germline telomerase activity.(276,280) This presumably allows the cancer cell to acquire an element of genomic instability whilst maintaining sufficient telomeric repeats to avoid replicative cell senescence. (274,281) A recent study found that prostate cancer patients with increased intercellular heterogeneity among telomere lengths had a significantly greater chance of developing advanced lethal disease.(282) Interestingly, telomeres are also shortened in PIN with congruent activation of telomerase – giving a molecular correlate in this supposed pre-malignancy.(277,279) Normal prostate epithelia, including the stem cells, lack telomerase, yet levels of the enzyme increase during disease

onset and progression into castrate resistant prostate cancer (CRPC). The shortened telomere lengths in cancer suggest a burst of expansion in the TERT⁻ stem cell pool during PIN, followed by maintenance of this length in TERT⁺ cancer cells.(283) The telomerase-positive signature is restricted to the aberrantly differentiated luminal cells of tumours, driving further clonal heterogeneity and maintaining viability whilst protecting against replicative senescence in this luminal cell pool.(284)

These recent observations from our lab may explain the aberrant differentiation seen in tumours that lose basal cells and become dominated by the luminal cell phenotype. **Figure 6.**

Basal cancer cells of the prostate do not express telomerase, thus experience replicative senescence and are removed from the tumour. The luminal cancer epithelia express active telomerase, allowing for maintenance of chromosome termini and cellular survival. Hypothetically, shortened telomeres in the basal CSC increases genomic instability, to facilitate the chromoplexy seen throughout tumours and, as the prostate is a small, relatively slow cycling organ, these cells never encounter replicative senescence or have alternative telomere lengthening mechanisms.(283,285)

The telomere lengths in fractionated BPH cells supports the existence of separate lineage progenitors for basal and luminal cell layers. The same may also be true in cancer, even though luminal cells can be derived from CB cells in normal prostate, (5) yet if the basal lineage becomes a “dead-end”, the shifting of asymmetric divisions to produce the luminal progenitor may become favoured from the CSC. (273)

Figure 6. Telomerase in Prostate Cancer Development

The TERT⁻ stem cell pool expands during PIN which shortens the telomeres. Progression into cancer occurs and telomerase activation in the luminal epithelial cells allows for maintenance of the shortened telomeres and prevention of replicative senescence in this mitotic cell population. Conversely the lack of telomerase in the basal cells of the prostate means that telomeres will continue to shorten. The lineage enters replicative senescence and would be lost from acini.

The role of this aberrant differentiation is still unclear, but as AR is the most potent neoplastic effector in the prostate, utilising this molecule in the dominant luminal cell type is advantageous for the cancer to increase in size and develop hetero-clonality.

Driver and Passenger Mutations in Carcinogenesis

Mutation is the most obvious and traditional route into, firstly, transformation of a normal cell and, secondly, the creation of intra-tumoural heterogeneity. With the advent of next generation sequencing, mapping the mutational landscapes of cancer has allowed identification of recurrently altered pathways and genes amongst different cancer types. (286,287) Driver mutations are defined as those that confer a proliferative advantage to the recipient cell – these are centred about 12 pathways encompassing cell cycle regulation, survival etc. and currently ~140 genes have been described as drivers in cancer.(22) It would be expected that such changes begin in cancer-initiating normal cells as part of pre-tumour and pre-malignant progression. Sequencing studies however pick up far more mutations in tumours than is necessary to transform a cell. These background changes are termed passenger mutations; they themselves do not grant the cell any micro-evolutionary benefit but are merely interred in the cells that have been selected through advantageous driver mutations and are “along for the ride”.(22) However such changes may become adaptive, and produce a favourable outcome in response to a selection pressure such as treatment, but otherwise would confer no advantage in the present micro-environment. **Figure 7.** Aberrant epigenetic events can also act as drivers. These permit mutagenic changes yet afford plasticity, allowing response to variant selection pressures such as micro-environmental alteration or treatment.(22) There is also evidence of, in pre-tumour development, epigenetic progenitors of cancer in which the initial tumourigenic changes aren't fixed mutations, but subtle changes in the expression levels of genes.(reviewed in 248) Analysis of late-stage tumours is thus a process with almost irreconcilable complexity, given the number of mutations. The key question when presented with the vast number of mutations present in cancers is; which drive the cancer and which are passively present?

Figure 7. Driver mutations in Cancer

- A. In a field of cells, two have acquired an identical driver mutation (red bolt) that confers a proliferative advantage over the non-affected cells.
- B. A cell in then accrues a secondary mutation (yellow bolt) that confers no advantage in the current micro-environment and the cell populations' proliferative indices remain the same.
- C. A micro-environmental change occurs, for example inflammatory stimuli or cancer treatment.
- D. In this new microenvironment, the previously passive mutation (yellow bolt) now confers a selective advantage over cells that have just the original driver mutation, demonstrating the appearance of adaptive mutations in cancer.

The theory of step-wise carcinogenesis is much like the clonal evolution model, in that it implies an initiating driver event which affords the originating cell the opportunity to form a neoplastic clonal field. Cells in this field can then undergo further selective mutations resulting in generation of a clonally diverse pre-malignancy and, providing the eventual acquisition of a minimal number of driver events, the first cancerous cell clone. The original linear step model proposed by Fearon & Vogelstein (20) however places metastatic potential as a final acquisition by cancer cells as the disease trends towards patient death. More recent evidence (288) suggests that metastasis may in fact occur much earlier in progression than initially thought, but remains undetectable due to the inefficiency of the process, development of required adaptive phenotypic changes for secondary site seeding and latency of micro-metastases. (116,289) This early EMT may in fact be stimulated by immune cell invasion of the primary tumour, (290) an event that occurs recurrently in prostate cancers.

