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Interrelated evaluation of WT1 and E-cadherin expressions in the prostate adenocarcinoma *

Objective: This study aimed to perform concomitant evaluation of the expressions of WT1 and E-cadherin in benign prostatic hyperplasia (BPH), high-grade prostate intraepithelial neoplasia (HGPIN), and prostate acinar adenocarcinoma (PAC) and analyze the relationships of these potential markers with clinicopathological parameters.

Materials and Methods: The study included 60 cases of 38 PAC, 10 HGPIN, and 12 benign BPH tissues. E-cadherin and WT1 stains were applied by immunohistochemical method.

Results: In the PAC group, a significant increase in WT1 expression was determined compared to BPH (p<0.01). E-cadherin levels decreased significantly (p<0.05) in the PAC group compared to BHP. In PAC, E-cadherin expression (p<0.001) was decreased and WT1 expression (p<0.05) increased compared to HGPIN. However, no significant relationship (p>0.05) was detected between HGPIN and BPH. Also, an important relationship was detected between the Gleason score and the expressions of E-cadherin and WT1 (p<0.05). A significant relationship was also detected between E-cadherin expression and pT (p<0.05). There was no significant correlation between both markers and perineural, lymphovascular, and extraprostatic invasions (p>0.05). In addition, a negative correlation was found between E-cadherin and WT1 in PAC (r=-0.341; p=0.008).

Conclusion: After evaluating all the data together, it was concluded that WT1 expressed in PAC epithelial cells could suppress E-cadherin and promote the progression of the PAC.

Key Words: *WT1, E-cadherin, prostate adenocarcinoma, high-grade prostatic intraepithelial neoplasia, benign prostatic hyperplasia*

Prostat Adenokarsinomunda WT1 ve E-cadherin Ekspresyonlarının Eş Zamanlı Değerlendirilmesi

Amaç: Bu çalışmada benign prostat hiperplazisi (BPH), yüksek dereceli prostat intraepitelyal neoplazisi (HGPIN) ve prostat asiner adenokarsinomunda (PAC) WT1 ve E-cadherin ekspresyonlarının eş zamanlı değerlendirilmesi ve bu potansiyel belirteçlerin klinikopatolojik parametrelerle ilişkilerinin analiz edilmesi amaçlandı.

Gereç ve Yöntem: Çalışmaya 38 PAC, 10 HGPIN ve 12 iyi huylu BPH dokusuna ait 60 olgu dahil edildi. E-cadherin ve WT1 boyamaları immünohistokimyasal yöntemle uygulandı.

Bulgular: PAC grubunda, BPH ile karşılaştırıldığında WT1 ekspresyonunda anlamlı bir artış belirlendi (p<0.01). E-cadherin düzeyleri PAC grubunda BHP ile karşılaştırıldığında anlamlı bir şekilde azaldı (p<0.05). PAC'de HGPIN'e kıyasla E-cadherin ekspresyonu (p<0.001) azaldı ve WT1 ekspresyonu (p<0.05) arttı. Ancak HGPIN ile BPH arasında anlamlı bir ilişki (p>0.05) saptanmadı. Ayrıca Gleason skoru ile E-cadherin ve WT1 ekspresyonları arasında anlamlı bir ilişki saptandı (p<0.05). E-cadherin ekspresyonu ile pT arasında da anlamlı bir ilişki saptandı (p<0.05). Her iki belirteç ile perinöral, lenfovasküler ve ekstraprostatik invazyonlar arasında anlamlı bir korelasyon saptanmadı (p>0.05). Ayrıca PAC'de E-cadherin ile WT1 arasında negatif bir korelasyon bulundu (r=-0,341; p=0.008).

Sonuç: Tüm veriler birlikte değerlendirildiğinde PAC epitel hücrelerinde eksprese edilen WT1'in E-cadherin'i baskılayıp PAC'nin ilerlemesini destekleyebileceği sonucuna varıldı.

