



CD44 rs13347C>T Variants in 3'UTR and Prostate Neoplasms: A Case-control Study and Bioinformatics Approach

Emadoddin Moudi¹,  Mohammadkazem Heydari^{2*},  Abasalt Hosseinzadeh Colagar² 

1. Clinical Research Development Unit of Shahid Beheshti Hospital, Babol University of Medical Sciences, Babol, Mazandaran, Iran.

2. Department of Molecular and Cell Biology, Faculty of Science, University of Mazandaran, Babolsar, PC: 47416-95447, Mazandaran, Iran.

Article type: ABSTRACT

Original Article

CD44, a cell-surface receptor and a key player in cellular signaling, can act as both tumor suppressor and promoter. This study aimed to investigate the association of *CD44* rs13347C>T variants with prostate neoplasms, including both benign prostatic hyperplasia (BPH) and prostate cancers using a case-control and bioinformatics approach. Genomic DNA was extracted from 545 blood samples (225 BPH, 225 prostate cancers, and 95 control) and the *CD44* rs13347C>T genotypes were identified using PCR-RFLP. We explored miRNA interactions using the miRNASNP-v3 database and GeneMANIA for co-expression networks. Results showed cancer patients had significantly higher PSA levels compared to both controls (p= 0.03) and BPH (p= 0.01). Additionally, digital rectal examination-positive and smoker BPH patients showed significantly the increased cancer risk (p= 0.004, p= 0.046). Prostate cancer group indicated significantly higher frequency of *CD44* rs13347C>T mutant allele compared to control and BPH groups, particularly in TT and CT+TT genotypes (p < 0.05). miRNA SNP-v3 database predicted the mutant allele of *CD44* rs13347C>T could lose 1 and gain 6 miRNAs for a new site created. Co-expression analysis revealed a direct interaction between CD44 and aryl hydrocarbon receptor (AHR), a gene known to be dysregulated in smokers. Furthermore, these genes alone display co-expression interactions with integrin subunit alpha 4 (ITGA4), protein plays a paradoxical role, both suppressing and promoting tumors. Based on the findings, the mutant allele of *CD44* rs13347C>T may disrupt miRNA binding, which may potentially impact CD44, AHR, and ITGA4 expression in smokers, possibly contributing to prostate cancer progression.

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*Corresponding: Mohammadkazem Heydari

Address: Department of Molecular and Cell Biology, Faculty of Science, University of Mazandaran, Babolsar, PC: 47416-95447, Mazandaran, Iran.

E-mail: Mohamadkazem_hs@yahoo.com



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Introduction

The prostate can develop two main types of abnormal cell growth including benign prostatic hyperplasia (BPH) and malignant tumors. BPH involves an enlargement of the prostate gland due to an increase in non-cancerous cells. Malignant tumors, like prostate cancer, are characterized by uncontrolled growth and division of abnormal cells, with the potential to spread beyond the prostate (1). Prostate cancer is a major cause of death among men in the developed countries, ranking as the fifth leading cause of death worldwide (1, 2). The risk factors for prostate cancer include differences in lifestyle, ethnicity, and family history, as well as other factors like hormonal factors and genetics (2, 3, 4). One of the most important genetic variations in disease and cancer susceptibility is a single nucleotide polymorphism (SNP). An SNP is a single base substitution within a gene sequence, which can potentially lead to unwanted phenotypes associated with various diseases (5). Previous studies have identified various SNPs linked to prostate cancer in genes such as *MMPs* (6), *IL-6* (7), *AR* (8), *CYP17* (9), *GSTT1* (10), and *CD44* (11).

CD44 is a type I single-pass transmembrane cell surface glycoprotein receptor with multiple names, including Homing cell adhesion molecule (HCAM), Phagocytic glycoprotein-1 (Pgp-1), Hermes antigen, and Lymphocyte homing receptor. The *CD44* gene spans approximately 443,500 base pairs (bp) and resides on the short arm of chromosome 11 (11p13) in humans. It encodes a transcript with 20 exons, of which the largest variant (NM_000610.4) includes 10 constant exons (1-5 and 16-20) and 10 alternatively spliced variable exons (6-15), resulting in a maximum length of 5437 bp. The *CD44* transcript containing only the 10 constant exons is known as standard-CD44 (sCD44). Each of the 10 variable exons (6-15) can be included or excluded through alternative splicing, generating distinct transcripts named v1-v10 of CD44, respectively (11).

CD44, a well-known cancer stem cell marker, acts as a cell adhesion molecule and a regulator of signaling pathways (12). Its extracellular region of this cell surface receptor can bind to various ligands, including protein receptors such as EGF and TGF- β (13, 14), as well as extracellular matrix components such as serglycin, osteopontin, and collagen (15, 16, 17). Although hyaluronan is considered the major ligand, the ability of CD44 to interact with this wide range of molecules highlights its versatility in mediating cell-cell and cell-matrix interactions (18). These interactions lead to the activation of MMPs, cleavage of the stem loop, and its free intracellular domain (19). The cleaved cytoplasmic domain of CD44 (CD44ICD) can translocate to the nucleus and influence cellular functions. Previous studies showed dual functions of CD44 in normal and cancerous cell such as survival, migration, homing, invasion and metastasis (18, 19). The specific interactions between CD44 and its ligands play a crucial role in its diverse functions (20). For example, high molecular weight HA (Hyaluronic acid) leads to cell-matrix adhesion in normal tissue, supporting structural integrity and cell communication. Conversely, low-molecular-weight HA fragments can activate different signaling pathways in cancer cells, potentially leading to enhanced survival, proliferation, migration, invasion, and metastasis (20).

