

Androgen receptor-driven chromatin looping in prostate cancer

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The androgen receptor (AR) is important for prostate cancer development and progression. Genome-wide mapping of AR binding sites in prostate cancer has found that the majority of AR binding sites are located within non-promoter regions. These distal AR binding regions regulate AR target genes (e.g. *UBE2C*) involved in prostate cancer growth through chromatin looping. In addition to long-distance gene regulation, looping has been shown to induce spatial proximity of two genes otherwise located far away along the genomic sequence and the formation of double-strand DNA breaks, resulting in aberrant gene fusions (e.g. *TMPRSS2-ERG*) that also contribute to prostate tumorigenesis. Elucidating the mechanisms of AR-driven chromatin looping will increase our understanding of prostate carcinogenesis and may lead to the identification of new therapeutic targets.

AR signaling in prostate cancer

Although significant strides have been made in prostate cancer research and treatment, prostate cancer remains one of the most commonly diagnosed cancers and the second leading cause of cancer deaths in American men [1]. The ‘male’ hormones or androgens, exerting their biological effect through androgen receptor (AR), play important roles in prostate cancer development and progression [2,3]. Therefore androgen ablation therapy, including surgical and chemical castration, is the first-line therapeutic approach for advanced androgen-dependent prostate cancer (ADPC). Although this therapy is initially effective, ADPC ultimately progresses into an incurable, castration-resistant stage of the disease (CRPC), involving the reactivation of AR signaling. The mechanisms for AR reactivation after castration include AR amplification, increased androgen sensitivity, increased intracellular synthesis of androgen, a constitutively active AR lacking the ligand binding domain (LBD), activation of growth factor pathways, and retinoblastoma (RB) loss-induced, E2F1-mediated AR overexpression [3–6]. Thus, aberrantly active AR signaling is present in both ADPC and CRPC.

AR is a ligand-dependent transcription factor belonging to the nuclear hormone receptor (NR) superfamily [7]. To

understand how AR signaling contributes to ADPC and CRPC, recent studies have utilized genome-wide chromatin immunoprecipitation (ChIP) techniques to define AR binding sites across the entire human genome in prostate cancer cells [8–13]. In these studies, AR ChIP-enriched DNA was amplified and hybridized to tiling DNA microarrays (ChIP-on-chip), or subjected to massively parallel high-throughput sequencing (ChIP-seq) [14,15]. These studies have greatly advanced understanding of AR binding to chromatin, the functional interplay between AR and chromatin, and AR-mediated regulation of target genes involved in prostate tumorigenesis. In this review we discuss how the majority of AR binding sites in the genome are located within non-promoter distal regions and the importance of this for transcriptional regulation. At these sites, several transcription factors act as positive or negative regulators of AR function and affect AR-mediated gene regulation. We then discuss two types of AR binding-driven chromatin looping in prostate cancer – the AR-driven chromatin looping leading to target gene expression (without genomic rearrangement) and AR-driven chromatin looping that results in gene fusion (with genomic rearrangement). The elucidation of the mechanisms of AR signaling in prostate cancer has translational implications in the development of new therapies for prostate cancer.

Features of the genome-wide AR binding atlas in prostate cancer

Distal AR binding sites: their location and importance

In the pre-genome-wide ChIP era, studies on the canonical AR target gene *PSA* (prostate specific antigen) found that AR primarily binds to the *PSA* enhancer rather than to the promoter region [16]. Consistent with the findings from the *PSA* gene, genome-wide mapping of AR binding sites in prostate cancer cells revealed that most AR binding sites are not within the promoter region of AR-regulated genes. Approximately 86–95% of AR binding sites identified in ADPC cell models (LNCaP [12,13] and VCaP [13]) and CRPC cell models (LNCaP-abl [12] and C4-2B [11]) are located within non-promoter regions. Interestingly, similar distal AR binding patterns are also observed in an immortalized normal human prostate epithelium cell line (HPr-1) [17], and non-prostate androgen-responsive cells (human primary skeletal muscle myoblasts [18]) or tissues

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(mouse epididymis [19]). These studies strongly indicate that distal binding may be a general rule for AR action.

Several lines of evidence suggest that at least part of these distal AR binding regions are important for transcriptional regulation. First, some AR binding regions, when cloned upstream of minimal promoters, function as enhancers in reporter gene assays [8,10,11]. Second, transcriptional coactivators [e.g. the histone acetyltransferase (HAT), BRM-containing chromatin-remodeling complex and the Mediator complex] and RNA polymerase II (Pol II) bind to some distal AR binding sites [8,10,12,13,20,21], indicating that these sites are transcriptionally active. Third, some distal AR binding sites are associated with active histone modifications, including H3K4 mono- and di-methylation (H3K4me1 and H3K4me2) and H3 acetylation (H3Ac) [10–13], which create a permissive chromatin environment that facilitates active transcription. Fourth, the nucleosomes present at some distal AR binding regions contain the H2A.Z variant [22], which has been reported to correlate with gene activation [23,24]. Collectively, these observations suggest that some distal AR binding sites act as transcriptional enhancers.

