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CEACAM5 is correlated with Angio/ Lymphangiogenesis of Prostatic Lesions

Research Article

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Abstract: Objective.The aim of this study was to examine the expression patterns of CEACAM5 in prostatic non-neoplastic and neoplastic lesions and further investigate its relationship with tumor microvessel density(MVD) and lymphatic vessel density(LVD). Methods. CEACAM5 expression was detected using immunohistochemical staining in a serial sections of the benign prostatic hyperplasia (BPH), prostate intraepithelial neoplasia (PIN) and prostate carcinoma (PCa) lesions. MVD and LVD were quantified in CEACAM5 positive areas by dual-labelling with CD34 and D2-40 respectively. Results. Both PIN and PCa had significantly higher expression for CEACAM5 than BPH which has no positive expression for CEACAM5($P<0.05$). In PIN and PCa, CEACAM5 staining showed different expression patterns in terms the of most of membranous staining for PIN, less membranous staining and more cytoplasmic staining for PCa. MVD results showed that PCa and PIN had more angiogenesis than BPH tissue. The value of MVD in PCa tissue was correlated with tumor Gleason grading ($P<0.05$). LVD results showed that neoplastic lesions had more lymphangiogenesis than non-neoplastic lesion. Conclusion. CEACAM5 had different expression patterns in prostatic non-neoplastic and neoplastic lesions, and these various expression patterns may be correlated with tumor progression through promoting tumorous angiogenesis or lymphangiogenesis.

Keywords: *Prostatic neoplastic and non-neoplastic lesions • CEACAM5 • Angiogenesis • Lymphangiogenesis*

1. Introduction

The human carcinoembryonic antigen (CEA) family has 7genes that belong to the CEACAM subgroup. These subgroup members are mainly associated with the cell membrane and exhibit complex expression patterns in normal and cancerous tissues. The CEACAM5 gene encoding CEA protein, also known as CD66e, was first described as a gastrointestinal oncofetal antigen [1]. CEACAM5 mainly serves as a cell adhesion molecule mediating intercellular contact by both homophilic (CEACAM5 to CEACAM5) binding and heterophilic binding (CEACAM5 to CEACAM1 or CEACAM6). These interactions are predominantly mediated by the N-terminal IgV-like domain [2], which is conserved among all the CEACAM family members. Besides its functions in cell adhesion and migration, CEACAM5 also inhibits anoikis [3], which is apoptosis in the absence of adhesive interactions with extracellular matrix (ECM).

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Since resistance to anoikis is a characteristic of tumor cells, inhibition of anoikis by CEACAM5 suggests it could facilitate tumorigenesis and metastasis. Indeed, the tumorigenic functions of CEACAM5 have been demonstrated in both 3D culture of colon carcinoma cell lines *in vitro* [4] and CEABAC transgenic mice *in vivo* [5,6]. However, a number of studies have indicated that CEACAM5 contributes to the tumor invasion and metastasis in human colorectal carcinoma [7,8]. Most of the current studies on CEACAM5 focus on gastrointestinal carcinoma.

Nevertheless, the role of CEACAM5 in prostate cancer is not clear. In our study, we compared the immunohistochemical expression profiles in a series of 10 cases of benign prostatic hyperplasia (BPH), 30 cases of prostatic intraepithelial neoplasia (PIN), and 37 cases of primary prostate adenocarcinoma (PCa) to investigate the expression patterns of CEACAM5 in the pathophysiology of prostate tumor and further provide some direct evidence on its potential as a diagnostic and/or prognostic biomarker for prostate cancer.

2. Materials and methods

2.1. Antibodies

The mouse anti-human CEACAM5 monoclonal antibody (PTG; 60053-1-Ig, dilution 1:500), the mouse anti-human monoclonal antibody CD34 (ab8536, dilution 1:1500) and mouse anti-human monoclnal antibody D2-40 (ab52092, dilution 1:500) were all purchased from Abcam plc. (Cambridge, UK).

