CANCER BIOLOGY & THERAPY
2020, VOL. 21, NO. 1, 34–42
https://doi.org/10.1080/15384047.2019.1665393

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RESEARCH PAPER

MiR-133a-5p inhibits androgen receptor (AR)-induced proliferation in prostate cancer cells via targeting FUsed in Sarcoma (FUS) and AR

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ABSTRACT

Androgens and androgen receptors are vital factors involved in prostate cancer progression, and androgen ablation therapies are commonly employed to treat advanced prostate cancer. Previously, FUsed in Sarcoma (FUS) was identified as an AR-interacting protein that enhances AR transcriptional activity. In the present study, we attempted to identify miRNAs that might target both FUS and AR to inhibit FUS and AR expression. Based on TCGA data and the online tools UALCAN, Kaplan Meier-plotter (KMplot), LncTar and miRWalk prediction, miR-133a-5p was selected. MiR-133a-5p expression was significantly downregulated in prostate cancer, and low miR-133a-5p expression was correlated with low survival probability. As predicted by LncTar and miRWalk, miR-133a-5p could bind to the 3′UTR of FUS and AR to inhibit their expression. MiR-133a-5p overexpression significantly suppressed the cell viability of the AR-positive prostate cancer cell lines VCaP and LNCaP, inhibited the expression of FUS, AR, as well as AR downstream targets IGF1R and EGFR. More importantly, miR-133a inhibition increased cancer cell proliferation as well as the expression of AR and AR downstream factors, while FUS knockdown exerted an opposite effect; the effect of miR-133a on cancer cell proliferation and AR could be significantly reversed by FUS knockdown. Moreover, IGF1R and EGFR knockdown reversed the effect of the miR-133a-5p inhibition. In summary, miR-133a-5p inhibits AR-positive prostate cancer cell proliferation by targeting FUS/AR, thus improving the resistance of prostate cancer to androgen ablation therapies, which requires further *in vivo* validation. We provided a novel miRNA regulation mechanism for proliferation regulation in AR-positive prostate cancer cells.

ARTICLE HISTORY
Received 14 December 2018
Revised 26 June 2019
Accepted 1 September 2019

KEYWORDS
Prostate cancer; androgen receptor (AR); FUsed in Sarcoma (FUS); miR-133a-5p; proliferation

Introduction

The androgen receptor (AR) is essential for the survival and growth of prostate cancer cells. Therefore, androgen ablation therapies are commonly employed to treat advanced prostate cancer. However, when treatments fail, prostate cancer will become lethal, namely, castration-resistant prostate cancer (CRPC).[1]

AR is involved in the most extensively supported mechanism for CRPC. The ligand-dependent/ligand-independent activity of the receptor requires the amino-terminal region of the AR.[2] Therefore, inhibiting androgen synthesis, increasing the affinity of anti-androgens that competitively bind to the AR ligand-binding domain (LBD),[3] and inhibiting the AR amino-terminal domain[4] have been regarded as more effective strategies for anti-CRPC drug development. FET/TET, a family of proteins includes FUsed in Sarcoma (FUS)/Translocated in Liposarcoma (TLS), EWig's Sarcoma protein (EWS), and TATA-binding protein-associated factor TAF15, may serve as endogenous AR interaction partner.[5,6] FUS, also known as TLS, was initially identified in human myxoid and round cell liposarcoma as a tumorigenic fusion with the DNA-binding transcription factor CHOP-induced by stress.[7,8] More importantly, FUS could interact with AR to increase AR transcriptional activity, while the androgen-dependent proliferation of LNCaP cells was inhibited by a decrease in FUS.[5] Blocking FUS and FUS-enhanced AR transcriptional activity may be novel strategies for CRPC treatment.

miRNAs are small, non-encoding, and genetically conserved single-stranded RNAs. miRNAs can target the 3′UTR of downstream mRNAs, leading to target gene mRNA degradation or translation inhibition; in this manner, mRNAs serve as gene expression regulators in cell metabolism, proliferation, differentiation, death and survival.[9,10] miRNA expression profiles in prostate cancer were of increasing importance based on their complicated functions on the diagnosis, staging, prediction prognosis and therapeutic response.[9,11] miRNA microarray profiling indicated that the expression of 75 miRNAs differed in primary prostate cancer compared with normal specimens; 88 were differentially-expressed in CRPC and normal specimens; 22 were different in primary prostate cancer and CRPC samples.[11] Taken together, the deregulations in miRNA may participate in the progression of CRPC.

In the present study, FUS and AR expression and protein levels were examined in cell lines. Based on TCGA data and online tool prediction results, miRNAs that were negatively correlated with FUS and AR were identified, and miR-133a-5p was selected. The predicted binding between miR-133a-5p and FUS/AR was

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validated. Finally, we evaluated the effect of miR-133a-5p overexpression and the dynamic effects of miR-133a-5p inhibition and FUS, IGFR, and EGFR knockdown on prostate cancer cell proliferation and AR signaling. In summary, we proposed a new mechanism that affected AR-positive prostate cancer cells through the regulation of miRNA.

Materials and methods

Cell lines and cell transfection

The human normal prostate epithelial cell line RWPE-1 and AR-positive prostate cancer cell lines VCaP and LNCaP were obtained from the ATCC (Manassas, VA, USA). RWPE-1 cells were maintained in keratinocyte serum-free medium (K-SFM). LNCaP and VcaP cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. All media was supplemented with 100 U/ml penicillin-streptomycin. All cells were maintained at 37°C and 5% CO_2.

Cell transfection

miR-133a-5p mimics and inhibitors were purchased from Genechem Co., Ltd. (China). The small interfering RNAs si-FUS, si-IGF1R, si-EGFR, and si-NC were purchased from Genepharm Co., Ltd. (China). Cell transfections were performed using Lipofectamine 2000.

Real-time PCR

Total RNA was extracted using TRIzol reagent. Total RNA was reverse transcribed with miRNA-specific primers, and a miScript Reverse Transcription kit was used for miRNA qRT-PCR; RNU6B expression was used as an endogenous control. SYBR Green PCR Master Mix was used for mRNA expression detection; GAPDH expression was used as an endogenous control. The $2^{-\Delta\Delta}$CT method was used to analyze the relative fold changes. The primers are listed in Table S1.

Cell viability determination by MTT assay

Cells were seeded into 96-well plates at a density of 5×10^3 cells/well, incubated for 24 h, and transfected and/or treated as described; then, 20 µl MTT solution (at a concentration of 5 mg/ml; Sigma-Aldrich, USA) was added 48 h after transfection. After another 4-h incubation, the supernatants were discarded, and 200 µl DMSO was added to dissolve the formazan. $OD_{490\ nm}$ values were measured. Cell viability in the different groups was calculated by setting the viability of the non-treated cells (control) as 100%.

