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RESEARCH ARTICLE

CORRELATION OF STROMAL CHANGES IN BENIGN PROSTATIC HYPERPLASIA AND LOCALIZED AND ADVANCED PROSTATIC CARCINOMA

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ABSTRACT

Introduction: As the prostatic carcinoma progresses, stromal changes become more pronounced. The aim of this study is to correlate stromal changes with tumor grade and extraprostatic extension of the disease.

Material and Methods: Stromal changes were quantified histochemically and immune histochemically in 70 patients treated with radical prostatectomy for clinically localized prostatic carcinoma and the results were correlated with areas of benign prostatic hypeplasia and with tumor grade and tumor stage.

Results: Spearman rank correlation showed significant correlation between Trichome Mallory intensity index and higher tumor grade (R=0,27 p=0,023) and higher tumor stage (R=0,24 p=0,049), between vimentin expression and higher tumor grade (R = 0,35 p = 0,003) and higher tumor stage of the disease (R = 0, 28 p = 0,019), and also significant inverse correlation between desmin expression and higher tumor grade (R = - 0,25 p=0,035).

Conclusion: Quantification of stromal changes, using a combination of histochemical stains and immune histochemical antibodies, could serve as an additional important complement in the already well established Gleason score for determining tumor grade and tumor stage of the disease.

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INTRODUCTION

The stroma is a broad term encompassing a wide range of cell types (fibroblasts, endothelial cells, smooth muscle cells, macrophages, mast cells and a number of cells that pass through the microenvironment via blood and lymph vessels) and extracellular matrix components. In the prostate, smooth muscles cells are the most abundant cell type and they derive from the mesenchyme of the urogenital sinus. These cells are also the most important cell type regarding prostatic development, maintenance and homeostasis. Thus changes in the smoth muscle cells could be important in the evolution of prostatic carcinogenesis. The differentiation of prostatic smooth muscle occurs in an orderly manner with the sequential expression of a number of characteristic markers, including vimentin, actin, desmin and vinculin. Following castration, rapid regression of prostatic epithelium is associated with an ordered loss of expression, in an opposite direction to that to which these markers were expressed during normal development (Grossfeld *et al*., 1988). There is a growing body of evidence suggesting that changes occur in the surrounding connective tissue stroma that may serve to enhance the malignant potential of the nearby epithelium (Ronnov-Jessen *et al*., 1996). Epigenetic influences derived from stromal cells may be crucial in determining whether a tumor will assume slowly growing or invasive phenotype (Cunha *et al*., 1996; Hayward *et al*., 1997). Genetic mutations in the prostatic epithelium could alter the signaling to nearby smooth muscle cells and, as a consequence, that may trigger stromal dedifferentiation toward fibroblast phenotype. Hence, this transformation may yield a change in the local microenvironment, from promotion of epithelial homeostasis, toward epithelial mitogenesis and this might enhance the invasive potential of genetically altered epithelial cells (Grossfeld *et al*., 1988). This reactive stroma, surrounding epithelial carcinoma cells, is not yet fully defined (Tuxhorn *et al*., 1992). There is a similarity of cancer stroma with the stroma involved in wound repair. A special cell type, called myofibroblast, is found in sites of pathologic tissue repair. In wound repair, myofibroblasts derive from granulation tissue fibroblasts and in cancer, carcinoma cells induce normal

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fibroblasts to the reactive myofibroblast phenotype (Sappino *et al*., 1990). Many authors suggested the term carcinoma associated fibroblasts (CAF) for these cells. So far, some characteristics of these carcinoma associated fibroblasts are established. These cells do not form tumors when grown in absence of epithelium, stimulate progression of genetically altered, non--tumorogenic human prostate epithelium, toward carcinomatous phenotype, and are unable to stimulate initiation of genetically normal prostatic epithelium (Grossfeld *et al*., 1988; Tuxhorn *et al*., 1992).

MATERIALS AND METHODS

The study included 70 consecutive patients, median age 67,66 [range 50-87] years, treated with radical prostatectomy for clinically localized prostate carcinoma at Clinical Hospital Acibadem/Sistina in Skopje Macedonia, between 1 May 2010 and 1 February 2015. Regarding the stage of the disease, 38,57% of patients were diagnosed with localized disease, while 61,43% of patients showed advanced tumor stage (extraprostatic extension of the disease) (Table 1).

