Regulation of metastasis-promoting *LOXL2* gene expression by antitumor microRNAs in prostate cancer

（転移促進型遺伝子である *LOXL2* は、前立腺癌において複数の癌抑制型マイクロ RNA により制御される）

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Abstract

Our recent studies of microRNA (miRNA) expression signatures of prostate cancer (PCa) showed that 6 miRNAs (specifically, *miR-26a*, *miR-26b*, *miR-29a*, *miR-29b*, *miR-29c* and *miR-218*) were markedly reduced in cancer tissues. Moreover, ectopic expression of these miRNAs suppressed PCa cell aggressiveness, indicating that these miRNAs acted in concert to regulate genes that promoted metastasis. Genome-wide gene expression analysis and *in silico* database analysis identified a total of 35 candidate genes that promoted metastasis and were targeted by these 6 miRNAs. Using luciferase reporter assays, we showed that the lysyl oxidase-like 2 (*LOXL2*) gene was directly controlled by these tumor-suppressive miRNAs in PCa cells. Overexpression of LOXL2 was confirmed in PCa tissues and knockdown of the *LOXL2* gene markedly inhibited the migration and invasion of PCa cells. Aberrant expression of *LOXL2* enhanced migration and invasion of PCa cells. Downregulation of anti-tumor miRNAs might disrupt the tightly controlled RNA networks found in normal cells. New insights into the novel molecular mechanisms of PCa pathogenesis was revealed by anti-tumor miRNAs-regulated RNA networks.

Key words:
microRNA, *LOXL2*, *miR-26a/b*, *miR-29 family*, *miR-218*, tumor-suppressor

Running title:
Regulation of *LOXL2* by miRNAs in PCa

Introduction

The current five-year survival rate of patients with localized prostate cancer (PCa) is almost 100% which is attributed to efficacy of treatments of early stage disease.[1] Although most patients with PCa are initially responsive to androgen deprivation therapy, their cancers eventually become resistant to this mode of treatment, and they progress to castration-resistant prostate cancer (CRPC).[1, 2] Unfortunately, most clinical trials for advanced PCa have shown limited benefits, eventually resulting in disease progression and metastasis.[1, 2] Distant metastasis of advanced PCa is common and leads to significant morbidity and mortality. Therefore, we have proposed that current therapies for advanced PCa could be improved by using new genomic approaches to better understand the molecular mechanisms of metastasis and disease progression.

MicroRNAs (miRNAs) are endogenous small non-coding RNA molecules (19~22 bases in length) that regulate the expression of protein-coding or protein non-coding genes in a sequence-specific manner.[3, 4] There is substantial evidence that miRNAs can be oncogenic or act to suppress tumours. Dysregulated expression of oncogenic miRNAs can disrupt the nomally controlled RNA networks present in normal cells and enhance the development of cancer.[5-8] Identification of aberrantly expressed miRNAs in cancer cells provides important new information regarding the initiation, progression and metastasis of cancer cells.

We have identified anti-tumor miRNAs and miRNA-mediated oncogenic genes in PCa cells by using our PCa miRNA expression signatures.[9, 10] Our recent studies showed that 6 miRNAs (*miR-26a*, *miR-26b*, *miR-29a*, *miR-29b*, *miR-29c* and *miR-218*) were downregulated in PCa tissues and restoration of these miRNAs markedly suppressed cancer cellaggressiveness.[11-13] Our data suggested that these 6 miRNAs act as anti-metastatic miRNAs in PCa cells. Therefore, we hypothesized that the genes regulated by these miRNAs significantly contributed to PCa metastasis. The aim of this study was to identify the genes targeted by these 6

anti-tumor miRNAs and to investigate their functional roles in migration and invasion in PCa cells.

Present study identified that a total of 35 putative metastasis-promoting genes were targeted by the 6 abovementioned miRNAs. Here, we focused on the lysyl oxidase-like 2 (*LOXL2*) gene, a member of the lysyl oxidase (LOX) family.[14] The function of *LOXL2* is to promote crosslinking of collagen and elastin in the extracellular matrix (ECM).[15-17] Past studies showed that *LOXL2* contributed to the regulation of extracellular and intracellular cell signaling pathways.[15-17] Our recent studies demonstrated that *LOXL2* was controlled by several tumor suppressive miRNAs and promoted cancer cell aggressiveness in renal cell carcinoma, head and neck cancer and lung cancer.[18-20] Furthermore, several studies demonstrated that upregulation of *LOXL2* occurred in many types of cancers and its expression contributed to cancer cell metastasis.[21-23] Our present data show that insights into the molecular mechanisms of PCa pathogenesis can be revealed by identification of anti-tumor miRNAs-regulated RNA networks.

