Genome-wide analyses of transcription factors and co-regulators across seven cohorts identified reduced PPARGC1A expression as a driver of prostate cancer progression

Manjunath Siddappa¹, Sajad A. Wani², Mark D. Long³, Damien A Leach⁴,
Ewy A. Mathé^{5,6}, Charlotte L. Bevan⁷, Moray J. Campbell^{1,8,9*}.

¹ College of Pharmacy, Pharmaceutics and Pharmaceutical Chemistry, 536 Parks Hall, 500 West 12th Ave.,
The Ohio State University, Columbus, OH 43210. smanju712@gmail.com

² College of Pharmacy, Pharmaceutics and Pharmaceutical Chemistry, 536 Parks Hall, 500 West 12th Ave.,
The Ohio State University, Columbus, OH 43210. wani.16@osu.edu

³ Department of Cancer Genetics and Genomics, Roswell Park Comprehensive Cancer Center (RPCCC),
Buffalo, NY, 14263, USA. Mark.Long@RoswellPark.org

⁴ Imperial Centre for Translational and Experimental Medicine, Department of Surgery & Cancer, Imperial
College London, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK.
damien.leach@imperial.ac.uk

⁵ Biomedical Informatics Department, College of Medicine, The Ohio State University, Columbus, Ohio, 43210,
USA.

⁶ Division of Preclinical Innovation, National Center for Advancing Translational Sciences, NIH, 9800 Medical
Center Dr., Rockville, MD, 20892, USA. ewy.mathe@nih.gov

1 ⁷ Imperial Centre for Translational and Experimental Medicine, Department of Surgery & Cancer, Imperial
2 College London, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK.
3 charlotte.bevan@imperial.ac.uk

4 ^{8,9} The James, Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, 43210, USA.
5 Biomedical Informatics Shared Resource, The Ohio State University, Columbus, Ohio, 43210, USA.
6 Campbell.1933@OSU.edu

7 *Corresponding author

8

9 Running title: Transcriptional co-regulators and prostate cancer

10

11

Acknowledgements

MJC acknowledges support in part from the Prostate program of the Department of Defense Congressionally Directed Medical Research Programs [W81XWH-14-1-0608], the National Institute of Health Cancer Center Support Grant (P30CA016058) to the OSUCCC The James. *EW* and *MJC* acknowledge seed-funding support from the Molecular Carcinogenesis program of the OSUCCC The James, CCSG. *CLB*, *MJC*, *DAL* acknowledge support in part from Prostate Cancer, UK [RIA18-ST2-022]. *EAM* acknowledges support The Ohio State University Translational Data Analytics Institute, startup funds from The Ohio State University.

Conflict of interest

The author certifies that he has NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

1 ABSTRACT

2 Defining altered transcription factor (TF) and coregulators that are oncogenic drivers remains a
3 challenge, in part because of the multitude of TFs and coregulators. We addressed this challenge by
4 using bootstrap approaches to test how expression, copy number alterations or mutation of TFs (n =
5 2662), coactivators (COA; n= 766); corepressor (COR; n = 599); mixed function coregulators (MIXED;
6 n = 511) varied across seven prostate cancer (PCa) cohorts (three of localized and four advanced
7 disease). COAS, CORS, MIXED and TFs all displayed significant down-regulated expression (q.value
8 < 0.1) and correlated with protein expression (ρ 0.4 to 0.55). Stringent expression filtering identified
9 commonly altered TFs and coregulators including well-established (e.g. ERG) and underexplored
10 (e.g. *PPARGC1A*, encodes PGC1 α) in localized PCa. Reduced *PPARGC1A* expression significantly
11 associated with worse disease-free survival in two cohorts of localized PCa. Stable
12 PGC1 α knockdown in LNCaP cells increased growth rates and invasiveness and RNA-Seq revealed
13 a profound basal impact on gene expression (~2300 genes; FDR < 0.05, logFC > 1.5), but only
14 modestly impacted PPAR γ responses. GSEA analyses of the PGC1 α transcriptome revealed that it
15 significantly altered the AR-dependent transcriptome, and was enriched for epigenetic modifiers.
16 PGC1 α -dependent genes were overlapped with PGC1 α -ChIP-Seq genes and significantly associated
17 in TCGA with higher grade tumors and worse disease-free survival. Together these data
18 demonstrate an approach to identify cancer-driver coregulators in cancer and that PGC1 α expression
19 is clinically significant yet underexplored coregulator in aggressive early stage PCa.

20

21

1 INTRODUCTION

2 Prostate cancer (PCa) is a high-profile hormone-responsive cancer that displays uncertain
3 progression risks. For men with localized PCa, it is unclear which patients will experience treatment
4 failure following either surgery or radiotherapy. In advanced PCa, it's similarly unclear which patients
5 will experience a sustained response to androgen deprivation therapy (ADT), and who will experience
6 treatment failure giving rise to ADT-recurrent PCa (ADT-RPCa).

7 Many of these prognostic uncertainties arise due to changes in the control of androgen receptor (AR)
8 signaling. In the normal prostate gland the AR functions in a large and dynamic multimeric complex to
9 regulate gene expression programs that control tissue homeostasis and epithelial cell fates^{27, 34}. In
10 PCa there are strong examples of how signaling is distorted due to changes in AR expression and
11 structural variation, alongside changes in the members of the AR complex^{9, 15, 26, 28, 35, 47, 58, 59, 64, 86, 93}.
12 Furthermore, epigenomic events^{2, 25, 33, 57, 96} alter enhancer accessibility of downstream AR target
13 genes and even upstream of the AR gene itself⁷⁹. As a result of these changes to the AR complex
14 and allied epigenetic events, the normal differentiation driving and growth limiting functions of the AR
15 are attenuated and there is enhanced regulation of genes that promote aggressive cancer
16 phenotypes^{3, 87, 9263}.

17 This re-wiring of AR signaling is mirrored by disrupted functions of other members of the nuclear
18 hormone receptor super-family^{35, 50}, and probably occurs more widely to alter how transcription
19 factors (TFs) function. Re-wiring of TFs changes the consequences of cell signaling and contributes
20 to changes in lineage plasticity that are also associated with aggressive PCa^{8, 40, 72}. Therefore
21 understanding how TF signaling occurs in PCa progression can lead to the development of strategies
22 for targeting these changes through targeted epigenetic therapies^{6, 21, 42}, or the downstream gene
23 networks may be uniquely drug-sensitive^{56, 68}. Furthermore disease-specific enhancers that are newly

1 activated provide rationale for targeted deep sequencing to dissect the interactions of germline and
2 structural variation at tumor-driving enhancers^{60, 75}. These studies have revealed a number of high
3 profile and common alterations in TFs and coregulators in PCa. For example, ERG⁶¹ and
4 NR3C1/GR³⁹ have been identified as commonly altered, whereas others such as the PPAR γ
5 coactivator PGC1 α ^{88, 89} are relatively under-investigated. However, given the multitude of TFs and
6 coregulators, many have not been investigated at all in the context of PCa.

7 The Cancer Genome Atlas (TCGA) and other consortia^{13, 23, 74} established mutation, copy number
8 variation and gene/protein expression in hundreds of tumors from both early and advanced PCa
9 along-side clinical features and patient outcome data. These studies have revealed powerful insights
10 into PCa drivers, and offer up the possibility for secondary analyses focusing specifically on TFs and
11 coregulators. In the current study TCGA and other genome-wide data were used specifically to test
12 how TFs and their co-regulators are altered and associate with PCa outcome.

13 We utilized seven PCa cohorts; three cohorts of localized PCa (TCGA¹⁰, MSKCC⁸¹, OICR⁷⁴) and four
14 cohorts of advanced PCa (MICH⁴¹ FHCRC⁴¹, BELT⁷ SU2C^{1, 66}). In these cohorts we examined the
15 expression and genetic changes in different classes of coregulators and TFs, and within each class
16 identified the most significant and clinically-relevant gene changes. These approaches established
17 the class-specific significant mRNA and protein down-regulation of TFs and co-regulators in localized
18 PCa, which were not further altered in advanced PCa, and are not altered significantly by either
19 mutation or copy number alterations (CNA).

