Title

Long non-coding RNA *MANCR* is a target of BET bromodomain protein BRD4 and plays a critical role in cellular migration and invasion abilities of prostate cancer

Authors

Masayuki Nagasawa [a, b, 1], Kosuke Tomimatsu [a, 1], Koji Terada [a], Kenta Kondo [a], Kazuko Miyazaki [c], Masaki Miyazaki [c], Daisuke Motooka [d], Daisuke Okuzaki [d], Tetsuya Yoshida [b], Susumu Kageyama [b], Hiroshi Kawamoto [c], Akihiro Kawauchi [b], Yasutoshi Agata [a, *]

[a] Department of Biochemistry and Molecular Biology, Shiga University of Medical Science, Shiga, Japan

[b] Department of Urology, Shiga University of Medical Science, Shiga, Japan

[c] Laboratory of Immunology, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

[d] Genome Information Research Center, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

[*]Correspondence: Yasutoshi Agata (yagata@belle.shiga-med.ac.jp)

[1] These authors contributed equally to this work.

ABSTRACT

Androgen receptor (AR)-negative castration-resistant prostate cancer (CRPC) is highly aggressive and is resistant to most of the current therapies. Bromodomain and extra terminal domain (BET) protein BRD4 binds to super-enhancers (SEs) that drive high expression of oncogenes in many cancers. A BET inhibitor, JQ1, has been found to suppress the malignant phenotypes of prostate cancer cells, however, the target genes of JQ1 remain largely unknown. Here we show that SE-associated genes specific for AR-negative CRPC PC3 cells include genes involved in migration and invasion, and that JQ1 impairs migration and invasion of PC3 cells. We identified a long non-coding RNA, *MANCR*, which was markedly down-regulated by JQ1, and found that BRD4 binds to the *MANCR* locus. *MANCR* knockdown led to a significant decrease in migration and invasion of PC3 cells. Furthermore, RNA sequencing analysis revealed that expression of the genes involved in migration and invasion was altered by *MANCR* knockdown. In summary, our data demonstrate that *MANCR* plays a critical role in migration and invasion of PC3 cells.

KEYWORDS

Prostate cancer; super-enhancer; BET protein inhibitor; lncRNA; *MANCR*; epithelial-mesenchymal transition

INTRODUCTION

Prostate cancer is the second most common cancer in men worldwide and is a major cause of cancer death [1]. For patients with metastatic prostate cancer expressing the androgen receptor (AR), androgen deprivation therapy is effective, however, most patients develop castration-resistant prostate cancer (CRPC). In particular, AR-negative CRPC is highly aggressive and resistant to most of the current therapies [2]. Therefore, it is desirable to develop a novel therapy for AR-negative CRPC.

Inhibitors of the bromodomain and extra terminal domain (BET) family proteins (BETi) are emerging as promising anti-cancer therapies for many types of cancer including prostate cancer [3][4]. BET bromodomain proteins (BRD2, 3, and 4) recognize acetylated histones and facilitate transcriptional activation [5][6]. In particular, BRD4 binds to super-enhancers (SEs), large clusters of enhancers that drive expression of genes that define cell identity and disease [7]. In diverse types of cancer, SEs are formed at critical oncogenes such as *MYC* and cause high expression of these oncogenes [7][8]. Because transcription of SE-associated genes is sensitively down-regulated by a BETi, JQ1 [8], this inhibitor has been used to suppress the malignant phenotypes of cancer cells. In particular for prostate cancers, recent studies have shown that JQ1 abrogates BRD4 localization to AR target loci and AR-mediated gene transcription [9], reduces cell

migration in multiple CRPC cell lines [10], and inhibits the expression of clinically relevant master regulator genes in CRPC [11]. Thus, several clinical trials for BETis have been initiated to determine a candidate for CRPC therapy [12].

Here we show that SE-associated genes that are specific to an AR-negative CRPC line, PC3 cells, include genes involved in the regulation of epithelial-mesenchymal transition (EMT). Indeed, EMT-related phenotypes, cell migration and invasion, of PC3 cells are inhibited by JQ1 treatment. We identified a long non-coding RNA (lncRNA), *MANCR*, which is sensitively down-regulated by JQ1, and found that *MANCR* is critical for migration and invasion of PC3 cells. Thus, our results imply that *MANCR* may serve as a potential therapeutic target for AR-negative CRPC.