Anahtar Kelimeler*:* *WT1, E-cadherin, prostat adenokarsinomu, yüksek dereceli prostat intraepitelyal neoplazi, benign prostat hiperplazisi*

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Introduction

Prostate acinar adenocarcinoma, (PAC), one of the most common malignancies of the urogenital tract and more common in older men, affects more than 200.000 men per year. PAC, the second most lethal cause of cancer among men, also shows significant prognosis differences among individuals. Therefore, it is important to determine the mechanisms that regulate the progression of PAC (1, 2). It is reported that the main cause of morbidity and mortality due to PAC is due to metastasis. Metastasis, which occurs at the stage after the initial neoplastic transformation of cells, involves a series of

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sequential steps including neoangiogenesis and lymphangiogenesis and entry into the systemic vascular system or lymphatics (3).

E-cadherin is a cell adhesion molecule that determines epithelial development in the embryo and maintains the differentiated epithelial pattern in the adult. Decreased E-cadherin expression has been reported to be associated with PAC progression (4). E-cadherin expression is also used to indicate the epithelial phenotype, and loss of its expression suggests epithelial-mesenchymal transition (EMT). EMT triggers tumor metastasis. EMT disruption can be demonstrated by loss of E-cadherin expression. However, the mechanisms associated with the exit from EMT are not well understood at present (5). Loss of E-cadherin expression is associated with increased migration and invasiveness of many cancer cells, including prostate (6).

Wilms tumor antigen 1 (WT1) is a transcription factor overexpressed in some leukemias and solid tumors. There is limited information on the expression and immunogenicity of WT1 in prostate cancer (7). Immunohistological studies of PAC cell lines have shown that WT1 protein expression is cytoplasmic. This suggests that the function of WT1 in prostate tumors may extend beyond the role of a transcription factor to post-transcriptional or translational modulations in the cell (2). It has been reported that WT1 can promote metastatic disease, suppress E-cadherin, and increase the motility of PAC cells with low migration and metastatic potential (8). Therefore, WT1 in prostate cancer may function as a novel oncogene facilitating the development of a lethal metastatic phenotype (6). This study aimed to evaluate the relationships between E-cadherin and WT1 proteins expressed in tissue samples from PAC cases and their relationships with histopathological data and prognosis.

Materials and Methods

Research and Publication Ethics: Ethical approval was obtained from the Kirikkale University Non-invasive Research Ethics Committee (2019.07.02).

A total of 38 primary PAC, 10 high-grade prostatic intraepithelial neoplasia (HGPIN) and 12 benign prostatic hyperplasia (BPH) tissues were included in this study. Histopathological samples were obtained from 29 radical prostatectomy (RP) and 9 transurethral resection (TUR) or suprapupic prostatectomy materials. HGPIN areas were obtained from PAC samples. Histopathological evaluation was performed on hematoxylin eosin (H&E) stained slides of PAC cases, and Gleason grading and scoring were performed according to the modified Gleason scoring system of the International Society of Urological Pathology (ISUP). Vascular and perineural invasion, HGPIN, and the presence or absence of extraprostatic invasion were also recorded in RP cases. In addition, the pT stages of RP cases were evaluated according to the TNM system. pT2 cases were collected in a single group for statistical analysis.

The preparations that best represented the tumor were selected and immunohistochemical staining of E-cadherin (clone EP700Y; Rabbit Monoclonal) and WT1 (clone 6F-H2; Mouse Monoclonal) was performed using an automatic immunostaining device (Benchmark ULTRA, Ventana® Medical Systems Inc., Tucson, AZ, USA). Kidney tissue was used as a positive control for WT1, and breast tissue was used as a positive control for e-cadherin. Scoring for E-cadherin expression was performed according to the presence and strength of membranous staining in each sample (absent: 0, weak membranous staining: 1 and strong membranous staining: 2); and for WT1, according to the presence of cytoplasmic positive staining (no staining: 0, staining: 1).

Statistical analysis was performed using the SPSS statistical software program (SPSS 20.0 Inc., Chicago, IL). The relationship between the results and clinicopathological parameters was evaluated using the Chi-square test. The significance of expression levels was determined using the Kruskal-Wallis test. Differences in expression between groups were examined using the Mann-Whitney U test. A 95% confidence interval (p<0.05) was considered significant.