Transcription factors that positively or negatively affect AR-mediated gene expression

De novo motif searching (i.e. searching for common sequence patterns) or known motif scanning within the AR binding regions across the genome has identified that, in addition to AR binding motifs, many other transcription factor motifs (e.g. Forkhead, GATA, OCT, ETS, and NKX3-1) are significantly enriched within the AR binding regions, compared with genomic background. These observations suggest that transcription factors recognizing these motifs may be recruited to AR binding regions and play important roles in AR-mediated gene expression in prostate cancer, either by enhancing or repressing AR action. Consistent with this hypothesis, the pioneer factors FoxA1 and GATA2 were reported to be recruited to a significant portion of AR binding sites in ADPC and CRPC cells, facilitating AR binding and enhancing AR-mediated transcription [8,11,13,22]. In addition, OCT1, a member of the POU domain family of transcription factors, may facilitate Pol II binding to AR-bound regions after short-term and prolonged androgen stimulation [8,22]. By contrast, E26 transformation-specific (ETS) family member ERG binding sites that significantly overlap with AR binding sites in VCaP cells repress AR action and AR target gene expression [13]. These studies suggest that AR-mediated gene expression in prostate cancer involves combinatorial transcriptional regulation.

AR-driven chromatin looping leads to target gene expression

Distal AR binding regions communicate with target gene promoters through chromatin looping

Because the majority of AR binding sites are located at distal regions, how these distal binding sites interact with their target gene promoters and regulate target gene expression remains an open question. The looping model proposes that distal transcription factor binding sites interact with the proximal promoter regions, looping out

the intervening DNA [25,26]. Chromosome conformation capture (3C) technology developed almost a decade ago [27] allows this proposed model to be tested. In this assay, the distal and proximal genomic regions, which have been cross-linked, are digested by the same restriction enzyme and re-ligated under extensively diluted DNA concentrations. The re-ligation of distal/proximal regions should be detected by PCR if the distal region is physically associated with the proximal region [27,28]. Using this assay and its derived ChIP-3C (ChIP combined with 3C) assay, recent studies have demonstrated that distal AR binding sites regulate well-known AR target genes such as *PSA* [16] and *TMPRSS2* [8,21] through chromatin looping. Chromatin looping also drives the expression of crucial AR target genes directly involved in prostate cancer growth. For example, the CRPC-specific AR target gene *UBE2C* (ubiquitin-conjugating enzyme E2C) has an essential role in promoting CRPC cell growth [12,21] by inactivating the M-phase checkpoint [29] or by increasing the pool of active anaphase-promoting complex/cyclosome (APC/C) [30]. It was recently demonstrated that two CRPC cell-specific AR-bound enhancers, located –32.8 kb and +41.6 kb from the transcription start site (TSS) of the *UBE2C* gene, interact with the *UBE2C* promoters through chromatin looping in CRPC but not in ADPC cells, and that this looping is required for *UBE2C* gene expression and CRPC growth [12,21].

Although the 3C assay is a powerful approach for studying enhancer/promoter interaction, it is not feasible to perform standard 3C for each AR binding site to identify or validate its target gene because there are more than 10 000 AR binding sites in the genome. The development of high-throughput adaptation of 3C assays, 4C (circular 3C or 3C-on-chip) [31], 5C (3C-carbon copy) [32], Hi-C [33] and CHIA-PET (chromatin interaction analysis by paired-end sequencing) [34], should allow for genome-wide assessment of AR-bound enhancer/promoter interactions in the near future.

Mechanisms for AR-mediated chromatin looping in CRPC

Chromatin looping is established and/or maintained by protein–protein interaction between enhancer-bound transcription factors and promoter-bound proteins [25,26,35]. Although the distally bound transcription factors may directly interact with promoter-bound proteins, in many cases transcriptional coregulators or Pol II facilitate and/or mediate such interactions [26,36]. Indeed, recent studies demonstrated that both transcription factors [e.g. AR, estrogen receptor (ER), FoxA1 and GATA-1] [8,16,21,34,37] and coactivators (e.g. Med12, SRC-1, p300/CBP and BRG1) [38–41] play essential roles in looping formation and/or maintenance. Among these coactivators, Mediator, an evolutionally conserved multiprotein complex including ~30 subunits [42], is of particular interest. Although previous studies imply that Mediator is important for chromatin looping at several loci [41,43], a recent study found that Mediator may mediate genome-wide enhancer/promoter interactions. The global Mediator-mediated looping is stabilized by cohesin, a protein which embraces the loop [39]. These studies suggest that Mediator is an essential

chromatin architectural factor. Consistent with this notion, a recent study demonstrated that silencing of the Mediator subunit MED1, an AR coactivator [16,44], significantly decreased the interaction between the AR-bound *UBE2C* enhancers and the *UBE2C* promoter in the CRPC cell model LNCaP-*abl*, leading to decreased *UBE2C* gene expression [21]. Importantly, phosphorylation of MED1 at Thr1032 by phosphatidylinositol 3-kinase (PI3K)/AKT was proposed as a mechanism for MED1-mediated looping in CRPC cells by enhancing interactions between enhancer-bound FoxA1/AR and promoter-bound Pol II and TATA binding protein [21]. Based on the findings from these studies, we propose a looping model for AR-mediated gene regulation in CRPC (Figure 1). Furthermore, it seems reasonable to hypothesize that other looping-related coactivators such as SRC-1, p300/CBP [38], and cohesin [39] may also be involved in AR-mediated looping in CRPC (Figure 1). Further studies are needed to test these hypotheses.

