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Inflammation as the Primary Aetiological Agent of Human Prostate Cancer: A Stem Cell Connection?

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ABSTRACT

Inflammation has been implicated for some time as a potential aetiological agent in human prostate cancer. Viral and bacterial infections or even chemical carcinogens such as those found in cooked meat have been proposed as the inflammatory stimuli, but the mechanism of cancer induction is unknown. Recent information about gene expression patterns in normal and malignant epithelial stem cells from human prostate provides a new hypothesis for inflammation-induced carcinogenesis. The hypothesis states that in the stem cells located in the basal cell compartment of the prostate, activated prostate epithelial stem cells acquire a survival advantage, by expressing one of more of the same cytokines such as IL6. The establishment of one or more autocrine signalling loops results in an expansion of these cells in the absence of inflammation, as a potential first stage in the development of the tumour. J. Cell. Biochem. 105: 931–939, 2008.

KEY WORDS: PROSTATE CARCINOMA; CANCER STEM CELLS; INFLAMMATION

The prostate is a small, walnut shaped (and sized) gland, which is located below the bladder in humans. The gland surrounds the urethra and has a fibromuscular function which acts to restrict urine flow, but its principal function is secretory, producing a number of essential proteins for the functioning of sperm, such as acid phosphatase, citric acid and bioavailable zinc. It makes some of the highest amounts of polyamines, which regulate the pH of sperm, preserving a mildly alkaline environment for the sperm within the acidic female cervix [Devens et al., 2000].

The human prostate consists of three zones, which are composed of relatively simple secretory epithelial structures: the central, peripheral and transitional zones [McNeal, 1980]. There have been distinctive gene expression differences noted between the various zones, and while the transitional zone is the location for the most common prostate proliferative disorder: Benign prostatic hyperplasia (BPH), most prostate cancers (80%) originate in the peripheral zone [Franks, 1954; McNeal et al., 1988]. In contrast, the murine prostate has a lobular anatomy, and does not have the same close relationship with the urethra [Abate-Shen and Shen, 2000]. Despite these differences there is considerable information concerning prostate development and prostate cancer development resulting from the modelling of human disease in the murine prostate [Pienta et al., 2008].

Although most males are born with a vestigial prostate, development (and growth) accelerates during puberty. Both the epithelial and stromal components of the prostate cellular structure express the receptor for male sex hormone, androgen [the androgen receptor (AR)]. In the absence of a functional AR, the prostate does not develop, therefore the prostate is considered an androgen dependent and regulated organ [Marker et al., 2003]. The prostate also shrinks and involutes after castration, but will regenerate after restoration of normal androgen levels [English et al., 1987]. The castration-resistant fraction of normal prostate epithelium (prostate epithelial stem cells) has been proposed to reside within the basal epithelial compartment, as first proposed by Isaacs and Coffey [1989], and reviewed recently by Collins and Maitland [2006], but the stimulus for re-growth may lie within the androgen responsive stromal environment [Cunha, 1984]. Such close interplay between the different cellular compartments also persists into prostate cancers [Olumi et al., 1999], where there is a substantial stromal component, whose influence decreases as the differentiated (structural) nature of the tumour changes [Cunha et al., 2003]. Such histological/anatomical features were precisely recorded in the 1960s by Gleason, who provided a grading system [Gleason, 1966], which is still an excellent, if imperfect, predictor of clinical outcome in prostate cancer. In the Gleason classification, the loss of glandular morphology and basement membrane which typifies grade 4 was associated with increased chance of metastatic spread, and hence a poor clinical outcome [Bostwick et al., 1995].

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DIFFERENTIATION IN THE PROSTATE