Materials and Methods

Prostate cancer cell lines and RNA extraction

Two human PCa cell lines (PC3 and PC3M) was obtained from ATCC (Manassas, VA, USA). The maintenance of PCa cells and RNA extraction procedures were described as previously.[11, 24]

Quantitative real-time reverse transcription-PCR

TaqMan probes and primers of *LOXL2* (P/N: Hs00158757_ml; Applied Biosystems, Foster City, CA, USA) and *GAPDH* (P/N: Hs02758991_gl; Applied Biosystems) which was used as an internal control. PCR quantification method was described as previously.[13, 25, 26]

Transfection with miRNA mimic and small-interfering RNA (siRNA)

The miRNAs mimics were used in the present analysis: Ambion Pre-miR miRNA precursor for *hsa-miR-26a-5p* (product ID: PM10249),

hsa-miR-26b-5p (product ID: PM12899), *hsa-miR-29a-3p* (product ID: MC12499), *hsa-miR-29b-3p* (product ID: MC10103), *hsa-miR-29c-3p* (product ID: MC10518) and *hsa-miR-218* (product ID: AM17100). Following si-RNAs were used in this study: *si-LOXL2* (P/N: HSS180848, HSS106125; Invitrogen), and negative control miRNA/small-interfering RNA (P/N: AM17111; Applied Biosystems). The procedures of transfection of miRNAs and siRNAs were described as previously.[12, 13]

Selection of putative *miR-26a/b*, *miR-29a/b/c* and *miR-218* regulated targets in PCa cells

To identify putative targets of these miRNAs, we carried out a combination of *in silico* database analysis and comprehensive gene expression analysis, described as previous studies.[12, 13, 25] Our strategy for identification of putative targets is shown in Figure 1.

Immunohistochemistry and Western blotting

A tissue microarray containing PCa, prostatic intraepithelial neoplasias (PIN) and prostatic hyperplastic tissues was obtained from Provitro (Berlin, Germany) (Cat. #401 2209, Lot #146.1 P020212, 26-46). The information about these tissues can be found at http://www.provitro.com/fileadmin/provitro-data/TMA/4012209.pdf. Tissue immunostaining of LOXL2 and scoring method were described previously.[24, 25]
The procedures of LOXL2 Western blotting were described as previously.[12, 13, 25]

Cell proliferation, migration and invasion assays in prostate cancer cells

The functional significance of *LOXL2* in PCa cells, we investigated the knockdown effects of *LOXL2* on cell proliferation, migration and invasion assays using *si-LOXL2*-transfected PC3 and PC3M cells. These assays were described as previously.[12, 13, 25]

Plasmid construction and dual-luciferase reporter assays

Partial wild-type sequences of the *LOXL2* 3′-UTR or those with deleted sequences of miRNAs (*miR-26a/b*, *miR-29a/b/c* and *miR-218* binding sites) were inserted in the hRluc gene in the psiCHECK-2 vector (C8021; Promega, Madison, WI, USA). The procedure for the dual-luciferase reporter assay was described as previously.[12, 13, 25]

Statistical analysis

Relationships between 2 or 3 variables and numerical values were investigated using the Mann-Whitney U test or Bonferroni adjusted Mann-Whitney U test. Expert StatView software, version 4, was used in these analyses.

Results

Identification of candidate targets regulated by anti-tumor miRNAs in PCa cells

To identify target genes of the 6 miRNAs, we carried out a combination of *in silico* database analysis and genome-wide gene expression analysis. Seed sequences of these miRNAs, *miR-26a* and *miR-26b* are identical, and *miR-29a*, *miR-29b* and *miR-29c* are identical (Figure 1). First, we screened putative target genes using the TargetScan database Release 6.2 (http://www.targetscan.org/) and selected 334 genes that had putative binding sequences for these miRNAs in their 3′-UTRs. Next, we narrowed down the list of genes by oligomicroarray analysis using PC3 cells and found that 35 genes were downregulated ($\log_2$ ratio < 0) following transfection with *miR-26a* or *miR-29a* or *miR-218* as compared with expression levels in miR-control transfected cells (Table 1). In this study, we focused on *LOXL2* because many reports showed that this gene had a pivotal role in cancer metastasis. Furthermore, we previously reported that *miR-29a/b/c* acted as anti-tumor miRNAs via targeting of *LOXL2* in lung squamous cell carcinoma and renal cell carcinoma.[18, 20]

Direct regulation of *LOXL2* by anti-tumor miRNAs in PCa cells

First, we investigated qRT−PCR and Western blotting in PC3 and PC3M cells transfected with *miR-26a, miR-26b*, *miR-29a, miR-29b, miR-29c* or *miR-218* to investigate whether *LOXL2* expression was downregulated by restoration of these miRNAs. The expression levels of *LOXL2* mRNA and protein were markedly suppressed in transfected cells with these miRNAs (Figures 2A,B, 3A,B and 4A,B). Next, we performed luciferase reporter assays in PC3 cells to determine whether *LOXL2* mRNA had actual target sites for these miRNAs. We used vectors encoding either a partial wild-type sequence or a sequence in which the miRNA binding sequence was deleted from *LOXL2* mRNA. The luminescence intensity was reduced by co-transfection with *miR-26a, miR-26b*, *miR-29a, miR-29b, miR-29c*, *miR-218* and the vector carrying the wild-type LOXL2 3’-UTR in PC3 cells (Figures 2C, 3C and 4C).

Effects of knockdown of *LOXL2* on cell proliferation, migration and invasion in PCa cell lines

To investigate the cancer cell promoting role of *LOXL2*, we applied to loss-of-function assays using *si-LOXL2* transfectants. Knockdown efficiency of *si-LOXL2* was evaluated in PC3 and PC3M transfectant cells. Both mRNA and protein, expression levels of LOXL2 were downregulated in PC3 and PC3M cells (Figures 5A, 5B). XTT assays, wound-healing assays and invasion assays demonstrated that cell proliferation, migration and invasion activities were significantly suppressed in *si-LOXL2* transfectant cells (Figures 5C,D,E).

