

GENOMIC AND EPIGENOMIC ABERRATIONS OF GENE REGULATION IN PROSTATE
CANCER AND LEUKEMIA

by

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A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
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2021

Abstract

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Chapter 1

Noncoding mutations target *cis*-regulatory elements of the *FOXA1* plexus in prostate cancer

This chapter is a version of the paper published in *Nature Communications* as follows:

Zhou, S., **Hawley, J. R.**, Soares, F., Grillo, G., Teng, M., Tonekaboni, S. A. M., Hua, J. T., Kron, K. J., Mazrooei, P., Ahmed, M., Arlidge, C., Yun, H. Y., Livingstone, J., Huang, V., Yamaguchi, T. N., Espiritu, S. M. G., Zhu, Y., Severson, T. M., Murison, A., Cameron, S., Zwart, W., van der Kwast, T., Pugh, T. J., Fraser, M., Boutros, P. C., Bristow, R. G., He, H. H., and Lupien, M. Noncoding mutations target *cis*-regulatory elements of the *FOXA1* plexus in prostate cancer. **Nature Communications**, 2020;11:1–13.

Contributions per the manuscript: S.Z. and M.L. conceptualized the study. S.Z. designed and conducted most of the experiments with help from F.S., G.G., M.T., K.J.K., J.T.H., C.A., H.Y.Y., Y.Z. and S.C. J.R.H. implemented most of the computational analyses and statistical approaches with help from S.A.M., P.M., M.A., A.M., V.H., T.N.Y., S.M.G.E., T.M.S. and J.L. under the supervision of W.Z., T.v.d.K., T.J.P., M.F., P.C.B., R.G.B., H.H.H., or M.L. Figures were designed by S.Z. with assistance from J.R.H. and S.A.M. The manuscript was written by S.Z., J.R.H. and M.L. with assistance from all authors. M.L. oversaw the study.

1.1 Abstract

Prostate cancer is the second most commonly diagnosed malignancy among men worldwide. Recurrently mutated in primary and metastatic prostate tumours, *FOXA1* encodes a pioneer transcription factor involved in disease onset and progression through both androgen receptor-dependent and androgen receptor-independent mechanisms. Despite its oncogenic properties however, the regulation of *FOXA1* expression remains unknown. Here, we identify a set of six *cis*-regulatory elements in the *FOXA1* regulatory plexus harboring somatic single-nucleotide variants in primary prostate tumours. We find that deletion and repression of these *cis*-regulatory elements significantly decreases *FOXA1* expression and prostate cancer cell growth. Six of the ten single-nucleotide variants mapping to *FOXA1* regulatory plexus significantly alter the transactivation potential of *cis*-regulatory elements by modulating the binding of transcription factors. Collectively, our results identify *cis*-regulatory elements within the *FOXA1* plexus mutated in primary prostate tumours as potential targets for therapeutic intervention.

1.2 Introduction

Prostate cancer is the second most commonly diagnosed cancer among men with an estimated 1.3 million new cases worldwide in 2018 [1]. Although most men diagnosed with primary prostate cancer are treated with curative intent through surgery or radiation therapy, treatments fail in 30% of patients within 10 years [2] resulting in a metastatic disease [3]. Patients with metastatic disease are typically treated with anti-androgen therapies, the staple of aggressive prostate cancer treatment [4]. Despite the efficacy of these therapies, recurrence ultimately develops into lethal metastatic castration resistant prostate cancer (mCRPC) [4]. As such, there remains a need to improve our biological understanding of prostate cancer development and find novel strategies to treat patients. Sequencing efforts identified coding somatic single-nucleotide variants (SNVs) mapping to *FOXA1* in up to 9% [5–10] and 13% [9–11] of primary and metastatic castration resistant prostate cancer (mCRPC) patients, respectively. These coding somatic SNVs target the Forkhead and transactivation domains of *FOXA1* [12], altering its pioneering functions to promote prostate cancer development [10, 13]. Outside of coding SNVs, whole genome sequencing also identified somatic SNVs and indels in the 3' UTR and C-terminus of *FOXA1* in 12% of mCRPC patients [14]. In addition to SNVs, the *FOXA1* locus is a target of structural rearrangements in both primary and metastatic prostate cancer tumours, inclusive of duplications, amplifications, and translocations [9, 10]. Taken together, *FOXA1*

is recurrently mutated taking into account both its coding and flanking noncoding sequences across various stages of prostate cancer development.