2.2. Patients

Analytical data and the summarized follow-up results were available for 77 patients. The patients were sampled in groups according to disease:

1. *PCa group* . A total of 37 PCa tissue samples of first surgical treatment made up this group. 37 patients with prostate carcinoma who received radical prostatectomy (primary surgical resection) between 2003 and 2012 at the Second Hospital of Shandong University;
2. *PIN group*. This group comprised samples with histopathological signs of intraepithelial neoplasia of the prostate (28 cases) or with atypic adenomatous hyperplasia (two cases) but without signs of PCa. All samples were taken by needle biopsy. The patients received transrectal 13-core prostatic biopsies with local anesthesia guided by transrectal ultrasound. These samples included 19 low grade and 11 high grade.
3. *BPH group.* A total of 8 tissue samples of benign prostate hyperplasia taken by transurethral resection (TURP) were available, in 2 cases by adenomectomy.

The patients of every group were followed up for 34 (range 3-64) months. The clinicopathologic information, including age, histological types, lymph node metastasis and tumor stage were obtained from the clinical records. All the diagnoses were made based on the Eble J.N. Sauter G., EpsteinJ.I. Sesterhenn I.A: World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs (IARC Press: Lyon 2004) and The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma [9] by three pathologists. This study was approved by the Moral and Ethical Committee of the Second Hospital of Shandong University.

2.3. Immunohistochemistry (IHC) staining

Immunohistochemistry was performed on 4μm-thick, routinely processed paraffin sections in series. CEACAM5 was detected with a mouse anti-human CEACAM5 -monoclonal antibody (PTG; 60053-1-Ig, dilution 1:500). The MVD and LVD were observed with a mouse anti-human CD34 ((ab8536, dilution 1:1500) and D2-40 (ab52092, dilution 1:500) respectively. Sections were dewaxed, and endogenous peroxidase was blocked by immersing the slides in a 3% solution of hydrogen peroxide in methanol for 10 minutes. This was followed by a step of antigen retrieval. Slides were immersed in 0.01 mol/L citrate buffer solution (pH 6.0) and placed in a microwave oven for 25 minutes. Following a wash in 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4), sections were covered with normal serum in a humidity chamber for 30 minutes at room temperature. Excess serum was rinsed off with 0.01 mol/L PBS, and sections were incubated with the primary antibody in a humidity chamber for 45 minutes at room temperature. Then, sections were rinsed with PBS before being incubated with the biotinylated second antibody in a humidity chamber for 40 minutes at 37°C. After rinsing with PBS, the streptavidin–peroxidase complex reagent (StrepABComplex/HRP Duet, DAKO) was added. Slides were incubated for 45 minutes at room temperature, then washed in 0.01 mol/L PBS, and covered with 3,3-diaminobenzidine tetrahydrochloride solution for 15 minutes under a microscope. Sections were then immersed in running tap water, counterstained with hematoxylin for 1minute, followed by tap water bath, immersion in a series of alcohol baths of increasing concentrations, and xylene, then covered with coverslips. Negative

controls were performed, in which the primary antibody was omitted.

2.4. Immunohistochemical staining evaluation

Evaluation of CEACAM5 staining was performed independently by three pathologists. Slides with equivocal evaluation were reevaluated, and a consensus was reached. For each sample, at least 3000 carcinoma cells were evaluated for the immunohistochemical staining. We examined the sections 200× magnification, and the carcinoma cells with cytoplasmic or membranous staining was determined.

2.5. MVD and LVD counting in CEACAM5-stained areas

Microvessel density (MVD) was assessed in the CEACAM5-positive carcinoma areas. Vessel counts were performed under light microscope based on staining of CD34. Five areas of maximal MVD were identified by screening (magnification 40×). The number of vessels was counted within a counting grid at 400× magnification (40× objective and 10× ocular). For the blood vessels counts, any stained endothelial cell or cell cluster separated from another microvessel structure was considered as a countable microvessel. Lumen was not necessary for a structure to be counted as a microvessel. The number of vessels was expressed as the mean value of counted microvessels in five evaluated grids in areas of maximum vessel density. Likewise, the LVD was accessed in CEACAM5-stained areas based on staining of D2-40. The methods of detecting both MVD and LVD were based on The Weidner [10]. Data were expressed as the mean ± SD (Table 1).

