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Expression of extracellular matrix proteins: tenascin-C, fibronectin and galectin-3 in prostatic adenocarcinoma

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ABSTRACT

Introduction: The interchanged stromal-epithelial relations and altered expression profiles of various extracellular matrix (ECM) proteins creates a suitable microenvironment for cancer development and growth. We support the opinion that remodeling of the extracellular matrix plays an important role in the cancer progression. The aim of this study was to examine the expression of ECM proteins tenascin-C, fibronectin and galectin-3 in prostatic adenocarcinoma.

Methods: Glands and surrounding stroma were analyzed in randomly selected specimens from 52 patients with prostate cancer and 28 patients with benign prostatic hyperplasia (BHP). To evaluate the intensity of tenascin-C, fibronectin and galectin-3 expression the percentage of positively immunostained stromal cells was examined.

Results: Compared to BPH, stroma of prostatic adenocarcinoma showed statistically significant increase in tenascin-C expression ($p < 0.001$), predominantly around neoplastic glands, while fibronectin ($p = 0.001$) and galectin-3 ($p < 0.001$) expression in the same area was decreased.

Conclusions: Our study confirms changes in the expression of ECM proteins of prostate cancer which may have important role in the cancer development.

Keywords: ECM remodeling; fibronectin; galectin-3; tenascin-C; prostatic adenocarcinoma

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INTRODUCTION

Cancers are heterogeneous entities with various cell types and cells of multiple lineages collaborating together in favor of their survival and dissemination. The interchanged stromal-epithelial relations and altered expression profiles of various extracellular matrix (ECM) proteins creates a suitable

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microenvironment for cancer cell proliferation, movement and differentiation (1-4). Fibroblasts, the predominant cells in tumorous stroma, are important because of their role in synthesis, deposition and remodeling of the ECM. The phenotype of fibroblasts changes in tumors, where they are known by different names, such as myofibroblasts, reactive stromal cells, peritumoral fibroblasts, carcinoma-associated fibroblasts (1,2,5). In prostate cancer, the stromal composition is modified, showing myofibroblast/fibroblast predominance with a significant decrease of fully differentiated smooth muscle cells (6-8).

The ECM proteins fibronectin, tenascin-C and galectin-3, are important for cell adhesion and signaling. It is thought that tenascin expression in normal prostatic stroma decreases or disappears when the maturation of the gland is completed, but seems to increase again in adenocarcinoma (7,9,10). Tenascin-C can act as an antiadhesion molecule by modulating cell to cell adhesion through fibronectin, important in the cell to ECM adhesion (11). Galectin-3 interacts with intracellular glycoproteins, cell surface molecules and extracellular matrix proteins and, according to present data, is downregulated in the prostate cancer (11,12).

In this study we compared the expression of fibronectin, galectin-3 and tenascin-C in prostatic adenocarcinoma to benign prostate hyperplasia (BPH).

METHODS

Patients

Neoplastic glands and surrounding stroma were analyzed in randomly selected specimens from 52 patients (aged 51–76 years, median age 63.3) who underwent radical prostatectomy in the period from 2005 to 2007. Adenectomy specimens from 28 patients with BHP (aged 53–82 years, median age 64.4) were used as controls and for comparison.

Methods

For each carcinoma case we analyzed between 11 and 30 slides and for each BHP cases between 4 to 11 slides, under light microscope. Specimens were taken from the archive at the Ljudevit Jurak Department of Pathology, Sestre milosrdnice University Hospital Center, Zagreb. Retrieval of archival tissue block was conducted under institutional review board approval.

Cancers were graded using the Gleason grading system. The distribution of Gleason scores is shown in Table 1. Pathological TNM status is shown in Table 2. None of the patients was treated with hormone or radiation therapy before radical prostatectomy, and none had secondary cancer or distant organ metastases.