20 These approaches revealed that *PPARGC1A* (encodes PGC1 α) was commonly down-regulated
21 associated with worse disease outcome in local PCa. Stable knockdown of PGC1 α in LNCaP cells
22 increased proliferation, led to a more invasive phenotype, and profoundly changed gene expression
23 patterns both positively and negatively. Combining these data with differentially regulated genes

1 between tumors with high or low *PPARGC1A* expression alongside PGC1 α ChIP-Seq data identified
2 a network of 60 genes that are both significantly bound and regulated by PGC1 α , and significantly
3 associated with higher Gleason Grade tumors. In parallel, these approaches establish a relatively
4 generic work-flow for analyses of gene families or functional groupings to allow investigators to
5 identify clinically-significant relationships which in turn support functional analyses.

6

1 MATERIALS AND METHODS

2 **Data analyses, integration and code availability.** PCa data was downloaded from cBioPortal¹³.
 3 Analyses was undertaken using R (version 3.6.2)^{43 43 43}.

4 **Cell culture and materials.** LNCaP cells (ATCC) were confirmed to be mycoplasma free everyon
 5 month and authenticated using STR profiling. Cells were treated with 5,8,11,14-Eicosatetraynoic acid
 6 (ETYA, Calbiochem).

7 **Cell viability and Scratch Assays.** Cellular viability was measured by bioluminescent detection of
 8 ATP (CellTitre-Glo® assay kit (Promega)). Cells were plated at 5×10^3 cells per well in 96-well, white-
 9 walled plates, allowed to adhere and treated with ETYA (10 μ M) or EtOH (vehicle control) to a final
 10 volume of 100 μ l for 96 hr. Each experiment was performed in triplicate in triplicate wells. For scratch
 11 assay, cells were seeded at 1×10^6 cells per well in a 6-well plate, allowed to adhere for 48 hr to a
 12 confluence of about 80% and then wounded by scratching with p200 sterile pipette tip. The debris
 13 were removed, and cells washed to make ensure the edges were smoothed with the same
 14 dimensions for experimental and control cells. Cells were incubated, and cell migration was assessed
 15 by monolayer gap closure after 48 hr.

16 **Stable knockdown of PGC1 α in LNCaP Cells** PGC1 α (encoded by PPARGC1A) was knocked-
 17 down with shRNA constructs (TG310260, Origene) in LNCaP cells (LNCaP_shPGC1A) or scrambled
 18 shRNA (LNCaP_shCtrl). Two PGC1A targeting constructs (shPGC1A-34 and shPGC1A-35) were
 19 selected and maintained in media containing puromycin (0.2ug/ml).

20 **Western Immunoblotting** Total cellular protein was isolated from exponentially growing cells and
 21 lysed in ice cold RIPA buffer containing 1x cOmplete Mini Protease Inhibitor Tablets (Roche). Protein
 22 concentration were quantified and 75 ug resolved (SDS-PAGE) using 10% polyacrylamide gels,
 23 transferred to PVDF membrane and probed with primary antibody against PGC1 α (PA5-72948,

1 Invitrogen) and Beta-Actin (ab8229, abcam) overnight at 4°C. Primary antibody was detected with
2 HRP-linked goat anti-rabbit IgG (abcam) and signal captured (ChemiDoc XRS+ system (Bio-Rad)).

3 **Unique lists of annotated transcription factors and co-regulators.** A comprehensive list of TFs
4 and co-regulators genes was developed by text-mining⁵⁴ Gene Ontology (GO) terms that contained
5 phrases including “positive control of transcription”, “negative control of transcription”, “co-activator”,
6 “co-repressor”. From these GO terms, the HGNC gene name and ENSEMBL transcript ids were
7 retrieved using biomaRt¹⁹ and combined with canonical lists of TFs from UniProt (n= 1994) and
8 FANTOM⁶⁷ (n = 1988).

9 Groups were cross-referenced for uniqueness and annotated as following. TFs (n = 2662);
10 coactivators were genes that exclusively associated with positive regulation of transcription (COA; n=
11 766); corepressors were genes that exclusively associated with negative regulation of transcription
12 (COR; n = 599); mixed function coregulators were genes with evidence of context dependent
13 negative and positive regulation of gene expression (MIXED; n = 511) (**Supplementary Table 1**).

14 **Testing family-wide changes in each gene category.** Bootstrapping permutation approaches
15 (boot), were used to test if the proportions of each gene group was altered more than predicted by
16 chance. For mRNA and protein expression, Z-scores were calculated for gene expression data. In
17 the cohorts of local tumor (MSKCC, TCGA) gene expression was calculated as Z-scores of tumor-
18 normal comparisons with genes detectable in at least 80% of samples. In the other data sets the
19 median expression data frame of RNA-Seq or Mass-Spec was converted to Z-scores. For copy
20 number alterations (CNA) (via the GISTIC 2 method⁵³) and mutations the data were used as obtained
21 from cBioPortal¹³. To establish mutation frequency, the coding length of all exons for a given gene
22 (including all alternative exons; BioMart) was calculated to yield the total gene CDS length. The

1 mutation frequency was then calculated as the number of gene-length normalized mutations per gene
2 and the square root of the summed mutation rate to control for the number of tumors in a cohort.
3 To test whether observed alteration frequencies within gene classes (e.g. COA, COR) was altered
4 significantly, a vector of changes for all genes was calculated and the observed values (e.g. mean Z-
5 score of expression) of a given class tested compared to all genes detected in each cancer cohort.
6 Random sampling method was applied 100,000 times to select gene sets of equivalent size to
7 simulate the distribution of changes across the genome for comparison²². Empirical p-values were
8 calculated based on the group position relative to the sampling distribution of the genome.

9 **Testing relationships between the most significantly altered transcription factor and co-**
10 **regulators and clinical outcome.** Gene expression levels in each gene family were filtered
11 (`genefilter`) to select for genes that were commonly and significantly altered e.g. 2 Zscores in x %
12 of tumors (figure legends). The expression and clustering of genes were then visualized with
13 `pheatmap`, and the intersections visualized with `UpSetR`¹⁷. The association of patient cluster
14 membership and clinical outcome (either categorical data or continuous data that was categorized)
15 was then tested using a Chi-squared test and Kaplan-Meier curves generated for individual genes
16 (`survival`)³⁶.

17 **Analyses of the PGC1 α -dependent transcriptome and cistrome in cells and TCGA cohorts.**
18 LNCaP_shPGC1A and LNCaP_shCtrl cells (1x10⁶ cells per well in a 6-well plate) adhered for 24 hr
19 then dosed with ETYA (10 μ M) or EtOH (vehicle control) and RNA extracted. All the samples were
20 prepared in triplicates for RNA sequencing. Paired end sequence reads were aligned to the
21 human genome (hg38) using `Rsubread`⁴⁶, (> 90% of ~ 25x10⁶ unique reads/sample mapped)
22 and translated to expression counts via `featurecounts`, followed by a standard `edgeR`
23 pipeline⁶⁷ to determine differentially expressed genes (DEGs), visualized with volcano plots

1 (ggplot2) and interpreted by gene set enrichment⁷⁷ and in particular the enrichment in the
 2 Hallmarks, Curated, GO and Reactome sets was analyzed.

3 Similarly, in the TCGA PRAD cohort tumors in the upper and lower quartile by PPARGC1A
 4 expression were identified and DEGs established. These DEGs were combined with publicly
 5 available PGC1 α ChIP-Seq data¹⁴, and the enriched regions overlapped with PGC1 α -
 6 dependent DEGs as indicated.

7

8

9

1 RESULTS

2 **Transcription factor and coregulator groups are significantly down-regulated in localized**
3 **prostate cancer cohorts** We generated unique gene lists of TFs (n = 2662); coactivators, (COA; n=
4 766); corepressors, (COR; n = 599); and mixed function coregulators, (MIXED; n = 511)
5 (**Supplementary Table 1**). These groups were used to test the family-wide significance of changes in
6 expression (mRNA or protein), CNA and mutation of each group in three local cancer (MSKCC,
7 PRAD, OICR) and four advanced (MICH, FH, NEURO and SU2C) cancer cohorts (**Table 1**).

8 Hypergeometric tests revealed that COAS, CORS and MIXED genes, but not TFs, were significantly
9 protected ($p < 0.01$) from loss of function structural variation in normal tissues (GNOMAD⁷⁰) and
10 significantly enriched in Pan-Cancer fitness genes⁵.