Another criticism of a linear route is that if mutations were to occur sequentially in cells, without concurrent dysregulation of apoptosis, then surely the cell would be deleted by still-functional "quality control" mechanisms. The accumulation of driver mutations in pre-tumoural development (34) would of course be separated temporally yet may not be presented by the affected stem cells until there was subsequent or indeed previous removal of signalling pathways that may result in cancer cell death. For illustrative purposes, HPV oncogenesis mediated by E7 and E6 proteins requires the presence of both proteins to escape cell deletion; E7 binding to Rb causes cell cycle dysregulation but needs E6 to inactivate p53 to prevent deletion of aberrant replicating cells. (291–293) However, most p53 mutations are regularly observed in CRPC only after treatment (294) and are uncommon in early disease.(295,296) Deep sequencing may soon be able to address this problem by detecting the presence of "final" mutations in minute cellular populations of early-stage cancers, but the current limit of such studies (0.02%) may be insufficient in detecting rare clones.(297) A

The definitive number of driver events that can separate normal and cancer do however seem to be tissue-specific. Early studies estimated that there needed to be 6-7 of these mutations to transform a cell (298) but current mutational modelling suggests a lower number of 3 key mutations in most solid tumours.(287) Leukaemias, on the other hand, may require only a single event such as the RUNX1-ETV5 or BCR-ABL fusions, providing a reasonable explanation for the childhood onset of blood cancers.(18,299) Sequential mutagenesis in prostate cancer mouse models has shown that between one and three specific mutations are required for carcinogenesis.(10)

Genetic and Epigenetic Aberrancy in Prostate Cancer

Prostate tumours differ from the vast majority of solid tumours in that they favour copy number variations to orchestrate cellular dysregulation, rather than targeted point mutations.(27,149,184,263,300,301) This "scrambling" of the prostate cancer genome occurs in a patterned and coordinated manner of chained events referred to as chromoplexy. The temporal frame of these linked deletions and translocations is unknown, but probably accumulate over a prolonged period, due to observed clonality of founder events (TMPRSS2-ERG fusion and NKX3.1 deletion) and sub-clonality of "later" alterations (PTEN and CDKN1B deletions). Deletions in the classic tumour suppressors, RB1 and TP53 are also observed, albeit in more advanced cancers.(184) Presumably prostate tumours' aberrant DNA repair mechanisms facilitate chromoplectic reorganisation of the genome and these changes also present in the CSC.(81,82)

Sequencing has also identified an enrichment of events that dysregulate chromatin modifying enzymes in prostate tumours. The H3K27 methyltransferase EZH2 is upregulated and its locus frequently amplified in

advanced cancers.(107,149) EZH2 expression can be activated by ERG and has been shown to influence a de-differentiation program,(302) by transcriptional silencing of critical genes, for example NKX3.1.(303) Recurrent mutation of MLL2, another H3 methyltransferase (Lysine 4), that interacts with and facilitates epigenetic transactivation of AR, has also been observed.(301) Interestingly these enzymes are involved in maintenance of bivalent chromatin,(176) where dual histone methylations mark a gene-set that becomes dysregulated in prostate cancer development.(154) Multiple methyltransferases and demethylases are also abrogated in disease, with alteration presumably compounding dysregulating effects on the transcription of target genes. (304–308) Specific epigenetic events certainly play a role in prostate tumourigenesis, however the extent to which the epigenome of prostate cancer is altered throughout disease development is only just being uncovered. (154,309,310)

Recent studies have also allowed the identification of distinct molecular subtypes of prostate cancer. (184,301) This implies variant genetic founder events (**Table 2**) and may in future be aligned to stratify different treatment programs. The ETS factor status of the tumour can provide one classification paradigm. The TMPRSS2-ERG fusion was identified as a recurrent event in prostate tumours just over a decade ago (311) and is an established early event in around half of all prostate cancers.(149,184) Presence of the fusion is also detected in putative tumour initiating cells of the prostate.(81,82) TMPRSS2-ERG positive tumours also have recurrent deletion of loci including; PTEN, TP53 and 3p14 – a region containing three proposed tumour suppressing genes.(149,184,263) Deletion of CHD1, a chromatin remodelling protein, in addition to SPOP mutation, are mutually exclusive of the fusion, suggesting a variant initiating event. CHD1 is thought to be involved in the production of the TMPRSS2-ERG fusion by its co-recruitment to androgen responsive promoters, thus loss of CHD1 abolishes the possibility of generating a fusion and creates a variant lineage of prostate cancer. (312,313) Although genetically disparate, fusion positive and SPOP mutated tumour classes exhibit biological convergence as both increase ERG protein expression (314,315) and display genomic instability.(149,316) Transcriptomics and genomics have allowed for further assimilation of comprehensive mutational and expression signatures that can be used to stratify prostate tumours. (149,317–319)

Table 2 – Molecular defects which can drive primary prostate cancer

These sequencing studies however fall short on two accounts; until recently they predominantly have used end-stage tumours but more importantly they all fail to sufficiently account for intra-tumoural heterogeneity.

Figure 8.

Most studies involve sequencing of advanced tumours, partially due to harvesting of tissue at autopsy but more effort to collect a wider range of samples of radical prostatectomies must be made to ensure that prostate cancer is mapped throughout each stage of disease. This would inform on the development of tumours at various prognostic levels and give us an extrapolated view to pin down true initiating events.

Figure 8. Understanding Heterogeneity – a tumour model to illustrate the difficulties of disseminating cellular diversity in prostate cancer

Prostate cancer is regularly multi-focal. These separate tumours are not identical and often have variant founder events and characteristic mutations. DNA/RNA sequencing of sections taken from different positions A-D of the prostate in the top left of the illustration will produce differing combinations and percentages of focal tumour tissue. This will give an averaged mutational signature between the foci (1, 2 and 3) and, without cellular fractionation, will not be able to distinguish between epithelial and stromal cell type profiles.

Heterogeneity of mutational foci can also exist within the tumour epithelium due to sustained sub-clonal expansion from different CSCs. Clonal mutations would also accumulate through cellular division along the acini (x -> y -> z). Branching of acini can show divergence of the parental tumour cell populations with a shared founder event, and the creation of sub-clonal pools with different mutations (slice E). The lower image (E) is a transverse section across the 2 divergent acini in slice E in the upper right representation of the tissue.