FOXA1 serves as a pioneer transcription factor (TF) that can bind to heterochromatin, promoting its remodelling to increase accessibility for the recruitment of other TFs [15]. FOXA1 binds to chromatin at cell-type specific genomic coordinates facilitated by the presence of mono- and dimethylated lysine 4 of histone H3 (H3K4me1 and H3K4me2) histone modifications [16, 17]. In prostate cancer, FOXA1 is known to pioneer and reprogram the binding of the Androgen Receptor (AR) alongside HOXB13 [18]. Independent from its role in AR signalling, FOXA1 also regulates the expression of genes involved in cell cycle regulation in prostate cancer [19, 20]. For instance, FOXA1 co-localizes with CREB1 to regulate the transcription of genes involved in cell cycle processes, nuclear division and mitosis in mCRPC [19–25]. FOXA1 has also been shown to promote feed-forward mechanisms to drive disease progression [26, 27]. Hence, FOXA1 contributes to AR-dependent and AR-independent processes favouring prostate cancer development.

Despite the oncogenic roles of FOXA1, therapeutic avenues to inhibit its activity in prostate cancer are lacking. In the breast cancer setting for instance, the use of cyclin-dependent kinases inhibitors have been suggested based on their ability to block FOXA1 activity on chromatin [28]. As such, understanding the governance of FOXA1 mRNA expression offers an alternative strategy to find modulators of its activity. Gene expression relies on the interplay between distal cis-regulatory elements (CREs), such as enhancers and anchors of chromatin interaction, and their target gene promoter(s) [29]. These elements can lie tens to hundreds of kilobases (kbp) away from each other on the linear genome but physically engage in close proximity with each other in the three-dimensional space [30]. By measuring contact frequencies between loci through the use of chromatin conformation capture-based technologies, it enables the identification of regulatory plexuses corresponding to sets of CREs in contact with each other [31, 32]. By leveraging these technologies, we can begin to understand the three-dimensional organization of the prostate cancer genome and delineate the FOXA1 regulatory plexus.

Here, we integrate epigenetics and genetics from prostate cancer patients and model systems to delineate CREs establishing the regulatory plexus of FOXA1. We functionally validate a set of six mutated CREs that regulate FOXA1 mRNA expression. We further show that SNVs mapping to these CREs are capable of altering their transactivation potential, likely through modulating the binding of key prostate cancer TFs.

1.3 Results

1.3.1 *FOXA1* is essential for prostate cancer proliferation

We interrogated FOXA1 expression levels across cancer types. We find that FOXA1 mRNA is consistently the most abundant in prostate tumours compared to 25 other cancer types across patients (fig1a), ranking in the 95th percentile for 492 of 497 prostate tumours profiled in TCGA (figs1a). Using the same dataset we also find that FOXA1 is the most highly expressed out of 41 other Forkhead Box (FOX) factors in prostate tumours (figs1b). We next analyzed expression data from DEPMAP and observed FOXA1 to be most highly expressed in prostate cancer cell lines compared to cell lines of other cancer types (figs2a). Amongst the eight prostate cancer cell lines in the dataset (22Rv1, DU145, LNCaP, MDA-PCa-2B, NCI-H660, PrECLH, PC3, and VCaP), FOXA1 mRNA abundance is above the 90th percentile in all but one cell line (PrECLH) compared to the > 56,000 protein coding and non-protein coding genes profiled (figs2b). These new results gained from the TCGA and DEPMAP validate previous understanding that FOXA1 is one of the highest expressed genes in prostate cancer [33].

Following up on FOXA1 mRNA expression levels, we interrogated the essentiality of FOXA1 for prostate cancer cell growth. RNAi-mediated essentiality screens compiled in DEPMAP show that FOXA1 lies in the 94th percentile across 6 of the 8 available prostate cancer cell lines: 22Rv1, LNCaP, MDA PCa 2B, NCI-H660, PC3, and VCaP cells (fig1b-c). The median RNAi-mediated essentiality score for all prostate cell lines is significantly lower than all other cell lines, suggesting that FOXA1 is especially essential for prostate cancer cell proliferation (permutation test, $p = 1 \cdot 10^{-6}$, see Methods) (figs3a). Growth assays in LNCaP and VCaP cells following FOXA1 knockdown using two independent siRNAs (fig1d, figs3b) show significant growth inhibition in LNCaP (siRNA #1: 4-fold, siRNA #2: 3.35-fold) and VCaP (siRNA #1: 8.7-fold, siRNA #2: 2-fold) cells five days post-transfection (Mann-Whitney U Test, $p < 0.05$; fig1e-f). In accordance with previous reports, our results using essentiality datasets followed by knockdown validation reveals that FOXA1 is oncogenic and essential for prostate cancer cell proliferation.

