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RESEARCH

Overexpression of CD73 in Prostate Cancer is Associated with Lymph Node Metastasis

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Abstract Prostate cancer is the most common malignancy in men in Europe and North America. At present, it is becoming an increasingly common cancer in China. CD73 (ecto-5′-nucleotidase) is a glycosylphosphatidylinositol (GPI)-linked 70-kDa cell surface enzyme. It is also broadly expressed in many types of tissues. Recent studies have showed that CD73 is widely expressed on malignancies and is up-regulated in cancerous tissues. Consequently, we analyzed the expression of CD73 in prostate cancer tissue. The expression of the CD73 protein was evaluated by Immunohistochemistry staining in 116 tissue specimens. The expression was further examined by quantitative real-time PCR (qRT-PCR) and Western blot in the same set of patients. The intense cell membrane staining for the CD73 protein was observed. The expression of CD73 in lymph node non-metastasizing prostate cancer tissues can be seen at low levels, and is generally undetectable. RT-PCR and Western blot showed that the expression of CD73 in lymph node metastasizing prostate cancer was higher compared with non-metastasizing ones. These results suggest that CD73 could be considered as a relevant-specific target for molecular therapy of prostate cancer metastasis.

Keywords Prostate cancer · CD73 · Immunohistochemistry · Lymph node · Metastasis

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Introduction

Prostate cancer is the most common malignancy in men in Europe and North America, and it is the second leading cause of cancer-related death in American men [1, 2]. At present, it is becoming an increasingly common cancer in China. Despite recently great progress in conventional therapies such as surgery and hormone treatment, it is quite difficult to conquer this malignant disease [3]. Looking for specific targets to prostate cancer might provide a straight effective way to diagnose and cure.

CD73 (ecto-5′-nucleotidase) is a glycosylphosphatidylinositol (GPI)-linked 70-kDa cell surface enzyme that catalyzes the dephosphorylation of extracellular AMP to adenosine [4, 5]. CD73 mainly expressed on endothelial and epithelial cells and on a subset of lymphocytes, especially on regulatory T cells. CD73, which was originally known as a lymphocyte differentiation antigen, is found to function as a signaling and adhesion molecule of lymphocytes [6]. It is also broadly expressed in many types of tissues. Recent studies have showed that CD73 is widely expressed on malignancies such as leukemia and certain cancers of epithelial cell origin, and is up-regulated in cancerous tissues [7–9]. However, the role of CD73 in cancer remains unclear.

Materials and Methods

Patients and Tissue Specimens

To detect the expression of CD73, 116 specimens from prostate cancer tissues were obtained from prostate cancer patients resected at Tianjin Medical University Cancer Hospital. The use of clinical specimens for this study was approved by the ethical committee of Tianjin Medical University Cancer Hospital. All tissues samples including non-metastasis or metastasis to lymph nodes were histologically confirmed. The

tissues were paraffin embedded, and cut at a thickness of 4 lm. The slices were mounted on microscope slides. The slices were then fixed in 4 % formalin (pH 7.0), and stained using H&E to determine the pathological type and grade. The histopathological grade and clinical stage of prostate cancer were defined according to the criteria of the World Health Organization (WHO, 2004). The study protocol was approved by the Ethics Committee of Tianjin Medical University Cancer Hospital, and informed consent was obtained from the included patients.

Immunohistochemistry

Immunohistochemical staining was carried out with Avidin Biotin Peroxidase system using antibodies against CD73 (Santa Cruz Biotechnology, 1:100 dilution). Briefly, 5-mm-thick paraffin sections were deparaffinized and hydrated through a graded series of alcohol. After inhibition of endogenous peroxidase activity by immersion in 3 % H_2O_2/methanol solution, antigen retrieval was conducted using citrate buffer (pH 6.0) in a microwave oven for 10 min at 120 ° C. Sections were incubated with primary antibodies, washed in phosphate-buffered saline (PBS), then incubated with biotinylated secondary antibody, followed by the avidin-horseradish peroxidase complex. Finally, immune complexes were visualized by incubation with diaminobenzidine chromogen. Scores of 0, 1+, 2+, and 3+ were given according to the summation of intensity and proportion of positive cells in immunohistochemically stained sections. Scores =2 were considered as positive.

