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Prostate Cancer Epigenetic Plasticity and Enhancer Heterogeneity: Molecular Causes, Consequences and Clinical Implications

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Abstract

Prostate cancer (PCa) proliferation is dictated by androgen receptor (AR) signaling, which regulates gene expression through *cis*-regulatory regions including proximal and distal enhancers. The repertoire of AR interactions at enhancers is dependent on tissue and cellular contexts and thus shape a spectrum of phenotypes through such epigenetic heterogeneity. Moreover, PCa is a multifocal disease and displays a high degree of intra- and inter-tumor heterogeneity, adding to the phenotypic complexity. It is increasingly becoming clear that PCa may be considered an epigenetic disease caused by various molecular causes with profound consequences and clinical implications which are underpinned by enhancer interaction heterogeneity.

In this review, we provide a detailed overview of molecular interactors that affect prostate cancer epigenetic heterogeneity, such as coding and non-coding somatic variants, large scale structural variations, pioneer factor binding at enhancers and various contexts that influence enhancer engagement heterogeneity in PCa development and progression. Finally, we explore how the vast heterogeneity in epigenetic profiles identified in recent omics studies results in distinct genomic subtypes which predict disease progression and thus offer opportunities in biomarker discovery and further personalizing cancer treatment. As such, heterogeneous enhancer interactions take center stage in elucidating mechanisms of prostate cancer progression, patient prognostication, therapy discovery and overcoming acquired treatment resistance.

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Keywords

Prostate cancer · Enhancers · Androgen receptor · Epigenetics · Heterogeneity · Cistrome plasticity · Personalized medicine

Abbreviations

AR	Androgen receptor
ARBS	Androgen receptor binding site
ARE	Androgen response element
ADT	Androgen deprivation therapy
ChIP-seq	Chromatin immunoprecipitation followed by sequencing
CRPC	Castration resistant prostate cancer
DHT	Dihydrotestosterone
GWAS	Genome wide association study
NR	Nuclear receptor
PCa	Prostate cancer
PDX	Patient derived xenograft
PIN	Prostatic intraepithelial neoplasia
PTM	Post-transcriptional modification
SE	Super enhancer
SNP	Single nucleotide polymorphism
TAD	Topologically associating domains
TF	Transcription factor
TSS	Transcriptional start site

15.1 Introduction

Enhancers were first discovered in 1981 when researchers of two independent groups found simian virus (SV40) DNA sequences 3kb distal to the SV40 promoter capable of stimulating transcriptional output of a linked β-globin gene by 200-fold when transfected in mammalian cells [1, 2]. These experiments generated a more complete understanding of how gene regulation emerges from an interplay between often distally located enhancers and proximal promoter regions. An onset of subsequent studies discovered not only that enhancers are general genomic features in a variety of organisms including mammals [3–7], but also that defects in enhancers can lead to pathogenesis [8–11]. Although the human genome contains approximately 20,000 protein coding genes [12], currently roughly fifty times more non-coding regulatory regions have been described across tissue types [13, 14], prompting a reassessment of non-coding genome functionality. Moreover, genome-wide association studies (GWAS) have shown that variants involved in human disease are enriched at non-coding regulatory elements over coding sequences [15]. Early genome-wide studies identified the total repertoire of promoter and enhancer sequences based on a combination of ChIP-seq and chromatin accessibility assays with specific histone modifications such as high H3K27ac signal [16–18], whereas later a high proportion of H3K4 mono- versus tri-methylation allowed researchers to separate enhancers from promoters [19, 20].

Successive research endeavors characterized enhancer sequences to have the following properties: (1) activated enhancers mediate strong transcriptional activation of the gene it controls [1, 2], (2) activation is independent from the orientation of the enhancer element [1, 2], (3) enhancers function in a tissue specific manner [3], (4) enhancer sequences are bidirectionally transcribed as short enhancer RNA (eRNA) transcripts [21], (5) enhancers possess regulatory multiplicity, in which a single enhancer can activate multiple promoters of linked genes, whereas multiple enhancers can also regulate a single promoter [22, 23], (6) activation can be exerted in *cis* over genomic distances up to megabases away [24], (7) enhancers are scattered throughout 98% of the non-coding human genome [25]. The last property was an unexpected finding of modern genome sequencing and annotation by the Encyclopedia of DNA Elements (ENCODE) project, showing that a large proportion of the non-coding genome has regulatory control over the expression of the coding genome [25]. Interestingly, changes in non-coding regulatory elements are frequently observed in oncogenesis [10, 11, 26].

15.2 Prostate Cancer as Enhancer-Driven Disease

Prostate cancer (PCa) is the second-most commonly diagnosed malignancy in men worldwide [27]. PCa is mainly driven by the nuclear receptor androgen receptor (AR) [28], that acts as a master transcription regulator of cell proliferation when bound to its cognate ligand dihydrotestosterone (DHT) [29, 30]. While blockade of the AR signaling axis using androgen deprivation therapy (ADT) as a first line of treatment is initially successful [31, 32], over time resistance to ADT inevitably occurs and remaining cancer cells rebound as lethal castration resistant prostate cancer (CRPC) [33, 34]. AR signaling persists during CRPC despite castration level circulating testosterone, which highlights the essentiality of AR signaling in PCa cells. Sustained successful PCa treatment is challenged by the heterogeneous nature of PCa, which is present on multiple levels (Fig. 15.1).

PCa is a multifocal disease with ~60–90% of patients presenting multiple independent primary tumor foci at time of diagnosis [35–38]. Such foci exhibit inter-lesion heterogeneity, which

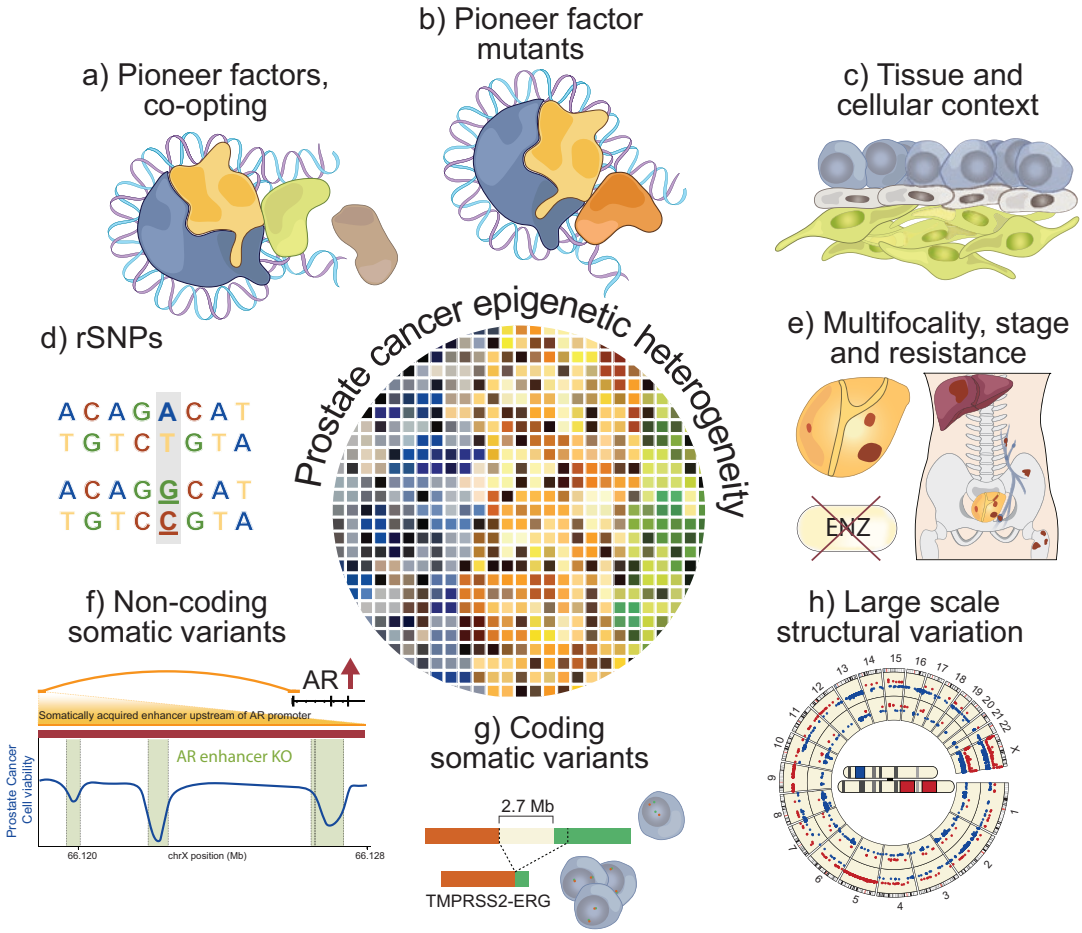

Fig. 15.1 Overview of sources of prostate cancer epigenetic heterogeneity: Epigenetic heterogeneity in AR enhancer interactions varies in different tissues and cellular contexts and can be induced by (**a**) pioneer factor binding at chromatin and (**b**) pioneer factor mutations altering chromatin binding properties, (**c**) tissue and cellular context, (**d**) germline PCa risk single nucleotide polymorphisms, (**e**) PCa multifocality, disease stage and acquirement of therapy resistance, (**f**) non-coding and (**g**) coding somatic variants and (**h**) large scale structural variations that amplify or delete genomic regions

manifests in differences in cell morphology, tumor microenvironment and degrees of aggressiveness [39, 40]. Contrastingly, metastatic PCa lesions were reported to predominantly share a homogeneous, monoclonal background [41]. While primary local interventions, such as radiotherapy and prostatectomy, affect the entire prostate and treat all foci successfully, these treatments are associated with significant adverse effects [42, 43]. An alternative approach revolves around limited local treatments that ablate only the largest tumor focus while sparing the prostate and limiting adverse effects. However, these strategies are complicated by PCa heterogeneity, as remaining lesions may still metastasize at a later stage [44, 45]. Second, intratumoral heterogeneity is observed in genetically diverse cell populations within a single tumor focus and arises from tumor microenvironmental cues, lineage plasticity, as well as genetic and epigenetic defects [46–50]. Genomic inter-tumor heterogeneity manifests itself in the shape of small-scale genetic mutations like single nucleotide variants (SNVs), while copy number alterations (CNAs) and translocations of large-scale genomic elements are even more likely to impact tumor development [46, 51, 52]. Third, such events also impact *cis*-regulatory elements such as enhancers that tightly control expression on the same DNA strand, which disrupts epigenetic regulatory networks leading to profound phenotypic differences and loss of cellular identity [53].