Immunostaining of *LOXL2* in PCa tissues

We validated the expression levels of *LOXL2* in PCa tissues using immunostaining. We used a tissue microarray containing 51 PCa, 10 prostatic intraepithelial neoplasias and 10 prostatic hyperplastic specimens. Upregulation of LOXL2 protein was confirmed in the PCa tissues compared with noncancerous tissues (Figure 6).

Discussion

Aberrantly expressed miRNAs might disturb normally regulated RNA networks and contribute to cancer cell pathology. Strategies to identify aberrant expression of miRNA-mediated RNA networks are being developed as a new direction in cancer research in the post-genome sequencing era. Based on the miRNA expression signature of PCa cells, we have continued the identification of tumour-suppressive miRNAs and their regulated novel PCa metastatic pathways.[10-13, 24-27] Our recent studies showed that restoration of anti-tumor miRNAs (*miR-26a*, miR-26*b*, *miR-29a, miR-29b, miR-29c* and *miR-218*) markedly suppressed PCa cell aggressiveness via targeting of La-related protein 1 (*LARP1*), laminin γ1 (*LAMC1*) and LIM and SH3 protein 1 (*LASP1*), respectively.[11-13] Moreover, these miRNA-targeted genes were overexpressed in PCa tissues and expression of these genes enhanced cancer cell migration and invasion.[11-13] LASP1 encodes a LIM motif at its N-terminus and a src homology 3 (SH3) domain at its C-terminus. In LASP1, the SH3 domain functions in protein-protein interactions[28, 29] and LASP1 acts as an actin-binding protein and overexpression of LASP1 enhancing cancer cell proliferation and invasion.[13, 28, 30] Our present data showed that *LASP1* is a putative candidate of these 6 anti-tumor miRNAs regulation, suggesting that LASP1 is a key molecule for cancer cell aggressiveness.

The *miR-26* family is comprised of three subtypes in the human genome: *miR-26a-1* (chromosome 3p22.2), *miR-26a-2* (12q14.1) and *miR-26b* (2q35). The seed sequences of these miRNAs are identical, suggesting that the *miR-26* family might regulate the same genes in human cells (miRBase: release 21; http://www.mirbase.org/). Downregulation of the *miR-26* family and its anti-tumor effects have reported in several cancers, such as bladder cancer, breast cancer, hepatocellular carcinoma, oral cancer and PCa.[31-34] Our past study showed that downregulation of these miRNAs enhanced cancer cell migration and invasion in oral cancer through direct regulation of

TMEM184B.[35] In PCa, EZH2, a histone-lysine N-methyltransferase enzyme, was directly regulated by *miR-26a* and *miR-26b*.[36] Overexpression of EZH2 is observed in several cancers and contributes to cancer aggressiveness.[36, 37]

The *miR-29* family consists of four miRNAs: *miR-29a*, *miR-29b-1*, *miR-29b-2* and *miR-29c*. These miRNAs are formed cluster miRNA on two human chromosome loci: *miR-29b-1* and *miR-29a* in 7q32 and *miR-29b-2* and *miR-29c* in 1q32 (miRBase: release 21; http://www.mirbase.org/). Our miRNA expression signatures showed that all member of *miR-29* family were downregulated in several cancers and that restoration of these miRNAs markedly inhibited cancer cell aggressiveness via targeting of ECM-integrin pathways.[12, 38] Anti-tumor roles of the *miR-29* family were demonstrated in several cancers.[12, 18, 20, 38, 39] Interestingly, recent studies demonstrated molecular mechanisms of silencing of *miR-29* family expression in cancer cells.[40] The genome structure of *miR-29b-1*/*miR-29a* promoter region contains two E-box (MYC-binding) sites and four NF-κB-binding sites such that overexpressed Myc and NF-κB inhibited the expression of *miR-29b-1*/*miR-29a* at the transcriptional level.[40] Overexpression of Myc was frequently observed in advanced PCa,[41] and this phenomenon might enhance PCa cell progression and metastasis.

The miRNA database indicates that the *miR-218* family is distributed between 2 human chromosomal loci: *miR-218-1* at 4p15.31 and *miR-218-2* at 5q35.1. Likewise *miR-26a*, *miR-26b* and *miR-29*-family, anti-tumor function of *miR-218* have been described in many types of cancers.[13, 42-44] Our past studies demonstrated that loss of *miR-218* enhanced cancer cell migration and invasion through dysregulation of genes involved in the focal adhesion pathway, including *CAV2*, *LAMA3* and *LASP1*.[13, 42, 44] Two miRNAs, *miR-218-1* and *miR-218-2,* are located on the introns of *SLIT2* and *SLIT3* genes, respectively, and expression control depends on the same promoter in their host genes.[45] Several reports showed that the promoter regions of these genes were frequently methylated in cancer cell lines and clinical specimens.[45, 46] Thus, hyper-methylation of their promoter regions

caused silencing of *miR-218-1* and *miR-218-2* expression in cancer cells.

The highly invasive properties of PCa cells cause distant metastasis in patients with PCa, and metastasis is the primary reason for the high mortality of advanced PCa.[1, 2] Studying the non-coding RNA networks could reveal the molecular mechanisms underlying metastatic pathways and facilitate the development of novel therapies to block progression of the disease. In this study, we hypothesized that several anti-tumor miRNAs (the *miR-26a/b*, the *miR-29* family and m*iR-218*) coordinately regulate genes that have key roles in PCa metastasis.