RT-PCR

RNA was extracted from prostate cancer tissues by TRIZOL (Invitrogen). A polymerase chain reaction (PCR) was used to detect the expression of CD73 gene (5′ primer: 5′-GCCTGGGAGCTTACGATTTTG-3′, and 3′ primer: 5′-TAGTGCCCTGGTACTGGTCG-3′).

As an internal control, the GAPDH-specific primers were included in the PCR (5′ primer: 5′-GGAGCGAGATCC CTCCAAAAT-3′, and 3′ primer: 5′-GGCTGTTGTCATACTTC TCATGG-3′). Cycling conditions included: 95 °C for 5 min; 30 cycles at 94 °C for 30 s, 60 °C for 150 s, and 72 °C for 30 s; 72 °C for 10 min. Reaction products were separated on a 2 % agarose gel and stained with ethidium bromide.

Western Blot

Cells were lysed with 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1 % Igepal, 0.5 % sodium deoxycholate, 0.1 % SDS, 10 % glycerol, 150 mM NaCl plus phosphatase and protease inhibitors for 30 min on ice. Nuclei were removed by centrifugation at 12,000 g at 4 °C for 15 min and protein concentration in the supernatants was determined by Protein Assay. Proteins were separated on a 12 % polyacrylamide gel, transferred to a polyvinylidene difluoride membrane and processed as described. 40 μg of total cellular protein were loaded for evaluation of CD73 expression. Actin was used as house-keeping protein for sample normalization. Goat anti-CD73 polyclonal antibody (Santa Cruz Biotechnology) and rabbit anti-GAPDH antibody (Santa Cruz Biotechnology) were used as primary antibodies. Protein presence was detected through the incubation with the respective horseradish peroxidase-labeled secondary antibodies (Santa Cruz Biotechnology) followed by a colorimetric reaction.

Statistical Methods

Data were analyzed using chi-square test and Fisher's exact test when appropriate. All tests were performed two tailed with a confidence interval (CI) of 95 %. Statistical calculations were carried out using Statistical Package for Social Sciences software (SPSS, Chicago, Illinois, USA).

Results

In total, 116 tissue specimens were evaluated for expression of the CD73 protein. Taking the tissue cores obtained from the primary prostate cancer specimens which metastasize in lymph node into consideration, an intense cell membrane staining for the CD73 protein was uniformly observed within simultaneously present. Examples of CD73 staining in lymph node metastasizing and non-metastasizing prostate cancer are shown in Fig. 1. The IHC reactions were evaluated on tumor cells. The expression of CD73 in non-metastasizing prostate cancer tissues can be seen at low levels, and is generally undetectable. Of the 72 cases of lymph node metastasizing prostate cancer, 68 (94 %) showed the expression of CD73. However, there are only 3 cases expressing CD73 in 34 ones of lymph node non-metastasizing prostate cancer (Table 1). The expression of CD73 is not showed the difference in other pathological characteristics such as age and T stage.

To further determine the role of CD73 in prostate cancer, we detect the expression of CD73 in prostate cancer by RT-PCR and Western blot analysis. RT-PCR showed that the expression of CD73 in lymph node metastasizing prostate cancer was higher compared with non-metastasizing ones (Fig. 2). The results of Western blot analysis also showed the higher expressing CD73. The mRNA and protein level both demonstrated the higher expressing CD73 was positively associated with lymph node metastasis of prostate cancer.

