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Original Article *Özgün Araştırma*

CD44 EXPRESSION IN PROSTATIC ADENOCARCINOMA: CORRELATION WITH GLEASON GRADES

Prostat Adenokarsinomunda CD44 Ekspresyonu: Gleason Derecesi ile Korelasyon

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ABSTRACT

Objective: CD44, a cell-surface adhesion protein, is a marker of stem cells and is involved in the structural maintenance of prostate gland basal cells. It plays an important role in prostate carcinogenesis; however, research results till date have been inconsistent. The current study was conducted to evaluate the expression level of CD44 among carcinomas with different Gleason growth patterns and hyperplastic glands.

Material and Methods: Eighty archival tissue samples from patients with either prostate carcinoma of differing Gleason patterns or benign prostate hyperplasia from the pathology archives of the Kırıkkale University, Faculty of Medicine, from 2011 to 2018 were included. Immunoreaction to CD44 antibodies was evaluated by calculating positively stained cell percentage and staining intensity. Mean values and significance were determined using the Kruskal–Wallis test. $p<0.05$ was considered significant.

Results: CD44 staining was more diffuse and intense in most of benign hyperplastic tissues compared to carcinoma cases (p values ranged from <0.01 to 0.005). Expression level gradually decreased with increasing severity of histologic pattern. Pattern 3 tumor showed higher percentage of positively stained cells than pattern 4 ($p<0.01$) There was no positivity with CD44 in grade 5 tumours. Also, in pattern 4 only few cells reacted with CD44. There was no statistically significant difference in the staining score between pattern 4 and 5 ($p=0.278$).

Conclusion: We conclude that once cancerous properties have been established, cells tend to lose the ability of CD44 expression. CD 44 expression in low grade carcinomas suggest that CD 44 maintains the tumor in a differentiated, gland forming state so it may not act as a cancer stem cell marker in prostate carcinogenesis.

Keywords: *Prostate carcinogenesis, stem cells, prostatic hyperplasia, keratins*

ÖZ

Amaç: Bir yüzey adezyon proteini olan CD44, kök hücre belirteci olup prostat glandlarının bazal hücrelerinin yapısal bütünlüğünü korumada görev alır. Prostat karsinogenezisinde de önemli bir rolü olduğu düşünülse de şu ana dek yapılan çalışmalarda elde edilen sonuçlar belirsizdir. Bu çalışma, farklı Gleason büyüme patternlerine sahip tümörlerde ve hiperplastik glandlarda CD44 ekspresyonunu değerlendirmek amacı ile yapılmıştır.

Gereç ve Yöntemler: Kırıkkale Üniversitesi, Tıp Fakültesi patoloji arşivine ait, 2011 ile 2018 yılları arasında tanı almış, farklı Gleason derecelerine sahip prostat karsinomlu ve benign prostat hiperplazili 80 olgu, çalışmaya dahil edilmiştir. CD44 ile olan immünreaksiyon, pozitif boyanan hücrelerin yüzdesi ile boyanma yoğunluğunun çarpımı olarak değerlendirilmiştir. Ortalama değerler ve anlamlılık Kruskal-Wallis testi kullanılarak belirlenmiş; $p<0.05$ anlamlı kabul edilmiştir.

Bulgular: Benign hiperplazi olgularında CD44 boyanması, karsinom olgularına göre daha diffüz ve yoğun idi (p değerleri <0.01-0.005). Ekspresyon seviyesi, histolojik derece arttıkça kademeli olarak düşüş gösterdi. Pattern 3 tümörler, pattern 4 tümörlerden daha yüksek oranda pozitif hücre sayısına sahipti ($p<0.01$). Pattern 5 tümörlerde CD44 ile boyanma olmadı. Pattern 4 tümörlerde ise az sayıda hücrede pozitiflik görüldü. Pattern 4 ve 5 arasında, CD44 boyanması bakımından anlamlı farklılık gözlenmedi (($p=0.278$).

Sonuç: Bu çalışmada, bir kez kanseröz özellikler kazanıldıktan sonra hücrelerin CD44 ekspresyonu kabiliyetini yitirme eğiliminde olduğu sonucuna vardık. Düşük dereceli kanserlerdeki CD44 ekspresyonunun, tümör dokusunu diferansiye ve gland oluşturabilen durumda kalmasını sağladığını, bu nedenle CD44'ün prostat karsinogenezisinde bir tümör kök hücre marker'ı gibi davranmayabileceğini düşündük.

