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Clinical significance of endothelial cell marker CD34 and mast cell marker CD117 in prostate adenocarcinoma

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ABSTRACT

Prostate cancer is the second cause of cancer-related deaths in men and this is attributed to its aggressiveness and metastatic identity. Our objective was to evaluate the expression patterns of endothelial cell marker CD34 and mast cell marker CD117 in prostate adenocarcinoma (PCa) compared to benign prostate tissue and their relation to the clinicopathological features. A total of 90 prostate samples, including 45 PCa and 45 benign prostate tissues were immunohistochemically examined for the detection of CD34 and CD117 markers. The expression of these markers was also correlated with clinicopathological parameters. Significant overexpression of CD34 was found in PCa group compared to benign prostate tissues ($P \leq 0.001$). The expression of CD34 and CD117 in PCa with advanced Gleason score was more than PCa with early Gleason score ($P = 0.02$ and $P = 0.005$, respectively). A significant positive correlation was observed between CD34 expression and the level of total serum prostate specific antigen (sPSA) ($P = 0.006$). In addition, CD34High/CD117High phenotype was frequently observed in PCa cases compared to benign prostate tissues ($P \leq 0.001$). There was a positive significant association between CD34High/CD117High phenotype with advanced Gleason score ($P \leq 0.001$) and total sPSA level ($P = 0.02$). Our findings showed that increased expression of CD34 and CD117 markers confer tumor progression and aggressiveness on PCa. These molecules may be good candidates for targeted therapy of PCa patients.

1. Introduction

Prostate cancer is the most common malignancy and the second death-leading cancer in men population. Adenocarcinoma is observed in >99% of prostate cancers [1,2]. Although the application of prostate-specific antigen (PSA) has enhanced the detection power of early stage prostate adenocarcinoma (PCa), approximately 40% of patients with localized disease manifested drug resistance, metastasis and distant seeding [3]. Since the incidence rate of PCa has shown an alarming trend in almost all countries; therefore, development of novel therapeutic approaches to overcome its cellular invasion and aggressiveness seems necessary and challenging [4].

CD34 is a member of the single-pass transmembrane sialomucin protein family and it was firstly used as a potent hematopoietic cancer stem cells (CSCs) marker [5]. Although the biological role of CD34 is still unknown, it plays a key role in angiogenesis and it is one of the hallmarks indicating tumor progression and metastasis [6]. Nassif et al. showed high CD34 expression in PCa cases with higher PSA level, advanced Gleason score and tumor recurrence [7]. Another previous study reported a significant negative correlation between CD34 expression and Gleason score [8]. Recently, increased expression of CD34 has been documented in an advanced stage and high Gleason score in non-neoadjuvant hormonal therapy (NHT) group of PCa cases, while there was no significant association between CD34 expression with pathological parameters in NHT group of PCa samples [9].

CD117 is a receptor tyrosine kinase protein type III and it is also named c-kit or stem cell growth factor receptor. It binds stem cell factor (SCF); subsequently, it is involved in cell survival, proliferation, and differentiation [10]. In a preliminary study, overexpression of CD117 was correlated with good prognosis in PCa

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patients, whereas Vatansever and colleagues showed that CD117 was up-regulated in an advanced stage of PCa [11,12]. They also demonstrated no expression of CD117 in benign prostate hyperplasia (BPH) and early stage of PCa [12]. In a recent study, circulating tumor cells expressing CD117 ($CD117^+$) in PCa cases showed a decrease after radical prostatectomy [13].

Co-expression of CD34 and CD117 has been demonstrated in myeloid progenitor cells and gastrointestinal stromal tumors (GISTs) [14–17]. This implicates that both of mentioned cell surface glycoproteins may have a relationship. For example, upstream regulatory factors regulate their expression levels and biological roles in normal and malignant cells [18,19]. In addition, mutations in the juxtamembrane – coding region of CD117 have been observed in mast cell tumors and GISTs [20]. Interest in studying of progression and angiogenesis in PCa and targeting the potential molecules or markers for tailored therapy, made us evaluate the expressions of CD34 and CD117 and clinicopathological significance. Also, the clinical significance of endothelial cell marker CD34 and mast cell marker CD117 in PCa is challenging [21,22]. Therefore, the present study was carried out to analyze the expression and distribution patterns of CD34 and CD117 markers in PCa tissues compared to benign prostate tissues. The possible relationship between the expression of these potential markers and clinicopathological characteristics were also analyzed. Finally, a combined analysis was utilized to precisely represent theses markers which are requisite for PCa invasiveness and angiogenesis.

