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1. Introduction

Prostate cancer has the highest incidence in the United States and the second highest in the world among cancers in the male population. It is also one of the leading causes of cancer deaths in males in the United States. Like other glandular organs, benign prostate has an epithelial compartment containing mainly secretory luminal cells outlined with basal cells and a stromal compartment including fibroblasts and smooth muscle cells. The development and function of the prostate is mediated by circulating androgens which act via androgen receptor (AR). Amongst the epithelial cells, AR is expressed only in secretory luminal cells, while in the stroma, AR is expressed primarily by fibroblasts and smooth muscle cells in adulthood. In the past, investigators mainly focused on studying epithelial AR function in prostate cancer, defined the involved mechanisms and developed numerous hypotheses which have been published and are widely accepted. However, limited data is available which can be used to describe the function of stromal AR in prostate cancer. This review of the literature examines the current knowledge and understanding of stromal AR function in prostate cancer and endeavors to illustrate its translational significance.

2. Stromal cells in prostate carcinogenesis

The role of stromal cells on the initiation and promotion of carcinogenesis has been studied over many years. This concept was pioneered from previous studies showing [1-3] that tumor stroma, termed as CAF (cancer associated fibroblast), TAS (tumor associated stroma), or RS (reactive stroma), is often different from the normal stroma [1]. Normal prostate stromal cells
play a protective role and maintain growth quiescence within the prostatic tissue. Some investigators have demonstrated in animal studies that when normal prostate stromal cells are associated with malignant epithelial cells, there is a decrease in the proliferation rate \[4,5\] and an apparent loss of former malignant properties of epithelial cells\[6\]. Some studies have also shown restriction of growth of epithelial cells and induction into a more differentiated phenotype \[7\]. Recombination studies using Dunning rat adenocarcinoma revealed that normal stromal environment may override the effects of oncogenic mutations in tumor cells \[8\]. Normal stromal cells therefore, retain properties of growth control and can prevent the proliferation of cells undergoing neoplastic transformation.

Modification of stromal environment is necessary for carcinogenesis and it is adequately evident on observation of stroma immediately adjacent to carcinoma cells in several tumors \[1\]. Recombination experiments by viral transfection of oncoproteins \textit{myc} and \textit{ras} into urogenital sinus mesenchyme and epithelium have illustrated that changes are required in both epithelium and stroma for prostatic carcinogenesis to occur \[9\]. The principal stromal cells – smooth muscle cells and fibroblasts undergo a phenotype switching to emerge as myofibroblasts during tumorigenesis. Morphologically and on the basis of cytoskeletal protein expression, myofibroblasts are an intermediate between fibroblasts and smooth muscle cells \[10,11\]. They are identified by increased expression of vimentin, alpha actin and decreased expression of calponin and smooth muscle myosin. Other phenotypic changes seen in the cancer associated stroma include abnormal migratory behavior \textit{in vitro}, alterations in the cell surface molecules, expression of prostaglandin synthesizing enzymes, alterations in extra cellular matrix (ECM) and altered expression of growth factors – platelet derived growth factor (PDGF), insulin-like growth factor (IGF) 1 & 2, transforming growth factor beta 1 (TGF-b1), hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) \[1\]. There are several possible factors which promote the modification of normal stromal cells into cancer associated stroma. Some signals from epithelial cancer cells to surrounding stromal cells have been shown to alter the function of stromal cells and ECM production, such as TGF-b1, which induces stromal secretion of ‘versican’ an extracellular chondroitin sulfate proteoglycan \[12\]. In a hormone sensitive cell model, variations in ECM have been shown to regulate stromal cell phenotype \[13\]. There is also evidence that the genetic modifications seen in the cancer associated stroma \[14\] are a result of epithelial to mesenchymal transitions of previously genetically abnormal epithelial cells. There is a genome-wide change in stromal genes associated with prostate cancer. In an analysis by Rowley et al. \[15\], when compared with normal stroma, a total of 544 unique genes were significantly higher in the reactive stroma and 606 unique genes were lower. Gene ontology analysis revealed significant alterations in a number of novel processes in prostate cancer reactive stroma, including neurogenesis, axonogenesis, and the DNA damage/repair pathways, as well as an evidence of increased number of stem cells in prostate cancer reactive stroma.

Alternatively, in the ‘reactive stroma’ hypothesis \[11\] the stroma of prostate cancer has been correlated with the granulation tissue in wound repair mechanism with reference to similar biological responses. As in any wound repair situation the microenvironment would be expected to be growth promoting which correlates with the promotion of survival and proliferation of carcinoma cells by stroma in prostatic carcinogenesis. Tissue recombination
studies have demonstrated that human prostatic tumor associated stroma can promote carcinogenesis in genetically initiated human prostatic epithelial cells [1,16]. The results of this experiment revealed an important inference that the cancer associated stroma, when formed, exhibit a significant role in the epithelial cells promoting prostate carcinogenesis.