By combining sequencing, immunohistochemistry with the separation of cellular populations, identification of the cell type in which mutation originated and tracking of said mutation through the lineage hierarchy in multi-focal prostate cancer will be possible.

Tumours are innately heterogeneous, composed of varying cancer cell types, cancer-associated cells and infiltrating immune cells. Processing an entire prostate tumour as a homogeneous entity allows no distinction between stroma, epithelia and the skewed epithelial populations. Ultimately the cells driving the cancer may be masked by bulk tumour cells. Multi-region sequencing of the primary tumour and metastases has provided some insights into the spatial and temporal evolution of cancers,(320,321) including that of the prostate. (322) Further improvements are required such as cell fractionation prior to sequencing efforts, and/or sufficiently deep coverage should in future be able to resolve the nature and origin of human prostate cancer heterogeneity. (323)

The Role of Androgens in Prostate Cancer Initiation

Neonatal morphogenesis of the prostate is directed by androgens, (324) as is growth of the organ during puberty, in what is thought to be an “imprinted” proliferative response.(325) Most cells of the prostate apart notably from stem cells express detectable levels of AR protein, however only the luminal cells depend upon the hormone for survival.(79) Expansion of the luminal cell monolayer in PIN and cancer strongly implies that AR is important in disease development. Indeed chemical castration by ablation of the androgen axis effectively de-bulks tumours, a realisation made over 70 years ago.(326)

Androgens have also been shown to induce *de novo* TMPRSS2-ERG fusions in LNCaP cells through interphase co-localisation of both loci (dictated by AR transcriptional activation) twinned with topoisomerase II recruitment.(327–329) The creation of the fusion is a gateway event for establishment of an inappropriate ERG profile towards a de-differentiated state and androgen independence of cancer cells.(302,330)

Aberrations of the AR gene itself are observed post treatment through locus amplification, (331) mutation (332) and aberrant splicing. (333) These events drive activation of the receptor in castrate levels of androgen. Prostate cancer also hosts frequent mutational and expressional changes of AR regulatory proteins including SPOP, (263,334) NCOA2, (149) FOXA1, (263) MLL2 (301) and RNF6.(335) This highlights the importance of androgen signalling for the fate of luminal cancer cells of the prostate. The dominant disease “signature” identified by these studies could also be due to the skewing of the prostate epithelial lineage in tumourigenesis, and it is therefore unsurprising that prostate cancer has perturbations in this neoplastic pathway.

Androgen ablation, is initially successful and causes involution of the normal prostate and a reduction in tumour mass yet, it almost inevitably fails at longer term. Relapse of CRPC occurs in almost every patient. AR changes through mutation and amplification in small sub-clones undoubtedly exist in the tumour prior to treatment for the changes to be so common after relapse, but these alterations are rarely selected for in hormone naïve cancers. (294)

Prostate CSCs express little or no AR protein and are independent of the androgen axis for survival, making the population a primary candidate for driving cancer relapse.(79,336) The castrate levels of androgen may select for AR⁺TA cells that have AR alterations, to kick-start tumour regrowth.

DNA Damage Repair and Response Defects

Genome instability is regarded as a hallmark of cancer as it allows for acceleration in the mutation rate that can either predispose or accelerate existing cancer development. Telomere shortening, defects in response and repair of DNA damage all contribute to instability and offer a worsened prognosis in prostate cancer. (282,337)

Similar to other malignancies, prostate cancer often presents with compromised DNA damage response pathways, (319,338) however tumours with true mutator phenotypes only ever present after treatment. (323,339) Population and familial studies have shown that a number of genes predispose for prostate cancer. Mutation of BRCA1 and 2, commonly related to ovarian and breast cancers in women, increases the risk of development of prostate cancer in men.(340,341) Chk2 mutations were also shown to be elevated in families with hereditary prostate cancer.(342) Numerous population studies examining the risk factors of single nucleotide polymorphisms in DNA repair genes found several allelic variants for genes such as PARP1, XRCC1

and ATM.(343) Defects in this quality control system are also observed in sporadic tumours with mutation of ATM and p53 in advanced disease.(344)

Oncogene-mediated replicative stress through AKT (ungated activation due to common PTEN loss (345)) and Myc further destabilise the genome.(346) AKT enhances non-homologous end joining (NHEJ) that, in the setting of prostate cancer, presumably serves to create mutagenic rearrangements.(184,338) AR is dependent on PARP1 for general transcription (347) and the nuclear receptor also responds to DNA damage by facilitating expression of XRCC2 and XRCC3 that function in homologous recombination repair alongside upregulation of DNA-PK_{CS}, a key NHEJ enzyme. (348)

Prostate tumours with mutator phenotypes have been identified and attributed to MSH2 and MSH6 mismatch repair enzyme mutation and rearrangements.(149,263,323,339) Whilst these defects present more frequently in advanced cancers, there is evidence that disruption of these enzymes is also present in primary tumours.(339) However the mutation may be a more recent event in establishing metastatic clones, rather than driving early disease.

Variants of DNA repair genes have also been shown to promote the formation of the TMPRSS2-ERG fusion. These genes were identified through linkage studies in ETS⁺ tumours and included BRCA2, ESCO1 and POLI. (349) Interestingly it was also shown that inducing genotoxic stress promotes fusion formation in a prostate cell specific manner, (328) due to transcriptional proximity of AR induced loci.

Conclusion – Current Evidence and Future Work

There are still no definitive epigenetic or genetic events that initiate prostate cancer. However it is clear that inflammation is involved in the development of putative precursor lesions, and carcinogenesis of the prostate. This observation holds true for many other cancers, including stomach and colorectal carcinomas. Increasing evidence that environmental factors can also overcome the supposed racial component of disease is also intriguing. There may be a culprit hiding in plain sight to account for the huge discrepancy of the disease incidence between men in well-developed and undeveloped regions in the world, aside from longevity and causes of premature death. Cancer found recurrently at non-related autopsy also suggests that prostate tumour development may be a case of when and not if.