2. Materials and methods

2.1. Patients and tumor characteristics

In this cross-sectional and hospital base study, formalin-fixed-paraffin-embedded (FFPE) blocks were collected from 90 patients diagnosed with PCa and benign prostate tissues at Hasheminejad hospital, a major university-based and referral Urology-Nephrology center in Tehran, Iran, during the years 2010–2015. The control group consisted of 45 specimens taken from patients with total serum PSA greater than 4 ng/ml. The patients who underwent a needle biopsy were included in this study. Medical records were reviewed for data on clinicopathological factors, including Gleason score and total serum prostate specific antigen (sPSA) level. The level of total sPSA was divided into 3 groups; less than 4 ng/ml (group 1), 4 – 10 ng/ml (group 2) and greater than 10 ng/ml (group 3). The Gleason scoring system was applied based on the International Society of Urological Pathology (ISUP) in 2014 and the new patient-centric grading system [23,24]. Hematoxylin and eosin (H&E) stained sections of the tissues were observed and each PCa case was graded according to the Gleason scoring system (group 1 = Gleason score ≤ 6, group 2 = Gleason score 3 + 4 = 7 and 4 + 3 = 7, and group 3 = Gleason score 8–10) [25]. All samples were made anonymous according to the ethical and legal standards.

2.2. Immunohistochemical (IHC) assay

Expression of CD34 and CD117 was examined in benign prostate tissues and PCa cases by immunohistochemistry technique, as described previously [26–28]. Briefly, paraffin-embedded tissues were cut in 5-micrometer sections and mounted onto Superfrost slides (Superfrost plus, Thermo Scientific, Germany), dewaxed at 60 °C for 30 min, deparaffinized in xylene and rehydrated in different concentrations of ethanol. The sections were immersed in 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase activity. For epitope demasking, the slides were treated by pressure heating using citrate buffer (pH = 6.0) as a target retrieval solution for 20 min and then were rinsed with Tris-buffered saline (TBS). Protein Block Serum-Free (Dako; code X0909; Denmark; Ready-to-use) was used to reduce nonspecific background staining. For primary staining, monoclonal mouse anti-human CD34 antibody (Dako; Class II; clone QBEnd-10; Denmark) and polyclonal rabbit anti-human CD117 (c-kit) (Dako; code A4502; Denmark) were added to slides in 1:600 dilution (2 h) and ready-to-use (2 h), respectively. For secondary staining, EnVision™/HRP, Rabbit/Mouse (ENV) reagent (Dako; code K5007; Denmark; Ready-to-use) which is a peroxidase-conjugated polymer was applied. Visualization was performed by incubation of the sections in Dako REAL™ DAB+ Chromogen (Dako; Denmark), according to the manufacturer's instructions. After washing, the sections were counter-stained with Mayer's hematoxylin dye (Dako; Denmark) for 15 min, dehydrated in different degrees of alcohol and cleared in xylene. Human tonsil and seminoma tissues were applied as positive controls of CD34 and CD117 antibodies, respectively. Negative control was considered as omitting of the primary antibodies. All steps of staining were carried out at room temperature with incubation in a moist chamber.

2.3. Scoring of immunohistochemical staining and interpretation

Immunostained slides of CD34 and CD117 were observed and evaluated under light microscope Olympus CX-31 by a trained observer (M.F.) in a blinded manner to the patient's outcome and other clinical findings. The obtained results were also confirmed by two specialists (M.M. and M.A.) and a consensus was achieved. For scoring the CD34 positive microvascular density (MVD), highly vascularized areas were selected at low magnification (40X) and microvessels were counted in 10 non-overlapping selected fields at high magnification (400X). A single countable microvessel was defined as any endothelial cell or endothelial cell cluster positive for CD34 and clearly separated from an adjacent cluster. For scoring mast cell any cell with membranous and cytoplasmic staining for CD117 was counted as mast cell. For a patient with PCa, the intratumoral area was selected for mast cell evaluation. The $CD34^+$ vessels and $CD117^+$ mast cells were counted in 10 high power fields (400X magnification) as described previously and the average number of positive mast cells and microvessels was finally evaluated [9,29]. The median of *H*-scores were considered as a cut-off value to classify the specimens as low or high expression levels of CD34 or CD117 which were 25 and 9, respectively [30,31].

2.4. Statistical analysis

All data were analyzed using the SPSS software version 20 (SPSS, Chicago, IL, USA). The relationship of CD34 and CD117 markers with clinicopathological factors was evaluated using Pearson's chi-square or Fisher's exact test. The association between CD34/CD117 phenotypes and clinicopathological factors were also examined using one-way ANOVA and Tukey post hoc analysis. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Clinicopathological characteristics of patients

The mean age of all prostate cases (45 benign prostate tissues and 45 PCa cases) was 65.9 ± 8.7 years (range 50–82). The mean level of total sPSA was 19.32 ± 29 ng/ml (range: 1.73–206.7). Of the 90 cases, 5 (6%) had total sPSA less than 4 ng/ml, whereas 47 (52%) and 38 (42%) had total sPSA 4–10 ng/ml and greater than 10 ng/ml, respectively. Gleason score 7 and greater than 8 were found in simi-