AR-driven chromatin looping results in gene fusion

Overview of AR-regulated fusion genes in prostate cancer

Although genomic rearrangements are important for physiological processes such as variable, diversity and joining [V(D)J] recombination and class switch recombination (CSR) in lymphocytes, aberrant genomic rearrangements could lead to gene fusions in prostate cells contributing to prostate cancer initiation and progression [45,46]. For example, the *TMPRSS2-ERG* fusion gene, which is expressed and functional in ~50% of primary prostate cancer and ~30% of CRPC patients [45,47,48], is generated by the juxtaposition of the 5' untranslated region of the AR target gene *TMPRSS2* (21q22.3) [8] and the 5' end of the oncogene *ERG* (21q22.2) [45,49]. The juxtaposition of

TMPRSS2 and *ERG* occurs either through balanced translocations with a 'closed chain' pattern (i.e. without loss of genetic material) or through interstitial deletions (Edel) [50–52].

The *TMPRSS2-ERG* fusion gene was first identified by Chinnaiyan and colleagues using an integrative computational and experimental approach [49]. An algorithm termed COPA (cancer outlier profile analysis) was used to identify *ERG* as an outlier gene (i.e. a gene overexpressed in a subset of patients) from gene expression datasets in the Oncomine database [53]. Exon-walk PCR and 5'-RNA-ligase-mediated rapid amplification of complementary DNA ends (5'-RLM-RACE) assays were then performed to identify the fusion between *TMPRSS2* and *ERG* [45,49]. Using the same approach, many other androgen-regulated genes fused with members of the ETS gene family (e.g. *TMPRSS2-ETV1* [49], *SLC45A3-ETV1* [54], and *CANT1-ETV4* [55,56]), have been identified. Recently, more androgen-regulated gene fusions including non-ETS fusions (e.g. *NDRG1-ERG* [57], *SLC45A3-BRAF* [58] and *TMPRSS2-FKBP5-ERG* [59]) have been identified in prostate cancer by using the newly developed paired-end RNA sequencing (PE RNA-seq) method [60]. In this approach total RNA is fragmented and converted into double-stranded cDNA. The cDNA fragments go through adapter ligation and PCR amplification processes, and the final cDNA library is used for paired-end high-throughput sequencing. By integrating publicly available AR ChIP-seq data [13] and standard AR ChIP data [8,61,62] with gene expression and FISH (fluorescence *in situ* hybridization) analysis data, we have summarized the published AR-regulated fusion genes (Table S1 in the supplementary material online).