Cellular Origins of Human Prostate Cancer; Summary and Further Work

Combination of CSC and stochastic/clonal models of disease is possible in prostate. The frequency of diagnosis of disease in older men, and the slow relapse of CRPC suggests a transformational event in the stem cells. Yet the aggressiveness of late-stage disease, reliance of CRPC on perturbed AR pathways and the sequential adaptive mutation seen in metastatic lesions suggests the runaway nature of dominant clone/s.

If we view primary tumours as dysregulated tissues, a hierarchical ordering of cells is necessary, but perturbed in cancer.(31) Tumour cells often show greater plasticity than normal counterparts, and the initiating stem-like cells prove the most flexible in their phenotype and in some cases are capable of trans-differentiation.(51) There is clear evidence of a CSC pool in the human prostate (32,83,139,350), however one unknown is whether this is a fixed population or whether it is maintained through constant plastic transitions into and from more differentiated “progeny” which really becomes an issue upon targeted treatments of CSCs. Here the adaptive nature of the CSCs may render them “untouchable” to targeted treatment, as they could fluctuate between cell-types.(351) There is also the observation that founder mutations such as the TMPRSS2-ERG fusion, although found in the CSC, are more easily formed in androgen responsive cells. Is it the mutation that allows for de-differentiation or is it accrued in a phenotypically fluctuating CSC? (302,327–330)

Further work also needs to be undertaken to dissect inter-CSC heterogeneity in the prostate. This will require discovery and utilisation of markers exclusive to separate CSC populations. Identification through deep-sequencing of fractionated CSCs will be the obvious route to this goal and will ideally identify metastatic and dormant sub-populations of progenitors. Markers can then be utilised in selective searches of CTCs and to validate CSC seeding of metastatic disease. Segregation of CSC populations on tissue Gleason grade (preferably from the same cancer which will prove a challenge) may also shed light on clonal or transitional progression of

prostate cancer in indolent and aggressive disease. Even current single-cell sequencing from tumours and blood circulation reveals an array of genotypes and phenotypes in a single patient. (158,162,164,352)

Building up a profile of prostate CSCs through Genome, RNA and ChIP sequencing will cut through the heterogeneity of prostate cancer and allow relevant genomic, transcriptomic and epigenetic data to be discerned without the CSCs being masked by overlying basal and luminal cell signatures. Single-cell data is also required and will yield information on 2 fronts; the assessment of chromoplexy to a greater detail with confirmation whether these catastrophic genome rearrangements are tolerated by the CSC, and the presence of bivalent genes in the CSC followed by monoallelic expression analysis in more differentiated progeny as an assessment of both expressional heterogeneity and contribution of the phenomenon to disease.

Generation of the same profiles for the more differentiated cells of the prostate will be key to identify variants that can be used in targeted therapies, and accrual of this data in temporally and clinically separated stages of disease will inform on progression and possible routes to block the advancement of prostate cancer. Dissemination of this heterogeneity will be especially useful in early stage primary tumours, where the genomic data is extremely convoluted, due to uncertainty of driving and passive events alongside dominant cell type “artefacts”.

Defining Prostate Cancer Pre-malignancies

PIA and PIN are, in the light of current evidence, genuine precursor lesions of prostate cancer. However definitive proof rather than transcriptomic congruence and histological adjacency is still required. Cytochrome oxidase deficiency has been used previously for stem cell lineage tracking in the prostate (7) and could be used in a similar manner to track clonality through PIA, PIN and into cancer. This experiment would require cross sectioning of prostate tissue that has adjacency of PIA, PIN and cancer through acini. Our methods in tracking telomerase activity and telomere length in BPH and cancer may also be of use here. (273,283)

Inflammation; importance and modelling of disease

To address the inflammatory nature of prostatic disorders and the contribution that this environment makes towards carcinogenesis, whole tissue sequencing studies should attempt to incorporate open searches for pathogenic organisms in addition to the primary study goal. This will allow functional correlates to be discerned between disease stage and presence of potentially oncogenic bacteria and viruses. The infiltration of immune cells in the prostate could also be interrogated by these studies by taking blocks of tissue aside from sequencing efforts. This will allow a web of pathogen-immune system interplay to be constructed which may be used clinically to infer invasive species or indeed appropriate and inappropriate lymphocytic responses in the prostate. The cytokine environment of prostate cancer is evidently an important factor in disease; recurrent finding of IL-6, and concomitant STAT3 phosphorylation, as a synergic driver of transformational events (190,191,205) makes the point that other important inflammatory molecules are very probably included in a melee of micro-environmental triggers contributing toward tumourgenesis.

The inflammatory noise of the *in vivo* environment is not re-encapsulated by current *in vitro* models. That and the lack of stromal co-culture marks a failure in disease which already has a shortage of cell lines. More effort is required to ensure that our mimics of prostate cancer are as close as feasibly possible to human tumours, in line with the adage; “All models are wrong, but some are *more* useful.” The lack of primary tumour and CRPC specific mutations in cell lines is also a problem. Currently only VCaP cells (353) harbour the TMPRSS2-ERG fusion and, until last year, no cell line contained an SPOP mutation or CHD1 deletion. The work by Gao *et al* in the generation of seven CRPC cell lines is an extremely valuable contribution to the field and will bring readily usable and relevant models to enhance understanding of end-stage disease. (354)

Castrate Resistant Prostate Cancer – a Cancer Stem Cell driven disease?