11 Next, a bootstrapping permutation approach was used to test if observed family-wide changes were
12 more than predicted by chance ⁴⁹. We calculated empirical p-values based on the position of the
13 family gene set (e.g. TF, COA etc) relative to the distribution of random gene sets of the same size.
14 The heatmap in **Supplementary Figure 1A** shows CORS expression in the TCGA cohort, indicating
15 the genes are commonly down-regulated. To test CORS down-regulation we identified the proportion
16 of these genes altered by > 2 Z-scores (vertical dashed red line, **Supplementary Figure 1A, right**
17 **panel**) and compared this to the proportion of all gene families down-regulated in sets of the same
18 size (green histogram); a similar analyses was applied to the up-regulated genes (red histogram).
19 The observed proportion of CORS down-regulated by > 2 Z-scores was significantly more (and
20 hence, to the right) than the estimated proportion of down-regulated groups of the same size and
21 randomly sampled ($p=1e^{-05}$). The CNA data and normalized exome mutation rate were treated in the
22 same manner. Positive controls included gene groups known to be significantly altered by expression

(nuclear hormone receptor (NR) down-regulation; HOX family (HOX)⁴⁹ up-regulation), mutation (Cosmic_mutant) (**Supplementary Figure 1B**) or CNA (COSMIC_CNA) (data not shown)⁸⁰.

To visualize significant results we plotted the negative log₁₀ of the FDR-corrected empirical p-values for each test across cohorts (**Figure 1**). The mRNA expression in MSKCC and TCGA, and protein expression in OICR were significantly more down-regulated and/or less up-regulated. For example, CORS were significantly more down-regulated (MSKCC, TCGA, FH) and less up-regulated (MSKCC, FH, OICR) than predicted by chance. Indeed, all groups were more down-regulated or less upregulated in at least 4 cohorts. Only two group/cohort tests revealed significant up-regulation; COR genes in MICH, and MIXED genes in OICR.

Therefore down-regulation of these groups was more common than predicted by chance in local tumors. The OICR cohort displayed significant protein down-regulation of TFs, although the protein detection of all genes is significantly reduced in number (**Table 1**) and therefore caution is needed to compare between patterns of RNA and protein expression. Nonetheless, the correlation between RNA (TCGA or SU2C) and protein (OICR) when considering RNA transcripts for detected proteins ranged from ρ 0.4 to 0.55 (**Supplementary Figure 2**).

By contrast to the findings on RNA and protein expression, the mutation and CNA data were largely negative; COAS and MIXED genes were more mutated than predicted by chance in the FH cohort only (**Supplementary Figure 1C**). These findings suggest that changing the stoichiometry of TF and coregulator interactions is potentially more impactful in local tumors than disruption through either mutation or CNA.

Reduced expression of transcription factors and coregulators associates with more aggressive PCa. Next, from each of the 15 significant RNA expression-cohort relationships we identified those genes with the most frequent and greatest expression change. For example, COAS in

the TCGA cohort, were filtered for genes altered by > 2 Z-scores in 35% of TCGA tumors. The filtered RNA (**Figure 2**) and protein expression (**Supplementary Figure 3**) were visualized as heatmaps. Shared targets between the TCGA and MSKCC cohorts were investigated further (**Figure 3A**). PubMed analyses (search terms given in Table Legend) revealed an uneven representation of these genes in the context of PCa (**Table 2**). These genes included those known to be commonly altered, including *ERG*, a frequently up-regulated a TF in PCa⁶¹ and common target of translocations with *TMPRSS2*^{69, 85}. By contrast, to date, the COA, PDLIM1 has not been investigated in the context of PCa, and others such as *PPARGC1A* have only been modestly investigated^{20, 38, 73, 83, 88, 90, 98}.

Next, we examined how expression related to PCa progression by generating Kaplan Meier estimates of the time to biochemical progression (**Table 3**). In both TCGA and MSKCC cohorts, *PPARGC1A* was the only gene to be commonly and significantly down-regulated, and significantly associated with reduced time to biochemical progression (**Figure 3B**).

Knockdown of PGC1 α increases proliferation and alters a large transcriptome. We generated stable PGC1 α knockdown clones in LNCaP cells and examined the cell phenotype, and basal and PPAR γ ligand ETYA transcriptome^{4, 91}. (**Figure 4A**). Reduced PGC1 α expression increased the basal cell proliferation rate compared to vector controls, but not the anti-proliferative effect of ETYA (**Figure 4B**). Reduced PGC1 α also increased the invasiveness of the cells as measured by a scratch assay (**Figure 4C**).

RNA-Seq revealed the impact of reduced PGC1 α expression in LNCaP cells had a profound impact on gene expression, reflecting the significant impact on proliferation the knockdown of PGC1 α (**Figure 4D, E**). These changes included 37 microRNA, 243 intra.microRNA, 343 lncRNA and 3600 protein coding genes significantly altered. By contrast the impact of ETYA was very modest. This

1 suggests that PGC1 α regulation of ligand-activated PPAR γ does not appear to be significant, at least
2 with respect to the impact of ETYA exposure (**Figure 4D**).

3 Supporting a coactivator function, PGC1 α knockdown down-regulated more than up-regulated genes.
4 For example, 30 miRNA were down-regulated (e.g. miR17 host gene) and only 7 up-regulated, and
5 similarly 1993 protein-coding genes were down-regulated and 1607 up-regulated. The fold change
6 was also skewed to down-regulation, with the mean logFC for down-regulated protein-coding genes
7 being -1.11, and 0.83 for up-regulated genes and similarly the mean logFC for down-regulated
8 lncRNA was -1.20, and 0.87 for up-regulated genes (**Figure 4D**).

9 We undertook GSEA analyses to classify the PGC1 α -dependent changes in gene expression ⁷⁶.
10 **Supplementary Figure 4** illustrates the top eight positive and negative significant (FDR < .1)
11 normalized enrichment scores (NES) in four gene set categories; Hallmarks, Curated, GO and
12 Reactome sets. These highly enriched gene patterns illuminate the cancer biology impact of reduced
13 PGC1 α expression. The NES plots and expression of the altered genes in each term is shown in
14 **Figure 4E**.

15 Analyses of the Hallmark gene sets revealed androgen response genes were negatively and
16 peroxisome genes positively enriched, respectively, suggesting that PGC1 α expression represses AR
17 signaling and enhances the activity of the peroxisome. AR target genes notably associated with
18 energy production were repressed, including the putative tumor suppressor hydroxyprostaglandin
19 dehydrogenase (*HPGD*)²⁹, as well as Acyl-CoA synthetase long chain family member 3 (*ACSL3*)⁵⁵
20 and Alpha-2-glycoprotein 1, zinc-binding (*AZGP1*)¹². Up-regulated peroxisome genes included
21 retinoic acid anabolizing enzyme, Dehydrogenase/Reductase 3 (*DHRS3*)⁴⁸, and the steroid
22 catabolizing enzyme UDP Glucuronosyltransferase Family 2 Member B17 (*UGT2B17*)⁹⁹. These

changes in hallmark gene sets suggests that altered PGC1 α disrupts the energetic utilization of the cell and distorts AR signaling.

This was also borne out by changes in Curated gene sets, with repression of Interferon signaling genes including retinoic acid inducible DExD/H-Box Helicase 58 (*DDX58*)¹⁶, and upregulation of IGF-signaling gene targets including the AR-target genes Homocysteine Inducible ER Protein With Ubiquitin Like Domain 1 (*HERPUD1*)⁷¹ and Growth Differentiation Factor 15 (*GDF15*), known to be a prostate mitogen⁹⁴. Similarly, Reactome gene set enrichment revealed repression of Interferon regulated genes including Interferon Induced Protein With Tetratricopeptide Repeats 1 (*IFIT1*)⁶², which is actually repressed by PPAR α ³¹. Interestingly, this was accompanied by upregulation of a number of histone modifying enzymes including lysine demethylases³¹ (*KDM1A*, *KDM4A*, *KDM6A*) suggesting a potential for significant changes in the methylation status of histone lysine modifications such as H3K4me3 and H3K27me3, and thereby impacting the epigenome.

Genes bound and regulated by PGC1 α associate with more aggressive PCa. Reduced PGC1 α expression impacted gene expression positively and negatively. Whether PGC1 α exerts both coactivator and corepressor functions is obscured by direct (*cis*) and indirect (*trans*) relationships between PGC1 α and the regulated genes captured by RNA-Seq. To identify PGC1 α *cis*-dependent gene expression we examined how genes bound by PGC1 α binding relate to changes in gene expression.

