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Original Article UROLOGY

# Expression of nectin-4 in prostate cancer

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## ABSTRACT

**OBJECTIVE:** Nectin-4 is a transmembrane protein belonging to the nectin family of immunoglobulin-like molecules which is found in the placenta and trachea under physiological conditions and its expression has been shown in many cancer types. We aimed to investigate for the 1st time nectin-4 expression in human prostate cancer tissues.

**METHODS:** We retrospectively analyzed the prostate pathology specimens of 82 patients who underwent initial transrectal ultrasound-guided prostate biopsy or transurethral prostate resection and were found to have atypical small acinar proliferation (ASAP) and incidentally prostate cancer. Tissue samples with prostatic cancer were used as a control for alpha-methylacyl-CoA racemase (AMACR), and benign prostatic glands in the same tissue provided the negative control. The intensity and extent of nectin-4 expression were determined microscopically using the histochemical scoring system which was defined as the product of the staining intensity (score: 0–3) and percentage of stained cells (0–100) at a given intensity.

**RESULTS:** We conducted immunohistochemical analysis of nectin-4 and AMACR expression in all 82 samples. While AMACR expression was positive in prostate cancer tissues with a GS of <7 (n=24, 100%), 7 (n=18, 100%), and ≥8 (n=15, 100%), it was negative in all ASAP samples (n=25, 100%) (p<0.001). Nectin-4 expression was not detected in any of the GS <7, GS 7, or GS ≥8 samples but was found in benign prostatic gland tissues and all 25 (100%) ASAP samples (p<0.001).

**CONCLUSION:** We found that nectin-4 was not expressed in prostate cancer tissues but was expressed in ASAP-and benign prostate gland containing tissues. We believe that prospective studies with more patients and samples including radical prostatectomy materials will reveal the relationship between nectin-4 and prostate cancer more clearly.

*Keywords: Atypical small acinar proliferation; expression; nectin-4; prostate cancer.*

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Prostate cancer is the second-most common cancer in men, with an incidence of 1.4 million worldwide in 2020 [1]. Its prevalence increases with age, as it occurs in <5% of those under the age of 30 years and in 60% by the age of 80 years and above [2, 3]. To manage this widespread disease, clinicians and researchers have focused on diagnosis and treatment, and research on cancer markers has recently become popular. Studies have focused on many new markers for prostate cancer, such as PCA3 and Prolaris [4]; however, the search for new markers for prostate cancer diagnosis continues.

Nectin-4, a transmembrane protein belonging to the nectin family of immunoglobulin-like molecules, is found in the placenta and trachea under physiological conditions and more rarely in tissues such as bladder, breast, stomach, and salivary glands [5]. Recently, nectin-4 expression has been shown in many cancer types, such as breast, cervical, ovarian, gastric, esoph-

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ageal cancer, head–neck cancer, lung cancer, melanoma, thyroid cancer, and hepatocellular and urothelial carcinomas [6]. In addition, nectin-4 is used as a target agent in chemotherapy-resistant urothelial carcinoma treatment by enfortumab vedotin antibody drug conjugate [7].

Although nectin-4 expression has been demonstrated in many cancer types, studies on nectin-4 expression in human prostate tissue are lacking. The present study for the 1st time investigated nectin-4 expression in human prostate cancer tissues diagnosed after transrectal ultrasound (TRUS)-guided prostate biopsy and transurethral resection of the prostate (TUR-P).

**Highlight key points**

- The expression of Nectin-4 in prostate cancer tissues was not detected, but it was expressed in ASAP and benign prostate gland–containing tissues.
- Nectin-4 can be used to make a definitive diagnosis in tissues suspected of containing ASAP.
- We believe that the findings of our study represent an important step for future studies investigating the relationship between nectin-4 and prostate cancer.

## MATERIALS AND METHODS

This study was conducted in accordance with the Declaration of Helsinki, and all patients provided written informed consent. Approval was granted by the Institutional Review Board of Aksaray University Training and Research Hospital (date: June 23, 2022; no. 2022/12-15). We retrospectively analyzed the prostate pathology specimens of 82 patients who underwent initial TRUS-guided prostate biopsy due to elevated prostate specific antigen (PSA) and suspicion of malignancy as well as TUR-P for benign prostatic obstruction between 2016 and 2021 at the Department of Pathology, Aksaray University Training and Research Hospital. We analyzed prostatic tissues with atypical small acinar proliferation (ASAP) and incidental prostate cancer with a Gleason score (GS) of <7, 7, and ≥8 according to the pathology results. None of the patients was metastatic at the time of diagnosis. The age, total PSA, and pathological data of the patients were recorded. Immunohistochemical staining for nectin-4 and alpha-methylacyl-CoA racemase (AMACR) was performed on the tissue sections from the prostate specimens, and the AMACR staining results were obtained from patients' pathology reports. Furthermore, basal cell marker 34bE12 (cytoplasmic) and p63 (nuclear) staining was used immunohistochemically in these biopsy quadrants when making ASAP diagnosis from pathology reports.