The inevitable development of fatal CRPC is very probably induced by current treatment strategies. The ablation of androgen-dependent luminal cells (326) and selection of basal and neuroendocrine lineages (28,79,355–357) alongside sub-clonal mutated populations (332,358–364) suggest that androgen deprivation therapy may actually be enhancing disease aggressiveness and disrupting cellular genotypes.(365) For example genomic instability, a hallmark of cancer, is rare in prostate cancer yet appears post-treatment in the form of

tumours with characteristic mutator phenotypes.(323,339) Perturbed AR signalling is important in this stage of prostate cancer (354,366,367) and does have the ability to drive metastatic clones (158,368,369) – indicated by presence of locus amplification (331,370) and splice variants (333,371–375) in these cells. Androgen signalling however isn't disrupted in development of the disease, where dysregulated ETS factors are more involved in progression.(107,184,301,314,315) 65% of CRPCs have detectable aberrations in androgen signalling, (294) but what is driving the cancers in the remaining 35%? This also doesn't account for intra-tumoural heterogeneity– some cells in the AR perturbed tumours won't be necessarily driven by AR aberration.(376) The current focus on the state of the AR in CRPC while, although important, perhaps masks other recurrently altered molecular targets that could be exploited, such as PI3K and Wnt signalling. (158,294,377,378) Selective luminal cell searching in CTC and metastatic cell studies completely excludes the possibility that other prostate cell types contribute to disease and biases any results – also what could these studies be missing through their selectivity? In this respect there may be a case for hypothesis driving results in CRPC, whilst any evidence against anti-androgen efficacy or dependence of cells on androgen is ignored.

Finally the presence of progenitor cells in metastases is yet to be determined in prostate cancer, although the possibility has been discussed.(379,380) Since prostate CSCs have enhanced invasive potential over metastatic cell lines,(32) express markers of EMT,(81) are androgen independent,(79) and the messenger and micro-RNA signatures of CRPC resembles that of the stem cells (381,382) suggests an involvement the in metastatic spread of prostate cancer. Could these cells be drivers of minimal residual disease and castrate resistance? Disseminating the heterogeneity of the CSC pool will determine possible existence of a metastatic sub-clone that can be detected in both the primary and secondary seeded cancers – inference of metastatic tissue tropism may also be attainable from this data. Metastatic spread, although initially thought to be a late event, (20) is now presumed to occur early in cancer development and lie periodically dormant as micro-metastases. Accumulating knowledge on and the targeting of these metastatic clones is therefore paramount in prostate cancer.

Concluding Remark

It is perhaps not sufficient to conclude that prostate cancer has a multifactorial aetiology and is characterised by genomic and phenotypic heterogeneity. In short, each man's cancer is somehow unique and attempts to characterise and design best fit treatments are naïve. Truly personalised medicine, based on understanding of the molecular and cellular characteristics, however heterogeneous, will provide the ultimate treatments.

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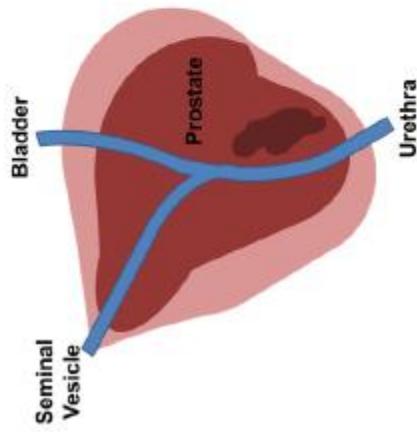
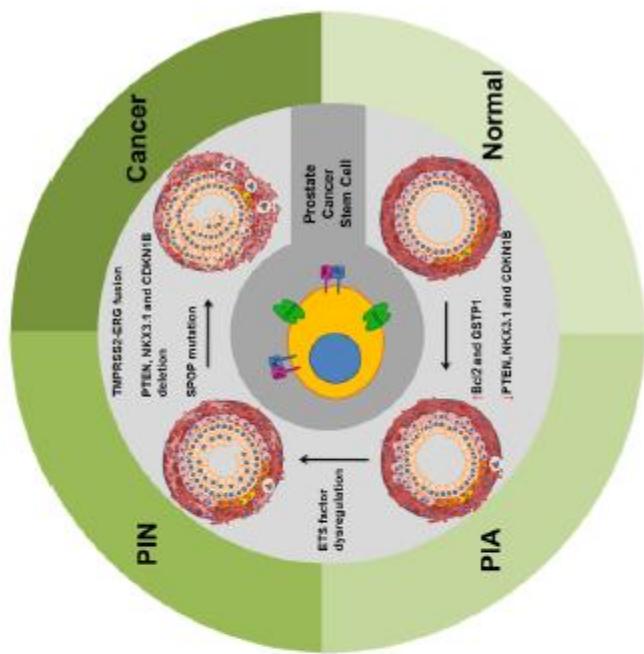
Table 1 –immunophenotypes of Human Cancer Stem Cells

Tissue	Immunophenotype	Ref
Acute Myeloid Leukaemia	CD34 ⁺ CD38 ⁻	(Bonnet & Dick 1997)(64)
Bladder	67LR ⁺ CEACAM6 ⁺ CK17 ⁺	(He 2009)(65)
Brain	CD133 ⁺	(Singh 2003)(66)
Breast	CD44 ⁺ CD24 ^{/lo}	(Al-Hajj 2003)(67)
Colon	CD133 ⁺	(Ricci-Vitiani 2007)(68)
Cervix	CD44 ⁺ CK17 ⁺	(Feng 2009)(69)
Endometrium	CD133 ⁺	(Rutella 2009)(70)
Head and Neck	CD44 ⁺ BMI1 ⁺	(Prince 2007)(71)
Liver	CD133 ⁺	(Ma 2007)(72)
Lung	CD133 ⁺	(Eramo 2008)(73)
Melanoma	CD20 ⁺	(Fang 2005)(74)
Multiple Myeloma	CD138 ⁻	(Matsui 2004)(75)
Pancreas	CD44 ⁺ CD24 ⁺ ESA ⁺	(Li 2007)(76)
Prostate	CD44 ⁺ $\alpha_2\beta_1^{hi}$ integrin CD133 ⁺	(Collins 2005)(32)
Ovary	CD44 ⁺ CD117 ⁺	(Zhang 2008)(77)

Table 2 – Molecular defects which can drive primary prostate cancer

Primary Prostate Cancer Molecular Defects	
Oncogenic Drivers	Tumour Suppressors
TMPRSS2-ETS fusions A translocation or interstitial deletion event places an ETS factor, most commonly ERG but also ETV1, ETV4 and ETV5, under the control of the TMPRSS2 promoter. The ETS proteins are developmental transcription factors that affect proliferation, migration and transformation of cells. These gene fusions are seen in over half of all prostate cancers.	PTEN deletion Heterozygous deletion of the PTEN gene is observed in ~40% of primary tumours. This causes haploinsufficiency of the gene product; PTEN is the reciprocal phosphatase of PI3K. Reduced removal of PI3K phosphorylations causes unchecked AKT activation that allows for increased cell survival and proliferation. Mutation of the gene is also observed.

