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Evaluation of Apelin/APJ and Fibronectin Expression in Genitourinary Tumors: An Immunohistochemical Analysis

Genitoüriner Tümörlerde Apelin/APJ ve Fibronektin Ekspresyonunun Değerlendirilmesi: Bir İmmünohistokimyasal Analiz

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Abstract

Aim: Cancer is a leading cause of death worldwide, making cancer research and the development of new treatment methods crucial. Bladder, endometrial, and prostate cancers are among the most prevalent forms of cancer. This study aimed to investigate the expression and distribution of endogenous apelin/APJ receptor and fibronectin in these genitourinary tumors and compare them to benign tissues to contribute new data to the literature.

Material and Method: Immunohistochemical methods were applied to 44 cases, including benign and malignant formalin-fixed paraffin-embedded tissues of the endometrium, prostate, and bladder.

Results: The findings showed a significant increase in apelin, APJ, and fibronectin expression in endometrioid adenocarcinoma, urothelial carcinoma, and prostatic adenocarcinoma compared to benign tissues. Moreover, the expression of these molecules had a direct correlation with each other in these tumors. However, as the tumor grade increased, the expression of these molecules decreased in prostatic adenocarcinoma and endometrioid adenocarcinoma.

Conclusion: This is the first study to examine the co-expression and distribution of endogenous apelin/APJ receptor and fibronectin in genitourinary tumors and compare them histologically with benign counterparts, to the best of our knowledge. This underscores the novelty and significance of our findings, providing a foundation for further exploration of the potential roles of these molecules in tumorigenesis and cancer therapies.

Keywords: Apelin, APJ, bladder carcinoma, endometrial carcinoma, fibronectin, genitourinary tumors, immunohistochemistry, prostate carcinoma

Öz

Amaç: Kanser, dünya genelindeki ölüm nedenleri arasında önde gelen bir durum olup, kanser araştırmaları ve yeni tedavi yöntemlerinin geliştirilmesi ciddi önem arz etmektedir. Mesane, endometrium ve prostat kanserleri, en yaygın kanser türleri arasında yer almaktadır. Bu çalışma, bu genitouriner tümörlerdeki endojen apelin/APJ reseptörü ve fibronektin ekspresyonunu ve dağılımını araştırmayı ve elde ettiği verileri benign dokularla karşılaştırarak literatüre katkı sunmayı amaçlamıştır.

Gereç ve Yöntem: Endometrium, prostat ve mesaneye ait formalin ile fikse parafine gömülü benign ve malign dokularını içeren 44 vakaya immünohistokimyasal analiz uygulanmıştır.

Bulgular: Endometrioid adenokarsinom, ürotelyal karsinom ve prostat adenokarsinomunda apelin, APJ ve fibronektin ekspresyonunda benign dokulara kıyasla önemli bir artış olduğu saptanmıştır. Ayrıca, bu tümörlerde bu moleküllerin ekspresyonu birbirleriyle doğrudan bir ilişki sergilemiştir. Bununla birlikte, prostat adenokarsinomunda ve endometrioid adenokarsinomunda tümör derecesi arttıkça, bu moleküllerin ekspresyonu azalmıştır.

Sonuç: Bu çalışma, literatürde genitouriner tümörlerdeki endojen apelin/APJ reseptörü ve fibronektin ekspresyonunun ve dağılımının histolojik olarak benign karşılıklarıyla kıyaslandığı ilk çalışmadır. Elde edilen bulguların, apelin/APJ reseptörü ve fibronektinin tümorigenez ve kanser tedavilerindeki potansiyel rolleri açısından planlanacak daha ileri araştırmalar için bir temel oluşturacağı kanısındayız.

Anahtar Kelimeler: Apelin, APJ, mesane karsinomu, endometrial karsinom, fibronektin, genitouriner tümörler, immünohistokimya, prostat karsinomu

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INTRODUCTION

Genitourinary system cancers account for approximately 25% of all tumors worldwide.[1] Prostate, bladder, and endometrium cancers are among the most common genitourinary system cancers leading to death globally.[2] The heterogeneity and variability in treatment and survival responses of different cancer types underscore the need to elucidate the biological mechanisms of tumor formation and progression. Consequently, researchers are investigating the targeting of certain molecules in the tumor microenvironment that may be involved in the pathogenesis and prognosis of cancer to discover more effective therapies. Identifying a general and appropriate target that is oncogenic or tumor suppressive will provide an ideal basis for designing and developing cancer therapeutic strategies. Apelin, angiotensin-like receptor 1 (APJ), and fibronectin are among the most attractive molecules for current studies evaluating their association with different tumors, but there is little data available for genitourinary tumors.[3]