Several studies demonstrate connections between various *CD44* variants and cancer (21, 22, 23). For instance, replacing sCD44 with v6CD44 in prostate cancer cells increased their survival and adhesion,

potentially facilitating disease progression (22). Additionally, high expression of v6CD44 serves as a marker for aggressive prostate cancer, highlighting its potential clinical relevance (23). Multiple studies have identified single nucleotide polymorphisms (SNPs) within the *CD44* gene as potential markers for progression in various cancers. These include rs187115T>C (24) associated with esophageal squamous cell carcinoma, rs353639T>G linked to bladder cancer (25), and rs8193C>T implicated in both gastric and prostate and (26, 11). The *CD44* rs13347C>T variant has been associated with high molecular weight HAh increased risk for developing various cancers, including head and neck cancers like nasopharyngeal carcinoma (27) and hepatocellular carcinoma (28), as well as other malignancies such as breast cancer (29), colorectal cancer (30), and bladder cancer (25).

This diverse range of tissues affected by the *CD44* rs13347C>T polymorphism suggests that it potentially regulates complex mechanisms in cancer development and potentially other pathways influencing tumorigenesis. Therefore, this study aims to address potential understudied aspects of this association in prostate neoplasms, including both BPH and prostate cancers, in a case-control and bioinformatics study conducted within the Mazandaran province (Northern Iran).

Materials and methods

Samples collection

Participants for the control group were asymptomatic individuals undergoing routine annual examinations. Selection criteria included documented absence of prostate-related symptoms and a serum PSA level below 4 ng/mL based on medical history review. Individuals with symptoms suggestive of prostate disease were evaluated by an urologist. Confirmed BPH cases were then selected for the BPH group based on medical history review and a documented PSA level below 4 ng/mL. Similarly, confirmed prostate cancer cases were selected for the cancer group based on a documented PSA level exceeding 4 ng/mL and concordant pathological findings. Additional relevant clinical data, such as age, BMI, digital rectal examination or DRE (categorized as negative for ≤ 3 and positive for > 3), smoking history, family history, and Gleason scores, were extracted from medical records for further analysis. To ensure reliable results, we determined the minimum sample size for this study based on the established prevalence of prostate cancer (31). Using a standard biostatistics equation ($n = [Z^2P(1-P)] \div d^2$), where P represents the disease prevalence (6.8% in this case), Z signifies the confidence level (95% here), and d indicates the margin of error (set at 0.05), we calculated that a minimum of 98 participants is required. A total of 545 blood samples were collected and analyzed in this study. The samples were derived from three distinct groups including the BPH group with $n = 225$, the prostate cancer group with $n = 225$, and the control group with $n = 95$. Peripheral blood samples (5 mL) were collected from all participants following venipuncture. The blood was collected in laboratory sample tubes containing EDTA_{Na2} as an anticoagulant and stored at -20 °C. All samples were obtained from hospitals in Babol, Mazandaran province, Iran, between September 2017 and May 2023. The participating hospitals were Shahid Beheshti, Babol Clinic, and Rhoani Hospital in Babol. Participants resided in Mazandaran province, primarily in the cities of Amol, Babol, and Qaem Shahr and all of them were over 50 years old. This study was approved by the Ethics Committee of Mazandaran University of

Medical Sciences (#IR.UMZ.REC.1397.056) and all subjects signed informed consent form before entering the study.

DNA extraction and genotyping

The first step involves isolating DNA from white blood cells (WBCs). Blood samples are first treated with red blood cell (RBC) lysis buffer (155 mM NH₄Cl, 12 mM NaHCO₃, 0.1 mM EDTA_{Na2}) to remove erythrocytes. Subsequently, DNA is extracted from the remaining WBCs using a standard phenol-chloroform extraction method (32). *CD44* rs13347C>T genotypes were identified using PCR-RFLP. PCR primers flanking the SNP were designed using Primer3Plus (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) based on data from the NCBI database (Table 1, Fig. 1A and B). Genotyping was achieved by digesting PCR products with the *HinIII* restriction enzyme (Fig. 1B). PCR fragments were obtained under table 1 conditions in 25 μl of PCR total volume, by DNA thermal cycler (Master Cycler Gradient; Eppendorf Co., Germany). All PCR reactants and chemical materials were purchased from SinaColon Co (Iran) and Merck Co. (Germany), respectively. PCR products and digested fragments were separated by electrophoresis on a 1.2% agarose gel stained with ethidium bromide (1 μg/mL) and visualized using a UV transilluminator (ProteinSimple Red SA-01587, Co, USA).