Table 1. Distribution of the stage of the disease in 70 patients with prostatic carcinoma

pT	N = 70(%)
2a	5 (7,14%)
2c	22 (31,43%)
3a	13 (18,57%)
3b	30 (42,86%)
(T1 and T2) localized	27 (38,57%)
(T3 and T4) advanced	43 (61,43%)

Concerning the tumor grade (Gleason grade) 17,14% were well differentiated neoplasms (Gleason score 6 or less), 67,14% were moderately differentiated neoplasms (Gleason score 7) and 15,71 were poorly differentiated neoplasms (Gleason score 8 or more) (Table 2).

Table 2. Distribution of Gleason score in 70 patients with Prostatic carcinoma

Gleason score (G)	N = 70 (%)
G1 (<=6) well differentiated	12(17,14%)
G2 (7) moderately differentiated	47(67,14%)
G3 (8=>) poorly diferentiated	11(15,71%)
mean±SD, median, rang	6,99±0,6 7,0 (6 – 8)

Specimens were fixed in 10% buffered formalin, embedded in paraffin, cut at 5 μm thickness, and routinely stained with haematoxylin and eosin. Samples were chosen from the periphery of the cancer containing relatively equal amounts of cancerous and benign prostatic tissue. First, sections were stained with Mallory Trichrome method, following standard procedure. Prostatic stromal smooth muscle cells stained red (Figure 1a) and carcinoma associated fibroblasts (CAF) stained blue (Figure 1b). Five consecutive areas with the most intensive blue staining were analyzed under high magnification (x400). The amount of CAFs in prostatic stroma was graded semiquantitatively and expressed as: negative 0 = no blue staining; 1 = weak blue staining; 2 = moderate blue staining; and 3 = strong blue staining.

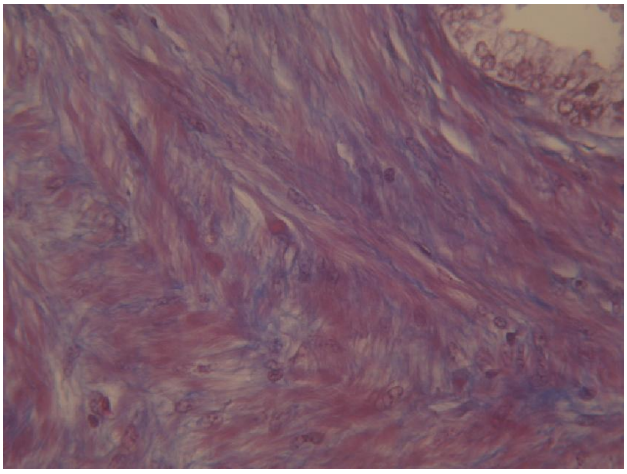

Figure 1a. Mallory Trichrome staining in stroma of benign prostatic hyperplasia

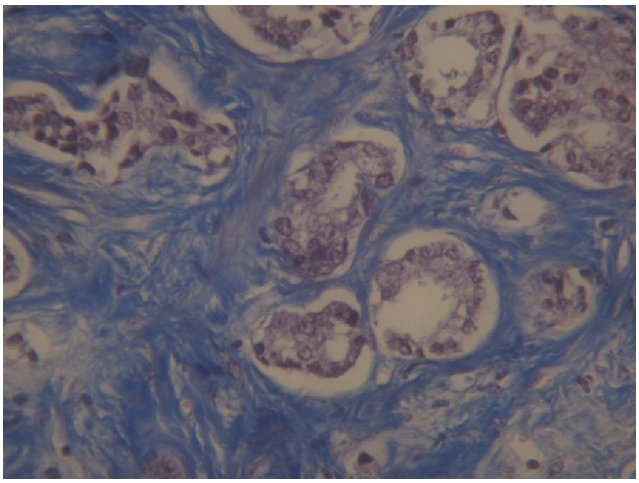

Figure 1b. Mallory Trichrome staining in stroma of prostatic carcinoma

For determination of the immunohistochemical profile of the stroma these antibodies were used: Vimentin, clone V9, IgG_1 class (DAKO), dilution: 1:100, Desmin, clone D33, IgG_1 class (DAKO), dilution 1:50 and α-SMA,clone HHF35, IgG_1 class (DAKO), dilution 1:100. Immunohistochemical staining was performed with the technique of Avidin – Biotin Immunoperoxydase Complex, using LSAB and En-Vision kit for visualiasation of the antigen – antibody complex. Immunohistochemically, smooth muscle cells of normal prostatic stroma are vimentin negative (Figure 2a) and desmin positive (Figure 3a), while carcinoma associated fibroblasts (CAFs) are characterized by vimentin expression (Figure 2b) and negative expression to desmin (Figure 3b). Both CAFs and normal prostatic stroma express smooth muscle actin (actin). To evaluate the intensity of stromal changes in prostatic carcinoma the percentage of stromal cells positive for vimentin, desmin and actin was quantified in five consecutive fields, previously chosen with the Trichrome Mallory stain, for each antibody under high magnification (400x). Scoring scale was established for frequency of positive cells from 0 to 3: 0 = 0% positive stromal cells; 1 = 1-33% positive stromal cells; 2 = 34-66% positive stromal cells; and 3 = 67-100% positive stromal cells. Than on the same fields the intensity of the

signal was analyzed using the following scale: 0 = no signal; 1 = weak signal detected on high magnification (400x); 2 = moderate signal detected at medium magnification (100x); and 3 = strong signal detected on low magnification (40x).

