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Review Article

# Stroma Features in Prostate Cancer Diagnosis and Prognosis

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**Abstract**

The oncogenesis of Prostate Cancer (PCa) is a process involving epithelial cells of the gland and their interaction with the stroma. Reactive stroma formation has been shown to be critical in the progression of many cancers. In PCa, the reactive stroma is unique when compared with other cancer types and characterized by replacing the normal well differentiated smooth muscle cells with fibroblasts and myofibroblasts. The Masson's trichrome stain and immunohistochemistry (IHC) / immunofluorescence studies characterized these changes and a grading system of reactive stroma has been developed. In this review, the changes of the molecular and morphometric features of the reactive stroma during the progress of PCa, and their use in clinical implications in the diagnosis, prognosis prediction and potential treatment of PCa are discussed.

## Introduction

### Prostate cancer

Prostate Cancer (PCa) is the most common non-cutaneous malignancy in men in the United States and is the second most common cause of cancer death in men. The estimated new cases are 233,000 and estimated prostate cancer deaths are 29,480 in 2014 in the US [1]. PSA screening and prostate biopsies resulted in an over-diagnosis and over-treatment of prostate cancer. The new American Urological Association (AUA) guidelines have limited the PSA screening for men age 55 to 69 years old, for whom benefits outweigh harms [2,3], however, these two problems still exist. Therefore, sensitive and specific biomarkers development to predict the progress of PCa, especially at early stages are still in great need for the effective management of PCa patients. The interaction between stroma and the glandular area plays pivotal role in the regulation of local cancer growth, invasion, and distant metastasis [4]. While there are tremendous studies of biomarkers on the epithelial cells of the prostate glandular area, researches in the stroma area during oncogenesis of PCa are relatively lagging behind. Recent years of studies in the field have unveiled the important roles of stroma and shown their great impact in inducing PCa progression. This review will focus on changes of the molecular and morphometric features of the stroma area during the progress of PCa, and their use in clinical implications in the diagnosis, prognosis prediction and potential treatment of PCa.

### Stroma of prostate

The prostate gland is composed of multiple cellular components and Extracellular Matrix (ECM). The cuboidal to columnar epithelial cells secrete products into the acinar lumen and are situated on basal lamina of basement membrane with basal cells in between these two components. There are also neuroendocrine cells interspersed to regulate the secretion of prostate gland and perhaps provide nutrient as well. Outside the acini is the stromal compartment, which is constituted of blood vessels, ECM and cells including smooth muscle cells, trace firbroblasts, autonomic nerve endings, immunological cells, etc. In addition to the primary support function of the stroma, more and more proof has shown that the stroma interacts with the acini and plays pivotal roles in the progression of PCa.

## Reactive Stroma and Molecular, Morphometric Features

Similar to chronic wound repair in other tissues in the body, when prostate encounter with infection, inflammation or other damages, the stroma responses to these changes and participate in the repair progress. The phenotypic and genotypic alterations during the damage response in prostate are referred to as reactive stroma [5].

In general, reactive stroma contains an increased amount of fibroblasts and myofibroblasts, replacing the dominant and well-differentiated smooth muscle cells in normal prostate. A series of immunohistochemistry or immunofluorescence experiments were used to identify the molecular features of reactive stroma. Fibroblasts are characterized by expression of vimentin without other smooth muscle markers; the predominant prostate smooth muscle cells coexpress Smooth Muscle (SM) α-actin, calponin, and desmin (the latter two are the markers of late-stage smooth muscle

differentiation with desmin as a muscle specific marker); while the induced myofibroblasts in reactive stroma coexpress vimentin, SM α-actin, prolyl 4-hydroxylase and but near a total loss of expression of cytokeratin, calponin and desmin [6-13]. These cellular component changes in reactive stroma were also verified by primary cell cultures from PCa tissues and interestingly, some SM α-actin positive cells expressing basal cytokeratin K14 were also identified and referred to as myoepithelial cells [14]. In parallel to these changes, reactive stroma showed an over expression of collagen type I, tenascin, and Fibroblast Activation Protein (FAP) [7].