Specimens were fixed in 10% buffered formaldehyde, embedded in paraffin, cut at 5 μm and routinely stained with H and E. We analyzed all slides under low magnification (40x) and selected tumorous tissue with at least 70% of the tumor in the material, as well as BPH slides without inflammation for immunohistochemistry.

Immunohistochemical staining for tenascin-C, fibronectin and galectin-3 was performed on DAKO Tech-Mate Horizon automated immunostainer (DAKO, Copenhagen, Denmark) with LSAB method for visualization system. We used primary monoclonal antibodies to tenascin-C (clone 49; dilution 1:100), fibronectin (clone AV61; dilution 1:100) and galectin-3 (clone 9C4; dilution 1:100) (all

TABLE 1. Distribution of Gleason scores in 52 cases of prostatic adenocarcinoma

Gleason score	Number	Proportion (%)	Cumulative proportion
5	3	5.8	5.8
6	12	23.1	28.8
7 (3+4)	21	40.4	69.2
7 (4+3)	7	13.5	82.7
8	9	17.3	100.0
Total	52	100.0	

TABLE 2. pTNM stage of 52 cases of prostatic adenocarcinoma

	Number	Proportion (%)
T classification		
2	35	67.3
3	17	32.7
N classification		
0	45	86.5
1	7	13.5
Total	52	100.0

purchased from Novocastra Laboratories, Great Britain).

As positive controls we used: squamous cell carcinoma (positive in stromal component) for tenascin-C, renal parenchyma (positive in stromal component) for fibronectin and large bowel (positive in epithelium, in cytoplasm and nucleus) for galectin-3. Replacement of the primary antibodies with isotype-matched IgG was used as a negative control.

To evaluate the intensity of tenascin-C, fibronectin and galectin-3 expression in prostatic carcinoma and in prostate glands with BPH, the percentage of positively stained stromal cells was examined for each antibody in 10 fields under high magnification (400x). The analyzing area was chosen as a "hot spot" under the low magnification (40x). All proteins were analyzed in the same, previously marked area. The staining intensity was graded on a scale of 1–3 and expressed as 1, up to 33% positive stromal cells; 2, >33–66% positive stromal cells; 3, more than 66% positive stromal cells. The immunohistochemistry results were evaluated by three independent observers (UM, TD, KB) and any difference was resolved by a joint review.

Statistical analysis

Statistical analysis was performed using Smimirnov-Kolmogorov test for distribution of the parameters, Fisher's exact test and Spearman's rank correlation test, the Chi-squared test, Mann-Whitney U test and test of proportions were also used. The level of significance was set at $P < 0.05$ in all cases. All analyses were performed with IBM SPSS Statistics version 19.0.0.1 (www spss.com).

TABLE 3. Expression of tenascin-C, fibronectin and galectin-3 in tumoral and BPH stroma

Group	Tenascin-C				Total
	0	1*	2**	3***	
Cancer					
N	1	13	26	12	52
%	1.9	25.0	50.0	23.1	100.0
BPH					
N	5	20	3	0	28
%	17.9	71.4	10.7	0.0	100.0
Total					
N	6	33	29	12	80
%	7.5	41.3	36.3	15.0	100.0
Group	Fibronectin				Total
	0	1*	2**	3***	
Cancer					
N	17	33	2	0	52
%	32.7	63.5	3.8	0.0	100.0
BPH					
N	4	15	9	0	28
%	14.3	53.6	32.1	0.0	100.0
Total					
N	21	48	11	80	21
%	26.3	60.0	13.8	100.0	26.3
Group	Galectin-3				Total
	0	1*	2**	3***	
Cancer					
N	21	29	2	0	52
%	40.4	55.8	3.8	0.0	100.0
BPH					
N	2	14	10	2	28
%	7.1	50.0	35.7	7.1	100.0
Total					
N	23	43	12	2	80
%	28.8	53.8	15.0	2.5	100.0

*1: ≤ 33% positive stromal cells, **2: ≥33–66% positive stromal cells, ***3: ≥66% positive stromal cells