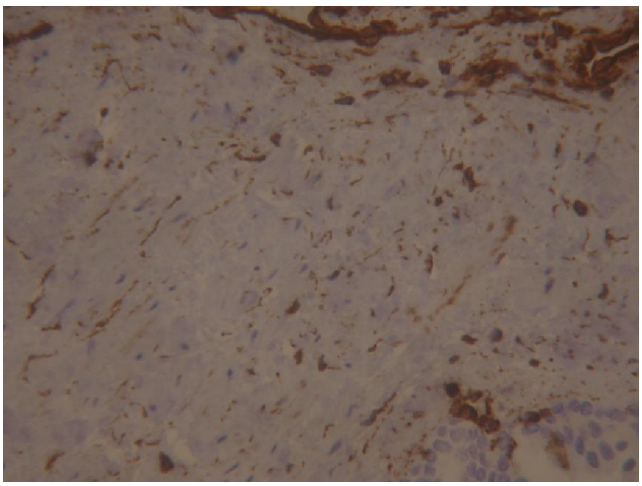

Figure 2a. Vimentin expression in stroma of benign prostatic hyperplasia

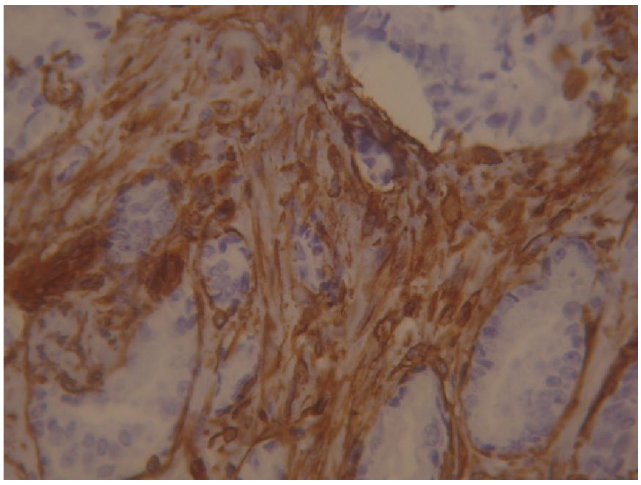

Figure 2b. Vimentin expression in stroma of prostatic carcinoma

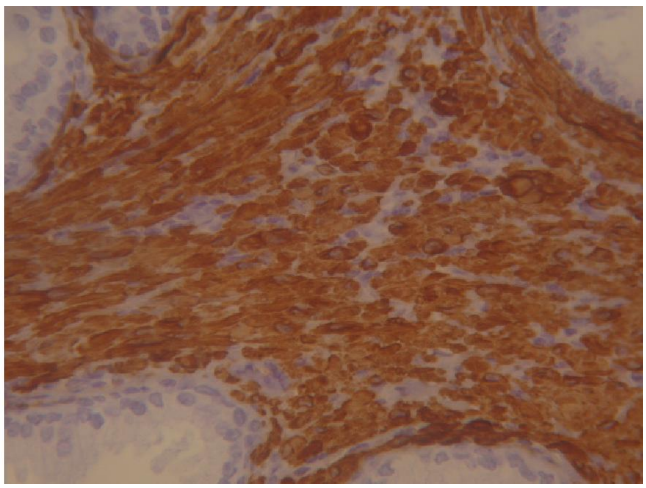

Figure 3a. Desmin expression in stroma of benign prostatic hyperplasia

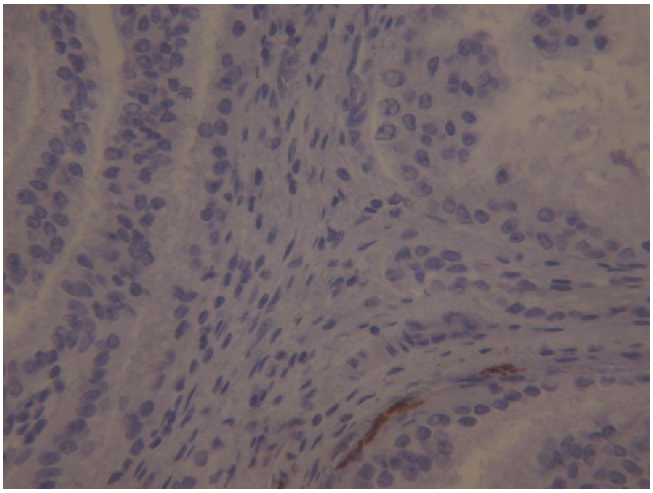

Figure 3b. Desmin expression in stroma of prostatic carcinoma

Than the percentage of positive cells was multiplied with the signal intensity to reach staining index: 0 = no signal; 1-2 low staining index; 3-4 = moderate staining index; and 6-9 = high staining index (Tuxhorn *et al*., 2002; Tomas *et al*., 2010; Tomas *et al*., 2004). Statistical analysis was performed using the chi square test and, for correlation between parameters, Spearman coefficient rang correlation was used. The levels of statistical significance was set at $p<0,05$. The statistical analysis was performed using the program SPSS for Windows 17,0.

RESULTS

Trichrome Mallory stain showed statistically significant change between stromal cancer cells (CAFs) and stromal cells in benign prostatic hyperplasia (Chi-square=31.71 df=1 $p<0.001$). Vimentin antibody had significantly (Chi-square=45,55 df=1 $p<0,001$) greater expression in CAFs compared to stromal cells in benign prostatic hyperplasia. There are significant (Chi-square=117,89 df=1 $p<0,001$) differences in the staining index for the antibody desmin in benign prostatic hyperplasia and prostatic carcinoma and in all cases stromal cells of benign prostatic hyperplasia showed a staining index of 3. The difference of actin expression in stromal cells of benign prostatic hyperplasia and prostatic carcinoma was not significant (Chi-square=0,2 df=1 p=0,65). The values of Spearman coefficient and p values show that the intensity of trichrome Mallory staining has