In contrast to cancer of breast, cervix, colon, lung, etc., the "lack of desmoplastic response" makes the diagnosis of small foci of PCa in biopsies difficult [8]. Masson's trichrome stain provides a clear cut morphology of reactive stroma: the normal prostate stroma will be stained red and shows orientation of fibers, whereas the reactive stroma contains a disorganized mixture of red and blue, which stains the collagen. The intensity of blue staining depends on the grade of reactive stroma [7,9].

## Clinical Implications of Stroma

### Morphometry changes of reactive stroma

The imbalanced and under-regulated repair progress is a critical step in tumor growth and progression. It was reported that the reactive myofibroblasts may prevent infiltration of the immune and inflammatory cells into the cancer area, protecting cancer cells from host immune responses [15]. The reactive stroma creates a microenvironment to promote tumorigenesis in many cancer types [16]. In prostate cancer, features of the reactive stroma was characterized by the replacement of predominant smooth muscle cells with mainly myofibroblasts and also fibroblasts, a progress regulated by TGF-β1 and other factors [5,7]. Interestingly, these features of reactive stroma could be detected in Prostatic Intraepithelial Neoplasia (PIN) [7], an early stages of PCa when the cancer cells remain limited by the intact basal lamina and the basement membrane morphologically but initiated genomic instability [17], suggesting the promising use of reactive stromal features to predict the progression of PCa in an early stage.

Considering the importance of reactive stroma, a pioneer scoring system named Reactive Stroma Grading (RSG) was developed based on Masson's trichrome stain [9]. In general, the criteria are based on the percentage volume of reactive stroma to that of total tumor area: RSG Grade 0: 0~5%; RSG Grade 1: 5~15%; RSG Grade 2: 15~50%; RSG Grade 3: >50% with at least a 1:1 ratio between stroma and epithelium. Applying these criteria to a group of 847 patients in prostatectomy TMAs, stroma volume was found as an independent predictor of disease-free survival [9]. A similar RSG was created based on H&E staining [8], the golden standard of current pathological diagnosis. Using this H&E RSG criteria and Biochemical Recurrence (BCR) as an end point, it was reported that RSG is an independent predictor of recurrence-free survival in prostatectomy samples, with PCa containing no reactive stroma and/or abundant reactive stroma has shorter BCR-free survival [9]. Further studies in diagnostic biopsies also show the use of RSG in the prediction of BCR-free survival independently, especially in patients with a Gleason score of 7, when heterogeneous groups of patients are grouped together [8]. In general, patients with RSG 0 and 3 are associated with increased BCR-free risk [8]. In primary cultures, the percentage of myofibroblasts was reported to be higher in higher-grade PCa compared to moderate and low-grade PCa cases. On the contrary, myoepithelial cells showed a reverse correlation with the grade of PCa [14]. Recent research results also show that there is a significant association between reactive stroma grade in tumors and the occurrence of Castration-Resistant PCa (CRPC) in patient with a Gleason score of 6-7. Also, higher expression of vimentin in tumor stroma (which may include fibroblasts and myofibroblasts) was found to be independently associated with poor outcomes with Gleason score 6-7, suggesting that it probably could be used a prognostic biomarker as well [18].

### Reactive stroma biomarkers

Reactive stroma showed parallel molecular changes in the expression of stromal biomarkers along with morphometric changes. Semi-quantification of desmin and SM α-actin (two of reactive stromal biomarkers) expression showed that both are significant and independent predictors of recurrence-free survival, while other biomarkers (e.g., vimentin, calponin, and pro-collagen I) did not show significant predictive capability in the cohort explored [9] although it is considered that vimentin is over expressed in PCa [13]. In another study of 52 PCa cases with Gleason score ≤7, SM α-actin did not show differences among PCa, Benign Prostatic Hyperplasia (BPH) and peritumor normal tissue [13]. However, considering these biomarkers are usually expressed in more than one cell types, confounding factors perhaps exist and masked their power for prediction of PCa progression. Double staining using fluorescence in combination with accurate quantification could be one solution to reveal the underlying relations.