1.3.2 Identifying putative *FOXA1* CREs

The interweaving of distal CREs with target gene promoters establishes regulatory plexuses with some to be ascribed to specific genes [125,165]. Regulatory plexuses stem from chromatin interactions orchestrated by various factors including ZNF143, YY1, CTCF and the cohesin complex

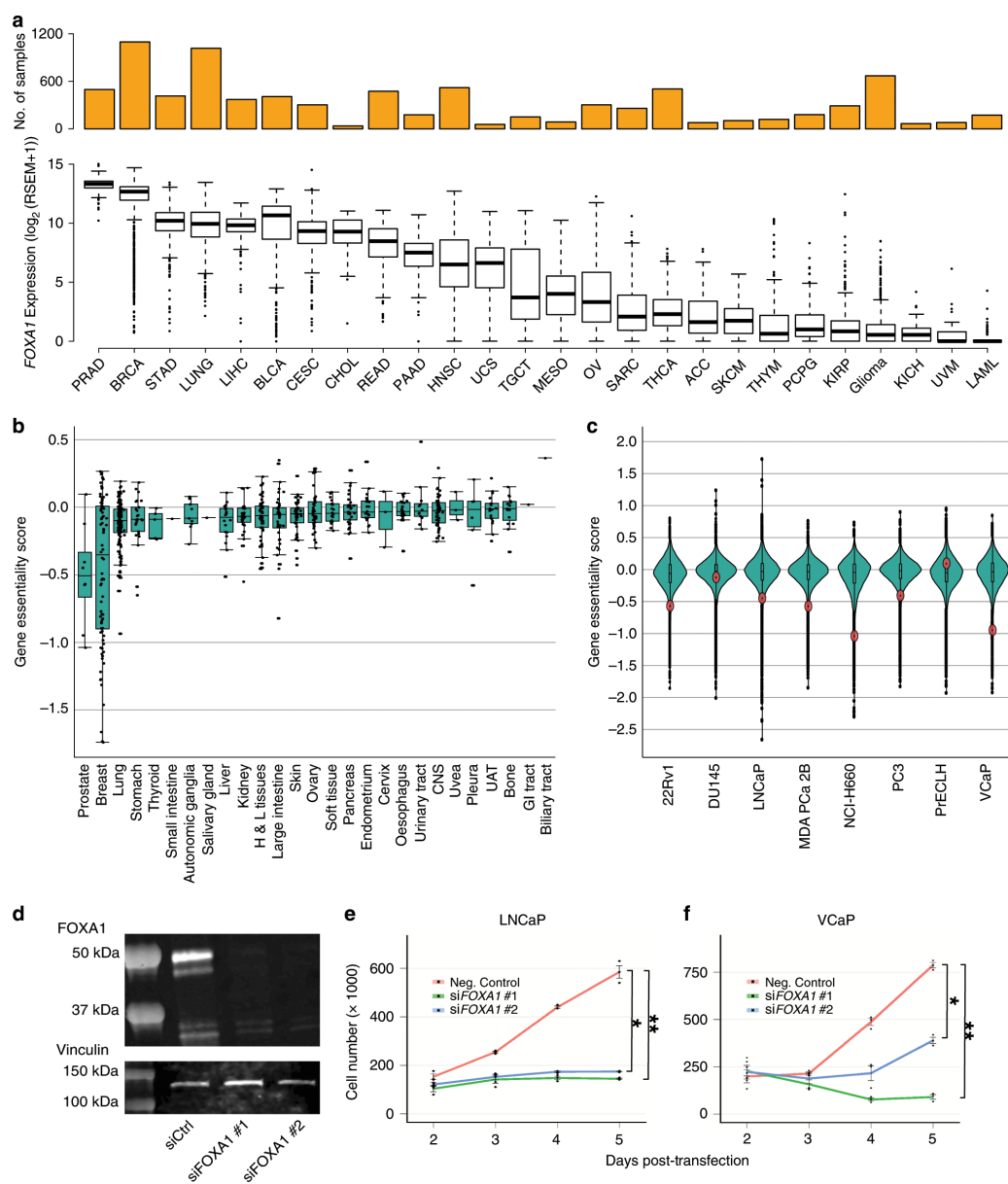


Figure 1.1: FOXA1 is highly expressed in prostate cancer and essential for prostate cancer cell proliferation.. **a.** The mRNA expression of FOXA1 across tumour types ($n = 26$) from RNA-seq data of TCGA. **b.** FOXA1 essentiality mediated through RNAi across various cell lines ($n = 707$) from DEPMAP. Gene essentiality scores are normalized Z-scores. Higher scores indicate less essential, and lower scores indicate more essential for cell proliferation. X-axis indicate tissue of origin for each cell line tested. Each dot indicates one cell line. **c.** Gene essentiality mediated through RNAi across prostate cancer cell lines ($n = 8$) from DEPMAP. Each dot indicates one gene, red indicates FOXA1. **d.** Representative Western blot against FOXA1 in LNCaP cells 5 days post-transfection of non-targeting siRNA and two independent siRNA targeting FOXA1. **e.** Cell proliferation assay conducted in LNCaP cells upon siRNA-mediated knockdown of FOXA1 across 5 days. **f.** Cell proliferation assay conducted in VCaP cells upon siRNA-mediated knockdown of FOXA1 across 5 days. Error bars indicate \pm s.d. $n = 3$ independent experiments. Mann-Whitney U test, * $p < 0.05$, ** $p < 0.01$.

141–143. Motivated by the oncogenic role of FOXA1 in prostate cancer, we investigated its regulatory plexus controlling its expression. According to chromatin contact frequency maps generated from Hi-C assays performed in LNCaP prostate cancer cells, FOXA1 lies in a 440 kbp TAD (chr14: 37720002-38160000 \pm 40 kbp adjusting for resolution) (Figure 3.2a). By overlaying DNase-seq data from LNCaP prostate cancer cells, there are a total of 123 putative CREs reported as DNase I Hypersensitive Sites (DHS) that populate this TAD (Figure 3.2a). We next inferred the regulatory plexus of FOXA1 