An increasing amount of evidence illuminates a role for heterogeneous epigenetic regulation in PCa through AR [37, 54, 55], but how can intra- and intertumoral heterogeneous enhancer interactions shape a spectrum of phenotypes and outcomes in PCa? As heterogeneity seems to be pervasive in tumors, one can ask the question what the contributions of different sources of heterogeneity in the progression of PCa are. Clearly, research questions and efforts have converged on elucidating the role of AR as oncogenic driver and the emergence of resistance. Can we apply such knowledge of AR chromatin interaction profiles and their dysregulation to attempt overcoming resistance by optimizing and personalizing PCa treatment based on heterogeneity? In this review, we aim to address these questions by providing a comprehensive overview of recent progress that has been made on this subject and indicate which therapeutic avenues future research might illuminate.

15.3 AR Biology and Enhancer Regulation in Prostate Cancer

Historically, nuclear receptors were investigated in the context of their activity at promoter elements. For AR and PCa, prostate specific antigen (PSA; encoded by *KLK3*) represents a highly characterized example of AR promoter binding, with specificity to prostate tissue and high androgen inducibility [56, 57]. However, later studies revealed that AR binding at promoters is an exception and represents a relatively rare event, as compared to AR binding at enhancers [58]. Activated steroidal (Type I), nuclear receptors like AR possess the capacity to regulate transcription of target genes through binding at enhancer elements that are located distally from a target gene's transcriptional start site (TSS) [59, 60]. Such distal regulation offers tight, but also highly modular control of transcription in response to hormonal cues, with many co-regulators involved in transcriptional output [61]. Specifically, AR becomes activated upon binding with its cognate ligand dihydrotestosterone (DHT) in the cytosol, dimerizes and subsequently translocates to the nucleus where it binds to AR binding sites (ARBS) [29, 62]. Although AR's DNA binding domain recognizes and binds androgen response elements (AREs) consisting of dihexameric palindromes on the DNA [63], ARE presence is not a strict requirement for DNA binding, since AR cooperates with interacting TFs bound at AP-1, MYC, KLF and SREBF motifs [64, 65].

Recruitment of co-factors to enhancers is required for DNA looping and subsequent enhancer-promoter interactions. Factors bound at enhancers provide scaffolding for the large mediator complex to bind transiently and further recruit the transcriptional machinery [66–69].

Indeed, mediator's MED1 subunit contains LXXLL binding motifs that strongly interacts with the AR-AF2 domain in a ligand-dependent manner [70] and recently a cryo-EM study reported steroid receptor coactivator 3 (NCOA3/SRC-3) to interact with an FXXLF binding motif in AR's N-terminal domain, enabling p300/CREB-binding protein (CBP) recruitment [71]. Since mediator recruits RNA polymerase II (RNAPII) and activates expression at promoters, enhancers can affect expression over large distances without direct promoter contact, which was demonstrated for PCa and AR by collaboration with ERG [72]. We provide a graphical overview of proteins involved in AR promoter-enhancer interactions in Fig. 15.2.

Additionally, transcription also occurs at enhancer loci when active AR complexes recruit RNAPII polymerases [73]. In contrast to RNAPII activity at gene-coding promoters resulting in mRNAs, bidirectional transcription at RNAPII-occupied enhancers gives rise to small, unstable eRNAs [21]. Ascribing specific functionality to a number of eRNAs has succeeded in the context of gene expression [74, 75] and fine-tuning co-activator function at gene promoters [76]. Although defining general functionality of eRNAs remains challenging, TF activity at enhancers can be inferred through RNAPII stochastic models quantifying co-localization of TF binding motifs and eRNAs [77, 78]. These findings were further corroborated by transgenic embryonic assays, showing that enhancer functionality can be predicted by the level and directionality of eRNA transcription [79]. Finally, combining RNA-seq with chromatin accessibility data through ATAC-seq has been used to map eRNA transcript abundance on a genome-wide scale in neuronal cell populations in different activation states, providing first evidence that eRNA function is dependent on genomic context and partially dependent on sequence [80]. Next to eRNA transcription at enhancers, other studies also revealed the existence of large and dynamic transcriptional hubs at highly active loci of TF binding [81–83]. Such loci containing many active enhancer elements often regulate key differentiation processes in development and tissue

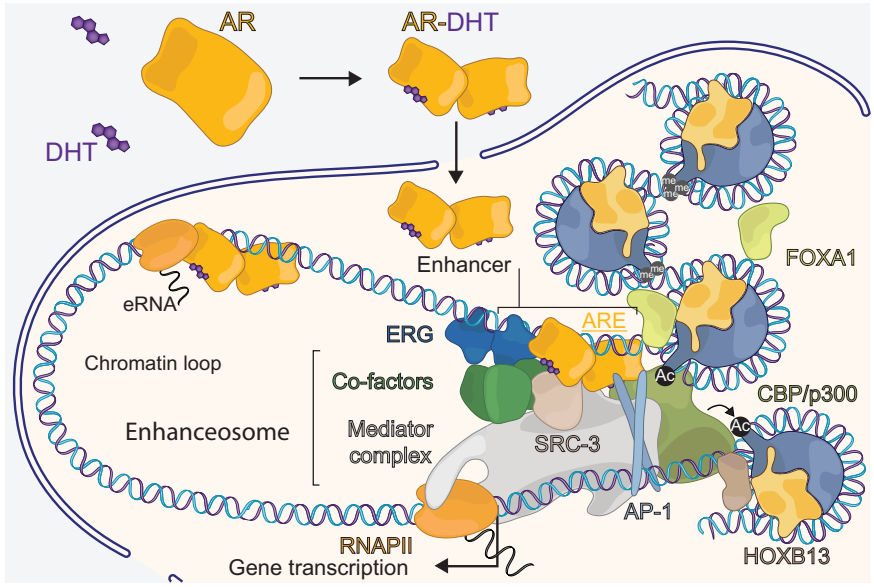

Fig. 15.2 Graphical overview of AR action at enhancers: AR binds DHT and dimerizes in the cytoplasm prior to nuclear translocation. Pioneer factor FOXA1 opens chromatin wrapped tightly around histones, allowing AR dimers to bind the chromatin through AREs and other regulatory elements. Co-factors and transcriptional machinery components such as SRC-3, CBP, p300, AP1, mediator complex and RNAPII are recruited to facilitate gene transcription, while RNAPII activity at AR-bound enhancers results in bidirectional transcription of eRNAs

identity and have been dubbed 'super enhancers' (SEs). Since clusters of enhancers in close proximity recruit many TFs, SEs form phase-separated condensates [84] with a local high-density biomolecule assembly of RNAPII [83], co-activators MED1, BRD4 [82, 83] and KLF4 [85]. However, the true number, distribution, and the proposed synergistic transcriptional activation of SEs is a matter of ongoing research and scientific debate [86].

15.4 PCa-Specific Pioneer Factors as Source of Regulatory Heterogeneity in AR Binding

Transcriptionally silent chromatin is required for maintaining correct cellular identity dictated by a specific subset of genes transcribed from active chromatin, tightly regulating cell fate decisions. Pioneer factors like forkhead box protein A (FOXA1) open up condensed chromatin [87], so that transcription factors (TFs) and ultimately transcriptional coactivator complexes such as CBP and p300 [88, 89] and other coregulators like SRC-3 can bind [90, 91]. Additionally, SWI/SNF chromatin remodelers (or human BAF complex: ATP-dependent BRG1/BRM associated factors) and other co-modulators can bind to activate and repress expression through inducing chromatin conformation changes [61].

Transcriptionally inactive chromatin or heterochromatin is nucleosome-dense and compactly folded DNA characterized mainly by histone tail post-transcriptional modifications (PTMs) of up to three methyl groups at histone H3 lysine 9 (H3K9me1-3) and H3 lysine 27 (H3K27me1-3) [92]. Consequently, gene transcription is silenced as TFs are physically blocked by nucleosomes from binding heterochromatin at enhancer elements [92]. However, pioneer factors open chromatin and enhancer sequences for TF binding [87]. In PCa development, FOXA1 and homeobox B13 (HOXB13) expression levels are increased while their mode of action is reprogrammed, allowing for altered regulation of AR-mediated transcription [61, 93]. Additionally, GATA2 and OCT1 have also been found to cooperate with AR to mediate androgen response in PCa growth [58, 94].

As a result of these functions, pioneer factors facilitate AR binding through nucleosome displacement, thereby inducing an open chromatin conformation which is characterized by 'active' enhancer histone modifications and which is permissive to TF binding [95–98]. AR binding at DNA is mostly pioneered by FOXA1 binding to chromatin, marked by hypomethylated DNA and presence of histone modifications H3K4me1 and H3K4me2 [99–101]. FOXA1 was first identified as an AR interactor when FOXA1 binding motifs were found located adjacent to ARBS in prostate gene regulatory regions for human PSA and rat probasin (PSA orthologue) [102]. Additionally, AR's DNA binding domain interacts directly with FOXA1's forkhead domain [102, 103]. Genome-wide FOXA1-bound sites were shown to be cell-line specific and differentially functional between breast and PCa cell lines [99, 104], with genome-wide FOXA1 binding at the majority of ARBS later confirmed specifically in PCa cell lines LNCaP and VCaP [105, 106]. Interestingly, silencing of FOXA1 triggers a switch in AR binding at ARBS, altering gene expression profiles in PCa cell lines [105–108]. As such, transcriptional activity of diverse gene networks resulting from FOXA1's pioneer factor activity, are tissue-specific and control cellular identity [87, 109].

Interestingly, ARBS are rarely found at promoters, as the vast majority of ARBS are found at putative enhancer sequences located distally of the target gene's locus depending on tissue and cellular context [93, 110]. Taken together, such distal *cis*-regulatory ARBS constitute the AR cistrome; the term cistrome was first coined in a 2008 study on FOXA1 and ERα binding sites in breast cancer [99]. As such, an AR cistrome is a collection of ARBS that describes the transcriptional regulatory potential of activated AR in a specific context, which have been extensively reported in many different contexts such as healthy prostate tissue, PCa cell lines and tissues from varying stages of PCa [61, 93, 110–114]. Additionally, AR cistromes also vary in different cell type contexts like fibroblasts [115], macro-

phages [116], male breast cancer [111] and female breast cancer [117]. In this review, we focus on AR function in prostate epithelial cells, mostly in the context of PCa. In the following section, we address the question of how context-dependent AR cistromes influence PCa heterogeneity and how shifts in AR cistromes affect tumor progression.