Anahtar Kelimeler*: Prostat karsinogenezi, kök hücreler, prostatik hiperplazi, keratinler*

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INTRODUCTION

Prostate adenocarcinoma (PCa) is the second most common cause of cancer and cancer-related deaths among men worldwide (1). Despite early detection in the localized stages by screening for prostate-specific antigen in the serum, some patients (approximately 20–30%) progress to an androgen receptor (AR)-independent or castration-resistant phenotype (2).

One of the mainstays for predicting the malignant potential of PCa is Gleason grading, which is based on the architectural patterns observed in hematoxylin and eosin sections. These models differ according to the degree of glandular lumen formation, which can range from individual and different glands to cribriform growths and solid forms.

There is likely a biological continuum from a polarized glandular state to a solid state, and alterations in the adhesion properties of neoplastic cells play a pivotal role in this multi-step transition. Among these adhesion molecules, CD44, a transmembrane glycoprotein that serves as the main receptor of glycosaminoglycan hyaluronan, has been demonstrated to modulate migration/invasion during cancer progression (3).

CD44, has also been considered to be a putative cancer stem cell marker in various tumors including PCa. Several studies have suggested that the mortality and heterogeneity of PCa are related to the expansion of cancer stem cells (CSC). CSC are rare, immortal cells within a tumor that can self-renew by dividing and can give rise to many cell types that constitute the tumor. CD44 in the benign prostate tumor is a marker of basal cells, the stem cell compartment of the epithelium (4,5).

In this study, we sought to investigate CD44 expression to evaluate its association with Gleason patterns and to assess the role of CD44 as a cancer stem cell marker in prostate carcinogenesis.

MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded tissues from 60 patients with definitive pathological diagnoses of PCas of Gleason pattern 3 (n=20), 4 (n=20), and 5 (n=20) and from 20 patients with benign prostate hyperplasia were retrieved from the pathology archives of the Kırıkkale University, Faculty of Medicine, from 2011 to 2018. Each individual in this study provided written informed consent prior to the surgery for pathological examination and further investigation. This study was approved by the local ethics committee (*Kırıkkale University Non-Interventional Research Ethics Committee, 24.07.2019-2019/11*).

Immunoreactivity with antibodies against CD44 (predilute, clone SP37, Ventana, Roche) was measured using a semi-quantitative scoring method based on both the proportion of positively stained tumor cells and their staining intensity. Two pathologists who were blinded to the pathologic data of the patients at the time of the analysis evaluated the staining. If the observers reported different results, they reviewed the slides using a microscope until a consensus was reached. Staining was evaluated for each tissue sample by calculating the total immunoreactive score (IRS) as the product of the proportion score and intensity scores according to previously reported criteria (6). Briefly, this proportion score reflected the estimated fraction of positively stained tumor cells (0%, none; 1, 1%–10%; 2, 11%–50%; 3, 51%–80%; and 4, 81%–100%). The intensity score represented the estimated staining intensity (0, no staining; 1, weak; 2, moderate; and 3, strong). Thus, total IRS ranged from 0 to 12.

All statistical analyses were performed using the SPSS 15.0® statistical software (SPSS Chicago, IL, USA). The Kruskal–Wallis test followed by Conover–Inman correction test was used for individual statistical comparisons.

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RESULTS

This study evaluated the expression levels of CD44 among 60 patients with PC and 20 cases of benign prostate hyperplasia. The carcinoma cases were divided into three categories that consisted of 20 patients in each group according to their Gleason growth patterns (patterns 3–5). The mean age of our patients was 65 years (range 49-89).

CD44 staining was more diffuse and intense in most benign hyperplastic tissues than in carcinoma tissues. Figure 1 shows a representative slide of diffuse and intense staining with anti-CD44 antibody in a specimen of benign hyperplasia. Staining was predominantly observed at the basolateral locations in basal cells. The percentage of cases exhibiting an overall strong positivity was the highest in this group.

Expression level gradually decreased with increasing histological pattern. Pattern 3 tumors exhibited higher percentages of positively stained cells than pattern 4 tumors; there was no CD44 staining in pattern 5 tumors. In pattern 4 tumors, only few cells were stained with CD44.

