Shaping Chromatin States in Prostate Cancer by Pioneer Transcription Factors

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ABSTRACT

The androgen receptor (AR) is a critical therapeutic target in prostate cancer that responds to antagonists in primary disease, but inevitably becomes reactivated, signaling onset of the lethal castration-resistant prostate cancer (CRPC) stage. Epigenomic investigation of the chromatin environment and interacting partners required for AR transcriptional activity has uncovered three pioneer factors that open up chromatin and facilitate AR-driven transcriptional programs. FOXA1, HOXB13, and GATA2 are required for normal AR transcription in prostate epithelial development and for oncogenic AR transcription during prostate carcinogenesis. AR signaling is dependent upon these three pioneer factors both before and after the clinical transition from treatable androgen-dependent disease to untreatable CRPC. Agents targeting their respective DNA binding or downstream chromatin-remodeling events have shown promise in preclinical studies of CRPC. AR-independent functions of FOXA1, HOXB13, and GATA2 are emerging as well. While all three pioneer factors exert effects that promote carcinogenesis, some of their functions may inhibit certain stages of prostate cancer progression. In all, these pioneer factors represent some of the most promising potential therapeutic targets to emerge thus far from the study of the prostate cancer epigenome.

Introduction

Prostate cancer is predominantly viewed as a relatively indolent and slow-progressing malignancy that presents clinically in a highly treatable localized stage. However, lethal and intractable forms of prostate cancer evolve through poorly understood mechanisms of therapeutic resistance and metastasis that have become subjects of intense research interest. Genetic studies have successfully pinpointed loci that acquire critical mutations during prostate cancer development, including the gene encoding the androgen receptor (AR). AR is the target of androgen deprivation therapy (ADT) during the treatable stages of the disease, and later acquires amplifications and mutations that confer ADT resistance and progression to a lethal castration-resistant state. AR activates a critical oncogenic transcriptional program in prostate cancer, but currently cannot be therapeutically targeted in the lethal stage of the disease.

Like most transcription factors, AR binds to specific target DNA motifs within regions of euchromatin, where nucleosomes have been shifted or depleted and these motifs are most accessible (1, 2). In contrast, transcription factors that can directly bind DNA motifs within condensed chromatin form a much smaller subset and are defined as pioneer factors (**Fig. 1**). Their existence was first recognized in the context of embryonic liver development, where the binding of FOXA1 to condensed chromatin opened the accessibility of the locus to facilitate the transcriptional activity of other transcription factors (3). Pioneer factors initiate a process of transcriptional activation, in part, by recruiting histone acetyltransferases (4, 5) and other chromatin remodelers (6) to render the locus accessible to accumulation of a transcriptionally active condensate consisting of RNA polymerase II and other factors at the promoter (7–9). Interestingly, genetic analyses of prostate cancer have detected a high frequency of mutations in the locus encoding FOXA1 (10–12), while transcriptomic analyses have identified progressive upregulation of the pioneer factors HOXB13 and GATA2 (13, 14). Thus, the landscape of aberrant transcription in prostate cancer is not driven by AR in isolation, but in conjunction with pioneer factors that modify chromatin to enable AR and other factors to access cancer-specific binding sites (**Fig. 1**). The genome-wide interplay between these factors, AR, and various epigenetic readers and writers has become increasingly recognized as a source of next-generation therapeutic targets to attenuate aberrant AR signaling in tumors that have evolved resistance to standard therapies.

This article will review the discoveries of FOXA1, HOXB13, and GATA2, the evidence linking each of them to prostate cancer, their contributions to disease trajectory and outcomes, and the potential for their therapeutic targetability.

Discovery and Functional Characterization of FOXA1

FOX proteins comprise a transcription factor family defined by a characteristic forkhead domain (also known as a winged helix) that enables each FOX protein to bind DNA as a monomer. FOXA1 (previously known as HNF3A) is one of three members of the FOXA subfamily and was the first family member to have a solved crystal structure of its forkhead domain, revealing similarity to the globular domain of linker histones (15). FOXA1 can stably bind and reposition nucleosomes *in vitro* while displacing linker histones (16, 17), indicating a tendency to counteract chromatin condensation. These characteristics provided early clues to the pioneer factor ability of FOXA1 to bind condensed chromatin and open a locus for access by other transcription factors (3, 18). FOXA1 binds to genomic sites matching the consensus sequence 5′-[A/C/G]A[A/T]T[A/G]TT[G/T][A/G][C/T]T[C/T]-3′ (19). In the context of liver development, FOXA1 is one of the earliest transcription factors to bind key loci and convert the surrounding chromatin into transcriptionally active

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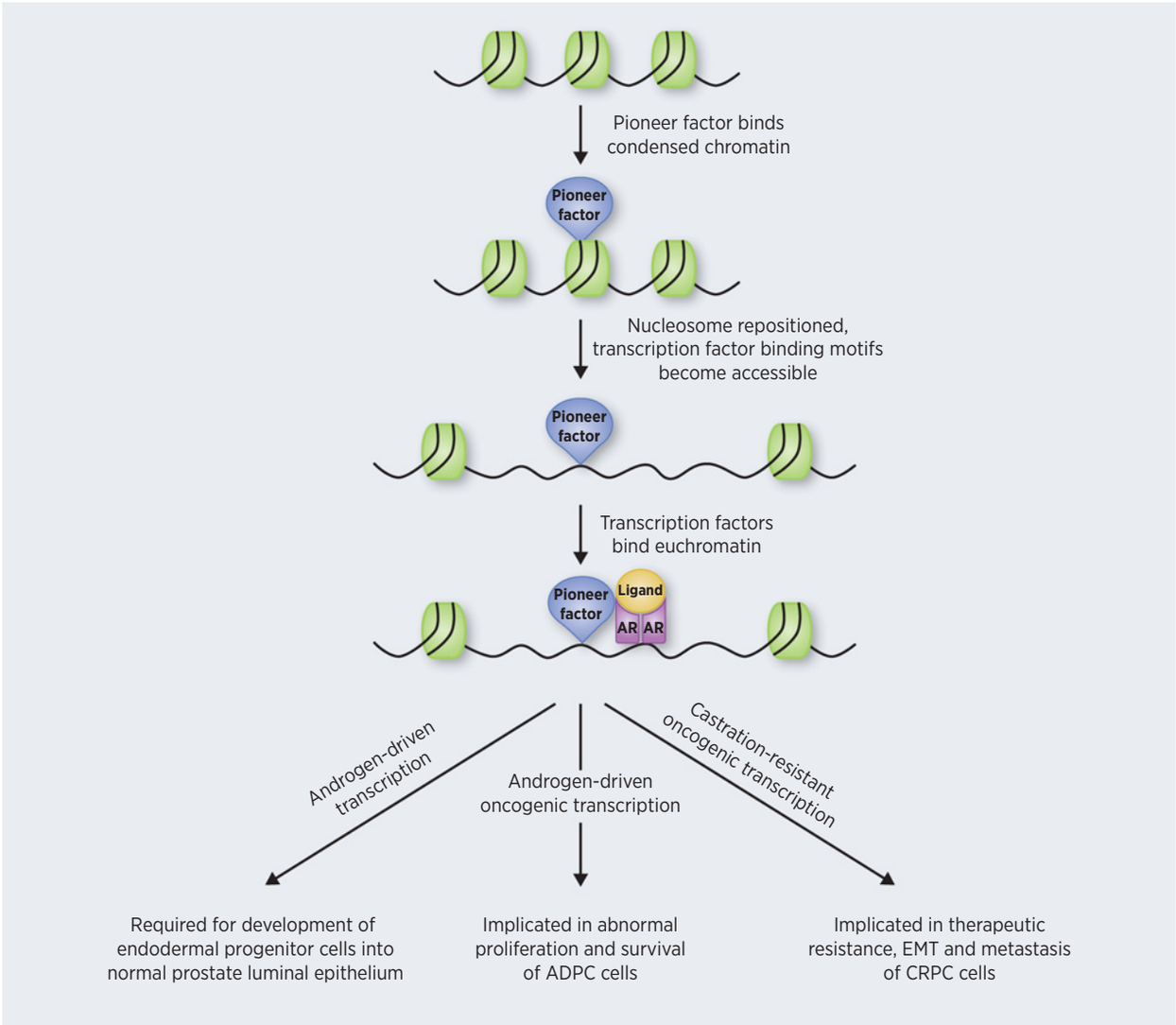