MATERIALS AND METHODS

Cell culture and drugs

LNCaP clone FGC cells were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). PC3 cells were provided by the RIKEN BioResource Research Center (BRC) through the National BioResource Project of the Ministry of Ministry of Education, Culture, Sports, Science, and Technology/Japan Agency for Medical Research and Development (MEXT/AMED). LNCaP and PC3 cells were

cultured in RPMI-1640 medium (FUJIFILM Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (Life Technologies, Carlsbad, CA, USA), 100 units/mL penicillin, 100 μg/mL streptomycin, and 292 μg/mL L-glutamine (Life Technologies) at 37°C in a humid 5% CO_2 atmosphere. A BET inhibitor, (+)-JQ1 (ab141498) was purchased from Abcam (Cambridge, UK).

Migration and invasion assay

Migration and invasion assays were performed in Transwell chambers. Polycarbonate filters (8 μm pore size, Kurabo, Osaka, Japan) in the chamber were coated with 1 μg fibronectin (Sigma-Aldrich, MO, USA) for migration assays and with 100 μL 0.15 mg/mL Matrigel (Corning, NY, USA) for invasion assays. Cells were pre-treated with phenol red-free RPMI containing charcoal-stripped serum (Life Technologies) for 48 h followed by DMSO or JQ1 treatment. The cells in 100 μL RPMI with DMSO or JQ1 were seeded at a density of 5×10^4 cells/well in the upper chamber, and 680 μL RPMI supplemented with 10% FBS was placed in the lower chamber as a chemotactic agent. The plates were incubated with 5% CO_2 at 37°C for 8 h for migration assays and for 16 h for invasion assays. The cells that migrated and invaded to the opposite side of the

membrane were fixed and stained using Diff-Quik Kit (Sysmex, Kobe, Japan). Five random fields were captured to count the number of viable cells.

Wound healing assay

PC3 and LNCaP cells were cultured in six-well plates until 100% confluence was achieved. The monolayers were scratched in a straight line with a 200 μL pipette tip and washed with PBS. The wounded monolayers were cultured for 24 h under starvation conditions in serum-free medium.

siRNAs, plasmid, and transfection

MANCR siRNAs and negative control siRNA were purchased from Life Technologies. siRNA sequences against *MANCR* were as follows:

MANCR siRNA#1 5′-GCUUGCUCUCACAGCCAUUTT-3′

MANCR siRNA#2 5′-GGCUGAGUCUAAGUGUACATT-3′

MANCR siRNA#3 5′-CACUUAAUGUUAGACAUUATT-3′

MANCR siRNAs and negative control siRNA were transfected into PC3 cells using Lipofectamine RNAiMAX (Life Technologies) according to the manufacturer's instructions. After 48-h transfection, the cells were collected for further experiments.

RT-qPCR

Total RNA was isolated using TRIzol reagent (Life Technologies), and cDNA was synthesized using the reverse transcriptase Superscript III kit (Life Technologies). Then, RT-qPCR was performed with SYBR Green Master Mix (Takara, Shiga, Japan). Data were normalized to *GAPDH* expression. The primers were as follows: *MANCR* forward, 5′-TGCGTTCAGTAAAACGGGCA-3′ and reverse, 5'-TGTGGGAAATGCAGGGTTCT-3′; *GAPDH* forward, 5'-TGCACCACCAACTGCTTAGC-3′ and reverse, GGCATGGACTGTGGTCATGAG-3′.

Data analysis of external ChIP-seq and RNA-seq datasets

H3K27ac ChIP-seq and the input datasets of LNCaP (dihydrotestosterone: DHT untreated) and PC3 cells were downloaded from the Gene Expression Omnibus (GEO) GSE51621 and GSE57498, respectively. Reads were aligned to the human reference genome sequences (hg19) using Bowtie2 [13]. The TagDirectories were generated using HOMER software [14]. SEs were identified using the H3K27ac ChIP-seq reads by running HOMER's findPeaks command with "-style super" option. Pathway analysis was performed using NCI-Nature 2016 provided on the web-tool 'Enrichr' [15]. RNA-seq and

BRD4 ChIP-seq datasets for untreated or JQ1-treated PC3 cells were downloaded from GSE98069 and analyzed using the Galaxy platform [16]. The BRD4 ChIP-seq data were visualized on the IGV genome browser [17]. *MANCR* expression in several cancer cell lines were analyzed using Expression Atlas [18].