Results

Clinicopathological features of the patients are summarized in Table 1. As to WT1, all BPH and HGPIN groups were scored as 0, and 39.5% of the PAC group was detected as score 1. Regarding E-cadherin, 41.7% of BPH was detected as a score of 2, 70% of HGPIN was a score of 1, and 52.6% of PAC was a score of 0 (Figure 1, Table 2).

Table 1. Clinicopathological features of the PAC group.

Age (mean)	54-92 (71.7±7.23)
Gleason Score	n (%)
6	12 (%31.6)
7	15 (%39.5)
8	3 (%7.9)
9	8 (%21.1)
LVI	
absent	33 (%86.8)
present	5 (%13.2)
PNI	
absent	21 (%55.3)
present	17 (%44.7)
pT Stage	
pT2	20 (%66.7)
pT3	10 (%33.3)

LVI: lymphovascular invasion, PNI: perineural invasion

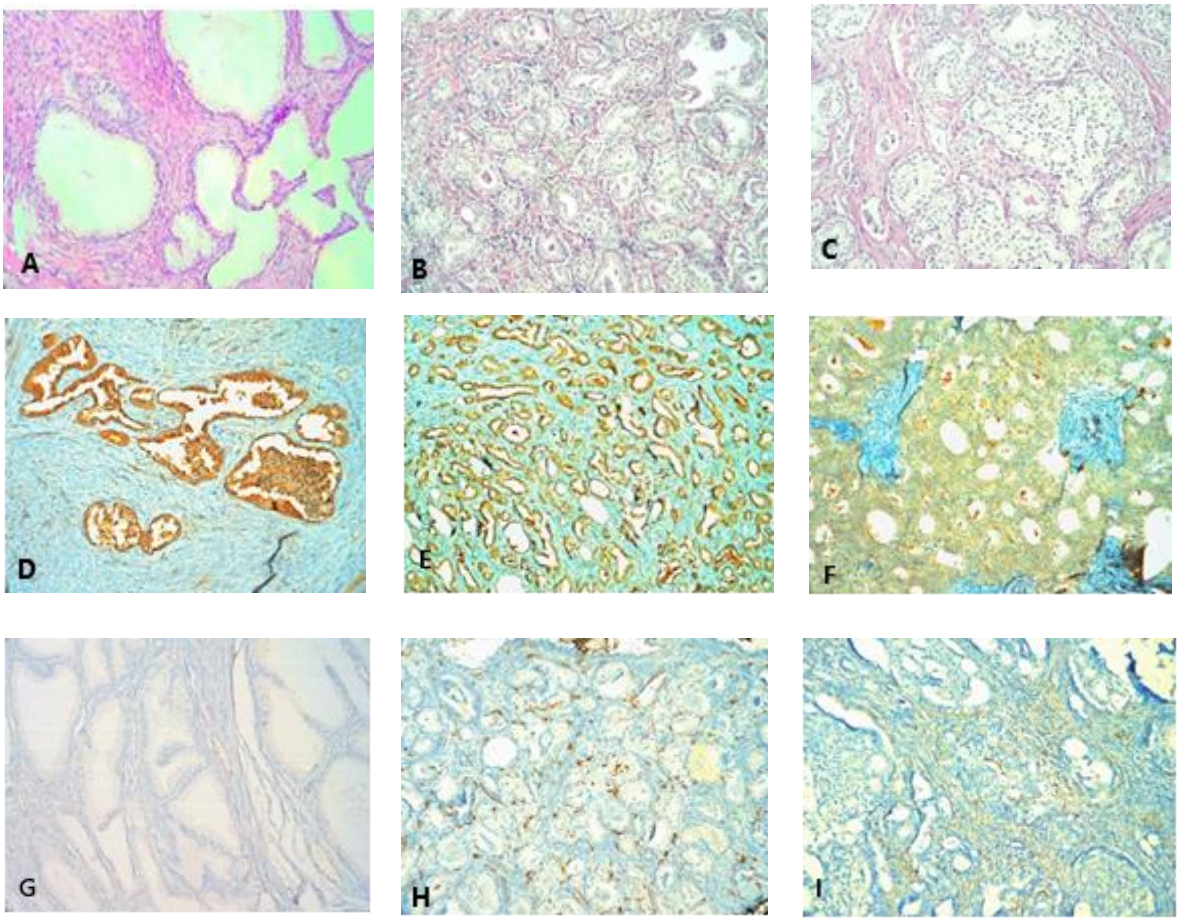

Figure 1. Histopathological images (*x200*). *Hematoxilen-Eosin Staining (A: Benign prostatic hyperplasia, B: Prostate acinar adenocarcinoma Gleason Score 3+3, C: Prostate acinar adenocarcinoma Gleason Score 4+4), E-cadherin Immunohistochemical Staining (D: Benign prostatic hyperplasia, E: Prostate acinar adenocarcinoma Gleason Score 3+3, F: Prostate acinar adenocarcinoma Gleason Score 4+4), and WT1 Immunohistochemical Staining (G: Benign prostatic hyperplasia, H: Prostate acinar adenocarcinoma Gleason Score 3+3, I: Prostate acinar adenocarcinoma Gleason Score 4+4).*

Table 2. Distribution of study groups according to WT1 and E-cadherin scores

	WT1=0.003		**E-cadherin <0.001**		
	0	**1**	**0**	**1**	**2**
BPH	12 (100%)	0	4 (33.3%)	3 (25%)	5 (%41.7)
HGPIN	10 (100%)	0	0	7 (%70)	3 (%30)
PAC	23 (60.5%)	15 (39.5%)	20 (52.6%)	18 (47.4%)	0

BPH: benign prostatic hyperplasia, HGPIN: high-grade prostatic intraepithelial neoplasia, PAC: prostatic acinar adenocarcinoma.