RESULTS

The expression profiles of tenascin-C, fibronectin and galectin-3 in BPH and prostatic carcinoma are shown in Table 3. Compared to BPH, the stroma of prostatic adenocarcinoma showed statistically significant increased tenascin-C ($p < 0.001$) and decreased fibronectin ($p = 0.001$) and galectin-3 ($p < 0.001$) expression. In 38 (73.1%) carcinoma patients compared to 3 (10.7%) BPH patients tenascin-C expression around neoplastic/hyperplastic glands was high (≥33% positive stromal cells), unlike fibronectin and galectin-3 expression which showed low expression around neoplastic glands. Immunohistochemical staining for fibronectin was positive in the stroma of 24 (85.7%) BPH patients and completely negative in even 17 (32.7%) carcinoma patients, whereas only 2 (3.8%) examined carcinomas showed more than 33% positive cells in the stroma. In BPH group galectin-3 expression was high in 12 (42.8%) patients compared to 2 (3.8%) carcinoma patients. Immunohistochemical reaction

for galectin-3 was negative in 21 (40.4%) carcinomas and only 2 (7.1%) BPH cases (Figure 1). Tenascin-C was predominantly expressed in stroma around neoplastic glands, while expression of fibronectin and galectin-3 was reduced in the same area (Figure 2).

The expression of the ECM proteins (tenascin-C, fibronectin, galectin-3) showed no statistically significant correlation to pTNM, serum PSA level, Gleason score or age.

DISCUSSION

Fávaro et al. studied the expression of different molecules in the stroma of benign and neoplastic prostate glands. According to their results, the lack of epithelial basal cells, dystroglycans and laminin and increased matrix metalloproteinase-2, fibroblast growth factor and insulin-like growth factor could be considered important in ECM remodeling and changes in tumorous stroma (13). Majority of these molecules are components of the extracellular

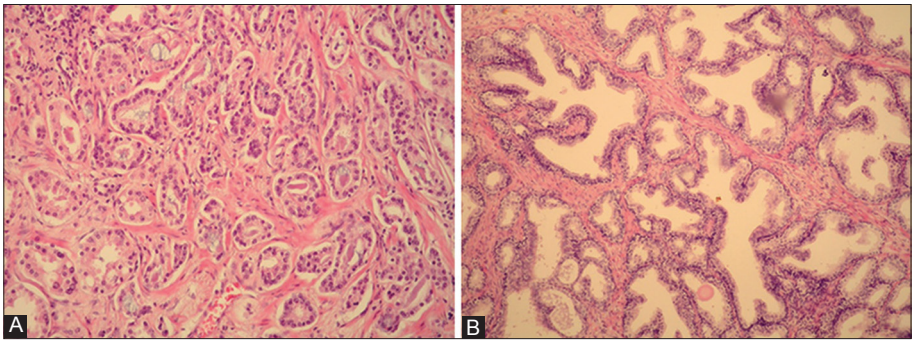

FIGURE 1. A) Prostatic adenocarcinoma (HEx200). B) Benign prostate hyperplasia (HEx100)