2.6. Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software package (SPSS Inc.,Chicago, IL) for Windows. Measures of central tendency and dispersion were determined. The data were analyzed by Pearson correlation coefficient, Fisher's Exact Test and *t*-test for significance (differences were considered significant at $p<0.05$).

3. Results

3.1. Detection of CEACAM5 expression

CEACAM5 was expressed in different patterns between non-neoplastic and neoplastic lesions. In the 10 cases of BPH, no positive staining was observed (Figure 1. A). Interestingly, 29 positive staining of all 30 cases of PIN, whether low grade or high grade, showed positive staining with membranous patterns while no case showed cytoplasmic staining. Further statistical analysis indicated that there was no significant difference between the high and low grade of PIN (Figure 1. B). PCa showed more CEACAM5 expression than in PIN and BPH. However in 37 cases of PCa, 33 CEACAM5 positive cases were classified as 11 cases of membranous staining, 21 cases of cytoplasmic staining and 3 cases of cytoplasmic staining along with little membranous staining. CEACAM5 expression patterns in BPH, PIN and PCa showed significant difference ($P<0.05$) (Figure 1. C).

CEACAM5 expression patterns showed significant difference between BPH and PCa, whether the expression patterns were of membranous distribution ($t=2.803$, $p=0.019<0.05$) or cytoplasmic distribution ($t=10.542$, $p=0.000<0.01$). Likewise, there was a significant difference in CEACAM5 expression patterns between BPH and PIN ($t=13.143$, $p=0.000<0.010$). Moreover, the PCa showed significantly different patterns from PIN with cytoplasmic CEACAM5 ($t=5.019$, $p=0.000<0.01$).

In PCa, CEACAM5 expression patterns were well correlated with tumor Gleason grading, and there was significant difference in expression patterns ($p=0.010$). According to the histological grading, poorly differentiated carcinomas showed more cytoplasmic staining [8], while the well differentiated displayed more membranous CEACAM5 staining (Figure 2A, B, C). The 9 cases with membranous staining have lower Gleason grading while the 24 cases with cytoplasmic staining have higher Gleason grading on average. The CEACAM5 expression were not connected with the clinical features including the patients' age, clinical stages and lymph node involvement.

MVD counting based on CD34 staining shows that neoplastic lesions have more angiogenesis than non-neoplastic lesion

Vessels evaluation with CD34 labelling showed significantly difference in BPH, PIN and PCa ($p<0.05$) (Figure 3A, B, C). PCa showed the most CD34-positive vessels (72.08±9.309), followed by PIN (51.33±9.07) and BPH (41.3±4.398). Meanwhile, the number of MVD labelled with CD34 in both poorly and moderately differentiated adenocarcinoma was significantly increased compared to that in well differentiated ones ($p=0.040$, 43.8±6.460 vs. 87.85±18.339; $p=0.029$, 43.8±6.460 vs. 70.8±11.353). However, there was no significant difference between moderately and poorly differentiated ($p=0.081$, 70.8±11.353 vs. 87.85±18.339) (Table 1.)

Figure 1. CEACAM5 is not expressed in BPH(A), expressed with membranous pattern in PIN, and overexpressed in PCa(C). A 100×, B and C 400×

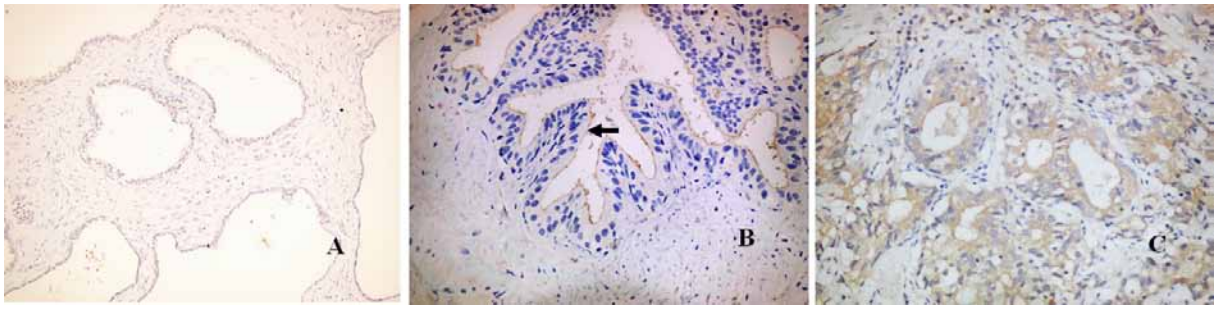

Figure 2. CEACAM5 is expressed with membranous pattern in well differentiated PCa(A), with cytoplasmic staining in moderately(B) and poorly differentiated PCa(C). 400×

