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Tenascin-C is a potential cancer-associated fibroblasts marker and predicts poor prognosis in prostate cancer

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ABSTRACT

Tenascin-C (TNC), as a member of the extracellular matrix (ECM), plays an important role in cancer cell proliferation and migration and tumor invasion in various types of cancer. Here, we attempted to investigate the role of TNC as a prognostic factor in prostate cancer. We studied TNC expression via immunohistochemistry in 145 prostate cancer tissue samples. The clinicopathological relevance of TNC expression was examined, as well as other cancer-associated fibroblasts (CAFs)-related factors. Our results showed that the high levels of TNC expression in prostate cancer stroma was significantly associated with lymph node metastasis ($P = 0.024$) and clinical stage ($P = 0.032$). Furthermore, TNC was positively correlated with increased micro-vessel density (MVD) ($P = 0.017$) and tumor associated macrophage (TAM) population ($P = 0.025$). In both univariate and multivariate Cox regression analyses, TNC ($P < 0.001$) was an independent poor prognostic factor for overall survival in prostate cancer patients. Moreover, over-expression of TNC ($P < 0.001$), SMA ($P = 0.042$) and vimentin ($P = 0.010$) were significantly correlated with the lower overall survival. In addition, TNC expression in prostate cancer stroma was significantly associated with FSP1 ($P = 0.011$), SMA ($P = 0.021$), and vimentin ($P = 0.002$). In conclusion, our study revealed that high level of TNC as a potential biomarker of CAFs was significantly correlated with the poor prognosis for prostate cancer patients.

1. Introduction

Prostate cancer is the most common male cancer and the second leading cause of male cancer death worldwide [1]. Metastasis is the most feared complication by far and the major cause of death for prostate cancer patients [2]. Although the tumor microenvironment plays an essential role in prostate cancer metastasis and offers novel opportunities to prevent and treat prostate cancer, the molecular mechanisms of recurrence and metastasis that cause the majority of prostate cancer-related deaths remain unknown and need to be clarified.

Tenascin-C (TNC), a member of the extracellular matrix (ECM), can directly or indirectly affect the invasiveness and metastatic potential of carcinoma tumors [3]. TNC expression has been correlated with lymph node metastasis in breast, colon, liver, and oral squamous cell carcinoma [4]. In addition, high expression of TNC could be a useful marker in cancer-associated fibroblasts (CAFs) for determining prognosis in esophageal squamous cell carcinoma (ESCC) [5].

Although TNC is important in many human cancers, the role of TNC in prostate tumor growth and progression is not well characterized and controversial. Some authors have proposed that TNC was involved in the maintenance of normal prostate stromal–epithelial homeostasis and protected against the effects of neoplasia [6]. However, another view of TNC expression in prostate carcinoma is that as a reactive stroma compartment, it could enhance tumour progression and promote cancer cell survival,

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proliferation and invasion [7]. It is necessary to investigate the role of TNC in prostate cancer. In this study, we examined TNC expression in prostate cancer stroma to investigate its potential value as a prognostic marker in prostate cancer patients, and to identify a novel CAFs marker for prostate cancer as a potential therapeutic target.

2. Materials and methods

2.1. Tissue specimens

A total 145 prostate cancer tissue samples were obtained from patients who underwent prostate surgery and were stored in Shanghai Outdo Biotech Co. Ltd. (Outdo Biotech). The Institutional Review Board of Yanbian University Medical College approved the study protocol and conducted in accordance with the 1996 Declaration of Helsinki. All patients provided written informed consent according to institutional guidelines. No patient received preoperative chemotherapy or radiotherapy. Pathological parameters, including age, histological grade, clinical stage, advanced tumor (pT) stage and lymph node metastasis were carefully reviewed in all 145 prostate carcinomas. All patients had follow-up records of more than 5 year, survival time was measured from the date of surgery to the follow-up deadline, or date of death. The pTNM classification was applied according to guidelines from the 2010 American Joint Committee on Cancer staging manual (AJCC 7th edition).