RNA-sequencing and data analysis

Biological triplicates of PC3 cells transfected with negative control siRNA and *MANCR* siRNA#1 were used for RNA-sequencing. Total RNA was isolated using TRIzol reagent from PC3 cells collected at 48 h after transfection. Library preparation was performed using a TruSeq stranded mRNA sample prep kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Sequencing was performed on an Illumina HiSeq 2500 platform in a 75-base single-end mode. Illumina Casava1.8.2 software was used for base calling. The sequenced reads were mapped to the human reference genome sequences (hg19) using TopHat version 2.0.13 in combination with Bowtie2 version 2.2.3 and SAMtools version 0.1.19. The fragments per kilobase of exon per million mapped fragments (FPKMs) were calculated using Cufflinks version 2.2.1. RNA-seq data are available at the Gene Expression Omnibus (GEO) with accession number GSE145081.

Statistical analyses

Statistical analyses were conducted using Graphpad Prism 8 (GraphPad Software, CA, USA). One-way ANOVA with Dunnett's multiple comparisons test was used for datasets with more than two conditions. Student's *t*-tests were used for two-condition comparisons. A *P*-value of 0.05 was used to indicate significance.

RESULTS

PC3-specific SE-associated genes include genes involved in cell migration and invasion

SEs are clusters of enhancers that drive high-level expression of genes that define cell identity. To investigate the differences in SE-associated genes between AR-dependent and -independent prostate cancer cell lines, we profiled SEs of androgen-dependent LNCaP and androgen-independent PC3 cells using public H3K27ac ChIP-seq datasets (GSE51621, GSE57498) [19][20]. We identified 1,078 and 929 SE-associated genes from LNCaP and PC3 cells, respectively (Fig. 1A). Although comparable numbers of SE-associated genes were obtained from LNCaP and PC3 cells, most genes did not overlap among the cells (Fig. 1B). We obtained 941 LNCaP-specific

and 792 PC3-specific SE-associated genes and performed a pathway analysis. The indicated terms from LNCaP cells are mainly related to AR activity and ErbB signaling. (Note that ErbB2 and ErbB3 genes are highly expressed in AR-positive cell lines [21].) In contrast, the terms from PC3 cells are related to EMT including cell migration and invasion ability of cancer cells such as HIF-1α transcription network, p38 signaling, SMAD2/3 signaling, and ATF2 transcription network [22][23] (Fig. 1C). These results suggest that androgen-dependent LNCaP and androgen-independent PC3 cells have a distinct SE-associated gene set that determines each cell identity, and that particularly in AR-negative PC3 cells, genes related to cell migration and invasion are potentially regulated by SEs.

A BETi, JQ1, decreases migration and invasion abilities of PC3 cells

To examine whether genes related to cell migration and invasion are under the regulation of SEs in PC3 cells, we used a BETi, JQ1, which blocks BRD4 binding to acetylated histones, thereby abrogating the formation of SEs. We first performed migration assays and found that JQ1 treatment significantly reduced migration of PC3 cells, but not LNCaP cells (Fig. 2A). Consistently, wound healing assays, which are used to measure cell migration activity, showed that wound healing was abrogated by JQ1 in

PC3 cells, while the wound healing activity was low in LNCaP cells with or without JQ1 (Fig. 2B). We next performed invasion assays using Matrigel-coated chambers and found that JQ1 significantly decreased the invasive ability of PC3 cells, but not LNCaP cells (Fig. 2C). These results suggest that genes involved in migration and invasion are regulated by SEs in PC3 cells.