Apelin is a bioactive peptide that binds to a G protein-coupled receptor called APJ.[3] Apelin encodes a secreted precursor called preproapelin, consisting of 77 amino acids. The preproapelin is cleaved by endopeptidases, generating several active forms of apelin.[3] The apelin/APJ axis is widely expressed in organs such as the heart, brain, kidney, placenta, etc.[3] It has been demonstrated that the apelin/APJ system plays a role in various physiological processes such as cardiovascular regulation, angiogenesis, pain, feeding behavior, etc.[3,4] Additionally, recent studies have revealed the potential role of the apelin/APJ system in tumorigenesis and mainly adverse prognosis of various tumors, including lung, skin, breast, kidney, ovary, bladder, endometrium, and prostate cancer, with some contradictory results.[3,5-12]

Fibronectin (FN) is a ~500 kDa glycoprotein located in a polymeric fibrillar network in the extracellular matrix (ECM).[13] Fibronectin functions are mediated by insoluble polymeric fibrils. The conversion of soluble fibronectin to fibronectin fibrils in the ECM is initiated by binding to cell surface integrins, other fibronectin subunits, collagen, heparin, fibrin, matrix metalloproteinases (MMPs), and growth factors, resulting in exposure of cryptic epitopes necessary for polymerization.[13] To the best of our knowledge, no literature is currently available that evaluates the potential relationship between the apelin/APJ axis and fibronectin in genitourinary tumors. Both molecules have been implicated in a range of cellular processes, including angiogenesis, cell adhesion, migration, and signaling. While fibronectin has been identified as a potential target for anti-cancer therapies, its precise role in tumor development and progression remains unclear, particularly in genitourinary tumors.[14] Interestingly, cancerous fibronectin appears to have a tumor-suppressive role, but may also be pro-metastatic and associated with poor prognosis.[15] In contrast, fibronectin deposited in the tumor microenvironment is paradoxically associated with a better prognosis.[15] In light of these findings, it is important to better understand how fibronectin affects tumor transformation and metastatic progression.

To address this gap in the literature, our study aims to investigate the expression and distribution of endogenous apelin/APJ receptor and fibronectin in endometrium, prostate, and bladder tumors, and compare these findings to their benign counterparts histologically.

MATERIAL AND METHOD

The study was carried out with the permission of Akdeniz University Clinical Research Ethics Committee (Date: 09.11.2022, Decision No: KAEK-661). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 44 formalin-fixed paraffin-embedded tissues containing endometrial curettage (8 endometrioid adenocarcinomas, 4 secretory phase endometrium, and 3 proliferative phase endometrium), prostate needle biopsy (11 acinar adenocarcinomas intermixed with benign tissues), and bladder transurethral resection material (9 urothelial carcinomas, and 9 benign bladder tissues) from the archive of the Department of Pathology at Alanya Education and Research Hospital were evaluated. The Hematoxylin&Eosin-stained slides were examined to confirm the diagnosis and tumor grade and paraffin blocks containing both tumors with different grades and benign epithelial areas were selected, if available. Immunohistochemical staining of 5 µm thick sections prepared from the selected paraffin blocks was performed using apelin, APJ, and fibronectin primary antibodies and appropriate secondary antibodies.

The sections were placed onto a superfrost slide and incubated in an oven at 56°C overnight. They were then rehydrated by passing them through a series of xylene and alcohol to deparaffinize them. To eliminate antigenic masking, they were boiled in citrate buffer (100244; Merck) and incubated with hydrogen peroxide (18312; Sigma) to remove endogenous peroxidase activity. The sections were then treated with a UV block (TA-125-UB Thermo Scientific) to prevent nonspecific immunoglobulin binding. Primary antibodies for Apelin (Thermo; PA5-114860, 1/150), Apelin Receptor (APJ) (Thermo; PA5-114830, 1/200), and Fibronectin (Abcam; ab2413, 1/200) and their respective secondary antibodies were added. The reaction was made visible with DAB chromogen (D4168; Sigma), and the sections were counterstained with Mayer's Hematoxylin (109249, Merck). Dehydrated sections were passed through a series of alcohol and xylene, and then closed with Entellan. The protein expression levels and localizations were then determined in these sections. Finally, the sections were measured using Image J (1.52 R, National Institutes of Health, USA) after photographing them with an Olympus CX43 Microscope (Japan) to visualize the protein localization.