Figure 1.
Pioneer factor functions in prostate development and carcinogenesis. Pioneer factors can bind target motifs within condensed chromatin by competing with and displacing linker histones. This repositions the nucleosome from the center of the binding site and counteracts chromatin condensation, facilitating access to adjacent binding motifs recognized by other transcription factors in the surrounding euchromatin. Pioneer factors play a critical role in facilitating access and AR binding to its target sites during prostate epithelial development and both the androgen-driven and castration-resistant stages of prostate carcinogenesis.

regions, epigenetically preparing endodermal cells to differentiate along the hepatic lineage upon receipt of further developmental signals (20). FOXA1 contributions to organ development are not limited to the liver, and *FOXA1*$^{-/-}$ mice die as neonates due to impaired development of the pancreas and defects in its endocrine functions (21, 22). Findings linking FOXA1 to regulation of the estrogen receptor (23–25), glucocorticoid receptor (26), and androgen receptor (27) have led to its recognition as a key modulator of nuclear receptor signaling. *FOXA1* mRNA is detectable in prostate, seminal vesicles, and bladder tissues at higher levels than in liver tissues, while Foxa1 protein in the mouse embryo specifically marks the epithelial rather than the stromal cells along the region that give rise to the urinary and reproductive organs (28, 29). Foxa1 can be detected in both benign and malignant mouse prostate tissues (30), while Foxa1-deficient mice exhibit highly abnormal prostate duct morphology characterized by an absence of luminal epithelial cells and relative thickening of adjacent stromal smooth muscle layers (31).

Discoveries Linking *FOXA1* to Prostate Cancer

The first mechanistic evidence of a collaboration between FOXA1 and AR emerged through chromatin immunoprecipitation (ChIP)-based genome-wide mapping of AR-binding sites, revealing significant enrichment of forkhead-binding motifs adjacent to androgen response elements (ARE; ref. 32). FOXA1 is recruited by lineage-specific patterns of dimethylated histone H3K4, supporting the conclusion

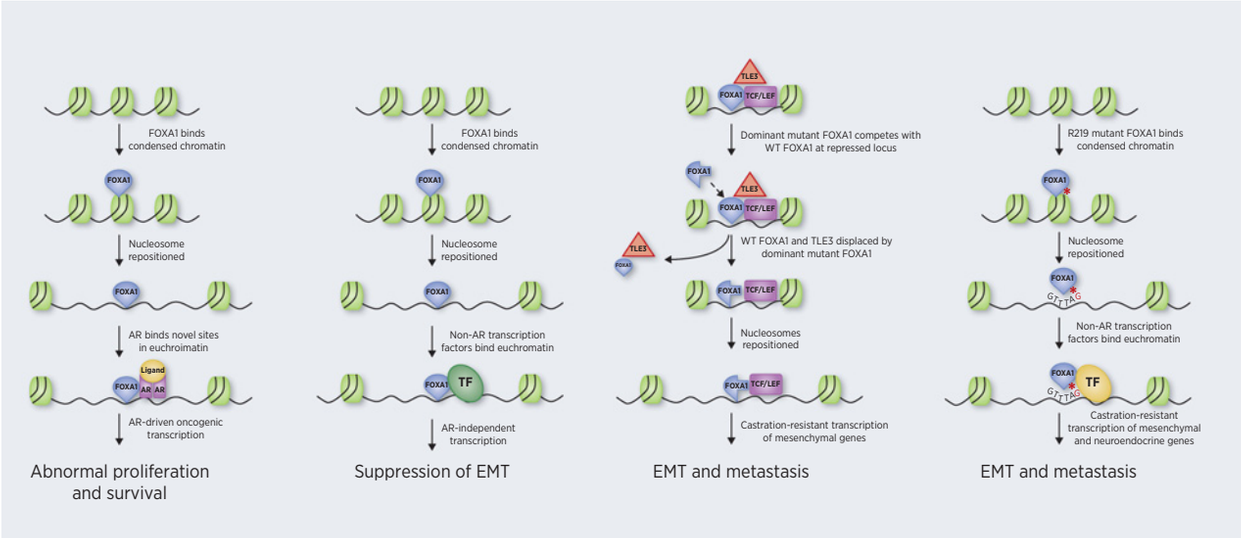

Figure 2.
Wild-type and mutant FOXA1 participate in diverse mechanisms in prostate carcinogenesis. Wild-type FOXA1 facilitates oncogenic AR signaling while suppressing genes associated with epithelial-to-mesenchymal transition independently of AR (41). Metastasis-enriched C-terminal truncation mutants in FOXA1 preserve the forkhead DNA-binding domain but activate an EMT- and metastasis-promoting gene expression signature by displacing the transcriptional corepressor TLE3 from WNT signaling-associated target genes (39). An AR-independent mesenchymal and neuroendocrine transcriptional program is driven by NEPC-enriched FOXA1 mutants at R219 (indicated by red asterisk), which recognize and bind a target motif that is modified from the forkhead motif recognized by wild-type FOXA1 (38). FOXA1 hotspot mutants are not depicted here due to the need to further clarify their mechanism(s) of action.

that FOXA1 functions to convert H3K4me2 signatures into transcriptional enhancers that help define specific lineages in development (33). Work in breast cancer models indicates that FOXA1 also promotes H3K4 methylation through recruitment of histone methyltransferase MLL3, forming a positive feedback loop in which FOXA1 and H3K4 methylation promote one another during the establishment of active enhancers (34). Like other pioneer factors, FOXA1 can bind compact chromatin but not silent heterochromatin characterized by repressive marks such as H3K9me2 and H3K9me3 (35). FOXA1-binding sites precisely coincide with loss of H3K4me2 ChIP sequencing (ChIP-seq) signal, revealing the displacement of a histone at the center of the binding site and stabilization of adjacent nucleosomes on either side (35, 36). The exact positioning of FOXA1 occupancy replacing the displaced histone core suggests a model in which FOXA1 approaches the nucleosome from one side along the chromatin axis and successfully competes with the linker histone for DNA binding (18). FOXA1 and AR physically interact and cooperatively promote differentiation within the developing prostate (**Fig. 2**; ref. 27). FOXA1 also facilitates changes in AR signaling that are characteristic of prostate tumorigenesis, is required for androgen response at key AR targets (27), and is present at more than half of AR-binding sites in LNCaP cells (33). When coexpressed with HOXB13 in an immortalized prostate cell line, FOXA1 reprograms genome-wide AR occupancy to a prostate tumor-like pattern (37). Wild-type FOXA1 canonical motif recognition is functionally correlated with enhanced luminal epithelial signaling through shared AR targets and increased cell proliferation (**Fig. 2**; refs. 37–41). In addition, AR-independent transcriptional functions of wild-type FOXA1 suppress invasion and metastasis by serving as a barrier to epithelial-to-mesenchymal transition (**Fig. 2**; ref. 41). FOXA1 is known to play a similar epithelial maintenance role in breast and pancreatic cancers, where it promotes expression of E-cadherin and suppresses that of mesenchymal markers (42, 43).