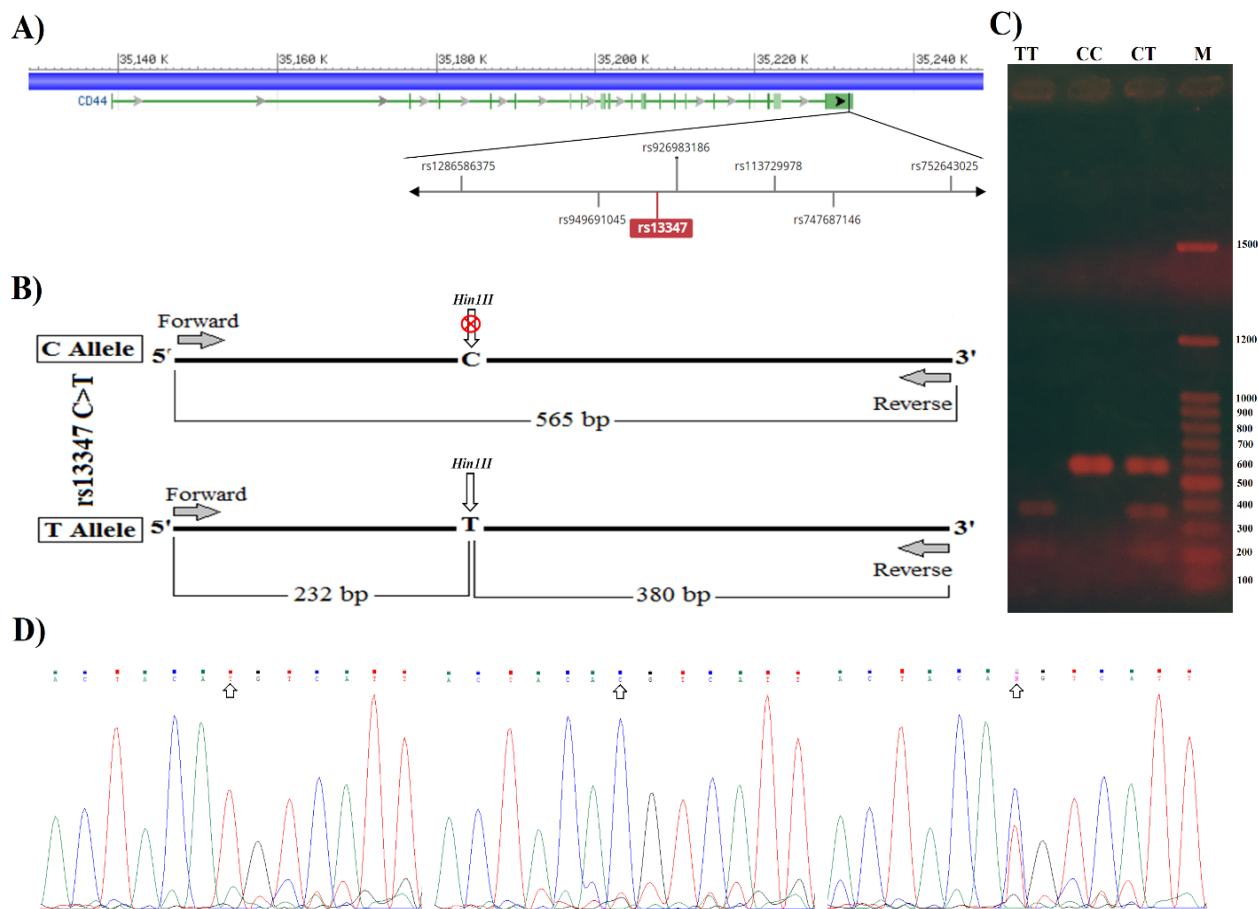


Fig. 1. CD44 rs13347C>T location, RFLP mapping, agarose gel profile, and DNA sequencing: A) The CD44 rs13347C>T is located in the 3'UTR (NM_000610.4); B) A schematic RFLP map of CD44 rs13347C>T and digestion of PCR products with *HinIII*

restriction enzymes; C) Digestion of PCR products with *HinIII* with distinct band patterns on a 1.2% agarose gel corresponding to CC, CT, and TT genotypes. Bands for each genotype were identified based on the 100-1500 bp DNA marker (SinaColon Co, Iran); D) Sequencing chromatograms for each PCR product with genotypes CC, CT, and TT.

Table 1. Oligomers used as primers and PCR conditions.

| Names | Oligomers (5'→3') | PCR conditions | Cycles and thermal conditions | PCR product |
|-----------------|----------------------------------|--|--|-------------|
| <i>F13347CT</i> | 5'- CTGTTGTAGTCCC TCACTTGG | 0.25 U, <i>Taq</i> polymerase; 0.2 mM, mix-dNTP; 0.0.5 μM each of forward and reverse primers; 3 mM, MgCl ₂ ; 2.5 μL of 10X PCR buffer (200mM, Tris-Cl, pH 8.4; 200 mM, 500 mM, KCl); and ~30 ng of t-DNA | 4 min. at 94 °C; 30 cycles (45 sec. at 94 °C, 30 sec. at 64 °C, 30 sec. 72 °C); Finally 10 min at 72°C | 565 bp |
| <i>R13347CT</i> | 5'- TTCCTCTCTCCTA CTCCTCTG | | | |

Oligomers: synthesized by CinnaClon Co, Iran; bp: base pair; t-DNA: template DNA

Data sources for in silico analysis

miRNA SNP-v3 database (<http://bioinfo.life.hust.edu.cn/miRNASNP>) was used to check if the *CD44* rs13347C>T creates or removes binding sites for microRNAs (miRNAs). This helps us understand how these SNPs might influence gene expression and potentially be involved in disease. To investigate the interactions of the *CD44* gene with other proteins, the GeneMANIA website (<https://genemania.org/>) was utilized.

Statistical analysis

In this study, Hardy-Weinberg equilibrium (HWE) was evaluated using a χ^2 -test to compare the expected genotype frequencies with observed genotype frequencies (<https://wpcalc.com/en/equilibrium-hardy-weinberg/>). The comparison between allele type and genotype distributions of *CD44* rs13347C>T, in the patients and healthy controls, was confirmed by logistic regression analyses. The odds ratios (ORs) and 95% confidence intervals (CIs) were computed of this analyses and p-value with less than 0.05 was considered significant. All statistical analyses were performed by SPSS ver. 19 (PSS Inc., IBM Corp Armonk, NY, USA).

Results

Comparison of some risk factors

The comparison of PSA levels as a risk factor revealed significant differences between the cancer group and for both the control (p= 0.03) and BPH (p= 0.01) groups. Conversely, no significant difference of PSA levels was observed between the control and BPH groups (p= 0.088). The study also identified a significant association between BPH and cancer in patients who were positive for DRE and smokers with p= 0.004 and 0.046 respectively. In addition, the results showed that the frequency of prostate cancer patients with a Gleason score ≥ 7 is higher (70.66%) than prostate cancer patients with a Gleason score < 7 (29.33%). On the other hand, no significant associations were observed between the groups in factors of age, body mass index (BMI), and family history (Table 2).