PGC1 α ChIP-Seq data¹⁴ were combined with the PGC1 α -dependent gene expression patterns and shared genes were identified. Given each protein-coding genes is regulated by multiple enhancers⁶⁵,⁸⁴ and promoter-capture Hi-C experiments revealed the median distance between enhancer and target gene is 158 kb³². Therefore PGC1 α ChIP-Seq peaks were segregated into different chromatin states identified in LNCaP cells (e.g. Promoter, Active Enhancer)⁷⁸, and annotated to known genes

1 within 100 kb. Thus, the 1304 PGC1 α ChIP-Seq peaks overlapped with 914 chromatin states
2 identified in LNCaP, and annotated to 2381 peak:gene relationships.

3 In the first instance we examined how *PPARGC1A* correlated either to genes that were differentially
4 regulated by PGC1 α knockdown in LNCaP cells (n=4187, **Figure 4D**) and bound by PGC1 α
5 overlapping with LNCaP chromatin states (n=2381). We compared the relationships between PGC1 α
6 binding and gene expression in the TCGA cohort. In the first instance cumulative correlation plots
7 were generated, which revealed that the empirical correlation between the expression of *PPARGC1A*
8 and genes regulated by *PPARGC1A* shRNA were significantly more positive than the background of
9 all expressed genes (KS test; p<1e-9) (**Supplementary Figure 5**, green symbol). This suggests that
10 there is a stronger positive correlation between PGC1 α expression and PGC1 α -dependent genes
11 than predicted by chance, and fits with a model of coactivator function. By contrast, the correlation
12 between the expression of *PPARGC1A* and genes directly bound by PGC1 α were significantly more
13 negative than predicted by chance and suggested the correlations were significantly more negative
14 (KS test; p<1e-9). This suggests that direct binding of PGC1 α is more nuanced in its direct
15 relationship with gene levels, with a more even distribution of negative and positive correlations with
16 expression.

17 Next, these 2381 PGC1 α peak:gene relationships linked to LNCaP chromatin states were filtered to
18 include genes that were: 1. differentially regulated by *PPARGC1A* shRNA in LNCaP cells (n = 4187,
19 **Figure 4**); 2. differentially expressed in the TCGA cohort between the top and bottom quartile
20 expressing *PPARGC1A* expression (n = 7324). This identified 160 genes bound by PGC1 α and
21 significantly altered by PGC1 α knockdown in LNCaP cells and significantly altered *PPARGC1A*-
22 dependent expression in TCGA, with the most altered 63 genes in the TCGA cohort shown in **Figure**
23 **5A**. Expression genes clustered higher Gleason grade tumors (X-squared = 5.0601, p-value = 0.025).

1 Interestingly, amongst the PGC1 α -dependent gene signature 25 genes were upregulated, and 38
2 genes were actually downregulated, again suggesting that the direct impact of PGC1 α on gene
3 expression is more nuanced and not exclusively a coactivator. Finally, we screened how expression
4 of these 63 PGC1 α -dependent genes related to tumor outcome by generating Kaplan–Meier plots.
5 The top five significant genes were *FAM57B*, *EME2*, *DDB2*, *PCP4L1* and *PBXIP1* (**Supplementary**
6 **Table 2**) and the plots for an upregulated gene (*FAM57B*) and a downregulated gene (*PCP4L1*) are
7 shown in **Figure 5B**. Interestingly, *FAM57B* is an established PPAR γ target gene that regulates
8 adipocyte differentiation⁹⁷. Similarly, *PCP4L1* is related to obesity induced phenotypes³⁷ and *PBXIP1*
9 plays a role in regulation of differentiation⁵¹ in part by regulating ER α signaling⁹⁵. By contrast *EME2*
10 and *DDB2* relate to DNA repair^{52, 82}.

11

12

13

DISCUSSION

The current study was undertaken to identify the most significantly altered and clinically-relevant TF and co-regulators in PCa. It was reasoned that this knowledge may help to explain disease progression risks, or to highlight novel therapeutic opportunities. Assessment of family-wide significance across seven PCa cohorts revealed that TFs, COAS, CORs and MIXED were more down-regulated and less up-regulated at the mRNA and protein level, most clearly in local tumor cohorts. In contrast, family-wide CNA and mutation levels were not significant. Filtering those group/cohort relationships identified the most altered genes in the local tumor cohorts (MSKCC and TCGA). Overlapping identified commonly altered genes associated with PCa including *ERG*⁶¹ (which was also altered at the protein level in the OICR cohort) and *FGFR2*⁴⁴, but also identified others that were relatively under-investigated such as *PPARGC1A*^{11, 88, 89} but associated with worse disease free survival, or uninvestigated in PCa, such as the metastasis suppressor *PDLIM1*³⁰. Stable knockdown of PGC1 α increased proliferation and invasion of LNCaP cells, and profoundly altered the transcriptome. Finally, PGC1 α bound and regulated genes associated with higher grade tumors in the TCGA cohort and individual genes such as the PPAR γ target gene *FAM57B* associated with worse disease-free survival.

The global down-regulation of TFs and co-regulators suggests that an initial event in PCa is disrupting the flexibility of gene regulatory signaling that may limit the permutations of TF co-regulator interactions or lessen the ability of the TF and co-regulators to form effective stoichiometric interactions for correct functioning. This may suggest that although these genes are reduced in expression they are not altered by structural variation, and therefore they remain functional, and in turn this may support the disrupted stoichiometry argument. The current findings would suggest that globally the flexibility of TF actions is disrupted. This may suggest that signaling is more restricted in

1 terms of capacity, and this is seen in the PCa AR transcriptome^{27,29}, but also in signaling via MYC
2 and AP1 (reviewed in⁷³).

3 The stringent filtering of these data and overlap between TCGA and MSKCC revealed commonly and
4 significantly altered genes but are unevenly investigated in PCa (**Table 2**). Genes that are only
5 identified in one cohort and whose expression is associated with more aggressive disease (**Table 3**)
6 reveals a number of genes that are potentially impactful in disease but are under-investigated or have
7 not been investigated at all in the context of PCa. *PPARGC1A* was selected for mechanistic study,
8 given it was down-regulated and significantly associated with worse disease-free survival in both
9 MSKCC and TCGA cohorts. Knockdown of PGC1 α in LNCaP cells increased proliferation and
10 migration, coupled with a profound impact on the transcriptome. The PGC1 α transcriptome was
11 enriched for terms associated with AR signaling as well overlaps with regulation of the peroxisome,
12 interferon signaling and epigenetic modifiers. These findings were further supported when
13 considering target genes that were bound by PGC1 α and differentially expressed in the TCGA cohort.
14 These genes distinguished aggressive tumors and included RAR γ /TACC1⁵⁰, which we have
15 previously established to act to antagonize AR signaling, as well as genes associated PPAR γ
16 signaling. Furthermore, motif analyses revealed that basic helix-loop-helix (b-HLH) motifs were
17 enriched in the PGC1 α -ChromHMM sites, as were sites for LXR binding. These findings suggest that
18 PGC1 α regulates signaling by a cohort of nuclear receptors including AR, RAR γ , PPAR γ as well
19 epigenetic process and interferon signaling.

20 Earlier studies by Carracedo and colleagues identified reduced expression of PGC1 α in PCa, and
21 these workers pursued a strategy to over-express PGC1 α in advanced PCa cell (PC-3) cells line and
22 analyzed these effects in two impactful studies^{88, 89}. Their findings support a role for PGC1 α to
23 regulate a transcriptome that is regulated by PGC1 α and ERR α cross-talk, which regulates MYC

1 signaling to regulate invasiveness; indeed in the current study b-HLH motifs were identified in the
 2 PGC1 α -binding sites identified in LNCaP cells. The PGC1 α -dependent transcriptome in PC-3 cells is
 3 ~ 900 genes (GSE75193) and few (n=119) appear to be shared with the RNA-Seq data generated in
 4 the current study, but none of the genes proposed by the authors as a PGC1 α -dependent signature
 5 overlap with the 63 PGC1 α -dependent and PGC1 α -bound genes significantly altered in TCGA cohort
 6 associated with more aggressive disease.