In CRPC models such as LNCaP-abl cells, FOXA1 helps AR regulate an androgen-independent gene expression pattern that is distinct from that of the parental LNCaP (ADPC) cell line, and AR transactivation of the *UBE2C* oncogene in the absence of androgen is FOXA1-dependent (44). While the FOXA1 cistrome is not dramatically changed in LNCaP-abl versus LNCaP cells, some differentially bound enhancers upregulate critical cell-cycle regulators, and *FOXA1* silencing in LNCaP-abl decreases cell proliferation by triggering defects in both G_1–S-phase and G_2–M-phase progression (45). Long-range chromatin loops linking FOXA1-bound enhancers with the *UBE2C* promoter are facilitated by phosphorylated coactivator MED1 (Thr1032), which is present at higher levels in CRPC models (46). FOXA1 expression appears to be increased in some IHC studies of CRPC metastatic samples (47, 48), but decreased in another IHC study of metastatic CRPC (49). Further research is necessary to validate these results and separately address the comparisons between FOXA1 expression in ADPC versus CRPC and between primary versus metastatic prostate cancer. *FOXA1* mRNA is also reduced in RNA sequencing (RNA-seq) data from human NEPC tumors relative to their adenocarcinoma counterparts (50). Several studies in LNCaP cells under hormone-depleted conditions that mimic androgen deprivation indicate that FOXA1 performs functions that are tumor-suppressive functions in some contexts. These functions include inhibition of G_1–S progression through FOXA1-dependent AR targets (51), and inhibition of AR binding through interactions with its DNA-binding domain (52), transcriptional repression of the *TGFB3* gene to prevent cell motility and epithelial-to-mesenchymal transition (49), and repression of the gene encoding IL8 to block tumor progression to the NEPC subtype (50).

Nevertheless, increasing the expression of FOXA1 in LNCaP cells expands AR chromatin binding into new areas of the genome (53). FOXA1 is particularly important for recruitment of AR to low-affinity AREs located near forkhead-binding motifs (52). This is relevant to the

emerging mechanism of antagonist-liganded AR transactivation of noncanonical targets, which is associated with regions of FOXA1 occupancy and may contribute to the development of resistance to ADT (54, 55).

Interestingly, a recent report that estrogens and glucocorticoids can partially redirect chromatin binding by FOXA1 in breast cancer cells have called into question the extent to which FOXA1 acts upstream as opposed to downstream of steroid hormone receptors to reprogram gene expression (56). This finding has proven controversial, as a replication study concluded that greater than 99% of FOXA1-binding sites across the genome remained constant following hormone treatments (57). A follow-up study reanalyzing and comparing data from both groups has arrived at the explanation that differences in data analysis and criteria used to define FOXA1-binding sites are at the heart of the opposing findings (58). However, in the absence of a more definitive resolution of which perspective is the correct one, it simply remains an interesting question.

Classification and Functional Characterization of *FOXA1* Mutations in Prostate Cancer

FOXA1 has been recognized for some time as one of the most frequently mutated loci in prostate cancer (12), and four recent studies have transformed our understanding of the types and functional consequences of *FOXA1* alterations throughout the stages of prostate carcinogenesis (Supplementary Table S1; refs. 38–40, 59). Mutations in *FOXA1* have generally been detected in 3%–5% of primary prostate cancers (10–12), but at a rate of 8%–9% in a large recent cohort (39). In comparison, 12%–13% of metastatic prostate cancers exhibit *FOXA1* mutations, with additional metastatic cancers harboring amplifications and other genomic rearrangements affecting the locus (39). Within primary disease, gene expression profiling defines *FOXA1*-mutant cancers as a distinct subtype characterized by very high AR transcriptional activity and genome-wide hypermethylation (12). In primary tumors, most *FOXA1* mutations occur within the forkhead domain (12), particularly within a hotspot in the Wing2 subdomain that harbors more than 50% of all *FOXA1* mutations (38). Hotspot *FOXA1* missense mutations are found at similar frequencies in primary and metastatic prostate cancer, and most alter contacts with the DNA backbone rather than sequence-specific DNA contacts (39).

Heterozygous C-terminal truncations represent approximately 20% of all FOXA1 mutants (38) and are found infrequently in primary disease but enriched in metastases (39). Truncated FOXA1 proteins exhibit stronger DNA-binding affinity and an expanded cistrome relative to wild-type FOXA1. New targets include activation of canonical WNT signaling, which contributes to enhanced invasion and metastasis (**Fig. 2**; ref. 39). Among 9 common prostate and prostate cancer cell lines, only LAPC4 expresses a truncated FOXA1 protein (39). Substitutions at R219 represent 5% of all *FOXA1* mutations and are also enriched specifically in metastatic tumors with neuroendocrine features (38). Unlike other missense mutants, R219 substitutions alter sequence-specific FOXA1 contacts with the major groove that block luminal epithelial signaling while opening up new regions of chromatin and transactivating mesenchymal and neuroendocrine genes (**Fig. 2**; ref. 38). A third class of alterations within the *FOXA1* locus is created by tandem duplication and translocation events that impact nearby enhancer elements and cause either overexpression of *FOXA1* or insertion of an oncogene upstream of *FOXA1* (39).

Metastasis-enriched C-terminally truncated FOXA1 mutants and NEPC-specific R219 FOXA1 mutants unambiguously represent drastic changes in DNA binding and function relative to wild-type FOXA1 (38). Hotspot mutants, on the other hand, have been universally found to be pathogenic but have exhibited quite different mechanisms of action from study to study, despite the use of similar or identical mutations. In one such study, multiple hotspot mutants were found to preserve wild-type FOXA1 motif recognition while expanding its cistrome, significantly enhancing the luminal epithelial transcriptional program associated with FOXA1 and AR in prostate cancer, and increasing cell proliferation in a mouse organoid model (38). A second study showed using fluorescence recovery after photobleaching (FRAP) assays and luciferase reporter assays that hotspot mutants exhibit increased nuclear mobility, more efficient scanning of the genome for binding sites, and enhanced transactivation (39). FOXA1 ChIP-seq indicated that these mutants retained wild-type motif recognition and gave rise to a cistrome that overlaps strongly with that of the wild-type, and they promoted proliferation in 22Rv1 cells only under androgen-depleted conditions (39).

While both of the above studies have characterized hotspot mutations as promoting FOXA1 binding and transactivation of AR-dependent target genes (38, 39), two other studies have reported that they inhibited those events and redirected binding to AR-independent loci (40, 59). In cells with both endogenous wild-type and inducible mutant FOXA1, expression of the mutant inhibited both FOXA1 and AR binding to known AR-dependent enhancers and instead transactivated an AR-independent program associated with epithelial-to-mesenchymal transition, leading to increased LNCaP invasion (59). This study also included a gel-shift assay indicating that the mutations increase binding affinity for a novel enriched DNA motif (59). A similar study showed reduced binding to a forkhead motif by gel shift-assays and to chromatin by FOXA1 ChIP-seq, as well as increased binding to AR, which decreased AR ChIP-seq signal at both FOXA1-dependent and -independent gene targets (40). Genes upregulated by hotspot mutants exhibited an epithelial-to-mesenchymal transition signature, and FOXA1 mutants triggered decreased proliferation in the presence of androgens but increased proliferation in the absence of androgens (40).

The four studies described above provide a fascinating but complex picture of FOXA1 hotspot mutants and their functions in prostate cancer progression. The opposite directions of certain effects may be attributable to differences in the specific assays, model systems, particular genes analyzed, and/or the challenges of interpreting overexpression effects. While each study provides evidence of a different mechanism of action, they collectively demonstrate the pathogenic nature of these acquired mutations.

The paralog FOXA2 has also emerged as a key player in prostate cancer. Foxa2 is expressed at low levels relative to Foxa1 in most epithelial regions of the adult mouse prostate (31, 60) and instead is preferentially expressed in another male reproductive accessory tissue, the epididymis (61). Interestingly, FOXA2 shows a similar ability to interact with AR (61) and can localize to and activate the AR target loci *Probasin* and *Fkbp5* when expressed as a transgene in a mouse prostate epithelial cell line (62). However, *Foxa2* expression in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model actually correlates inversely with AR expression (63). Under hypoxic conditions that can drive prostate cancer progression to the castration-resistant NEPC phenotype, FOXA2 regulates gene expression in collaboration with HIF1α (64). IHC has revealed that FOXA2 is expressed in neuroendocrine prostate tumors (65) and that it serves as a highly specific marker for NEPC (66). Thus, while FOXA1 and

FOXA2 are largely expressed in distinct cellular contexts within prostate development and prostate cancer, both represent important drivers and potential therapeutic targets.