Immunohistochemical staining intensity of apelin, APJ, and fibronectin was scored using the following criteria: score 0 for no staining, score 1 for mild staining, score 2 for moderate staining, and score 3 for strong staining. Clinicopathologic parameters were obtained from patient records for each sample.

Statistical Analysis

Statistical analysis was performed using Image J software (version 1.52 R; National Institutes of Health) on three randomly selected photographs from each experimental group. Differences in expression between groups were calculated using ANOVA and Sidak's test for multiple comparisons. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 8. The difference between groups and the p-value summary of the difference was marked with an asterisk/asterisks.

RESULTS

Comparison of Endometrioid Adenocarcinoma and Benign Endometrial Tissues

The mean age of patients with endometrioid adenocarcinoma, secretory phase endometrium, and proliferative phase endometrium were 58.3±4.9 (range, 43-69), 43.7±2.1 (range, 40-47), and 46.3±1.4 (range, 40-50), respectively. According to the International Federation of Gynecology and Obstetrics (FIGO) system, 3 out of 8 endometrioid adenocarcinomas were grade 1 (low grade), and the remaining 5 cases were grade 2 (high grade). The expression of apelin, APJ, and fibronectin was found to be significantly higher in endometrioid adenocarcinoma than in benign tissues (p<0.05) (**Figures 1, 2, 3,** and **4**). There was a higher expression of apelin, APJ, and fibronectin in low-grade endometrioid adenocarcinoma than in high-grade tumors (p<0.05) (**Figures 1, 2, 3**, and **4**). The benign endometrial tissues exhibiting secretory phase features showed higher expression of apelin and APJ, prominent in the apical parts of the glands, compared to those exhibiting the proliferative phase (p<0.05) (**Figures 1, 2,** and **3**).

Comparison of Bladder Carcinoma and Benign Bladder Tissues

The mean age of patients with urothelial carcinoma and benign bladder tissues was 69.2 ± 8.4 (range, 56-77) and 63.6 ± 6.3 (range, 47-74), respectively. According to the WHO 2016 classification, there were 5 high-grade and 4 low-grade urothelial carcinomas. Five tumors were non-infiltrative, and 4 tumors were infiltrative. The male-to-female ratio in patients with urothelial carcinoma and benign bladder tissue was 2 (n=6/3) and 8 (n=8/1), respectively.

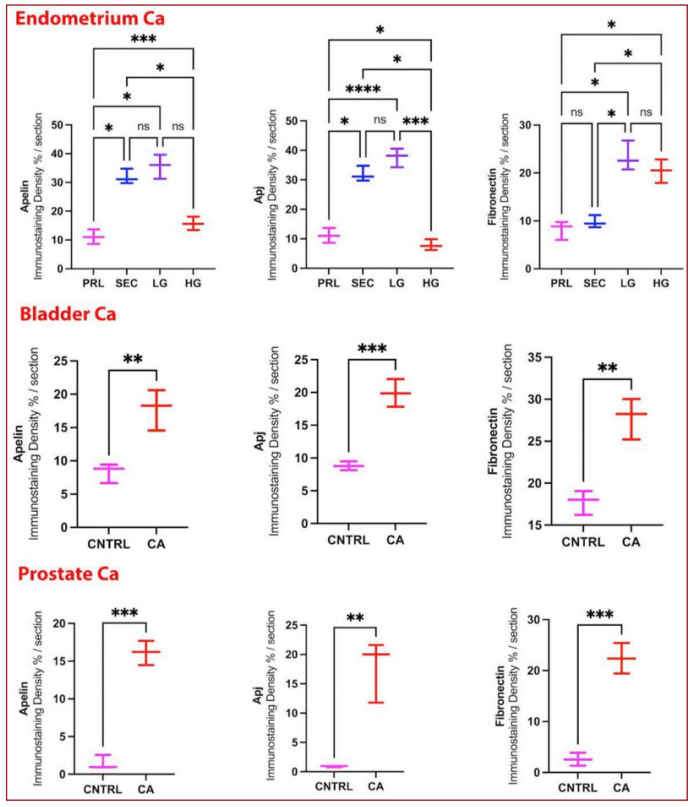

Figure 1. Immunohistochemical density of apelin, APJ, and fibronectin expression in endometrium, bladder, and prostate cancer; PRL: proliferative phase, SEC: secretory phase, LG: low-grade, HG: high-grade, CNTRL: control, Ca/CA: cancer, ns: no statistical difference, *: statistically significant difference, p<0.05.