MYC overexpression MYC is seen to be upregulated in early prostate tumours and its locus is frequently amplified in advanced cancers. The gene encodes a transcription factor that has well characterised transformative properties due to its role in cell cycle progression.	CDKN1B deletion CDKN1B is deleted in ~20% of primary tumours. The protein acts as a CDK inhibitor that controls the G ₁ cell cycle checkpoint, loss of the protein allows for easier commitment to the cell cycle and thus promotes increased proliferation. Mutation of the gene is also observed.
Telomerase activation The protection of chromosome ends by inappropriate activation of telomerase prevents replicative senescence in highly proliferative cancer cells. Telomerase expression is switched occurs during hgPIN and early prostate cancer.	NKX3.1 deletion NKX3.1 is heterozygously deleted in up to 85% of prostate cancers and is downregulated in PIN. The protein is a homeobox transcription factor that regulates prostate epithelial development.
IL-6 addiction Inflammation causes upregulation of both IL-6R and oncostatin M, an IL-6 signal transducer, in the prostate stem cell population. An increase of systemic IL-6 in prostate cancer patients creates a positive feedback loop favourable towards transformation through enhanced STAT3 activation and associated downstream gene expression.	SPOP mutation SPOP is mutated in 6-15% of primary cancers. This is a molecular event that is mutually exclusive of TMPRSS2-ERG fusions. SPOP is an adaptor protein for the Cullin3 E3 Ubiquitin Ligase and directs the proteasomal degradation of oncogenic proteins such as AR, DEK and ERG, and is also involved in the DNA damage response and cellular senescence. Mutations cluster in the substrate binding domain of the protein and abrogate SPOP effectiveness.
	CHD1 deletion CHD1 is commonly deleted in primary tumours, correlates with SPOP mutation and is thus mutually exclusive from the TMPRSS2-ERG fusion. The protein is involved in chromatin remodelling.