In contrast, some investigators [17] have shown that tumor associated stromal cells inhibit epithelial cell growth by production of a specific inhibitory factor termed as prostatic epithelium inhibiting factor (PEIF). The expression of this factor by stromal cells was only in the conditioned media collected from isolated stromal cell subcultures. Later in another experiment [18], stromal cells derived from surgically obtained prostatic carcinoma specimens were co-cultured with PC-3 cells using double layer soft agar system. It was noticed that growth of PC-3 cells was inhibited by the stromal cells.

The diversity in stromal cell function in inhibiting or promoting epithelial cell growth may be explained by the heterogeneity of stromal cells in the stromal compartment. During carcinogenesis, the stromal cells display heterogeneity in their morphology as smooth muscle cells, fibroblasts and myofibroblasts. Also, they are heterogenous in AR expression as AR positive and AR negative cells. It may be possible that the presence and absence of AR in stromal cells can dictate cancer epithelial cell proliferation or growth suppression.

3. Progressive loss of AR expression

Numerous studies have focused on AR expression in the epithelial cells during prostate carcinogenesis and the progression of prostate cancer from primary to metastatic cancer and from hormone sensitive to castration resistant prostate cancer (CRPC). It has been established that epithelial AR is continuously expressed throughout prostate cancer disease progression. Increased AR expression has been associated with aggressive disease and decreased progression free survival (PFS) in patients [19].

The expression and function of stromal AR may be distinct from epithelial AR. As a result of the structural, genetic and genomic [11,15] modifications of the stromal cells, there are behavioral modifications expressed in tumor associated stroma. AR expression in stroma is progressively decreased during the transition from benign tissue to cancer and during progression of prostate cancer from low grade to high grade, primary to metastatic, hormone sensitive to CRPC, as well as aggressive prostate cancer in African Americans.

In immunohistochemistry (IHC) studies, some investigators [20] found that AR expression declines in the peri-epithelial stroma as early as in high grade prostatic intraepithelial neoplasia (HGPIN) compared to normal prostate. In their analysis using tissue samples of HGPIN, expression of AR was found to be absent in 80% and weak in 20% of peri-epithelial stromal cell sections.

Analysis of stromal tissue of prostate cancer showed that loss of AR expression increased linearly with higher histological grades in several studies. AR expression was absent in 67% of peri-epithelial stromal tissue in well differentiated (Gleason score 2-4), 91% in moderately
differentiated (Gleason score 5-7) and 94% in poorly differentiated (Gleason score 8-10) prostate cancer [20]. In our study [21], we have shown a statistically significant decrease of stromal AR expression (p < 0.001) in the areas of prostate cancer compared with benign prostate with up to a 6% decrease in stromal AR expression. When stratified with Gleason score, we established a trend of greater decrease of AR-positive stromal cells in cancerous areas compared to benign areas with increased tumor grade. Later on, other investigators have also demonstrated that magnitude of loss of stromal AR is directly proportional to advanced pathological stage along with higher Gleason scores [22]. By AR antibody immunostaining of TURP (Trans Urethral Resection of Prostate) specimens obtained from patients with varying Gleason scores and pathological stages, they found lower expression of AR in tumor stroma compared to areas with normal stroma. This difference was notable (p < 0.05) in tumor specimens of stage T2 and tumors with Gleason score of 7, while it was more statistically significant (p <0.01) in tumor stage T3 and T4 and in specimens with Gleason score of 8-10.

Decreased stromal AR expression has also been correlated to disease progression including metastasis and androgen-independence. Bergh et al. showed [22] that specimens with metastatic disease displayed significantly lower (p < 0.01) stromal AR expression. The AR staining was only 1.6% in metastatic tumor stroma compared to 18 % in normal stroma which was equivalent to a loss of expression by 11 fold. While in the non-metastatic disease specimens, the AR staining was 13% in tumor stroma compared to 48 % in normal stroma, equivalent to a loss of expression by 3.5 fold. Evidence is available [21] that during transition of prostate cancer from hormone sensitive to CRPC, there is a significant decrease in stromal AR expression. AR levels were determined in the prostate stroma of 44 cases of hormone sensitive prostate cancer and in 22 cases of CRPC by IHC analysis using affinity purified polyclonal AR antibodies. Scoring was performed by selecting three areas with 100 cells each in benign and cancerous regions in prostate stromal tissue sections to determine the relative percentages of stromal cells that were AR-positive and AR-negative, respectively. The levels of stromal AR expression were expressed as an average percentage of AR-positive stromal cells. When comparing hormone sensitive and CRPC tumor sections, a statistically significant 3-fold decrease of AR-positive stromal cells was observed, from 4 % in hormone sensitive to 12 % in CRPC tumors. Most importantly, some investigators have also reported an association of loss of stromal AR expression with clinical outcome or prostate cancer specific death in patients [25].