positive correlation with vimentin expression (R=0,68 $p<0,001$), Gleason score (R=0,27 p=0,023) and extraprostatic extension of the disease (R=0,24 p=0,049), while there is inverse correlation with desmin expression (R= - 0,28 p=0,023). The intensity of the blue staining using trichrome Mallory in the cancer stroma grows with the intensity of vimentin expression and with the increase of Gleason score and tumor stage (extraprostatic spread of the disease) (Table 3).

Vimentin expression in stromal cells of prostatic carcinoma shows significant correlation with Mallory Trichrome staining (R = 0,68 $p<0,001$), Gleason score (R = 0,35 p = 0,003) and extraprostatic extension of the disease (R = 0, 28 p = 0,019). Vimentin expression increases in stromal cells of prostatic

cancer together with the increase of tumor grade and tumor stage (Table 4).

Table 3. Correlation of Trichrome staining index in prostatic carcinoma with other parameters

Ca Trichrome co	Spearman Rank R	p-value
Ca Vimentin	R = 0,68	t = 7,67 p<0,001**
Ca Desmin	R = - 0,28	t = 2,39 p=0,02*
Ca Actin	R = 0, 068	t = 0,56 p=0,57 NS
G score	R = 0,27	t = 2,32 p=0,023*
Extraprostatic extension	R = 0,24	t = 1,99 p=0,049*

*p<0,05 **p<0,01 NS – not significant

Table 4. Correlation of Vimentin expression in prostatic carcinoma and other parameters

Ca Vimentin co	Spearman Rank R	p-value
Trichrome	R = 0,68	t = 7,67 p<0,001**
Ca Desmin	R = - 0,15	t = 1,28 p=0,2 NS
Ca Actin	R = 0, 002	t = 0,016 p=0,99 NS
G score	R = 0,35	t = 3,03 p=0,003**
Extraprostatic extension	R = 0, 28	t = 2,4 p=0,019*

*p<0,05 **p<0,01 NS – not significant

Desmin expression showed significant inverse correlation with Mallory trichrome stain (R = - 0,28 p=0,02) and Gleason score (R = - 0,25 p=0,035). The correlation of desmin expression with extraprostatic extension of the disease was not significant (R = - 0, 2 p=0,095) (Table 5).