7 Thus, whilst the current study and the Carracedo studies both identify important roles for PGC1 α to
 8 regulate tumor aggressiveness, the mechanisms appear to be different. This may arise for technical
 9 reasons (for example, cell background and transcriptomic approach) and may reflect the differential
 10 impact of knockdown versus over-expression selecting for different transcriptionally-dependent
 11 events. Certainly, the fact that PGC1 α -dependent and PGC1 α -bound genes favor down-regulated
 12 over up-regulated genes (~ 2:1, **Supplementary Table 3**) suggests that PGC1 α participates in
 13 diverse transcriptional events. Outside of PCa, in renal disease it has been proposed that
 14 PGC1 α determines phenotypic consequences by selecting which TFs to interact with, and that
 15 RARs/RXRs, PPARs is associated with fatty acid metabolism⁴⁵. It is tempting to speculate the
 16 phenotype is current study is associated with similar energetic changes.

17 A final comment concerns the scale of the analyses and the relatively generic approach. This
 18 approach considers all genes in a class or superfamily to identify which may have merit to investigate.
 19 This is an important step for many wet-lab approaches, given that it can often take considerable
 20 resources to dissect a gene function. For example, the 20,000 ft view approach to cancer genomics in
 21 PCa integrates genome-wide data to identify novel networks^{18,58}. Traditionally trained wet-lab based
 22 investigators often face challenges in assimilating these findings and may rather search for gene(s) in
 23 the supplementary data that are from the family on which they study; this can be considered as a 200

1 ft view. The current approach is neither the 200 ft nor 20,000 ft views, but rather a 2,000 ft view. That
2 is, the approach allows an investigator to remain focused in their research arena, for example TF co-
3 regulators, but ask the global question over how these are altered and at what stage of cancer
4 progression. This is a relatively generic approach and may find traction with investigators across
5 disease types.

6

7

1 Figure legends

2 **Figure 1. Family-wide analyses of transcription factors (TFs), coactivators (COAS),**
3 **corepressors (CORS) and mixed function coregulators (MIXED) in PCa cohorts.** The mean rank
4 of the genes in each family were compared to the distribution of ranking of the same number of genes
5 randomly sampled 100,000 times. The empirical p-values were -log10 transformed and FDR
6 corrected for mRNA expression (A) and proteins (B).

7 **Figure 2. Expression of transcription factors (TFs), coactivators (COAS), corepressors**
8 **(CORS) and mixed function coregulators (MIXED) in PCa cohorts.** Genes in each group were
9 filtered (`genefilter`; >2.5 Z scores in 35% tumors) to reveal the most frequently and strongly
10 altered and genes and tumors (columns) were visualized (`pheatmap`).

11 **Figure 3. Identification of commonly altered transcription factors (TFs), coactivators (COAS),**
12 **corepressors (CORS) and mixed function coregulators (MIXED) in the MSKCC and TCGA**
13 **cohorts. A.** Genes commonly altered in both cohorts were identified and visualized (`UpsetR`). **B.**
14 Kaplan–Meier plots of the relationship between tumors with lower and upper quartile *PPARGC1A*
15 expression and time to biochemical progression.

16 **Figure 4. Stable knockdown of PGC1 α in LNCaP cells changes proliferation and gene**
17 **expression patterns. A.** LNCaP cells were each stably transfected with two shRNA constructs (sh-
18 PGC1A 34 and 35) targeting *PPARGC1A* resulting in reduced PGC1 α expression (sh-PGC1A)
19 compared to empty vector (sh-CTRL) at protein level as detected in western blotting. **B.**
20 Measurements of cellular levels of ATP, as an indicator of cell viability was detected in the vector
21 controls and knockdown cells. Each measurement was performed in biological triplicates and in
22 triplicate wells. Cells were treated in triplicate with exogenous PGC1 α ligand ETYA (10 μ M, 96hr) or
23 EtOH vehicle control. Increased cell proliferation was seen in sh_PGC1A cells after treatment with

1 ETYA at 96 hr. **C.** Time course scratch closure of sh-Control and sh-PPARGC1A cells mechanically
2 wounded with p200 sterile pipette tip, sh-PPARGC1A after 48 hr showed increased cell migration
3 compared to sh-Control. **D.** LNCaP sh-PGC1A and sh-CTRL cells were treated with ETYA (10 μ M,
4 24hr) or EtOH vehicle control and total RNA expression and RNA-Seq undertaken according to the
5 edgeR pipeline. Volcano plots depicting expression changes upon PGC1 α knockdown or in response
6 ETYA in the indicated classes of RNA in color ($-\log_{10}(p.adj) > 1$, $abs(\log_2(\text{fold change}))$). **E.**
7 Summary of significantly enriched pathways from gene set enrichment analyses (GSEA) (FDR $q.val <$
8 0.05) associated with reducing PGC1 α expression levels.

9 **Figure 5. Expression of PGC1 α -regulated and PGC1 α -bound genes in the TCGA cohort. A.**
10 PGC1 α ChIP-Seq data (GSE75193) were annotated to genes within 100 kb, to yield PGC1 α
11 peak:gene relationships. These were then filtered to include only those that overlapped by 1 bp with
12 chromatin states in LNCaP derived using ChromHMM to yield PGC1 α peak:gene relationships. Thus,
13 the 1304 PGC1 α ChIP-Seq peaks overlapped with 914 specific LNCaP chromatin states and
14 annotated to 2381 peak:gene relationships. In turn these further filtered to include only those genes
15 that were: 1. differentially regulated by PPARGC1A shRNA in LNCaP cells ($n = 4187$, **Figure 4**); 2.
16 differentially expressed in the TCGA cohort between the top and bottom quartile expressing
17 PPARGC1A expression ($n = 7324$), resulting in 160 genes that were significantly bound by PGC1 α
18 and whose expression was significantly altered by both shRNA PPARGC1A in LNCaP cells and
19 significantly altered in TCGA tumors in PPARGC1A-dependent manner. The top 63 genes are
20 illustrated (heatmap) and their expression patterns clustered higher Gleason grade tumors (X-
21 squared = 5.0601, $p\text{-value} = 0.025$). **B.** Kaplan–Meier plots of the relationship between tumors with
22 lower and upper quartile expression of the indicated genes and time to biochemical progression.

23

1

2

1
2
3
4
5
6

Table 1: Summary of the prostate cancer cohorts. The number of tumors and the number for genes detected from each category in the unique gene lists (TFs (n = 2662); coactivator (COA; n= 766); corepressor (COR; n = 599); mixed function coregulator (MIXED; n = 511).

Cohort	TumorType	Material	Tumors	COAS	CORS	MIXED	TFs
MSKCC	Local	RNA, CNA	152	696	439	457	1506
TCGA	Local	RNA, CNA, Exome muts	498	663	415	414	1305
OICR	Local	Protein	76	320	185	156	295
FH	Metastatic	RNA, CNA, Exome muts	148	712	445	465	1384
MICH	Metastatic	RNA, CNA, Exome muts	95	276	179	169	740
SU2C	Metastatic	RNA, CNA, Exome muts	271	543	343	384	1122
NEURO	Metastatic	RNA, CNA, Exome muts	51	725	466	485	1518

1 **Table 2: Number of publications addressing the top altered genes in both the TCGA and**
2 **MSKCC cohorts.** The genes were mined in PubMed using the following search term (prostate
3 cancer[Title/Abstract]) AND *gene name*[Title/Abstract])

SharedGenes	Group	Publications
ERG	TFs	1057
HOXD13	TFs	20
ID4	TFs	0
L3MBTL4	TFs	3
ZNF154	TFs	0
ZNF655	TFs	0
PDLIM1	COA	0
PPARGC1A	COA	7
ROR2	COA	4
SMARCD3	COA	0
DKK3	COR	13
FHL2	COR	16
FGFR2	MIXED	45

4
5
6
7

Table 3. Relationships between altered expression and time to disease progression. Kaplan–Meier plots of the relationship between tumors with lower and upper quartile expression of the indicated gene and time to biochemical progression were generated and the FDR-corrected pvalues indicated.