Discovery and Functional Characterization of HOXB13

HOXB13 is one of 39 human members of the Hox subgroup of the homeobox family of pioneer factors, which are characterized by a homeobox DNA-binding domain and best known for their regulation of anterior/posterior patterning in vertebrate development. The homeobox DNA-binding domain recognizes A/T-rich sequences, often with a core sequence of 5′-TAAT-3′. The binding affinity and sequence specificity of their DNA binding is supplemented by their interactions with homeobox transcription factors of the three amino acid loop extension (TALE) family (67), which includes the MEIS, PBX, TGIF, and IRO transcription factor subgroups. Whereas many Hox proteins bind DNA cooperatively with the TALE family member PBX1, HOXB13 does not interact with PBX1 but rather with MEIS1 (68). Similar to other TALE family members, MEIS1 exhibits limited DNA-binding activity and specificity on its own, but strong ability to bind a specific target sequence when in a complex with a Hox protein (68). HOXB13 contains two MEIS interaction domains (69). HOXB13 is also part of a subgroup of Hox proteins that can repress transcription independently of either TALE cofactors or DNA binding by interacting with CBP or p300 and inhibiting their histone acetyltransferase activities (70).

HOXB13 expression was first identified within posterior regions of human and mouse embryos such as the tailbud and parts of the spine and digestive tract, as well as the urogenital sinus which gives rise to the prostate (71). *Hoxb13* is expressed in both the developing prostate and adult prostate (72), particularly within luminal epithelial cells lining prostate ducts (73), and promotes differentiation into the luminal epithelial cell fate (73, 74). Mice harboring homozygous mutations disrupting the homeobox domain of Hoxb13 exhibit defective ventral prostate lobe morphology, including loss of luminal epithelial cell polarity and complete absence of secretory functions (73). Double mutants in *Hoxb13* and *Hoxd13* have revealed additional functions in ventral prostate duct formation that are masked by redundancy of function (73).

HOXB13 was first linked to cancer through overexpression studies in prostate and colorectal cell lines lacking endogenous HOXB13 expression, causing growth inhibition in both models, in part, through inhibition of the WNT signaling pathway (75, 76). In contrast, reversing *HOXB13* overexpression in endometrial and ovarian cancer cells through RNA interference was found to reduce invasive ability (77, 78). These studies collectively provided the first evidence of aberrant *HOXB13* overexpression or loss of HOXB13 expression in cancer and their potential contributions to tumorigenesis through multiple downstream pathways.

Discoveries Linking HOXB13 to Prostate Cancer

HOXB13 has long been recognized as overexpressed in prostate cancer (13). Like FOXA1, HOXB13 physically interacts with AR (79, 80) and colocalizes to shared AR target loci, where it enhances AR transactivation of shared targets that harbor AREs adjacent to HOXB13-response elements (81). As previously mentioned, exogenously expressed HOXB13 and FOXA1 reprogram the AR cistrome of transformed prostate epithelial cells to a prostate tumor-like pattern (37). However, while FOXA1-binding motifs are enriched among both AR-binding regions in normal prostate epithelium and tumor-specific AR-binding regions, HOXB13 motifs are only enriched in tumor-specific AR-binding regions alone (37). Unlike FOXA1, HOXB13 also negatively regulates AR transactivation of many shared targets such as *KLK3* (which encodes PSA; ref. 79). Because HOXB13 specifically interacts with the DNA-binding domain of AR, it interferes with transactivation of a subset of AR targets within loci such as *KLK3* that contain an ARE but lack a HOXB13-response element (81).

Functional studies in LNCaP cells have created some debate around whether HOXB13 plays an oncogenic or tumor suppressive role in ADPC. HOXB13 overexpression suppresses colony formation in LNCaP cells, which can be overcome by increased expression of AR (79). On the other hand, *HOXB13* knockdown attenuates the ability of androgen treatment to stimulate proliferation, migration, and intracellular lipid production (81). Despite their differences, both of these findings provide strong evidence that HOXB13 contributes to fine-tuning of the AR cistrome in ADPC.

In LNCaP cells grown under hormone-free conditions, cell proliferation is repressed by *HOXB13* silencing and enhanced by HOXB13 expression, in part, through HOXB13 inhibition of p21 expression and resulting activation of RB-E2F signaling (82). This finding indicates that HOXB13 may retain its ability to promote prostate cancer growth in the context of hormone-independent disease, in part, through its transcriptional repressor function. HOXB13 does, in fact, exhibit a high degree of overexpression in castration-resistant prostate cancer (CRPC) tissues relative to ADPC tissues (82), and plays a less ambiguous tumor-promoting role in this context. For example, HOXB13 interacts with the hormone-independent AR variant AR-V7 and extensively colocalizes with AR-V7–binding sites across the CRPC genome, to a much greater degree than with wild-type AR (83). HOXB13 is required for AR-V7 transactivation of target genes, and *HOXB13* silencing inhibits CRPC cell growth through an AR-V7–dependent mechanism (83). A mechanism behind the closer association of HOXB13 with AR-V7 rather than with full-length AR was revealed by data from the Assay for Transposase-Accessible Chromatin by sequencing (ATAC-seq) showing that chromatin accessibility was higher at AREs within HOXB13/AR-V7–binding regions than at AREs bound by full-length AR, indicating that HOXB13 may open chromatin (83). *HOXB13* mRNA levels in circulating tumor cells from the serum of patients with CRPC are significantly higher for the AR-V7–positive subset (83).

HOXB13 Mutations in Prostate Cancer

While *HOXB13* is not considered a frequent target of somatic mutations in prostate cancer, an inherited variant coding for a G84E substitution has been found to confer predisposition to develop prostate cancer (Supplementary Table S1; ref. 84). The variant is predominantly found among individuals of European descent, with an initially reported prevalence of less than 0.1% in this general population compared with a frequency of 1.4% in a group of unrelated patients with prostate cancer (84). While the initial study indicated a more than 10-fold increase in risk, subsequent case–control studies in other cohorts have calculated approximately 3%–5% increase in relative risk due to HOXB13 G84E (85, 86). Patients with prostate cancer carrying the mutation exhibit clinical profiles characteristic of high-risk disease, including relatively early onset (84), higher PSA levels and Gleason scores at diagnosis, and more frequent positive surgical margins in comparison with noncarriers (85). Similar studies

are underway in a number of populations to examine the relationships between other rare HOXB13 variants and prostate cancer risk (87).

The substitution occurs within one of the MEIS-interacting domains of HOXB13 (84). As mentioned previously, HOXB13 and MEIS1 are known interacting partners, and Hox family members such as HOXB13 generally require cooperation with a TALE family member such as MEIS1 to enhance DNA-binding affinity and sequence specificity. Preliminary investigations of G84E mechanism have so far determined that expression of the MEIS1 and MEIS2 homeobox cofactors progressively decreases during prostate carcinogenesis, and that *MEIS1/2* silencing in LAPC-4 cells increases xenograft growth (88). Paradoxically, *MEIS1* silencing has separately been shown to inhibit LAPC-4 proliferation and to enhance the antiproliferative effects of *HOXB13* silencing in this model (89). Surprisingly, the HOXB13 G84E variant exhibits wild-type stability and ability to interact with MEIS1 (89), defying efforts to understand its contributions to prostate carcinogenesis. It is important to note that the previously described roles of HOXB13 in CRPC are performed by the wild-type protein, as one study failed to detect the G84E substitution in any of 135 patients (83) predominantly from two published CRPC cohorts (90, 91). At this point, the mechanism by which the inherited G84E substitution increases the risk of prostate cancer incidence is still unclear, and it remains an important priority to continue to investigate the role of TALE family members in mediating HOXB13 activity in prostate cancer.

