Aberrant Expression of Forkhead Box Proteins in Prostate Cancer Development

Forkhead Box Proteinlerinin Prostat Kanser Gelişiminde Değişmiş İfadeleri

Cigdem Donmez, Ece Konac

Department of Medical Biology and Genetics, Faculty of Medicine, Gazi University, Besevler, 06510, Ankara, Turkey

ABSTRACT

Prostate cancer is one of the most common types of cancer in men. The evolutionarily-conserved Forkhead-box (FOX) family proteins are able to bind to promoters and enhancers in a sequence-specific way to regulate gene expression. FOX proteins participate in the most diverse range of biological functions from genetic diseases to cancers. Recent studies over the past few years demonstrated the dysregularity of the FOX family proteins which lead to prostate cancer pathogenesis and their role as an oncogene or tumor suppressor in prostate cancer. In addition, experimental studies have shown that the dysregulation of FOX proteins is associated with cancer initiation, proliferation, migration, invasion, metastasis and survival. In this review, we summarized the roles of FOX proteins in the pathogenesis of prostate cancer and evaluated their potential as targets for therapeutic intervention.

Key Words: Forkhead box protein, oncogene, tumor suppressor, prostate cancer

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ÖZET

Prostat kanseri erkeklerde en sık görülen kanser türlerinden biridir. Evrimsel olarak korunmuş olan Forkhead-box (FOX) ailesi proteinleri, gen ekspresyonunu düzenlemek için promotörler ve güçlendiriciler üzerine diziye özgü bir şekilde bağlanabilir. FOX proteinleri genetik hastalıklardan kanserlere kadar çok çeşitli biyolojik fonksiyonlara katılır. Son birkaç yıldaki çalışmalar, prostat kanseri patogenezine yol açan FOX ailesi proteinlerinin düzensizliğini ve onların prostat kanserinde onkogen veya tümör baskılayıcı rollerini göstermiştir. Ayrıca, deneysel çalışmalar FOX proteinlerinin düzensizliğinin kanser başlatma, proliferasyon, migrasyon, invazyon, metastaz ve sağkalım ile ilişkili olduğunu göstermiştir. Bu derlemede, prostat kanseri patogenezinde FOX proteinlerinin rollerini özetleyerek, terapötik müdahale hedefleri olarak potansiyellerini değerlendirdik.

Anahtar Sözcükler: Forkhead box protein, onkogen, tümör supresör, prostat kanser

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ORCID IDs: C.D.0000-0002-2310-2718, E.K. 0000-0001-5129-2515

Address for Correspondence / Yazışma Adresi: Prof. Dr. Ece Konac (Ph. D.) Department of Medical Biology and Genetics, Faculty of Medicine, Gazi University, Besevler, 06500, Ankara, Turkey E-mail: ecemercanoglu@yahoo.com

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INTRODUCTION

Prostate cancer is one of the leading causes of cancer-related deaths among men in the world. According to Global Cancer Statistics 2018 from GLOBOCAN, approximately 1.3 million men are diagnosed annually with prostate cancer worldwide and more than 300,000 die despite treatment (1). Prevalence of prostate cancer is related to advanced age, ethnicity, family history, genetic and epigenetic factors, hormones, eating habits and environmental carcinogens. Despite recent improvements in the management of prostate cancer, its treatment is still a challenging issue in the clinical setting due to metastasis and recurrence. Therefore, it is necessary to understand better the molecular mechanisms in initial development and progression of prostate cancer in order to identify appropriate biomarkers for early diagnosis and treatment methods.

Forkhead box (FOX) proteins form a large family of transcription factors (TFs) that are characterized by approximately 110 evolutionary-conserved amino-acid-long winged helix DNA-binding domain (DBD) called forkhead or winged-helix because of its butterfly-like winged structure. The DBD of the FOX proteins consists of two wing-like loops (W1 and W2), three α-helices (H1, H2 and H3) and three β-sheets (S1, S2 and S3). It is also involved in chromatin remodeling and nuclear localization (2). A research conducted by Weigel et al. in 1989 identifies the first FOX gene in *Drosophila melanogaster*. This gene is called forkhead (FKH) as it takes the shape of a fork-head in *Drosophila* when mutated (3). Since then, fifty different FOX proteins identified in humans have been divided into 19 subfamilies (FOXA to FOXS) based on their protein sequence homology (2). It has been shown that FOX proteins family play important roles in a wide variety of biological processes, such as cell proliferation, differentiation, migration, invasion, survival, apoptosis and DNA damage repair. FOX proteins family is expressed differentially in prostate cancer. For this reason, dysregulation of some FOX proteins contribute to the pathogenesis of the cancer.