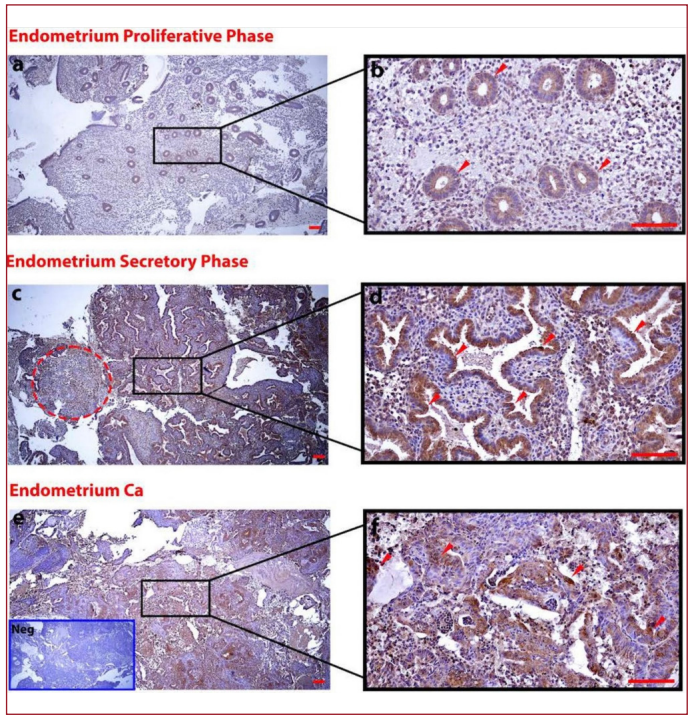

Figure 2. Immunohistochemical expression of apelin in benign endometrial tissues (a-d), and endometrioid carcinoma (e-f); red arrows indicate positive staining in endometrial glands and epithelial structures, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

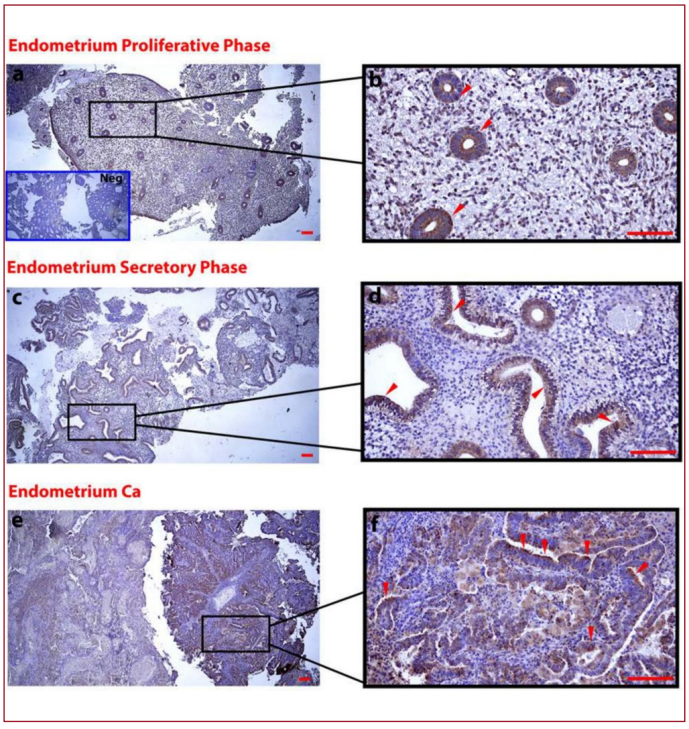

Figure 3. Immunohistochemical expression of APJ in benign endometrial tissues (a-d), and endometrioid adenocarcinoma (e-f); red arrows indicate positive staining in endometrial glands and epithelial structures, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