Within the epithelial compartment there are several epithelial cell subtypes. In its simplest form the prostatic epithelium can be divided into basal and luminal compartments [reviewed recently by Maitland and Collins, 2008] as illustrated in Figure 1A. Probably the most homogeneous is the luminal compartment. These cells are the synthetic 'factory' of the prostate, generating the secretory products, which appear in prostatic fluid. Gene expression in the luminal compartment is controlled by AR. The 'prostate-specific' genes such as prostatic acid phosphatase, prostate specific antigen and polyamines found in luminal cells are amongst the most highly expressed sequence tags in total gene expression profiles [Clegg et al., 2004]. Their expression as epithelial genes is modulated by the presence of response and binding sites for the AR within the transcriptional control sequences. These have been located to an imperfect canonical sequence of 5′-AGAACAnnnTGTACC-3′ [Roche et al., 1992] which can be arranged in both direct and inverted repeat orientations [Verrijdt et al., 2003], a recognition specificity which has recently been extended to include the necessity for binding in some genes to an ets1 transcription factor binding site [Massie et al., 2007]. Basal transcriptional control can be seen the on–off switch (including epigenetic control by methylation), whereas the differentiation-linked androgen response is a rheostat to control *magnitude* of transcription when the switch is on. In keeping with this, the AR-responsive genes are amongst the highest expressed tissue-specific genes, comparable only to the proteolytic enzymes found in human pancreas [Adams et al., 1995].

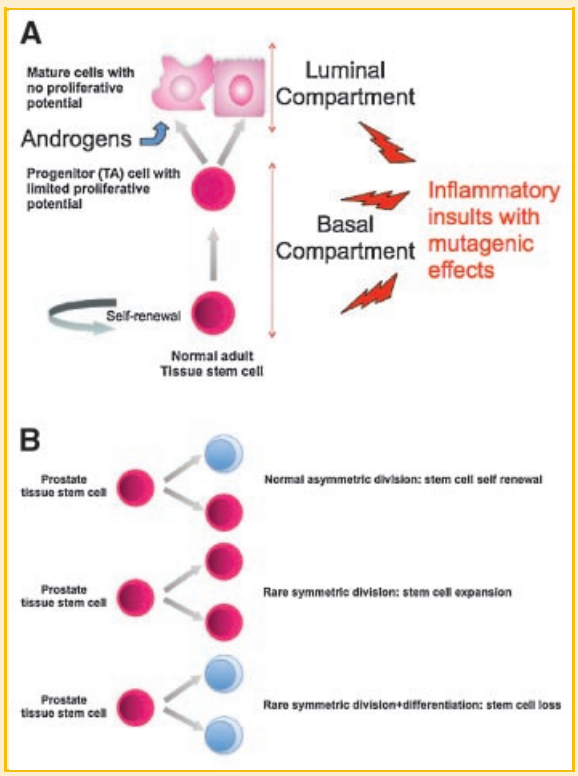

Fig. 1. A: Differentiation of prostate epithelium. The human prostate consists of two epithelial compartments: basal and luminal. Cell turnover is more rapid in the terminally differentiated luminal compartment, whereas cells are longer lived (and hence could accumulate mutations) in the basal compartment, which contains the stem cell fraction. Direct action of androgens is only seen in the luminal compartment. The point of damage from inflammation (indicated in red) is not known. B: Stem cell fate. Within the basal cells in human prostate lies the stem cell compartment. There are three possible fates for a stem cell on mitotic division: (a) Asymmetric division leading to stem cell self-renewal and a progeny Transit Amplifying (TA) cell (b) Symmetric division, resulting in two new stem cells and expansion of the stem cell compartment or (c) Symmetric division and differentiation, where two TA cells are produced resulting in loss of a stem cell. It is important to note that there are most likely to be several stem cells in the stem cell compartment, and that loss of one cell need not result in extinction. Indeed it is unlikely that a single stem cell is immortal, but rather the content of the stem cell compartment remains fairly constant, unless affected by outside agents such as inflammatory cytokines or toxic stimuli.

DIFFERENTION, LIFE SPAN AND CARCINOGENESIS

In contrast to the secretory luminal compartment in the prostatic acini, the basal compartment does not show the same extremes of gene expression, and shares many of its essential expressed genes with basal epithelium from other secretory glands. Whereas luminal epithelium exists to secrete, the cells are terminally differentiated and have little capacity to divide [de Marzo et al., 1998]. Rather, renewal of the luminal cells is achieved by amplification, replacement and differentiation of mature basal cells. While the precise determination of luminal cell life span in prostate lags behind that in the intestine [Potten and Grant, 1998] a higher fraction of the luminal cells is apoptotic compared to extremely low rates in the basal compartment, and there are at least 10-fold more proliferating cells as measured by Ki67/PCNA expression within the basal compartment relative to the luminal compartment.