using the Cross Cell-Type Correlation based on DNA accessibility (C3D) method 311. C3D aggregates and draws correlation of DHS signal intensities between the cell line of choice and the DHS signal across all systems in the database 311. Anchoring our analysis to the FOXA1 promoter and using accessible chromatin regions defined in LNCaP prostate cancer cells identified 55 putative CREs to the FOXA1 regulatory plexus ($r > 0.7$) (Figure 3.2b).

1.3.3 Putative *FOXA1* CREs harbour TF binding sites and SNVs

To delineate the CREs that could be actively involved in the transcriptional regulation of FOXA1, we annotated the DHS with available ChIP-seq data for histone modifications and TFs conducted in LNCaP, 22Rv1, VCaP prostate cancer cell lines and primary prostate tumours (Figure 3.2b) 186,232. Close to 60% (33/55) of the putative FOXA1 plexus CREs are positively marked by H3K27ac profiled in primary prostate tumours 232, indicative of active CREs in tumours (Figure 3.2b) 140. Next, considering that noncoding SNVs can target a set of CREs that converge on the same target gene in cancer 125, we overlapped the somatic SNVs called from the whole-genome sequencing across 200 primary prostate tumours to the 33 H3K27ac-marked DHS predicted to regulate FOXA1 214,234. This analysis identified 6 out of the 33 DHS marked with H3K27ac (18.2%) harboring one or more SNV(s) (10 total SNVs called from 9 tumours) (Figure 3.2b). We observe that these 6 CREs can be bound by multiple TFs in prostate cancer cells, including FOXA1, AR and HOXB13 (Figure 3.2b, Appendix II Supplemental Figure 3.4a-f). The Hi-C data from the LNCaP prostate cancer cells corroborates the C3D predictions as demonstrated by the elevated contact frequency between the region harboring the FOXA1 promoter and where the 6 CREs are located, compared to other loci in the same TAD (Figure 3.3a). The 6 CREs lie in intergenic or intronic regions (Figure 3.3b-h). Together, histone modifications, TF binding sites and noncoding SNVs support that these 6 putative CREs are active in primary prostate cancer. The Hi-C and C3D predictions suggest that they regulate FOXA1 expression.