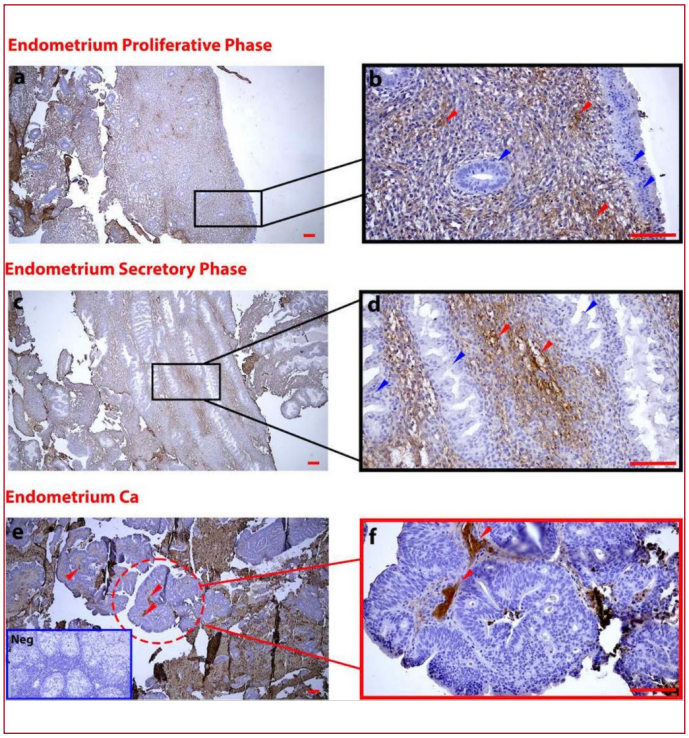

Figure 4. Immunohistochemical expression of fibronectin in benign endometrial tissues (a-d), and endometrioid carcinoma (e-f); red arrows indicate positively stained endometrial glands and epithelial structures, the blue arrows indicate negatively stained endometrial glands and epithelial structures, the red dashed circle with red arrows represents densely positively stained blood vessels in endometrial cancer, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

The expression of apelin, APJ, and fibronectin was significantly higher in urothelial carcinoma compared to benign tissues ($p<0.05$) (**Figures 1, 5, 6,** and **7**). The staining intensity of apelin, APJ, and fibronectin was higher in high-grade invasive bladder carcinomas compared to low-grade ones (**Figures 1, 5, 6,** and **7**). Invasive urothelial carcinomas exhibited higher expression of apelin, APJ, and fibronectin than non-invasive ones of the same grade. Apelin showed more intense staining in the upper 1/3 of the tumoral epithelium. In benign urothelial epithelium, apelin was intensely expressed in the plaque region of umbrella cells, whereas the plaque region was negative for apelin and thinner compared to the benign epithelium (**Figure 5**). The increase in apelin expression, especially in vessels adjacent to the tumor cells feeding urothelial carcinoma, was remarkable.

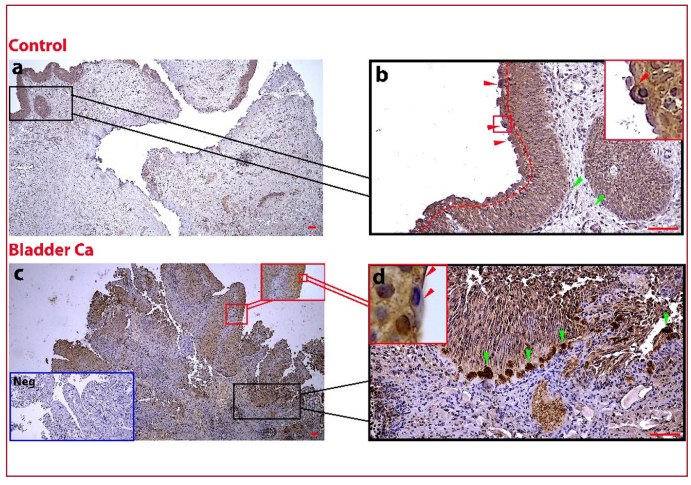

Figure 5. Immunohistochemical expression of apelin in benign bladder tissues (a-b), and urothelial carcinoma (c-d); red arrows indicate umbrella cells, green arrows indicate blood vessels, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