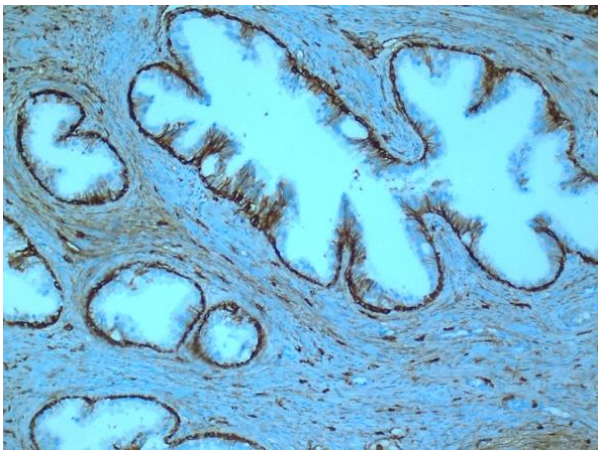

Figure 1: Immunostaining with CD44 is evident as a continuous layer in the basal compartment of the hyperplastic glands (Magnification: ×100).

There was heterogeneity in CD44 expression in specimens of Gleason pattern 3 tumors and reduced expression was noted in specimens of invasive glands (Figure 2). Most pattern 4 tumors did not exhibit reactivity to CD44, although weak staining was observed in some fused glands and cribriform structures (Figure 3). None of the pattern 5 tumors, which comprised solid nests or islands, expressed CD44 (Figure 4).

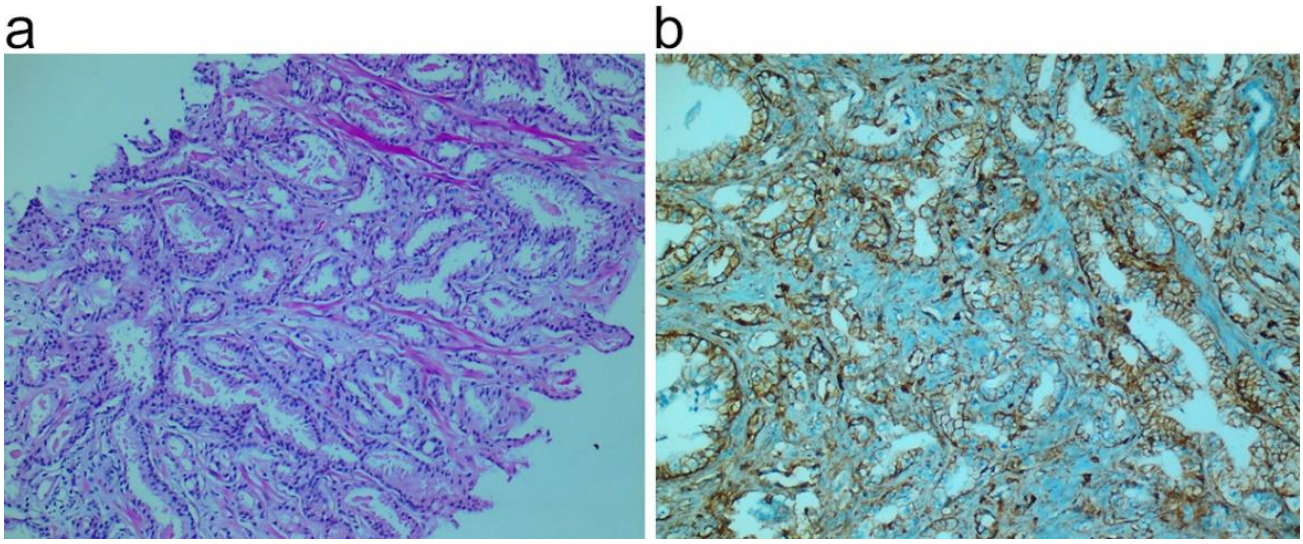

Figure 2: a. A grade 3 tumor consists of variably sized neoplastic glands with flocculated secretions within the lumen (HE staining, magnification: ×100).
b: Moderate and weak basolateral CD44 staining in grade 3 tumors (Magnification: ×100).