In this review, we aim to explain the roles played by FOX proteins in the pathogenesis of prostate cancer. We mainly focused on the relationship between prostate cancer and the FOX subfamilies (FOXA, FOXM, FOXO and FOXP) that appear to be the most relevant and studied. The other FOX subfamilies are mentioned only briefly because the literature about them is very limited. Furthermore, we evaluated the emerging role of FOX proteins in prostate cancer as targets for therapeutic intervention.

FOXA in the Biology of Prostate Cancer

FOXA1, FOXA2 and FOXA3, also known as hepatocyte nuclear factor 3 (HNF3) α, HNF3β and HNF3γ respectively, are members of the FOXA subfamily and play important roles in the development and maintenance of the endoderm-derived organs and regulation of gene transcription. FOXA proteins can interact with chromatin and directly modulate the chromatin structure, thereby facilitating the binding of other transcription factors to DNA. Therefore, they function as a pioneer transcription factor (4). Moreover, it has been reported that FOXA1 participates in androgen receptor (AR)-mediated gene regulation in prostate cancer. Two independent groups of scholars reported similar findings for how FOXA1 controls the AR cistrome in prostate cancer (5, 6). In both studies, the FOXA1 gene was silenced by siRNA in prostate cancer cell lines and then AR ChIP-seq was performed. Depending on the loss of FOXA1, loss of about 50% AR-binding events was observed while the remaining 50% of AR-binding events still existed independent of FOXA1. Interestingly, as many as three times more new AR-binding events have occurred while FOXA1 was absent. Hence, it has been suggested that FOXA1 has a dual role in prostate cancer as it can both mediate AR-binding events and prevent additional AR-binding events by simultaneously anchoring AR to cognate loci and restricting AR from other ARE-containing loci in the human genome (5, 6). Moreover, Jin et al. have reported that low FOXA1 levels alter the AR cistrome and lead to AR reprogramming (7). In addition to its androgen receptor-mediated functions, FOXA1 has another function in prostate cancer. Jin et al. have identified that FOXA1 inhibited tumor metastasis in prostate cancer independently of AR (8).

FOXA1 has been extensively studied in prostate cancer development and progression due to its relationship with AR. Studies demonstrated that FOXA1 and FOXA2 have been upregulated in prostate cancer (9, 10). However, the expression and role of FOXA3 in prostate cancer remain unknown. FOXA1, one of the three members of FOXA subfamily, is essential for prostate development because it regulates prostate morphogenesis and cell differentiation. Mirosevich et al. have investigated FOXA protein expression in prostate cells in a mouse model and human prostate cancer tissue. They have reported that FOXA1 was expressed in both pre-neoplastic lesions and adenocarcinomas while FOXA2 was only expressed in prostate neuroendocrine carcinoma (11).

Another study conducted by Jain et al. investigated FOXA1 protein levels in primary and metastatic prostate tumors. They found high level FOXA1 expression in 25 of the 28 metastatic prostate cancer specimens, and FOXA1 expression was positively correlated with AR and tumor size. They suggested that overexpression of FOXA1 in prostate cancer was related to development of metastatic prostate cancer, and FOXA1 might act as a marker for poor prognosis (9). In one of our previous studies, we investigated the changes occurring in protein expression levels of some apoptotic, metastatic and invasion-related genes via knock-out of the FOXA1 gene in androgen-dependent LNCaP prostate cancer cell. We found decrease in the expression of CCND1 protein and we suggested that FOXA1 might be a drug target for prostate cancer treatment due to its crucial role in cell cycle G1/S transition (12).