Colony formation assay

Cells were suspended in DMEM containing 0.35% low-melting agarose and plated onto 0.6% agarose in six-well culture plates at a density of 1×10^5 cells per dish. The plates were incubated for two weeks at 37°C in a 5% CO_2 incubator, and the number of colonies was counted after staining with a 0.1% crystal violet solution. Colonies with more than 50 cells were counted manually.

Luciferase reporter assay

To generate wild-type luciferase reporter vectors, we cloned the FUS 3′UTR or AR 3′UTR into the psiCHECK2 vector (Promega) and named them wt-FUS 3′UTR or wt-AR 3′UTR. For the mutant reporter, 6 or 9 bp in the predicted miR-133a-5p sequence within the FUS 3′UTR or AR 3′UTR were mutated; these vectors were named mut-FUS 3′UTR or mut-AR 3′UTR. HEK293 cells (ATCC) were co-transfected with the abovementioned vectors and miR-133a-5p mimics/inhibitor. Luciferase activity was then detected by the Dual-Luciferase Reporter Assay System (Promega). Renilla luciferase activity was normalized to the firefly luciferase activity for each transfected well.

Immunoblotting analysis

Briefly, cell lysates were prepared with RIPA buffer (Sigma-Aldrich) and Complete Protease Inhibitor Cocktail (Roche, Basel, Switzerland) and then transferred to 1.5-mL tubes and kept at −20°C. Protein separation was performed using SDS-PAGE. After separation, the proteins were separated on an SDS-PAGE minigel, transferred onto PVDF membranes, and then probed with the following antibodies at 4°C overnight: anti-FUS (ab23439, Abcam, Cambridge, CA, USA), anti-AR (ab9474, Abcam), anti-IGF1R (ab182408, Abcam), anti-EGFR (ab52894, Abcam), and anti-GAPDH (1:5000, ab8245, Abcam); then, the membranes were incubated with an HRP-conjugated secondary antibody. The signals were visualized using an ECL substrate (Millipore, Germany). The protein level of GAPDH was used as an endogenous normalization factor.

Statistical analysis

The data are expressed as the means ± SD of at least three independent experiments and statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests or independent sample *t*-tests using SPSS Statistics 17.0 software. The level of significance was based on the probability of $P < .05$, $P < .01$.

Results

FUS and AR mRNA expression and protein levels are upregulated in prostate cancer tissues and cell lines

In prostate cancer cells, FUS interacts with AR and is a co-activator of AR.[5] According to the data in the TCGA database, the expression of FUS is significantly higher in primary tumor tissue samples (n = 497) than in non-cancerous tissue samples (n = 52) (Figure 1A). FUS expression significantly increased with the progression of prostate cancer (Gleason score) (Figure 1A). Patients with high FUS expression (>median value) had a significantly lower survival probability than patients with low FUS expression (<median value) (Figure 1A). Consistently, FUS and AR mRNA expression and protein levels were significantly

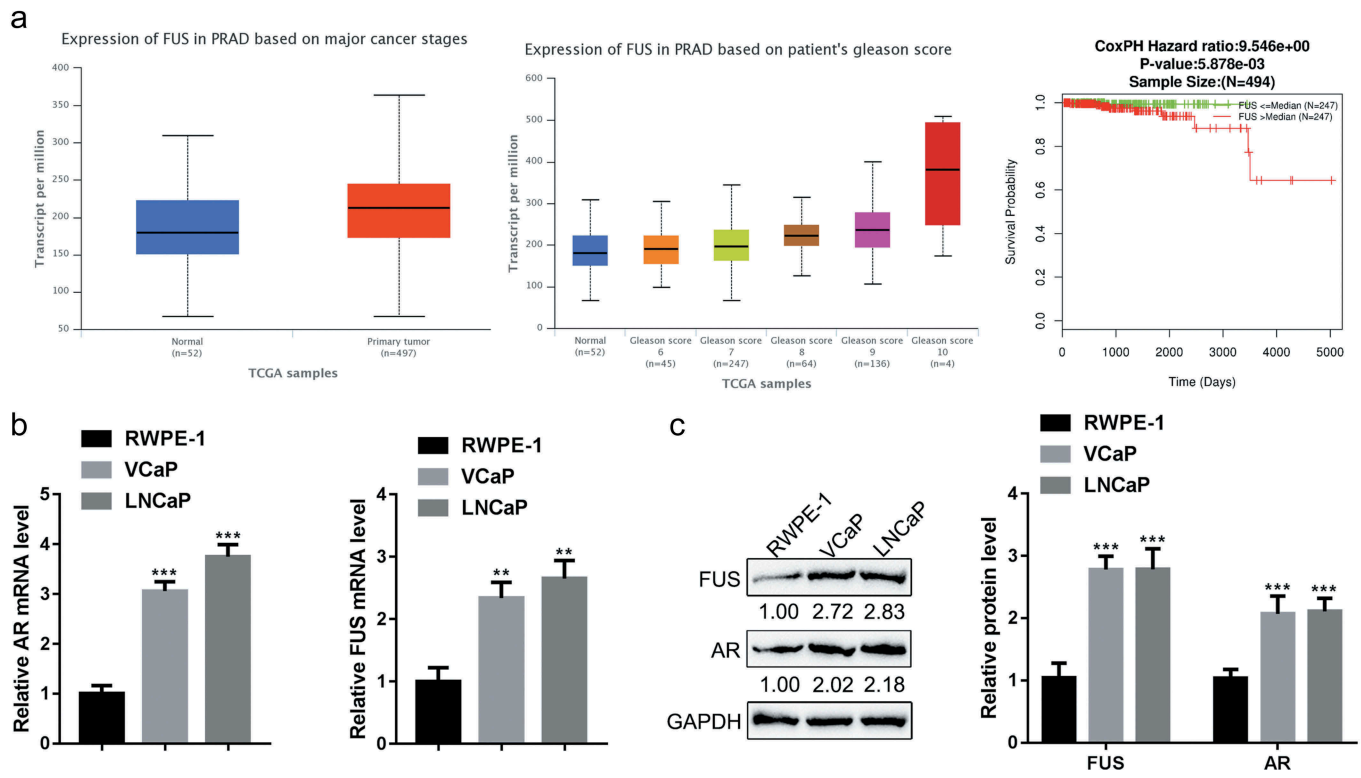

Figure 1. The expression and protein levels of FUS and AR are increased in prostate cancer tissues and cell lines.

(A) FUS expression in prostate cancer and non-cancerous tissues based on the TCGA database was analyzed using the online tool UALCAN Kaplan Meier-plotter (KMplot) for survival overall analysis. (B) FUS and AR mRNA expression in RWPE-1, VCaP, and LNCaP cell lines examined by real-time PCR. (C) FUS and AR protein levels in RWPE-1, VCaP, and LNCaP cell lines examined by immunoblotting. * $P < .05$, ** $p < .01$, *** $p < .001$, compared to the RWPE-1 group.

higher in the AR-positive prostate cancer cell lines VCaP and LNCaP than in the normal cell line RWPE-1 (Figure 1B-C).