Graphical Abstract

Figure 1

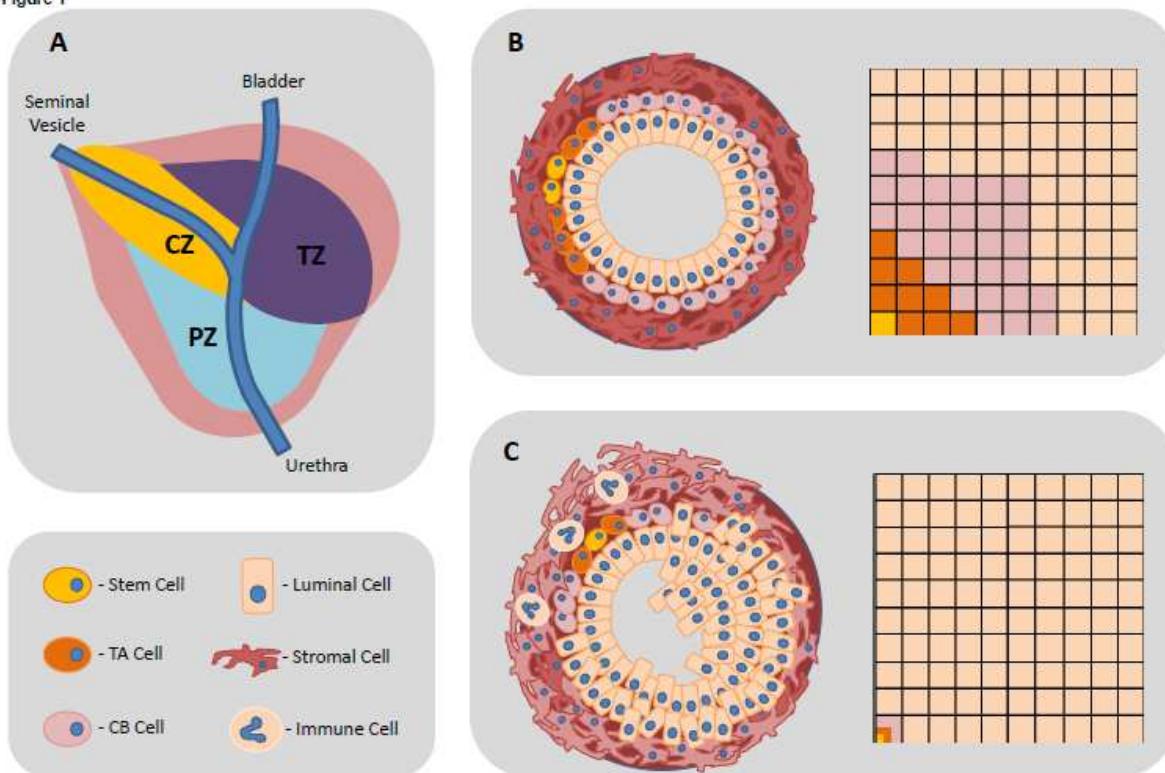


Figure 2

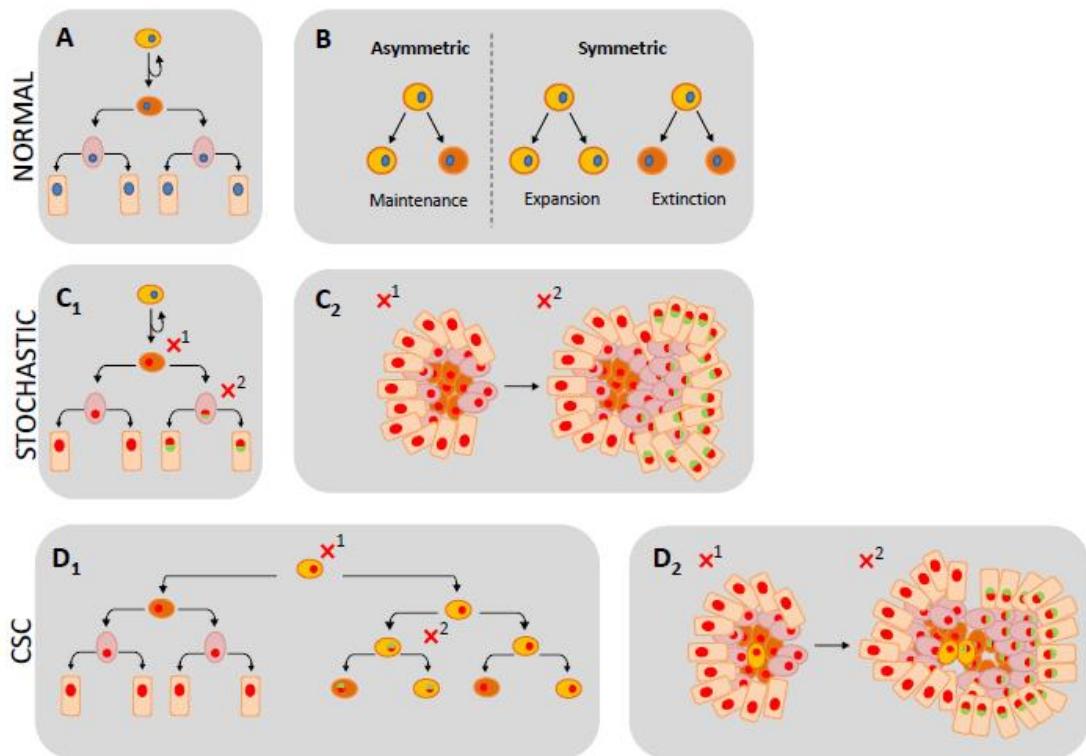


Figure 3

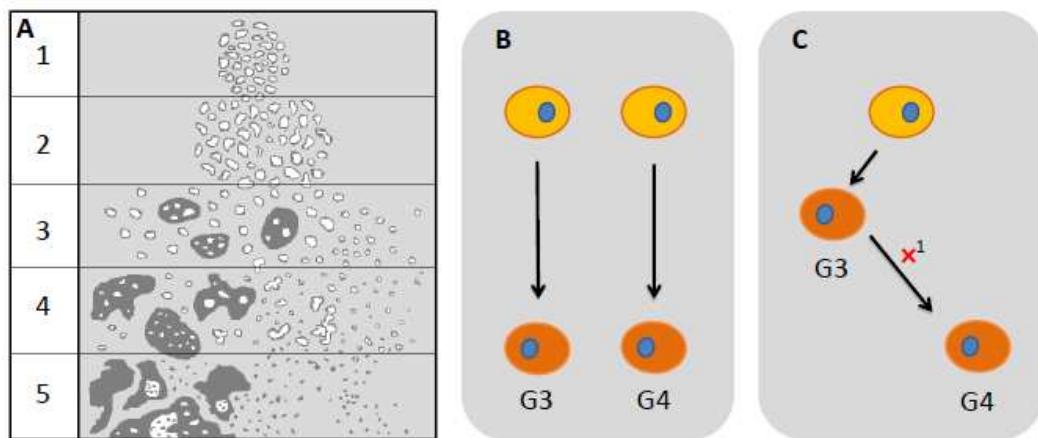


Figure 4