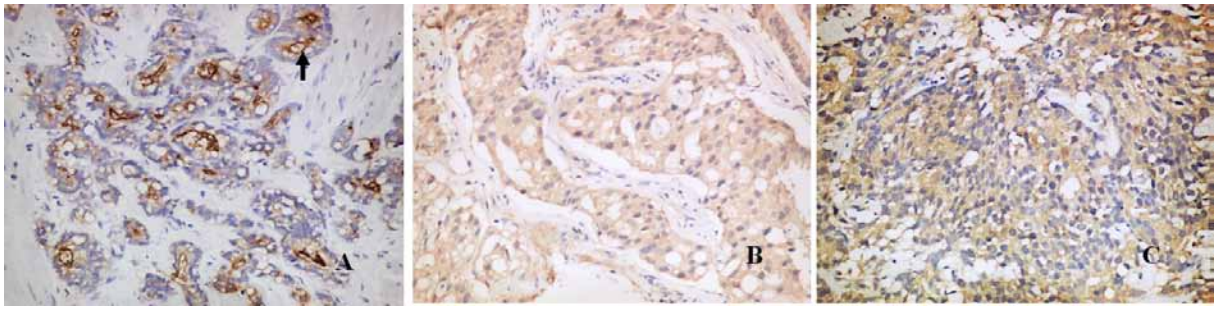

Figure 3. In CEACAM5 positive areas, MVD shows significant difference in BPH(A), PIN(B) and PCa(C). 400×

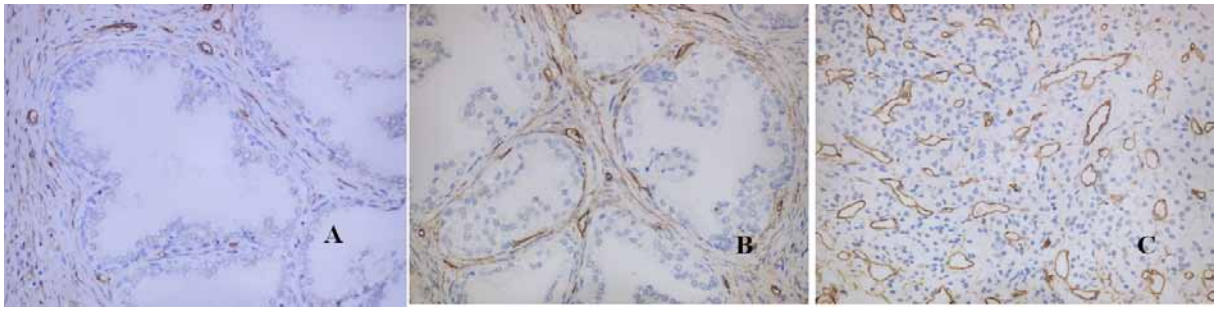

Figure 4. In CEACAM5 expression areas, LVD shows significant difference in BPH(A), PIN(B) and PCa(C). 400×