Here, we focused on the *LOXL2* gene as a putative regulatory target of these anti-tumor miRNAs in PCa cells. Recently, we showed that *LOXL2* was directly regulated by the *miR-29a, miR-29b and miR-29c* and its expression promoted cancer cell aggressiveness in renal cell carcinoma, non-small cell lung cancer and head and neck squamous cell carcinoma.[18-20] Present data demonstrated that 6 anti-tumor miRNAs (*miR-26a*, *miR-26b*, *miR-29a*, *miR-29b*, *miR-29c* and *miR-218*) directly regulated *LOXL2* in PCa cells. Moreover, overexpression of LOXL2 was confirmed in PCa tissues and knockdown of *LOXL2* markedly impaired cancer cell aggressiveness. Notably, we showed that LOXL2 regulation by these miRNAs was also observed in HNSCC cells.[20]

The lysyl oxidase (LOX) protein family is comprised of five proteins (LOX and LOXL1-L4). Their primary functions appear to be covalent crosslinking of collagen to elastin in the ECM.[14-17] Overexpression of the LOX family was observed in several cancers,[18-20] indicating that dysregulated expression of the LOX family enhances ECM deposition and subsequent tissue stiffness. Overexpression of ECM component proteins is frequently observed in many types of cancer tissues. This aberration promotes cancer cell aggressiveness by dysregulation of cell adhesion and ECM remodeling.[14] Thus, aberrant expression of the LOX family is a trigger of malignant transformation of cancer cells through ECM dysregulation. Several studies indicated that high expression LOXL2 was correlated with poor prognosis in patients with

gastric, breast, lung and laryngeal cancers.[19-22] Interestingly, transcriptional control of *LOXL2* was regulated by HIF1. LOXL2 directly interacts with transcriptional factor SNAIL1 in the nucleus and repressed expression of E-cadherin.[47, 48] Thus, hypoxic conditions induced LOXL2 expression, and the accumulating LOXL2 enhanced the aberrant activation of epithelial-mesenchymal transition (EMT) signaling in cancer cells.

In conclusion, direct regulation of *LOXL2* by anti-tumor miRNAs (*miR-26a, miR-26b*, *miR-29a*, *miR-29b*, *miR-29c* and m*iR-218*) was observed in PCa cells. Overexpression of LOXL2 was validated in PCa tissues and aberrantly expressed LOXL2 enhanced PCa cell aggressiveness. Understanding of novel RNA networks regulated by the anti-tumor miRNAs may lead to a better understanding of PCa metastasis.

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Disclosure statement

The authors declare that they have no conflicts of interest.

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Figure Legends

Figure 1.

Workflow of the strategy for selection of putative target genes regulated by tumor-suppressive miRNAs (*miR-26a/b*, *miR-29s* and *miR-218*) in PCa. We screened candidate targets using the TargetScan database. In total, 334 genes were identified as putative targets containing binding sites for *miR-26a/b*, *miR-29s* and *miR-218.* Among these, 35 genes were downregulated in *miR-26a*, *miR-29a* and *miR-218* transfected PC3 cells ($\log_2$ ratio < 0).

The sequences of *miR-26a/b*, *miR-29* family and *miR-218* miRNAs. Seed sequences are shown by red letters.

Figure 2.

Direct regulation of *LOXL2* by *miR-26a* and *miR-26b* in PCa cell lines.

(A) *LOXL2* mRNA expression 72 h after transfection with *miR-26a* or *miR-26b*. *GAPDH* expression was used for normalization. *, $P < 0.0001$.

(B) LOXL2 protein expression 72 h after transfection with *miR-26a/b*. GAPDH was used as a loading control.

(C) The *miR-26a* and *miR-26b* binding site in the 3’-UTR of *LOXL2* mRNA. Luciferase reporter assays were performed using vectors that included (WT) or lacked (DEL) the wild-type sequences of the putative *miR-26a* and *miR-26b* target site. *, $P < 0.0001$.

Figure 3.

Direct regulation of *LOXL2* by *miR-29a*, *miR-29b* and *miR-29c* in PCa cell lines.

(A) *LOXL2* mRNA expression 72 h after transfection with *miR-29s*. *GAPDH* expression was used for normalization. *, $P < 0.0001$.

(B) LOXL2 protein expression 72 h after transfection with *miR-29s*. GAPDH was used as a loading control.

(C) The *miR-29s* binding site in the 3’-UTR of *LOXL2* mRNA. Luciferase reporter assays were performed using vectors that included (WT) or lacked (DEL) the wild-type sequences of the

putative *miR-29s* target site. *, $P < 0.0001$.

Figure 4.
Direct regulation of *LOXL2* by *miR-218* in PCa cell lines.
(A) *LOXL2* mRNA expression 72 h after transfection with *miR-218*. *GAPDH* expression was used for normalization. *, $P < 0.0001$. **, $P = 0.0002$.
(B) LOXL2 protein expression 48 h after transfection with *miR-218*. GAPDH was used as a loading control.
(C) The *miR-218* binding site in the 3'-UTR of *LOXL2* mRNA. Luciferase reporter assays were performed using vectors that included (WT) or lacked (DEL) the wild-type sequences of the putative *miR-218* target site. *Renilla* luciferase assays were normalized to firefly luciferase values. *, $P < 0.0001$.