Selection of mirnas that may be related to FUS and AR in prostate cancer

Next, we analyzed differentially-expressed miRNAs that may correlate with FUS and AR levels in prostate cancer based on the TCGA database. As shown in Figure 2A, a total of 16 miRNAs were negatively correlated with FUS (Table S2, R<-0.15, $P < .05$); a total of 100 miRNAs negatively associated with AR are listed in Table S2 (Table S3, R<-0.15, $P < .05$). Via cross-checks, miR-133a-5p and miR-222 were both negatively correlated with FUS and AR (Figure 2A). More importantly, according to the TCGA database, miR-133a-5p expression was lower in tissue samples resistant to radiation therapy (Fig. S1A), samples with more lymph node metastases (Fig. S1B), and samples from advanced N and T stage patients (Fig. S1C-D). Patients with low miR-133a expression (<median value) had a low survival probability (Fig. S1E). Large sample size data from TCGA indicated that miR-133a-5p was negatively correlated with both FUS and AR (Figure 2B-C). Based on the above-described reasons, miR-133a-5p was selected for further experiments.

FUS and AR are direct downstream targets of mir-133a-5p

We first examined the binding of miR-133a-5p to the FUS 3′UTR and AR 3′UTR predicted by the LncTar and miRWalk online tools and then investigated the value of miR-133a-5p for treating prostate cancer. We conducted miR-133a-5p inhibition by transfecting a miR-133a-5p inhibitor, and real-time PCR was performed to verify the transfection efficiency (Figure 3A). Then, two types of FUS 3′UTR and AR 3′UTR luciferase reporter vectors, wild- and mutant (mut)-type, were constructed. Within the putative miR-133a-5p binding site of the mutant-type vectors, 6 or 9 bases were mutated (Figure 3B and D). Then, we co-transfected these vectors into HEK293 cells with miR-133a-5p mimic and miR-133a-5p inhibitor and measured the luciferase activity. According to Figure 3C and E, the luciferase activity of the wild-type vectors (FUS 3′UTR-wt and AR 3′UTR-wt) could be remarkably downregulated by the overexpression of miR-133a-5p and upregulated by the inhibition of miR-133a-5p; mutating the putative miR-133a-5p binding site in the 3′UTR of FUS and AR could eliminate the alterations in luciferase activity. These findings reveal that FUS and AR are direct downstream targets of miR-133a-5p.

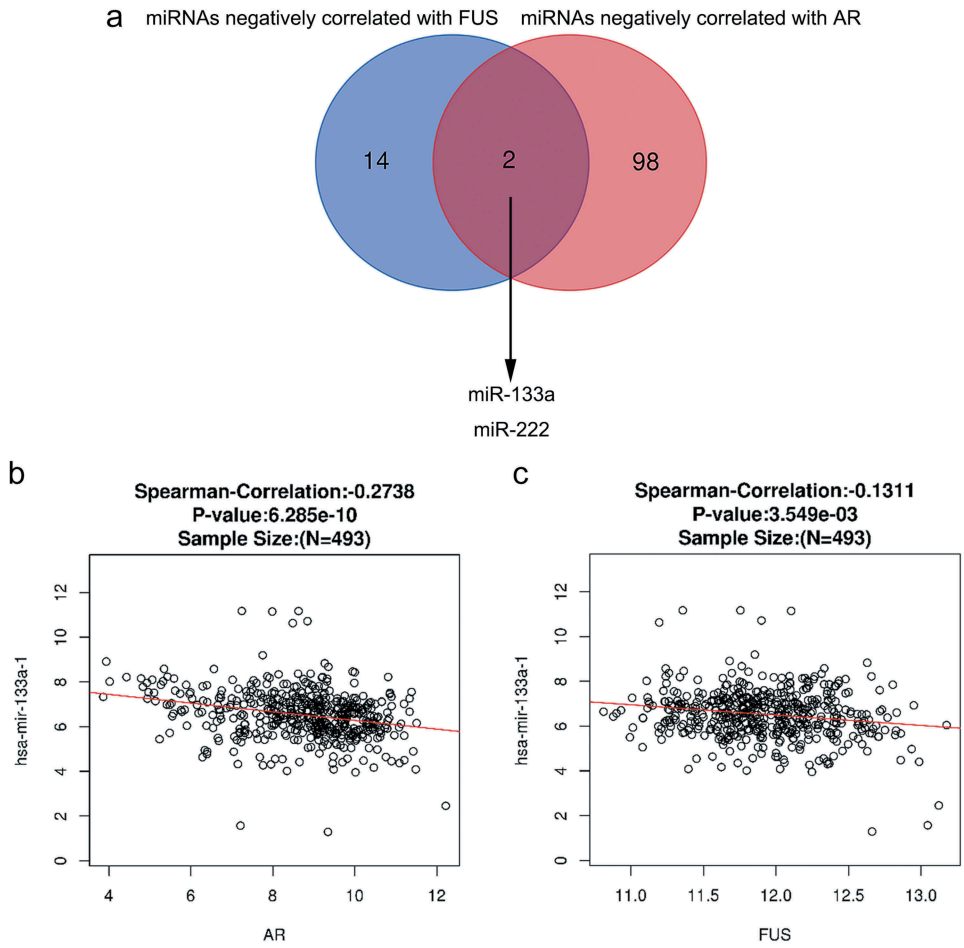

Figure 2. Selection of miRNAs that may be related to FUS and AR in prostate cancer.
(A) A schematic diagram showing the processing of selecting miRNAs related to FUS and AR in prostate cancer. (B-C) Correlation of miR-133a-5p with FUS and AR in tissue samples. The data were obtained from the TCGA database and analyzed with the LinkedOmics online tool.

miR-133a-5p inhibits prostate cancer cell proliferation through FUS and AR

We examined the cell viability of miR-133a-overexpressing VCaP and LNCaP cells to study the role of miR-133a-5p in prostate cancer growth. In miR-133a-overexpressing VCaP cells, the cell viability and colony formation capacity were both significantly suppressed, with or without androgen mibolerone (MIB) stimulation (Figure 4A-B). Moreover, the mRNA expression and protein levels of FUS, AR, IGF1R and EGFR could be dramatically increased by MIB and decreased in miR-133a-overexpressing VCaP and LNCaP cells (Figure 4C-D), indicating that miR-133a-5p could inhibit prostate cancer cell proliferation and FUS, AR, and AR downstream targets.

miR-133a-5p modulates FUS co-activated AR transcription and affects AR downstream target genes

To further validate the regulation of FUS/AR by miR-133a-5p, we achieved FUS knockdown in VCaP cells by transfecting si-FUS, and performed real-time PCR and immunoblotting to verify the transfection efficiency (Figure 5A-B). Then, we co-transfected VcaP cells with an inhibitor of miR-133a-5p and si-FUS and evaluated the expression of AR, PSA, IGF1R, and EGFR. Consistent with the above-described results, the expression of AR, PSA, IGF1R, and EGFR was remarkably upregulated by the miR-133a-5p inhibitor but significantly downregulated by FUS knockdown. Moreover, FUS knockdown remarkably attenuated the effect of the miR-133a-5p inhibitor (Figure 5C).