1.3.4 Disruption of CREs reduces *FOXA1* mRNA expression

We next assessed the role of CREs toward FOXA1 expression using LNCaP and 22Rv1 clones stably expressing the wild-type Cas9 protein (Figure 3.4a-b). Guide RNAs (gRNAs) designed against the FOXA1 gene (exon 1 and intron 1) served as positive controls while an outside-TAD region (i.e. termed Chr14 (-)), a region on a different chromosome (the human AAVS1 safe-harbor site at the PPP1R12C locus 232,312), and three regions within the TAD predicted to be excluded from the FOXA1 plexus served as negative controls. Individual deletion of the FOXA1 plexus CREs through transient transfection of gRNAs into the LNCaP cells (See Methods) led to significantly decreased FOXA1 mRNA expression (Δ CRE1 $29.3 \pm 8.3\%$, Δ CRE2 $40.1 \pm 11.8\%$, Δ CRE 3 $30.6 \pm 9.1\%$, Δ CRE4 $23.6 \pm 8.2\%$, Δ CRE5 $25.3 \pm 6.6\%$, Δ CRE6 $24.5 \pm 10.2\%$ and Δ FOXA1 (exon 1 and intron 1) $87.4 \pm 8.8\%$ reduction relative to basal levels) (Figure 3.4c, Appendix II Supplemental Figure 3.5a-f). In contrast, deletion of several negative control regions within the same TAD did not significantly reduce FOXA1 mRNA level (Figure 3.4c, Appendix II Supplemental Figure 3.5g-i). Similar results were observed in 22Rv1 prostate cancer cells (Figure 3.4d). As each clone expressed Cas9 protein at different levels, there may be a difference between genome editing efficiencies between the clones. We compared the CRISPR/Cas9 on-target genome editing efficiency across the five LNCaP cell line-derived clones with the relative FOXA1 mRNA levels, and indeed observe a significant inverse correlation across all CREs (Pearson's correlation $r = 0.49, p < 0.005$) (Appendix II Supplemental Figure 3.6a) and agreeing trends for each individual CRE (Appendix II Supplemental Figure 3.6b).

Complementary to our findings using the wild-type CRISPR/Cas9 system, we next generated four LNCaP and four 22Rv1 cell line-derived dCas9-KRAB fusion protein expressing clones (Figure 3.4e-f). Transient transfection of the same gRNAs used in the wild-type Cas9 experiments, targeting the six FOXA1 plexus CREs into our dCas9-KRAB LNCaP clones significantly decreased FOXA1 expression relative to basal levels (iCRE1 $24.6 \pm 6.2\%$, iCRE2 $42.2 \pm 10.8\%$, iCRE3 $25.3 \pm 9.2\%$, iCRE4 $23.3 \pm 4.3\%$, iCRE5 $30.2 \pm 3.4\%$ and iCRE6 $23.1 \pm 8.1\%$ reduction). Similarly, gRNAs targeting the dCas9-KRAB fusion protein to FOXA1 decreased its expression (iFOXA1 $81.6 \pm 11.8\%$ reduction; Student's t-test, $p < 0.05$, Figure 3.4g). Analogous results were also observed in our four clonal 22Rv1 dCas9-KRAB cell lines (Student's t-test, $p < 0.05$, Figure 3.4h). Collectively, our results suggest that the six CREs control FOXA1 expression.

We further assessed the regulatory activity of the six FOXA1 plexus CREs by testing the consequent mRNA expression on other genes within the same TAD, namely MIPOL1 and TTC6. Δ

CRE1 and Δ CRE2 significantly reduced MIPOL1 mRNA expression by $38.4 \pm 6.4\%$ and $48.4 \pm 9\%$, respectively relative to basal levels, whereas deletion of the other four CREs did not result in any significant MIPOL1 expression changes (Student's t-test, $p < 0.05$, Appendix II Supplemental Figure 3.7a). On the other hand, deletion of CREs each significantly reduced TTC6 mRNA expression relative to its basal levels (Δ CRE1 $52.9\% \pm 6.4\%$, Δ CRE2 $66 \pm 11.3\%$, Δ CRE3 $55.5 \pm 12.8\%$, Δ CRE4 $44.9 \pm 10.6\%$, Δ CRE5 $43.1 \pm 11.9\%$ and Δ CRE6 $52.2 \pm 7.3\%$ reduction (Student's t-test, $p < 0.05$, Appendix II Supplemental Figure 3.7b), in agreement with the fact that TTC6 shares its promoter with FOXA1 as both genes are transcribed on opposing strands (Appendix II Supplemental Figure 3.7c).