These studies suggest that there is a natural selection of stromal AR negative cells over AR positive cells as the tumor progresses. With these results, we established that stromal AR expression proportionately decreases as tumor grade increases and as cancer advances towards metastatic and androgen independent disease. The mechanism behind the loss of AR expression in the peri-epithelial stroma is not well understood. It has been attributed that during the malignant transformation of epithelial cells, there is a shift in AR axis from stromal cell dependent paracrine pathways to autocrine dependent pathways [23] and is increased during tumor progression. When these cancer cells shift to autocrine mechanism of proliferation, it appears that epithelial AR regulates a new series of genes for survival and proliferation, not normally expressed by prostate epithelial cells [7]. The consequence of this may be that
malignant epithelial cells no longer depend upon stromal-epithelial interactions and stromal AR mediated growth factors for their survival and proliferation.

4. Stromal AR inhibits cancer epithelial cells

We have observed and previously demonstrated by co-culture experiments using well characterized stromal cell lines, both in vitro and in vivo that, in the presence of androgen, stromal cells expressing AR decrease the growth and invasive ability of prostate cancer epithelial cells. It was hypothesized that this distinct effect of AR in stromal cells is due to the involvement of paracrine factors/mechanisms regulated by both the epithelial and stromal cells.

The analysis was established [21] by using a well characterized prostate stromal cell line morphologically similar to the tumor stroma. We constructed an immortalized stromal cell line from prostate with BPH, termed as PShTert, stably expressing the human telomerase catalytic subunit – hTert. Morphologically and ultra structurally, the cells expressed typical characteristics of myofibroblasts. IHC showed diffuse, strongly positive stain for Vimentin with a strong SMA staining in 25% of cells, and negative staining for Desmin. Together these data support the myofibroblastic phenotype of the PShTert stromal cells. Western blot analysis showed the absence of AR in these cell lines. We transduced this cell line with pBabeAR retroviral vector and selected stable clonal cell lines expressing AR, termed as PShTertAR. Functionality of the ectopic AR was confirmed by in vivo dual luciferase assay eliciting ligand dependent transcriptional activation in the presence of androgens.

For in vitro analysis, transwell indirect co-culture assays using these two stromal cell lines with PC3 cells were performed. In the presence of androgen, co-culture with PShTertAR resulted in inhibition of PC3 cell proliferation compared to PC3 cell growth when cultured alone (p = 0.045). In contrast, co-culture with AR negative PShTert cells resulted in enhancement of growth rate of PC3 cells compared to PC3 cells grown alone (p = 0.03). Flow cytometric analysis revealed that PC3 cells co-cultured with PShTertAR showed 20% S-phase cells, decreased from the 27% S-phase cells measured in PC3 cells co-cultured with PShTert cells. We examined the expression of cell cycle genes, including cyclin A, cyclin B, p21 and p27, and the expression of Skp2, and all were decreased in PC3 cells co-cultured with PShTertAR compared with PC3 cells co-cultured with PShTert cells.

However, with co-cultures in androgen free media, both PShTert and PShTertAR cells stimulated the growth of PC3 cells. Similarly in vivo analysis by co-injecting PC3 cells with PShTert subcutaneously in the flank region of nude male mice resulted in development of tumors twice as large as when PC3 was injected alone. On the other side, co-injection of PC3 cells and PShTertAR cell line resulted in statistically significant reductions of tumor growth and size.

There were two important observations drawn from the analysis. Firstly, both AR negative and AR positive stromal cells promote growth of prostate cancer epithelial cells in the absence
of androgen by secretion of a paracrine factor which is independent of AR. Secondly, AR positive stromal cells secrete another paracrine factor which is growth inhibitory for prostate cancer epithelial cells and is dependent on the presence of androgen and AR.

5. Conclusion

With reference to our hypothesis that AR positive stromal cells inhibit the growth of PC3 cells in the presence of androgen, we also analyzed and found similar results while using LNCaP cells. However, the magnitude of growth inhibition was less significant in LNCaP cells as compared to PC3 cells.

Therefore, there is a need to re-identify the role of continued androgen deprivation therapy (ADT) during progression to CRPC. It may be possible that due to androgen deprivation, the growth promoting stromal effects counteract the apoptotic effects of androgen ablation on epithelial cells. On the contrary, the growth inhibiting effects of the stromal AR are lost during ADT. The permanent methods of androgen ablation such as surgical castration can be replaced by reversible methods of castration such as medical castration with LHRH analogues. Interestingly, some investigators have even observed that using androgen replacement therapy (ART) in metastatic CRPC displayed biochemical improvement in patients [24]. Newer therapies targeting the prostate cancer stromal cells should be evaluated.

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