Knockdown of IGF1R or EGFR inhibits mir-133a-5p-induced prostate cancer cell proliferation

We achieved IGF1R or EGFR knockdown in VCaP cells by transfecting si-IGF1R or si-EGFR and performed real-time PCR and immunoblotting to verify the transfection efficiency (Figure 6A-B). Then, we co-transfected VcaP cells with an inhibitor of miR-133a-5p and si-IGF1R or EGFR and then evaluated the mRNA expression of IGF1R and EGFR. The expression of IGF1R and EGFR was remarkably upregulated by the miR-133a-5p inhibitor but significantly downregulated by small RNA interference (Figure 6C). MTT assay results showed that si-IGFR and si-EGFR inhibited VCaP cell growth. However, the miR-133a-5p inhibitor attenuated the growth inhibition effect (Figure 6D).

Discussion

Herein, FUS and AR mRNA and protein expression levels were significantly increased in the AR-positive prostate cancer

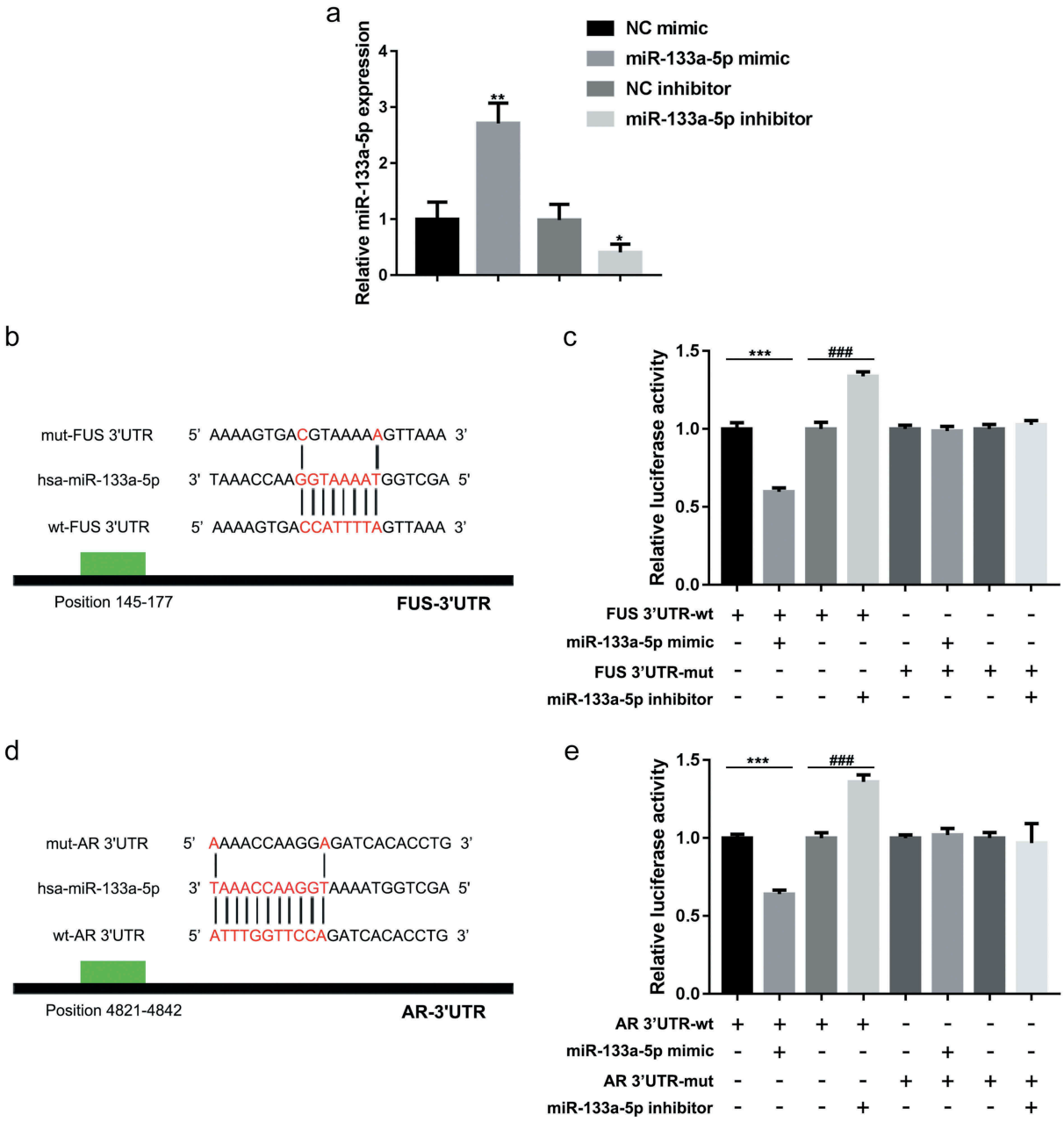

Figure 3. miR-133a-5p directly targets the FUS 3′UTR and AR 3′UTR.

(A) miR-133a-5p expression in VCaP cells was achieved by miR-133a-5p mimic/miR-133a-5p inhibitor transfection, as confirmed by real-time PCR. (B and D) Schematic diagrams showing the structures of wild-type and mutant-type FUS 3′UTR and AR 3′UTR luciferase reporter vectors. Mutant-type reporters contained a 6 or 9 bp mutation in the predicted miR-133a-5p binding site. (C and E) The above-described vectors were co-transfected in HEK293 cells with miR-133a-5p mimic/inhibitor. Then, the luciferase activity was determined. *p < .05, ** $p < .01$, *** $p < .001$.

cell lines, VCaP, and LNCaP. According to TCGA data and TagetScan and LncTar predictions, the miR-133a-5p expression is decreased in prostate cancer tissues, and miR-133a-5p may target FUS and AR simultaneously. miR-133a-5p is negatively correlated with FUS and AR. miR-133a-5p directly binds to the FUS 3′UTR and AR 3′UTR; miR-133a-5p inhibition promotes the expression of FUS and AR, as well as prostate cancer cell proliferation and AR downstream targets IGF1R and EGFR. More importantly, knockdown of FUS, IGF1R, or EGFR yields an opposite effect on VCaP cell proliferation and significantly attenuates the effect of miR-133a-5p inhibition.