Table 5. Correlation of Desmin expression in prostatic carcinoma with other parameters

Ca Desmin co	Spearman Rank R	p-value
Ca Trichrome	R = - 0,28	t = 2,39 p = 0,02*
Ca Vimentin	R = - 0,15	t = 1,28 p = 0,2 NS
Ca Actin	R = 0, 13	t = 1,11 p = 0,27 NS
G score	R = - 0,25	t = 2,14 p = 0,035*
екстрапростатична екстензија	R = - 0, 2	t = 1,7 p = 0,095 NS

*p<0,05 NS – not significant

Actin expression in stromal cells of prostatic cancer showed only significant correlation (R = - 0,35 p = 0,005) with tumor grade (Gleason score).

DISSCUSSION

Genetic mutations are the basis of the process of carcinogenesis. Mutations are a direct cause of so called sporadic cancers that encompass around 95% of human malignant neoplasms. This theory of somatic mutations is established in the last four decades, but novel ideas question this theory (Hahn *et al*., 2002; Soto *et al*., 2004; Van Regenmortel *et al*., 2004; Fukino *et al*., 2004). In spite of aggressive attempts in the laboratories worldwide the theory of somatic mutations cannot be firmly substantiated. Growing evidence suggest a role of tissue interactions in carcinogenesis (Olumi *et al*., 1999; Barcellos-Hoff *et al*., 2000; Maffini *et al*., 2004). The role of oncogene / suprepressor gene concept, as the last incarnation of the somatic theory, is questioned many times (Olumi *et al*., 1999; Barcellos-Hoff *et al*., 2000). Tissue related concepts were introduced as corrections in the current theory of somatic mutations rather than discarding completely this somatic mutation theory (Weinstein 2002, Sonnenschein *et al*., 2000). As a complement to mutations, the final resolution of malignant neoplastic phenotype had to accommodate the role of stromal-epithelial interactions, and a new hybrid theory for carcinogenesis emerged, that incorporated elements of theory of somatic mutations and the role of stromal-epithelial interactions in the process of carcinogenesis (Bissell *et al*., 2001). This hybrid theory is the epigenetic theory of carcinogenesis that implies tissue based phenomena in modifications of epigenetic gene expression. Alternative theories to somatic mutation emerged that state carcinogenesis as a problem of normal histogenesis and tissue repair (Maffini *et al*., 2004). This new approach assumes that proliferation is the basic state of cells (Maffini *et al*., 2002). This view is diametrically opposite to the theory of somatic mutations, where quiescence is the basic state of cells in multicellular organisms. This alternative theory, that incorporates the tissues as a target of carcinogens, and proliferation as the basic state of cells, is called tissue organization field theory (Sonnenschein *et al*., 2005). In practice, stromal changes are currently thoroughly investigated in order to relate those changes with tumor grade and tumor stage. Authors design various methods of quantification of these stromal changes (Tuxhorn *et al*., 2002; Tomas *et al*., 2010; Tomas *et al*., 2004). These studies have shown that grading stromal changes can predict tumor aggressiveness and tumor recurrence. The value of the well established Gleason grading system, that analyses the morphology of the epithelial malignant cells, is unquestionable. However, assessment of stromal changes as well, could serve as a valuable complement to the Gleason grading system. When comparing patients with identical Gleason pattern, the intensity of vimentin expression could identify patients with higher risk of disease recurrence (Tomas *et al*., 2010). Also Mallory trichrome staining can be used in everyday practise in interpretation of difficult cases of prostatic carcinoma in needle core biopsies (Tomas *et al*., 2004).

Our study confirmed significant correlation of the intensity of trichrome Mallory staining with Gleason grade and tumor stage (extraprostatic extension of the disease). Also, there was a significant correlation of the expression of vimentin antibody with Gleason grade and extraprostatic extension of the disease. Desmin expression showed significant inverse correlation only with Gleason grade. Our study analyzed specimens from radical prostatectomies where the whole prostate was sampled for clinically localized prostatic carcinoma but a number of cases showed microscopic foci of extraprostatic extension of the disease. However these results can be utilized when reviewing needle core biopsies (Tomas *et al*., 2010; Ayala *et al*., 2003; Yanagisawa *et al*., 2007). This field of research is relatively new and additional studies like these are required in order to resolve several issues concerning the interpretation of the findings. Several methods of quantification of stromal changes have been proposed and a unifying concept has to emerge, concerning the questions of tumor heterogeneity, tumor volume, finding adequate fields of assessment of these changes and so on. Further studies are needed to assess the correlation of stromal changes with other clinical parameters like the level of prostate specific antigen (PSA) and tumor stage.

Conclusion

Assessment of stromal changes in prostatic carcinoma, in the future, might serve as an additional diagnostic and prognostic tool in everyday practice. Further basic research studies might elucidate this changes on molecular level, concerning the stromal cells in malignant prostatic carcinoma, and mutations of stromal cells could be targeted with novel monoclonal gene therapy.

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