Gene	Group	TCGA.Adj.Pval	MSKCC.Adj.Pval
KAT2A	COAS	0.001	NS
PPARGC1A	COAS	0.057	0.061
NTF3	COAS	0.057	NS
MED21	COAS	0.057	NS
ELF1	TFs	0.059	NS
ZNF655	TFs	NS	0.034
LMO4	COAS	NS	0.034
MAML2	COAS	NS	0.034
HOXD11	TFs	NS	0.034
MYOCD	TFs	NS	0.034
TSHZ3	TFs	NS	0.034
SNAI2	CORS	NS	0.047
HOXD10	TFs	NS	0.047
MEIS2	TFs	NS	0.049
FHL2	CORS	NS	0.061
FGFR2	MIXED	NS	0.061
TRIM29	CORS	NS	0.061
CAV1	MIXED	NS	0.061
CCND2	TFs	NS	0.061
ID4	TFs	NS	0.071
HOXC6	TFs	NS	0.071
SAFB2	TFs	NS	0.087
HOXD13	TFs	NS	0.094

- 1
- 2 1 Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I *et al.* Genomic correlates of clinical
3 outcome in advanced prostate cancer. Proc Natl Acad Sci U S A 2019; 116: 11428-11436.
4
- 5 2 Armenia J, Wankowicz SAM, Liu D, Gao J, Kundra R, Reznik E *et al.* The long tail of oncogenic
6 drivers in prostate cancer. Nat Genet 2018; 50: 645-651.
7
- 8 3 Barfeld SJ, Urbanucci A, Itkonen HM, Fazli L, Hicks JL, Thiede B *et al.* c-Myc Antagonises the
9 Transcriptional Activity of the Androgen Receptor in Prostate Cancer Affecting Key Gene Networks.
10 EBioMedicine 2017; 18: 83-93.
11
- 12 4 Battaglia S, Maguire O, Thorne JL, Hornung LB, Doig CL, Liu S *et al.* Elevated NCOR1 disrupts
13 PPARalpha/gamma signaling in prostate cancer and forms a targetable epigenetic lesion. Carcinogenesis
14 2010; 31: 1650-1660.
15
- 16 5 Behan FM, Iorio F, Picco G, Goncalves E, Beaver CM, Migliardi G *et al.* Prioritization of cancer
17 therapeutic targets using CRISPR-Cas9 screens. Nature 2019; 568: 511-516.
18
- 19 6 Bell CC, Fennell KA, Chan YC, Rambow F, Yeung MM, Vassiliadis D *et al.* Targeting enhancer
20 switching overcomes non-genetic drug resistance in acute myeloid leukaemia. Nat Commun 2019; 10:
21 2723.
22
- 23 7 Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J *et al.* Divergent clonal evolution of
24 castration-resistant neuroendocrine prostate cancer. Nat Med 2016; 22: 298-305.

1

2 8 Beltran H, Hruszkewycz A, Scher HI, Hildesheim J, Isaacs J, Yu EY *et al.* The role of lineage plasticity

3 in prostate cancer therapy resistance. Clin Cancer Res 2019.

4

5 9 Blessing AM, Ganesan S, Rajapakshe K, Ying Sung Y, Reddy Bollu L, Shi Y *et al.* Identification of a

6 Novel Coregulator, SH3YL1, That Interacts With the Androgen Receptor N-Terminus. Mol Endocrinol

7 2015; 29: 1426-1439.

8

9 10 Cancer Genome Atlas Research N. The Molecular Taxonomy of Primary Prostate Cancer. Cell 2015;

10 163: 1011-1025.

11

12 11 Canto P, Granados JB, Feria-Bernal G, Coral-Vazquez RM, Garcia-Garcia E, Tejeda ME *et al.*

13 PPARGC1A and ADIPOQ polymorphisms are associated with aggressive prostate cancer in Mexican-

14 Mestizo men with overweight or obesity. Cancer Biomark 2017; 19: 297-303.

15

16 12 Cao R, Ke M, Wu Q, Tian Q, Liu L, Dai Z *et al.* AZGP1 is androgen responsive and involved in AR-

17 induced prostate cancer cell proliferation and metastasis. J Cell Physiol 2019; 234: 17444-17458.

18

19 13 Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA *et al.* The cBio cancer genomics portal:

20 an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012; 2: 401-

21 404.

22

- 14 Charos AE, Reed BD, Raha D, Szekely AM, Weissman SM, Snyder M. A highly integrated and complex PPARGC1A transcription factor binding network in HepG2 cells. *Genome Res* 2012; 22: 1668-1679.
- 15 Chen Z, Wu D, Thomas-Ahner JM, Lu C, Zhao P, Zhang Q *et al.* Diverse AR-V7 cistromes in castration-resistant prostate cancer are governed by HoxB13. *Proc Natl Acad Sci U S A* 2018; 115: 6810-6815.
- 16 Chowdhari S, Saini N. Gene expression profiling reveals the role of RIG1 like receptor signaling in p53 dependent apoptosis induced by PUVA in keratinocytes. *Cell Signal* 2016; 28: 25-33.
- 17 Conway JR, Lex A, Gehlenborg N. UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* 2017; 33: 2938-2940.
- 18 DeDiego ML, Nogales A, Martinez-Sobrido L, Topham DJ. Interferon-Induced Protein 44 Interacts with Cellular FK506-Binding Protein 5, Negatively Regulates Host Antiviral Responses, and Supports Virus Replication. *mBio* 2019; 10.
- 19 Durinck S, Spellman PT, Birney E, Huber W. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nat Protoc* 2009; 4: 1184-1191.
- 20 Dwyer MA, Joseph JD, Wade HE, Eaton ML, Kunder RS, Kazmin D *et al.* WNT11 expression is induced by estrogen-related receptor alpha and beta-catenin and acts in an autocrine manner to increase cancer cell migration. *Cancer Res* 2010; 70: 9298-9308.

1

2 21 Flavahan WA, Drier Y, Johnstone SE, Hemming ML, Tarjan DR, Hegazi E *et al.* Altered chromosomal
3 topology drives oncogenic programs in SDH-deficient GISTs. *Nature* 2019.

4

5 22 Franke J, Neumann MH. Bootstrapping neural networks. *Neural computation* 2000; 12: 1929-1949.

6

7 23 Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO *et al.* Integrative analysis of complex
8 cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013; 6: p11.

9

10 24 Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S *et al.* Bioconductor: open
11 software development for computational biology and bioinformatics. *Genome Biol* 2004; 5: R80.

12

13 25 Geybels MS, Wright JL, Bibikova M, Klotzle B, Fan JB, Zhao S *et al.* Epigenetic signature of Gleason
14 score and prostate cancer recurrence after radical prostatectomy. *Clin Epigenetics* 2016; 8: 97.

15

16 26 Gu H, Liu T, Cai X, Tong Y, Li Y, Wang C *et al.* Upregulated LMO1 in prostate cancer acts as a novel
17 coactivator of the androgen receptor. *Int J Oncol* 2015; 47: 2181-2187.

18

19 27 Henry GH, Malewska A, Joseph DB, Malladi VS, Lee J, Torrealba J *et al.* A Cellular Anatomy of the
20 Normal Adult Human Prostate and Prostatic Urethra. *Cell Rep* 2018; 25: 3530-3542 e3535.

21

22 28 Henzler C, Li Y, Yang R, McBride T, Ho Y, Sprenger C *et al.* Truncation and constitutive activation of
23 the androgen receptor by diverse genomic rearrangements in prostate cancer. *Nature communications*
24 2016; 7: 13668.

- 1
- 2 29 Huang X, Taeb S, Jahangiri S, Korpela E, Cadonic I, Yu N *et al.* miR-620 promotes tumor
3 radioresistance by targeting 15-hydroxyprostaglandin dehydrogenase (HPGD). *Oncotarget* 2015; 6:
4 22439-22451.
- 5
- 6 30 Huang Z, Zhou JK, Wang K, Chen H, Qin S, Liu J *et al.* PDLIM1 Inhibits Tumor Metastasis Through
7 Activating Hippo Signaling in Hepatocellular Carcinoma. *Hepatology* 2019.
- 8
- 9 31 Janssen AW, Betzel B, Stoopen G, Berends FJ, Janssen IM, Peijnenburg AA *et al.* The impact of
10 PPARalpha activation on whole genome gene expression in human precision cut liver slices. *BMC*
11 *Genomics* 2015; 16: 760.
- 12
- 13 32 Jung I, Schmitt A, Diao Y, Lee AJ, Liu T, Yang D *et al.* A compendium of promoter-centered long-
14 range chromatin interactions in the human genome. *Nat Genet* 2019; 51: 1442-1449.
- 15
- 16 33 Kang Z, Janne OA, Palvimo JJ. Coregulator recruitment and histone modifications in transcriptional
17 regulation by the androgen receptor. *Mol Endocrinol* 2004; 18: 2633-2648.
- 18
- 19 34 Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging
20 implications in tissue kinetics. *Br J Cancer* 1972; 26: 239-257.
- 21
- 22 35 Khanim FL, Gommersall LM, Wood VH, Smith KL, Montalvo L, O'Neill LP *et al.* Altered SMRT
23 levels disrupt vitamin D3 receptor signalling in prostate cancer cells. *Oncogene* 2004; 23: 6712-6725.
- 24