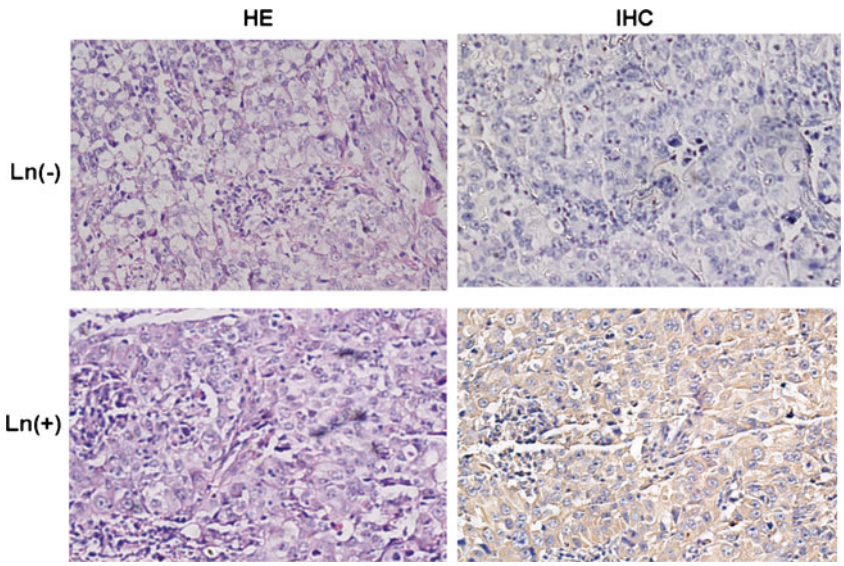

Fig. 1 CD73 immunostaining in lymph node metastasizing and non-metastasizing prostate cancer tissues. To investigate the role of CD73 in prostate cancer, 106 specimens were stained for CD73 by immunohistochemistry

Discussion

There are studies reported that CD73 participates in a variety of physiological functions such as epithelial ion and fluid transport, epithelial barriers, hypoxia and vascular leak [5, 10, 11]. Interestingly, CD73 seems to be associated with tumor promotion. The participation of CD73 as a proliferative factor is involved in the control of cell growth, differentiation, invasion, migration and metastasis processes. CD73 is up-regulated in many human tumors including breast, colon, lung, pancreas and ovary [12, 13]. CD73 promotes invasion, migration, adhesion and metastasis of human breast cancer cells [12, 14]. In a melanoma model, the growth of primary tumors and formation of metastasis were significantly attenuated in mice lacking CD73 [15]. CD73 expression in CRC was significantly higher than in normal colorectal tissues. High expression of CD73 may become a poor prognostic biomarker in human colorectal cancer [16, 17]. CD73 may modulate glioma cell adhesion and tumor cell-extracellular matrix interactions [18]. Expression of CD73 and its ecto-5′-nucleotidase activity are elevated in papillary thyroid carcinomas [19].

In this study, 116 tissue specimens were evaluated for expression of the CD73 protein. The positive expression of CD73 in lymph node non-metastasizing prostate cancer tissues can be seen lower compared to metastasizing prostate cancer tissues by IHC. Furthermore, we found that the expression of CD73 in lymph node metastasizing prostate cancer was higher compared with non-metastasizing ones. These results are in accordance with the previous reports. Evidence from many experiments suggests that expression of CD73 may be an important player in increasing the metastatic properties of some cancer cells and overexpression of CD73 may contribute to progression of cancer [20]. Stagg et al. showed that CD73 expression on nonhematopoietic cells, most likely endothelial cells, was critical for promoting lung metastasis in a manner independent from immunosuppressive effects [21]. Further study showed that anti-CD73 antibody therapy inhibits breast tumor growth and metastasis [22]. Lee et al.

Table 1 Analyses of CD73 expression in prostate cancer patients

Variable	*N*	Median percentage of CD73-positive cells	*P*-value
Age			
<60 years	48	14(34)	0.443
≥60 years	58	21(37)	
T stage			
T1 + T2	51	16(35)	0.729
T3 + T4	55	19(36)	
N stage			
LN(−)	34	31(3)	<0.001
LN(+)	72	4(68)	

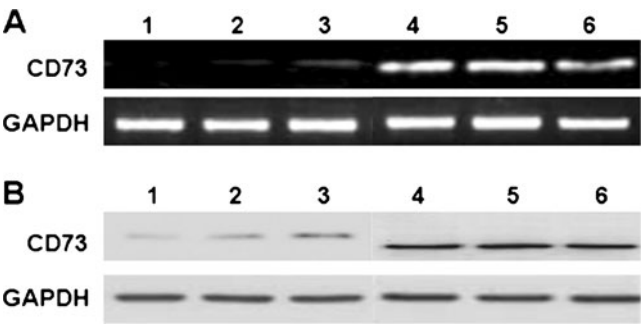

Fig. 2 Expression profile of CD73 in prostate cancer was further detected. **a** The mRNA level of CD73 was detected by RT-PCR. **b** The protein level of CD73 was determined by Western blot

found that CD73 protein is also upregulated in breast tumors and that its expression is elevated in the lymph node metastases [23].

In conclusion, we show that the expression of CD73 in lymph node metastasizing prostate cancer was higher compared with non-metastasizing ones. CD73 might be a new potential therapeutic target for prostate cancer metastasis.

Disclosure Statement No competing financial interests exist.

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