E-cadherin expression was significantly increased in the BPH group compared to the PAC group (p<0.05). A significant decrease in E-cadherin expression was detected (p<0.001) when the PAC group was compared to the HGPIN group (Table 3). In contrast, WT1 expression was significantly higher in the PAC group compared to the BPH group (p<0.01). The PAC group also showed an increase in WT1 expression (p<0.05) compared to the HGPIN group (Table 4). Significant correlations were found between the Gleason score and both E-cadherin and WT1 expressions (p<0.05) and between E-cadherin expression and the pT stage (p<0.05). A negative correlation was also determined between E-cadherin and WT1 in PAC (r=-0.341; p=0.008). No significant difference was observed between HGPIN and BPH with either marker (p>0.05). There was also no significant relation between the clinicopathological markers (p>0.05).

Table 3. Distribution of clinicopathological parameters in prostate aciner adenocarcinoma according to E-cadherin score

E-cadherin	0	1	*p*
Gleason Score			
6-7	11 (28.9%)	16 (42.1%)	<0.05
8-9	9 (23.6%)	2 (5.2%)	
Grade Group			
1-2	8 (21%)	11 (28.9%)	<0.05
3-4-5	12 (31.6%)	7(18.4%)	
LVI			
present	4 (10.5%)	1 (2.6%)	>0.05
absent	15 (39.5%)	17 (44.7%)	
PNI			
present	8 (21.1%)	9 (23.7%)	<0.05
absent	12 (31.6%)	9 (23.7%)	
EPI			
present	4 (10.5%)	3 (7.9%)	<0.05
absent	15 (39.5%)	14 (36.8%)	
pT			
2	5 (13.2%)	15 (39.5%)	*<0.05*
3	7 (18.4%)	3 (7.9%)	

LVI: lymphovascular invasion, PNI: perineural invasion, EPI:extraprostatic invasion.

Table 4. Distribution of clinicopathological parameters in prostate aciner adenocarcinoma according to WT1 score.