Figure 5.
Effects of si-*LOXL2* transfection on cell proliferation, migration, and invasion in PCa cell lines.
(A) *LOXL2* mRNA expression levels were measured by qRT-PCR 72 h after transfection with 10 nM *si-LOXL2*. *GAPDH* was used for normalization. *, $P < 0.0001$.
(B) LOXL2 protein expression 72 h after transfection with 10 nM *si-LOXL2*. GAPDH was used as a loading control.
(C) Cell proliferation was determined by XTT assay 72 h after transfection with 10 nM *si-LOXL2*. *, $P < 0.0001$.
(D) Cell migration activity was determined by migration assay 48 h after transfection with 10 nM *si-LOXL2*. *, $P < 0.0001$.
(E) Cell invasion activity was determined by Matrigel invasion assay 48 h after transfection with 10 nM *si-LOXL2*. *, $P < 0.0001$.

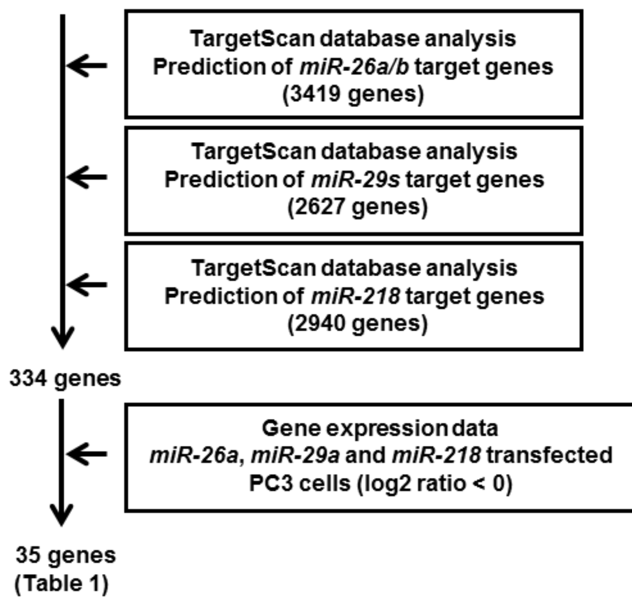
Figure 6.
Immunohistochemical staining of LOXL2 in PCa clinical specimens. LOXL2 was expressed more strongly in several cancer lesions, weakly stained in PIN lesions than in normal tissues. (A) Prostate cancer, pT4N0, Grade3a, Gleason score 3+4; (B) Prostate

cancer, pT2bN0, Grade3a, Gleason score 4+3; (C) PIN; (D) normal prostate tissue. (E) Quantification of LOXL2 expression. Expression of LOXL2 was upregulated in PCa and PIN specimens compared with normal prostate tissues.

Table 1.
Putative target genes regulated by *miR-26a*, *miR-29a* and *miR-218* in PCa cells.

Table 2.
Immunohistochemical status and characteristics of PCa, PIN and normal prostate cases.

Figure 1



Seed sequences

miR-26a	UUCAAGUAAUCCAGGAUAGGCU
miR-26b	UUCAAGUAAUUCAGGAUAGGU
miR-29a	UAGCACCAUCUGAAAUCGGUUA
miR-29b	UAGCACCAUUUGAAAUCAGUGUU
miR-29c	UAGCACCAUUUGAAAUCGGUUA
miR-218	UUGUGCUUGAUCUAACCAUGU