Many attempts have been made to derive subtypes from the cells within the basal compartment. The use of cytokeratin antibodies is a common tool in such efforts and several distinct populations expressing CK 5,14 in the most primitive cells and CK 8, 18 (and 19) in the most differentiated were shown. However, increased resolution in the analyses resulted in the identification of intermediate types with atypical partnerships of CK [Hudson et al., 2001; van Leenders et al., 2001]. These 'intermediate cells' remain difficult to define and a more plausible explanation is for the existence of a spectrum of differentiation, in which the most mature basal cells are para-luminal, and probably express initiating amounts of AR.

In terms of carcinogenesis, it has been assumed that because the major phenotype of prostate cancer is an AR+/PSA-secreting cell, that the normal AR+/PSA+ luminal cell is the primary target for oncogenic changes in the prostate. This hypothesis states that extension of lifespan by telomerase activation [Meeker et al., 2004], reduced dosage or inactivation of key tumour suppressor proteins, for example PTEN [Li et al., 1997] and ultimately relief of cell constraints on unscheduled DNA synthesis leading to apoptosis [Osman et al., 1999] result in an altered luminal cell with an extended lifespan and the ability to invade and re-colonise new cellular environments such as bone marrow, the preferred site of fatal prostate cancer metastases [Morrissey and Vessella, 2007].

There is also an extensive literature implicating the action of AR, which is expressed in the presumed luminal target cell in the oncogenic process, to the point where the AR has been considered to be an oncogene [Han et al., 2005; Taplin, 2007] and able to stimulate the initiation of DNA replication [Litvinov et al., 2006].

MUTATION AND STEM CELLS IN THE GENESIS OF PROSTATE CANCER

In a traditional view of tumour induction, Ashley [1969] calculated that between 3 and 7 mutations were required for tumour induction [Cairns, 1975] also discussed the temporal restrictions and concluded that the mutation rate required to achieve 3–7 critical mutations even in a long lived, primitive epithelial stem cell compartment would be 100-fold greater than his theoretical estimates of 10^{-6}. Normal human mutagenesis unaided by any mutator phenotype such as mismatch repair, is between 10^{-7} and 10^{-9} per generation. This is of course dependent on the target cells undergoing regular mitosis, something which is poorly understood for cells in the human prostate, but well studied in epithelial cells of the colon [Tomlinson et al., 1996].

However such calculations should be tempered by recent studies of actual genetic lesions in human cancer cell lines [Sjöblom et al., 2006]. Even allowing for polymorphisms, Sjöblom et al. found that in 22 commonly used colon and breast cancer cell lines there were in total 189 genes found to be mutated, with a mean of 90 in each cell line, of which only an average of 11 were predicted to be 'essential'. Many more 'silent mutations' are also predicted [Tomlinson et al., 1996]. The contribution of extensive in vitro culture was also taken into account, although the necessity to undergo primary chromosomal rearrangements such as seen in both glioblastoma cell lines [Lee et al., 2006] and in some embryonic stem cell cultures [Draper et al., 2004] was not directly considered. If we consider that five changes are the minimum requirement then with an unchanged mutation rate tumour development in a multihit stochastic model would require more than 10^{25} generations! A human lifespan has been predicted to span 10^{14} stem cell divisions based on one division/48 h and a total stem cell content of 10^{10} stem cells [Cairns, 1998]. The relative influences of genomic instability discussed recently by Bodmer [2008] and the mutator phenotype [Loeb et al., 2008] are still controversial, but both could contribute to an acceleration of the carcinogenic process. Studies of established and primary human prostate cancers are suggestive of, but have not provided conclusive evidence of the necessary genomic instability [Joshua et al., 2008], but mutator phenotypes as seen later in colon cancer development are found less frequently. This would argue for a Darwinian selective mechanism [Greaves, 2007; Bodmer, 2008] combined with a 'normal' mutation frequency. However, this mechanism would take no account of a 'protected' stem cell genotype, which would seem to desirable, for example apoptotic loss of damaged cells.