FUS was reported to be among a set of biomarkers that could predict prostate cancer aggressiveness and lethality.[12] More importantly, FUS/TLS was identified as an AR co-activator in prostate cancer cells.[5] According to TGCA data, FUS expression was remarkably higher in prostate cancer tissue specimens, especially in those from advanced stage patients. Patients with high FUS levels had a low survival probability. Similar to a previous study, AR mRNA and protein expression were significantly upregulated in an AR-positive prostate cancer cell line, which indicates that FUS and AR could cooperate to promote the progression of prostate cancer.

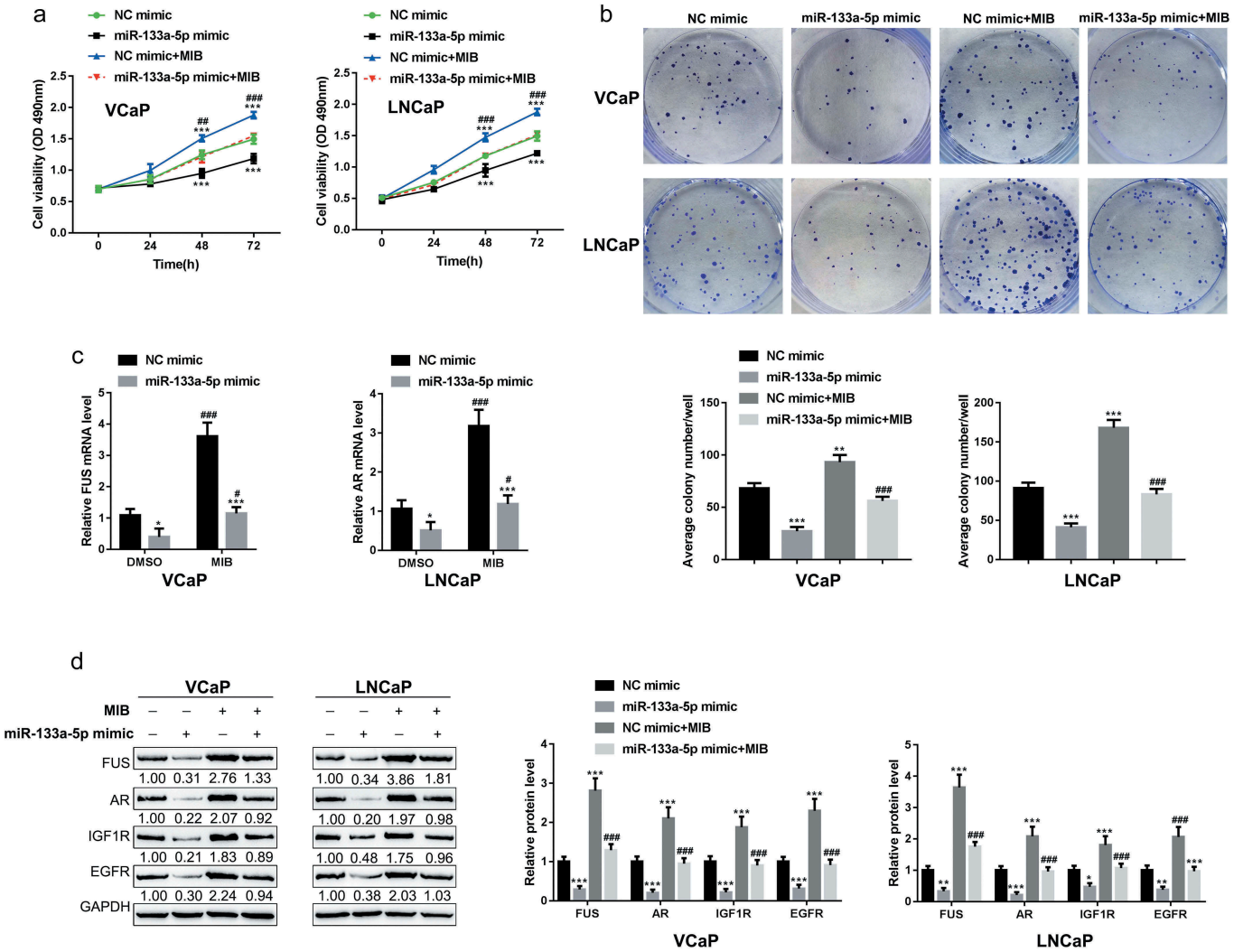

Figure 4. miR-133a-5p inhibits prostate cancer cell proliferation via FUS and AR.

(A-B) After miR-133a-5p mimic or NC mimic transfection, the cell viability and colony formation capacity of VCaP and LNCaP cells with or without 10 nM MIB treatment were examined by MTT and colony formation assays. (C) The mRNA expression of FUS, AR, IGF1R, and EGFR in miR-133a-5p mimic-transfected VCaP and LNCaP cells with or without MIB treatment was determined by real-time PCR. (D) The protein levels of FUS, AR, IGF1R, and EGFR in miR-133a-5p-inhibited VCaP and LNCaP cells with or without MIB treatment were determined by immunoblotting analyses. * $P < .05$, ** $p < .01$, *** $p < .001$, compared to the NC mimics group, $p < .001$, compared to the NC mimics +MIB group.

The analysis of anti-tumor miRNAs and their regulated genes could contribute to the discovery of new regulatory pathways in cancer. A number of miRNAs, including miR-26a, miR-26b, miR-218, miR-223, and the miR-29 family, might be downregulated in prostate cancer tissues; these miRNAs could bind to genes involved in the extracellular matrix (ECM), therefore inhibiting the migration and invasion of cancer cells.[13–15] miR-150-3p and miR-145-3p serve as anti-tumor miRNAs in naive prostate cancer and CRPC.[16,17] Based on these previous studies, we also analyzed data from the TCGA database to identify miRNAs associated with both FUS and AR in prostate cancer and CRPC cells. Of the miRNAs analyzed, miR-133a-5p was negatively correlated with both FUS and AR. More importantly, miR-133a-5p may target FUS and AR. Previous studies have shown that miR-133a-5p acts as an anti-tumor miRNA in prostate cancer. Via targeting the tumorigenic function of purine nucleoside phosphorylase (PNP), miR-133a-5p inhibited the proliferation, migration, and invasion of PC3 and DU145 cells.[18] The miR-133a gene is located at Chr18q11.2 and is transcribed to pre-miR-133a and then cleaved to miR-133a-5p and miR-133a-3p. Downregulation of miR-133a-3p could lead to the progression, recurrence, and distant metastasis of prostate cancer via PI3K/AKT signaling.[19] Herein, FUS and AR were predicted to be the direct downstream targets of miR-133a-5p; miR-133a-5p bound to the 3′UTR of FUS and AR to inhibit their mRNA expression and protein levels. In addition, miR-133a-5p inhibition also promoted the proliferation of VCaP cells, as well as the expression of AR downstream factors, IGF1R and EGFR.