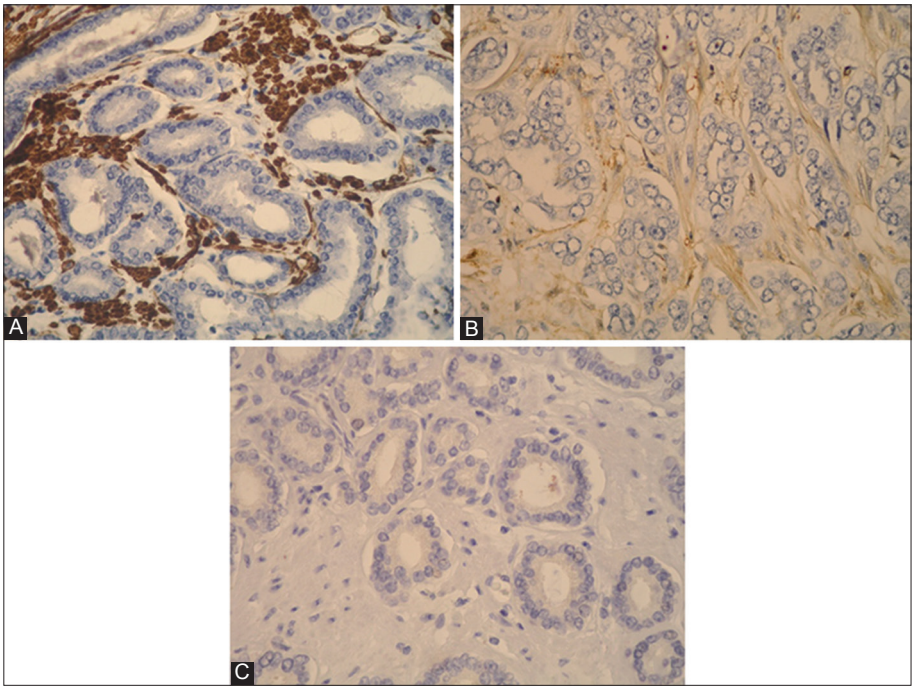

FIGURE 2. A) Immunohistochemical staining with tenascin-C, strong reaction (400x); B) fibronectin, moderate reaction (400x) and C) galectin-3, negative reactions

matrix (ECM) that is subject to constant remodeling, a process that involves breakdown of existing, and synthesis and deposition of new ECM proteins (1,2,5,14). In this study, we investigated the expression of the extracellular matrix (ECM) proteins tenascin-C, fibronectin and galectin-3 in the neoplastic and BPH glands. The expression of these proteins differs in the stroma of prostatic carcinoma compared to BPH glands. In prostatic carcinoma, the stroma showed statistically significant increase in tenascin-C and decrease in fibronectin and galectin-3 expression.

The role of tenascin in tumour growth and progression is still controversial. Tenascin-C expression in prostatic tissue disappears after maturation of the gland and reappears in the stroma of adenocarcinoma (7,8,10). In available studies, different stages of the prostatic carcinoma were examined. Gleason grade 3 tumors had the most pronounced tenascin-C expression, while in Gleason 4 and 5 it was weak. Expression of tenascin-C increased from low to high grade PIN and to Gleason grade 3 (15,16). Our results were similar, the stroma of prostatic adenocarcinoma showed statistically significant increase in tenascin-C expression, predominantly around neoplastic glands.

Tenascin-C interacts with fibronectin and through its expression modulates cell adhesion. In some *in vitro* studies on prostatic cancer cell lines, it was noted that adhesion of tumour cells was afected with inhibition of fibronectin, motility of the tumour cells was higher, as it was local invasion (17-19). Galectin-3 also participates in cell migration and adhesion to ECM. Van der Brule et al. suggested that galectin-3 might have an anti-tumour role when present in the nucleus, whereas it could favour tumor progression when expressed in the cytoplasm of the epithelial tumorous cells (20). Other autors did not confirm these results, but it was suggested that the expression of galectin-3 in cytoplasm correlates positively with tumour progression (21). Research on human prostate cancer LNCaP cells indicated that galectin-3 inhibits anticancer drug-induced apoptosis through regulation of Bad protein and suppression of the mitochondrial apoptosis pathway (22). In breast cancer, galectin-3 expression was examined in both epithelium and stroma, with results showing no prognostic correlation to either cytoplasmic or nuclear expression. The presence of galectin-3 in the stroma, however, indicated an unfavorable prognosis (23).

CONCLUSION

Our study confirmed that the stroma of prostate cancer is changing, which includes remodeling of the ECM matrix and altered expression of the different proteins. Expression of all three proteins in stroma differs in prostate cancer compared to BPH.

These or similar proteins may eventually be used in prediction of cancer progression or the possibility of recurrence. Future studies should be directed toward identifying specific markers of reactive stroma.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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