Reduction in FOXA1 mRNA expression resulting from the deletion of FOXA1 plexus CREs may also impact gene expression downstream of FOXA1, we assessed the mRNA expression of several FOXA1 target genes, namely SNAI2, ACPP, and GRIN3A. Deletion of CREs resulted in significant change in SNAI2 (up-regulation; Δ CRE1 190%, Δ CRE2 162.8%, Δ CRE3 147.5%, Δ CRE4 133.3%, Δ CRE5 137.3%, Δ CRE6 120.8%, Δ FOXA1 266.7%), ACPP (down-regulation; Δ CRE1 73.5%, Δ CRE2 62.5%, Δ CRE3 69.6%, Δ CRE4 75.6%, Δ CRE5 70.9%, Δ CRE6 74.6%, Δ FOXA1 52.2%) and GRIN3A expression (up-regulation; Δ CRE1 138.2%, Δ CRE2 168.8%, Δ CRE3 144.6%, Δ CRE4 132.1%, Δ CRE5 131.4%, Δ CRE6 127%, Δ FOXA1 228%) (Student's t-test, $p < 0.05$, Appendix II Supplemental Figure 3.7d-f). Collectively, our results support the restriction of most FOXA1 plexus CREs towards FOXA1 and its target genes.

1.3.5 *FOXA1* CREs collaborate to regulate its expression

Expanding on the idea that multiple CREs can converge to regulate the expression of a single target gene 125,165,313, we asked whether the CREs we identified collaboratively regulate FOXA1 mRNA expression. Here, we applied a transient approach that delivers Cas9 protein:gRNA as a ribonucleoprotein (RNP) complex formed prior to transfection that would avoid the heterogeneity of Cas9 protein expression across the prostate cancer cell clones (See Methods). We first validated this system through single CRE deletions, where we transiently transfected a set of gRNA targeting the CRE of interest. In accordance with data from our prostate cancer cell clones stably expressing wild-type Cas9 and dCas9-KRAB, individual CRE deletion resulted in a significant reduction in FOXA1 mRNA expression: (Δ CRE1 $29.3 \pm 7.3\%$, Δ CRE2 $36 \pm 11.8\%$, Δ CRE3 $30.6 \pm 12.7\%$, Δ CRE4 $24.5 \pm 6.1\%$, Δ CRE5 $23.7 \pm 13.2\%$, Δ CRE6 $26.8 \pm 14.2\%$ and Δ FOXA1 $96.2 \pm 1.4\%$ reduction (Student's t-test, $p < 0.05$, Figure 3.5a, Appendix II Supplemental Figure

3.8a-f). Next for combinatorial deletions, we prioritized the CREs that harbor more than 1 SNV (i.e CRE1, CRE2, CRE4), and transiently transfected RNP complexes that target both CREs in various combinations (i.e CRE1 + CRE2, CRE1 + CRE4, CRE2 + CRE4), and assessed FOXA1 mRNA expression. Compared to negative control regions, the combinatorial deletion of Δ CRE1 + Δ CRE2, Δ CRE1 + Δ CRE4, and Δ CRE2 + Δ CRE4 resulted in a significant $48.5 \pm 4.5\%$, $50.4 \pm 2.9\%$ and $45.2 \pm 5.5\%$ reduction in FOXA1 mRNA expression, respectively (Student's t-test, $p < 0.05$, Figure 3.5b, Appendix II Supplemental Figure 3.9a-f) a fold reduction greater than single CRE deletions (Student's t-test, Appendix II Supplemental Figure 10, $p < 0.05$). These results together demonstrate that these CREs collaboratively contribute to the establishment and regulation of FOXA1 expression in prostate cancer.