15.5 AR Cistromes are Heterogeneous Between Different Tissue, Cellular and Tumor Contexts

Prostate development is a complex process dependent on the presence of androgens and developmental pathways requiring activation of diverse genes at different stages and tissue identities [118]. As such, various types of prostate tissue are thought to be driven by different AR cistromes during development and tissue maintenance, but also during tumor initiation [119, 120]. One of the first studies to dissect the differences between AR cistromes in prostate tissue compared histologically normal prostate tissues with prostate cancers, which were both enriched in epithelial cell content [93]. A core set of tumor associated ARBS (T-ARBS) was found to co-localize with FOXA1 and HOXB13 binding, which was absent at normal associated ARBS (N-ARBS), providing the first clinical evidence of AR cistrome reprogramming [93].

Furthermore, overexpression of FOXA1 and HOXB13 in benign prostate cells induced a change in AR cistrome reminiscent of reprogramming in PCa cells, showing that in tumorigenesis HOXB13 may act as a pioneer factor and induces different AR cistromic repertoires that influence disease progression [121, 122]. This finding was later confirmed by a study that found somatic structural variants to impact master TF *cis*-regulatory regions, altering binding for various factors including AR, FOXA1, HOXB13 and SOX9, which in turn may influence prostate oncogenesis [123]. Additionally, such a malignancy-associated shift in AR signalling can also be pioneered by GATA2 and c-JUN [58, 124, 125]. GATA2 is a zinc-finger TF that normally regulates developmental gene expression but also influences AR chromatin binding by enabling access to additional putative ARBS prior to androgen stimulation [94]. Newly accessible ARBS include those near the AR locus, resulting in a GATA2-pioneered elevation of AR expression, which can further be enhanced by co-occupancy by FOXA1 at GATA2-pioneered sites [94]. c-JUN dimerizes with FOS to form the AP-1 complex which transactivates gene expression of PCa driver ETV1 [124]. Moreover, c-JUN's expression levels were found to correlate with AR transcriptional activity and knockdown of c-JUN abrogated AR-dependent PCa cell proliferation [64, 126]. Although c-JUN can control AR binding and has been implicated in AR malignancy shift, pioneering activity by c-JUN has not been formally proven. Taken together, an ensemble of TFs modulates AR through enabling chromatin accessibility at newly activated ARBS, thereby expanding the repertoire of possible AR cistromes that are associated with a context-dependent PCa AR signalling malignancy shift.

Acquired cancer therapy resistance is deeply rooted in inter- and intra-tumor heterogeneity, in which a certain cell population manages to overcome and adapt to therapy-induced selection over other populations [49]. In androgen-depleted conditions, PCa cell subpopulations that lose prostate differentiation while gaining resistance to AR signaling inhibition have been shown to survive and acquire an aggressive pathological phenotype [127]. As such, tumor progression can be viewed as an evolutionary dynamic process, in which tumor cells not only reprogram epigenetic control of cell identity or acquire a new phenotype, but also communicate differentially with their tumor microenvironment (TME) [50, 128]. While PCa cell lines -mostly derived from patients with advanced disease- are typically typical studied in the absence of a TME context, recently a push has been made to boost the diversity of clinical stages represented in PCa models in which a TME is present, using patient-derived xenografts (PDXs) [129].

Diverse PCa cell lines and PDX models contain ARBS that are shared, but there are also ARBS that are specifically found in a single cell line, that partly recapitulate the intrinsic interpatient heterogeneity [113]. Although AR cistromes in prostatic epithelial cells and tissues take center stage, AR cistromes are also heterogeneous between cell types of the prostate TME, which can interact with tumors and influence growth [47]. AR cistromes of PCa stroma constituent cells like fibroblasts and macrophages have been dissected and were found to deviate from AR cistromes reported in epithelial cells [115, 116, 130]. The context dependency of the AR cistrome in these TME-associated cell constituents functionally contributes to PCa progression by affecting PCa migration potential or by supporting PCa invasiveness through AR signaling.

On a final note, diverse AR cistromes are also found in both ER^+ and ER^- (molecular apocrine) breast cancer. AR cistromes in both breast cancer subtypes are also facilitated by FOXA1, yet with opposing forces on tumor driving potential, with AR acting as driver in ER^- but as tumor suppressor in ER^+ breast cancers [111, 117, 131, 132]. Clearly, the topic of cancer cistrome heterogeneity is wide-ranging and has been reviewed previously [133–135]. Therefore, we will focus on which AR cistromic heterogeneity occurs within the different stages of PCa progression from initiation to development of metastatic CRPC.

15.6 AR Cistromic Heterogeneity Progressively Develops from PCa Initiation to Neuroendocrine Differentiation

Early stage primary PCa is confined to the prostate, with lesions initiating in the glandular tissue lesions in the form of prostatic intraepithelial neoplasia (PIN) lesions in which DNA damage caused by oxidative stress and inflammation in the prostate gland plays an important role [136–138]. PCa tumorigenesis is genomically characterized by the occurrence of SNVs, small deletions and gene fusions, while AR activity is highly heterogeneous among tumors [37, 54]. Interestingly, different primary tumor foci in the same prostate rarely share SNVs or structural variation at regulatory elements, further highlighting the multiclonal heterogeneous nature of primary tumors [123]. SNV accumulation in tumor foci was also found to rarely drive pro-oncogenic processes, providing a potential explanation for PCa indolence [51, 139, 140]. However, the myriad of SNVs present at regulatory elements alter the transactivation potential of enhancers, especially of those regulating master TF activity [123]. Moreover, primary tumors do have an enrichment of SNVs in ARBS that are somatically acquired in tumors, thus providing a source of genetic heterogeneity in PCa that may affect epigenetic regulation [123]. To study epigenetic regulation in PCa, we previously undertook epigenetic analyses to dissect AR cistrome heterogeneity in primary tumors by integrating gene expression data with AR cistrome data with enhancer-mapping histone modification marks (H3K27ac, H3K27me3 and H3K4me3) [55].

Three major epigenetic subtypes were revealed in primary PCa tissues, two of which were dominated by TMPRSS2-ERG fusion status, while a third was characterized by low activity and chromatin binding of AR, but with high WNT and FGF signalling [55]. TMPRSS2-ERG fusions lead to a particularly reprogrammed cistrome, as evidenced by a different H3K27ac profile that enables co-opting of ERG of AR, FOXA1 and HOXB13 resulting in AR cistromic heterogeneity [141]. Although AR profiles in primary disease do not appear to have prognostic potential by themselves, AR cistrome reprogramming continuously occurs during disease progression [55]. Somatic structural variants, such as either TMPRSS2-ERG gene fusions or coding mutations in FOXA1 and SPOP are also found associated with AR cistrome plasticity and are discussed in-depth later.

15.7 Metastatic PCa Heterogeneity

PCa mortality is predominantly caused by metastatic disease, in which tumor cells preferentially spread from a primary lesion to locoregional lymph nodes and bones [27, 142, 143]. Somatically acquired large-scale structural enhancer variants are common in cancer [144]; a process which accelerates in metastatic disease [145] and affects TF binding, chromatin organization and gene expression [146]. In metastatic PCa, large scale structural variations at either coding or cis-regulatory sequences represent a class of key oncogenic events often coupled with copy number alterations (CNAs) such as gains at critical oncogenes including AR, MYC, CDK12, or losses at tumor-suppressor genes including TP53 and BRCA2 [147]. Recently, a study was reported that integrated pan-cancer genomics data with clinical information and functional genome-scale CRISPRi screens in metastatic PCa models to discover additional drivers of metastatic PCa, revealing that KIF4A knockdown alters genome-wide chromatin accessibility and acts as a driver of metastatic PCa aggressiveness with concomitant poor prognosis [148].

Prognostication of PCa patients based on pathological and genomic biomarkers could distinguish those patients with high-risk of developing aggressive disease over those with indolent PCa, paving the way for prognostication based on epigenetic status [149–151]. Another study from our group compared genome-wide AR binding, chromatin accessibility and gene expression between primary PCa and ADT-resistant tumors and integrated these with publically available clinical and genomic cancer databases [151]. The resulting gene expression signature could predict outcome in primary PCa patients in independent cohorts, suggesting that an underlying pro-metastatic AR cistrome may already be present in patients with primary patients whose disease eventually progressed [151]. This notion was further supported by a study that epigenetically profiled tissues in the disease progression spectrum from normal prostate epithelium to primary PCa to metastatic disease [121]. Normal prostate epithelium already displays regulatory elements that are prepopulated by FOXA1 and HOXB13, which AR later binds in metastatic PCa to drive fetal prostate developmental programs [121]. These two studies together underline the relevance of studying PCa state transitions epigenetically as a crucial method to understand molecular underpinnings underlying PCa progression and it critically suggests that inter-tumor PCa heterogeneity is strongly associated with cistromic heterogeneity.

Difficult to treat metastatic castration resistant prostate cancer (mCRPC) arises once metastatic PCa growth has been restored through reactivation of AR signaling pathways in an ADT-induced, low testosterone environment [152]. mCRPC is characterized by a distinct AR cistrome that is reprogrammed by CRPC specific TFs such as STAT, MYC and E2F, while such heterogeneity is not captured by cell lines but only found in tissues [113]. Later, a first report on AR, FOXA1 and CTCF binding in multiple metastatic tumors in an individual patient confirmed a robust, metastasis-specific transcriptional program despite few inter-lesion differences in the AR cistrome, showing that the metastatic AR cistrome between different affected organs is surprisingly similar [153]. Potent AR inhibitors such as enzalutamide and darolutamide are administered to suppress the AR signaling axis after CRPC emerges [154, 155]. Under the pressure of such therapies, mCRPC can further differentiate towards lethal neuroendocrine prostate cancer (NEPC) in the last stages of PCa, which rarely arises *de novo* and is characterized by absent AR signaling, neuroendocrine marker expression and loss of TP53 and RB1 [156, 157]. Additionally, neuroendocrine differentiation is characterized by a concomitant aberrant global shift in DNA methylation and altered expression of epigenetic modifiers and TFs [156, 158, 159]. Support for such epigenetic deregulation in NEPC was recently reported in genetically engineered NEPC mouse model by using single cell transcriptomics and chromatin accessibility methods, which revealed that *Ascl1* and *Pou2f3* are differentially regulated in dedifferentiated cell populations marked by shifts in global DNA methylation

[160]. Moreover, the FOXA1 cistrome is extensively reprogrammed during NEPC [161]. Taken together, an image emerges in which enhancer plasticity in each of the different PCa stages leads to adaptation and progression through rewiring of AR cistromes.