## Discussion

PCa is pathologically diagnosed using Gleason grading system [19], which primarily focusing on the morphological changes of prostate gland and has recently been updated into a new grading system [20]. In the new system, Grade Group1 includes Gleason score ≤ 6; Grade Group2 includes Gleason score 3+4 = 7 only; Grade Group3 includes Gleason score 4+3 = 7 only; Grade Group 4 includes all those with a Gleason score of 8; and Grade Group 5 includes Gleason score 9-10 [20]. The new system separate Gleason score 7 into two groups and more accurately reflects prostate cancer biology than the Gleason system, considering that majority of patients were diagnosed as Gleason score 7 in clinic. However, the importance of stromal changes was not taken into consideration and the inherent inter-observer reproducibility [21] limited their power in the diagnosis and prediction of progression of PCa.

The stromas interact with the epithelial cells and are critical factors in the induction of PCa progression. Reactive stroma under effective regulation is a key step in the oncogenesis of PCa. Myofibroblasts in the reactive stroma participate in the progression and metastasis of PCa, modulating inflammation, angiogenesis and epithelial cancer cell prolifereation [14]. The reactive stromal changes could be clearly detected by Masson's trichrome staining. H&E staining could also be used as an alternative.

The advantages of trichrome stain is that it provides a clear cut morphology of reactive stroma: the normal prostate stroma will be stained red whereas the reactive stroma will be stained with red and blue with the latter dominated [7,9]. H&E stain, the golden standard for pathological diagnosis, could also be used for reactive stroma studies,

but to a less content in recognition of reactive stroma compared with Masson's trichrome stain. In H&E staining, normal prostate stroma has a thick smooth muscle bundles with ample eosinophilic cytoplasm while the tumor reactive stroma stained similar color but has fibrillary quality and sometimes high power is needed for better differentiation of the reactive stroma from that of normal stroma [22]. Therefore, it will be important and easier to use Masson's trichrome stain for accurate studies of prediction PCa progression although H&E could be used as an alternative especially in special situations such as lack of cancer area in further sections after the H&E section. Moreover, the categorical RSG system has limitations. Considering high-grade PCa could be categorized as RSG 0 or 1, similar to that of low-grade PCa, quantification of many reactive stroma features' change in PCa might be bell-shaped with the progression of PCa and current categorical methods will lose important histological information without taking clinicopathological information into consideration. Also, the proportion of total stroma area within the whole tumor area is a confounding factor and should be taken into consideration since proportion of total stroma area could be either increased or decreased with PCa progression. Stratification by whole stroma area followed by reactive stroma grading may provide better correlation with Gleason score and other clinical information (TNM stage, etc.).

Biomarkers study including the parallel molecular changes in stroma will also increase the power in the diagnosis and prognosis prediction of PCa. Stratification of biomarkers is important for personalized treatment. The critical question will be which biomarker is important in the stratification? Not like breast cancer, Her2/neu has provided a successful strategy, we currently do not have such good biomarkers in prostate cancer management partially because PCa usually occurred in multiple loci and is quite heterogeneous [23].

Lastly but importantly, although Masson's trichrome staining, H&E staining, Feulgen staining for morphometry and biomarkers stained with IHC or immunofluorescence have been exist for decades, analysis of these staining results are mostly by categorical methods in publications, which is partially subjective and more accurate quantification is needed for the analysis of these staining results [24,25]. No accurate quantification of the size, shape, intensity of trichrome stain/H&E stain has been done so far, which will provide more accurate and comprehensive information of reactive stroma during PCa progression. Our lab applied ImagePro Premier software, AutoCyte™ pathology workstation, etc. to autonomously quantify nuclear morphometry changes during PCa progression and showed the power of these accurate quantification in the prediction of BCR, PCa aggressiveness, etc [26-32]. In general, these software involves firstly selection of Region of Interest (ROI) on the scanned images after staining (H&E, Feulgen, IHC, etc.), followed by autonomous quantification of nuclei (Feulgen staining) or whole regions with parameters of size, shape, staining intensity, roundness, clusterness, etc. of the nuclei or a region based on a macro to define the threshold of each parameter. Future application of accurate quantification tools in the study of reactive stroma morphometric changes and cellular composition alterations (reactive stroma has a higher cellular number than normal stroma with increased fibroblasts and myofibroblasts [8]) may provide an improvement of RSG system. The new system will provide more accurate information of morphometric changes and along with the compliment clinicopathological & epithelial/stromal biomarkers information could be used in the accurate diagnosis and prediction of PCa progression.

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