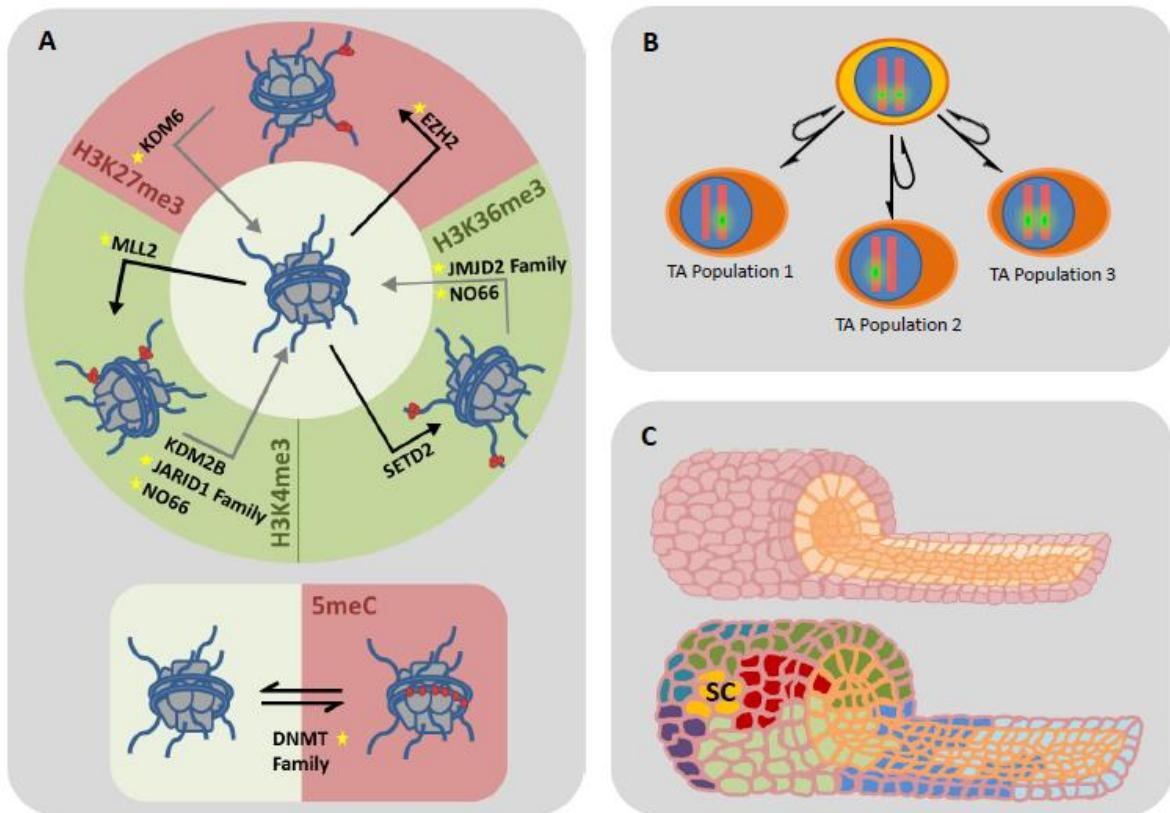


Figure 5

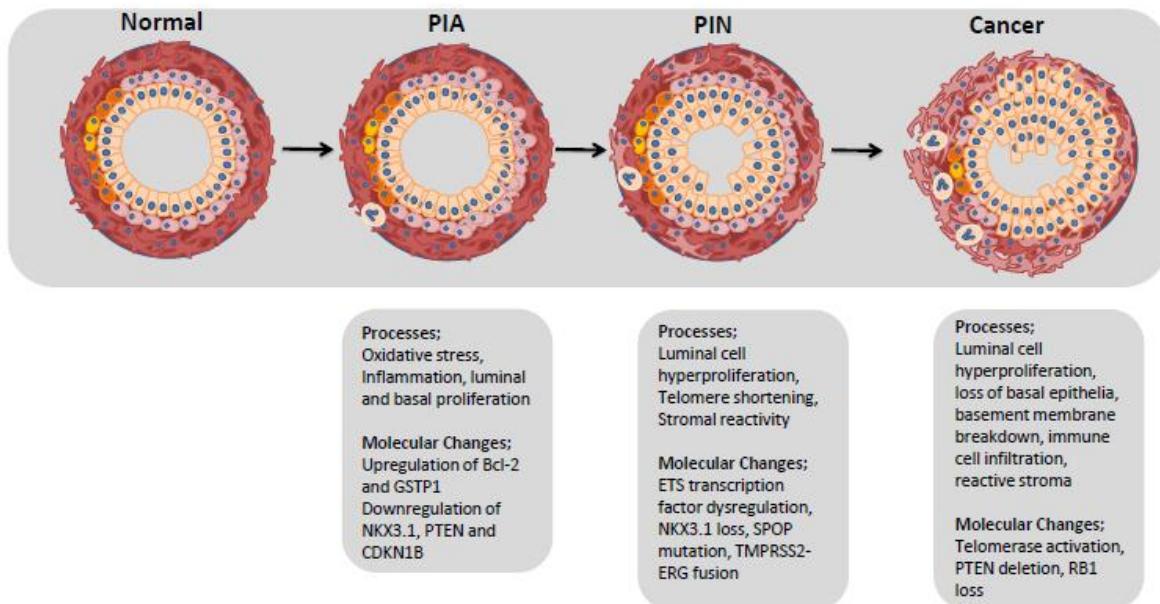


Figure 6

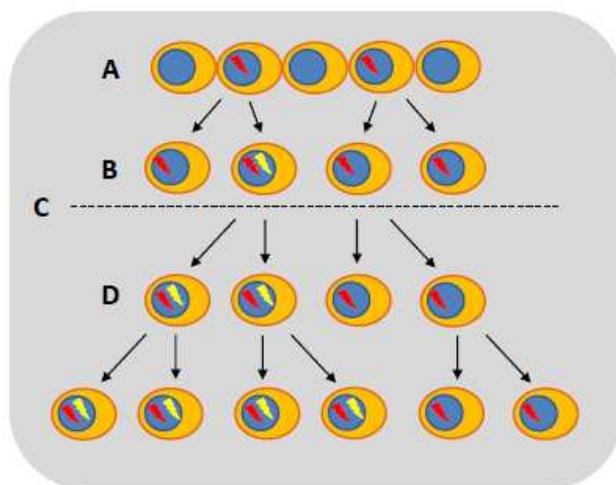


Figure 7

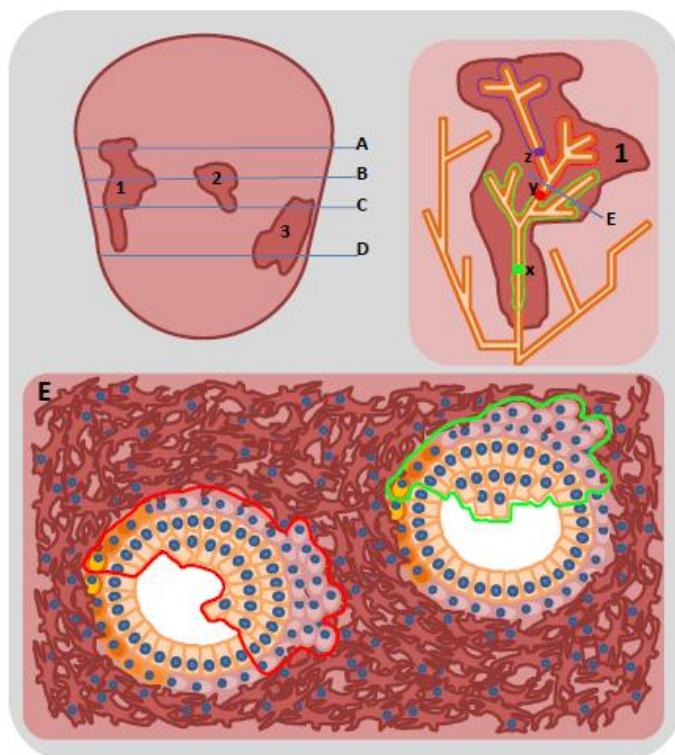


Figure 8