15.8 Non-coding and Protein Coding Somatic Mutations Induce AR Cistromic Heterogeneity

Somatic mutations are a prominent feature of metastatic PCa [147, 162–164], in which AR plays a key role. A multitude of studies reported that in the metastatic disease setting CNAs can lead to the amplification of a SE cluster driving AR expression, providing evidence for *de novo* rewiring of the AR cistrome as a powerful oncogenic driver [147, 162, 165]. Moreover, it was recently reported that AR binding sites are highly mutated in PCa, potentially due to faulty base excision repair at abasic sites [166]. Similarly, during NEPC differentiation, the FOXA1 promoter loses regulatory contact with its key enhancer while simultaneously acquiring *de novo* regulation from a further distally located super-enhancer [161]. Therefore, somatic mutations in pioneer factor binding sites represent another distinct class of non-coding somatic mutations causing epigenetic heterogeneity in PCa.

Conversely, FOXA1 protein coding somatic mutations are frequently occurring across disease stages [54, 167], with a substantial subset of primary PCa, mCRPC and NEPC tumors harboring recurrent SNVs in the FOXA1 coding sequence [168–170]. SNVs in FOXA1 that alter its pioneering function are mostly truncations, indels and missense mutations that converge on three mutational hotspots: the Wing2 region, the forkhead DNA binding domain and C-terminal truncations [171, 172]. Firstly, Wing2 hotspot mutants make up roughly half of all FOXA1 coding mutations which are enriched in the primary stage of PCa, suggesting emergence during localized disease. Moreover, Wing2 mutants exhibit greater pioneering activity than the effect of overexpression of wild-type FOXA1 [171, 172]. Secondly, forkhead DNA binding domain mutation R219 affects a highly conserved part of the forkhead domain that contacts the DNA, altering pioneering activity and activating a mesenchymal/neuroendocrine transcriptional program driven by WNT-signaling [171, 172]. Interestingly, $FOXA1^{R219}$ is acquired in PCa transitioning from primary to metastatic disease and its binding motifs differ markedly from canonical FOXA1-binding motifs, shutting down normal luminal differentiation programmes [171, 172]. Finally, 20% of FOXA1 mutations are frameshift truncations that result in loss of FOXA1's C-terminal transactivating domain. Such mutants show markedly higher DNA binding affinity resulting in altered chromatin binding, engaging an expanded total cistrome for FOXA1 [171–174]. Taken together, FOXA1 mutations are powerful drivers of AR cistromic reprogramming and plasticity by co-opting novel ARBS and transcriptional programs.

Another powerful and frequently recurring oncogenic driver in AR cistromic rewiring is the TMPRSS2-ERG fusion event that occurs in ~50% of patients and is a common initiator of prostate tumorigenesis [175–178], while tumor suppressor PTEN loss co-occurs with TMPRSS2-ERG in aggressive metastatic PCa [179–182]. Specifically, the promoter of TMPRSS2 is fused to the proto-oncogenic transcription factor ERG (ETV1, 4 or 5), causing aberrant overexpression of ERG that in turn drives a PCa oncogenic transcriptional program through ERG-mediated AR recruitment at novel and existing ARBS [110, 141, 178, 183]. Moreover, overexpressed ERG was recently reported to co-opt AR and FOXA1 bound sites to drive expression of DLX, a homeobox-containing TF whose elevated expression is linked to aggressive metastatic disease [184]. These findings further highlight the biological role of TMPRSS2-ERG fusions in advanced PCa beyond its better-understood role in primary disease.

Moreover, mutations occurring in speckle-type pox virus and zinc finger protein (SPOP) were proposed to further exacerbate ERG-driven PCa [185], since the E3 ubiquitin ligase SPOP is a tumor suppressor gene and frequently mutated in PCa [168, 186, 187]. Wild-type SPOP promotes ubiquitination and subsequent proteolytic degradation of critical PCa drivers including ERG [185, 188], AR [189, 190], Myc [191], BRD4 [192, 193] and SRC-3 [194], while SPOP's suppressing function is disrupted by binding cleft mutations [90, 189, 194], leading to a reprogrammed AR cistrome [195]. For instance, SRC-3's oncogenic role as steroid receptor coactivator in PCa is supported by its association with poor prognosis and aggressive phenotype [90, 91, 196, 197]. SRC-3 was proven to associate with AR at enhancers under androgen stimulation, increasing PSA expression [198] and later to be involved in expression of many AR-driven genes [199]. Many proliferation pathways are activated by SRC-3, amongst which MAPK/ERK signaling [200, 201] and Akt-mTOR signaling in PCa cells [91], while homozygous SRC-3 knockout in mice leads to PCa tumor growth arrest and prolonged survival [202].

Interestingly, co-occurring SPOP and ERG mutations are mutually exclusive [203] and the initially proposed SPOP-mutant stabilization was later explained as case of synthetic lethality that prevents appearance of this phenotype [204]. Bromodomain histone reader ZMYND11 is stabilized by mutated SPOP which in turn represses ERG function [204], further corroborating earlier observed paradoxal antagonism of ERG on AR signaling through auto-inhibitory PRMT5 methylation of AR [110, 205]. Additionally, an LXXLL AR interacting motif in the ETS domain of ERG was identified with affinity similar to AR coactivating peptides [206] through mutational studies and ERG-stimulated AR activation, suggesting that AR and ERG can directly interact resulting in a reprogrammed AR cistrome [207].

15.9 Risk SNPs and Somatic Mutations are Enriched at AR-Bound Enhancers

Another source of heterogeneity in AR cistromics comes in the form of germline and somatic sequence variation. With 80% of the cancer risk single nucleotide polymorphisms (rSNPs) [208] mapping to intronic and intergenic regions, a relatively large subset of these are enriched in *bona fide* enhancer elements over other non-coding regions when correcting for size [15, 26]. PCa genome-wide association studies (GWASs) and subsequent studies functionally annotated rSNPs as risk enhancers [209], associated rSNPs with higher risk of developing disease [210] and catalogued rSNPs found from a large pool of PCa tumors [211]. All studies report overrepresentation of rSNPs in enhancer elements that are linked to PCa master TFs with potential transcriptionally altering consequences. Further screening using high-throughput measurement of protein-bound oligo retention times, in which TFs in nuclear extracts bound to SNP-containing oligos are pulled down, found that 20 rSNPs were associated with decreased AR binding in LNCaP [212]. Interestingly, one rSNP was located at the center of a cluster of AR, HOXB13 and FOXA1 binding sites, of which specifically FOXA1 binding was decreased which translated to lower regulatory and transcriptional potential of PCa oncogene RGS17 [212].

Similarly, some PCa rSNPs within well-characterized enhancers influence PCa cell viability [123], as exemplified by enhancers that are located in a single topological associating domain regulating MYC [213, 214]: PCAT1 and PCAT2 [215–218]. Another high-throughput epigenomic study provides evidence that rSNPs create or perturb TF binding sites including AR, as exemplified by a rSNP abrogating AR-mediated repression of the putative oncogene CDKN2B-AS1 which influences cell cycle regulation [219]. Generally, heritable PCa risk is associated with a strong enrichment of PCa rSNPs in prostate-lineage specific enhancers

[121]. As such, rSNPs contribute to AR cistromic heterogeneity by perturbing and creating TF binding sites that affect PCa progression.

15.10 Clinical Implications and Biomarker Development of Heterogeneity in Epigenetic Subtypes

It is increasingly becoming more apparent that PCa may be considered an epigenetic disease in which many key cell identity processes are disrupted and different transcriptional programs are initiated through AR cistromic rewiring, orchestrated by reprogrammed FOXA1 and HOXB13 [121, 161, 220]. The future clinical potential of targeting enhancer-gene pairs in cancer is promising, as such interactions have been systematically charted for in the TCGA pan-cancer dataset, with aberrant enhancer activation observed in most cancers [221]. Since aberrant enhancer activation and cistromic heterogeneity appears to be a key feature of PCa, specific epigenetic states and biomarkers ensuing from such states offer great opportunities for informed clinical decisions based on epigenetic subtypes.

Our previous integrative epigenetic profiling study in primary prostate cancer has revealed a PCa subtype independent of TMPRSS2-ERG status, characterized by low mutational burden together with neutral copy number and AR expression but a contrastingly low AR activity and chromatin binding [55]. Since this subtype with heterogeneous TMPRSS2-ERG status is potentially driven by NGF, FGF and WNT signaling and associated with poor outcome [119], therapeutic opportunities may exploit applying small molecule inhibitors (SMIs) targeting these pathways [222–224], particularly for this subpopulation of patients. Further comparing AR chromatin binding patterns between disease states and contexts allows for the dissection of heterogeneous epigenetic subtypes and may accelerate PCa progression biomarker discovery [151, 225], expanding cistromic studies to other proteins such as CTCF [226, 227], ETS [178, 228], FOS [229, 230], HOXB13 [151, 225], KLF9 [151, 231, 232], SP1 [233, 234], SPOP [204, 228] and XBP1 [113, 151, 235].

Another distinct class of SMIs are epigenetic drugs targeting histone deacetylases (HDACs) expressed highly in primary PCa [236] and the enzymatic subunit of the polycomb repressive complex EZH2, which is overexpressed in CRPC [237] and co-occupies reprogrammed AR cistromes in NEPC [238]. Both HDAC and EZH2 promote transcriptional silencing through remodeling chromatin conformation, either deacetylation or methylation of histone tail modifications. Inhibition of EZH2 with SMIs [239] could help overcome ADT resistance and increase effectiveness of AR inhibition in CRPC patients and is suggested to potentiate PCa tumors to PD-1 checkpoint inhibition [240]. Although the HDAC inhibitor vorinostat is an effective inhibitor of PCa proliferation by synergizing with AR antagonists in cells and *in vivo* [241, 242], HDAC inhibition is associated with significant toxicity in patients which currently prevents phase III clinical investigation for PCa [243, 244]. Alternatively, FOXA1 chromatin binding can be indirectly repressed through inhibition of H3K4 demethylation by transcriptional repressor KDM1A (LSD1), which synergizes with AR antagonists *in vivo* and associates with FOXA1 [245]. Contrastingly, direct inhibition of FOXA1 with the SMI JQ1 abrogates FOXA1 binding with co-repressors, which alleviates repression of gene pathways associated with PCa invasion [246].

Finally, PCa's inclination towards inter- and intra-tumor heterogeneity necessitates enhanced minimally-invasive biomarker detection relying on a combination of classic and novel urine- or blood-based prognostic biomarkers [247, 248], which can be highly impactful by preventing the reported systematic overtreatment of patients with indolent disease [139, 211, 249, 250].