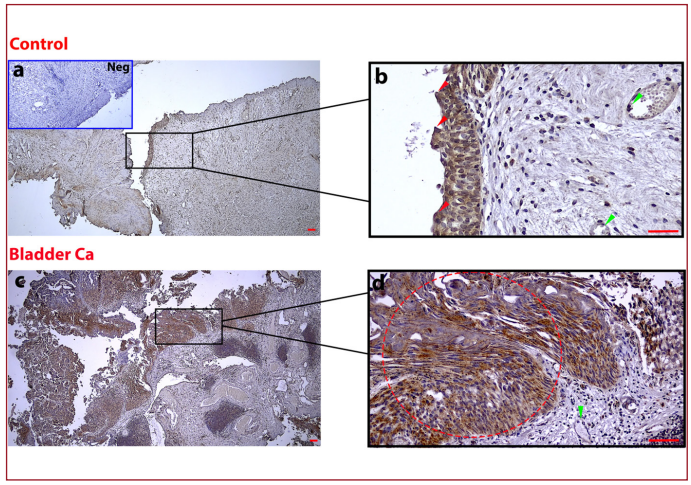

Figure 6. Immunohistochemical expression of APJ in benign bladder tissues (a-b), and urothelial carcinoma (c-d); red arrows indicate umbrella cells, green arrows indicate blood vessels, the red dashed circle represents positively stained transitional epithelium, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

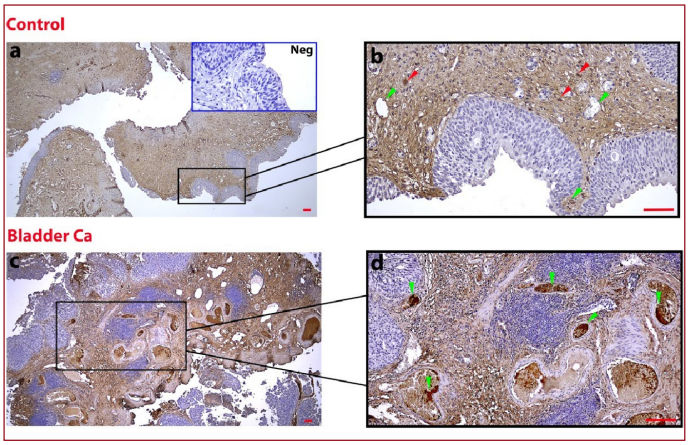

Figure 7. Immunohistochemical expression of fibronectin in benign bladder tissues (a-b), and urothelial carcinoma (c-d); red arrows indicate stromal cells, green arrows indicate blood vessels, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

Comparison of Prostate Adenocarcinoma and Benign Prostatic Tissues

The mean age of patients with prostate adenocarcinoma was 68.9±6.3 (range, 58-77). According to the WHO 2016 classification system, there were 3 acinar adenocarcinomas with Gleason's grade 3, 8 acinar adenocarcinomas with Gleason's grade 4, and 5 acinar adenocarcinomas with Gleason's grade 5. Significantly higher levels of apelin, APJ, and fibronectin were observed in prostatic adenocarcinoma compared to benign tissues (**Figures 1, 8, 9,** and **10**). In glands of prostatic adenocarcinoma and muscles, apelin and APJ were expressed intensely compared to the benign epithelium (**Figures 1, 8,** and **9**). Fibronectin expression was observed in the tumoral stroma and intra-tumoral nerves, but it was negative in the tumoral epithelium except for some tumor cells showing a cribriform pattern (**Figure 10**). The expression of apelin, APJ, and fibronectin decreased as the tumor grade increased, similar to endometrioid carcinoma.

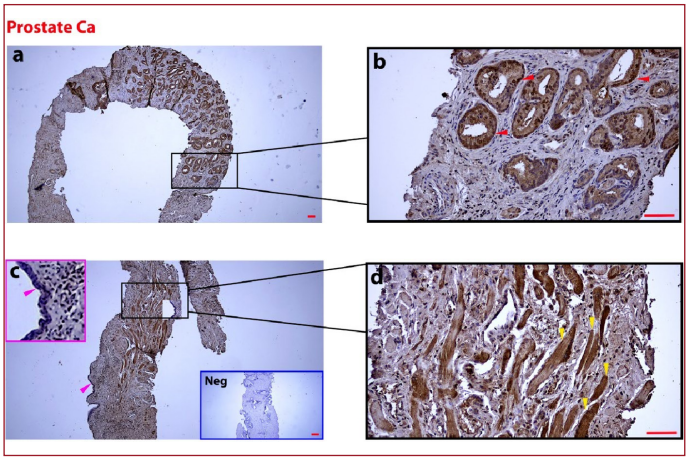

Figure 8. Immunohistochemical expression of apelin in Gleason's grade 3 prostate cancer (a-d); red arrows indicate positive staining in glands, pink arrows indicate negatively stained control epithelium, yellow arrows indicate positively stained muscles, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