The growth of prostate cells and the development of prostate cancer are mediated by androgens, as well as by the functional insulin receptor (IR) and IGF1R signaling. This association has been widely reported in previous studies, in which the relationship between high insulin and IGF-1 levels

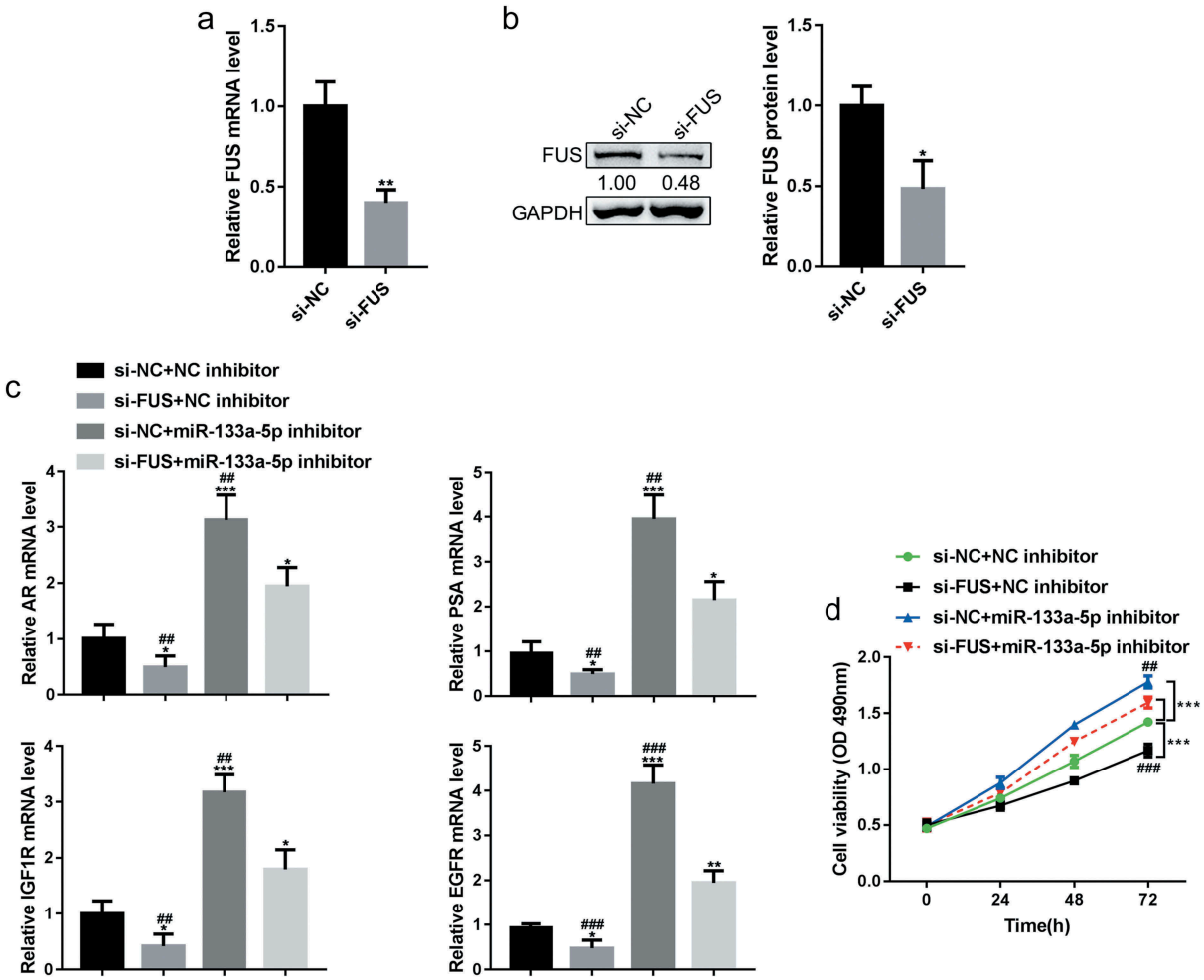

Figure 5. miR-133a-5p modulates FUS co-activated AR transcription and affects AR downstream target genes.

(A-B) FUS knockdown in VCaP cells achieved by transfection of si-FUS was confirmed by real-time PCR and immunoblotting. (C) VCaP cells were co-transfected with miR-133a-5p inhibitor and si-FUS and examined for the expression of AR, PSA, IGF1R, and EGFR. (D) Cell proliferation was examined by MTT assay. * $P < .05$, ** $p < .01$, *** $p < .001$, compared to the si-NC+NC inhibitor group, ## $p < .01$, $p < .001$ compared to the si-FUS+ miR-133a-5p inhibitor group.

and prostate cancer cell growth was revealed.[20–23] Moreover, AR could increase IGF1R expression and sensitize prostate cancer cells to IGF-1.[24] Several preclinical studies have indicated the importance of the IGF axis in PC progression and provided the basis for clinical trials with IGF1R as an anticancer target, alone or in combination with conventional therapies.[25] Reciprocal activation has been observed between IGF1R and AR signaling pathways, supporting the rationale of simultaneous inhibition in cancer treatment.[26,27] EGFR and its ligands could not only take the place of androgens to promote androgen receptor phosphorylation but also serve as co-regulators of androgen receptors to enhance the activation of downstream target genes.[28] Dual suppression of EGFR and HER-2 could impair PC tumor cell proliferation and survival.[29,30] Moreover, multiplex kinase activity profiling revealed that EGFR/ERBB2 kinase activity was remarkably enhanced in LNCaP cells co-cultured with osteoblasts. According to this study, the activity of EGFR is not only stimulated by tumor-associated bone cells[31] but also mediated by type 1 insulin-like growth factor (IGF) and ECM produced by the tumor-associated microenvironment during prostate cancer metastases into bone marrow.[32] In the present study, the protein levels of IGF1R and EGFR were remarkably enhanced by miR-133a-5p inhibition and dramatically inhibited by FUS knockdown. More importantly, FUS, IGFR, and EGFR knockdown remarkably attenuated the effect of miR-133a-5p. In conclusion, miR-133a-5p could regulate the proliferation of prostate cancer cells by targeting the 3′UTR of FUS and AR, subsequently affecting downstream IGF1R and EGFR.

In summary, we demonstrate that miR-133a-5p inhibits AR-positive prostate cancer cell proliferation by targeting FUS and AR, thereby affecting AR downstream signaling (Figure 7).