If we assume that a stem cell (or a primitive amplifying cell) in contrast to the luminal cell in the prostate is the target for such mutagenic changes, there is both sufficient time and an alternative view of oncogenesis [Cairns, 1975]. At each division there are three possible choices in cell fate for tissue stem cells as shown in figure 1B. When such a stem cell undergoes mutagenic change, the relative frequency of the three choices may be perturbed. Most such changes will be *silent* allowing the predicted *asymmetric* self-renewing division recognised as a core property of normal tissue stem cells [Calabrese et al., 2004]. Less likely, but still possible are mutations, which promote *symmetric* division: resulting in either stem cell *expansion* (two daughter stem cells) or *extinction* (two amplifying cells with loss of a stem cells from the protective niche). The latter state could also be achieved by lethal mutations.

It is important here to consider the types of mutations, which are important in the stem cell compartment. Until now, the *growth promoting* properties of mutations have been most studied in the multi-hit model of carcinogenesis [Fearon and Vogelstein, 1990; Hanahan and Weinberg, 2000] relative to *survival* or death preventing changes. Recently, a number of physical chromosomal fusions have been detected in human prostate cancers, most notably a fusion between the TMPRSS2 promoter and the erg oncogene on chromosome 21 [Mehra et al., 2007]. Considerable emphasis has been placed on the androgen regulation of the fusion gene [Hermans et al., 2006], and in fractionated human prostate epithelial cells we have been able to see TMPRSS2-erg expression in the most differentiated fractions. However, to our surprise, we also detected expression in the AR- stem cell population [Birnie et al., in press; see below]. Such fusions have been detected and linked to stem cell expansion in haematopoietic cancers, such as chronic myeloid leukaemia. Survival and/or expansion of the stem cell pool could require elements of this, but as the stem cell mitotic rate is presumed to be low, rapid mitosis is not an absolute requirement. Rather, mutations which relieve the restrictions (as yet poorly defined) of the stem cell niche [Scadden, 2006] might be favoured. Should such changes promote unscheduled divisions, then changes associated with apoptosis protection and telomere preservation would be important. Finally, need the essential changes be mutations? If survival in the stem cell niche is important, then perhaps flexibility would best be provided by epigenetic changes, which would nevertheless be heritable [Nelson et al., 2007].

INFLAMMATION AS AN AETIOLOGICAL AGENT IN HUMAN PROSTATE CANCER

Inflammation is a common occurrence in the human prostate, and it is emerging as a strong candidate for the primary aetiological event in development of the tumour [see a recent review by De Marzo et al., 2007]. The origin of the inflammatory infiltrates in prostate could be infection by viral (as described below) or bacterial agents [Krieger et al., 2000; Elkahwaji et al., 2006] but could also be a result of chemical damage [Nakai et al., 2007]. For example the frequency of both viral and bacterial infections is probably underestimated in the human prostate, and prostatitis is one of the main prostatic disorders diagnosed, much more frequently than either BPH or indeed cancer. It has long been accepted that chemical carcinogens such as the products of cigarette smoke [Haverkos, 2004], can be found in human cervical secretions. Smoking has not been implicated in prostate cancer, but dietary factors have and recent data has

shown that by-products of roasted meat (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) were present in prostate and could initiate both inflammation and tumorigenic changes in a mouse model of the disease [Nakai et al., 2007].

These ideas have produced an improved hypothesis of prostate cancer development, where the recognised premalignant state of prostatic intraepithelial neoplasia, or PIN is preceded by an inflammatory atrophy or PIA, in which prostatic epithelial cells show an increased Ki67-marked proliferation [De Marzo et al., 1999]. However the direct relationship between PIA and PIN, and its predisposing relationship to tumour development remains somewhat controversial, as lineage experiments are difficult to carry out in man [Postma et al., 2005].

VIRAL INFECTION OF THE PROSTATE AS A PRIMARY SOURCE OF INFLAMMATION

In a traditional viral oncogenesis mechanism, the transfer of growth promoting viral oncogenes into the tumour cells is predicted [reviewed by Butel, 2000]. Repeated studies of human prostate tissues for the presence and expression of virally encoded oncogenes has met with varying success, particularly for genital human papillomavirus, and the consensus presently lies against such a simple viral oncogenesis [Korodi et al., 2005], such as found in human cervical epithelium [zur Hausen, 2002]. A confounding factor in such studies is the transurethral route of biopsy, which could allow contamination via the penis, where HPV infection is frequent. However, it has long been supposed that the male is a reservoir for the oncogenic human papillomaviruses, and the secretion of virus into prostatic fluid in the prostate is a persuasive hypothesis for sexual transmission to the female cervix. A number of other potentially carcinogenic viruses have been detected in human prostatic tissues, and recently Das et al. [2008] have followed up earlier publications indicating the presence of the oncogenic human papovavirus BK in premalignant prostate lesions [Zambrano et al., 2002; Das et al., 2004]. The presence of a p53/pRb110 inactivating gene such as BK T antigen would remove the need for mutagenic inactivation of these powerful tumour suppressor genes. Mutational inactivation of p53 and pRb110 is extremely rare in the early stages of prostate cancer [Osman et al., 1999].