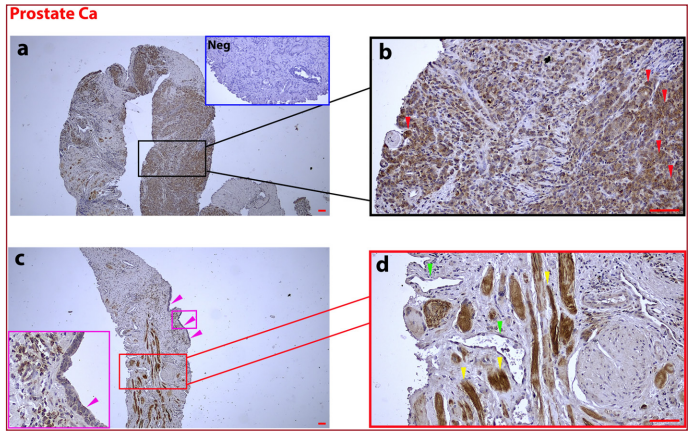

Figure 9. Immunohistochemical expression of APJ in Gleason's grade 5 prostate cancer (a-d); red arrows indicate positively stained glands and cells, pink arrows indicate negatively stained control epithelium, green arrows indicate blood vessels, yellow arrows indicate positively stained muscles, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

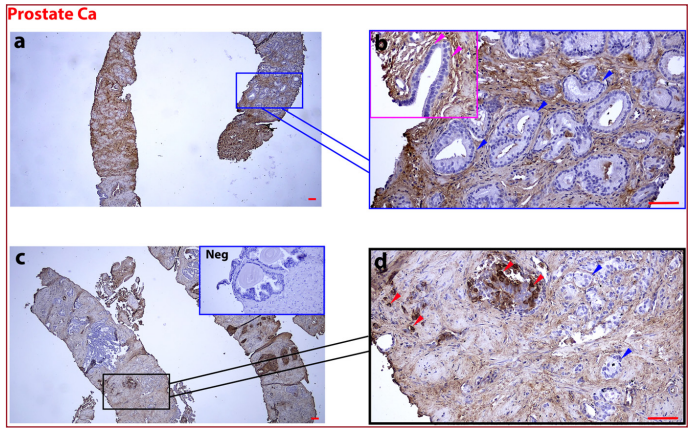

Figure 10. Immunohistochemical expression of fibronectin in Gleason's grade 3 prostate cancer (a-d); red arrows indicate positively stained glands and cells, pink arrows indicate negatively stained control epithelium, blue arrows indicate negatively stained glands, Ca: cancer, neg: negative control, magnification: 5X, 20X, scale bar: 100 µm.

DISCUSSION

The literature suggests that the apelin/APJ axis is associated with a poorer prognosis in certain genitourinary cancers such as bladder cancer, based on only a few studies.[3,9,16] Overexpression of apelin has been linked to higher tumor stage, vascular invasion, and distant metastasis in bladder cancer.[3] In our study, we found that higher levels of apelin and APJ expression were present in invasive urothelial carcinomas of the same grade compared to noninvasive ones. Additionally, the staining intensity of both apelin and APJ was higher in high-grade tumors than in low-grade tumors. Yang et al. reported higher immunohistochemical expression of apelin in tumor cells compared with adjacent benign tissues in bladder cancer with muscle invasion.[3,12] Similarly, in our current study, we observed higher expression of both apelin and APJ in urothelial carcinoma compared to benign tissues. Apelin showed more intense staining in the upper one-third of the tumoral epithelium. In the benign

urothelial epithelium, apelin was intensely expressed in the plaque region of umbrella cells, while in urothelial carcinoma, the plaque region was thinner compared to the benign epithelium. Umbrella cells, along with the plaque region containing uroplakin, provide a barrier by changing shape as the bladder fills and empties.[17]

The findings mentioned in the previous paragraph may be due to either tumor-related content change, architectural deformation, or dysfunction of the plaque region. The increase in apelin expression was notable, particularly in vessels located adjacent to the tumor cells that fed urothelial carcinoma. This increase may be related to neoangiogenesis, as suggested by a study conducted by Yang et al., which proposed that apelin might function as a pro-angiogenic factor.[12,18] The authors also claim that high levels of apelin are associated with a poor prognosis due to high tumor stage, distant metastasis, and vascular invasion. Our study found higher levels of apelin and APJ expression in high-grade tumors, which are known to have an unfavorable prognosis.