15.11 Future Outlook

The dissection of heterogeneity among populations of tumor cells and their TME has recently made exceptional progress through the implementation of single-cell omics technologies [251, 252]. First, a massive transcriptomic heterogeneity was found within tumors, with multiple distinct transcriptional programs and cellular subsets associated with PCa progression [253]. Second, persistent resistant cells without stem cell properties were found to repopulate tumors upon treatment [254], with high cell cycle turnover in resistant cells showing a heterogeneous response towards ADT therapies, such as with enzalutamide [255]. Finally, single cell epigenomics and cistromics studies are yet to be reported for PCa, but such technologies have been applied for identifying heterogeneous chromatin states in breast cancer [256] and were demonstrated to infer single cell heterogeneity in chromatin accessibility [257, 258]. These studies uncover the clinical impact of shifts in heterogeneous cell populations under therapeutic pressure, and underline how single-cell genomics and transcriptomics have improved our understanding of intra-tumor heterogeneity. Clearly, the future application of single cell epigenomics and cistromics technologies would provide a formidable tool to understand the consequences of epigenetic heterogeneity in the context of cancer and facilitate the identification of novel drug targets.

Tracing multiple foci in patients using their genomic profiles allows for dissection of heterogeneous patterns of metastatic spread [259]. It is becoming increasingly clear that PCa metastatic seeding occurs heterogeneously through asynchronous and cross-metastatic seeding [260, 261] with tumor lineages evolving differently [143, 262], which may have direct consequences on the level of epigenetic heterogeneity [153] as well as clinical decision-making [45]. As such, longitudinal sampling might offer the most comprehensive and dynamic view of heterogeneity in AR cistromes during the course of PCa, which to date has only been applied for blood-derived cfDNA methylomes [263]. Although currently unreported, we anticipate longitudinal translational studies with coupled single cell epigenomics and cistromics, so that epigenetic developments become embedded as an intrinsic component of clinical trials, allowing for a precise identification of the dynamics and heterogeneity of epigenetic subtypes to ultimately contribute to improved data-driven clinical decision-making.

Concluding, PCa presents many heterogeneous facets that diverge in AR cistromic reprogramming and contribute to PCa development, progression and therapy response. Taken together, there appear to be distinct and programmatic epigenetic alterations in which normal enhancer binding is altered during PCa initiation and progression, ultimately leading to heterogeneous AR cistromes between tumors, dictating markedly different transcriptional programs with different prognostication between patients. Future technological developments may facilitate a full epigenomic and cistromic characterization of PCa heterogeneity in patient samples, ultimately contributing to personalized medicine. Knowledge gained from such cistromic studies may facilitate the discovery of novel biomarkers for tailored therapeutics and lead to better patient prognostication. As such, AR cistrome heterogeneity in PCa resembles a shifting fingerprint of the tumor: personal and reflective of a specific transcriptional regulatory potential, yet dynamic and subject to change over time.

References

1. Banerji J, Rusconi S, Schaffner W (1981) Expression of a beta-globin gene is enhanced by remote SV40 DNA sequences. Cell 27:299–308
2. Moreau P et al (1981) The SV40 72 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. Nucleic Acids Res 9:6047–6068
3. Banerji J, Olson L, Schaffner W (1983) A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell 33:729–740
4. Gillies SD, Morrison SL, Oi VT, Tonegawa S (1983) A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. Cell 33:717–728
5. Mercola M, Wang XF, Olsen J, Calame K (1983) Transcriptional enhancer elements in the mouse

immunoglobulin heavy chain locus. Science 221:663–665
6. Struhl K (1984) Genetic properties and chromatin structure of the yeast gal regulatory element: an enhancer-like sequence. Proc Natl Acad Sci U S A 81:7865–7869
7. Shepherd B, Garabedian MJ, Hung MC, Wensink PC (1985) Developmental control of Drosophila yolk protein 1 gene by cis-acting DNA elements. Cold Spring Harb Symp Quant Biol 50:521–526
8. Kioussis D, Vanin E, deLange T, Flavell RA, Grosveld FG (1983) Beta-globin gene inactivation by DNA translocation in gamma beta-thalassaemia. Nature 306:662–666
9. Driscoll MC, Dobkin CS, Alter BP (1989) Gamma delta beta-thalassemia due to a de novo mutation deleting the 5' beta-globin gene activation-region hypersensitive sites. Proc Natl Acad Sci U S A 86:7470–7474
10. Erikson J, ar-Rushdi A, Drwinga HL, Nowell PC, Croce CM (1983) Transcriptional activation of the translocated c-myc oncogene in burkitt lymphoma. Proc Natl Acad Sci U S A 80:820–824
11. Wiman KG et al (1984) Activation of a translocated c-myc gene: role of structural alterations in the upstream region. Proc Natl Acad Sci U S A 81:6798
12. Nurk S et al (2021) The complete sequence of a human genome. 2021.05.26.445798 https://www.biorxiv.org/content/10.1101/2021.05.26.445798v1. https://doi.org/10.1101/2021.05.26.445798
13. Moore JE et al (2020) Expanded encyclopaedias of DNA elements in the human and mouse genomes. Nature 583:699–710
14. Domcke S et al (2020) A human cell atlas of fetal chromatin accessibility. Science 370:eaba7612
15. Maurano MT et al (2012) Systematic localization of common disease-associated variation in regulatory DNA. Science 337:1190–1195
16. Furey TS (2012) ChIP-seq and beyond: new and improved methodologies to detect and characterize protein-DNA interactions. Nat Rev Genet 13:840–852
17. Klemm SL, Shipony Z, Greenleaf WJ (2019) Chromatin accessibility and the regulatory epigenome. Nat Rev Genet 20: 207–220
18. Mp C et al (2010) Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci U S A 107:21931–21936
19. Heintzman ND et al (2007) Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet 39:311–318
20. Robertson AG et al (2008) Genome-wide relationship between histone H3 lysine 4 mono- and tri-methylation and transcription factor binding. Genome Res 18:1906–1917
21. Kim T-K et al (2010) Widespread transcription at neuronal activity-regulated enhancers. Nature 465:182–187
22. Mohrs M et al (2001) Deletion of a coordinate regulator of type 2 cytokine expression in mice. Nat Immunol 2:842–847
23. Fulco CP et al (2019) Activity-by-Contact model of enhancer-promoter regulation from thousands of CRISPR perturbations. Nat Genet 51:1664
24. Fulco CP et al (2016) Systematic mapping of functional enhancer–promoter connections with CRISPR interference. Science 354:769–773
25. ENCODE Project Consortium (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489:57–74
26. Sur I, Taipale J (2016) The role of enhancers in cancer. Nat Rev Cancer 16:483–493
27. Sung H et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71:209–249
28. Yang Q, Fung K-M, Day WV, Kropp BP, Lin H-K (2005) Androgen receptor signaling is required for androgen-sensitive human prostate cancer cell proliferation and survival. Cancer Cell Int 5:8
29. Brinkmann AO et al (1999) Mechanisms of androgen receptor activation and function. J Steroid Biochem Mol Biol 69:307–313
30. Heinlein CA, Chang C (2004) Androgen receptor in prostate cancer. Endocr Rev 25:276–308
31. Prostate Cancer Trialists' Collaborative Group (2000) Maximum androgen blockade in advanced prostate cancer: an overview of the randomised trials. Lancet 355:1491–1498
32. Ohlson N, Wikström P, Stattin P, Bergh A (2005) Cell proliferation and apoptosis in prostate tumors and adjacent non-malignant prostate tissue in patients at different time-points after castration treatment. Prostate 62:307–315
33. Oh WK, Kantoff PW (1998) Management of hormone refractory prostate cancer: current standards and future prospects. J Urol 160:1220–1229
34. Chi KN et al (2009) Castration-resistant prostate cancer: from new pathophysiology to new treatment targets. Eur Urol 56:594–605
35. Aihara M, Wheeler TM, Ohori M, Scardino PT (1994) Heterogeneity of prostate cancer inradical prostatectomy specimens. Urology 43:60–66
36. Andreoiu M, Cheng L (2010) Multifocal prostate cancer: biologic, prognostic, and therapeutic implications. Hum Pathol 41:781–793
37. Espiritu SMG et al (2018) The evolutionary landscape of localized prostate cancers drives clinical aggression. Cell 173:1003–1013.e15
38. Carm KT et al (2019) Interfocal heterogeneity challenges the clinical usefulness of molecular classification of primary prostate cancer. Sci Rep 9:13579
39. Algaba F, Montironi R (2010) Impact of prostate cancer multifocality on its biology and treatment. J Endourol 24:799–804
40. Cyll K et al (2017) Tumour heterogeneity poses a significant challenge to cancer biomarker research. Br J Cancer 117:367–375