2.2. Immunohistochemical staining procedure

A total of 145 prostate specimens were fixed in formalin, embedded in paraffin and cut into 3-μm serial sections. Sections on microslides were deparaffinized with xylene, hydrated using a diluted alcohol series, and immersed in 3% H_2O_2 in methanol to quench endogenous peroxidase activity. Then sections were heated in an autoclave with TE buffer (1 mM EDTA and 10 mM Tris, pH 9.2) at 98 °C for 30 min. To reduce nonspecific staining, each section was blocked with 4% bovine serum albumin in TBS with 0.1% Tween 20 for 30 min. The sections were incubated with anti-TNC (1:100, Abcam, EPR4219(ab108930), Rabbit monoclonal, Cambridge, UK), anti-fibroblast- stimulating protein-1(FSP1) (1:100, ZSGB-BIO, ZA-0257, Rabbit polyclonal, Beijing, China), anti-smooth muscle actin (SMA) (1:100, ZSGB-BIO, ZM-0003, Mouse monoclonal, Beijing, China), anti-vimentin (1:100, ZSGB-BIO, ZM-0260, Mouse monoclonal, Beijing, China) in TBST containing 3 mg/ml goat globulin (Sigma, St. Louis, MO, USA) for 2 h at room temperature, followed by three washes with TBST buffer. Then sections were incubated with an anti-mouse/rabbit antibody (Envision plus, Dako, Denmark) for 30 min at room temperature. A chromogen was used to create red staining with ImmPACT AEC Peroxidase Substrate (VECTOR Laboratories, Burlingame, CA, USA) for 20 min. Counterstaining was performed with Meyer's hematoxylin for 1 min. After reading and taking photograph of the slide (Model: ECLIPSE Ni-U; Manufacturer: Nikon; Location: Japan), then used the stripping buffer (20% SDS, 0.5 M Tris, and mercaptoethanol) to remove the original antibody for 1 h in a water bath at 56°C, and next for 10 min of dehydrated alcohol to remove the red reaction, so that the sections could be used again. Omitting the original antibody provided negative controls for immunostaining.

The double immunostaining procedure was performed using a two-step method with anti-TNC (developed with 3, 3′-diaminobenzidine) (a brown reaction product), anti-CD105 antibodies (1:250, Abcam, EPR10145-10(ab170943), Rabbit monoclonal, Cambridge, UK) and anti-CD68 antibodies (1:100, ZSGB-BIO, ZM-0464, Mouse monoclonal, Beijing, China) (developed with ImmPACT AEC Peroxidase Substrate) (a red reaction product), to observe the correlation between the expression of TNC/CD105 and TNC/CD68 in prostate cancer. Firstly, for the TNC protocols, except that the chromogen with the 3, 3'-diaminobenzidine (Dako) for 10 min, all steps are the same as before. Then, the subsequent staining of the same section was performed after incubating the samples with an antibody to CD105 or CD68 by ImmPACT AEC Peroxidase Substrate for 20 min.

2.3. Evaluation of the immunohistochemical analysis

Two pathologists (YHX & WDN) evaluated the immunohistochemical results with no prior knowledge of clinical data results, and discussed any discrepancies in scores until a consensus was reached. CD105 positive individual microvessel counts were performed on 200 × fields and three area microvessel numbers were averaged as the micro-vessel density (MVD), and CD68 as a marker of tumor associated macrophage (TAM) [8]. Moreover, according to the staining intensity and the proportion of positive stromal cells, immunohistochemical scores was measured, as follows: [IHC score 1], weak staining in <50% or moderate staining in <20% of stromal cells; [IHC score 2], weak staining in ≧50%, moderate staining in 20–50% or strong staining in <20%; [IHC score 3], moderate staining in ≧50% or strong staining in ≧20%. Cases with score 2 and 3 were regarded as positivity for each protein expression [5].

2.4. Statistical analysis

Correlations were assessed using Pearson's chi-square test as appropriate. Overall survival (OS) was determined using the Kaplan–Meier method and were compared using the log-rank test. Survival was measured from the date of surgery. The Cox proportional hazards model was used for multivariate analysis.

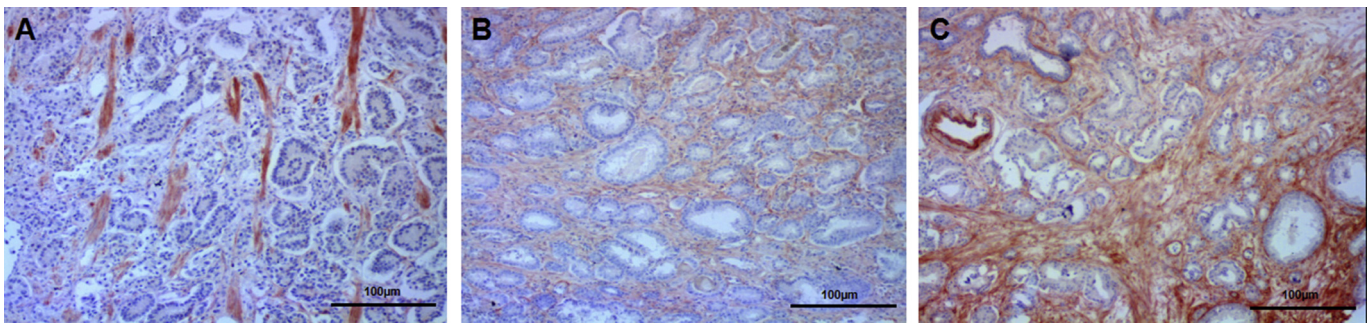

Fig. 1. The expression of TNC in prostate cancer stroma tissues, and the immunohistochemical staining scores according to the staining intensity and the proportion of positive stromal cells: weak staining(A), moderate staining(B), and strong staining (C).

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