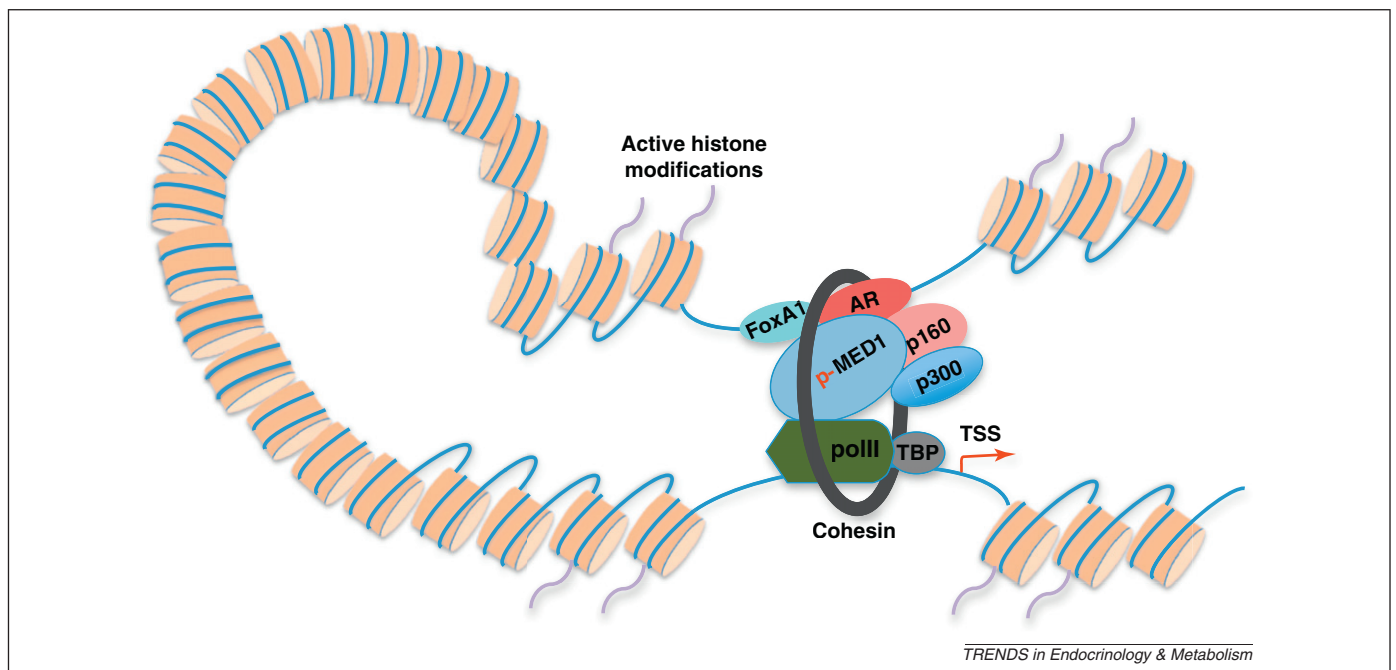


Figure 1. A proposed looping model for AR-regulated gene expression in CRPC. Active histone modifications (e.g. H3K4me1 and H3K4me2) facilitate the binding of AR and its collaborating transcription factors (e.g. FoxA1) to distal nucleosome-depleted regions [12,22]. p-MED1 mediates chromatin looping by facilitating interactions between distal transcription factors and the basal transcriptional apparatus [21,39]. Other coactivators (e.g. p160 coactivators and p300 [38]) may also enhance chromatin looping. Cohesion may stabilize chromatin looping by embracing the loop [39]. AR, androgen receptor; H3K4me1 and H3K4me2, H3K4 mono- and di-methylation; p-MED1, PI3K/AKT phosphorylated MED1.

In addition to AR-regulated fusion genes, many non-AR regulated fusion genes have been reported. For example, *ETV1* was found to be fused with the housekeeping gene *HNRPA2B1* [54]. In addition, none of the most recently identified 5' gene fusion partners (e.g. *ALG5*, *PIGU*, and *TNPO1*) by PE RNA-seq are androgen-regulated [59]. These findings suggest that there are multiple mechanisms for the regulation of fusion gene expression in prostate cancer.

Function of AR-regulated fusion genes

The binding of liganded AR to the regulatory elements of the 5' partner of a fusion gene drives the overexpression of the 3' partner, which is often an oncogene (e.g. *ERG*), thus contributing to prostate carcinogenesis. For example, it has been reported that AR-driven expression of the ETS family members *ETV1*, *ETV5* and *ERG* promotes invasion of benign prostate cells (e.g. RWPE, PrEC and BPH-1) and ADPC cells (e.g. LNCaP and VCaP) by activating an invasion-associated transcriptional program and thus inducing the plasminogen activator pathway [13,54,63–65]. Although overexpression of *ETV1* and *ETV5* showed no effect on cell proliferation [54,63], recent studies found that *ERG* overexpression also suppresses the AR-mediated differentiation program to promote LNCaP and VCaP cell growth [13,65]. Although *ERG* expression is necessary for prostate cancer cell invasion and growth, transgenic overexpression of *ERG* in mouse prostate is insufficient to induce prostatic intraepithelial neoplasia (PIN) and prostate cancer [64,65], suggesting that *ERG* overexpression cooperates with other genetic alterations in prostate tumorigenesis. Consistent with this hypothesis, recent studies using mouse models have indicated that loss of the tumor suppressor gene *PTEN* collaborates with *ERG* overexpression to develop PIN and prostate cancer [66,67]. In addition, assessment of human prostate samples identified an association of *TMPRSS2-ERG* with deletion of chromosome 3p14, which includes the potential tumor suppressor genes *FoxP1*, *RYBP* and *SHQ1*, suggesting a potential new cooperation in prostate tumorigenesis [68].

In addition to AR-regulated *ETS* fusion genes, AR-driven non-*ETS* fusion genes also have a crucial role in prostate cancer growth and invasion. For example, the AR target gene *SLC45A3* was reported to be fused with *BRAF*, a gene encoding a serine/threonine-specific protein kinase involved in the mitogen-activated protein kinase (MAPK) signaling pathway, in less than 2% of prostate cancer patients. Overexpression of *SLC45A3-BRAF* in RWPE cells increases cell proliferation and invasion, and leads to the formation of small tumors in immunodeficient mice. Although ETS fusion genes are considered to be poor therapeutic targets, the function of the *SLC45A3-BRAF* fusion gene can be readily inhibited by RAF and mitogen-activated protein kinase kinase (MAP2K1) inhibitors, indicating that this fusion gene is a druggable target [58].

Mechanisms for AR-mediated gene fusion

In general, gene fusions require the spatial proximity of two genomic regions otherwise located far apart on the genome, the formation of double-strand DNA breaks (DSBs), and error-prone DNA repair, which together might

lead to illegitimate DNA recombination [46,69]. In addition to the ability of AR to regulate the expression of fusion genes directly, as described above, recent studies focusing on the *TMPRSS2-ERG* fusion have found that androgen and/or AR might contribute to the processes that drive gene fusion. For example, androgen stimulation increases the spatial association of *TMPRSS2* and *ERG*. Inspired by the finding that estrogen is able to induce chromatin looping [38], two independent studies [61,70] found that dihydrotestosterone (DHT) treatment of LNCaP cells induces colocalization of *TMPRSS2* and *ERG*. Further studies found that this androgen-induced chromatin looping is mediated by AR, which is recruited to the *TMPRSS2* and *ERG* breakpoints upon DHT treatment [61,62] (Table S1). In agreement with the notion that breakpoints are associated with active histone modifications [46], these AR binding regions at the *TMPRSS2* and *ERG* breakpoints are enriched in histone H3K79 methylation and H4K16 acetylation [61]. A recent study that combined prostate cancer whole-genome sequencing data with VCaP ChIP-seq data [13] further revealed a genome-wide association of AR binding and active histone modifications (H3K4me3, H3K36me3 and H3Ac) near breakpoints in *TMPRSS2-ERG* positive patients [50]. These active histone modifications could facilitate AR binding and gene fusions.