Initial Discovery of GATA2 and Characterization of Its Normal Functions

GATA2 is one of six vertebrate members of the GATA pioneer transcription factor family, named after their shared DNA-binding sequence 5′-(T/A)GATA(A/G)-3′ (92). As in the case of FOXA1, the ability of GATA family members to bind to condensed chromatin and open the accessibility of a locus to other transcription factors was first recognized in embryonic liver development (3). Its expression is highest in hematopoietic cell types, particularly in early progenitors (93) where it drives proliferation and inhibits differentiation (94). Germline loss-of-function *GATA2* mutations are responsible for a spectrum of haploinsufficiency disorders collectively known as GATA2 deficiency, with symptoms including predisposition to cancers of the blood and lymphatic systems (95–97). GATA2 is required to produce and expand hematopoietic stem cells and other immune cell types during development as well as to maintain these lineages in the adult bone marrow (95–97). *GATA2*$^{-/-}$ mice exhibit mid-gestation lethality from hematopoietic failure (97), and tissue-specific rescue of this hematopoietic defect extends survival only until the neonate stage, when death occurs from blocked urine excretion and is accompanied by evidence of extensive genitourinary malformation (98). As expected, GATA2 is expressed in urogenital tissues including the prostate (99).

The Role of GATA2 in Prostate Cancer

Blood and lymphatic cancers such as ALL frequently exhibit downregulation of *GATA2* (100), yet its expression progressively increases throughout prostate carcinogenesis (32, 101, 102). GATA2 was first linked to prostate cancer in cell lines, where it enables optimal androgen-stimulated transcription of the gene encoding PSA (99) and interacts with AR in an androgen-dependent manner (32). This was confirmed at the genome-wide level through ChIP-based genome-wide mapping of AR-binding sites, which revealed significant enrichment of GATA transcription factor–binding motifs in areas adjacent to AREs (32). GATA2 binds canonical AR enhancers regulating the expression of PSA and TMPRSS2 (32, 99) and promotes chromatin accessibility and chromatin looping even before androgen stimulation (101), and AR recruitment to these regions is GATA2-dependent (32). Genome-wide overlap between GATA2, FOXA1 and AR ChIP-seq datasets reveals that GATA2 and FOXA1 occupy approximately 55% of AR-binding sites prior to AR itself, dramatically impacting its genomic distribution (101). *GATA2* silencing in prostate cancer cells not only triggers drastic transcriptional changes but also inhibits proliferation, migration, invasion, and the breakdown of focal adhesions (103).

The importance of GATA2 in promoting AR activity continues following prostate cancer progression from ADPC to CRPC, as it promotes AR transcription under low-androgen conditions, and its expression correlates with elevated risk of biochemical recurrence and distant metastasis (14). High GATA2 protein expression also correlates with tumor stage, Gleason score, and positive lymph nodes, and is very strongly linked to AR-positive cancers (104). The ability of GATA2 to generate highly accessible chromatin states may also facilitate transcriptional events that promote the progression to CRPC. Administration of second-generation AR-targeted therapies triggers GATA2 to direct antagonist-liganded AR binding to and transactivation of an alternative set of target genes (55). The increase in GATA2 expression characteristic of CRPC also results in upregulation of its AR-independent targets such as the growth hormone IGF2, contributing further to the evolution of chemotherapeutic resistance (102). While the data on GATA2 argue for a consistent role in promoting prostate carcinogenesis, the *GATA2* locus has not been listed among frequent targets of pathogenic mutations in prostate cancer.

Interestingly, the prostate exhibits high expression not only of GATA2 but also of GATA3, relative to other family members (99). While prostate-specific mouse knockouts of *Gata2* and *Gata3* reveal some redundancy of function, GATA3 has not been investigated to the same extent due to its low expression in prostate cancer cell lines (105).

Potential Therapeutic Implications and Future Directions

FOXA1, HOXB13, and GATA2 shape the prostate cancer transcriptome during both androgen-dependent and castration-resistant stages, indicating potential utility as therapeutic targets in CRPC. While it is difficult to target transcription factors pharmacologically, mechanistic studies have identified chromatin-remodeling complexes and other potentially targetable components that function downstream of each of these pioneer factors to render the surrounding loci accessible to AR binding. Early attempts to target these components have yielded interesting preliminary results.

FOXA1 interacts with the PARP2 enzyme, and PARP1/2 inhibitors enhance this interaction while blocking FOXA1 binding to chromatin (106). This reduces AR occupancy and transcriptional activity of shared target enhancers (106), helping to explain the known sensitivity of CRPC cell lines to PARP1/2 inhibitors (107, 108). Preclinical research indicates that cancers harboring hotspot mutations in *FOXA1* may also exhibit unique therapeutic susceptibilities due to their transactivation of targets in the EMT pathway, as upregulation of the *MET* proto-oncogene results in sensitivity to the c-Met kinase inhibitor crizotinib (59).

The GATA inhibitor K7174 inhibits GATA2 DNA binding and transactivation of target genes (109, 110). K7174 treatment of prostate cancer cells reduces expression of AR and its splice variants associated with CRPC, as well as the expression of downstream AR target genes (111). It has also demonstrated the ability to reduce tumor volume in a mouse xenograft model of CRPC (111) and to sensitize cells to cotreatment with next-generation AR antagonists (55). A second emerging option for GATA2 targeting in prostate cancer is to take advantage of its regulation through acetylation, which facilitates its interactions with bromodomain and extraterminal (BET) proteins (112). GATA2 DNA binding in CRPC exhibits significant overlap with gene targets of the BET proteins, and GATA2 occupancy of enhancer regions regulating PSA and TMPRSS2 expression is required to enable binding of the BRD4 component (113). Their interaction can be abolished with JQ1, a BET-inhibitor that blocks GATA2 DNA binding and transcriptional output (113).

HOXB13 activity is also dependent on BET family members such as BRD4; however, they appear to regulate it at the level of *HOXB13* transcription (114). The inhibitor JQ1 can block the localization of BRD4 to the *HOXB13* promoter, triggering apoptosis in prostate cancer cells and counteracting growth in xenograft models (114). Other related agents from this emerging class of BET inhibitors are designed to simultaneously inhibit oncogenic tyrosine kinases such as JAK2 (115), and are predicted to exhibit greater efficacy by combining the ability to suppress *HOXB13* transcription with other benefits (114).

In considering FOXA1, HOXB13, and GATA2 as potential therapeutic targets, it is important to note the caveat that their target genes are highly cell type-specific (116), such that their functions in other cell types outside of the prostate can in some cases be tumor suppressive. For example, the aforementioned role of FOXA1 in epithelial maintenance and suppression of mesenchymal markers in pancreatic cancer (43) and ER-negative breast cancer (117) results in decreased migration and may explain the association of higher FOXA1 levels with better outcomes in these contexts (42, 43). Similarly, HOXB13 has been demonstrated to perform tumor suppressive functions in colorectal cancer (118), while loss of GATA2 expression has been linked to the development of blood and lymphatic cancers (100). These findings collectively encourage those researchers and clinicians working to develop such potential therapeutics to be attentive to effects on other tissues and to continue to strategize about how to deliver these agents to tumor cells as specifically as possible.