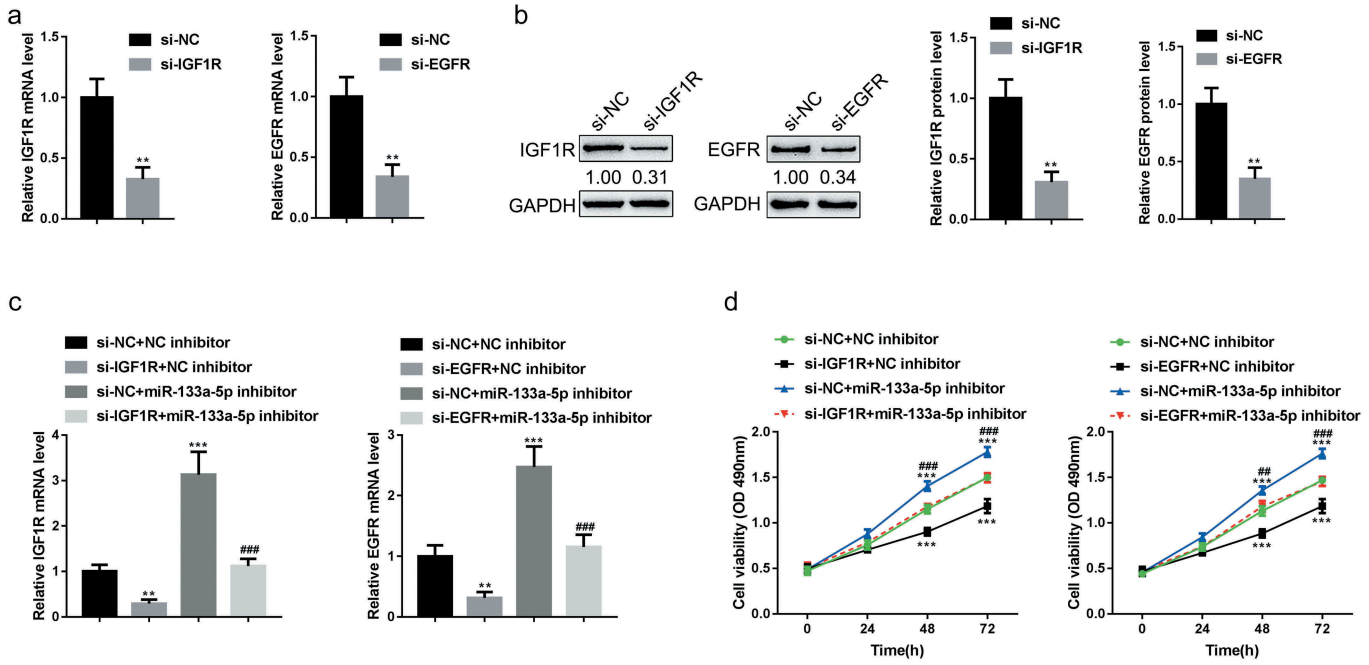

Figure 6. IGFR or EGFR knockdown-induced prostate cancer cell growth inhibition could be rescued by a miR-133a-5p inhibitor.

(A and B) IGF1R and EGFR knockdown in VCaP cells was achieved by transfection of si-IGF1R or EGFR and confirmed by real-time PCR and immunoblotting. (C) VCaP cells were co-transfected with miR-133a-5p inhibitor and si-IGF1R or si-EGFR. The mRNA expression of IGF1R and EGFR was determined by real-time PCR. (D) Cell proliferation was examined by MTT assay. ** $p < .01$, *** $p < .005$, compared to the si-NC+NC inhibitor group, ## $p < .01$, $p < .005$ compared to the si-IGF1R or si-EGFR+ miR-133a-5p inhibitor group.

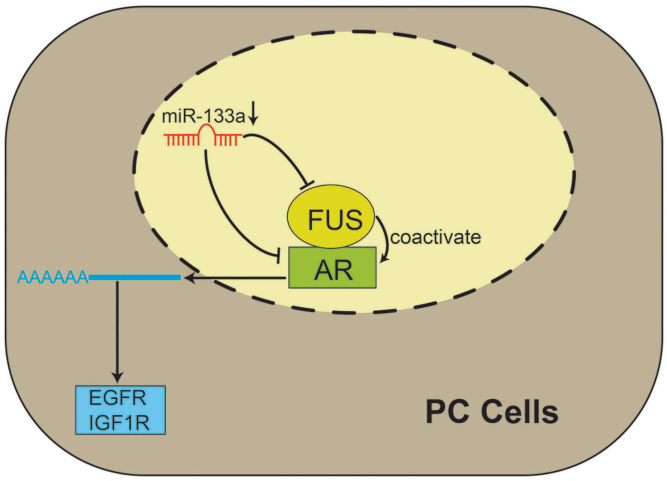

Figure 7. Schematic diagram showing the mechanism by which miR-133a-5p/FUS/AR modulates the proliferation of AR-positive prostate cancer cells via IGF1R and EGFR.

Funding

This study was supported by the Natural Science Foundation of Hunan Province (2015JJ4054).

References

1. Fujita K, Nonomura N. Role of Androgen receptor in prostate cancer: a review. World J Mens Health. 2019;37(3):288–295.
2. Wang G, Sadar MD. Amino-terminus domain of the androgen receptor as a molecular target to prevent the hormonal progression of prostate cancer. J Cell Biochem. 2006;98:36–53. doi:10.1002/jcb.20802.
3. Molina A, Belldegrun A. Novel therapeutic strategies for castration resistant prostate cancer: inhibition of persistent androgen production and androgen receptor mediated signaling. J Urol. 2011;185:787–794. doi:10.1016/j.juro.2010.10.042.
4. Sadar MD. Small molecule inhibitors targeting the "achilles' heel" of androgen receptor activity. Cancer Res. 2011;71:1208–1213. doi:10.1158/0008-5472.CAN_10-3398.
5. Haile S, Lal A, Myung JK, Sadar MD. FUS/TLS is a co-activator of androgen receptor in prostate cancer cells. PLoS One. 2011;6:e24197. doi:10.1371/journal.pone.0024197.
6. Law WJ, Cann KL, Hicks GG. TLS, EWS and TAF15: a model for transcriptional integration of gene expression. Brief Funct Genomic Proteomic. 2006;5:8–14. doi:10.1093/bfgp/ell015.
7. Rabbitts TH, Forster A, Larson R, Nathan P. Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. Nat Genet. 1993;4:175–180. doi:10.1038/ng0693-175.
8. Crozat A, Aman P, Mandahl N, Ron D. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature. 1993;363:640–644. doi:10.1038/363640a0.
9. Cannistraci A, Di Pace AL, De Maria R, Bonci D. MicroRNA as new tools for prostate cancer risk assessment and therapeutic intervention: results from clinical data set and patients' samples. Biomed Res Int. 2014;2014:146170. doi:10.1155/2014/146170.
10. Kojima S, Goto Y, Naya Y. The roles of microRNAs in the progression of castration-resistant prostate cancer. J Hum Genet. 2017;62:25–31. doi:10.1038/jhg.2016.69.
11. Jalava SE, Urbanucci A, Latonen L, Waltering KK, Sahu B, Janne OA, Seppälä J, Lähdesmäki H, Tammela TLJ, Visakorpi T. Androgen-regulated miR-32 targets BTG2 and is overexpressed in castration-resistant prostate cancer. Oncogene. 2012;31:4460–4471. doi:10.1038/onc.2011.624.
12. Shipitsin M, Small C, Choudhury S, Giladi E, Friedlander S, Nardone J, Hussain S, Hurley AD, Ernst C, Huang YE, et al. Identification of proteomic biomarkers predicting prostate cancer aggressiveness and lethality despite biopsy-sampling error. Br J Cancer. 2014;111:1201–1212. doi:10.1038/bjc.2014.396.