PC3-specific SE-associated lncRNA *MANCR* is down-regulated by JQ1

To identify genes that are down-regulated by JQ1 treatment in PC3 cells, we analyzed the expression of 792 PC3-specific SE-associated genes shown in Figure 1B using the published RNA-seq dataset for PC3 cells treated with DMSO or 500 nM JQ1 (GSE98069) (Fig. 3A) [24]. We selected 58 genes that were considerably expressed in control cells (Log2 FPKM >1 on DMSO) and were down-regulated by JQ1 to less than half that of control cells (Log2 fold change <−1 on JQ1/DMSO). Among them, we focused on a long non-coding RNA, mitotically-associated lncRNA (*MANCR*), because it has been reported that *MANCR* is up-regulated in aggressive breast cancer and is involved in cell proliferation, viability, and genomic stability [25]. To validate the RNA-seq data, we performed RT-qPCR analysis for *MANCR* expression in PC3 cells and confirmed that *MANCR* expression was markedly suppressed by JQ1 (Fig. 3B). To

assess the binding profile of BRD4 in the *MANCR* locus, we analyzed published BRD4 ChIP-seq dataset for PC3 cells (GSE98069) [24] and found that BRD4 binding was detected in the *MANCR* locus and was clearly lost by JQ1 (Fig. 3C). These data indicate that *MANCR* is sensitively down-regulated by JQ1, and thus is under the regulation of SEs via BRD4 in PC3 cells.

To investigate *MANCR* expression in various cancer cell lines, we analyzed the Expression Atlas database [18] and found that *MANCR* is selectively expressed in AR-negative prostate cancer cell lines such as PC3 and DU145 cells [26] (Fig. 3D). Interestingly, among several breast cancer cell lines, *MANCR* is expressed in triple-negative breast cancer (TNBC) cell lines, MDA-MB-231, MDA-MB468, and CAL-85-1, which do not express estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 [27] (Fig. 3D). These data suggest that *MANCR* might be commonly up-regulated in hormone-independent cancer cells with more aggressive phenotypes.

MANCR **plays a critical role in migration and invasion of PC3 cells**

To investigate whether *MANCR* is functionally involved in aggressive phenotypes such as migration and invasion of PC3 cells, we sought to knock down *MANCR* using

three siRNAs targeting *MANCR* (siRNA#1, siRNA#2, and siRNA#3). RT-qPCR analysis showed that all of three siRNAs significantly reduced *MANCR* expression in PC3 cells when compared with control siRNA (Fig. 4A). *MANCR* knockdown led to a significant decrease in migration and invasion of PC3 cells (Fig. 4B-D). To further investigate the genes whose expression is altered by *MANCR* knockdown, we performed RNA-seq analysis using biological triplicates of PC3 cells transfected with control siRNA and *MANCR* siRNA#1. We selected 94 genes that were considerably expressed in control cells (Log2 FPKM >1 on Ctrl) and were down-regulated by *MANCR* knockdown to less than half that of control cells (Log2 fold change <−1 on *MANCR* knockdown/Ctrl) including *MANCR* itself (Fig. 4E). The selected gene set included several critical EMT-related genes such as Snail (*SNAII*) and vimentin (*VIM*). In marked contrast to these mesenchymal marker genes, epithelial marker genes such as E-cadherin (*CDH1*) and Claudin1 (*CLDN1*) were up-regulated by *MANCR* knockdown (Fig. 4F). Gene Ontology (GO) analysis also indicated these genes were enriched within terms as ‘Positive regulation of EMT’ and ‘Regulation of EMT’ (Fig. 4G). Consistent with a previous report [25], GO terms related to mitosis and cell cycle were also included, however, cell proliferation was not significantly inhibited by *MANCR* knockdown in PC3 cells (Fig. S1A). The function of *MANCR* was further investigated using

androgen-dependent LNCaP cells, which express very low levels of *MANCR* (Fig. S2A). We overexpressed *MANCR* in LNCaP cells at a similar expression level in PC3 cells (Fig. S2A). We found that expression of *MANCR* significantly increased migration and invasion of LNCaP cells (Fig. S2B and C). Collectively, these data indicate that *MANCR* has the ability to enhance EMT-related functions such as migration and invasion in prostate cancer cells.