### Immunohistochemistry and Evaluation

Tissue sections were freshly cut (3 μm), mounted on Super Frost Plus slides (Thermo Fisher), and rehydrated in descending gradient alcohols. Immunohistochemical staining was carried out on the Ventana BenchMark Ultra automated staining system (Ventana) and visualized with the Ventana amplifier detection kit using the following antibodies: clone sp263 and AMACR (504s) SP 116 (Roche) as well as nectin-4, clone ab192033 (Abcam 1/1000). These antibodies were shipped ready to use. Tissue samples with prostatic cancer were used as a control for AMACR, and benign prostatic glands in the same tissue provided the negative control. Tumor cytoplasmic granular staining was considered positive for AMACR; it was negative in benign prostate gland tissue. Weak granular staining was seen with AMACR in ASAP cases, but this staining was considered negative when compared with cancer cases.

The intensity and extent of nectin-4 expression were determined microscopically using the histochemical scoring system (H-score), defined as the product of the staining intensity (score: 0–3) and percentage of stained cells (0%–100%) at a given intensity [8]. Specimens were then classified as negative (0; H-score: 0–14), weak (1+; H-score: 15–99), moderate (2+; H-score: 100–199), or strong (3+; H-score: 200–300). We also performed immunohistochemical staining of human placenta tissues as a positive control for nectin-4. The immunohistochemical staining and analyses were performed by an experienced pathologist.

### Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, USA) was used for statistical analysis of the research data. The descriptive statistics section presents the categorical variables as numbers and percentages, and continuous variables are shown as medians (smallest–largest value). Pearson's Chi-square test was used to compare nectin-4 and AMACR between pathological samples. The statistical significance level was determined at $p<0.05$.

**Table 1.** Clinicopathological characteristics of patients with prostate cancer and atypical small acinar proliferation

| | n | % |
|---|---|---|
| Age, years, median (min–max) | 67.5 (40–88) | |
| PSA, ng/dL, median (min–max) | 9.4 (1.1–149.7) | |
| Pathology | | |
| ASAP | 25 | 30.5 |
| PCa, Gleason score | | |
| <7 | 24 | 29.3 |
| 7 | 18 | 22 |
| ≥8 | 15 | 18.2 |
| Nectin-4 expression (+/-) | 25 | 30.5 |
| AMACR expression (+/-) | 57 | 69.5 |

Min: Minimum; Max: Maximum; PSA: Prostate specific antigen; ASAP: Atypical small acinar proliferation; Pca: Prostate cancer.

## RESULTS

This study examined the prostate samples of 82 male patients with a median age of 67.5 years (range: 40–88). The median PSA value was 9.4 (1.1–149.7) ng/dL. We found 25 (30.5%) tissue samples containing ASAP and 57 tissue samples containing prostate cancer, with 24 of the total samples (29.3%) having GS of <7, 18 (22%) having GS of 7, and 15 (18.2%) having GS of ≥8 (Table 1). We conducted immunohistochemical analysis of nectin-4 and AMACR expression in all 82 samples. The AMACR staining in ASAP, prostate cancer tissues, and benign prostatic tissues is shown in Fig. 1A–C. The association of nectin-4 and AMACR expression with pathological samples is presented in Table 2. While AMACR expression was positive in prostate cancer tissues with GS of <7 (n=24, 100%), 7 (n=18, 100%), and ≥8 (n=15, 100%), it was negative in all ASAP samples (n=25, 100%) (p<0.001). The typical pattern of nectin-4 expression in ASAP and benign prostatic tissue samples is shown in Fig. 1D–F. While nectin-4 expression was not detected in any of the GS <7, GS 7, or GS ≥8 samples, it was found in all 25 (100%) ASAP samples (p<0.001) (Fig. 1G and H).