Figure 2

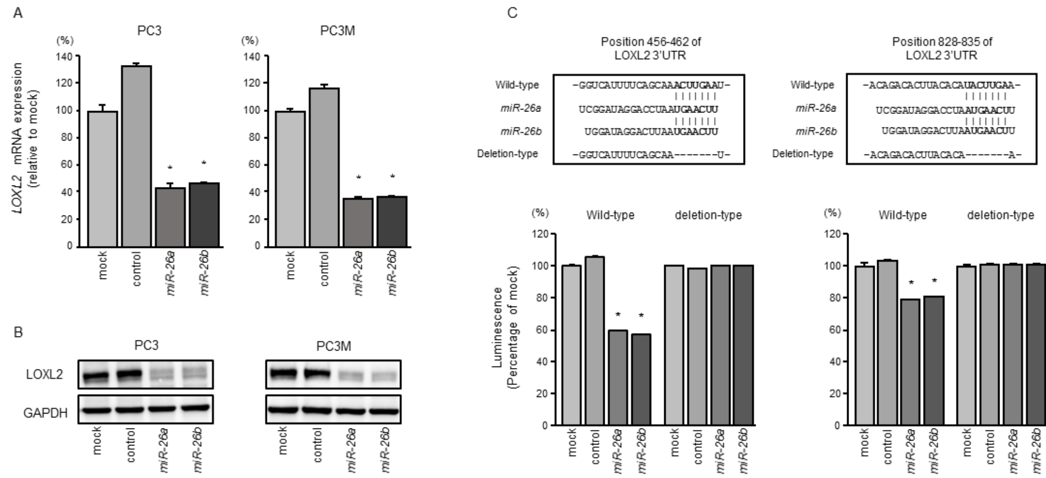


Figure 3

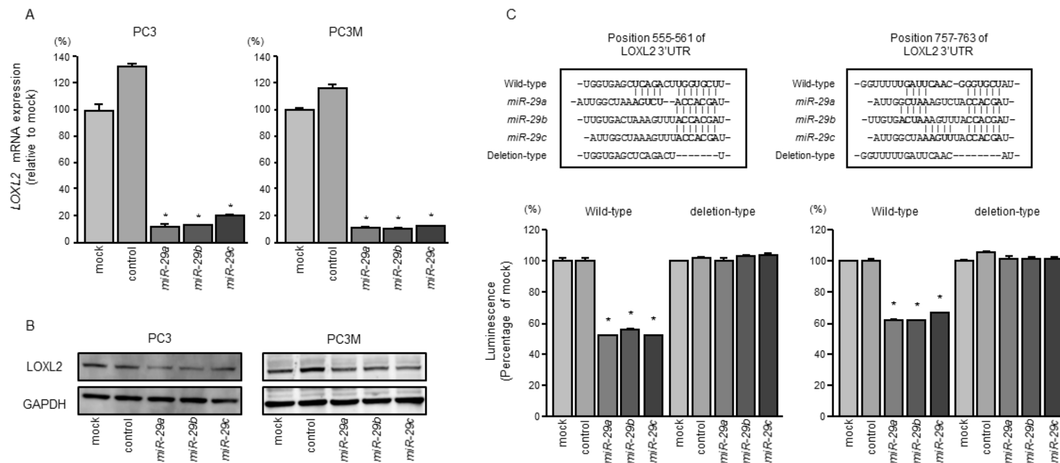


Figure 4

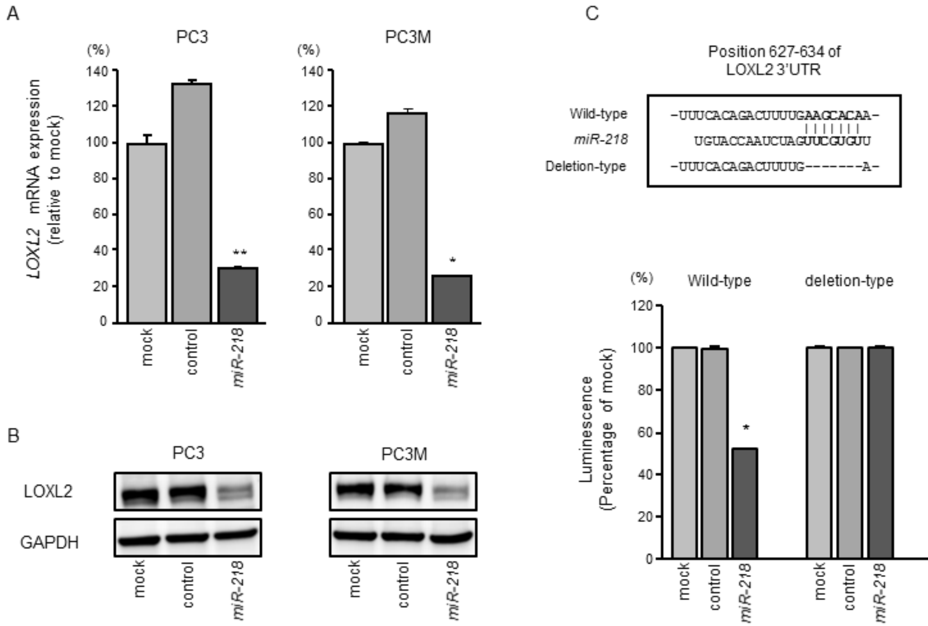


Figure 5

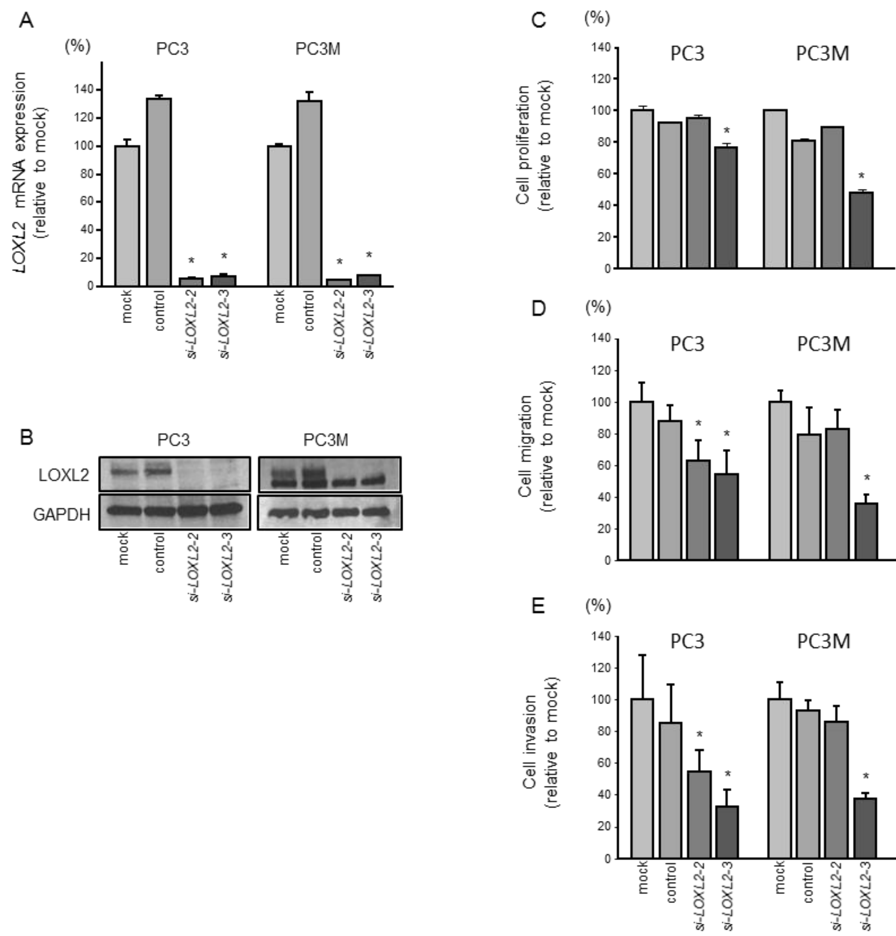


Figure 6

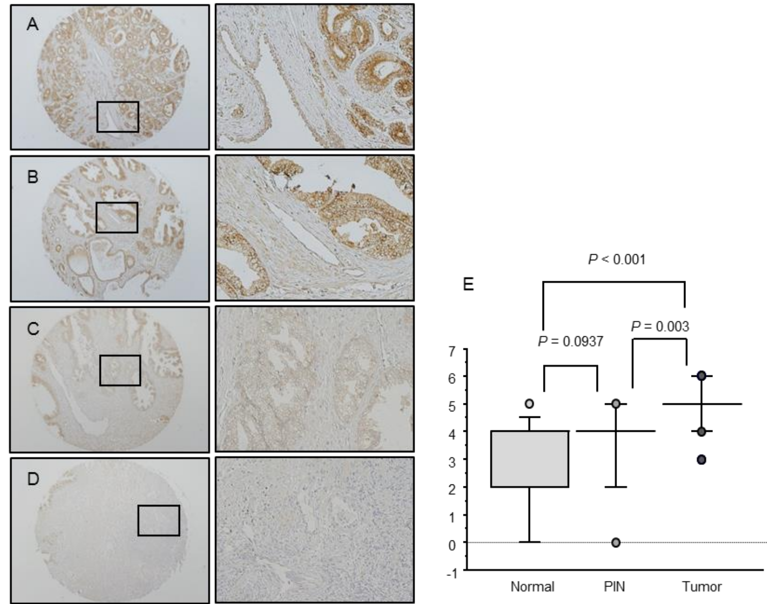


Table1. Putative target genes regulated by *miR-26a*, *miR-29a* and *miR-218* in PCa cells.

Gene symbol	Representative transcript	gene name	*miR-26a* transfection	*miR-29a* transfection	*miR-218* transfection	*miR-26ab*		*miR-29abc*		*miR-218*	
						conserved	poorly	conserved	poorly	conserved	poorly
HAS3	NM_001199280	hyaluronan synthase 3	-1.56	-2.10	-3.25	1	0	3	0	0	1
LOXL2	NM_002318	lysyl oxidase-like 2	-2.09	-2.18	-2.12	2	0	1	1	0	1
LASP1	NM_006148	LIM and SH3 protein 1	-0.51	-0.49	-1.96	1	0	2	0	1	2
SLC4A7	NM_003615	solute carrier family 4, sodium bicarbonate cotransporter, member 7	-0.30	-1.35	-1.95	0	1	0	1	0	1
LIF	NM_002309	leukemia inhibitory factor (cholinergic differentiation factor)	-1.02	-0.13	-1.91	1	0	1	0	0	1
VAMP7	NM_001145149	vesicle-associated membrane protein 7	-0.43	-1.24	-1.86	0	2	1	0	2	1
TRAK2	NM_015049	trafficking protein, kinesin binding 2	-0.18	-0.66	-1.67	0	1	0	1	0	1
RBBP9	NM_006606	retinoblastoma binding protein 9	-0.80	-0.32	-1.57	0	2	0	1	0	3
EMP2	NM_001424	epithelial membrane protein 2	-0.61	-0.32	-1.42	0	1	1	0	0	1
PTP4A1	NM_003463	protein tyrosine phosphatase type IVA, member 1	-0.73	-0.12	-1.05	1	0	1	0	2	0
ZHX3	NM_015035	zinc fingers and homeoboxes 3	-0.04	-0.88	-0.95	0	1	1	0	0	3
EPB41L1	NM_012156	erythrocyte membrane protein band 4.1-like 1	-0.57	-0.64	-0.73	0	1	0	2	1	0
SP100	NM_001080391	SP100 nuclear antigen	-0.30	-0.31	-0.72	0	1	0	1	0	1
LARP4B	NM_015155	La ribonucleoprotein domain family, member 4B	-0.54	-0.22	-0.69	2	0	1	0	1	0