DISCUSSION

BET protein inhibition exhibits anti-tumor activities in a wide range of cancers by suppressing the transcription of critical oncogenes driven by SEs. However, it remains to be elucidated as to which genes are down-regulated by BETi and are associated with the tumor phenotypes. In this study, we found that the genes involved in cell migration and invasion were selectively regulated by SEs in AR-negative CRPC PC3 cells. Indeed, in vitro assays confirmed that JQ1 treatment suppressed migration and invasion abilities of PC3 cells. In a further analysis, we identified the lncRNA *MANCR*, which was remarkably down-regulated by JQ1, and found that BRD4 binds to the *MANCR* locus. Additionally, *MANCR* knockdown reduced migration and invasion of PC3 cells. Consistent with these phenotypic alterations, RNA-seq analysis revealed that EMT-promoting genes were

down-regulated in *MANCR* knocked-down cells, and GO analysis indicated that 'positive regulation of EMT' was ranked among the top biological terms. Collectively, our data demonstrate that *MANCR* plays a critical role in cellular migration and invasion abilities of AR-negative CRPC PC3 cells.

MANCR was originally identified as a lncRNA that is up-regulated in aggressive breast cancer and is involved in cell proliferation, viability, and genomic stability [25]. Besides breast cancer, recent studies have reported that *MANCR* is also up-regulated and is involved in cell proliferation in thyroid cancer, gastric cancer, and mantle cell lymphoma (MCL) [28][29][30]. In the prostate cancer cell line PC3, we found that *MANCR* knockdown did not affect cell proliferation, however, *MANCR* is critical for cell migration and invasion. Consistent with our findings, it has been reported that *MANCR* overexpression had no significant effects on cell proliferation, but promoted migration and invasion of hepatocellular carcinoma (HCC) [31]. In thyroid cancer cells, *MANCR* depletion inhibited both cell proliferation and invasion [28]. Thus, *MANCR* may play distinct roles in tumorigenesis and cancer progression in different types of cancer [31].

Previous studies have shown that many lncRNAs serve as miRNA sponges to down-regulate tumor-suppressive miRNAs in cancer [32], suggesting that *MANCR* may also function as an miRNA sponge. In line with this notion, distinct miRNAs, *miR-218*,

miR-101, and *miR-122a*, have been proposed as targets of *MANCR* in MCL, gastric cancer, and HCC, respectively [29–31]. It is conceivable that certain types of miRNA may be specific targets of *MANCR* in prostate cancer. Further studies are required to clarify this possibility.

DECLARATION OF COMPETING INTERESTS

The authors have no conflict of interest to declare.

ACKNOWLEDGEMENTS

We thank Ms. N. Tanoue, N. Takano for technical assistance and all members of the Agata laboratory for helpful discussions. This work was supported by JSPS KAKENHI Grant Numbers JP18K07171, JP19K18557, and JP19K07664.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA. Cancer J. Clin. 68 (2018) 394–424. https://doi.org/10.3322/caac.21492.

[2] P.S. Nelson, Molecular states underlying androgen receptor activation: A framework for therapeutics targeting androgen signaling in prostate cancer, J. Clin. Oncol. 30 (2012) 644–646. https://doi.org/10.1200/JCO.2011.39.1300.

[3] J.E. Bradner, D. Hnisz, R.A. Young, Transcriptional Addiction in Cancer, Cell. 168 (2017) 629–643. https://doi.org/10.1016/j.cell.2016.12.013.

[4] A. Stathis, F. Bertoni, BET proteins as targets for anticancer treatment, Cancer Discov. 8 (2018) 24–36. https://doi.org/10.1158/2159-8290.CD-17-0605.

[5] G. LeRoy, B. Rickards, S.J. Flint, The Double Bromodomain Proteins Brd2 and Brd3 Couple Histone Acetylation to Transcription, Mol. Cell. 30 (2008) 51–60. https://doi.org/10.1016/j.molcel.2008.01.018.

[6] S. Rahman, M.E. Sowa, M. Ottinger, J.A. Smith, Y. Shi, J.W. Harper, P.M. Howley, The Brd4 Extraterminal Domain Confers Transcription Activation Independent of pTEFb by Recruiting Multiple Proteins, Including NSD3, Mol. Cell. Biol. 31 (2011) 2641–2652. https://doi.org/10.1128/mcb.01341-10.