1.3.6 Disruption of *FOXA1* CREs reduces prostate cancer cell growth

As FOXA1 is essential for prostate cancer growth (Figure 3.1b-e), we next sought to assess the importance of the six FOXA1 plexus CREs towards prostate cancer cell growth. We adapted a lentiviral-based approach that expressed both the Cas9 protein and two gRNA that target each CRE for deletion (See Methods). Upon lentiviral transduction with subsequent selection, we separated LNCaP prostate cancer cells for RNA, DNA and for cell proliferation. We first tested the system by measuring FOXA1 mRNA expression, and independently observed significant reductions of FOXA1 mRNA expression (Δ CRE1 18%, Δ CRE2 30%, Δ CRE3 15%, Δ CRE4 12%, Δ CRE5 35%, Δ CRE6 46% and Δ FOXA1 (exon 1 and intron 1) 48% reduction (Student's t-test, $p < 0.05$, Figure 3.5c, Appendix II Supplemental Figure 11a-f). We then seeded these cells at equal density. Six days post-seeding, we harvested the cells and observed a significant reduction in cell growth upon deleting any of the six FOXA1 plexus CREs (Δ CRE1 42%, Δ CRE2 28%, Δ CRE3 33%, Δ CRE4 27%, Δ CRE5 42%, Δ CRE6 44% and Δ FOXA1 (exon 1 and intron 1) 50% reduction (Student's t-test, $p < 0.05$, Fig 5d). These results suggest that the six FOXA1 plexus contribute to prostate cancer etiology, in agreement with their ability to regulate FOXA1 expression and the essentiality of this gene in prostate cancer cell growth.

1.3.7 SNVs mapping to *FOXA1* CREs can alter their activity

SNVs can alter the transactivation potential of CREs 125,127,145,147,155,314–318. In total, we found 10 SNVs called from 9 out of the 200 tumours that map to the six FOXA1 plexus CREs (Figure 3.6a). To assess the impact of these noncoding SNVs, we conducted luciferase assays comparing

differential reporter activity between the variant and the wild-type allele of each CRE (Figure 3.6b-k). We found that the variant alleles of 6 of the 10 SNVs displayed significantly greater luciferase reporter activity when compared to the wild-type alleles (Mann-Whitney U test, $p < 0.05$). Specifically, we observed the following fold-changes: chr14:37,887,005 A > G (1.65-fold), chr14:37,904,343 A > T (1.35-fold), chr14:37,905,854 A > G (1.28-fold), chr14:37,906,009 T > C (1.71-fold), chr14:38,036,543 A > G (1.44-fold), chr14:38,055,269 C > G (1.39-fold) (Figure 3.6b, d-h). These results indicate that these SNVs can alter the transactivation potential of FOXA1 plexus CREs in prostate cancer cells.

1.3.8 SNVs mapping to *FOXA1* CREs can modulate the binding of TFs

We next assessed if the changes in transactivation potential induced by noncoding SNVs related to changes in TF binding to CREs by allele-specific ChIP-qPCR 125,155,315 in LNCaP prostate cancer cells. We observed differential binding of FOXA1, AR, HOXB13, GATA2 and FOXP1 for the chr14:37887005 (A > G) SNV found in CRE1; the chr14:37904343 (A > T), chr14:37905854 (A > G) and chr14:37906009 (T > C) SNVs found in CRE2; and the chr14:38055269 (C > G) SNV found in CRE4 (Student's t-test, $p < 0.05$, Figure 3.6l-p). In contrast, SOX9 and NKX3.1 binding was unaffected by these SNVs (Figure 3.6l-q). Compared to the wild-type sequence, chr14:37,887,005 A > G significantly increased AR binding (1.31-fold increase), GATA2 binding (1.25-fold increase) and FOXP1 binding (1.23-fold increase); chr14:37,904,343 A > T significant increased AR binding (1.30-fold increase), GATA2 (1.25-fold increase) and FOXP1 (1.33-fold increase); chr14:37,905,854 A > G significantly increased FOXA1 binding (1.41-fold increase) and AR binding (1.33-fold increase); chr14:37,906,009 T > C significantly increased the binding of FOXA1 (1.29-fold increase), AR (1.31-fold increase), HOXB13 (1.13-fold increase) and FOXP1 (1.25-fold increase); and chr14:38,055,269 C > G significantly increased FOXA1 binding (1.20-fold increase). Notably all six SNVs increased the binding of the TFs known to bind at these CREs. In contrast, none of the SNVs significantly decreased the binding of these TFs. Our observations suggest that gain-of-function SNVs populate the FOXA1 plexus CREs.