Genotype frequency

The results of digesting the amplified fragments with the *HinIII* enzyme in agarose gel revealed three genotypes of *CD44* rs13347C>T, including CC (565 bp), TT (380 and 232 bp), and CT (565, 380, and 232

Table 2. Comparison of some risk factors between control and prostate patient groups (BPH and cancer).

| | Control (n= 95) | BPH (n= 225) | P-value (Control and BPH) | Cancer (n= 225) | P-value (Control and Cancer) | P-value (BPH and Cancer) |
|----------------------------|--------------------|-----------------|---------------------------------|--------------------|------------------------------------|--------------------------------|
| Age (years) | 67.9 ± 1.96* | 70.53 ± 8.81* | 0.365 [#] | 68.38 ± 7.89 | 0.73 [#] | 0.383 [#] |
| BMI | 25.52 ± 1.26* | 25.35 ± 2.60* | 0.849 [#] | 26.15 ± 3.76 | 0.62 [#] | 0.580 [#] |
| PSA (ng ml ⁻¹) | 1.02 ± 0.63* | 1.71 ± 1.1* | 0.088 [#] | 29.23 ± 39.59 | 0.03[#] | 0.01[#] |
| DRE | | | | | | |
| Negative | - | 195 (86.66%) | - | 85 (37.77%) | - | 0.004[†] |
| Positive | - | 30 (13.33%) | - | 140 (62.23%) | - | |
| Smoking | | | | | | |
| Yes | - | 42 (18.66%) | - | 91 (40.44%) | - | 0.046[†] |
| No | - | 183 (81.34%) | - | 134 (59.56%) | - | |
| Familial history | | | | | | |
| Yes | - | 49 (21.77%) | - | 97(43.11) | - | 0.05 [†] |
| No | - | 176 (78.23%) | - | 128 (56.89) | - | |
| Gleason scores | | | | | | |
| <7 | - | - | - | 66 (29.33%) | - | - |
| ≥ 7 | - | - | - | 159 (70.66%) | - | - |

bp), which were confirmed by sequencing (Fig. 1C and 1D). The statistical analyses indicated a significant difference in TT genotypes between the control and cancer groups, as well as between the BPH and cancer groups, with p-values of 0.019 and 0.048, respectively (Table 3). Moreover, it demonstrated a significant difference in the mutant allele T between the cancer group (with a frequency of 34%) and both the control (with a frequency of 22.11%) and BPH (with a frequency of 27.56%) groups, with p-values of 0.003 and 0.038, respectively (Table 3). In contrast, there was no significant correlation observed for the CT genotype between the groups.

In silico analysis

In silico analysis with the miRNASNP-v3 database revealed a potentially significant impact of the *CD44* rs13347C>T polymorphism on miRNA binding. Notably, our results suggest the mutant T allele exhibits a markedly increased capacity for miRNA binding compared to the wild-type C allele, interacting with six predicted miRNAs compared to only one (Table 4).

The results of the gene network interaction between *CD44* and *AHR* on the GeneMANIA website showed that in addition, these two genes have a direct co-expression relationship, they can have direct and

indirect co-expression interaction with other genes (Fig. 2A). *CD44* can directly interact with four genes: *AHR*, *AIP*, *HYAL2*, and *ITGA4* (Fig. 2B). Additionally, *AHR* can directly interact with five proteins: *CD44*, *SELE*, *CD9*, *MAF*, and *ITGA4* (Fig. 2C).

Table 3. Genotype and allele frequencies of *CD44* rs13347C>T were analyzed in case and control samples.

| Genotype | Control (n=95) | BPH (n=225) | P-value OR (95% CI) | Cancer (n=225) | P-value OR(95% CI) | P-value (BPH and Cancer) OR(95% CI) |
|----------|----------------|--------------|------------------------------|----------------|-------------------------------------|-------------------------------------|
| CC | 59 (62.10%) | 121 (53.77%) | Ref | 104 (46.22%) | Ref | Ref |
| CT | 30 (31.57%) | 84 (37.33%) | 0.241 1.365(0.812-2.297) | 89 (39.55%) | 0.51 1.638 (0.998-2.839) | 0.302 1.223 (0.829-1.833) |
| TT | 60 (6.33%) | 20 (8.90%) | 0.323 1.625 (0.620-4.242) | 32 (14.22%) | 0.019 3.026 (1.195-7.658) | 0.048 1.862 (1.004-3.450) |
| CT+TT | 36 (37.90%) | 104 (46.23%) | 0.171 1.409 (0.863-2.300) | 121 (53.77%) | 0.010 1.907 (1.168-3.114) | 0.109 1.354 (0.934-1.961) |
| C-allele | 148 (77.89%) | 326 (72.44%) | Ref | 297 (66%) | Ref | Ref |
| T-allele | 42 (22.11%) | 124 (27.56%) | 0.151 1.340 (0.898-2.000) | 153 (34%) | 0.003 1.815 (1.224-2.693) | 0.038 1.350 (1.016-1.794) |

Table 4. *In silico* analysis of effect CD44 rs13347C>T on the miRNA binding by miRNASNP-v3 database.