In addition, DHT-bound AR recruits enzymes capable of inducing DSB. DHT stimulation has been found to induce AR-mediated recruitment of topoisomerase II beta (TOP2B) to breakpoints [62], an enzyme producing transient DSB that are required for ER- [71,72] and AR-regulated transcription [62]. Of note is also the observation that, in the presence of exogenous genotoxic stress (e.g. irradiation), DHT treatment markedly increases the mRNA and protein expression of activation-induced cytidine deaminase (AID) [61], a lymphoid-specific enzyme that changes cytosine to uracil and could ultimately lead to DSB [73]. AR then recruits AID to chromatin via its coactivator Gadd45 [61]. Along these lines, genotoxic stress also increases the expression of long interspersed nuclear element-1 (LINE-1) retroelement encoded open reading frame 2 (ORF2) endonuclease. Although ORF2 is recruited to translocation regions in a DHT-dependent manner, no physical interaction between AR and ORF2 has been detected [61].

Finally, androgen stimulation increases recruitment of proteins involved in non-homologous end joining (NHEJ). There are two main pathways for DSB repair, namely the homologous recombination (HR) pathway and the NHEJ pathway. The error-prone NHEJ is the major pathway for the repair of DSB in eukaryotes [74]. It has been reported that, upon DHT stimulation and increased DSB, several proteins involved in NHEJ including Ku70-Ku80 and ataxia telangiectasia-mutated protein (ATM) are recruited to the breakpoints, resulting in *TMPRSS2-ERG* fusion [61,62]. Significantly, pharmacologic inhibitors and small interfering RNAs (siRNAs) targeting NHEJ pathway components have been shown to reduce the formation of *de novo* *TMPRSS2-ERG* gene fusions [62]. Based on these findings, a model for AR-mediated *TMPRSS2-ERG* fusion in prostate cancer is proposed (Figure 2). Evidence suggests that this model is generalizable to other AR-regulated fusion genes such as *SLC45A3-ETV1* [61].