41. Liu W et al (2009) Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. Nat Med 15:559–565
42. Chade DC et al (2012) Cancer control and functional outcomes of salvage radical prostatectomy for radiation-recurrent prostate cancer: a systematic review of the literature. Eur Urol 61:961–971
43. Mottet N et al (2017) EAU-ESTRO-SIOG guidelines on prostate cancer. Part 1: screening, diagnosis, and local treatment with curative intent. Eur Urol 71:618–629
44. van der Poel HG et al (2018) Focal therapy in primary localised prostate cancer: The European Association of Urology position in 2018. Eur Urol 74:84–91. https://doi.org/10.1016/j.eururo.2018.01.001
45. Kneppers J et al (2019) Frequent clonal relations between metastases and non-index prostate cancer lesions. JCI Insight 4: e124756
46. Martinez P et al (2013) Parallel evolution of tumour subclones mimics diversity between tumours. J Pathol 230:356–364
47. Berglund E et al (2018) Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity. Nat Commun 9:2419
48. Fane M, Weeraratna AT (2020) How the ageing microenvironment influences tumour progression. Nat Rev Cancer 20:89–106
49. Marusyk A, Janiszewska M, Polyak K (2020) Intratumor heterogeneity: the Rosetta stone of therapy resistance. Cancer Cell 37:471
50. Bozic I, Wu CJ (2020) Delineating the evolutionary dynamics of cancer from theory to reality. Nat Can 1:580–588
51. Boutros PC et al (2015) Spatial genomic heterogeneity within localized, multifocal prostate cancer. Nat Genet 47:736–745
52. Bakhoum SF, Cantley LC (2018) The multifaceted role of chromosomal instability in cancer and its microenvironment. Cell 174:1347–1360
53. Flavahan WA, Gaskell E, Bernstein BE (2017) Epigenetic plasticity and the hallmarks of cancer. Science 357: eaal2380
54. (2015) The molecular taxonomy of primary prostate cancer. Cell 163:1011–1025
55. Stelloo S et al (2018) Integrative epigenetic taxonomy of primary prostate cancer. Nat Commun 9:1–12
56. Wang MC, Valenzuela LA, Murphy GP, Chu TM (1979) Purification of a human prostate specific antigen. Investig Urol 17:159–163
57. Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J (1991) The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol 5:1921–1930
58. Wang Q et al (2007) A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. Mol Cell 27:380
59. Carroll JS et al (2006) Genome-wide analysis of estrogen receptor binding sites. Nat Genet 38:1289–1297
60. Massie CE et al (2011) The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis. EMBO J 30:2719–2733
61. Stelloo S et al (2018) Endogenous androgen receptor proteomic profiling reveals genomic subcomplex involved in prostate tumorigenesis. Oncogene 37:313–322
62. Itkonen H, Mills IG (2012) Chromatin binding by the androgen receptor in prostate cancer. Mol Cell Endocrinol 360:44–51
63. Roche PJ, Hoare SA, Parker MG (1992) A consensus DNA-binding site for the androgen receptor. Mol Endocrinol 6:2229–2235
64. Hsu C-C, Hu C-D (2013) Transcriptional activity of c-Jun is critical for the suppression of AR function. Mol Cell Endocrinol 372:12
65. Wilson S, Qi J, Filipp FV (2016) Refinement of the androgen response element based on ChIP-Seq in androgen-insensitive and androgen-responsive prostate cancer cell lines. Sci Rep 6:1–15
66. Kornberg RD (2005) Mediator and the mechanism of transcriptional activation. Trends Biochem Sci 30:235–239
67. Kagey MH et al (2010) Mediator and cohesin connect gene expression and chromatin architecture. Nature 467:430–435
68. Borggrefe T, Yue X (2011) Interactions between subunits of the Mediator complex with gene-specific transcription factors. Semin Cell Dev Biol 22:759–768
69. Zhao H et al (2021) Structure of mammalian Mediator complex reveals Tail module architecture and interaction with a conserved core. Nat Commun 12:1355
70. Chen W, Roeder R (2011) Mediator-dependent nuclear receptor functions. Semin Cell Dev Biol 22:749
71. Yu X et al (2020) Structural insights of transcriptionally active, full-length androgen receptor coactivator complexes. Mol Cell 79:812–823.e4
72. Zhang Z et al (2019) An AR-ERG transcriptional signature defined by long range chromatin interactomes in prostate cancer cells. Genome Res 29:223–235
73. De Santa F et al (2010) A large fraction of extragenic RNA pol II transcription sites overlap enhancers. PLoS Biol 8:e1000384
74. Shii L, Song L, Maurer K, Zhang Z, Sullivan KE (2017) SERPINB2 is regulated by dynamic interactions with pause-release proteins and enhancer RNAs. Mol Immunol 88:20–31
75. Austenaa LMI et al (2015) Transcription of mammalian cis-regulatory elements is restrained by actively enforced early termination. Mol Cell 60:460–474
76. Aguilo F et al (2016) Deposition of 5-methylcytosine on enhancer RNAs enables the coactivator function of PGC-1α. Cell Rep 14:479–492

77. Azofeifa JG, Dowell RD (2017) A generative model for the behavior of RNA polymerase. Bioinformatics 33:227–234
78. Azofeifa JG et al (2018) Enhancer RNA profiling predicts transcription factor activity. Genome Res 28:334–344
79. Mikhaylichenko O et al (2018) The degree of enhancer or promoter activity is reflected by the levels and directionality of eRNA transcription. Genes Dev 32:42–57
80. Carullo NVN et al (2020) Enhancer RNAs predict enhancer–gene regulatory links and are critical for enhancer function in neuronal systems. Nucleic Acids Res 48:9550–9570
81. Chong S et al (2018) Imaging dynamic and selective low-complexity domain interactions that control gene transcription. Science 361:eaar2555
82. Sabari BR et al (2018) Coactivator condensation at super-enhancers links phase separation and gene control. Science 361:eaar3958
83. Cho W-K et al (2018) Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science 361:412–415
84. Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA (2017) A phase separation model for transcriptional control. Cell 169:13–23
85. Sharma R et al (2021) Liquid condensation of reprogramming factor KLF4 with DNA provides a mechanism for chromatin organization. Nat Commun 12:5579
86. Choi J et al (2021) Evidence for additive and synergistic action of mammalian enhancers during cell fate determination. elife 10:e65381
87. Zaret KS (2020) Pioneer transcription factors initiating gene network changes. Annu Rev Genet 54:367–385
88. Visel A et al (2009) ChIP-seq accurately predicts tissue-specific activity of enhancers. Nature 457:854–858
89. Wang Z et al (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. Cell 138:1019–1031
90. Gnanapragasam VJ, Leung HY, Pulimood AS, Neal DE, Robson CN (2001) Expression of RAC 3, a steroid hormone receptor co-activator in prostate cancer. Br J Cancer 85:1928–1936
91. Zhou H-J et al (2005) SRC-3 is required for prostate cancer cell proliferation and survival. Cancer Res 65:7976–7983
92. Allshire RC, Madhani HD (2018) Ten principles of heterochromatin formation and function. Nat Rev Mol Cell Biol 19:229
93. Pomerantz MM et al (2015) The androgen receptor cistrome is extensively reprogrammed in human prostate tumorigenesis. Nat Genet 47:1346–1351
94. Wu D et al (2014) Three-tiered role of the pioneer factor GATA2 in promoting androgen-dependent gene expression in prostate cancer. Nucleic Acids Res 42:3607–3622
95. Cirillo LA et al (2002) Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. Mol Cell 9:279–289
96. ENCODE Project Consortium (2011) A user's guide to the encyclopedia of DNA elements (ENCODE). PLoS Biol 9:e1001046
97. Kharchenko PV et al (2011) Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. Nature 471:480–485
98. Mayran A, Drouin J (2018) Pioneer transcription factors shape the epigenetic landscape. J Biol Chem 293:13795–13804
99. Lupien M et al (2008) FoxA1 translates epigenetic signatures into enhancer driven lineage-specific transcription. Cell 132:958
100. Wang Q et al (2009) Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell 138:245–256
101. Sérandour AA et al (2011) Epigenetic switch involved in activation of pioneer factor FOXA1-dependent enhancers. Genome Res 21:555
102. Gao N et al (2003) The role of hepatocyte nuclear factor-3 alpha (Forkhead Box A1) and androgen receptor in transcriptional regulation of prostatic genes. Mol Endocrinol 17:1484–1507
103. Yu X et al (2005) Foxa1 and Foxa2 interact with the androgen receptor to regulate prostate and epididymal genes differentially. Ann N Y Acad Sci 1061:77–93
104. Eeckhoute J et al (2009) Cell-type selective chromatin remodeling defines the active subset of FOXA1-bound enhancers. Genome Res 19:372–380
105. Wang D et al (2011) Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. Nature 474:390–394
106. Sahu B et al (2011) Dual role of FoxA1 in androgen receptor binding to chromatin, androgen signalling and prostate cancer. EMBO J 30:3962–3976
107. Sahu B et al (2013) FoxA1 specifies unique androgen and glucocorticoid receptor binding events in prostate cancer cells. Cancer Res 73:1570–1580
108. Jin H-J, Zhao JC, Wu L, Kim J, Yu J (2014) Cooperativity and equilibrium with FOXA1 define the androgen receptor transcriptional program. Nat Commun 5:3972
109. Iwafuchi-Doi M, Zaret KS (2016) Cell fate control by pioneer transcription factors. Development (Cambridge, England) 143:1833
110. Yu J et al (2010) An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. Cancer Cell 17:443–454
111. Severson TM et al (2018) Characterizing steroid hormone receptor chromatin binding landscapes in male and female breast cancer. Nat Commun 9:482
112. Mei S et al (2017) Cistrome Data Browser: a data portal for ChIP-Seq and chromatin accessibility data in human and mouse. Nucleic Acids Res 45:D658–D662