[7] D. Hnisz, B.J. Abraham, T.I. Lee, A. Lau, V. Saint-André, A.A. Sigova, H.A. Hoke, R.A. Young, Super-enhancers in the control of cell identity and disease, Cell. 155 (2013) 934. https://doi.org/10.1016/j.cell.2013.09.053.

[8] J. Lovén, H.A. Hoke, C.Y. Lin, A. Lau, D.A. Orlando, C.R. Vakoc, J.E. Bradner, T.I. Lee, R.A. Young, Selective inhibition of tumor oncogenes by disruption of super-enhancers., Cell. 153 (2013) 320–34. https://doi.org/10.1016/j.cell.2013.03.036.

[9] I.A. Asangani, V.L. Dommeti, X. Wang, R. Malik, M. Cieslik, R. Yang, J. Escara-Wilke, K. Wilder-Romans, S. Dhanireddy, C. Engelke, M.K. Iyer, X. Jing, Y.M. Wu, X. Cao, Z.S. Qin, S. Wang, F.Y. Feng, A.M. Chinnaiyan, Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer, Nature. 510 (2014) 278–282. https://doi.org/10.1038/nature13229.

[10] J.S. Shafran, G.P. Andrieu, B. Györffy, G. V. Denis, BRD4 regulates metastatic potential of castration-resistant prostate cancer through AHNAK, Mol. Cancer Res. 17 (2019) 1627–1638. https://doi.org/10.1158/1541-7786.MCR-18-1279.

[11] D.J. Coleman, L. Gao, C.J. King, J. Schwartzman, J. Urrutia, A. Sehrawat, J. Tayou, A. Balter, J. Burchard, K.E. Chiotti, D.S. Derrick, D. Sun, Z. Xia, L.M. Heiser, J.J. Alumkal, BET bromodomain inhibition blocks the function of a critical AR-independent master regulator network in lethal prostate cancer., Oncogene. 38 (2019) 5658–5669. https://doi.org/10.1038/s41388-019-0815-5.

[12] A. Alqahtani, K. Choucair, M. Ashraf, D.M. Hammouda, A. Alloghbi, T. Khan, N. Senzer, J. Nemunaitis, Bromodomain and extra-terminal motif inhibitors: A review of preclinical and clinical advances in cancer therapy, Futur. Sci. OA. 5 (2019). https://doi.org/10.4155/fsoa-2018-0115.

[13] B. Langmead, S.L. Salzberg, Fast gapped-read alignment with Bowtie 2, Nat. Methods. 9 (2012) 357–359. https://doi.org/10.1038/nmeth.1923.

[14] S. Heinz, C. Benner, N. Spann, E. Bertolino, Y.C. Lin, P. Laslo, J.X. Cheng, C. Murre, H. Singh, C.K. Glass, Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities, Mol. Cell. 38 (2010) 576–589. https://doi.org/10.1016/j.molcel.2010.05.004.

[15] M. V. Kuleshov, M.R. Jones, A.D. Rouillard, N.F. Fernandez, Q. Duan, Z. Wang, S. Koplev, S.L. Jenkins, K.M. Jagodnik, A. Lachmann, M.G. McDermott, C.D. Monteiro, G.W. Gundersen, A. Ma'ayan, Enrichr: a comprehensive gene set enrichment analysis web server 2016 update, Nucleic Acids Res. 44 (2016) W90–W97. https://doi.org/10.1093/nar/gkw377.

[16] E. Afgan, D. Baker, B. Batut, M. Van Den Beek, D. Bouvier, M. Ech, J. Chilton, D. Clements, N. Coraor, B.A. Grüning, A. Guerler, J. Hillman-Jackson, S. Hiltemann,

V. Jalili, H. Rasche, N. Soranzo, J. Goecks, J. Taylor, A. Nekrutenko, D. Blankenberg, The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update, Nucleic Acids Res. 46 (2018) W537–W544. https://doi.org/10.1093/nar/gky379.

[17] J.T. Robinson, H. Thorvaldsdóttir, W. Winckler, M. Guttman, E.S. Lander, G. Getz, J.P. Mesirov, Integrative Genome Viewer, Nat. Biotechnol. 29 (2011) 24–6. https://doi.org/10.1038/nbt.1754.Integrative.