VPS13D	NM_015378	vacuolar protein sorting 13 homolog D (S. cerevisiae)	-0.26	-1.31	-0.55	0	1	0	1	0	1
PBX2	NM_002586	pre-B-cell leukemia homeobox 2	-0.48	-0.53	-0.54	0	1	0	1	1	0
NRAS	NM_002524	neuroblastoma RAS viral (v-ras) oncogene homolog	-0.01	-0.71	-0.52	1	2	1	0	0	4
ZNF592	NM_014630	zinc finger protein 592	-0.19	-0.79	-0.47	0	1	0	1	0	1
MYO5A	NM_000259	myosin VA (heavy chain 12, myoxin)	-0.09	-0.34	-0.44	0	1	0	2	0	1
ACER3	NM_018367	alkaline ceramidase 3	-0.64	-0.02	-0.42	1	2	0	1	0	1
WDR33	NM_018383	WD repeat domain 33	-0.52	-0.24	-0.42	1	1	0	2	1	0
HDAC4	NM_006037	histone deacetylase 4	-0.69	-0.50	-0.37	0	1	1	0	0	1
PIKFYVE	NM_015040	phosphoinositide kinase, FYVE finger containing	-0.47	0.00	-0.27	0	3	0	1	0	1
DTWD2	NM_173666	DTW domain containing 2	-0.18	-0.71	-0.27	0	1	1	0	0	1
SERBP1	NM_001018067	SERPINE1 mRNA binding protein 1	-1.25	-0.73	-0.27	2	1	1	0	1	0
RBM33	NM_053043	RNA binding motif protein 33	-0.34	-0.72	-0.27	0	1	0	1	0	1
REPS2	NM_001080975	RALBP1 associated Eps domain containing 2	-0.76	-0.76	-0.24	1	1	1	0	2	2
GTF2H5	NM_207118	general transcription factor IIH, polypeptide 5	-0.10	-0.02	-0.21	0	1	0	1	0	1
CACNA1C	NM_000719	calcium channel, voltage-dependent, L type, alpha 1C subunit	-0.15	-0.02	-0.20	1	1	0	2	0	1
EXD2	NM_001193360	exonuclease 3'-5' domain containing 2	-1.16	-0.03	-0.15	0	1	0	1	1	0
USP31	NM_020718	ubiquitin specific peptidase 31	-0.88	-0.56	-0.14	0	1	1	0	1	1
TDP1	NM_001008744	tyrosyl-DNA phosphodiesterase 1	-0.41	-0.08	-0.14	0	1	0	1	0	1
YPEL1	NM_013313	yippee-like 1 (Drosophila)	-0.24	-0.37	-0.14	1	3	0	1	0	1
IQCJ	NM_001042706	IQ motif containing J	-0.13	-0.11	-0.14	0	1	0	1	0	1
LOX	NM_001178102	lysyl oxidase	-0.25	-1.06	-0.03	0	1	3	0	1	1