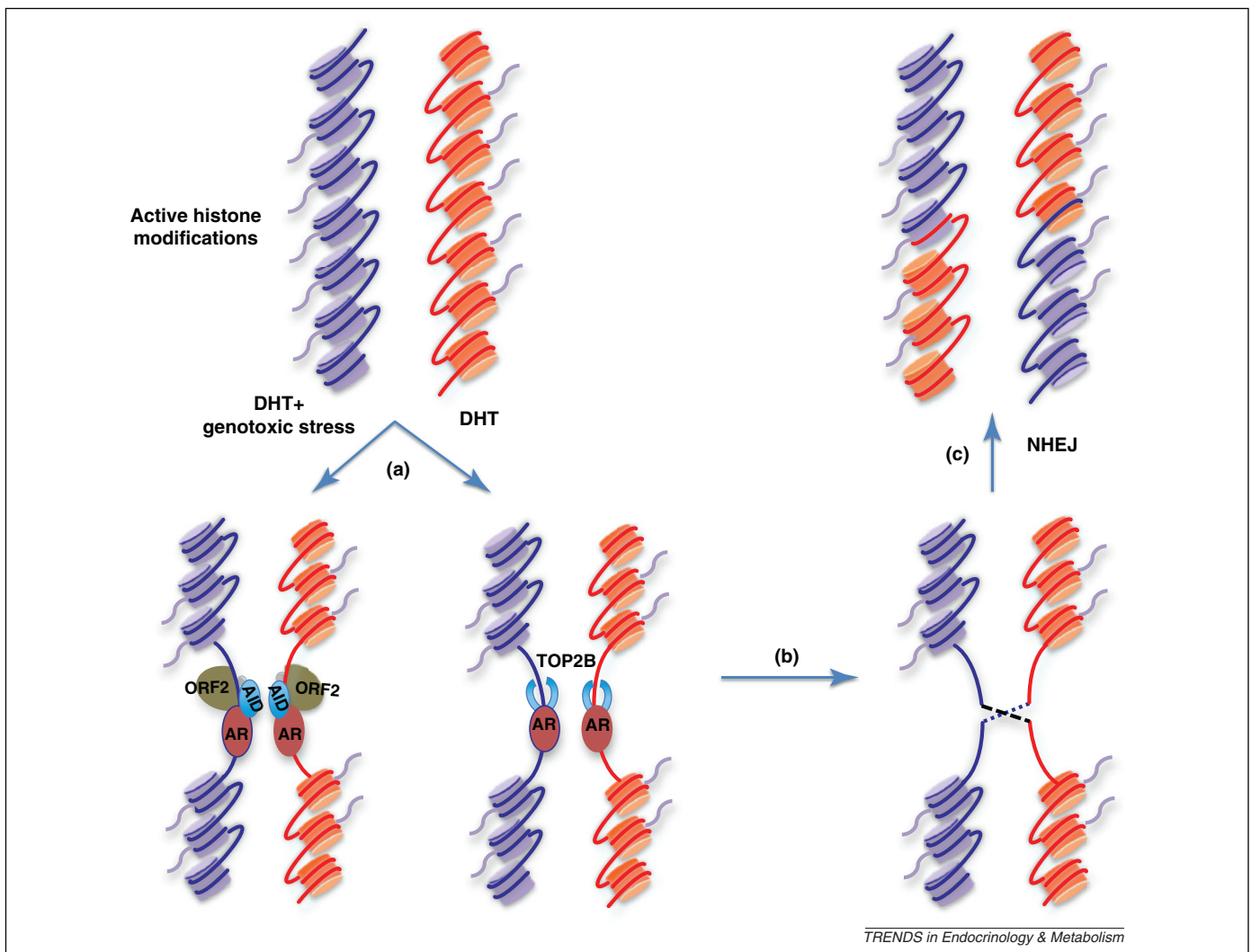


Figure 2. AR-mediated *TMPRSS2-ERG* gene fusion in prostate cancer cells. **(a)** In the presence of genotoxic stress, AR binds to *TMPRSS2* and *ERG* breakpoints and recruits AID upon DHT treatment. Exposure of prostate cancer cells under genotoxic stress to DHT also increases ORF2 recruitment to breakpoints [61]. DHT stimulation only leads to AR-TOP2B complex loading on breakpoints [62]. **(b)** The recruitment of AID, ORF2 and TOP2B to breakpoints causes DSB [61,62]. **(c)** NHEJ machinery is recruited to the breakpoints, leading to error-prone DSB repair and gene fusions such as *TMPRSS2-ERG* gene fusion [61,62]. AID, activation-induced cytidine deaminase; DHT, dihydrotestosterone; NHEJ, non-homologous end joining; ORF2, open reading frame 2; TOP2B, topoisomerase II beta.

Concluding remarks

AR expression and functionality have been well documented in both ADPC and CRPC [2,3]. Recent studies have found that the distal-binding AR transcription complex, including AR and associated transcription factors and coactivators, regulates the expression of several AR target genes involved in prostate cancer growth through chromatin looping. By using a global 3C assay, future studies should address whether such a long-range combinatorial regulation can be generalized to include other AR-dependent genes in the genome. This would allow the identification of all AR direct target genes involved in prostate tumorigenesis and might lead to the development of new therapies for the disease. In addition to the looping mechanism, future studies should also examine whether other mechanisms for enhancer function (e.g. spreading and non-coding RNA [26]) participate in AR-mediated long-range gene regulation.

With regard to AR-mediated gene fusion, significant progress has been made in our understanding of the mechanisms of AR-driven chromatin looping that leads to gene

fusions. The finding that inhibition of the NHEJ pathway reduces *TMPRSS2-ERG* gene fusion [62] suggests that future clinical trials may consider combining agents targeting both the AR and NHEJ pathway proteins. Despite progress on gene fusion mechanisms, it is still unclear on why gene fusions only occur in a limited number of genomic regions. It has been proposed that histone modifications, coactivators or noncoding RNAs may assist in the selection of these regions [75]. Future work should test these possibilities. Finally, because gene fusion at the RNA level without genomic rearrangement has recently been reported [59,76,77], future investigations should include whether AR has a role in driving the formation of such chimeric RNAs that also promote prostate carcinogenesis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tem.2011.07.006](https://doi.org/10.1016/j.tem.2011.07.006).