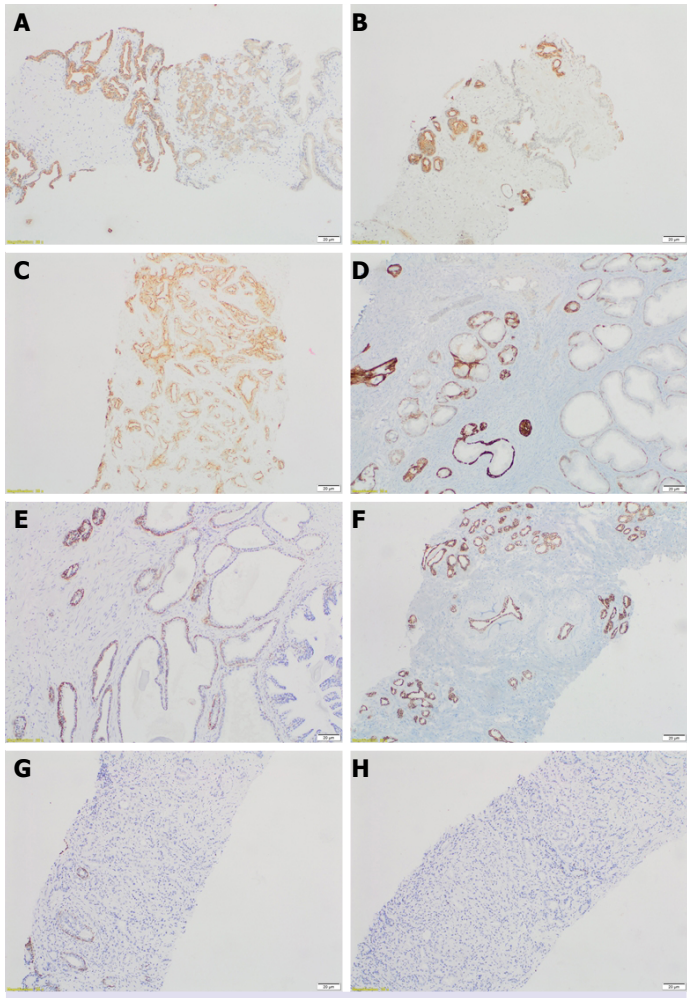


**Figure 1.** **(A)** ASAP foci with weak granular staining with AMACR (×20). **(B)** Prostate adenocarcinoma foci staining positive with AMACR and negative staining in the surrounding benign prostate gland (×20). **(C)** Positive staining in prostate adenocarcinoma with AMACR (×20). **(D)** Benign prostate glands stained with nectin-4 (×20). **(E)** ASAP foci stained with nectin-4 (×40). **(F)** ASAP foci stained with nectin-4 (×20). (**G** and **H**) No staining with nectin-4 in prostate adenocarcinoma foci, staining with nectin-4 in benign prostate gland around the tumor and within the tumor (×20).

## DISCUSSION

In the recent years, nectin-4 has been shown to be a potential cancer biomarker in many types of human cancer and has emerged as a promising target agent in cancer therapy [7, 9, 10]. To our knowledge, although nectin-4 expression in prostate tissue has been investigated in an animal model, its expression in human prostate tissue has not been investigated before this study. Unlike in other cancer types, we detected no nectin-4 expression in human prostate cancer tissue, while, interestingly, we found nectin-4 expression only in ASAP-containing human prostate tissues.

The nectin family of proteins is immunoglobulin-like cell adhesion molecules that are involved in many physiological processes, such as adhesion, dif-

Table 2. Association of nectin-4 and AMACR expression with pathological samples

| | ASAP (n=25) | GS <7 (n=24) | GS 7 (n=18) | GS ≥8 (n=15) | p |
|---|---|---|---|---|---|
| Nectin-4 expression | | | | | |
| + | 25 (100) | 0 (0) | 0 (0) | 0 (0) | <**0.001** |
| - | 0 (0) | 24 (100) | 18 (100) | 15 (100) | |
| AMACR expression | | | | | |
| + | 0 (0) | 24 (100) | 18 (100) | 15 (100) | <**0.001** |
| - | 25 (100) | 0 (0) | 0 (0) | 0 (0) | |

AMACR: Alpha-methylacyl-CoA racemase; ASAP: Atypical small acinar proliferation; GS: Gleason score.

ferentiation, immunomodulation, and migration [5, 11]. Nectin-4, a member of this family, triggers tumor angiogenesis, proliferation, and epithelial-to-mesenchymal transition and thus leads to tumor migration, invasion, and metastasis [9]. In breast and oral cancers, research has shown that the extracellular domain of nectin-4 induces angiogenesis through AKT/PI3k in neighboring cells and increases nitric oxide (NO) levels by regulating eNOS signaling, leading to vascularization and angiogenesis [12, 13]. In addition, nectin-4 is known to promote cancer cell survival by translocating to the nucleus and potentializing DNA repair [6]. It is also responsible for the formation of distant metastasis by some pathways in lymphatic endothelial cells [6], and it has been found to be a risk factor for relapse in breast cancer [14]. Nectin-4 is also known that it worsens the prognosis of urothelial carcinoma and causes resistance to chemotherapy by acting through the PI3K/AKT pathway [10].