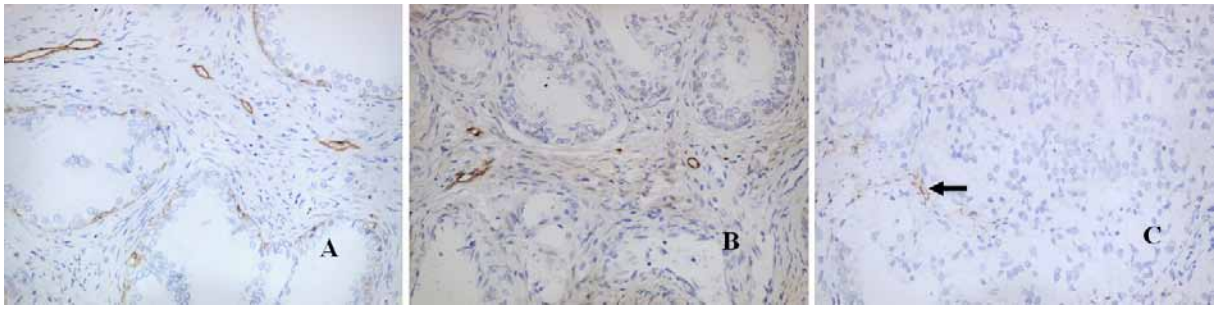

Table 1. çCEACAM5 expression patterns and its relationship with angiogenesis and lymphangiogenesis in prostatic non-neoplastic and neoplastic lesions

Variables	No. of patients	MVD	LVD	Staining patterns of CEACAM5			PSA(toal)
		(Mean±SD)		M	C	Negative	
Benign hyperplasia[1,2]	10	41.3±4.398	5.16±0.842	0	0	10	6.13±3.220
PIN[1,3]	30	51.13±9.077	4.68±1.170	29	0	1	7.73±4.012
High grade	11	55.12±12.292	5.291±1.126	11	0	0	7.19±3.009
Low grade	19	49.72±8.894	4.211±1.399	18	0	1	7.96±4.719
PCa[2,3]	37	72.08±9.309[5]	1.33±0.859[7]	9	24	4	16.90±9.397
Gleason classification							
Lower grading (<7)[4]							
Well differentiated							
(2-4 score)	10	43.8±6.460[6]	1.58±0.721	8	2	0	15.41±4.205
Moderately differentiated							
(5-6 score)	7	70.8±11.353	1.4±0.966	0	7	0	15.95±8.004
Higher grading(>=7)[4]							
Poorly differenciated							
(7-10 score)	20	87.85±18.339	1.18±1.072	1	15	4	17.20±6.715
Stage[8]							
I + II	12	58.94±3.18	2.18±0.372	2	9	1	13.38±7.036
III+IV	25	67.71±6.62	1.59±1.330	7	15	3	22.54±5.026
Lymph node involvement[9]							
Yes	14	38.61±5.81	0.73±0.085	3	8	3	17.92±8.859
No	23	30.44±4.82	1.52±0.194	6	16	1	16.15±5.599
Age (Adenocarcinoma)							
≤ 59 Y	14	38.8±4.407	2.16±1.021	3	9	2	14.41±6.600
≥ 60 Y	23	42.6±2.713	1.72±0.485	6	15	2	19.09±5.101

[1] Compared with BPH and PIN, there is significant difference in CEACAM5 expression staining with membranous patterns (P=0.000<0.05).
[2] Compared with BPH and PCa, there is significant difference in difference in CEACAM5 expression (P=0.000<0.05).
[3] Compared with PIN and PCa, the CEACAM5 expression patterns with either membranous patterns and cytoplasmic patterns is significantly different(P=0.000<0.05).
[4] Compared with higher Gleason grading(Gleason score >=7, poorly differentiated) with lower grading (Gleason score <7, including moderately and well differentiated, CEACAM5 expression pattern is significantly different with membranous expression (P=0.000<0.05).
[5] Compared with PIN and BPH, the MVD showed significant difference (P=0.043<0.05).
[6] Compared with poorly and moderately differentiated carcinoma, the MVD showed significant difference (P=0.028<0.05).
[7] Compared with PIN and BPH, the LVD showed significant difference (P=0.014<0.05).
[8] LVD showed significant difference in clinical stage(P=0.013<0.05).
[9] LVD showed significant difference in lymph node metastasis(P=0.029<0.05).

LVD counting based on D2-40 staining shows that neoplastic lesions have more lymphangiogenesis than non-neoplastic lesion

Similar to MVD counting, lymphatic vessel density (LVD) evaluation with D2-40 labelling showed significant difference between BPH, PIN and PCa (Figure 4 A, B,C). LVD labeled with D2-40 in PCa was significantly lower than that in BPH and PIN (p=0.018, 1.33±0.859 vs. 4.68±1.170; p=0.009, 1.33±0.859 vs. 5.16±0.842). Moreover, LVD in PCa showed significant difference between the clinical stage I,II and the stage III and IV (p=0.033, 2.18±0.372 vs. 1.59±1.330). Similarly, LVD also was significantly different in PCa whether with lymph node metastasis or not (p=0.027, 0.73±0.085 vs. 1.52±0.194). However, it had no significant difference in age and cancer histodifferentiation (p=0.070, p=0.061).