In conclusion, several pioneer factors interact with one another and with AR to shape the ADPC and CRPC transcriptomes and to drive prostate cancer progression in both androgen-dependent and -independent ways. Genetic inhibition has revealed their clinical relevance as potential therapeutic targets, while emerging pharmacologic inhibitors have shown preclinical promise to justify further study in clinical trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Knezetic JA, Luse DS. The presence of nucleosomes on a DNA template prevents initiation by RNA polymerase II *in vitro*. Cell 1986;45:95–104.
2. Lorch Y, LaPointe JW, Kornberg RD. Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. Cell 1987;49:203–10.
3. Cirillo LA, Lin FR, Cuesta I, Friedman D, Jarnik M, Zaret KS. Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. Mol Cell 2002;9:279–89.
4. Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, et al. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 1996;84:843–51.
5. Utley RT, Ikeda K, Grant PA, Cote J, Steger DJ, Eberharter A, et al. Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. Nature 1998;394:498–502.
6. Kraus WL, Kadonaga JT. p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation. Genes Dev 1998;12:331–42.
7. Cisse II, Izeddin I, Causse SZ, Boudarene L, Senecal A, Muresan L, et al. Real-time dynamics of RNA polymerase II clustering in live human cells. Science 2013;341:664–7.
8. Cho WK, Spille JH, Hecht M, Lee C, Li C, Grube V, et al. Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science 2018;361:412–5.
9. Sabari BR, Dall'Agnese A, Boija A, Klein IA, Coffey EL, Shrinivas K, et al. Coactivator condensation at super-enhancers links phase separation and gene control. Science 2018;361. DOI: 10.1126/science.aar3958.
10. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012;44:685–9.
11. Robinson JL, Holmes KA, Carroll JS. FOXA1 mutations in hormone-dependent cancers. Front Oncol 2013;3:20.
12. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015;163:1011–25.
13. Edwards S, Campbell C, Flohr P, Shipley J, Giddings I, Te-Poele R, et al. Expression analysis onto microarrays of randomly selected cDNA clones highlights HOXB13 as a marker of human prostate cancer. Br J Cancer 2005;92:376–81.
14. Bohm M, Locke WJ, Sutherland RL, Kench JG, Henshall SM. A role for GATA-2 in transition to an aggressive phenotype in prostate cancer through modulation of key androgen-regulated genes. Oncogene 2009;28:3847–56.
15. Clark KL, Halay ED, Lai E, Burley SK. Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. Nature 1993;364:412–20.
16. Cirillo LA, McPherson CE, Bossard P, Stevens K, Cherian S, Shim EY, et al. Binding of the winged-helix transcription factor HNF3 to a linker histone site on the nucleosome. EMBO J 1998;17:244–54.
17. Cirillo LA, Zaret KS. An early developmental transcription factor complex that is more stable on nucleosome core particles than on free DNA. Mol Cell 1999;4:961–9.
18. Ye Z, Chen Z, Sunkel B, Frietze S, Huang TH, Wang Q, et al. Genome-wide analysis reveals positional-nucleosome-oriented binding pattern of pioneer factor FOXA1. Nucleic Acids Res 2016;44:7540–54.
19. Overdier DG, Porcella A, Costa RH. The DNA-binding specificity of the hepatocyte nuclear factor 3/forkhead domain is influenced by amino-acid residues adjacent to the recognition helix. Mol Cell Biol 1994;14:2755–66.
20. Gualdi R, Bossard P, Zheng M, Hamada Y, Coleman JR, Zaret KS. Hepatic specification of the gut endoderm *in vitro*: cell signaling and transcriptional control. Genes Dev 1996;10:1670–82.
21. Kaestner KH, Katz J, Liu Y, Drucker DJ, Schutz G. Inactivation of the winged helix transcription factor HNF3alpha affects glucose homeostasis and islet glucagon gene expression *in vivo*. Genes Dev 1999;13:495–504.

22. Shih DQ, Navas MA, Kuwajima S, Duncan SA, Stoffel M. Impaired glucose homeostasis and neonatal mortality in hepatocyte nuclear factor 3alpha-deficient mice. Proc Natl Acad Sci U S A 1999;96:10152–7.
23. Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, et al. Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell 2005;122:33–43.
24. Laganiere J, Deblois G, Lefebvre C, Bataille AR, Robert F, Giguere V. From the cover: location analysis of estrogen receptor alpha target promoters reveals that FOXA1 defines a domain of the estrogen response. Proc Natl Acad Sci U S A 2005;102:11651–6.
25. Bernardo GM, Lozada KL, Miedler JD, Harburg G, Hewitt SC, Mosley JD, et al. FOXA1 is an essential determinant of ERalpha expression and mammary ductal morphogenesis. Development 2010;137:2045–54.
26. Roux J, Pictet R, Grange T. Hepatocyte nuclear factor 3 determines the amplitude of the glucocorticoid response of the rat tyrosine aminotransferase gene. DNA Cell Biol 1995;14:385–96.
27. Gao N, Zhang J, Rao MA, Case TC, Mirosevich J, Wang Y, et al. The role of hepatocyte nuclear factor-3 alpha (Forkhead Box A1) and androgen receptor in transcriptional regulation of prostatic genes. Mol Endocrinol 2003;17:1484–507.
28. Peterson RS, Clevidence DE, Ye H, Costa RH. Hepatocyte nuclear factor-3 alpha promoter regulation involves recognition by cell-specific factors, thyroid transcription factor-1, and autoactivation. Cell Growth Differ 1997;8:69–82.
29. Kopachik W, Hayward SW, Cunha GR. Expression of hepatocyte nuclear factor-3alpha in rat prostate, seminal vesicle, and bladder. Dev Dyn 1998;211:131–40.
30. Mirosevich J, Gao N, Gupta A, Shappell SB, Jove R, Matusik RJ. Expression and role of Foxa proteins in prostate cancer. Prostate 2006;66:1013–28.
31. Gao N, Ishii K, Mirosevich J, Kuwajima S, Oppenheimer SR, Roberts RL, et al. Forkhead box A1 regulates prostate ductal morphogenesis and promotes epithelial cell maturation. Development 2005;132:3431–43.
32. Wang Q, Li W, Liu XS, Carroll JS, Janne OA, Keeton EK, et al. A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. Mol Cell 2007;27:380–92.
33. Lupien M, Eeckhoute J, Meyer CA, Wang Q, Zhang Y, Li W, et al. FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. Cell 2008;132:958–70.
34. Jozwik KM, Chernukhin I, Serandour AA, Nagarajan S, Carroll JS. FOXA1 Directs H3K4 Monomethylation at enhancers via recruitment of the methyltransferase MLL3. Cell Rep 2016;17:2715–23.
35. Iwafuchi-Doi M, Zaret KS. Pioneer transcription factors in cell reprogramming. Genes Dev 2014;28:2679–92.
36. He HH, Meyer CA, Shin H, Bailey ST, Wei G, Wang Q, et al. Nucleosome dynamics define transcriptional enhancers. Nat Genet 2010;42:343–7.
37. Pomerantz MM, Li F, Takeda DY, Lenci R, Chonkar A, Chabot M, et al. The androgen receptor cistrome is extensively reprogrammed in human prostate tumorigenesis. Nat Genet 2015;47:1346–51.
38. Adams EJ, Karthaus WR, Hoover E, Liu D, Gruet A, Zhang Z, et al. FOXA1 mutations alter pioneering activity, differentiation and prostate cancer phenotypes. Nature 2019;571:408–12.
39. Parolia A, Cieslik M, Chu SC, Xiao L, Ouchi T, Zhang Y, et al. Distinct structural classes of activating FOXA1 alterations in advanced prostate cancer. Nature 2019;571:413–8.
40. Xu B, Song B, Lu X, Kim J, Hu M, Zhao JC, et al. Altered chromatin recruitment by FOXA1 mutations promotes androgen independence and prostate cancer progression. Cell Res 2019;29:773–5.
41. Jin HJ, Zhao JC, Ogden I, Bergan RC, Yu J. Androgen receptor-independent function of FoxA1 in prostate cancer metastasis. Cancer Res 2013;73:3725–36.
42. BenAyed-Guerfali D, Dabbeche-Bouricha E, Ayadi W, Trifa F, Charfi S, Khabir A, et al. Association of FOXA1 and EMT markers (Twist1 and E-cadherin) in breast cancer. Mol Biol Rep 2019;46:3247–55.
43. Song Y, Washington MK, Crawford HC. Loss of FOXA1/2 is essential for the epithelial-to-mesenchymal transition in pancreatic cancer. Cancer Res 2010;70:2115–25.
44. Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell 2009;138:245–56.
45. Zhang C, Wang L, Wu D, Chen H, Chen Z, Thomas-Ahner JM, et al. Definition of a FoxA1 Cistrome that is crucial for G1 to S-phase cell-cycle transit in castration-resistant prostate cancer. Cancer Res 2011;71:6738–48.
46. Chen Z, Zhang C, Wu D, Chen H, Rorick A, Zhang X, et al. Phospho-MED1-enhanced UBE2C locus looping drives castration-resistant prostate cancer growth. EMBO J 2011;30:2405–19.
47. Gerhardt J, Montani M, Wild P, Beer M, Huber F, Hermanns T, et al. FOXA1 promotes tumor progression in prostate cancer and represents a novel hallmark of castration-resistant prostate cancer. Am J Pathol 2012;180:848–61.
48. Jain RK, Mehta RJ, Nakshatri H, Idrees MT, Badve SS. High-level expression of forkhead-box protein A1 in metastatic prostate cancer. Histopathology 2011;58:766–72.
49. Song B, Park SH, Zhao JC, Fong KW, Li S, Lee Y, et al. Targeting FOXA1-mediated repression of TGF-beta signaling suppresses castration-resistant prostate cancer progression. J Clin Invest 2019;129:569–82.
50. Kim J, Jin H, Zhao JC, Yang YA, Li Y, Yang X, et al. FOXA1 inhibits prostate cancer neuroendocrine differentiation. Oncogene 2017;36:4072–80.
51. Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, et al. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. Nature 2011;474:390–4.
52. Jin HJ, Zhao JC, Wu L, Kim J, Yu J. Cooperativity and equilibrium with FOXA1 define the androgen receptor transcriptional program. Nat Commun 2014;5:3972.
53. Robinson JL, Hickey TE, Warren AY, Vowler SL, Carroll T, Lamb AD, et al. Elevated levels of FOXA1 facilitate androgen receptor chromatin binding resulting in a CRPC-like phenotype. Oncogene 2014;33:5666–74.
54. Chen Z, Lan X, Thomas-Ahner JM, Wu D, Liu X, Ye Z, et al. Agonist and antagonist switch DNA motifs recognized by human androgen receptor in prostate cancer. EMBO J 2015;34:502–16.
55. Yuan F, Hankey W, Wu D, Wang H, Somarelli J, Armstrong AJ, et al. Molecular determinants for enzalutamide-induced transcription in prostate cancer. Nucleic Acids Res 2019;47:10104–14.
56. Swinstead EE, Miranda TB, Paakinaho V, Baek S, Goldstein I, Hawkins M, et al. Steroid receptors reprogram FoxA1 occupancy through dynamic chromatin transitions. Cell 2016;165:593–605.
57. Glont SE, Chernukhin I, Carroll JS. Comprehensive genomic analysis reveals that the pioneering function of FOXA1 is independent of hormonal signaling. Cell Rep 2019;26:2558–65.
58. Paakinaho V, Swinstead EE, Presman DM, Grontved L, Hager GL. Meta-analysis of chromatin programming by steroid receptors. Cell Rep 2019;28:3523–34.
59. Gao S, Chen S, Han D, Barrett D, Han W, Ahmed M, et al. Forkhead domain mutations in FOXA1 drive prostate cancer progression. Cell Res 2019;29:770–2.
60. Mirosevich J, Gao N, Matusik RJ. Expression of Foxa transcription factors in the developing and adult murine prostate. Prostate 2005;62:339–52.
61. Yu X, Gupta A, Wang Y, Suzuki K, Mirosevich J, Orgebin-Crist MC, et al. Foxa1 and Foxa2 interact with the androgen receptor to regulate prostate and epididymal genes differentially. Ann N Y Acad Sci 2005;1061:77–93.
62. Connelly ZM, Yang S, Chen F, Yeh Y, Khater N, Jin R, et al. Foxa2 activates the transcription of androgen receptor target genes in castrate resistant prostatic tumors. Am J Clin Exp Urol 2018;6:172–81.
63. Huss WJ, Gray DR, Tavakoli K, Marmillion ME, Durham LE, Johnson MA, et al. Origin of androgen-insensitive poorly differentiated tumors in the transgenic adenocarcinoma of mouse prostate model. Neoplasia 2007;9:938–50.
64. Qi J, Nakayama K, Cardiff RD, Borowsky AD, Kaul K, Williams R, et al. Siah2-dependent concerted activity of HIF and FoxA2 regulates formation of neuroendocrine phenotype and neuroendocrine prostate tumors. Cancer Cell 2010;18:23–38.
65. Gupta A, Wang Y, Browne C, Kim S, Case T, Paul M, et al. Neuroendocrine differentiation in the 12T-10 transgenic prostate mouse model mimics endocrine differentiation of pancreatic beta cells. Prostate 2008;68:50–60.
66. Park JW, Lee JK, Witte ON, Huang J. FOXA2 is a sensitive and specific marker for small cell neuroendocrine carcinoma of the prostate. Mod Pathol 2017;30:1262–72.
67. Mann RS, Affolter M. Hox proteins meet more partners. Curr Opin Genet Dev 1998;8:423–9.
68. Shen WF, Montgomery JC, Rozenfeld S, Moskow JJ, Lawrence HJ, Buchberg AM, et al. AbdB-like Hox proteins stabilize DNA binding by the Meis1 homeodomain proteins. Mol Cell Biol 1997;17:6448–58.