Table 2. Clnical characteristics and IHC score of LOXL2 in tissue microarray

No.	Diagnosis	Age	Gleason score	Stage pT	Stage pN	IHC score of LOXL2
1	PCa	64	4+3	3b	0	5
2	PCa	67	3+4	2b	0	5
3	PCa	58	3+4	2b	0	5
4	PCa	63	7	3b	0	6
5	PCa	65	3+3	2b	0	5
6	PCa	61	4+4	3b	×	5
7	PCa	62	3+4	2b	×	4
8	PCa	66	4+4	2b	×	4
9	PCa	61	3+4	3a	×	4
10	PCa	74	4+3	2b	×	5
11	PCa	54	3+4	2c	×	5
12	PCa	68	3+4	3a	0	5
13	PCa	58	3+4	3a	0	5
14	PCa	65	3+3	2a	0	5
15	PCa	77	3+4	4	0	5
16	PCa	58	3+4	3a	0	5
17	PCa	50	4+3	2b	0	5
18	PCa	53	3+3	2b	0	5
19	PCa	59	4+5	3a	0	5
20	PCa	70	2+3	2b	0	5
21	PCa	65	5+4	3a	0	4
22	PCa	57	3+5	2b	0	4
23	PCa	68	4+4	2b	0	5
24	PCa	58	3+3	2b	0	5
25	PCa	63	3+4	2b	0	5
26	PCa	56	3+4	2b	0	5
27	PCa	63	5+3	3a	0	3
28	PCa	64	3+5	3a	0	5
29	PCa	60	3+4	2b	0	5
30	PCa	60	3+3	3a	0	4
31	PCa	57	3+2	2b	0	4
32	PCa	50	3+3	2a	0	5
33	PCa	68	3+3	3a	0	4

34	PCa	65	3+4	3b	1	3
35	PCa	69	5+5	3a	1	3
36	PCa	51	2+3	2b	0	4
37	PCa	62	3+3	3a	0	5
38	PCa	61	3+4	3a	0	4
39	PCa	53	4+4	3b	1	3
40	PCa	56	4+3	2b	0	4
41	PCa	59	2+3	2b	0	3
42	PCa	61	3+4	2b	0	4
43	PCa	62	3+4	3b	1	3
44	PCa	66	3+3	3a	0	5
45	PCa	62	3+3	2b	0	5
46	PCa	56	3+3	2b	0	5
47	PCa	58	3+3	3a	0	5
48	PCa	66	5+4	3a	0	4
49	PCa	55	3+4	3a	0	4
50	PCa	67	2+3	2b	0	4
51	PCa	61	3+5	2b	0	4
52	PIN	59	-	-	-	0
53	PIN	58	-	-	-	2
54	PIN	62	-	-	-	0
55	PIN	51	-	-	-	2
56	PIN	58	-	-	-	3
57	PIN	68	-	-	-	3
58	PIN	64	-	-	-	4
59	PIN	56	-	-	-	5
60	PIN	61	-	-	-	3
61	PIN	51	-	-	-	4
62	non-PCa	70	-	-	-	4
63	non-PCa	63	-	-	-	0
64	non-PCa	62	-	-	-	2
65	non-PCa	81	-	-	-	0
66	non-PCa	67	-	-	-	2
67	non-PCa	76	-	-	-	0
68	non-PCa	66	-	-	-	3
69	non-PCa	69	-	-	-	3

70	non-PCa	63	-	-	-	3
71	non-PCa	71	-	-	-	4

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