In an animal study in canines, Della Salda et al. [15] investigated nectin-4 expression in normal prostate, benign prostatic hyperplasia (BPH), primary prostate cancer, and metastatic prostate cancer tissues. High m-nectin-4 (membranous) and moderate c-nectin-4 (cytoplasmic) staining were found in tissues with metastatic prostate cancer. C-nectin-4 expression was significantly increased in tissues with primary prostate cancer and metastatic prostate cancer compared to BPH cases ($p<0.0001$). Of note, the study found moderate expression in normal and BPH tissues. Therefore, the authors suggest that m-nectin-4 may play a role in canine prostate tumorigenesis and metastasis. In contrast to Della Salda et al.'s [15] study, we detected nectin-4 expression in ASAP-containing tissues but not in cancerous prostatic tissues. This discrepancy may be explained by the difference in antigenicity in human and animal tissues or by the clone difference of the immunohistochemical stain used.

ASAP, a pathological entity defined as the proliferation of small acini without sufficient histological atypia to warrant a definitive diagnosis of prostate adenocarcinoma, is seen in 1.5–9% of primary prostate biopsies [16–18]. The presence of this lesion may be a source of concern for both the pathologist and the urologist in terms of making the correct diagnosis, detecting the cancer and determining follow-up intervals. ASAP, which is not a precancerous lesion, is actually a cause of high suspicion for prostate carcinoma when histopathological data are not sufficient to make a diagnosis of the prostate cancer or the tissue cannot be adequately sampled [19]. The most important reason for this situation is that ASAP shows a low degree of atypia and has very few atypical glands [17]. In the presence of such a suspicious lesion in prostate biopsy, pathologists should examine nuclear enlargement, nucleolus prominence, presence of hyperchromasia, cytoplasmic amphophilia, luminal acellular secretions, crystalloids, infiltrative growth pattern, and atrophy parameters. To clarify the diagnosis of ASAP, clinicians should examine the suspicious focus for nuclear enlargement ≥2-fold compared to the surrounding benign epithelium, shrinkage of the glands, and prominence of the nucleolus in focal areas. When these areas are immunohistochemically evaluated with basal cell markers and AMACR stainings, some loss of basal cell markers and weak granular staining with AMACR are revealed. This staining is considered negative compared to cancer tissues. It should be noted that the evaluation of these criteria is influenced by factors such as fixation, section thickness, and routine staining procedures [20].

The present study found nectin-4 staining in ASAP and benign prostate tissues but not in prostate cancer cases. There are several possible explanations for this outcome. It may be explained by the fact that nectin-4 is an adhesion molecule, and basal cells are not completely lost in ASAP cases. Another possible explanation is that these suspicious glands with ASAP have not completed their carcinogenesis. With better prostatic tissue sampling and additional immunohistochemical staining, differentiating the diagnosis of ASAP from cancer may become an easier matter for the pathologist, enabling the urologist to continue patient follow-up and treatment more confidently, and the diagnosis can be made without the need for repeat biopsies. As we found in our study, nectin-4 can be used to make a definitive diagnosis in tissues suspected of containing ASAP. We believe that the findings of our study represent an important step for future studies investigating the relationship between nectin-4 and prostate cancer.

### Study Limitations

Our study has some limitations that should be highlighted. First, it had a retrospective design. Second, we analyzed a limited number of cases. Third, the prostate tissues analyzed in our study were TRUS biopsy and TUR-P materials, not radical prostatectomy materials. Last but not least, we did not evaluate the relationship between nectin-4 and the patients' prognosis. Further studies using radical prostatectomy material and including patient prognosis will contribute to a better understanding of the relationship between prostate cancer and nectin-4 expression.

### Conclusion

This retrospective study did not detect the expression of nectin-4 in prostate cancer tissues but found that it was expressed in ASAP and benign prostate gland-containing tissues. We believe that prospective studies with more patients and with samples that include radical prostatectomy materials will more clearly reveal the relationship between nectin-4 and prostate cancer.

**Ethics Committee Approval:** The Aksaray University Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 23.06.2022, number: 2022/12-15).

**Conflict of Interest:** No conflict of interest was declared by the authors.

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