- 36 Kivela T, Grambsch PM. Evaluation of sampling strategies for modeling survival of uveal malignant melanoma. *Invest Ophthalmol Vis Sci* 2003; 44: 3288-3293.
- 37 Kluth O, Matzke D, Kamitz A, Jahnert M, Vogel H, Scherneck S *et al.* Identification of Four Mouse Diabetes Candidate Genes Altering beta-Cell Proliferation. *PLoS Genet* 2015; 11: e1005506.
- 38 Kresovich JK, Joyce BT, Gao T, Zheng Y, Zhang Z, Achenbach CJ *et al.* Promoter methylation of PGC1A and PGC1B predicts cancer incidence in a veteran cohort. *Epigenomics* 2018; 10: 733-743.
- 39 Krishnan AV, Zhao XY, Swami S, Brive L, Peehl DM, Ely KR *et al.* A glucocorticoid-responsive mutant androgen receptor exhibits unique ligand specificity: therapeutic implications for androgen-independent prostate cancer. *Endocrinology* 2002; 143: 1889-1900.
- 40 Ku SY, Rosario S, Wang Y, Mu P, Seshadri M, Goodrich ZW *et al.* Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. *Science* 2017; 355: 78-83.
- 41 Kumar A, Coleman I, Morrissey C, Zhang X, True LD, Gulati R *et al.* Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. *Nat Med* 2016; 22: 369-378.
- 42 Labbe DP, Sweeney CJ, Brown M, Galbo P, Rosario S, Wadosky KM *et al.* TOP2A and EZH2 Provide Early Detection of an Aggressive Prostate Cancer Subgroup. *Clin Cancer Res* 2017; 23: 7072-7083.

- 43 Leman ES, DeMiguel F, Gao AC, Getzenberg RH. Regulation of androgen and vitamin d receptors by
1,25-dihydroxyvitamin D3 in human prostate epithelial and stromal cells. *J Urol* 2003; 170: 235-240.
- 44 Leung HY, Mehta P, Gray LB, Collins AT, Robson CN, Neal DE. Keratinocyte growth factor
expression in hormone insensitive prostate cancer. *Oncogene* 1997; 15: 1115-1120.
- 45 Li SY, Susztak K. The Role of Peroxisome Proliferator-Activated Receptor gamma Coactivator 1alpha
(PGC-1alpha) in Kidney Disease. *Semin Nephrol* 2018; 38: 121-126.
- 46 Liao Y, Smyth GK, Shi W. The R package Rsubread is easier, faster, cheaper and better for alignment
and quantification of RNA sequencing reads. *Nucleic Acids Res* 2019; 47: e47.
- 47 Liu S, Kumari S, Hu Q, Senapati D, Venkadakrishnan VB, Wang D *et al.* A comprehensive analysis of
coregulator recruitment, androgen receptor function and gene expression in prostate cancer. *Elife* 2017;
6.
- 48 Lochbaum R, Schilpp C, Nonnenmacher L, Frick M, Dietl P, Wittekindt OH. Retinoic acid signalling
adjusts tight junction permeability in response to air-liquid interface conditions. *Cell Signal* 2020; 65:
109421.
- 49 Long MD, Campbell MJ. Pan-cancer analyses of the nuclear receptor superfamily. *Nucl Receptor Res*
2015; 2.

- 1 50 Long MD, Singh PK, Russell JR, Llimos G, Rosario S, Rizvi A *et al.* The miR-96 and RARgamma
2 signaling axis governs androgen signaling and prostate cancer progression. *Oncogene* 2019; 38: 421-
3 444.
4
- 5 51 Manavathi B, Lo D, Bugide S, Dey O, Imren S, Weiss MJ *et al.* Functional regulation of pre-B-cell
6 leukemia homeobox interacting protein 1 (PBXIP1/HPIP) in erythroid differentiation. *J Biol Chem*
7 2012; 287: 5600-5614.
8
- 9 52 Matsumoto S, Cavadini S, Bunker RD, Grand RS, Potenza A, Rabl J *et al.* DNA damage detection in
10 nucleosomes involves DNA register shifting. *Nature* 2019; 571: 79-84.
11
- 12 53 Mermel CH, Schumacher SE, Hill B, Meyerson ML, Beroukhi R, Getz G. GISTIC2.0 facilitates
13 sensitive and confident localization of the targets of focal somatic copy-number alteration in human
14 cancers. *Genome Biol* 2011; 12: R41.
15
- 16 54 Mi H, Muruganujan A, Ebert D, Huang X, Thomas PD. PANTHER version 14: more genomes, a new
17 PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic Acids Res* 2019; 47:
18 D419-D426.
19
- 20 55 Migita T, Takayama KI, Urano T, Obinata D, Ikeda K, Soga T *et al.* ACSL3 promotes intratumoral
21 steroidogenesis in prostate cancer cells. *Cancer Sci* 2017; 108: 2011-2021.
22

- 1 56 Nerlakanti N, Yao J, Nguyen DT, Patel AK, Eroshkin AM, Lawrence HR *et al.* Targeting the BRD4-
2 HOXB13 Coregulated Transcriptional Networks with Bromodomain-Kinase Inhibitors to Suppress
3 Metastatic Castration-Resistant Prostate Cancer. *Mol Cancer Ther* 2018; 17: 2796-2810.
4
- 5 57 Nickerson ML, Das S, Im KM, Turan S, Berndt SI, Li H *et al.* TET2 binds the androgen receptor and
6 loss is associated with prostate cancer. *Oncogene* 2017; 36: 2172-2183.
7
- 8 58 Niu Y, Yeh S, Miyamoto H, Li G, Altuwaijri S, Yuan J *et al.* Tissue prostate-specific antigen facilitates
9 refractory prostate tumor progression via enhancing ARA70-regulated androgen receptor
10 transactivation. *Cancer Res* 2008; 68: 7110-7119.
11
- 12 59 Paltoglou S, Das R, Townley SL, Hickey TE, Tarulli GA, Coutinho I *et al.* Novel Androgen Receptor
13 Coregulator GRHL2 Exerts Both Oncogenic and Antimetastatic Functions in Prostate Cancer. *Cancer*
14 *Res* 2017; 77: 3417-3430.
15
- 16 60 Patten DK, Corleone G, Gyorffy B, Perone Y, Slaven N, Barozzi I *et al.* Enhancer mapping uncovers
17 phenotypic heterogeneity and evolution in patients with luminal breast cancer. *Nat Med* 2018; 24: 1469-
18 1480.
19
- 20 61 Petrovics G, Liu A, Shaheduzzaman S, Furusato B, Sun C, Chen Y *et al.* Frequent overexpression of
21 ETS-related gene-1 (ERG1) in prostate cancer transcriptome. *Oncogene* 2005; 24: 3847-3852.
22

- 62 Pidugu VK, Wu MM, Yen AH, Pidugu HB, Chang KW, Liu CJ *et al.* IFIT1 and IFIT3 promote oral
squamous cell carcinoma metastasis and contribute to the anti-tumor effect of gefitinib via enhancing p-
EGFR recycling. *Oncogene* 2019; 38: 3232-3247.
- 63 Pomerantz MM, Li F, Takeda DY, Lenci R, Chonkar A, Chabot M *et al.* The androgen receptor
cistrome is extensively reprogrammed in human prostate tumorigenesis. *Nat Genet* 2015; 47: 1346-
1351.
- 64 Poukka H, Aarnisalo P, Santti H, Janne OA, Palvimo JJ. Coregulator small nuclear RING finger protein
(SNURF) enhances Sp1- and steroid receptor-mediated transcription by different mechanisms. *J Biol*
Chem 2000; 275: 571-579.
- 65 Roadmap Epigenomics C, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A *et al.* Integrative
analysis of 111 reference human epigenomes. *Nature* 2015; 518: 317-330.
- 66 Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM *et al.* Integrative clinical
genomics of advanced prostate cancer. *Cell* 2015; 161: 1215-1228.
- 67 Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression
analysis of digital gene expression data. *Bioinformatics* 2010; 26: 139-140.
- 68 Rotinen M, You S, Yang J, Coetzee SG, Reis-Sobreiro M, Huang WC *et al.* ONECUT2 is a targetable
master regulator of lethal prostate cancer that suppresses the androgen axis. *Nat Med* 2018; 24: 1887-
1898.