[18] R. Petryszak, M. Keays, Y.A. Tang, N.A. Fonseca, E. Barrera, T. Burdett, A. Füllgrabe, A.M.P. Fuentes, S. Jupp, S. Koskinen, O. Mannion, L. Huerta, K. Megy, C. Snow, E. Williams, M. Barzine, E. Hastings, H. Weisser, J. Wright, P. Jaiswal, W. Huber, J. Choudhary, H.E. Parkinson, A. Brazma, Expression Atlas update - An integrated database of gene and protein expression in humans, animals and plants, Nucleic Acids Res. 44 (2016) D746–D752. https://doi.org/10.1093/nar/gkv1045.

[19] D.J. Hazelett, S.K. Rhie, M. Gaddis, C. Yan, D.L. Lakeland, S.G. Coetzee, B.E. Henderson, H. Noushmehr, W. Cozen, Z. Kote-Jarai, R.A. Eeles, D.F. Easton, C.A. Haiman, W. Lu, P.J. Farnham, G.A. Coetzee, Comprehensive Functional

Annotation of 77 Prostate Cancer Risk Loci, PLoS Genet. 10 (2014). https://doi.org/10.1371/journal.pgen.1004102.

[20] P.C. Taberlay, A.L. Statham, T.K. Kelly, S.J. Clark, P.A. Jones, Reconfiguration of nucleosome-depleted regions at distal regulatory elements accompanies DNA methylation of enhancers and insulators in cancer, Genome Res. 24 (2014) 1421–1432. https://doi.org/10.1101/gr.163485.113.

[21] A.W. Grasso, D. Wen, C.M. Miller, J.S. Rhim, T.G. Pretlow, H.J. Kung, ErbB kinases and NDF signaling in human prostate cancer cells, Oncogene. 15 (1997) 2705–2716. https://doi.org/10.1038/sj.onc.1201447.

[22] E.B. Rankin, A.J. Giaccia, Hypoxic control of metastasis, Science (80-.). 352 (2016) 175–180. https://doi.org/10.1126/science.aaf4405.

[23] S. Lamouille, J. Xu, R. Derynck, Molecular mechanisms of epithelial-mesenchymal transition, Nat. Rev. Mol. Cell Biol. 15 (2014) 178–196. https://doi.org/10.1038/nrm3758.

[24] D.J. Coleman, L. Gao, J. Schwartzman, J.E. Korkola, D. Sampson, D.S. Derrick, J. Urrutia, A. Balter, J. Burchard, C.J. King, K.E. Chiotti, L.M. Heiser, J.J. Alumkal, Maintenance of MYC expression promotes de novo resistance to BET

bromodomain inhibition in castration-resistant prostate cancer, Sci. Rep. 9 (2019) 1–9. https://doi.org/10.1038/s41598-019-40518-5.

[25] K.M. Tracy, C.E. Tye, P.N. Ghule, H.L.H. Malaby, J. Stumpff, J.L. Stein, G.S. Stein, J.B. Lian, Mitotically-associated lncRNA (MANCR) affects genomic stability and cell division in aggressive breast cancer, Mol. Cancer Res. 16 (2018) 587–598. https://doi.org/10.1158/1541-7786.MCR-17-0548.

[26] A. Van Bokhoven, M. Varella-Garcia, C. Korch, W.U. Johannes, E.E. Smith, H.L. Miller, S.K. Nordeen, G.J. Miller, M.S. Lucia, Molecular Characterization of Human Prostate Carcinoma Cell Lines, Prostate. 57 (2003) 205–225. https://doi.org/10.1002/pros.10290.

[27] X. Dai, H. Cheng, Z. Bai, J. Li, Breast cancer cell line classification and Its relevance with breast tumor subtyping, J. Cancer. 8 (2017) 3131–3141. https://doi.org/10.7150/jca.18457.

[28] W. Lu, Y. Xu, J. Xu, Z. Wang, G. Ye, Identification of differential expressed lncRNAs in human thyroid cancer by a genome-wide analyses, Cancer Med. 7 (2018) 3935–3944. https://doi.org/10.1002/cam4.1627.

