CD24 expression in benign prostate hyperplasia low and high grade PIN and prostate adenocarcinoma

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Özet

Benign prostat hiperplazisi düşük ve yüksek dereceli PIN ve prostat adenokarsinomunda CD24 ekspresyonu

Amaç: CD24 aktive endotel hücreleri ve trombositler üzerinde eksprese edilen bir adezyon reseptörü olan P-selektin'in alternatif ligandı olarak fonksiyon gören son zamanlarda tanımlanan yeni bir prognostik belirleyici gendir. CD24'ün çeşitli solid tümörlerin metastatik tümör gelişiminde rol oynadığı düşünülmektedir. Çalışmamızda prostat adenokarsinomlarında, düşük ve yüksek dereceli prostat intraepitelyal neoplazilerde (PIN) ve benign prostat hiperplazisinde (BPH) CD24 ekspresyonunun histopatolojik parametreler ve serum PSA seviyesi ile ilişkisini değerlendirmeyi amaçladık. Gereç ve Yöntem: Yetmişaltı prostat adenokarsinomu, 16 düşük dereceli PIN, 16 yüksek dereceli PIN ve 14 BPH vakasında immunohistokimyasal olarak CD24 ekspresyonunu inceleyerek bulgularımızın histopatolojik parametreler ve serum PSA seviyesi ile ilişkisini değerlendirdik. Bulgular: Prostat adenokarsinomu ve PIN materyallerinde CD24 ekspesyonu saptanırken benign prostat dokularında ekspresyon izlenmedi. 76 prostat adenokarsinomu olgusunun 48'inde (%63.2) CD24 immunoreaktivitesi saptandı. CD24 ekspresyonu prostat adenokarsinomu ve yüksek dereceli PIN gruplarında BPH grubu ie karşılaştırıldığında anlamlı şekilde yüksek bulundu (p=0,000, p=0,040). Prostat adenokarsinomunda sitoplazmik CD24 ekspresyonu Gleason skoru (p=0.000, r= 0.465) ve PSA seviyesi (p=0.022, r=0.263) ile anlamlı şekilde pozitif korele bulundu. Sonuçlar: Bulgularımız prostat adenokarsinomlarında pozitif CD24 ekspresyonunun yüksek Gleason skoru ve yüksek PSA seviyesi ile birlikteliğini gösterdi. CD24'ün prostat kanseri için önemli bir moleküler belirleyici olabileceği kanısındayız.

Anahtar kelimeler: Prostat adenokarsinomu, PIN, CD24, immunohistokimya

Abstract

Purpose: CD24 recently identified novel prognostic marker gene that functions as an alternative ligand of P-selectin, an adhesion receptor expressed on activated endothelial cells and platelets. It has been implicated in metastatic tumor progression of various solid tumors. We aimed to investigate CD24 expression in prostate adenocarcinomas, low and high grade prostatic intraepithelial neoplasias (PINs) and benign prostate hyperplasias (BPHs), and to evaluate its relationship with histopathological parameters, and serum PSA level. Material and Methods: We examined immunohistochemically the expression of CD24 protein in 76 prostate adenocarcinomas, 16 low grade PIN, 16 high grade PIN, and 14 BPH and correlated our findings with histopathological parameters, and serum PSA level. Results: While we detected CD24 expression in prostat adenocarcinomas and PIN specimens, there was no expression in benign prostate tissues. CD24 immunoreactivity was detected in 48 (63.2%) out of 76 prostate adenocarcinomas. Expression of CD24 was significantly higher in prostate adenocarcinoma and high grade PIN compared with BPH (p=0,000, p=0,040). In prostate adenocarcinomas cytoplasmic CD24 expression was significantly positively correlated with Gleason score (p=0.000, r= 0.465) and PSA level (p=0.022, r=0.263). Conclusions: Our findings demonstrated that positive CD24 expression occurs in prostate adenocarcinomas with high Gleason score, and high PSA level. We suggest that CD24 might constitute an important moleculer marker for prostate cancer

Key words: Prostate adenocarcinoma, PIN, CD24, immunohistochemistry

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Müracaat tarihi: 05.12.2009
Kabul tarihi: 14.06.2010

This article was presented as a poster in 21st European Congress of Pathology which was organized in Istanbul, Turkey at Istanbul Convention & Exhibition Center on September 8-13, 2007.

Introduction

Adenocarcinoma of the prostate is one of the most frequently diagnosed cancers of men in the Western hemisphere and is second only to lung cancer for male cancer mortality (1). Established conventional prognostic markers for prostate cancer are tumor stage, histological grade, patient age, and residual tumor (2). Although serum PSA is widely recognized as the best prostate tumor marker currently available, levels between 4 and 10 ng/ml are seen in men with obstructive or inflammatory uropathies, lowering the specificity of PSA as a cancer marker Additionally, molecular markers are being sought to improve accuracy of outcome prediction (2).