The relationship of CEACAM5 expression and MVD/LVD

The Pearson correlation coefficient test has documented that in PCa, the CEACAM5 expression patterns had positive linear correlation with MVD (r=0.511, p=0.008), but had negative linear correlation with LVD (r=-0.702, p=0.016).

4. Discussion

Nowadays many studies are researching the new tumor markers that was correlated to the diagnosis and prognosis of the prostatic carcinoma [11-12]. CEACAM5 is a tumor-associated antigen that plays an important regulatory role in cell adhesion and in tumor cell chemosensitivity [13-16]. CEACAM5 has been revealed to be involved in both homophilic and heterophilic interactions and is showed to be an intercellular adhesion molecule involved in cancer invasion and metastasis [17-21]. These reactions are completely suppressed by the Fab' fragment of an anti-CEACAM5 antibody [22].

Our study showed that CEACAM5 had different expression patterns in prostatic non-neoplastic and neoplastic lesions, and the different expression patterns should be involved in the tumor progression. There is no CEACAM5 positive expression in BPH tissue, but in almost all neoplastic lesions, whether in PIN or PCa, CEACAM5 was expressed with different patterns and intensity. CEACAM5 expression was detected in PIN with membranous pattern and with weak intensity. In well differentiated carcinoma, CEACAM5 was mainly expressed with membranous pattern, whereas, in intermediately and poorly differentiated carcinoma, CEACAM5 was mainly expressed with cytoplasmic or cytoplasmic mixed with little membranous pattern. This might suggest that CEACAM5 positive expression and transformation of expression patterns should promote tumor progression.

Briganti A's study showed the clinical stage, primary biopsy Gleason grade, and percentage of positive cores were independent predictors of lymph node invasion in patients with PCa [23]. In our study, the CEACAM5 in PCa was significantly associated with the score of the Gleason grading, the pathological stage, and the MVD/LVD. Many researches have proved that MVD remained significant in predicting recurrence and MVD was an important predictor of metastatic disease and an independent predictor of tumor [24-26]. Just like MVD, many studies have demonstrated that LVD is an independent prognostic factor in many malignant tumors too [27-28]. But the relationship between LVD and the tumor metastasis remains controversial.

CEACAM5 has complicated roles with the genesis of canalis haemalis. In our study, we found the expression of CEACAM5 in prostate carcinoma correlated with MVD and LVD, which demonstrated that the expression of CEACAM5 might promote the angiogenesis but inhibit the lymphangiogenesis. CEACAM5 might be used to quantify MVD and LVD in prostate cancer and stratify patients at greatest risk of recurrence after radical prostatectomy. Interestingly, we found that along with BPH, PIN and PCa, CEACAM5 expression upregulated with MVD increasing, on the contrary, with LVD oppositely decreasing. Moreover, in PCa poorly differentiated carcinoma showed more MVD, but less LVD. Advanced carcinoma and carcinoma with lymph node metastasis LVD showed less LVD. This might be attributed to, in contrast to the stimulated angiogenesis of blood vessels in PCa, the destruction of lymphatic vessels rather than lymphangiogenesis [29-23]. The result might indicate that CEACAM5 promote angiogenesis, but inhibit lymphangiogenesis. Based upon our results, CEACAM5 should significantly be associated with angiogenesis and lymphangiogenesis in different expression patterns. However, the mechanism that allowed of CEACAM5 to promote or inhibit angiogenesis and lymphangiogenesis in prostate carcinoma remains unknown. We do not know whether the angiogenesis or lymphangiogenesis of prostate carcinoma would probably be changed if the CEACAM5 expression was inhibited. The mechanism should be further investigated. Conclusively, lessening the expression of CEACAM5 might have opportunities to attenuate the tumor invasion and migration and then might alleviate the progression of prostate cancer.

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