In addition to studies related to FOXA expression in prostate cancer, several genomic studies dwelled on the mutations occurring in the FOXA subfamily members and how these mutations affected prostate cancer progression and patient outcome. In one of these studies, Barbieri et al. have performed whole exome sequencing on 112 prostate tumor and normal tissue pairs and RNA-seq analysis on 22 exome-sequenced tumors and 41 independent samples. After evaluating the whole exome sequencing and RNA-seq analysis results, they identified FOXA1 as one of the mutated and highly expressed genes in prostate cancer (13). Similarly, in another exome-sequencing study, FOXA1 mutation has been found in 3.4% of all specimens in both localized prostate cancer and castration-resistant prostate cancer. It has also been reported that the FOXA1 mutation suppressed the androgen signaling and increased tumor growth (14). Annala et al. have investigated the mutation rate in untranslated regions (UTRs) in metastatic castration-resistant prostate cancer (mCRPC) patients. According to sequence analyses results, FOXA1 3′-UTR mutations (insertions/deletions) were detected at approximately 12% of patients (15). In addition, Wedge et al. carried out whole-genome analysis of 112 primary and metastatic prostate cancer specimens. They identified coding or noncoding mutations in 30 candidate driver genes, one of which was FOXA1 (16). Zhao et al. used four computational tools to detect driver genes on 332 prostate adenocarcinoma samples. They identified 10 driver genes, including FOXA1, which were significantly mutated in prostate adenocarcinoma samples (17). These studies have reported mutations that usually occurred in FOXA1, but the impact of these mutations in prostate cancer development remained unexplained. Nonetheless, two independent groups (18, 19) have investigated the impact of FOXA1 mutations in prostate cancer development using a large cohort of primary and metastatic prostate cancer patients. They have detected FOXA1 mutations in both primary and metastatic cancers and reported that these mutations contributed to the prostate cancer progression and that FOXA1 acted as an oncogene in prostate cancer.

FOXM in the Biology of Prostate Cancer

The FOXM1 transcription factor plays an important role in prostate cancer development and progression. Previous studies have shown that FOXM1 was highly expressed in prostate cancer and overexpression of FOXM1 was associated with poor prognosis (20-22). The first study in the literature on a relationship between FOXM1 (FoxM1b) and prostate cancer was reported by Kalin et al. (20). The finding of Kalin et al. showed that overexpression of FOXM1 accelerated development, proliferation and growth of prostatic tumors in mouse models. Moreover, increased FOXM1 expression in prostate cancer cells were correlated with cell proliferation while silencing of FOXM1 by siRNA resulted in the reduction of cell proliferation. Their work suggests that FOXM1 plays a crucial role in prostate cancer progression. Furthermore, FOXM1 was reported to be among the most upregulated genes in metastatic prostate tumor samples in a microarray gene expression analysis study (21). In 2014, Wang et al. revealed that FOXM1 was expressed more in prostate cancer tissues in comparison to BPH tissues. Besides, this study demonstrated that treatment with metformin suppressed epithelial mesenchymal transition by inhibiting FOXM1 in prostate cancer cells (22). Aytes et al. (23) analyzed gene expression profiles of human and mouse prostate cancer using an integrative computational approach to compare the gene regulatory networks and concluded that FOXM1 and centromere protein F (CENPF) genes worked synergistically to drive prostate cancer. To confirm their results, they silenced either or both FOXM1 and CENPF genes in human and mice prostate cancer cells.

Although individual silencing of the genes causes a modest reduction in tumor growth and tumor weight, their co-silencing lead to more effective results in terms of reducing tumor growth and tumor weight in mice. The results from *in vitro* and *in vivo* studies seem to confirm this synergistic interaction. They also performed microarray assay to evaluate FOXM1 and CENPF gene expression in tumor tissue samples obtained from 916 prostate cancer patients. The results of the study indicates that co-expression of FOXM1 and CENPF is associated with prostate cancer malignancy. In another study, the potential mechanisms through which increased expression of FOXM1 and c-Myc contribute to the development of prostate cancer were examined. It was found that FOXM1 and c-Myc gene expression was higher in prostate cancer tissue samples when compared to normal prostate tissue samples. In addition, c-Myc bonded directly with the promoter of FOXM1 and regulated its expression. Lastly, the same study shows that FOXM1 is implicated in the pathogenesis of prostate cancer (24).