CD24 is a small, heavyl glycosylated protein core that consists of 27 amino acids and is attachted to cell membrane by a phosphoatidylinositol anchor (3,4). Functionally, it is considered to play a critical role in the metastasis of tumor cells through P-selectin (5). It was first identified as the marker of B cells (6). Although CD24 is not present in adult human tissues it is expressed in many human carcinomas (6). CD24 expression was noted in various hematologic malignancies and solid tumors such as lung cancer, breast cancers, prostate cancers and ovary cancers (4,7-10). It functions as an alternative ligand of P-selectin, an adhesion receptor expressed on activated endothelial cells and platelets, and could thus enhance the metastatic potential of CD24 expressing tumor cells (11). The major ligand of P-selectin is a cell surface mucin P-selectin glycoprotein ligand-1 (PSGL-1), and certain PSGL-1-negative tumor cell lines can bind P-selectin through surface mucin CD24 (12). It is conceivable that CD24-expressing tumor cells can spread more easily due to their capacity to form thrombi with activated platelets or to adhere to endothelia in the bloodstream, which has been shown for CD24 expressing breast cancer cells (3).

In this study we aimed to examine CD24 expression in prostate adenocarcinomas, low and high grade PIN's and BPH's, to investigate the possible role of this protein during malignant transformation and to evaluate its relationship with histopathological parameters, and serum PSA level.

Materials and Methods

Using immunohistochemistry, the expression of CD24 was investigated in prostate tissue samples comprising of BPH (n =14), low grade PIN (n = 16), high grade PIN (n=16), and prostate adenocarcinoma (n = 76). All tissue samples were selected from archival material of the Department of Pathology, Suleyman Demirel University School of Medicine, including radical prostatectomy (n=42) and transurethral resection (n=80) specimens. Glass slides were reviewed for histological classification according to the World Health Organization (WHO) (13). Low grade PIN is identified by the presence of architecturally benign prostatic acini or ducts lined by cytologically atypical cells with slight enlargement of nuclei and stratification, and no prominent nucleoli. High grade PIN is identified by the presence of nuclear atypia (enlarged nuclei, hyperchromatism, overlapping nuclei, prominent nucleoli) with architectural abnormalities.

Immunohistochemistry

Immunohistochemical analysis for CD24 was performed on formalin fixed, parafin-embedded archival tissue using the streptavidin-biotin-peroxidase technique. For all cases, 4µm histologic section was deparaffinized in xylene and rehydrated in descending dilution of ethanol. For the antigen retrieval, slides were treated by microwave heating in citrate buffer (pH 6.0) for 20 min. Antigen retrieval was achieved by PT module at 90°C for 20 min. (Citrat buffer pH=6.0). The immunostaining was performed by Labvision autostainer 360 at immunohistochemical mode. Slides were incubated with monoclonal mouse antiCD24 antibody (1:100, Clone 24C02, Neomarkers). Streptavidin-biotin-peroxidase kit wasused and the reaction product was detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's hematoxylin, and mounted with mounting medium. The positive control for CD24 was inflamed granulation tissue. The presence of cytoplasmic staining of CD24 were evaluated separately and scored semiquantitatively as CD24 negative, weak, moderate, or strongly positive. Negative cases had to show definitely no CD24 immunoreactivity in any part of the tumor. Weak staining was defined by positive immunoreactivity in up to 10% of the tumor. Moderate

staining was defined by positive immunoreactivity in 11-50% of the tumor, and strong staining was defined by positive immunoreactivity in >50% of the tumor.

Statistical Analysis

For statistical evaluation the SPSS software version 11 was used. The non-parametric Mann-Whitney test was used to evaluate the expression of CD24 among the groups. The Pearson Chi-Square and Spearman's rank correlation coefficient tests were used to assess the relationship between CD24 expression and histopathological parameters and pre-operative PSA level. *P* values < 0.05 were considered significant.

Results

CD24 expression pattern in BPH

The age of BPH patients ranged from 56-76, with a mean of 65 years. No expression was found in BPH spesimens except few atrophic glands showed apical membranous CD24 reactivity. Secretion material in the gland lumen was also positive (Figure 1).