WT1	0	1	
Gleason Score			
6-7	19 (50%)	8 (21.1%)	>0.05
8-9	4 (10.5%)	7 (18.4%)	
Grade Group			
1-2	15 (39.5%)	4 (10.5%)	<0.05
3-4-5	8 (21.1%)	11 (28.9%)	
LVI			
present	2 (5.3%)	3 (7.9%)	>0.05
absent	21 (55.3%)	12 (31.6%)	
PNI			
present	11 (28.5%)	6 (15.8%)	>0.05
absent	12 (31.6%)	9 (23.7%)	
EPI			
present	4 (10.5%)	3 (7.9%)	>0.05
absent	16 (42.1%)	6 (15.8%)	
pT			
2	14 (36.4%)	5 (13.2%)	<0.05
3	6 (15.8%)	4 (10.5%)	

LVI: lymphovascular invasion, PNI: perineural invasion, EPI:extraprostatic invasion

Discussion

In the present study, we provided evidence for increased WT1 levels and decreased E-cadherin levels in PAC cells. We also observed an inverse relationship between WT1 and E-cadherin immunohistochemistry levels in the tumor tissue. It was understood that there was an increase in WT1 and a decrease in E-cadherin levels with the progression from HGPIN to PAC in tumor progression. It was reported that the upregulation of WT1 provides an experimental basis for clinically targeted therapy of cervical cancer by suppressing cervical cancer development via miR-205 (9). On the other hand, complex interactions have been identified between WT1 and IGFBP1, FBN1, SERPINA1, and 20 other genes, and it has been reported that WT1 has a new therapeutic potential in ovarian cancer (10). Another study in ovarian carcinoma revealed that WT1 plays a tumor-supporting role and increases EMT through negative modulation of ERK1/2 signaling and E-cadherin expression, and it was reported that WT1 may be a new therapeutic target that can improve the prognosis of ovarian carcinoma (11).

Many studies have evaluated the clinical significance of EMT markers at various stages of human PAC. Loss of E-cadherin staining in PAC has been reported to be associated with higher Gleason score, advanced clinical stage, and poor prognosis (12). E-cadherin expression is used to monitor the epithelial phenotype, and its loss suggests EMT (13). WT1 has been reported to be required for suppression of the epithelial phenotype during embryonic stem cell differentiation through direct transcriptional regulation of E-cadherin-related genes of EMT (14). These new insights into the molecular mechanisms regulating EMT may help in the development of cell-based therapies.

In recent years, new grade groups and American Joint Committee on Cancer stage groups have been used to predict prognosis (1). Although pathological stage seems to be one of the best prognostic factors available to date, the value of the degree of disease and clinical stage is controversial. In this study, E-cadherin immunostaining was associated with Gleason score and tumor grade, in line with the literature (Bengallo et al., 2016). However, unlike literature, significant associations were also found between E-cadherin expression and tumor stage and perineural invasion (15). Methods that allow more accurate prediction of clinical behavior are urgently needed. In this regard, investigating alternative molecular prognostic markers is now attracting significant attention.

It has been reported that E-cadherin suppresses in vitro invasion and the presence of E-cadherin staining in tumor tissue is associated with longer overall survival (16). Loss of E-cadherin has also been shown to reduce metastatic potential in invasive ductal carcinomas (17). In an overview of all data obtained, the findings suggest that E-cadherin plays opposing roles in tumor progression by suppressing cancer cell invasion.

E-cadherin, an important gene in epithelial differentiation and neoplastic transformation, represents a downstream target gene that links the roles of WT1 in differentiation and growth control (18). WT1 expression transcriptionally regulates the expression of growth control genes. WT1 is reported to be expressed in PAC epithelial cells and regulates PAC critical genes. There are reports that WT1 may also promote metastasis and suppress E-cadherin. It is reported that WT1 may activate angiogenesis in PAC cells to increase tumor growth and progression to metastatic disease (19). In this study, WT1 was not associated with the advanced pathological tumor stage. Considering the potential of WT1 to promote PAC invasion, it was evaluated that it may affect genes that promote PAC progression. Thus, it was concluded that therapies targeting WT1 in PAC may reduce metastatic spread and increase overall survival.

Consequently, it was thought that WT1 expressed in PAC epithelial cells suppresses E-cadherin and promotes progression to more advanced pathological stages, thus increasing the poor prognosis of PAC cells. It could be suggested that the potential of WT1 to promote PAC cell migration may be realized by regulating genes that stimulate the aggressive behavior of PAC.

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