69. Williams TM, Williams ME, Innis JW. Range of HOX/TALE superclass associations and protein domain requirements for HOXA13:MEIS interaction. Dev Biol 2005;277:457–71.
70. Shen WF, Krishnan K, Lawrence HJ, Largman C. The HOX homeodomain proteins block CBP histone acetyltransferase activity. Mol Cell Biol 2001;21: 7509–22.
71. Zeltser L, Desplan C, Heintz N. Hoxb-13: a new Hox gene in a distant region of the HOXB cluster maintains colinearity. Development 1996;122: 2475–84.
72. Sreenath T, Orosz A, Fujita K, Bieberich CJ. Androgen-independent expression of hoxb-13 in the mouse prostate. Prostate 1999;41:203–7.
73. Economides KD, Capecchi MR. Hoxb13 is required for normal differentiation and secretory function of the ventral prostate. Development 2003; 130:2061–9.
74. Huang L, Pu Y, Hepps D, Danielpour D, Prins GS. Posterior Hox gene expression and differential androgen regulation in the developing and adult rat prostate lobes. Endocrinology 2007;148:1235–45.
75. Jung C, Kim RS, Lee SJ, Wang C, Jeng MH. HOXB13 homeodomain protein suppresses the growth of prostate cancer cells by the negative regulation of T-cell factor 4. Cancer Res 2004;64:3046–51.
76. Jung C, Kim RS, Zhang H, Lee SJ, Sheng H, Loehrer PJ, et al. HOXB13 is downregulated in colorectal cancer to confer TCF4-mediated transactivation. Br J Cancer 2005;92:2233–9.
77. Zhao Y, Yamashita T, Ishikawa M. Regulation of tumor invasion by HOXB13 gene overexpressed in human endometrial cancer. Oncol Rep 2005;13:721–6.
78. Yamashita T, Tazawa S, Yawei Z, Katayama H, Kato Y, Nishiwaki K, et al. Suppression of invasive characteristics by antisense introduction of overexpressed HOX genes in ovarian cancer cells. Int J Oncol 2006;28: 931–8.
79. Jung C, Kim RS, Zhang HJ, Lee SJ, Jeng MH. HOXB13 induces growth suppression of prostate cancer cells as a repressor of hormone-activated androgen receptor signaling. Cancer Res 2004;64:9185–92.
80. Norris JD, Joseph JD, Sherk AB, Juzumiene D, Turnbull PS, Rafferty SW, et al. Differential presentation of protein interaction surfaces on the androgen receptor defines the pharmacological actions of bound ligands. Chem Biol 2009;16:452–60.
81. Norris JD, Chang CY, Wittmann BM, Kunder RS, Cui H, Fan D, et al. The homeodomain protein HOXB13 regulates the cellular response to androgens. Mol Cell 2009;36:405–16.
82. Kim YR, Oh KJ, Park RY, Xuan NT, Kang TW, Kwon DD, et al. HOXB13 promotes androgen independent growth of LNCaP prostate cancer cells by the activation of E2F signaling. Mol Cancer 2010;9:124.
83. Chen Z, Wu D, Thomas-Ahner JM, Lu C, Zhao P, Zhang Q, et al. Diverse AR-V7 cistromes in castration-resistant prostate cancer are governed by HoxB13. Proc Natl Acad Sci U S A 2018;115:6810–5.
84. Ewing CM, Ray AM, Lange EM, Zuhlke KA, Robbins CM, Tembe WD, et al. Germline mutations in HOXB13 and prostate-cancer risk. N Engl J Med 2012; 366:141–9.
85. Storebjerg TM, Hoyer S, Kirkegaard P, Bro F, LuCamp Study G, Orntoft TF, et al. Prevalence of the HOXB13 G84E mutation in Danish men undergoing radical prostatectomy and its correlations with prostate cancer risk and aggressiveness. BJU Int 2016;118:646–53.
86. Kote-Jarai Z, Mikropoulos C, Leongamornlert DA, Dadaev T, Tymrakiewicz M, Saunders EJ, et al. Prevalence of the HOXB13 G84E germline mutation in British men and correlation with prostate cancer risk, tumour characteristics and clinical outcomes. Ann Oncol 2015;26:756–61.
87. Lin X, Qu L, Chen Z, Xu C, Ye D, Shao Q, et al. A novel germline mutation in HOXB13 is associated with prostate cancer risk in Chinese men. Prostate 2013; 73:169–75.
88. Bhanvadia RR, VanOpstall C, Brechka H, Barashi NS, Gillard M, McAuley EM, et al. MEIS1 and MEIS2 expression and prostate cancer progression: a role for HOXB13 binding partners in metastatic disease. Clin Cancer Res 2018;24: 3668–80.
89. Johng D, Torga G, Ewing CM, Jin K, Norris JD, McDonnell DP, et al. HOXB13 interaction with MEIS1 modifies proliferation and gene expression in prostate cancer. Prostate 2019;79:414–24.
90. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016;22:298–305.
91. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161: 1215–28.
92. Orkin SH. Globin gene regulation and switching: circa 1990. Cell 1990;63: 665–72.
93. Mouthon MA, Bernard O, Mitjavila MT, Romeo PH, Vainchenker W, Mathieu-Mahul D. Expression of tal-1 and GATA-binding proteins during human hematopoiesis. Blood 1993;81:647–55.
94. Briegel K, Lim KC, Plank C, Beug H, Engel JD, Zenke M. Ectopic expression of a conditional GATA-2/estrogen receptor chimera arrests erythroid differentiation in a hormone-dependent manner. Genes Dev 1993;7: 1097–109.
95. Tsai FY, Orkin SH. Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. Blood 1997;89:3636–43.
96. Ling KW, Ottersbach K, van Hamburg JP, Oziemlak A, Tsai FY, Orkin SH, et al. GATA-2 plays two functionally distinct roles during the ontogeny of hematopoietic stem cells. J Exp Med 2004;200:871–82.
97. Tsai FY, Keller G, Kuo FC, Weiss M, Chen J, Rosenblatt M, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature 1994;371:221–6.
98. Zhou Y, Lim KC, Onodera K, Takahashi S, Ohta J, Minegishi N, et al. Rescue of the embryonic lethal hematopoietic defect reveals a critical role for GATA-2 in urogenital development. EMBO J 1998;17:6689–700.
99. Perez-Stable CM, Pozas A, Roos BA. A role for GATA transcription factors in the androgen regulation of the prostate-specific antigen gene enhancer. Mol Cell Endocrinol 2000;167:43–53.
100. Minegishi N, Morita S, Minegishi M, Tsuchiya S, Konno T, Hayashi N, et al. Expression of GATA transcription factors in myelogenous and lymphoblastic leukemia cells. Int J Hematol 1997;65:239–49.
101. Wu D, Sunkel B, Chen Z, Liu X, Ye Z, Li Q, et al. Three-tiered role of the pioneer factor GATA2 in promoting androgen-dependent gene expression in prostate cancer. Nucleic Acids Res 2014;42:3607–22.
102. Vidal SJ, Rodriguez-Bravo V, Quinn SA, Rodriguez-Barrueco R, Lujambio A, Williams E, et al. A targetable GATA2-IGF2 axis confers aggressiveness in lethal prostate cancer. Cancer Cell 2015;27:223–39.
103. Chiang YT, Wang K, Fazli L, Qi RZ, Gleave ME, Collins CC, et al. GATA2 as a potential metastasis-driving gene in prostate cancer. Oncotarget 2014;5: 451–61.
104. Buscheck F, Zub M, Heumann A, Hube-Magg C, Simon R, Lang DS, et al. The independent prognostic impact of the GATA2 pioneering factor is restricted to ERG-negative prostate cancer. Tumour Biol 2019;41: 1010428318824815.
105. Xiao L, Feng Q, Zhang Z, Wang F, Lydon JP, Ittmann MM, et al. The essential role of GATA transcription factors in adult murine prostate. Oncotarget 2016;7: 47891–903.
106. Gui B, Gui F, Takai T, Feng C, Bai X, Fazli L, et al. Selective targeting of PARP-2 inhibits androgen receptor signaling and prostate cancer growth through disruption of FOXA1 function. Proc Natl Acad Sci U S A 2019;116: 14573–82.
107. Kounatidou E, Nakjang S, McCracken SRC, Dehm SM, Robson CN, Jones D, et al. A novel CRISPR-engineered prostate cancer cell line defines the AR-V transcriptome and identifies PARP inhibitor sensitivities. Nucleic Acids Res 2019;47:5634–47.
108. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med 2015;373:1697–708.
109. Imagawa S, Nakano Y, Obara N, Suzuki N, Doi T, Kodama T, et al. A GATA-specific inhibitor (K-7174) rescues anemia induced by IL-1beta, TNF-alpha, or L-NMMA. FASEB J 2003;17:1742–4.
110. Umetani M, Nakao H, Doi T, Iwasaki A, Ohtaka M, Nagoya T, et al. A novel cell adhesion inhibitor, K-7174, reduces the endothelial VCAM-1 induction by inflammatory cytokines, acting through the regulation of GATA. Biochem Biophys Res Commun 2000;272:370–4.
111. He B, Lanz RB, Fiskus W, Geng C, Yi P, Hartig SM, et al. GATA2 facilitates steroid receptor coactivator recruitment to the androgen receptor complex. Proc Natl Acad Sci U S A 2014;111:18261–6.
112. Hayakawa F, Towatari M, Ozawa Y, Tomita A, Privalsky ML, Saito H. Functional regulation of GATA-2 by acetylation. J Leukoc Biol 2004;75: 529–40.