113. Sharma NL et al (2013) The androgen receptor induces a distinct transcriptional program in castration-resistant prostate cancer in man. Cancer Cell 23:35–47
114. Chen Z et al (2015) Agonist and antagonist switch DNA motifs recognized by human androgen receptor in prostate cancer. EMBO J 34:502–516
115. Cioni B et al (2018) Loss of androgen receptor signaling in prostate cancer-associated fibroblasts (CAFs) promotes CCL2- and CXCL8-mediated cancer cell migration. Mol Oncol 12:1308–1323
116. Cioni B et al (2020) Androgen receptor signalling in macrophages promotes TREM-1-mediated prostate cancer cell line migration and invasion. Nat Commun 11:4498
117. Hickey TE et al (2021) The androgen receptor is a tumor suppressor in estrogen receptor–positive breast cancer. Nat Med 27:310–320
118. Toivanen R, Shen MM (2017) Prostate organogenesis: tissue induction, hormonal regulation and cell type specification. Development (Cambridge, England) 144:1382
119. Zhao SG et al (2017) Associations of luminal and basal subtyping of prostate cancer with prognosis and response to androgen deprivation therapy. JAMA Oncol 3:1663–1672
120. Li F et al (2020) ERG orchestrates chromatin interactions to drive prostate cell fate reprogramming. J Clin Invest 130:5924–5941
121. Pomerantz MM et al (2020) Prostate cancer reactivates developmental epigenomic programs during metastatic progression. Nat Genet 52:790–799
122. Brechka H, Bhanvadia RR, VanOpstall C, Vander Griend DJ (2017) HOXB13 mutations and binding partners in prostate development and cancer: function, clinical significance, and future directions. Genes Dis 4:75–87
123. Mazrooei P et al (2019) Cistrome partitioning reveals convergence of somatic mutations and risk variants on master transcription regulators in primary prostate tumors. Cancer Cell 36:674–689.e6
124. Cai C, Hsieh C-L, Shemshedini L (2007) c-Jun has multiple enhancing activities in the novel cross talk between the androgen receptor and Ets variant gene 1 in prostate cancer. Mol Cancer Res 5:725–735
125. Copeland BT, Du J, Pal SK, Jones JO (2019) Factors that influence the androgen receptor cistrome in benign and malignant prostate cells. Mol Oncol 13:2616
126. Chen S-Y et al (2006) c-Jun enhancement of androgen receptor transactivation is associated with prostate cancer cell proliferation. Oncogene 25:7212–7223
127. Nouri M et al (2017) Therapy-induced developmental reprogramming of prostate cancer cells and acquired therapy resistance. Oncotarget 8:18949–18967
128. Faivre EJ et al (2020) Selective inhibition of the BD2 bromodomain of BET proteins in prostate cancer. Nature 578:306–310
129. Navone NM et al (2018) Movember GAP1 PDX project: an international collection of serially transplantable prostate cancer patient-derived xenograft (PDX) models. Prostate 78:1262–1282
130. Leach DA et al (2017) Cell-lineage specificity and role of AP-1 in the prostate fibroblast androgen receptor cistrome. Mol Cell Endocrinol 439:261–272
131. Robinson JLL et al (2011) Androgen receptor driven transcription in molecular apocrine breast cancer is mediated by FoxA1. EMBO J 30:3019–3027
132. Michmerhuizen AR, Spratt DE, Pierce LJ, Speers CW (2020) ARe we there yet? Understanding androgen receptor signaling in breast cancer. NPJ Breast Cancer 6:47
133. Guo M, Peng Y, Gao A, Du C, Herman JG (2019) Epigenetic heterogeneity in cancer. Biomarker Res 7:23
134. Carter B, Zhao K (2021) The epigenetic basis of cellular heterogeneity. Nat Rev Genet 22:235–250
135. Flach KD, Zwart W (2016) The first decade of estrogen receptor cistromics in breast cancer. J Endocrinol 229:R43–R56
136. Bostwick DG (2000) Prostatic intraepithelial neoplasia. Curr Urol Rep 1:65–70
137. Gupta-Elera G, Garrett AR, Robison RA, O'Neill KL (2012) The role of oxidative stress in prostate cancer. Eur J Cancer Prev 21:155–162
138. Papachristodoulou A et al (2021) NKX3.1 localization to mitochondria suppresses prostate cancer initiation. Cancer Discov 11:2316–2333
139. Daskivich TJ et al (2011) Overtreatment of men with low-risk prostate cancer and significant comorbidity. Cancer 117:2058–2066
140. Løvf M et al (2018) Multifocal primary prostate cancer exhibits high degree of genomic heterogeneity. Eur Urol 75: 498–505
141. Kron KJ et al (2017) TMPRSS2–ERG fusion co-opts master transcription factors and activates NOTCH signaling in primary prostate cancer. Nat Genet 49:1336–1345
142. Datta K, Muders M, Zhang H, Tindall DJ (2010) Mechanism of lymph node metastasis in prostate cancer. Future Oncol 6:823–836
143. Mangiola S et al (2016) Comparing nodal versus bony metastatic spread using tumour phylogenies. Sci Rep 6:33918
144. Beroukhim R et al (2010) The landscape of somatic copy-number alteration across human cancers. Nature 463:899–905
145. Stopsack KH et al (2019) Aneuploidy drives lethal progression in prostate cancer. PNAS 116:11390–11395
146. Albert FW, Kruglyak L (2015) The role of regulatory variation in complex traits and disease. Nat Rev Genet 16:197–212
147. Quigley DA et al (2018) Genomic hallmarks and structural variation in metastatic prostate cancer. Cell 174:758–769.e9
148. Das R et al (2021) An integrated functional and clinical genomics approach reveals genes driving

aggressive metastatic prostate cancer. Nat Commun 12:4601
149. Irshad S et al (2013) A molecular signature predictive of indolent prostate cancer. Sci Transl Med 5:202ra122
150. Lalonde E et al (2014) Tumour genomic and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: a retrospective cohort study. Lancet Oncol 15:1521–1532
151. Stelloo S et al (2015) Androgen receptor profiling predicts prostate cancer outcome. EMBO Mol Med 7:1450–1464
152. Kirby M, Hirst C, Crawford ED (2011) Characterising the castration-resistant prostate cancer population: a systematic review. Int J Clin Pract 65:1180–1192
153. Severson TM et al (2021) Epigenetic and transcriptional analysis reveals a core transcriptional program conserved in clonal prostate cancer metastases. Mol Oncol 15:1942–1955
154. Scher HI et al (2012) Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 367:1187–1197
155. Fizazi K et al (2019) Darolutamide in nonmetastatic, castration-resistant prostate cancer. N Engl J Med 380:1235–1246
156. Beltran H et al (2016) Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 22:298–305
157. Puca L, Vlachostergios PJ, Beltran H (2019) Neuroendocrine differentiation in prostate cancer: emerging biology, models, and therapies. Cold Spring Harb Perspect Med 9:a030593
158. Ruan L, Wang L, Wang X, He M, Yao X (2017) SIRT1 contributes to neuroendocrine differentiation of prostate cancer. Oncotarget 9:2002–2016
159. Reina-Campos M et al (2019) Increased serine and one-carbon pathway metabolism by PKCλ/ι deficiency promotes neuroendocrine prostate cancer. Cancer Cell 35:385–400.e9
160. Brady NJ et al (2021) Temporal evolution of cellular heterogeneity during the progression to advanced AR-negative prostate cancer. Nat Commun 12:3372
161. Baca SC et al (2021) Reprogramming of the FOXA1 cistrome in treatment-emergent neuroendocrine prostate cancer. Nat Commun 12:1979
162. Viswanathan SR et al (2018) Structural alterations driving castration-resistant prostate cancer revealed by linked-read genome sequencing. Cell 174: 433–447
163. van Dessel LF et al (2019) The genomic landscape of metastatic castration-resistant prostate cancers reveals multiple distinct genotypes with potential clinical impact. Nat Commun 10:5251
164. Mayrhofer M et al (2018) Cell-free DNA profiling of metastatic prostate cancer reveals microsatellite instability, structural rearrangements and clonal hematopoiesis. Genome Med 10:85
165. Takeda DY et al (2018) A somatically acquired enhancer of the androgen receptor is a noncoding driver in advanced prostate cancer. Cell 174:422–432.e13
166. Morova T et al (2020) Androgen receptor-binding sites are highly mutated in prostate cancer. Nat Commun 11:832
167. Robinson D et al (2015) Integrative clinical genomics of advanced prostate cancer. Cell 161:1215–1228
168. Barbieri CE et al (2012) Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 44:685–689
169. Grasso CS et al (2012) The mutational landscape of lethal castration-resistant prostate cancer. Nature 487:239–243
170. Beltran H et al (2020) Circulating tumor DNA profile recognizes transformation to castration-resistant neuroendocrine prostate cancer. J Clin Invest 130:1653–1668
171. Adams EJ et al (2019) FOXA1 mutations alter pioneering activity, differentiation and prostate cancer phenotypes. Nature 571:408–412
172. Parolia A et al (2019) Distinct structural classes of activating FOXA1 alterations in advanced prostate cancer. Nature 571:413–418. https://doi.org/10.1038/s41586-019-1347-4
173. Gao S et al (2019) Forkhead domain mutations in FOXA1 drive prostate cancer progression. Cell Res 29:770
174. Iwafuchi M et al (2020) Gene network transitions in embryos depend upon interactions between a pioneer transcription factor and core histones. Nat Genet 52:418–427
175. Tomlins SA et al (2005) Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310:644–648
176. Tomlins SA et al (2008) Role of the TMPRSS2-ERG gene fusion in prostate cancer. Neoplasia 10:177–188
177. Klezovitch O et al (2008) A causal role for ERG in neoplastic transformation of prostate epithelium. PNAS 105:2105–2110
178. Chen Y et al (2013) ETS factors reprogram the androgen receptor cistrome and prime prostate tumorigenesis in response to PTEN loss. Nat Med 19:1023–1029
179. Krohn A et al (2012) Genomic deletion of PTEN is associated with tumor progression and early PSA recurrence in ERG fusion-positive and fusion-negative prostate cancer. Am J Pathol 181:401–412
180. Ahearn TU et al (2016) A prospective investigation of PTEN loss and ERG expression in lethal prostate cancer. J Natl Cancer Inst 108:djv346
181. Punnoose EA et al (2015) PTEN loss in circulating tumour cells correlates with PTEN loss in fresh tumour tissue from castration-resistant prostate cancer patients. Br J Cancer 113:1225–1233
182. Salami SS et al (2019) Circulating tumor cells as a predictor of treatment response in clinically localized prostate cancer. JCO Precis Oncol 3:PO.18.00352

