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Prognostic significance of epithelial/stromal caveolin-1 expression in prostatic hyperplasia, high grade prostatic intraepithelial hyperplasia and prostatic carcinoma and its correlation with microvessel density

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ABSTRACT

Caveolin-1 may play a role in cancer development and progression. The aim was to record the expression and localization of caveolin-1 in benign prostatic hyperplasia (BPH), high grade prostatic intraepithelial neoplasia (HGPIN) and prostatic carcinoma (PCa). Microvessel density was evaluated with CD34 immunostain. Correlations with known prognostic factors of PCa were recorded. Immunohistochemical expression of caveolin-1 and the MVD was evaluated in 65 cases; BPH (25), HGPIN (20) and PCa (20). Stromal caveolin-1expression was significantly higher in BPH than HGPIN and PCca. There was significant inverse relation between stromal caveolin-1 expression and extension to lymph node and seminal vesicle in carcinoma cases. Epithelial caveolin-1 was significantly higher in carcinomas than in BPH and HGPIN. Epithelial expression in carcinoma was significantly associated with preoperative PSA, Gleason score and lymph node extension. MVD was significantly higher in PCa than in BPH and HGPIN. There were significant relations between MVD and preoperative PSA, Gleason score, lymph node and seminal vesicle extension. Stromal caveolin-1 was associated with low MVD while epithelial caveolin-1 with high MVD. *Conclusions*: Caveolin-1 plays an important role in prostatic carcinogenesis and metastasis. Stromal expression of caveolin-1 in PCa is lowered in relation to BPH and HGPIN. In PCa; stromal caveolin-1 was associated with good prognostic parameters. Epithelial caveolin-1 is significantly increased in PCa than BPH and HGPIN. It is associated with clinically aggressive disease. Caveolin-1 may play a role in angiogenesis.

Introduction

Prostatic carcinoma (PCa) is the second diagnosed malignancy and the sixth cause of cancer mortality in men. Incidence rates vary more than 25-fold worldwide, with the highest rates in the developed countries this may be due to the widespread use of prostate-specific antigen screening and prostatic tissue biopsy [1]. Overall, the complex morphology, histologic heterogeneity, and the early metastatic ability of localized PCa show the requirement for extra clinical and pathologic tests for the evaluation of PCa and its early diagnosis [2].

Distant spread of primary malignant cells to local and distant sites was explained by several models. Cumulative research shows a pivotal role of tumor microenvironment in the process of malignant cell dissemination. This role occurs through angiogenesis and epithelial-stromal interactions which induce an epithelial-mesenchymal transition which result in increased migration of epithelial cells [3].

Caveolin protein is an important component of caveolae structure that are found in smooth muscle cells, adipocytes and endothelium. Caveolin-1 was overexpressed in mouse and human metastatic malignant prostate cells. Moreover, decreased caveolin-1 expression with stably transfected antisense caveolin-1 cDNA converted androgen-insensitive metastatic mouse prostate cancer cells to an androgen-sensitive phenotype [4]. Accordingly, Caveolin-1 was considered a metastasis-related gene as well as a representative gene for hormone-resistant human prostate cancer. Recent studies suggested a possible role for caveolin-1 in the resistance of various malignances to multiple antineoplastic drugs [5].

Previous reports have shown different roles of caveolin protein in tumor stroma and epithelium. In breast cancer, absence of stromal caveolin-1 is associated with ductal carcinoma in situ (DCIS) progression and metastatic breast carcinoma [6]. While in PCa,

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mouse models studies found overexpressed epithelial caveolin-1 gene [7].

Angiogenesis is common denominator between benign prostatic hyperplasia (BPH) and PCa. It is the process of new blood vessel formation. Angiogenesis is important for tumor growth and metastasis in various malignancies. Non-vascular cells in tumoral microenvironments such as macrophages, mast cells and fibroblasts can regulate angiogenesis by secreting angiogenic factors. Microvessel density (MVD) is an indicator of the neoangiogenetic process, which helps local growth and distant metastases of malignant cells. Microvessel density (MVD) denotes the number of small blood vessels in BPH, high grade prostate intraepithelial neoplasia (HGPIN) and PCa and is evaluated by endothelial marker CD34 immunostaining [8].

Aim of the study was to record the expression and localization of caveolin-1 in BPH, HGPIN and PCa. Microvessel density was evaluated with CD34 expression. Correlations with known prognostic factors of PCa were recorded.

Material and methods

The present retrospective study included 25 cases of BPH, 20 cases of HGPIN and 20 cases of PCa surgically removed by radical prostatectomy. Formalin fixed paraffin embedded blocks were obtained from archives of pathology department, Tanta university in the period from 2012 till 2015. Data about preoperative PSA, Gleason score, seminal vesicle invasion and lymph node metastasis were obtained from clinical and pathologic files of the patients. All cases were subjected to routine hematoxylin and eosin staining and examined by two pathologists to confirm the diagnosis.