References

- Jemal, A. *et al.* (2010) Cancer statistics, 2010. *CA Cancer J. Clin.* 60, 277–300
- Heinlein, C.A. and Chang, C. (2004) Androgen receptor in prostate cancer. *Endocr. Rev.* 25, 276–308
- Knudsen, K.E. and Penning, T.M. (2010) Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer. *Trends Endocrinol. Metab.* 21, 315–324
- Feldman, B.J. and Feldman, D. (2001) The development of androgen-independent prostate cancer. *Nat. Rev. Cancer* 1, 34–45
- Attard, G. *et al.* (2009) Steroid hormone receptors in prostate cancer: a hard habit to break? *Cancer Cell* 16, 458–462
- Sharma, A. *et al.* (2010) The retinoblastoma tumor suppressor controls androgen signaling and human prostate cancer progression. *J. Clin. Invest.* 120, 4478–4492
- Mangelsdorf, D.J. *et al.* (1995) The nuclear receptor superfamily: the second decade. *Cell* 83, 835–839
- Wang, Q. *et al.* (2007) A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. *Mol. Cell* 27, 380–392
- Massie, C.E. *et al.* (2007) New androgen receptor genomic targets show an interaction with the ETS1 transcription factor. *EMBO Rep.* 8, 871–878
- Takayama, K. *et al.* (2007) Identification of novel androgen response genes in prostate cancer cells by coupling chromatin immunoprecipitation and genomic microarray analysis. *Oncogene* 26, 4453–4463
- Jia, L. *et al.* (2008) Genomic androgen receptor-occupied regions with different functions, defined by histone acetylation, coregulators and transcriptional capacity. *PLoS ONE* 3, e3645
- Wang, Q. *et al.* (2009) Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. *Cell* 138, 245–256
- 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
- Wang, Q. and Brown, M. (2009) Mapping the androgen receptor cisrome. In *Androgen Action in Prostate Cancer* (Tindall, D. and Mohler, J., eds), pp. 663–680, Springer
- Chen, Z. *et al.* (2010) Histone modifications and chromatin organization in prostate cancer. *Epigenomics* 2, 551–560
- Wang, Q. *et al.* (2005) Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. *Mol. Cell* 19, 631–642
- Bolton, E.C. *et al.* (2007) Cell- and gene-specific regulation of primary target genes by the androgen receptor. *Genes Dev.* 21, 2005–2017
- Wyce, A. *et al.* (2010) Research resource: the androgen receptor modulates expression of genes with critical roles in muscle development and function. *Mol. Endocrinol.* 24, 1665–1674
- Hu, S. *et al.* (2010) Research resource: genome-wide mapping of *in vivo* androgen receptor binding sites in mouse epididymis. *Mol. Endocrinol.* 24, 2392–2405
- Makkonen, H. *et al.* (2009) Long-range activation of *FKBP51* transcription by the androgen receptor via distal intronic enhancers. *Nucleic Acids Res.* 37, 4135–4148
- Chen, Z. *et al.* (2011) Phospho-MED1-enhanced *UBE2C* locus looping drives castration-resistant prostate cancer growth. *EMBO J.* 30, 2405–2419
- He, H.H. *et al.* (2010) Nucleosome dynamics define transcriptional enhancers. *Nat. Genet.* 42, 343–347
- Barski, A. *et al.* (2007) High-resolution profiling of histone methylations in the human genome. *Cell* 129, 823–837
- Wang, Z. *et al.* (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat. Genet.* 40, 897–903
- Dekker, J. (2008) Gene regulation in the third dimension. *Science* 319, 1793–1794
- Bulger, M. and Groudine, M. (2011) Functional and mechanistic diversity of distal transcription enhancers. *Cell* 144, 327–339
- Dekker, J. *et al.* (2002) Capturing chromosome conformation. *Science* 295, 1306–1311
- Hagege, H. *et al.* (2007) Quantitative analysis of chromosome conformation capture assays (3C-qPCR). *Nat. Protoc.* 2, 1722–1733
- Reddy, S.K. *et al.* (2007) Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature* 446, 921–925
- van Ree, J.H. *et al.* (2010) Overexpression of the E2 ubiquitin-conjugating enzyme UbcH10 causes chromosome missegregation and tumor formation. *J. Cell Biol.* 188, 83–100
- Zhao, Z. *et al.* (2006) Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. *Nat. Genet.* 38, 1341–1347
- Dostie, J. *et al.* (2006) Chromosome Conformation Capture Carbon Copy (5C): a massively parallel solution for mapping interactions between genomic elements. *Genome Res.* 16, 1299–1309
- Lieberman-Aiden, E. *et al.* (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326, 289–293
- Fullwood, M.J. *et al.* (2009) An oestrogen-receptor- α -bound human chromatin interactome. *Nature* 462, 58–64
- Miele, A. and Dekker, J. (2008) Long-range chromosomal interactions and gene regulation. *Mol. Biosyst.* 4, 1046–1057
- Nolis, I.K. *et al.* (2009) Transcription factors mediate long-range enhancer-promoter interactions. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20222–20227
- Vakoc, C.R. *et al.* (2005) Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. *Mol. Cell* 17, 453–462
- Hu, Q. *et al.* (2008) Enhancing nuclear receptor-induced transcription requires nuclear motor and LSD1-dependent gene networking in interchromatin granules. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19199–19204
- Kagey, M.H. *et al.* (2010) Mediator and cohesin connect gene expression and chromatin architecture. *Nature* 467, 430–435
- Kim, S.I. *et al.* (2009) BRG1 requirement for long-range interaction of a locus control region with a downstream promoter. *Proc. Natl. Acad. Sci. U.S.A.* 106, 2259–2264
- Park, S.W. *et al.* (2005) Thyroid hormone-induced juxtaposition of regulatory elements/factors and chromatin remodeling of *Crabp1* dependent on MED1/TRAP220. *Mol. Cell* 19, 643–653
- Malik, S. and Roeder, R.G. (2010) The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. *Nat. Rev. Genet.* 11, 761–772
- Degenhardt, T. *et al.* (2009) Population-level transcription cycles derive from stochastic timing of single-cell transcription. *Cell* 138, 489–501
- Wang, Q. *et al.* (2002) A coregulatory role for the TRAP-mediator complex in androgen receptor-mediated gene expression. *J. Biol. Chem.* 277, 42852–42858
- Kumar-Sinha, C. *et al.* (2008) Recurrent gene fusions in prostate cancer. *Nat. Rev. Cancer* 8, 497–511
- Mani, R.S. and Chinnaiyan, A.M. (2010) Triggers for genomic rearrangements: insights into genomic, cellular and environmental influences. *Nat. Rev. Genet.* 11, 819–829
- Cai, C. *et al.* (2009) Reactivation of androgen receptor-regulated *TMPRSS2:ERG* gene expression in castration-resistant prostate cancer. *Cancer Res.* 69, 6027–6032
- Attard, G. *et al.* (2009) Characterization of *ERG*, *AR* and *PTEN* gene status in circulating tumor cells from patients with castration-resistant prostate cancer. *Cancer Res.* 69, 2912–2918
- Tomkins, S.A. *et al.* (2005) Recurrent fusion of *TMPRSS2* and *ETS* transcription factor genes in prostate cancer. *Science* 310, 644–648
- Berger, M.F. *et al.* (2011) The genomic complexity of primary human prostate cancer. *Nature* 470, 214–220
- Mehra, R. *et al.* (2008) Characterization of *TMPRSS2-ETS* gene aberrations in androgen-independent metastatic prostate cancer. *Cancer Res.* 68, 3584–3590
- Mehra, R. *et al.* (2007) Heterogeneity of *TMPRSS2* gene rearrangements in multifocal prostate adenocarcinoma: molecular evidence for an independent group of diseases. *Cancer Res.* 67, 7991–7995
- Rhodes, D.R. *et al.* (2004) ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 6, 1–6