| | miRNA | ΔG^* Duplex | ΔG^* Binding | ΔG^* Open | TargetScan score | AU content | Exact probability |
|-------------------------|-----------------|---------------------|----------------------|-------------------|------------------|------------|-------------------|
| Gain¹ | hsa-miR-643 | -11.70 | -9.99 | 10.88 | 23.15 | 0.65 | 0.02 |
| | hsa-miR-3647 | -10.40 | -8.32 | 10.88 | 21.05 | 0.55 | 0.02 |
| | hsa-miR-7852-3p | -9.80 | -9.30 | 9.25 | 20.52 | 0.52 | 0.01 |
| | hsa-miR-425-5p | -8.70 | -9.21 | 9.45 | 21.66 | 0.66 | 0.01 |
| | hsa-miR-3668 | -7.80 | -6.79 | 9.25 | 20.05 | 0.55 | 0.02 |
| | hsa-miR-489-3p | -7.70 | -8.85 | 9.45 | 21.74 | 0.74 | 0.01 |
| Loss² | hsa-miR-4757-3p | -13.30 | -13.56 | 8.91 | 21.62 | 0.62 | 0.00 |

* ΔG energy =kCal/mol; 1= SNP in gene 3'UTR causes target gain; 2= SNP in gene 3'UTR causes target loss; Data retrieved from miRNA SNP-v3 database (<http://bioinfo.life.hust.edu.cn/miRNASNP>) at 18 January 2024.

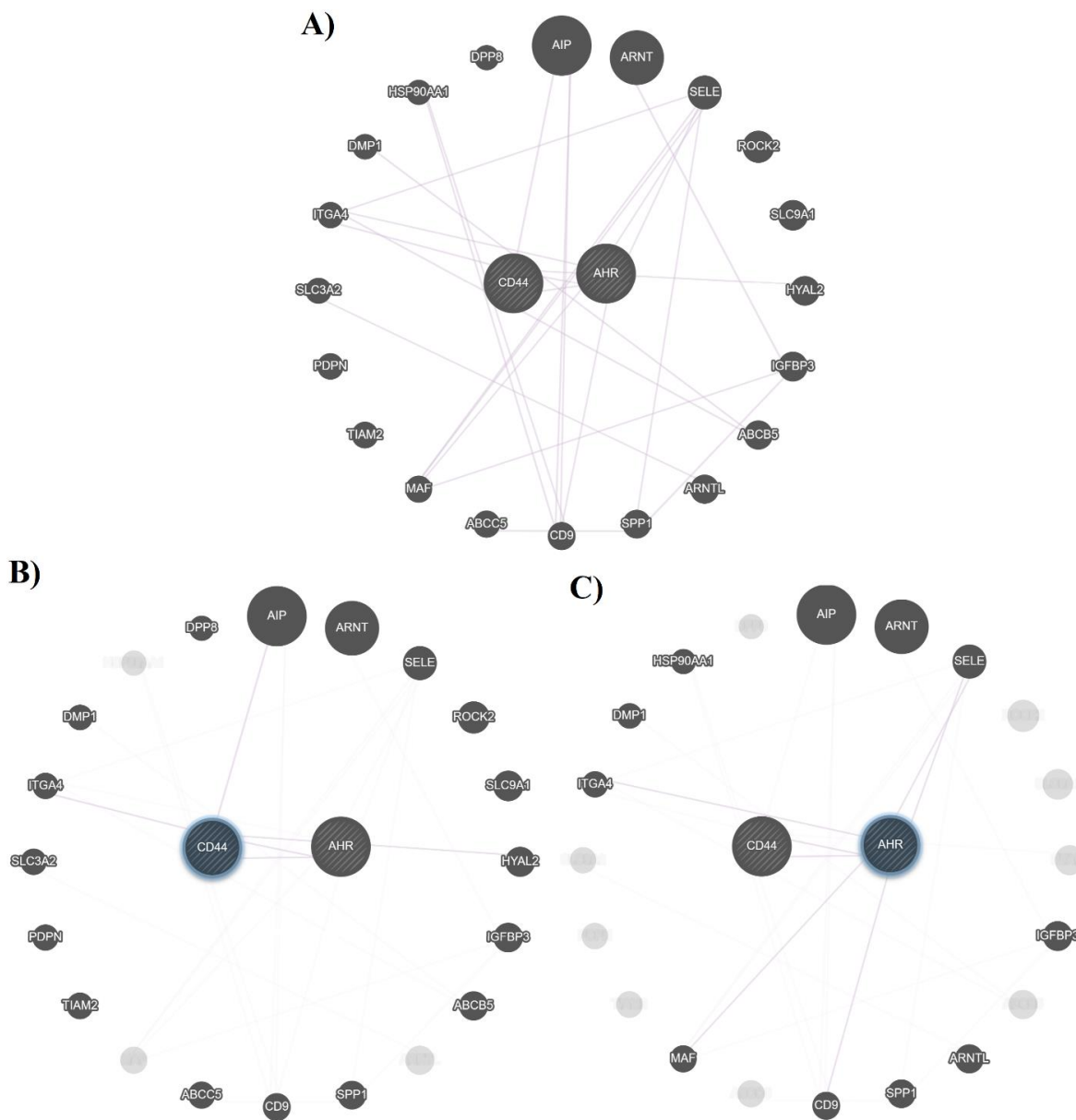


Fig. 2. Co-expression network interaction between CD44 and AHR genes on the GeneMANIA website: A) Co-expression network interaction of CD44 and AHR genes together; B) The direct co-expression network interaction of CD44; C) The direct co-expression network interaction of AHR. Data retrieved from GeneMANIA website (<https://genemania.org/>) at 20 January 2024.