FOXO in the Biology of Prostate Cancer

In mammals, FOXO subfamily consists of FOXO1 (previously known as FKHR), FOXO3 (previously known as FOXO3a or FKHRL1), FOXO4 (previously known as AFX) and FOXO6. FOXOs generally function as tumor suppressors in prostate cancer because they are frequently deleted or inactivated in prostate cancer and they inhibit tumor development. Several studies have revealed that FOXOs are widely downregulated in prostate cancer and play critical roles in cell proliferation, migration, invasion and apoptosis (25-28).

A number of factors, such as miRNAs (miR-96, miR-182, miR-370 and miR-592), astrocyte-elevated gene-1 (AEG-1), bromodomain-containing protein 4 (BRD4) and cyclin-dependent kinase 1 (CDK1) participate in the regulation of FOXO expression. For instance, Haflidadóttir et al. have found that miR-96 was upregulated while FOXO1 was downregulated in human prostate cancer cell lines and tissues. As per their findings, they have suggested that miR-96 could enhance cellular proliferation and regulate apoptosis in prostate cancer via downregulation of FOXO (25). Wu et al. have shown that miR-370 directly targeted 3'-untranslated region of FOXO1. The decreased expression of FOXO1 in five different prostate cancer cell lines was inversely correlated with miR-370 expression, thereby inducing the proliferation of human prostate cancer cells. In addition, they have demonstrated that overexpression of miR-370 in prostate cancer cell lines could lead to decreased expression of cyclin-dependent kinase (CDK) inhibitors, p27Kip1 and p21Cip1, and increased expression of cell-cycle regulator cyclin D1 (26). Lv et al. have revealed that miR-592 was upregulated in prostate cancer cell lines and tissues. Their experiment has shown that overexpression of miR-592 in prostate cancer repressed FOXO3 expression, which in turn resulted in increased expression of cyclin D1 and decreased expression of p21, inducing prostate cancer cell proliferation (27). Another study has reported that miR-182 suppressed the expression of FOXO1, thereby increased prostate cancer proliferation, migration and invasion (28). Liu et al. have demonstrated that cyclin-dependent kinase 1 (CDK1) that is frequently overexpressed in prostate cancer phosphorylated FOXO1 at S249, which in turn led to tumorigenesis through inhibition of FOXO1 (29). Results of another study showed that FOX1-6nls, a FOXO1-derived peptide, could inhibit CDK1- and CDK2-mediated phosphorylation. Hence, they have suggested that suppressing the phosphorylation of FOXO1 could restore its tumor suppressor function (30). Additionally, it has been reported that β-arrestin2 and β-arrestin1, which are negative regulators of G-protein-coupled receptor (GPCR) signaling, affected the progression of prostate cancer via inhibition or downregulation of FOXO1 and FOXO3a expression, respectively (31, 32). Tan et al. have found that BRD4 expression was upregulated in prostate cancer cells and prostate cancer specimens. They have also reported that silencing of BRD4 using shRNA decreased cell proliferation, promoted apoptosis and induced G0/G1 cell arrest in prostate cancer through the enhancement of FOXO1 expression (33). A series of studies have proposed several approaches differing from one another in terms of their pharmacological or chemical strategies in increasing or decreasing the FOXO activity in prostate cancer. For instance, Chen et al. have reported that resveratrol, a natural-derived phytopolyphenol compound, inhibited phosphorylation of FOXO1, FOXO3A and FOXO4 proteins and induced cell growth arrest and apoptosis through activation of FOXO transcription factors in LNCaP cells (34). In an animal model study, Ganapathy et al. have revealed that resveratrol induced the activation of FOXO-3A transcription factor and its target genes, such as Bim, TRAIL, p27, and cyclin D1. They have also suggested that this activation led to an enhancement in the pro-apoptotic potential of TNF-related apoptosis-inducing ligand (TRAIL) in prostate cancer (35).

In addition, Zhang et al. have demonstrated that treatment with methylseleninic acid (MSA) caused the increase of FOXO1 expression in prostate cancer cells (36). Another study tested the effects of statins on cell proliferation and apoptosis on human prostate cancer cell lines. In this study, it was observed that AKT/FOXO1 phosphorylation was downregulated in prostate cancer cells. Furthermore, statins decreased cell proliferation and induced apoptosis in prostate cancer cells in a dose- and time-dependent manner (37).