113. Chaytor L, Simcock M, Nakjang S, Heath R, Walker L, Robson C, et al. The pioneering role of GATA2 in androgen receptor variant regulation is controlled by bromodomain and extraterminal proteins in castrate-resistant prostate cancer. Mol Cancer Res 2019;17:1264–78.
114. Nerlakanti N, Yao J, Nguyen DT, Patel AK, Eroshkin AM, Lawrence HR, et al. Targeting the BRD4-HOXB13 coregulated transcriptional networks with bromodomain-kinase inhibitors to suppress metastatic castration-resistant prostate cancer. Mol Cancer Ther 2018;17:2796–810.
115. Ember SW, Lambert QT, Berndt N, Gunawan S, Ayaz M, Tauro M, et al. Potent dual BET bromodomain-kinase inhibitors as value-added multi-targeted chemical probes and cancer therapeutics. Mol Cancer Ther 2017; 16:1054–67.
116. Zhang G, Zhao Y, Liu Y, Kao LP, Wang X, Skerry B, et al. FOXA1 defines cancer cell specificity. Sci Adv 2016;2:e1501473.
117. Liu YN, Lee WW, Wang CY, Chao TH, Chen Y, Chen JH. Regulatory mechanisms controlling human E-cadherin gene expression. Oncogene 2005;24:8277–90.
118. Xie B, Bai B, Xu Y, Liu Y, Lv Y, Gao X, et al. Tumor-suppressive function and mechanism of HOXB13 in right-sided colon cancer. Signal Transduct Target Ther 2019;4:51.