Discussion

Prostate cancer, one of the most common cancers worldwide, ranks second among cancers diagnosed in Iranian men. The number of new cancer cases in Iran is expected to increase to 160,000 in 2025, potentially changing the age-standardized rate (ASR) for prostate cancer to 26.2 from 18.3 cases per 100,000

(33). Although both BPH and prostate cancer share common risk factors such as increasing age, family history of prostate cancer, and hormonal alterations, particularly changes in androgen levels, there are additional factors specific to each condition (2, 3, 4). For BPH, a dietary pattern high in red meat and processed foods may be a contributing factor (2, 3). Conversely, prostate cancer risk exhibits a strong association with race and ethnicity, with African American and Caribbean men demonstrating a higher susceptibility compared to Caucasians (2, 3, 4). Furthermore, obesity is linked to an increased risk of aggressive prostate cancer. Additionally, lifestyle factors such as a diet rich in saturated fats and deficient in fruits and vegetables, coupled with a sedentary lifestyle, might contribute to an elevated risk of prostate cancer development (3, 4).

Despite the benign nature of BPH, studies reveal some factors that might link it to prostate cancer. This suggests that individuals with BPH may have a higher lifetime risk of prostate cancer due to common stimulating factors influencing both conditions (34, 35). This study found that the average age of patients with prostate cancer (68.38 ± 7.89) was younger than those with BPH (70.53 ± 8.81). Although there was no statistically significant association between having cancer and BPH and control groups, this might be due to more severe symptoms prompting earlier referral in cancer patients, compared to those with BPH. Multiple clinical indicators, including PSA levels, DRE findings, and Gleason scores, offer invaluable insights into the malignancies of prostate cancer and inform early detection strategies. Understanding a patient's family history and genetic risk factors, such as ethnicity or specific gene variations, can further tailor screening decisions and treatment plans.

CD44 as a cell-surface glycoprotein is a fascinating protein with diverse roles that contribute to a variety of activities. It interacts with molecules like hyaluronic acid, influencing cell movement and adhesion, which can be crucial in different contexts. This receptor can have contrasting roles depending on the context. In some cases, it can promote cancer progression by aiding cell migration and growth. In others, it can act as a tumor suppressor by limiting cell movement and promoting cell death. Additionally, CD44 acts as a hub for receiving signals from outside the cell, influencing various cellular processes (19).

Numerous variants of *CD44* have been reported so far. Among these, the *CD44* rs13347C>T polymorphism has been associated with several types of cancer (27, 28, 29, 30, 25). Our study found a significant association between the mutant allele of *CD44* rs13347C>T and prostate cancer compared to both the control and BPH groups. High expression of specific *CD44* gene variants has been linked to an increased risk of prostate cancer. Wu et al. (2015) found significantly higher CD44 expression in cancer tissues with the mutant allele of *CD44* rs13347C>T compared to those with the C allele. Wu et al. (2015) and Jiang et al. (2012) found that cancer patients with TT or CT genotypes of *CD44* rs13347C>T had a significantly higher CD44 expression compared to CC genotype. They attributed this increase to the presence of the SNP in the 3'UTR region. This region plays a crucial role in regulating gene expression by serving as a binding site for microRNAs (miRNAs) and transcription factors. They found that the *CD44* rs13347C>T variant disrupts the binding site for miR-509-3p, a miRNA that normally suppresses CD44 expression. This disruption potentially leads to increased CD44 expression in cancer tissue. Our study showed that prostate cancer patients had a significantly higher frequency of the TT and CT+TT genotypes of *CD44* rs13347C>T compared to the control and BPH groups. Notably, this difference was also observed

between prostate cancer and BPH groups. This suggests that this specific nucleotide transition may contribute to the increased expression of CD44 variants associated with prostate cancer development. Furthermore, *in silico* analysis revealed that the *CD44* rs13347C>T variant disrupts the binding site for miR-4757-3p, a miRNA potentially involved in inhibited cancer cell migration and invasion (36). Additionally, it creates binding sites for several miRNAs, including the well-studied hsa-miR-425-5p, a miRNA that potentially may contribute to the malignancy progression of prostate cancer (37, 38). These findings, combined with the observed genotype frequency differences, suggest that this SNP may influence CD44 expression through altered miRNA binding, potentially contributing to prostate cancer progression.

Multiple studies have established CD44 as a key signaling pathway in cancer progression, particularly for its role in cell growth, proliferation, and motility (18, 19). Interestingly, our study identified a statistically significant correlation between the prostate cancer group and the BPH group ($p=0.004$) in abnormality of DRE, abnormal DRE findings include a hard mass or nodule, induration, or asymmetry. Also, previous studies have demonstrated that the level of PSA alone may not reliably predict the prognosis of prostate cancer, as it is influenced more by factors related to the organ rather than by the tumor itself (39, 40). However, the findings of our study indicate a significant difference in average PSA levels between the cancer group and both the normal group ($p=0.03$) as well as the BPH group ($p=0.01$). These findings may be linked to increased CD44 gene expression associated with the *CD44* rs13347C>T variant. A previous study reported that over 80% of patients with high PSA levels also exhibited high CD44 expression (41). In addition, the results of this study showed that in the studied population, there is a significant relationship between the cancer group and BPH in smokers ($p=0.046$). Co-expression interaction was used to investigate the relationship between the *CD44* and *AHR* gene as known to be dysregulated in smokers (42, 43). Our study revealed a potential link between specific gene expressions and a critical pathway involved in cancer progression. The results revealed a direct co-expression pattern between the *CD44* and *AHR* genes, suggesting they may mutually influence each other's expression levels. This finding is particularly intriguing because previous research has established the roles of both genes in cancer development. CD44 is known to be involved in cell adhesion, migration, and proliferation (18, 19), while AHR plays a complex role in various cellular processes, including inflammation, metabolism, and cancer progression (44, 45). Our data suggests that the *CD44* rs13347 C>T variant might influence AHR expression through a cascade of events involving miRNA binding. This specific variant could potentially alter the binding sites for miRNAs that regulate *CD44* expression. Consequently, this altered miRNA binding could lead to changes in *CD44* expression levels, which in turn could trigger downstream signaling pathways affecting *AHR* expression. This potential interplay between *CD44*, *AHR*, and the *CD44* rs13347 C>T variant becomes even more relevant when considering the downstream effects on another gene, *ITGA4*. Interestingly, our findings suggest that alterations in *CD44* and *AHR* expression might influence *ITGA4* expression as well. *ITGA4* holds a unique position in cancer progression, exhibiting a seemingly contradictory dual role (46). On one hand, it can act as a tumor suppressor by hindering cancer cell detachment and invasion. Conversely, it can also facilitate cancer metastasis by enabling binding to specific molecules on endothelial cells.