FOXP in the Biology of Prostate Cancer

Forkhead box P (FOXP) subfamily members consist of FOXP1, FOXP2, FOXP3 and FOXP4 in vertebrates. The studies investigating the roles played by the members of this subfamily in prostate cancer have revealed that each FOXP transcription factor possesses a different role in prostate cancer. It has been put forward that FOXP1, FOXP3 and FOXP4 act as tumor suppressors, whereas FOXP2 acts as an oncogene in prostate cancer (38-42).

In a study conducted by Banham et al., FOXP1 was frequently overexpressed in both nuclear and cytoplasmic compartments in prostate tumors compared to their normal counterparts (38). A tissue microarray study made on 11,000 normal prostate epithelium and cancerous tissues demonstrated that FOXP2 upregulation was associated with poor prognosis in ERG-negative prostate cancer (40). In contrast to oncogenic role of FOXP2, Takayama et al. have reported that FOXP1 acted as a tumor suppressor through inhibiting cell proliferation and migration in prostate cancer. In this study, decreased expression of FOXP1 in prostate cancer tissue samples were associated with poor prognosis and had a prognostic value in prostate cancer (41). Single nucleotide polymorphisms (SNPs) in FOXP3 (rs3761548) and FOXP4 (rs1983891) genes may have an association with susceptibility to prostate cancer (42, 43). Furthermore, a systematic meta-analysis from at least three independent population-based case-control studies performed by Hao et al. have explained that 20 genetic variants in 19 different genes, including FOXP4, had significant association with prostate cancer risk (44). Furthermore, it has been reported that FOXP proteins interact with a variety of signaling pathways and different molecules. It has been shown that miR-146a/b induced by FOXP3 exhibited tumor-suppressive activity during tumor initiation in prostate cancer (45). Song et al. have shown that overexpression of miR-618 inhibits prostate cancer migration and invasion through the FOXP2 gene (46). Hieronymus et al. have shown that deletion of both FOXP1-SHQ1 and PTEN were correlated with prostate oncogenesis (47). Wang et al. have demonstrated that c-MYC transcription, which was frequently upregulated in prostate cancer, was repressed by FOXP3 in prostate cells. They have also reported that FOXP3 played a suppressive role in prostate cancer development by modulating the expression of c-MYC (39). Lastly, in a recent study conducted by Wu et al. it has been reported that loss of FOXP3 and TSC1 promoted prostate cancer progression via transcriptional and post-translational regulation of c-MYC (48).

Other Important FOX Transcription Factors in Prostate Cancer

In addition to the FOX proteins mentioned above, there are several other FOX proteins that have been reported to play a role in prostate cancer. However, since there are not a sufficient number of studies about these proteins, we have not covered them in detail in this review. For instance, it has been reported that FOXC1, FOXC2, FOXJ2 and FOXQ1 were upregulated in prostate cancer tissues and cell lines (49-52). In addition, the studies about FOXJ1 expression as a potential cause of prostate cancer put forward controversial results. While some studies assert that FOXJ1 is highly expressed in prostate cancer tissues, another study showed that FOXJ1 was downregulated in prostate cancer tissues (53, 54). Zhang et al. postulated that FOXJ3 was the downstream target of miR-425-5p and high miR-425-5p expression was associated with prostate cancer development via FOXJ3 (55).

CONCLUSION

Evidence from experimental and clinical studies indicate that aberrant expressions of FOX family transcription factors promote the progression of prostate cancer. Furthermore, FOX family transcription factors can act both as an oncogene and as a tumor suppressor in prostate cancer. 50 FOX transcription factors have been identified in humans, but the role of many in prostate cancer still remains unknown. Hence, precise mechanisms through which FOX proteins affect prostate cancer and the unexplained roles of many FOX proteins in prostate cancer need to be investigated further in the future.

In addition, recent studies demonstrate that miRNAs are involved in the regulation of FOX proteins in prostate cancer. Therefore, comprehensive investigation of FOX-related miRNAs appears to be promising to provide novel therapeutic strategies for prostate cancer in the future. Lastly, the identification of small molecules that selectively block the functioning of FOX proteins and their adoption with siRNAs for suppressing the expression of FOX proteins can help increase the effectiveness of prostate cancer treatment.

Conflict of interest

No conflict of interest was declared by the authors.

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