A further viral candidate: a human gammaretrovirus, Xenotrophic MuLV-related virus (XMRV), was reported in prostate tissues [Urisman et al., 2006], most notably from patients with an RNAseL R462Q polymorphism on chromosome 1q25, the HPC1 locus which pre-disposed to development of familial, early onset prostate cancer [Carpten et al., 2002]. RNase L is one of the major cellular enzymes induced by the action of interferons in virus infected cells [Zhou et al., 1993]. There are several paradoxes in this study, as the retrovirus was detected principally in the stromal component of tissues, but also in the (epithelial) cell line LNCaP [Urisman et al., 2006]. The XMRV was extremely sensitive to interferon action (related to the reduced anti-viral capabilities of the RNaseL variant in vivo) and only in DU145 cells where the interferon response had been rendered RNaseL deficient by Si RNA treatment would the virus infect and express its genes [Dong et al., 2007].

The prospect of an infection in non-epithelial tissues does not eliminate an infectious aetiology, as such an infection would inevitably lead to localised inflammation. As described later, such a localised inflammatory response predisposes to localised tissue damage and cell turnover, which could be an unconventional infectious aetiological route.

INFLAMMATION AND THE CELL TYPE OF ORIGIN FOR HUMAN PROSTATE CANCER

Importantly, for prostate cancer, there may not be a single inflammatory stimulus, but rather it is the consequences of the inflammation, which promote the cancer initiating changes [Coussens and Werb, 2002]. As discussed above, the cell of origin is most likely to be a long-lived epithelial cell from the stem cell compartment. However there are other possibilities. Recent studies on gastric cancer in mice [Houghton et al., 2004] have suggested that mesenchymal stem cells (MSCs), which infiltrate during inflammation, can differentiate into gastric epithelial cells in response to potent local environmental signals and bacterial infection [Fox and Wang, 2007]. The non-gastric origin of the infiltrating cells was determined using GFP tagged cells from a recipient mouse. Whether this can be extrapolated to human tissues remains to be determined. In the skin and the prostate, infiltration of inflammatory cells and MSCs has also been shown [Palapattu et al., 2006] and destruction of the prostate tissue architecture is required to permit a colonisation (again in murine experiments). Interestingly this re-colonisation of the mouse prostate did not happen during regeneration after castration, but only after viral induced destruction (and associated inflammation). Under these circumstances one would expect indiscriminiate destruction of luminal and basal cells by viral infection, whereas the effects of castration should be limited to the AR-expressing luminal cells. If this were to happen in humans, then a long-lived cell with extensive differentiation potential and a degree of niche independence would be located in the prostate and subject to the androgen-induced differentiating influences of the prostatic stroma.

A more conventional consequence of inflammation would be tissue destruction, and disruption of the epithelial stem cell niche, requiring regeneration and stem cell (or more extensive transit amplifying cell) mitosis as part of the repair process. The infiltrating cells from the immune system also produce a variety of reactive, cytotoxic and mutagenic substances such as reactive oxygen species and nitric oxide [reviewed in De Marzo et al., 2007]. Multiple pro- and anti-inflammatory cytokines are also released by the cells from the innate immune system, resulting in the attraction of new partners to this dance of destruction. As a consequence there is clearly capacity for adaption of the primitive epithelium under these conditions. Any mutation which favours survival in this altered environment would be advantageous, resulting in a 'new' stem cell type occupying the niche (see Fig. 2A). These changes could include translocations, point mutations or even epigenetic inactivations. Mathematical calculations as to the likelihood of such an event transforming stem cell niches in the colon have been made and, depending on both the number of mitoses, and the power of selection, such a change could result in a completely transformed