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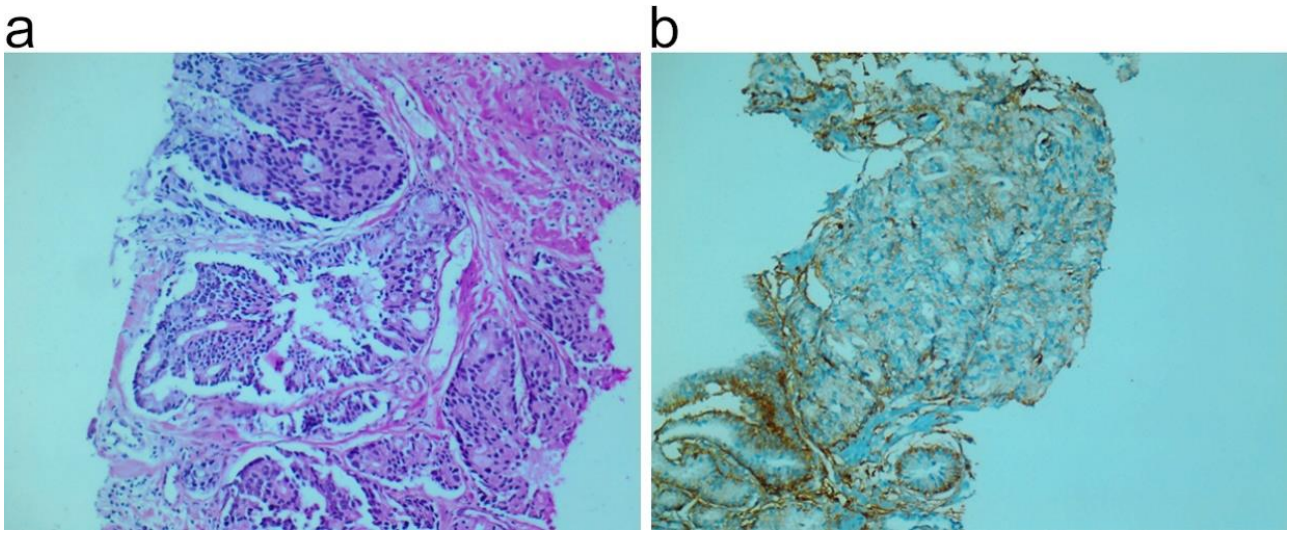

Figure 3: a. A grade 4 tumor with cribriform structures and fused glands (HE staining, magnification: ×100).
b: Mostly weak CD44 staining is observed in grade 4 tumors (Magnification: ×100)

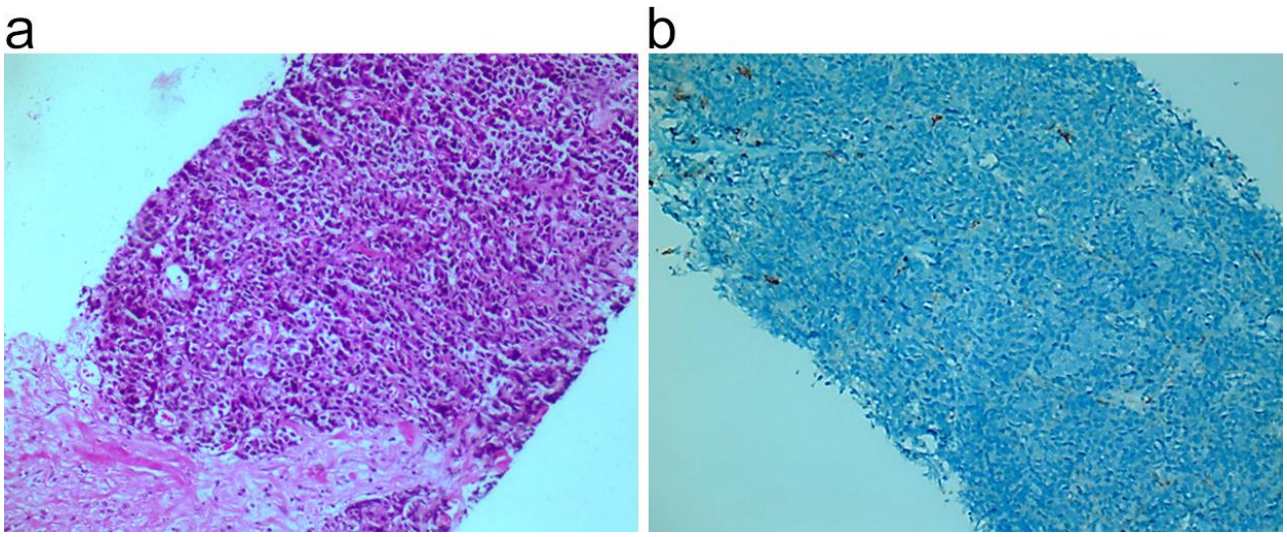

Figure 4: a. A grade 5 tumor exhibiting diffuse growth with nesting or islands (HE staining, magnification: ×100).
b: No CD44 staining is observed in grade 5 tumors (Magnification: ×100).

