Identification of potential targets of microRNA-101-3p in prostate cancer by bioinformatics analysis

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Research

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Abstract

Background: MiR-101-3p, a tumor suppressor, has been implicated as a tumor suppressor miRNA in multiple primary malignancies including prostate cancer (PCa). This study aimed to explore target genes and relevant signaling pathways regulated by microRNA-101-3p (miR-101-3p) for further researches in PCa with bioinformatics analysis.

Results: 565 target genes were appeared in all databases and enriched in positive regulation of transcription, which were mainly enriched in axon guidance and MAPK pathway. Two important modules were detected from PPI network. Ten hub genes were selected, including MAPK1, PIKfyve, EGFR, SMARCA4, TOP2B, GSK3B, FOS, RAC1, BCL2 and TAF1. After thoroughly reviewing published literature, we found that 10 target genes and six signaling pathways were truly inhibited by miR-101-3p in various tissues or cells; some of these verified targets were in accordance with our present prediction.

Conclusion: This study demonstrated that miR-101-3p target hub genes, including MAPK1, PIKfyve, EGFR, SMARCA4, TOP2B, GSK3B, FOS, RAC1, BCL2 and TAF1, might promote the development of PCa. However, further experiments are still required to confirm potential functions of these miR-101-3p target genes and pathways in PCa.

Introduction

Prostate cancer (PCa) is the most common malignant cancer and the second leading cause of cancer-related death in men worldwide [1]. In recent years, the number of PCa patients has been significantly increased in China, which may be due to the huge population, ageing, changes of environment and life styles [2]. In the past few decades, improvements in screening, diagnostics and treatment have led to a consistent decrease in PCa mortality and an increase in overall survival rate [3]. However, more than 650,000 men are diagnosed with PCa annually, and this constitutes almost 10% of all new cancer cases in men worldwide [4]. Thus, exploring novel target genes, molecular mechanisms and generating therapeutic approaches are urgently needed.

MicroRNAs (MiRNAs) are small, noncoding, single-stranded RNAs of ~ 22 nucleotides that negatively regulate gene expression at the posttranscriptional level, primarily through base pairing to the 3' untranslated region (UTR) of target mRNAs [5]. MiR-101-3p, a tumor suppressor, has been implicated as a tumor suppressor miRNA in a number of malignancies, such as non-small-cell lung cancer (NSCLC) [6], breast cancer [7], and glioma [8]. However, only few studies have assessed such a connection between miR-101-3p and its target genes in PCa [9]. Thus, it is important to identify comprehensive downstream target genes of miR-101-3p with bioinformatics analysis in PCa, which may be helpful for future therapy development of PCa. In the present study, we sought to unveil the role of miR-101-3p in PCa through identification of putative molecular targets by bioinformatics analysis. Meanwhile, we established PPI network of miR-101-3p target genes and picked out hub genes with high degree of connectivity. Besides, analysis of biological process (BP), molecular function (MF), cellular component (CC) and Kyoto

Encyclopedia of Genes and Genome (KEGG) pathways of miR-101-3p target genes and two modules were performed. Furthermore, the direct downstream targets and relevant signaling pathways regulated by miR-101-3p in PCa were extracted in the published literature.

Results

Prediction of target genes for miR-101-3p

As shown in Fig. 1, the number of predicted target genes of miR-101-3p in miRWalk, miRanda, RNA22, and Targetscan databases was 12106, 5426, 4250 and 10540, respectively. There were 10795 target genes supported by at least two databases, 4461 target genes predicted by at least three databases and 565 target genes appeared in all four databases. The target genes of miR-101-3p predicted in all four databases are listed in Table 1 and were used for further analyses.

Table 1

The target genes of miR-101-3p predicted in all four databases

ABCC5 ABHD17B ABHD17C ACSS2 ADAMTS12 ADAMTS17 ADAMTS3 ADAMTSL3 ADCYAP1 ADRB1 AFAP1L2 AFF4
AGPS AKAP11 AMER2 AMIGO2 ANK3 ANKRD50 ANKRD52 AP3D1 AP3M2 ARAP2 ARFIP1 ARHGEF10
ARHGEF3 ARID1A ARID5B ARL2BP ARL4C ASAP1 ASXL2 ATP2B2 ATP8A1 ATRX ATXN1 BAAT
BBX BCKDHB BCL2 BCL2L11 BDP1 BEGAIN BICD2 BMP6 BMPR2 BRD3 BTBD3 C11orf30
C11orf87 C17orf102 C17orf51 C3orf38 C4orf32 C7orf49 C8orf76 C9orf72 CACNB2 CACNB4 CACUL1 CAMK2D
CAPN14 CASP3 CBFA2T2 CBWD6 CBX5 CCDC170 CCNG2 CCNT2 CCP110 CDH5 CDH6 CDK6
CDS2 CDYL CELF2 CEP350 CEP63 CEP85L CHAC2 CHM CHST11 CLEC7A CMIP CNNM4
CNOT11 CNOT6L COG5 COL10A1 COL12A1 COL4A3BP COTL1 CPEB3 CREBRF CRLS1 CSRNP3 CSRP2
CTDSPL CTTNBP2 CXADR CYB561D2 DAZAP2 DCAF5 DCAF7 DCBLD2 DCP2 DCUN1D3 DGCR2 DIP2B
DIXDC1 DLG5 DLGAP3 DMXL2 DNAJC15 DNM1L DPY19L2 DR1 DSC1 DUSP1 DYRK1A EAF1
EBAG9 EBF2 EFNA5 EGFR EIF5 EIF5A2 ELF5 EMP1 EMP2 ENY2 EPHA7 EPT1
ERBB2IP ERBB4 ERLIN2 ERO1LB ESCO1 EVI5 EXOC5 EYA1 EZH2 FAM114A1 FAM169A FAM179B
FAM199X FAM203A FAM222A FAM46A FAM73A FAM78A FAR1 FAT3 FAXDC2 FBN2 FEM1C FGA
FKTN FLVCR1 FOS FOXN3 FREM1 FRYL FUT1 FYTTD1 FZD6 GABBR2 GABRA2 GABRA4
GABRB2 GCC2 GDE1 GFRA1 GID4 GLCCI1 GLTSCR1 GOLGA8B GRAMD1C GRIN2A GRSF1 GSK3B
GZF1 HDAC8 HEG1 HELZ HIVEP3 HMBOX1 HMGB3 HNF1B HSD11B2 HTRA3 ICK IFFO2
IGFBP5 ING3 INO80D INPP4B IPMKIPO11 IPO5 IPO9 IRF2BP2 ITGA2 JAK2 JAKMIP3
JDP2 JHDM1D KAT6B KAT7 KBTBD8 KCNJ3 KCTD12 KIAA0556 KIAA1377 KIAA1462 KIAA1549 KIAA1598
KIAA1737 KIAA1804 KIAA1841 KIAA1958 KIDINS220 KIF11 KIF1A KIF3A KLF3 KLF6 KLF7 KMT2A