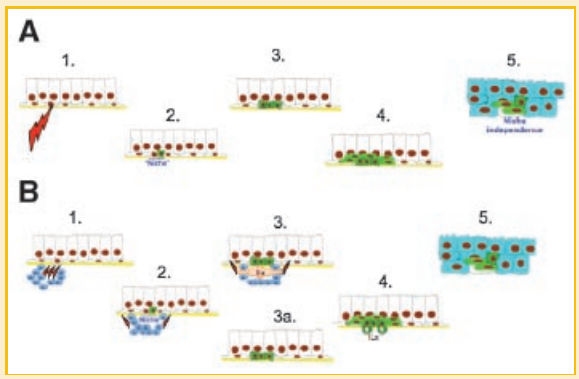

Fig. 2. A: Stem cell mechanism of carcinogenesis in prostate epithelium. (1) Tissue stem cell targeted and activated, can accumulate multiple mutations/epigenetic changes. (2) To allow amplification there is no need for the stem cell to replicate more rapidly, but it may be necessary to destroy the niche controls. (3) 'Activated' tissue stem cells can repopulate the glands, perhaps under local stromal cell influence. (4) Transit amplifying cells can replicate and allow mutations to become established. (5) Replication advantage/extended lifespan (as a result of further mutation?) becomes important as the TA cells differentiate abnormally to produce a mass of growing (abnormal) luminal cells. B: Inflammation-mediated carcinogenic changes in human prostate. (1) Tissue stem cell, targeted by mutagenic stimuli from inflammatory infiltration, can accumulate multiple mutations/epigenetic changes. (2) Rare cells acquire phenotypic changes (either through mutation or epigenetic activation), which could confer a survival advantage in the presence of inflammatory destruction. (3) An 'activated' tissue stem cell which responds positively to inflammatory cytokine(s) for example ILx, will repopulate the niche, perhaps under local stromal cell influence. (3a) On resolution of inflammation, the 'activated' stem cells have no selective advantage and will either atrophy or remain in a quiescent state. (4) As a result of further changes, either mutagenic or epigenetic, a rare cell acquires the ability to secrete the pro-inflammatory cytokine (ILx). These stem cells can now give rise to altered TA progeny, neither of which are recognisable tumour cells. (5) As a result of further changes (pre-tumour progression) replication advantage/extended lifespan becomes important as the TA cells differentiate abnormally to produce a mass of growing luminal cells.

stem cell population in only a few generations [Calabrese et al., 2004]. On removal of the inflammatory stimuli, however, these altered stem cells would have little advantage over any others. The altered stem cells would remain quiescent, awaiting a further inflammatory stimulus (Fig. 2B) or could indeed be deleted.

INFLAMMATION AND STEM CELL PHENOTYPE

When the different basal epithelial cells in a human prostate were separated according to their expression of the CD133 antigen, after an initial fractionation with respect to rapid collagen adhesion (high expression of the $\alpha 2\beta 1$integrin) a highly clonogenic population was obtained [Richardson et al., 2004]. The same fractionation, applied to human prostate cancers, also resulted in a highly clonogenic population, but with a higher cell output and a tumorigenic phenotype [Collins et al., 2005; Maitland et al., 2006; Birnie et al., 2008]. Stem like cells with a similar phenotype were also obtained from cell lines such as DU145 [Wei et al., 2007]. The primary cell populations were now relatively homogeneous and after a brief amplification by culture in a serum free media in vitro, to select for epithelial colonies and remove any residual contaminating luminal cells and haematopoietic cell infiltrates the raw material for detailed global gene expression studies was obtained. These cultures were further fractionated to enrich the CD133+/$\alpha 2\beta 1$ integrin hi phenotype (stem cell fraction) and a more differentiated fraction (or transit amplifying basal cells). Two comparisons were achieved. Firstly, changes in gene expression between malignant cells (from prostate biopsies containing a Gleason pattern 4 histopathology) and non-malignant cells (taken from non-malignant Central zone) formed a distinct 'signature' compared to the second comparison, between the stem and TA populations (differentiation signature) [Birnie et al., 2008]. The latter signature contained multiple known markers of prostate basal differentiation, such as microseminoprotein B (MSMB) and prostate stem cell antigen (PSCA) [Green et al., 1990; Tran et al., 2002] and multiple carcinoembryonic antigens in the TA population, with a lower expression of these genes in the stem population, irrespective of cancer phenotype. In some control fractionations from disaggregated human tissues without the in vitro amplification, we also observed a luminal signature in the 'stem' fraction, but here a contaminating luminal (AR+) cell fraction of 1–2% was responsible, where the prostate-specific AR and PSA gene expression was $3–4 \times 10^4$ times higher per cell. This small very luminal cell population was identified by FACS analysis of replicate cell populations, and serves to emphasise the stringent requirements for cell purity in gene expression analyses of minor cell populations. The luminal gene expression pattern equates to the 'gene signatures' currently in the literature and databases for prostate cancer: here, the predominant signature derives from the 99.9% of cells in the tumour with the luminal phenotype [LaTulippe et al., 2002].