Immunohistochemical procedures

Heat-mediated antigen retrieval was performed using a citrate buffer after initial deparaffinization and rehydration. Afterwards to inactivate endogenous peroxidase, sections were treated for 10 min with 2% hydrogen peroxide and blocked with 3% normal goat serum in 0.2 M phosphate-buffered saline, then incubated for 2 h with rabbit polyclonal antibody to caveolin-1 (anti-Caveolin-1 antibody (ab2910) Abcam UK, dilution of 1:10000) and CD-34 (Rabbit monoclonal antibody Biogenex, San Ramon, CA, USA, diluted 1:150). Standard avidin–biotin immunostaining was performed.

Epithelial caveolin-1 evaluation

Sections were evaluated at a high power. Positive epithelial caveolin-1 staining was considered if 50% of the tumor cells in any microscopic field showed caveolin-1 positive granular brown stain in their cytoplasm.

Stromal caveolin-1 evaluation

Caveolin-1 stromal expression was scored as negative (0; no staining), weak (1; either diffuse weak staining or strong staining in less than 30% of stromal cells per core), or strong (2; defined as strong staining of 30% or more of the stromal cells).

MVD evaluation by CD34 expression

Tumor sections stained for CD34 were examined using computer-aided image analysis [ImageJ software (version 4.10.03, Nikon, Tokyo, Japan)] to count CD34 +ve vessels per high-power field. 4–5 areas that had the greatest density of positively stained vessels (hot spots) were labeled. The median value of microvessel count (MVC) was 90 microvessels. For statistical analysis, MVD was divided into two groups: low (median or less) and high (greater than the median).

Statistical analysis of the collected data

Results were collected, tabulated and statistically analyzed with SPSS statistical package version 20 (SPSS Inc. Released 2011. IBM SPSS statistics for windows, version 20.0, Armnok, NY: IBM Corp.).

Two types of statistical analysis were done:

a) Descriptive statistics e.g. was expressed in: Number (No), percentage (%) mean ($\bar{x}$) and standard deviation (SD).
b) Analytic statistics e.g.

- Chi-square test (χ^2) was used to study association between qualitative variables. Whenever any of the expected cells were less than five, Fischer's Exact test was used.
- P-value of <0.05 was considered statistically significant.

Results

Clinicopathologic results

In PCa patients, the mean age was 62.4 (54–73). The mean PSA value was 12 ng/mL, range (1–75). Lymph node metastasis was detected in 80% of carcinoma cases. Seminal vesicle extension was found in 70%. PCa cases were histologically classified into 3 cases of low grade carcinomas (Gleason score 6), 4 cases of moderate grade carcinomas (Gleason score7) and 13 cases of high grade carcinomas (Gleason score 8–10).

Analysis of stromal caveolin-1 immunostaining

Stromal caveolin-1 expression was significantly higher in BPH vs. HGPIN and PCa (p value < 0.001) (Table 1).

Twenty-two cases (88%) of BPH were positive for stromal caveolin-1; including 13 cases with strong stain (score 2), and 9 showing weak stain (score 1). Only 3 benign cases (12%) were negative (score 0). In HGPIN cases; 5 cases (25%) were stromal caveolin-1 positive; 1 with strong stain (score 2). The majority of positive cases (4/5) exhibited weak expression (score 1). All positive cases showed evident epithelial expression. Among PCa cases; only 3 were positive for stromal caveolin-1; 1 showed strong staining (score 2) and the remaining 2 positive cases exhibited weak staining (score 1) (Fig. 1).

The correlation between stromal caveolin-1 expression and clinico-pathological factors of PCa are shown in Table 2. Most stromal caveolin-1 positive tumors (2\3) had a low preoperative PSA level <4 ng/mL, while none of the included 10 cases with high preoperative PSA >10 ng/mL showed stromal caveolin-1 expression. Regarding the Gleason score; all 3 positive cases were moderate and high Gleason score. There were no significant relations between stromal caveolin-1 expression and the preoperative PSA level or the Gleason score (*p value > 0.05*).

Stromal caveolin-1 positive tumors had no extension to lymph nodes or seminal vesicles. There were significant inverse relations between stromal caveolin-1 expression and lymph node metastasis and seminal vesicle extension.

Analysis of epithelial caveolin-1 immunostaining

In studied cases of BPH, 20% were positive for epithelial caveolin-1. Whereas; 60% of HGPIN cases and 90% of PCa cases were positive. Epithelial caveolin-1 expression was significantly higher in PCa than HGPIN and BPH (p value < 0.001). (Table 1) (Fig. 2).

The association between epithelial caveolin-1 expression and the clinicopathologic data are shown in Table 3. Cases with high preoperative PSA exhibited higher epithelial caveolin-1 expression then low and moderate groups. This relation was statistically significant (p value < 0.05). Epithelial caveolin-1 positivity was

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