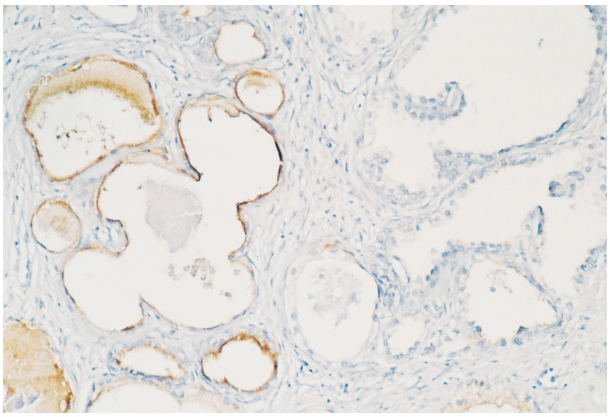

Figure 1. Representative immunostaining for CD24 in BPH. Few atrophic glands showed apical CD24 reactivity. Secretion material in the gland lumen was also positive, proliferative glands showed no immunoreactivity (DABX200)

CD24 expression pattern in PIN

The age of low PIN and high PIN patients ranged from 52-89, with a mean of 71 years and, 54-74, with a mean of 63.5 years respectively. Cytoplasmic CD24 expression was detected in 1 (6.3%) low PIN case, and 7 (43.8%) high PIN cases. The level of immunostaining in low PIN group was +1 cytoplasmic CD24 expression. The level of immunostaining in high PIN group was as follows; 1 (6.3%) case showed +1 and, 6 (37.5%) cases showed 2+ cytoplasmic CD24 expression .

CD24 expression pattern in prostat adenocarcinoma

The age of prostate adenocarcinoma patients ranged from 54-90, with a mean of 69.5 years. Among 76 prostate adenocarcinomas studied, 20 (26.3%) were diagnosed as well differentiated; Gleason score 6, 24 (31.6%) moderately differentiated; Gleason score 7 and 32 (42.1%) poorly differentiated; Gleason score 8,9,10. Fourty eight of 76 (63.2%) prostate adenocarcinoma cases showed CD24 immunoreactivity. The level of immunostaining was as follows; 6 (7.9%), 18 (23.7%), 24 (31.6%) cases showed +1, +2, and +3 cytoplasmic CD24 expression. CD24 expressions in high grade PIN (Figure 2), low grade and high grade prostate adenocarcinoma tissues (Figure 3a, 3b respectively) were shown in Figures 2,3.

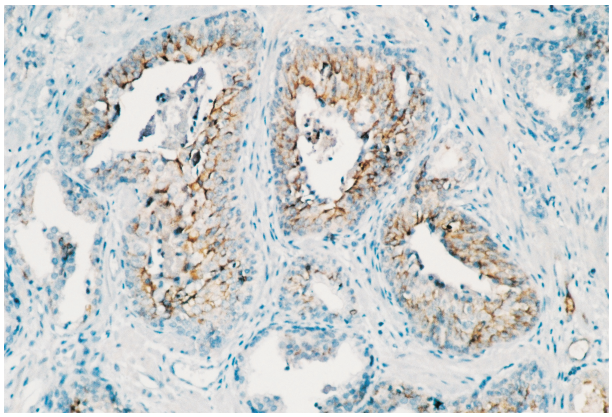

Figure 2. CD24 expression in high grade PIN. Displastic epithelium showed cytoplasmic immunoreactivity (DAB X200)

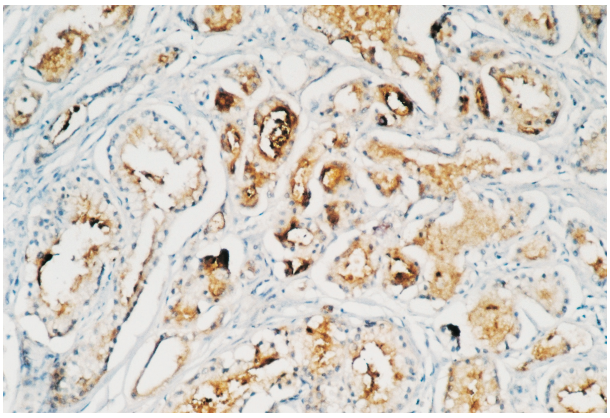

Figure 3a

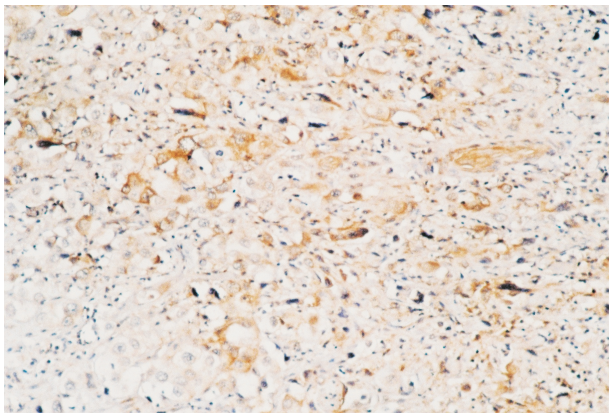

Figure 3b

Figure 3. CD24 expression in low grade (a) and high grade (b) prostate adenocarcinoma. (DAB X200)

Expression of CD24 was significantly higher in prostate adenocarcinoma and high grade PIN compared with BPH (p=0,000, p=0,040). Such a statistically significant relation was found between prostate adenocarcinoma and high grade PIN (p=0.005).