1.4 Discussion

Modern technologies and understanding of the epigenome allow the possibility of probing CRE(s) involved in regulating genes implicated in disease. Despite FOXA1 being recurrently mutated 177,203,204,214,297 and playing potent oncogenic roles in prostate cancer etiology 180,189,191, the

CREs involved in its transcriptional regulation are poorly understood. Understanding how FOXA1 is expressed can provide a complementary strategy to antagonize FOXA1 in prostate cancer.

We used the DHS profiled in prostate cancer cells to identify putative FOXA1 CREs through annotating these regions with five different histone modifications, TF binding sites and noncoding SNVs profiled in prostate cancer cells and primary prostate tumours. Our efforts identified and validated a set of six active CREs involved in FOXA1 regulation, agreeing with a recent report where a subset of our CREs map to loci suggested to be in contact with the FOXA1 promoter 319. The disruption of these six distal CREs each significantly reduced FOXA1 mRNA levels, similar to what has been demonstrated for ESR1 in luminal breast cancer 125, MLH1 in Lynch syndrome 320, MYC in lung adenocarcinoma and endometrial cancer 175 and AR in mCRPC 321,322. Through combinatorial deletion of two CREs, FOXA1 mRNA levels were further reduced in comparison with single CRE deletions, raising the possibility of CRE additivity 124. The deletion of the FOXA1 plexus CREs also significantly reduced prostate cancer cell proliferation at levels comparable to what has been reported upon deletion of the amplified CRE upstream of the AR gene in mCRPC 321, suggestive of onco-CREs as reported in lung 175 and prostate 321 cancer.

More than 90% of SNVs found in cancer map to the noncoding genome 215,323 with a portion of these SNVs mapping to CREs altering their transactivation potential 125,145,147,315 and/or downstream target gene expression 127,146,215. We extended this concept with SNVs identified from primary prostate tumours mapping to FOXA1 plexus CREs. We observed that a subset of these SNVs can alter transactivation potential by modulating the binding of specific TFs whose cistromes are preferentially burdened by SNVs in primary prostate cancer 323. Our findings complement recent reports of SNVs found in the noncoding space of FOXA1 that could affect its expression 193,324. The FOXA1 plexus CREs we identified here are also reported to be target of structural variants in both the primary and metastatic settings 179,180, including tandem duplication in 14% (14/101) mCRPC tumours over CRE2 179, amplification, duplication and translocation over CRE3, 4, 5 180. Notably, the translocation and duplication defining the FOXMIND enhancer driving FOXA1 expression reported in primary and metastatic settings harbors the CRE3 element we characterized 180. Collectively, these studies combined with our discoveries reveal the fundamental contribution of the FOXA1 plexus in prostate cancer etiology. As a whole, our findings in conjunction with recent reports suggest that CREs involved in the transcriptional regulation of FOXA1 may be hijacked in prostate tumours through various types of genetic alterations.

Despite initial treatment responses from treating aggressive primary and metastatic prostate cancer through castration to suppress AR signalling 296, resistance ensues as 80% of mCRPC tu-

mours harbor either AR gene amplification, amplification of a CRE upstream of AR, or activating AR coding mutations 179,204,321. Given the AR-dependent 186,201 and AR-independent 304 oncogenic activity of FOXA1 in prostate cancer, its inhibition is an appealing alternative therapeutic strategy. Our dissection of the FOXA1 cis-regulatory landscape complement recent findings through revealing loci that are important for the regulation of FOXA1. Theoretically, direct targeting of the CREs regulating FOXA1 would down-regulate FOXA1 levels and could therefore serve as a valid alternative to antagonize its function.

Taken together, we identified FOXA1 CREs targeted by SNVs that are capable of altering transactivation potential through the modulation of key prostate cancer TFs. The study supports the importance of considering CREs not only as lone occurrences but as a team that works together to regulate their target genes, particularly when considering the impact of genetic alterations. As such, our work builds a bridge between the understanding of FOXA1 transcriptional regulation and new routes to FOXA1 inhibition. Aligning with recent reports 180,189,191, our findings support the oncogenic nature of FOXA1 in prostate cancer. Gaining insight on the cis-regulatory plexuses of important genes such as FOXA1 in prostate cancer may provide new avenues to inhibit other drivers across various cancer types to halt disease progression.

1.5 Methods

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Appendices

.1 Supplemental Figures for Chapter 2