Regarding prostate cancer, Wan et al. have reported that overexpression of apelin is linked to disease progression and poor prognosis and that miR-224 regulates it inversely.[10] Additionally, a few studies suggest that apelin-13 promotes the proliferation of prostate cancer cells similar to androgens, and contributes to tumorigenesis.[19,20] In our study, we found that the expression of apelin and APJ was significantly higher in prostatic adenocarcinoma glands and muscles, compared to benign epithelium, which is consistent with the report of Soylu et al. using immunohistochemistry.[18]

Altinkaya et al.[5] recently presented an association between higher serum apelin levels and an elevated risk of endometrial cancer in obese patients. They demonstrated higher serum levels of apelin compared to the controls. In the current study, the expression of apelin, along with APJ, was detected to be higher in endometrioid adenocarcinoma than in benign control tissues. The benign glands in the secretory phase showed a higher expression of apelin and APJ, mainly in the apical parts of the glands, than those in the proliferative phase. Similar to prostate cancer, apelin expression was inversely associated with tumor grade in endometrial cancer, suggesting a possible interaction of apelin with hormones such as estrogen, progesterone, or androgen.

On the other hand, fibronectin showed a parallel pattern to apelin and APJ in all 3 genitourinary tumors examined in the study.

The prostate has organ-specific ECM components such as fibronectin, laminin, chondroitin, heparan sulfate proteoglycan, etc.[21] It is claimed that prostate stroma may induce non-prostate epithelial cells to differentiate into a prostatic phenotype.[21,22] Furthermore, the prostate cancer cell line (LNCaP) is known to secrete fibronectin.[23] A study using reverse transcription-polymerase chain reaction reported higher fibronectin expression in prostate cancer cell lines than benign prostatic hyperplasia.[23] In our study, fibronectin showed abundant positivity in the stroma of benign prostate tissue rather than cancer cells, similar to other studies in the literature.[23,24] These data might indicate the up-regulation of fibronectin in the tumor foundation of prostate cancer. Interestingly, a study by Jia et al.[25] showed that fibronectin expression was focal and significantly lower in high-grade prostate cancer than in low-grade ones, which seems to point out the down-regulation, similar to the current study.

Several studies in the literature on bladder cancer have reported a correlation between high levels of fibronectin expression and poor prognosis and increased invasiveness in the disease.[19] Arnold et al.[26] have similarly shown that total fibronectin expression is significantly higher in patients with bladder cancer than in normal controls, which is consistent with our study. They have also investigated the link between urinary oncofetal ED-A fibronectin and poor prognosis in bladder cancer patients.[19] Moreover, urine fibronectin has been proposed as a potential diagnostic and prognostic biomarker in bladder cancer.[27,28] However, further research is required to ascertain the precise role of fibronectin in bladder cancer progression and its potential as a therapeutic target.

The literature contains only a limited number of studies on fibronectin expression in endometrial cancer.[29] Our study produced similar findings to a previous investigation by Futyma et al. regarding the upregulation of fibronectin genes by PCR in endometrial cancer tissues compared to normal endometrial tissues.[28,29] Similarly, another Northern blotting study found higher fibronectin expression in endometrial hyperplasia and carcinoma compared to benign endometrial specimens.[19] These results suggest a potential association between fibronectin upregulation and carcinogenesis in the endometrium.

CONCLUSION

To summarize, our study demonstrated a significant increase in apelin, APJ, and fibronectin expression in endometrioid adenocarcinoma, urothelial carcinoma, and prostatic adenocarcinoma compared to benign tissues. Furthermore, the expression of apelin, APJ, and fibronectin exhibited a positive correlation with each other in these tumors. In opposite to urothelial carcinoma, as the grade of prostatic adenocarcinoma and endometrioid adenocarcinoma increased, the expression of apelin, APJ, and fibronectin decreased, which seemed to be related to hormonal issues.

To the best of our knowledge, our study is the first to investigate the co-expression and distribution of endogenous apelin/APJ receptors and fibronectin in genitourinary tumors and compare them histologically with benign counterparts. This underscores the novelty and significance of our findings and suggests that the Apelin/APJ axis and its interaction with fibronectin could be considered useful targets for future studies to clarify their possible association with tumorigenesis and develop more effective cancer therapies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Akdeniz University Clinical Research Ethics Committee (Date: 09.11.2022, Decision No: KAEK-661).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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