INFLAMMATION IN STEM CELLS FROM PROSTATE CANCER

The gene expression data was analysed based on both individual genes (verified in most cases by quantitative RT-PCR and protein detection with commercial antibodies where available), and by association of genes with specific signalling pathways using the Gene Ontology and similar tools. From this analysis there emerged three dominating pathways associated with the stem cell phenotype. While both *wnt* signalling and focal adhesion terms were over-represented in the CSC phenotype, multiple gene markers associated with inflammation were also recorded, including active NFκB signalling, interferon kappa (epithelial secreted interferon), and interferon gamma receptor expression; Jak/STAT signalling and IL6 expression [Birnie et al., 2008]. There are three obvious explanations for these observations.

Firstly, like the luminal cell contamination of stem cells occasionally seen during expression phenotyping, some residual inflammatory cells might have remained in the cultured epithelial stem cells from the human tissue biopsies, after cell fractionation and culture. Since the chosen culture medium was designed to maintain primitive cells of most lineages this would be theoretically

possible. However the prostate CSC's grew to form epithelial colonies from rare cells and the potential for adherent contamination was reduced after time in culture. Equally, and more importantly for a contamination hypothesis, as cells from other lineages do express the CD133 antigen, there was no evidence of multiple known markers of haematopoietic (CD14, 34, 35) or indeed endothelial cells (e.g., CD105 or VEGF receptors) and precursors or infiltrating macrophages (marked by EMR-1 or CD68) in the expression data. MSC markers such as CD44 and CD29 were found in the Prostate CSC, but the MSC marker CD90 was not expressed.

Secondly, given that the Gleason pattern 4 specifies a more invasive phenotype, a fusion could have occurred between inflammatory or MSCs with prostate epithelial cells. This has recently been proposed as a primary mechanism for metastatic spread of tumours [reviewed by Pawelek and Chakraborty, 2008]. Since the genotype of the CSC's was largely diploid, despite some evidence of genomic instability [Collins et al., 2005] and that no 'hybrid' antigens such as those discussed earlier were over-expressed in the selected CD133 population, the fusion hypothesis could be rejected, at least for the population whose expression phenotype was determined.

Thirdly, and the most likely source of the 'inflammatory phenotype' is that the CSC's have been selected to survive in the presence of inflammatory stimuli, produced as a result of chemical or infectious insults. Some evidence of this was revealed by the upregulation of a primary defence mechanism against reactive oxygen species such as produced by the respiratory burst in phagocytic macrophages: peroxyredoxin 2, in the CSC phenotype. The closely related peroxyredoxin 1 has previously been found in association with AR in prostate cancer cells [Park et al., 2007]. In the presence of the inflammatory stimulus, the presence of the correct receptors (interferon gamma and IL6R) in the stem cells would allow stem cells to respond positively and confer a selective advantage, but when inflammation is resolved, then cells which can establish an autocrine stimulatory loop, by producing their own cytokines, would be at the greatest selective advantage (Fig. 2B). IL6 synthesis by prostate cancer cells, in comparison to their normal equivalents, has long been known [Hobisch et al., 2000], but is usually discussed in terms of non-steroidal activation of the AR [Culig et al., 2002]. IL6 activation therefore persists into the tumour cell mass population. In the stem cell compartment, there is of course no AR, and IL6 expression is both higher in the stem cells and in malignancy [Birnie et al., 2008]. There is also evidence that the downstream signalling pathways are activated, Jak/STAT for IL6 and NFkappaB for interferon signalling [Birnie et al., 2008]. In addition there is elevated expression in the CSC phenotype of other interferon related proteins such as the poly(rC)-binding protein, which has also been seen in established prostate cancer cell lines [Molinaro et al., 2006]. However, no upregulation of the RNaseL component of the oligoadenylate synthase system was seen in the CSC cells, in keeping with current thinking about the role of RNaseL as an inducer of apoptosis, and its frequent mutation and downregulation in total prostate cancer cell populations, especially familial cases [Carpten et al., 2002]. The presence of an activated interferon response could also be seen as a pre-emptive mechanism for stem cells to resist viral infections.