IRS scores were significantly lower for pattern 3, 4, and 5 tumors ($p=0.005$, $p<0.001$, and $p<0.001$, respectively) than those for benign hyperplasia. In addition, pattern 4 and 5 tumors had lower staining scores than pattern 3 tumors ($p<0.001$ and $p<0.001$, respectively). There was no statistically significant difference in the staining score of pattern 4 and 5 tumors ($p=0.278$; Figure 5 and Table 1).

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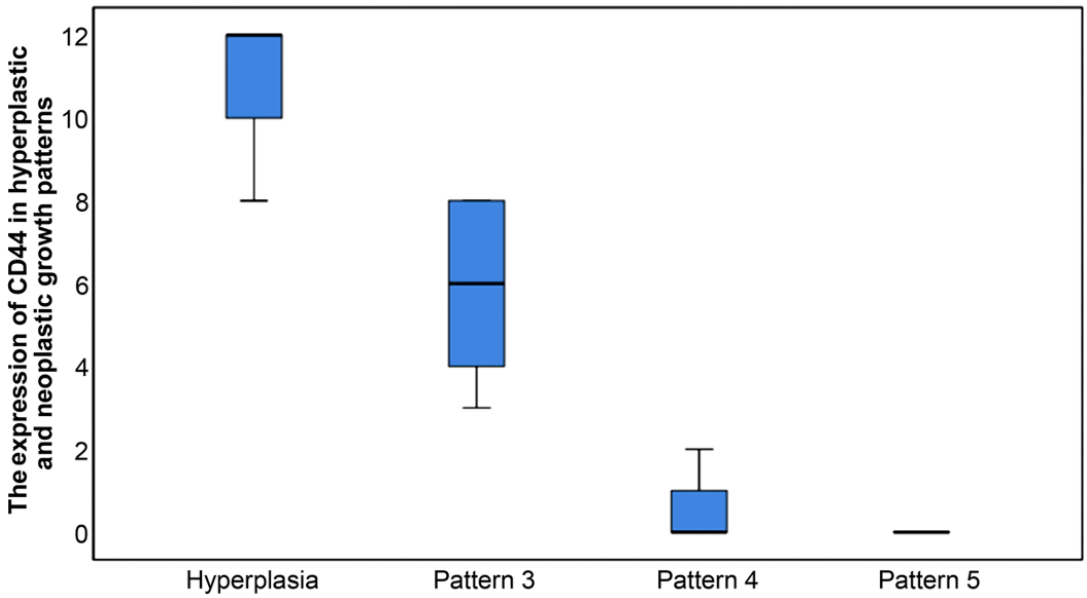

Figure 5: Box plots of immunohistochemical staining scores for CD44 staining in different specimen categories. Horizontal lines inside the boxes represent the median value; box limits indicate the 25th and 75th percentiles; and whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles.

Table 1: Multiple comparisons of CD44 immunohistochemical staining scores between groups

	Staining score
Hyperplasia (n=20)	12 (9-12)
Pattern 3 (n=20)	6 (4-8)
p-value	0.005
Hyperplasia (n=20)	12 (9-12)
Pattern 4 (n=20)	0 (0-1)
p-value	<0.001
Hyperplasia (n=20)	12 (9-12)
Pattern 5 (n=20)	0 (0-0)
p-value	<0.001
Pattern 3 (n=20)	6 (4-8)
Pattern 4 (n=20)	0 (0-1)
p-value	<0.001
Pattern 3 (n=20)	6 (4-8)
Pattern 5 (n=20)	0 (0-0)
p-value	<0.001
Pattern 4 (n=20)	0 (0-1)
Pattern 5 (n=20)	0 (0-0)
p-value	0.278

DISCUSSION

Approximately 150 years ago, the concept that cancer arises from a rare population of cells with stem cell properties was proposed. According to this hypothesis, tumor growth is driven by a rare population of select cells, designated as CSCs (7). Many of the markers, including CD44, used to define CSC are being studied to appropriately identify this sub-population in hematological and solid tumors. Several studies have demonstrated that CD44 overexpression is associated with poor prognosis in colorectal, lung, breast, ovarian, and gastric cancers (8). However, there have been limited studies till date that correlate Gleason grade and CD44 expression. We observed that CD44 expression was significantly reduced in higher histological grade tumors, whereas the expression was intense in the basal compartments of benign glands and pattern 3 tumors.