[29] L. Yao, J. Yan, L. Gan, S. Huang, F. Cheng, N. Fang, Upregulation of MANCR predicts poor survival in patients with gastric cancer, Oncol. Lett. 18 (2019) 6801–6806. https://doi.org/10.3892/ol.2019.11026.

[30] S. Wen, M. Zeng, Y. Li, X. Hu, S. Li, X. Liang, L. Zhu, S. Yang, Downregulation of MANCR inhibits cancer cell proliferation in mantle cell lymphoma possibly by interacting with RUNX2, Acta Biochim. Biophys. Sin. (Shanghai). 51 (2019) 1142–1147. https://doi.org/10.1093/abbs/gmz114.

[31] X. Zhang, H. Li, M. Mao, X. Wang, J. Zheng, S. Yang, Mitotically-associated long non-coding RNA promotes cancer cell proliferation in hepatocellular carcinoma by downregulating miR-122a., Oncol. Lett. 18 (2019) 6237–6242. https://doi.org/10.3892/ol.2019.10947.

[32] F.J. Slack, A.M. Chinnaiyan, The Role of Non-coding RNAs in Oncology, Cell. 179 (2019) 1033–1055. https://doi.org/10.1016/j.cell.2019.10.017.

FIGURE LEGENDS

Figure 1. PC3-specific SE-associated genes include genes involved in cell migration and invasion. (A) Numbers of SE-associated genes in the indicated cell lines.

(B) Venn diagram showing the numbers of SE-associated genes in LNCaP and PC3 cells. **(C)** Pathway analysis was performed using the LNCaP- and PC3-specific SE-associated gene sets; the top 10 terms are indicated.

Figure 2. A BETi, JQ1, decreases migration and invasion abilities of PC3 cells. (A) Migration and **(C)** invasion assays using LNCaP and PC3 cells. Cells were treated with DMSO (Ctrl) or 100 nM or 500 nM JQ1 and incubated in the chamber for 8 h for migration assays and 16 h for invasion assays. Error bars indicate mean ± S.E.M. (n=3 biological replicates). ***P<0.001, **P<0.01; one-way ANOVA. Scale bar, 200 μm. **(B)** Wound healing assay using LNCaP and PC3 cells treated with 500 nM JQ1. Scale bar, 500 μm.

Figure 3. PC3-specific SE-associated lncRNA *MANCR* is down-regulated by JQ1. **(A)** Scatter plot comparing Log2 FPKM expression values for PC3-specific SE-associated genes as identified in Figure 1B. An RNA-seq dataset for untreated (Ctrl) and JQ1-treated PC3 cells were used (GSE98069). **(B)** RT-qPCR analysis of *MANCR* expression in PC3 cells treated with JQ1. Error bars indicate mean ± S.E.M. (n=3 biological replicates). ***P<0.001; unpaired t-test. **(C)** Genome browser images of BRD4 ChIP-seq in PC3 cells. ChIP-seq dataset for untreated (Ctrl) and JQ1-treated PC3 cells

were used (GSE98069). **(D)** Comparative analysis of *MANCR* expression in several cancer cell lines. Expression data were downloaded from Expression Atlas (http://www.ebi.ac.uk/gxa).

Figure 4. *MANCR* plays a critical role in migration and invasion of PC3 cells. (A) RT-qPCR analysis of *MANCR* expression in PC3 cells transfected with control or *MANCR*-targeted siRNA. **(B)** Representative images of migration and invasion assays using PC3 cells transfected with the indicated siRNA. Scale bars, 100 μm. **(C, D)** Quantification of the migration (C) and invasion assays (D). **(A, C, D)** Error bars indicate mean ± S.E.M. (n=3 biological replicates). ***P<0.001, **P<0.01, *P<0.05; one-way ANOVA. **(E)** Scatter plot displaying mRNA expression levels in *MANCR*-knocked down PC3 cells compared with the control. Red dots indicate down-regulated genes (Log2 FPKM >1 in Ctrl, Log2 FC <−1 by *MANCR* knockdown). **(F)** Heat map showing expression levels of genes involved in EMT. **(G)** Gene Ontology analysis using the gene set indicated by red dots in **(E)**. Top 10 terms are indicated.