CANCER PREVENTION AND A STEM CELL ORIGIN FOR PROSTATE CANCER

Human prostate cancer is an extremely common human disease for which no firm aetiological basis, apart from ageing, has been determined. While the link between inflammation and the disease has been discussed for many years and has been reviewed extensively [Coussens and Werb, 2002; De Marzo et al., 2007; Fox and Wang, 2007] and summarised in the preceding sections, the actual mechanism of carcinogenesis is not known. All of our current thinking about aetiology in prostate as been constrained by the need to involve AR. The existence of an AR-stem cell population in normal and malignant prostate epithelial cells offers a new perspective on tumorigenesis. By considering the effects over many years of highly localised elevated levels of pro-inflammatory cytokines, on long-lived epithelial stem cells, we can begin to form hypotheses to test for alternative explanations. In a stem cell derived tumour hypothesis, as has now been largely proven for haematopoietic stem cells [Bonnet and Dick, 1997; Greaves, 2007] and strongly inferred for both neurological and many epithelial tumours [Al-Hajj et al., 2003; Singh et al., 2003; Kim et al., 2005; O'Brien et al., 2007; Ricci-Vitiani et al., 2007] the power of natural selection in a genetically unstable environment is likely to be important [see review by Greaves, 2007]. Inflammation provides just such a selective environment, including cellular damage, death and a milieu of potent cytokines and enzymes, which can saturate the long lived stem cells over a period of time. Similar ideas have been promoted in other tumour types, particularly hepatocellular and stomach cancers, in addition to the better understood (at the molecular level) tumours in the small intestine [Coussens and Werb, 2002]. The relationship between inflammation, cellular responses and the initiation of the carcinogenic process remains a complex system to understand not only in prostate and its relationship to activation by both exogenous and endogenous viral infection, as described earlier is clearly worthy of closer examination, perhaps in representative animal models and in human tissue reconstructions rather than established cancer cell lines, where the damage was done many years previously.

If indeed the 'driver' cell for prostate carcinogenesis is AR- and basal in nature, this could have consequences for currently topical chemoprevention strategies which are largely directed against the luminal phenotype, including 5-alpha reductase inhibitors [Goetzl and Holzbeierlein, 2006] and metal supplements such a zinc and selenium. Perhaps a simpler strategy would be the provision of anti-inflammatories. There is already some evidence that NSAIDS can delay the progression of prostate cancer (i.e. suppress malignancy) according to Jacobs et al. [2007]. In the latter study, long-term (>5 years) daily aspirin use was associated with a statistically significant reduction in risk of prostate cancer. However, the time of application of such measures might be important. The selective advantage conferred on a stem cell which can respond positively to inflammatory cytokines would form a core part of the pre-tumour development hypothesis [Calabrese et al., 2004] as developed for colon cancer, where the relative ease with which tissue stem cells with an added survival advantage can take over and occupy the stem cell niche in a reasonable time scale has been calculated.

Equally, if there is a period of time when the stem cell compartment contains inapparent cancer [the pre-tumour development phase according to Calabrese et al., 2004] it is likely that the first pre-cancer changes will develop following the development of the mature prostate (i.e. in the late teens and early 20s). Theoretical calculations also provide evidence for carcinogenic mutations arising during fetal development [Frank and Nowak, 2003]. For the prostate, the major developmental 'burst' and cell expansion occurs at puberty. Thus one conclusion might be that the first genetic changes leading to prostate cancer begin at this time. If inflammation is indeed a predisposing event to the development of prostate cancer, and is acting through primitive basal/stem cells rather than luminal secretory cells, then current intentions to apply preventative measures from age 40 could easily be too late.

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