In this study, we focused on the histopathological growth patterns that principally determine tumor aggressiveness. Although prostate cancer is itself diagnosed by the loss of basal cell markers, grade 3

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glands still contain a CD44 (+) stem-like cell sub-population that may drive cellular differentiation and the acquisition of a luminal phenotype, which may be expected in benign glands. These self-renewing cells may be residual or transformed stem cells, and we believe that their quantity is sufficient to establish an acinar structure in low-grade tumors.

In contrast to rapidly dividing epithelial tissues, such as in the epidermis and gut, there is little information available regarding stem cells of the prostate. It has been proposed that basally located androgen-independent progenitor cells give rise to basal or luminal cells by altering their keratin expression profiles (9,10). These cells lose simple keratin 5, 8, 18, and 19 expressions, resulting in only keratins 5 and 14 being expressed. If luminal cells develop, they lose basal cell keratins 5 and 14 and other basal cell markers such as p63. Based on its lack of keratin expression, we can assume that PCa does not undergo this hypothetical complex differentiation pathway. In low-grade tumors, in the presence of CD44 (+) cells, the process directs toward to more rapidly dividing amplifying population and ends with luminal cells. Normally, these cells divide asymmetrically, generating one stem cell and one cell committed to differentiation. However, in glands involved in the progression of PCa, the path that generates stem cell might be suppressed and all CD44 (+) cells enter a transit stage in which cells have proliferative and maturation capacity. No healthy basal cell is formed in this sudden transitioning stage. However, the exact location of basal cells in this scheme whether they represent the suppressed pathway or bypassed differentiated progenitor remains to be determined because these cells have dual cell properties, with stem cell associated genes such as CD44, CD133, bcl-2, telomerase, and p63 and properties of differentiated cells such as keratin profiles (11). As mentioned earlier, in low-grade tumors, these CD44 (+) cells are probably host stem cells that already existed and their numbers gradually decreased with the increase in tumor grade.

The other function attributed to CD44 is the interaction with various stromal ligands including glycosaminoglycan hyaluronan, collagens, and matrix metalloproteinases. Loss of CD44 causes easy detachment of cells from the basement membrane and allows malignant cells to escape from their site of origin by degrading the extracellular matrix and acquiring a more motile and invasive phenotype. Draffin and Lokeshwar et al determined that prostate cancer cells that express CD44 constitute an aggressive sub-population with an increased potential to metastasize to bones. It has been suggested to relate to elevated hyaluronic acid levels in prostate cancer. (12,13,14). Shang et al claimed that CD44 (+) stem-like cells are the initiators of an epithelial–mesenchymal transition, which is regulated by the TGFbeta1–CD44 signaling pathway that forces cell to adhere to bone marrow endothelial cells, thereby facilitating metastasis (14). However, *in vitro* experiments have shown that the loss of CD44 expression in human prostate carcinoma cell lines is correlated with the methylation of the CD44 promoter region, which is also detected in metastatic lymph nodes (15). We believe that CD44 surface expression depends on the amount of available ligands. As malignant clones proliferate in a limited microenviroment such as the prostate gland, the formation of cribriform structures is inevitable. Moreover, there is no necessity for stromal interaction via CD44 glycoprotein. This theory can be applied to metastatic tumor cells in stroma-poor lymph nodes and also high-grade solid tumor islands. Peritumoral desmoplastic reaction in PCa sometimes reflects as a periacinar retraction; however, it is not significant as in gastrointestinal adenocarcinomas. Thus, tumor-promoted poorly vascularized fibrosis that may not be the fundamental defense mechanism against PCa can alter CD44 interaction.

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Based on our findings, we conclude that once cancerous properties have been established, cells tend to lose the ability of CD44 expression. Loss of CD44 expression is a hallmark of prostate carcinogenesis, but the nature of cells exhibiting CD44 positivity in low-grade tumors remains uncertain, maintain the tumor in a differentiated, gland forming state. We also suggest that CD44 silencing in tumors may be an adaptation to the degradation of the basement membrane and the disruption of stromal–epithelial communication.

Disclosure: All authors have no potential conflicts of interest.

Ethics Committe Approval: (Kırıkkale University Non-Interventional Research Ethics Committee, 24.07.2019-2019/11.

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