Our study found the *CD44* rs13347C>T mutant allele associated to prostate cancer, with higher frequency of TT and CT+TT genotypes compared to control and BPH groups. Further, this mutant allele

creates binding site for several miRNAs including hsa-miR-425-5p, a miRNA that may be associated with prostate cancer malignancy. Co-expression analysis revealed *CD44* and *AHR*, a gene dysregulated in smokers, directly interact. The potential *CD44* and *AHR* interaction holds greater importance due to its impact on the expression of *ITGA4*, a protein with a contradictory dual role, acting as both a tumor suppressor and a promoter of progression. Therefore, *CD44* rs13347C>T variant's impact on miRNA binding potentially alters *CD44*, *AHR*, and ultimately *ITGA4* expression.

Declaration of Competing Interest

The authors declare that there are no competing interests associated with the manuscript.

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References

1. Wang L, Lu B, He M, et al. Prostate Cancer Incidence and Mortality: Global Status and Temporal Trends in 89 Countries From 2000 to 2019. *Front Public Health* 2022;10:811044.
2. Leitao C, Matos B, Roque F, et al. The Impact of Lifestyle on Prostate Cancer: A Road to the Discovery of New Biomarkers. *J Clin Med* 2022;11.
3. Ebbesen M, Sorensen KD, Pedersen BG, et al. Ethical Principles in the Analysis of Prostate Cancer Diagnostics. *Cancer Invest* 2022;40:799-810.
4. Sekhoacha M, Riet K, Motloug P, et al. Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches. *Molecules* 2022;27.
5. Allemailem KS, Almatroudi A, Alrumaihi F, et al. Single nucleotide polymorphisms (SNPs) in prostate cancer: its implications in diagnostics and therapeutics. *Am J Transl Res* 2021;13:3868-89.
6. Zhou H, Zhu X. Association between matrix-metalloproteinase polymorphisms and prostate cancer risk: a meta-analysis and systematic review. *Cancer Manag Res* 2018;10:5247-59.
7. Liu TZ, Guo ZQ, Wang T, et al. Meta-analysis of the role of IL-6 rs1800795 polymorphism in the susceptibility to prostate cancer: Evidence based on 17 studies. *Medicine (Baltimore)* 2017;96:e6126.
8. Weng H, Li S, Huang JY, et al. Androgen receptor gene polymorphisms and risk of prostate cancer: a meta-analysis. *Sci Rep* 2017;7:40554.
9. Xu Wz, Xiao L, Yang WU, et al. Association Between CYP17 rs743572 Polymorphism and Prostate Cancer Risk: Systematic Review With Meta-Analysis and Trial Sequential Analysis. *J Int Transl Med* 2018;6:45-54.
10. Zhou TB, Drummen GP, Jiang ZP, et al. GSTT1 polymorphism and the risk of developing prostate cancer. *Am J Epidemiol* 2014;180:1-10.
11. Heydari M, Hosseinzadeh Colagar A, Moudi E. Mutant Allele of CD44 (rs8193C>T) and Pum2 Regulatory Element as A Prognosis Factor of Prostate Neoplasms: A Case-Control and In Silico Studies. *Cell J* 2022;24:723-31.
12. Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 2003;4:33-45.
13. Yin J, Zhang H, Wu X, et al. CD44 inhibition attenuates EGFR signaling and enhances cisplatin sensitivity in human EGFR wild-type non-small-cell lung cancer cells. *Int J Mol Med* 2020;45:1783-92.