- 54 Tomlins, S.A. *et al.* (2007) Distinct classes of chromosomal rearrangements create oncogenic *ETS* gene fusions in prostate cancer. *Nature* 448, 595–599
- 55 Han, B. *et al.* (2008) A fluorescence *in situ* hybridization screen for E26 transformation-specific aberrations: identification of DDX5-ETV4 fusion protein in prostate cancer. *Cancer Res.* 68, 7629–7637
- 56 Hermans, K.G. *et al.* (2008) Two unique novel prostate-specific and androgen-regulated fusion partners of *ETV4* in prostate cancer. *Cancer Res.* 68, 3094–3098
- 57 Pflueger, D. *et al.* (2009) N-myc downstream regulated gene 1 (*NDRG1*) is fused to *ERG* in prostate cancer. *Neoplasia* 11, 804–811
- 58 Palanisamy, N. *et al.* (2010) Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. *Nat. Med.* 16, 793–798
- 59 Pflueger, D. *et al.* (2011) Discovery of non-*ETS* gene fusions in human prostate cancer using next-generation RNA sequencing. *Genome Res.* 21, 56–67
- 60 Maher, C.A. *et al.* (2009) Chimeric transcript discovery by paired-end transcriptome sequencing. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12353–12358
- 61 Lin, C. *et al.* (2009) Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. *Cell* 139, 1069–1083
- 62 Haffner, M.C. *et al.* (2010) Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. *Nat. Genet.* 42, 668–675
- 63 Helgeson, B.E. *et al.* (2008) Characterization of *TMPRSS2:ETV5* and *SLC45A3:ETV5* gene fusions in prostate cancer. *Cancer Res.* 68, 73–80
- 64 Klezovitch, O. *et al.* (2008) A causal role for ERG in neoplastic transformation of prostate epithelium. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2105–2110
- 65 Tomlins, S.A. *et al.* (2008) Role of the *TMPRSS2-ERG* gene fusion in prostate cancer. *Neoplasia* 10, 177–188
- 66 Carver, B.S. *et al.* (2009) Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. *Nat. Genet.* 41, 619–624
- 67 King, J.C. *et al.* (2009) Cooperativity of *TMPRSS2-ERG* with PI3-kinase pathway activation in prostate oncogenesis. *Nat. Genet.* 41, 524–526
- 68 Taylor, B.S. *et al.* (2010) Integrative genomic profiling of human prostate cancer. *Cancer Cell* 18, 11–22
- 69 Nussenzweig, A. and Nussenzweig, M.C. (2010) Origin of chromosomal translocations in lymphoid cancer. *Cell* 141, 27–38
- 70 Mani, R.S. *et al.* (2009) Induced chromosomal proximity and gene fusions in prostate cancer. *Science* 326, 1230
- 71 Ju, B.G. *et al.* (2006) A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. *Science* 312, 1798–1802
- 72 Perillo, B. *et al.* (2008) DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. *Science* 319, 202–206
- 73 Nambiar, M. and Raghavan, S.C. (2011) How does DNA break during chromosomal translocations? *Nucleic Acids Res.* 39, 5813–5825
- 74 Lieber, M.R. (2008) The mechanism of human nonhomologous DNA end joining. *J. Biol. Chem.* 283, 1–5
- 75 Mathas, S. and Misteli, T. (2009) The dangers of transcription. *Cell* 139, 1047–1049
- 76 Maher, C.A. *et al.* (2009) Transcriptome sequencing to detect gene fusions in cancer. *Nature* 458, 97–101
- 77 Kannan, K. *et al.* (2011) Recurrent chimeric RNAs enriched in human prostate cancer identified by deep sequencing. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9172–9177