183. Tomlins SA et al (2009) ETS gene fusions in prostate cancer: from discovery to daily clinical practice. Eur Urol 56:275–286
184. Goel S et al (2021) Transcriptional network involving ERG and AR orchestrates Distal-less homeobox-1 mediated prostate cancer progression. Nat Commun 12:5325
185. Gan W et al (2015) SPOP promotes ubiquitination and degradation of the ERG oncoprotein to suppress prostate cancer progression. Mol Cell 59:917–930
186. Nagai Y et al (1997) Identification of a novel nuclear speckle-type protein, SPOP. FEBS Lett 418:23–26
187. Zhuang M et al (2009) Structures of SPOP-substrate complexes: insights into molecular architectures of BTB-Cul3 ubiquitin ligases. Mol Cell 36:39–50
188. An J et al (2015) Truncated ERG oncoproteins from TMPRSS2-ERG fusions are resistant to SPOP-mediated proteasome degradation. Mol Cell 59:904–916
189. An J, Wang C, Deng Y, Yu L, Huang H (2014) Destruction of full-length androgen receptor by wild-type SPOP, but not prostate-cancer-associated mutants. Cell Rep 6:657–669
190. Geng C et al (2014) Androgen receptor is the key transcriptional mediator of the tumor suppressor SPOP in prostate cancer. Cancer Res 74:5631–5643
191. Geng C et al (2017) SPOP regulates prostate epithelial cell proliferation and promotes ubiquitination and turnover of c-MYC oncoprotein. Oncogene 36:4767–4777
192. Janouskova H et al (2017) Opposing effects of cancer-type-specific SPOP mutants on BET protein degradation and sensitivity to BET inhibitors. Nat Med 23:1046–1054
193. Dai X et al (2017) Prostate cancer-associated SPOP mutations confer resistance to BET inhibitors through stabilization of BRD4. Nat Med 23:1063–1071
194. Geng C et al (2013) Prostate cancer-associated mutations in speckle-type POZ protein (SPOP) regulate steroid receptor coactivator 3 protein turnover. Proc Natl Acad Sci U S A 110:6997–7002
195. Grbesa I et al (2021) Reshaping of the androgen-driven chromatin landscape in normal prostate cells by early cancer drivers and effect on therapeutic sensitivity. Cell Rep 36:109625
196. Tien JC-Y et al (2013) The steroid receptor coactivator-3 is required for the development of castration-resistant prostate cancer. Cancer Res 73:3997
197. Yan J et al (2008) Steroid receptor coactivator-3/AIB1 promotes cell migration and invasiveness through focal adhesion turnover and matrix metalloproteinase expression. Cancer Res 68:5460
198. Louie MC et al (2003) Androgen-induced recruitment of RNA polymerase II to a nuclear receptor–p160 coactivator complex. PNAS 100:2226–2230
199. Zhou XE et al (2010) Identification of SRC3/AIB1 as a preferred coactivator for hormone-activated androgen receptor. J Biol Chem 285:9161
200. Migliaccio A et al (2000) Steroid-induced androgen receptor–oestradiol receptor β–Src complex triggers prostate cancer cell proliferation. EMBO J 19:5406–5417
201. Migliaccio A et al (2007) Inhibition of the SH3 domain-mediated binding of Src to the androgen receptor and its effect on tumor growth. Oncogene 26:6619–6629
202. Chung AC-K et al (2007) Genetic ablation of the amplified-in-breast cancer 1 inhibits spontaneous prostate cancer progression in mice. Cancer Res 67:5965–5975
203. Shoag J et al (2018) SPOP mutation drives prostate neoplasia without stabilizing oncogenic transcription factor ERG. J Clin Invest 128:381–386
204. Bernasocchi T et al (2021) Dual functions of SPOP and ERG dictate androgen therapy responses in prostate cancer. Nat Commun 12:734
205. Mounir Z et al (2016) ERG signaling in prostate cancer is driven through PRMT5-dependent methylation of the Androgen Receptor. elife 5:e13964
206. Hsu C-L et al (2014) Identification of a new androgen receptor (AR) co-regulator BUD31 and related peptides to suppress wild-type and mutated AR-mediated prostate cancer growth via peptide screening and X-ray structure analysis. Mol Oncol 8:1575–1587
207. Wasmuth EV et al (2020) Modulation of androgen receptor DNA binding activity through direct interaction with the ETS transcription factor ERG. PNAS 117:8584–8592
208. Welter D et al (2014) The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res 42:D1001–D1006
209. Hazelett DJ et al (2014) Comprehensive functional annotation of 77 prostate cancer risk loci. PLoS Genet 10:e1004102
210. Chen H et al (2015) Systematic enrichment analysis of potentially functional regions for 103 prostate cancer risk-associated loci. Prostate 75:1264–1276
211. Whitington T et al (2016) Gene regulatory mechanisms underpinning prostate cancer susceptibility. Nat Genet 48:387–397
212. Zhang P et al (2018) High-throughput screening of prostate cancer risk loci by single nucleotide polymorphisms sequencing. Nat Commun 9:1–12
213. Ahmadiyeh N et al (2010) 8q24 prostate, breast, and colon cancer risk loci show tissue-specific long-range interaction with MYC. PNAS 107:9742–9746
214. Wasserman NF, Aneas I, Nobrega MA (2010) An 8q24 gene desert variant associated with prostate cancer risk confers differential in vivo activity to a MYC enhancer. Genome Res 20:1191–1197
215. Guo H et al (2016) Modulation of long noncoding RNAs by risk SNPs underlying genetic predispositions to prostate cancer. Nat Genet 48:1142–1150
216. Han Y et al (2016) Prostate cancer susceptibility in men of African ancestry at 8q24. J Natl Cancer Inst 108: djv431

217. Chung S et al (2011) Association of a novel long non-coding RNA in 8q24 with prostate cancer susceptibility. Cancer Sci 102:245–252
218. Kim T et al (2014) Long-range interaction and correlation between MYC enhancer and oncogenic long noncoding RNA CARLo-5. Proc Natl Acad Sci U S A 111:4173
219. Wang T et al (2021) Integrative epigenome map of the normal human prostate provides insights into prostate cancer predisposition. Front Cell Dev Biol 9:723676
220. Augello MA et al (2019) CHD1 loss alters AR binding at lineage-specific enhancers and modulates distinct transcriptional programs to drive prostate tumorigenesis. Cancer Cell 35:603
221. Chen H et al (2018) A Pan-cancer analysis of enhancer expression in nearly 9000 patient samples. Cell 173:386–399.e12
222. Chen W-Y et al (2021) Nerve growth factor interacts with CHRM4 and promotes neuroendocrine differentiation of prostate cancer and castration resistance. Commun Biol 4:1–14
223. Zhang Z et al (2018) Inhibition of the Wnt/β-catenin pathway overcomes resistance to enzalutamide in castration-resistant prostate cancer. Cancer Res 78:3147–3162
224. Capozzi M et al (2019) Lenvatinib, a molecule with versatile application: from preclinical evidence to future development in anti-cancer treatment. Cancer Manag Res 11:3847
225. Jeong T-O et al (2012) Evaluation of HOXB13 as a molecular marker of recurrent prostate cancer. Mol Med Rep 5:901–904
226. Taslim C et al (2012) Integrated analysis identifies a class of androgen-responsive genes regulated by short combinatorial long-range mechanism facilitated by CTCF. Nucleic Acids Res 40:4754–4764
227. Höflmayer D et al (2020) Expression of CCCTC-binding factor (CTCF) is linked to poor prognosis in prostate cancer. Mol Oncol 14:129–138
228. Liu D et al (2021) Tumor subtype defines distinct pathways of molecular and clinical progression in primary prostate cancer. J Clin Invest 131: e147878
229. Shemshedini L, Knauthe R, Sassone-Corsi P, Pornon A, Gronemeyer H (1991) Cell-specific inhibitory and stimulatory effects of Fos and Jun on transcription activation by nuclear receptors. EMBO J 10:3839–3849
230. Lu H et al (2016) αvβ6 integrin promotes castrate-resistant prostate cancer through JNK1-mediated activation of androgen receptor. Cancer Res 76:5163–5174
231. Shen P et al (2014) KLF9, a transcription factor induced in flutamide-caused cell apoptosis, inhibits AKT activation and suppresses tumor growth of prostate cancer cells. Prostate 74:946–958
232. Shen P et al (2021) KLF9 suppresses cell growth and induces apoptosis via the AR pathway in androgen-dependent prostate cancer cells. Biochem Biophys Rep 28:101151
233. Lu S, Jenster G, Epner DE (2000) Androgen induction of cyclin-dependent kinase inhibitor p21 gene: role of androgen receptor and transcription factor Sp1 complex. Mol Endocrinol 14:753–760
234. Bedolla RG et al (2012) Predictive value of Sp1/Sp3/FLIP signature for prostate cancer recurrence. PLoS One 7:e44917
235. Sheng X et al (2019) IRE1α-XBP1s pathway promotes prostate cancer by activating c-MYC signaling. Nat Commun 10:1–12
236. Weichert W et al (2008) Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy. Br J Cancer 98:604–610
237. Varambally S et al (2002) The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 419:624–629
238. Davies A et al (2021) An androgen receptor switch underlies lineage infidelity in treatment-resistant prostate cancer. Nat Cell Biol 23:1023–1034
239. Bai Y et al (2019) Inhibition of enhancer of zeste homolog 2 (EZH2) overcomes enzalutamide resistance in castration-resistant prostate cancer. J Biol Chem 294:9911–9923
240. Morel KL et al (2021) EZH2 inhibition activates a dsRNA–STING–interferon stress axis that potentiates response to PD-1 checkpoint blockade in prostate cancer. Nat Cancer 2:444–456
241. Marrocco DL et al (2007) Suberoylanilide hydroxamic acid (vorinostat) represses androgen receptor expression and acts synergistically with an androgen receptor antagonist to inhibit prostate cancer cell proliferation. Mol Cancer Ther 6:51–60
242. Butler LM et al (2000) Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. Cancer Res 60:5165–5170
243. Bradley D et al (2009) Vorinostat in advanced prostate cancer patients progressing on prior chemotherapy (National Cancer Institute Trial 6862). Cancer 115:5541–5549
244. Rana Z, Diermeier S, Hanif M, Rosengren RJ (2020) Understanding failure and improving treatment using HDAC inhibitors for prostate cancer. Biomedicines 8
245. Gao S et al (2020) Chromatin binding of FOXA1 is promoted by LSD1-mediated demethylation in prostate cancer. Nat Genet 52:1011–1017
246. Wang L, Xu M, Kao C-Y, Tsai SY, Tsai M-J (2020) Small molecule JQ1 promotes prostate cancer invasion via BET-independent inactivation of FOXA1. J Clin Invest 130:1782–1792
247. Narayan VM (2020) A critical appraisal of biomarkers in prostate cancer. World J Urol 38:547–554
248. Koo KM, Mainwaring PN, Tomlins SA, Trau M (2019) Merging new-age biomarkers and nanodiagnostics for precision prostate cancer management. Nat Rev Urol 16:302–317

249. Antonelli A et al (2018) Biological effect of neoadjuvant androgen-deprivation therapy assessed on specimens from radical prostatectomy: a systematic review. Minerva Urol Nefrol 70:370–379
250. Loeb S et al (2014) Overdiagnosis and overtreatment of prostate cancer. Eur Urol 65:1046–1055
251. Shalek AK et al (2013) Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature 498:236
252. Buenrostro JD et al (2015) Single-cell chromatin accessibility reveals principles of regulatory variation. Nature 523:486–490
253. Chen S et al (2021) Single-cell analysis reveals transcriptomic remodellings in distinct cell types that contribute to human prostate cancer progression. Nat Cell Biol 23:87–98
254. Karthaus WR et al (2020) Regenerative potential of prostate luminal cells revealed by single-cell analysis. Science 368:497–505
255. Taavitsainen S et al (2021) Single-cell ATAC and RNA sequencing reveal pre-existing and persistent cells associated with prostate cancer relapse. Nat Commun 12:5307
256. Grosselin K et al (2019) High-throughput single-cell ChIP-seq identifies heterogeneity of chromatin states in breast cancer. Nat Genet 51:1060–1066
257. Ramani V et al (2017) Massively multiplex single-cell Hi-C. Nat Methods 14:263
258. Zhang R, Zhou T, Ma J (2022) Multiscale and integrative single-cell Hi-C analysis with Higashi. Nat Biotechnol 40: 254–261
259. ICGC Prostate UK Group et al (2015) The evolutionary history of lethal metastatic prostate cancer. Nature 520:353–357
260. Hong MKH et al (2015) Tracking the origins and drivers of subclonal metastatic expansion in prostate cancer. Nat Commun 6: 6605
261. Macintyre G et al (2017) How subclonal modeling is changing the metastatic paradigm. Clin Cancer Res 23:630–635
262. Haffner MC et al (2013) Tracking the clonal origin of lethal prostate cancer. J Clin Invest 123:4918–4922
263. Silva R et al (2021) Longitudinal analysis of individual cfDNA methylome patterns in metastatic prostate cancer. Clin Epigenetics 13:168