13. Kato M, Goto Y, Matsushita R, Kurozumi A, Fukumoto I, Nishikawa R, Sakamoto S, Enokida H, Nakagawa M, Ichikawa T, et al. MicroRNA-26a/b directly regulate La-related protein 1 and inhibit cancer cell invasion in prostate cancer. Int J Oncol. 2015;47:710–718. doi:10.3892/ijo.2015.3043.
14. Kurozumi A, Goto Y, Matsushita R, Fukumoto I, Kato M, Nishikawa R, Sakamoto S, Enokida H, Nakagawa M, Ichikawa T, et al. Tumor-suppressive microRNA-223 inhibits cancer cell migration and invasion by targeting ITGA3/ITGB1 signaling in prostate cancer. Cancer Sci. 2016;107:84–94. doi:10.1111/cas.12842.
15. Nishikawa R, Goto Y, Sakamoto S, Chiyomaru T, Enokida H, Kojima S, Kinoshita T, Yamamoto N, Nakagawa M, Naya Y, et al. Tumor-suppressive microRNA-218 inhibits cancer cell migration and invasion via targeting of LASP1 in prostate cancer. Cancer Sci. 2014;105:802–811. doi:10.1111/cas.12441.
16. Goto Y, Kurozumi A, Arai T, Nohata N, Kojima S, Okato A, Kato M, Yamazaki K, Ishida Y, Naya Y, et al. Impact of novel miR-145-3p regulatory networks on survival in patients with castration-resistant prostate cancer. Br J Cancer. 2017;117:409–420. doi:10.1038/bjc.2017.191.
17. Okato A, Arai T, Kojima S, Koshizuka K, Osako Y, Idichi T, Kurozumi A, Goto Y, Kato M, Naya Y, et al. Dual strands of pre-miR-150 (miR-150-5p and miR-150-3p) act as antitumor miRNAs targeting SPOCK1 in naïve and castration-resistant prostate cancer. Int J Oncol. 2017;51:245–256. doi:10.3892/ijo.2017.4008.
18. Kojima S, Chiyomaru T, Kawakami K, Yoshino H, Enokida H, Nohata N, Fuse M, Ichikawa T, Naya Y, Nakagawa M, et al. Tumour suppressors miR-1 and miR-133a target the oncogenic function of purine nucleoside phosphorylase (PNP) in prostate cancer. Br J Cancer. 2012;106:405–413. doi:10.1038/bjc.2011.462.
19. Tang Y, Pan J, Huang S, Peng X, Zou X, Luo Y, Ren D, Zhang X, Li R, He P, et al. Downregulation of miR-133a-3p promotes prostate cancer bone metastasis via activating PI3K/AKT signaling. J Exp Clin Cancer Res. 2018;37:160. doi:10.1186/s13046-018-0813-4.
20. Venkateswaran V, Haddad AQ, Fleshner NE, Fan R, Sugar LM, Nam R, Klotz LH, Pollak M. Association of diet-induced hyperinsulinemia with accelerated growth of prostate cancer (LNCaP) xenografts. J Natl Cancer Inst. 2007;99:1793–1800. doi:10.1093/jnci/djm231.
21. Polychronakos C, Janthly U, Lehoux JG, Koutsilieris M. Mitogenic effects of insulin and insulin-like growth factors on PA-III rat prostate adenocarcinoma cells: characterization of the receptors involved. Prostate. 1991;19:313–321. doi:10.1002/pros.2990190405.
22. Weroha SJ, Haluska P. The insulin-like growth factor system in cancer. Endocrinol Metab Clin North Am. 2012;41:335–350. vi. doi:10.1016/j.ecl.2012.04.014.
23. LeRoith D, Roberts CT Jr. The insulin-like growth factor system and cancer. Cancer Lett. 2003;195:127–137. doi:10.1016/s0304-3835(03)00159-9.
24. Pandini G, Mineo RF, Jr RC, Marcelli M, Vigneri R, Belfiore A. Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. Cancer Research. 2005;65:1849–1857. doi:10.1158/0008-5472.CAN-04-1837.
25. Heidegger I, Massoner P, Sampson N, Klocker H. The insulin-like growth factor (IGF) axis as an anticancer target in prostate cancer. Cancer Lett. 2015;367:113–121. doi:10.1016/j.canlet.2015.07.026.
26. Pandini G, Mineo R, Frasca F, Roberts CT Jr., Marcelli M, Vigneri R, Belfiore A. Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. Cancer Res. 2005;65:1849–1857. doi:10.1158/0008-5472.CAN-04-1837.
27. Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res. 1994;54:5474–5478.
28. Pignon JC, Koopmansch B, Nolens G, Delacroix L, Waltregny D, Winkler R. Androgen receptor controls EGFR and ERBB2 gene expression at different levels in prostate cancer cell lines. Cancer Res. 2009;69:2941–2949. doi:10.1158/0008-5472.CAN-08-3760.
29. Chen L, Mooso BA, Jathal MK, Madhav A, Johnson SD, van Spyk E, Mikhailova M, Zierenberg-Ripoll A, Xue L, Vinall RL, et al. Dual EGFR/HER2 inhibition sensitizes prostate cancer cells to androgen withdrawal by suppressing ErbB3. Clin Cancer Res. 2011;17:6218–6228. doi:10.1158/1078-0432.CCR-11-1548.
30. Day KC, Lorenzatti Hiles G, Kozminsky M, Dawsey SJ, Paul A, Broses LJ, Shah R, Kunja LP, Hall C, Palanisamy N, et al. HER2 and EGFR overexpression support metastatic progression of prostate cancer to Bone. Cancer Res. 2017;77:74–85. doi:10.1158/0008-5472.CAN-16-1656.
31. Bratland A, Boender PJ, Hoifodt HK, Ostensen IH, Ruijtenbeek R, Wang MY, Berg JP, Lilleby W, Fodstad Ø, Ree AH. Osteoblast-induced EGFR/ERBB2 signaling in androgen-sensitive prostate carcinoma cells characterized by multiplex kinase activity profiling. Clin Exp Metastasis. 2009;26:485–496. doi:10.1007/s10585-009-9248-9.
32. Chott A, Sun Z, Morganstern D, Pan J, Li T, Susani M, Mosberger I, Upton MP, Bubley GJ, Balk SP. Tyrosine kinases expressed in vivo by human prostate cancer bone marrow metastases and loss of the type 1 insulin-like growth factor receptor. Am J Pathol. 1999;155:1271–1279. doi:10.1016/S0002-9440(10)65229-7.