The relation between CD24 expressions in BPH, PIN and prostate adenocarcinoma were shown in Table.

Table : CD24 expressions in BPH, PIN and Prostat Adenocarcinoma

	CD24 expression			p
	1 (+)	2(+)	3(+)	
BPH	0	0	0	0.285*
Low PIN	1(6.25%)	0	0	0.040**
High PIN	1(6.25%)	6(37.5%)	0	0.005***
Prostat Adenocarcinoma	6 (7.9%)	18 (23.7%)	24 (31.6%)	0.000****

* BPH vs. low grade PIN
** BPH vs. High grade PIN
*** High grade PIN vs. Prostate adenocarcinoma
****Prostate adenocarcinoma vs. BPH

In the prostate adenocarcinoma group 4 (20%) of 20 Gleason score 6 cases showed cytoplasmic CD24 expression. Among 24 Gleason score 7 cases we observed 16 (66.7%) cases showed cytoplasmic expression. Also 28 (87.5%) out of 32 Gleason score 8,9,10 cases showed cytoplasmic CD24 expression.. Cytoplasmic CD24 expression and the level of immunopositivity of the protein was positively associated with Gleason scores (p=0,000, r=0.465 and p=0,000, r=0.473 respectively).

The pre-operative PSA level of prostate adenocarcinoma patients ranged from 0.03-52.12, with a mean of 7.45 in well differentiated group, 4,26-52 with a mean of 11,6 in moderately differentiated group, 5,5-112 with a mean of 24,06 in poorly differentiated group We also detected that cytoplasmic CD24 expression and the level of immunopositivity of the protein was positively associated with pre-operative PSA level (p=0,022, r=0.263, p=0,015, r=0.278).

Discussion

In recent years, new molecular markers for many tumors, including prostate cancer, have been searched. CD24 is a molecule that has recently attracted considerable attention in tumor biology. In vitro data demonstrate that the small mucin-like adhesion molecule CD24 increases tumor cell proliferation and supports cell motility as well as spreading, thus promoting tumor invasion and metastasis in experimental animals (14). On the contrary, loss of CD24 function in cell lines derived from common tumor types is associated with significant change in cell morphology and the actin cytoskeleton, decreases rates of cell proliferation and induction of apoptosis (15). In this immunohistochemistry-based study we analyzed the expression of CD24 in benign and malignant prostate tissue and assess their possible association with different clinical parameters. Also this is the first study comparing CD24 expression in prostate adenocarcinomas, PINs, and BPHs and searching the correlation with Gleason score and serum PSA levels.

Recently, in the studies of Welsh et al. (16) and Singh et al. (17) CD24 is significantly upregulated in prostate adenocarcinoma with rates of 33% and 46%, respectively. Kristiansen et al. (9) immunostained 102 adenocarcinomas of the prostate and found CD24 was heterogeneously expressed, with a rate of 48% positive cases. In their study CD24 positivity was significantly related to younger patient age, higher pT stages and a higher 3-year PSA relapse rate of 47.7% compared with 11.7% for CD24- negative tumours (9).

In this study we compared the CD24 expression of benign and malignant prostate tissue with the aim of eveluating the role of CD24 in tumorigenesis, progression and malignant transformation of prostate adenocarcinoma. Also we examined the differences in CD24 expression based on the clinicopathologic parameters (Gleason score, PSA level) of patients with prostate adenocarcinoma We found CD24 expression rate was 63.2% in prostate adenocarcinoma cases. Expression of CD24 was significantly higher

in prostate adenocarcinoma and high grade PIN compared with BPH cases. Also the difference between high grade PIN and prostate adenocarcinoma was significant. We also observed that cytoplasmic CD24 expression and the level of immunopositivity of the protein was positively associated with high Gleason scores (high grade tumors) and high PSA levels in prostate adenocarcinomas. These findings may confirm that CD24 expression may play a role in prostate tumorigenesis, and may contribute to the formation of malignant tumors.

In immunohistochemistry- based studies, some authors have reported that the staining quality of CD24 expression might reflect a pathologic condition. The intracytoplamic CD24 expression is significantly correlated with lymph node status and the patient's prognosis (18). Moreover, in several tumor entities higher rates of CD24 expression or CD24 positivity were significantly associated with shorter patient survival (5,19). In the study of Kristiansen et al. (9) CD24 expression was strongly linked to significantly earlier disease progression, which was especially pronounced in organ confined, or moderately differentiated primary prostate tumors. They also suggest that CD24 is an important prognostic tissue marker for prostate cancer which could help to define patients of low or high risk of recurrence (9). In conclusion we suggest that CD24 expression in prostate adenocarcinoma correlates with high Gleason scores (high grade tumors) and high PSA levels associated with aggressive tumor behaviour.

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