1

2 69 Sanda MG, Feng Z, Howard DH, Tomlins SA, Sokoll LJ, Chan DW *et al.* Association Between

3 Combined TMPRSS2:ERG and PCA3 RNA Urinary Testing and Detection of Aggressive Prostate

4 Cancer. JAMA Oncol 2017.

5

6 70 Scheps KG, Hasenahuer MA, Parisi G, Targovnik HM, Fornasari MS. Curating the gnomAD database:

7 Report of novel variants in the globin-coding genes and bioinformatics analysis. Hum Mutat 2020; 41:

8 81-102.

9

10 71 Segawa T, Nau ME, Xu LL, Chilukuri RN, Makarem M, Zhang W *et al.* Androgen-induced expression

11 of endoplasmic reticulum (ER) stress response genes in prostate cancer cells. Oncogene 2002; 21: 8749-

12 8758.

13

14 72 Sheahan AV, Ellis L. Epigenetic reprogramming: A key mechanism driving therapeutic resistance. Urol

15 Oncol 2018; 36: 375-379.

16

17 73 Shiota M, Yokomizo A, Tada Y, Inokuchi J, Tatsugami K, Kuroiwa K *et al.* Peroxisome proliferator-

18 activated receptor gamma coactivator-1alpha interacts with the androgen receptor (AR) and promotes

19 prostate cancer cell growth by activating the AR. Mol Endocrinol 2010; 24: 114-127.

20

21 74 Sinha A, Huang V, Livingstone J, Wang J, Fox NS, Kurganovs N *et al.* The Proteogenomic Landscape

22 of Curable Prostate Cancer. Cancer Cell 2019; 35: 414-427 e416.

23

- 1 75 Stelloo S, Nevodomskaia E, Kim Y, Schuurman K, Valle-Encinas E, Lobo J *et al.* Integrative epigenetic
2 taxonomy of primary prostate cancer. Nat Commun 2018; 9: 4900.
3
- 4 76 Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA *et al.* Gene set
5 enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles.
6 Proc Natl Acad Sci U S A 2005; 102: 15545-15550.
7
- 8 77 Subramanian A, Kuehn H, Gould J, Tamayo P, Mesirov JP. GSEA-P: a desktop application for Gene Set
9 Enrichment Analysis. Bioinformatics 2007; 23: 3251-3253.
10
- 11 78 Taberlay PC, Achinger-Kawecka J, Lun AT, Buske FA, Sabir K, Gould CM *et al.* Three-dimensional
12 disorganization of the cancer genome occurs coincident with long-range genetic and epigenetic
13 alterations. Genome Res 2016; 26: 719-731.
14
- 15 79 Takeda DY, Spisak S, Seo JH, Bell C, O'Connor E, Korthauer K *et al.* A Somaticallv Acquired
16 Enhancer of the Androgen Receptor Is a Noncoding Driver in Advanced Prostate Cancer. Cell 2018;
17 174: 422-432 e413.
18
- 19 80 Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N *et al.* COSMIC: the Catalogue Of
20 Somatic Mutations In Cancer. Nucleic Acids Res 2019; 47: D941-D947.
21
- 22 81 Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS *et al.* Integrative genomic
23 profiling of human prostate cancer. Cancer Cell 2010; 18: 11-22.
24

Techer H, Koundrioukoff S, Carignon S, Wilhelm T, Millot GA, Lopez BS *et al.* Signaling from Mus81-Eme2-Dependent DNA Damage Elicited by Chk1 Deficiency Modulates Replication Fork Speed and Origin Usage. *Cell Rep* 2016; 14: 1114-1127.

Tennakoon JB, Shi Y, Han JJ, Tsouko E, White MA, Burns AR *et al.* Androgens regulate prostate cancer cell growth via an AMPK-PGC-1alpha-mediated metabolic switch. *Oncogene* 2014; 33: 5251-5261.

Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E *et al.* The accessible chromatin landscape of the human genome. *Nature* 2012; 489: 75-82.

Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW *et al.* Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 2005; 310: 644-648.

Toropainen S, Malinen M, Kaikkonen S, Rytinki M, Jaaskelainen T, Sahu B *et al.* SUMO ligase PIAS1 functions as a target gene selective androgen receptor coregulator on prostate cancer cell chromatin. *Nucleic Acids Res* 2015; 43: 848-861.

Toropainen S, Niskanen EA, Malinen M, Sutinen P, Kaikkonen MU, Palvimo JJ. Global analysis of transcription in castration-resistant prostate cancer cells uncovers active enhancers and direct androgen receptor targets. *Sci Rep* 2016; 6: 33510.

1 88 Torrano V, Valcarcel-Jimenez L, Cortazar AR, Liu X, Urosevic J, Castillo-Martin M *et al.* The
2 metabolic co-regulator PGC1alpha suppresses prostate cancer metastasis. Nat Cell Biol 2016; 18: 645-
3 656.
4
5 89 Valcarcel-Jimenez L, Macchia A, Crosas-Molist E, Schaub-Clerigue A, Camacho L, Martin-Martin N *et*
6 *al.* PGC1alpha Suppresses Prostate Cancer Cell Invasion through ERRalpha Transcriptional Control.
7 Cancer Res 2019; 79: 6153-6165.
8
9 90 Valcarcel-Jimenez L, Macchia A, Crosas-Molist E, Schaub-Clerigue A, Camacho L, Martin-Martin N *et*
10 *al.* PGC1alpha suppresses prostate cancer cell invasion through ERRalpha transcriptional control.
11 Cancer Res 2019.
12
13 91 Vu-Dac N, Schoonjans K, Kosykh V, Dallongeville J, Fruchart JC, Staels B *et al.* Fibrates increase
14 human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated
15 receptor. J Clin Invest 1995; 96: 741-750.
16
17 92 Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J *et al.* Androgen receptor regulates a distinct transcription
18 program in androgen-independent prostate cancer. Cell 2009; 138: 245-256.
19
20 93 Wang T, Song W, Chen Y, Chen R, Liu Z, Wu L *et al.* Flightless I Homolog Represses Prostate Cancer
21 Progression through Targeting Androgen Receptor Signaling. Clin Cancer Res 2016; 22: 1531-1544.
22

- 94 Wang W, Yang X, Dai J, Lu Y, Zhang J, Keller ET. Prostate cancer promotes a vicious cycle of bone metastasis progression through inducing osteocytes to secrete GDF15 that stimulates prostate cancer growth and invasion. *Oncogene* 2019; 38: 4540-4559.
- 95 Wang X, Yang Z, Zhang H, Ding L, Li X, Zhu C *et al.* The estrogen receptor-interacting protein HPIP increases estrogen-responsive gene expression through activation of MAPK and AKT. *Biochim Biophys Acta* 2008; 1783: 1220-1228.
- 96 Wang Y, Jadhav RR, Liu J, Wilson D, Chen Y, Thompson IM *et al.* Roles of Distal and Genic Methylation in the Development of Prostate Tumorigenesis Revealed by Genome-wide DNA Methylation Analysis. *Sci Rep* 2016; 6: 22051.
- 97 Yamashita-Sugahara Y, Tokuzawa Y, Nakachi Y, Kanesaki-Yatsuka Y, Matsumoto M, Mizuno Y *et al.* Fam57b (family with sequence similarity 57, member B), a novel peroxisome proliferator-activated receptor gamma target gene that regulates adipogenesis through ceramide synthesis. *J Biol Chem* 2013; 288: 4522-4537.
- 98 Yu S, Wong YC, Wang XH, Ling MT, Ng CF, Chen S *et al.* Orphan nuclear receptor estrogen-related receptor-beta suppresses in vitro and in vivo growth of prostate cancer cells via p21(WAF1/CIP1) induction and as a potential therapeutic target in prostate cancer. *Oncogene* 2008; 27: 3313-3328.
- 99 Zhu Z, Chung YM, Sergeeva O, Kepe V, Berk M, Li J *et al.* Loss of dihydrotestosterone-inactivation activity promotes prostate cancer castration resistance detectable by functional imaging. *J Biol Chem* 2018; 293: 17829-17837.

1

2

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5