14. Park NR, Cha JH, Jang JW, et al. Synergistic effects of CD44 and TGF-beta1 through AKT/GSK-3beta/beta-catenin signaling during epithelial-mesenchymal transition in liver cancer cells. *Biochem Biophys Res Commun* 2016;477:568-74.
15. Toyama-Sorimachi N, Sorimachi H, Tobita Y, et al. A novel ligand for CD44 is serglycin, a hematopoietic cell lineage-specific proteoglycan. Possible involvement in lymphoid cell adherence and activation. *J Biol Chem* 1995;270:7437-44.
16. Weber GF, Ashkar S, Glimcher MJ, et al. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). *Science* 1996;271:509-12.
17. Knutson JR, Iida J, Fields GB, et al. CD44/chondroitin sulfate proteoglycan and alpha 2 beta 1 integrin mediate human melanoma cell migration on type IV collagen and invasion of basement membranes. *Mol Biol Cell* 1996;7:383-96.
18. Chen C, Zhao S, Karnad A, et al. The biology and role of CD44 in cancer progression: therapeutic implications. *J Hematol Oncol* 2018;11:64.
19. Senbanjo LT, Chellaiiah MA. CD44: A Multifunctional Cell Surface Adhesion Receptor Is a Regulator of Progression and Metastasis of Cancer Cells. *Front Cell Dev Biol* 2017;5:18.
20. Monslow J, Govindaraju P, Pure E. Hyaluronan - a functional and structural sweet spot in the tissue microenvironment. *Front Immunol* 2015;6:231.
21. Todaro M, Gaggianesi M, Catalano V, et al. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. *Cell Stem Cell* 2014;14:342-56.
22. Gupta A, Zhou CQ, Chellaiiah MA. Osteopontin and MMP9: Associations with VEGF Expression/Secretion and Angiogenesis in PC3 Prostate Cancer Cells. *Cancers (Basel)* 2013;5:617-38.
23. Ni J, Cozzi PJ, Hao JL, et al. CD44 variant 6 is associated with prostate cancer metastasis and chemo-/radioresistance. *Prostate* 2014;74:602-17.
24. Liu Y, Qing H, Su X, et al. Association of CD44 Gene Polymorphism with Survival of NSCLC and Risk of Bone Metastasis. *Med Sci Monit* 2015;21:2694-700.
25. Verma A, Kapoor R, Mittal RD. Cluster of Differentiation 44 (CD44) Gene Variants: A Putative Cancer Stem Cell Marker in Risk Prediction of Bladder Cancer in North Indian Population. *Indian J Clin Biochem* 2017;32:74-83.
26. Mokhtarian R, Tabatabaeian H, Saadatmand P, et al. CD44 Gene rs8193 C Allele Is Significantly Enriched in Gastric Cancer Patients. *Cell J* 2020;21:451-8.
27. Lou F, Ma HN, Xu L, et al. Two polymorphisms of CD44 3'UTR weaken the binding of miRNAs and associate with nasopharyngeal carcinoma in a Chinese population. *Eur Rev Med Pharmacol Sci* 2014;18:2444-52.
28. Deng Y, Chen ZJ, Lan F, et al. Association of CD44 polymorphisms and susceptibility to HBV-related hepatocellular carcinoma in the Chinese population. *J Clin Lab Anal* 2019;33:e22977.
29. Jiang L, Deng J, Zhu X, et al. CD44 rs13347 C>T polymorphism predicts breast cancer risk and prognosis in Chinese populations. *Breast Cancer Res* 2012;14:R105.
30. Wu XM, Yang HG, Zheng BA, et al. Functional Genetic Variations at the microRNA Binding-Site in the CD44 Gene Are Associated with Risk of Colorectal Cancer in Chinese Populations. *PLoS One* 2015;10:e0127557.
31. Hosseinzadeh Colagar A, Arjmand M, Heydari M, et al. COL12A1-rs970547, Mutant Allele Advantage by Being Protective against Recurrent Spontaneous Abortion. *J Genet Resour* 2023;9:59-68.
32. Sambrook J, Russell DW. *Molecular cloning: a laboratory manual*. 3th ed. New York: Cold Spring Harbor Laboratory; 2013.
33. Roshandel G, Ferlay J, Ghanbari-Motlagh A, et al. Cancer in Iran 2008 to 2025: Recent incidence trends and short-term predictions of the future burden. *Int J Cancer* 2021;149:594-605.

34. Miah S, Catto J. BPH and prostate cancer risk. *Indian J Urol* 2014;30:214-8.
35. Taheri M, Habibi M, Noroozi R, et al. HOTAIR genetic variants are associated with prostate cancer and benign prostate hyperplasia in an Iranian population. *Gene* 2017;613:20-4.
36. Zhao P, Zhao Q, Chen C, et al. miR-4757-3p Inhibited the Migration and Invasion of Lung Cancer Cell via Targeting Wnt Signaling Pathway. *J Oncol* 2023;2023:6544042.
37. Zhang JY, Su XP, Li YN, et al. MicroRNA-425-5p promotes the development of prostate cancer via targeting forkhead box J3. *Eur Rev Med Pharmacol Sci* 2019;23:547-54.
38. Rode MP, Silva AH, Cisilotto J, et al. miR-425-5p as an exosomal biomarker for metastatic prostate cancer. *Cell Signal* 2021;87:110113.
39. Kim HW, Ko YH, Kang SH, et al. Predictive Factors for Prostate Cancer in Biopsy of Patients with Prostate-Specific Antigen Levels Equal to or Less Than 4 ng/ml. *Korean J Urol* 2011;52:166-71.
40. Choi MH, Ha US, Park YH, et al. Combined MRI and PSA Strategy Improves Biopsy Decisions Compared with PSA Only: Longitudinal Observations of a Cohort of Patients with a PSA Level Less Than 20 ng/mL. *Acad Radiol* 2023;30:509-15.
41. Damarasingu PV, Das S, Mh S, et al. Evaluation of CD44 Expression in Prostatic Adenocarcinoma: An Institutional Study. *Cureus* 2023;15:e40510.
42. Anderson RA, McIlwain L, Coutts S, et al. Activation of the aryl hydrocarbon receptor by a component of cigarette smoke reduces germ cell proliferation in the human fetal ovary. *Mol Hum Reprod* 2014;20:42-8.
43. Szymanowska-Narloch A, Jassem E, Skrzypski M, et al. Molecular profiles of non-small cell lung cancers in cigarette smoking and never-smoking patients. *Adv Med Sci* 2013;58:196-206.
44. Ide H, Lu Y, Yu J, et al. Aryl hydrocarbon receptor signaling involved in the invasiveness of LNCaP cells. *Hum Cell* 2017;30:133-9.
45. Opitz CA, Holfelder P, Prentzell MT, et al. The complex biology of aryl hydrocarbon receptor activation in cancer and beyond. *Biochem Pharmacol* 2023;216:115798.
46. Klemke M, Weschenfelder T, Konstandin MH, et al. High affinity interaction of integrin alpha4beta1 (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1) enhances migration of human melanoma cells across activated endothelial cell layers. *J Cell Physiol* 2007;212:368-74.