ABCC5 ABHD17B ABHD17C ACSS2 ADAMTS12 ADAMTS17 ADAMTS3 ADAMTSL3 ADCYAP1 ADRB1 AFAP1L2 AFF4
KPNA1 KRBOX4 KTI12 LANCL3 LCORL LGALSL LGI2 LIMS1 LIN28B LONRF2 LPIN2 LRAT
LRP2 LRRRC8B LRRN1 MAGI2 MAK MAP3K13 MAP3K2 MAP3K7 MAP9 MAPK1 MAST4 MBNL1
MCF2L MCL1 MECP2 MED13 MED23 MED6 METAP1 MEX3B MGAT4A MGAT5 MIEF1 MKLN1
MMGT1 MMP15 MOB4 MON2 MORN4 MPP5 MPP7 MRGBP MRPS9 MTFR1 MTMR1 MTMR9
MXD1 MYCN MYRIP MYSM1 NAA15 NAA25 NACC2 NCEH1 NDC1 NDUFB5 NEK4 NEK7
NEUROD1 NFAT5 NFIB NKTR NLK NONO NOS1 NOVA1 NPNT NR4A3 NR6A1 NRP1
NRP2 NRXN3 NSD1 NUDT11 NUDT16 NUDT21 NUFIP2 NUMB NUP62CL NUPL2 ONECUT2 OPA3
ORAI2 OTUD3 PAFAH1B1 PALM2 PAPOLB PBX3 PCDH20 PCDH7 PCDH8 PCDHB14 PDE4A PDE4D
PDP1 PDPK1 PDS5B PEAK1 PEBP1 PGR PHACTR2 PHF20L1 PHTF1 PIK3C2B PIKFYVE PIP5K1C
PITPNB PLCB1 PLCG1 PLEKHA1 PLEKHH2 PLXNA1 PLXNB1 POMP POP1 POU2F1 POU3F2 PPARGC1B
PPFIA1 PPFIA4 PPIG PPM1A PPP1R8 PPP6C PRICKLE1 PRKAA1 PRKD3 PRKG1 PROK2 PROX1
PRRC2CPRRG4 PSEN1 PSMD9PTBP3 PTCH1 PTER PTGER4 PTGS2 PUM2 QKI RAB11FIP4
RAB1A RAB27A RAB3GAP1 RAB3GAP2 RAC1 RALGPS2 RAP1B RAP2B RAPH1 RASD2 RASGRF2 RASGRP3
RBM25 RBM33 RC3H1 RC3H2 RCN RFX3 RFX4 RGS1 RHOU RIMBP2 RIN2 RNF213
RNF38 ROBO2 RPL10L RSF1 RXRB RYR3 SACM1L SCN2A SCNN1G SDC2 SEMA3A SEMA3G
SENP6 SERPINB8 SERTAD2 SETD5 SGMS2 SGPL1 SH2B3 SIK3 SIX4 SLC12A2 SLC19A2 SLC1A1
SLC22A23 SLC26A2 SLC2A11 SLC2A13 SLC30A7 SLC33A1 SLC35D1 SLC36A1 SLC39A10 SLC39A6 SLC5A3 SLC7A11
SLC9A2 SLIT1 SLIT3 SLMO2 SMAD2 SMAD9 SMARCA1 SMARCA4 SOAT1 SOCS2 SOCS5 SOGA3
SORL1 SOX11 SOX6 SP2 SPAG9 SPARC SPATA2 SPCS2 SPG11 SPN SPRED1 SRGAP1
SRRSRSF6 SSX2IP ST7 STAMBP STC1 STC2 STK4 STX6 STYX SUB1 SULT4A1

ABCC5 ABHD17B ABHD17C ACSS2 ADAMTS12 ADAMTS17 ADAMTS3 ADAMTSL3 ADCYAP1 ADRB1 AFAP1L2 AFF4
SURF4 TAF1 TAF5L TAGAP TAPT1 TBC1D12 TCF4 TCP11L2 TEAD1 TEAD3 TET2 TEX2
TFIP11 TGFA TGFBR1 TGFBR3 THRB TIA1 TK2 TLR6 TMED5 TMEFF1 TMEM170B TMEM182
TMEM260 TMEM50B TMEM64 TMF1 TMOD2 TMTC1 TMX3 TNFAIP1 TNFRSF11A TNFSF4 TNKS2 TNPO1
TNRC18 TNS3 TOP2B TRERF1 TRIM2 TRIM24 TRPS1 TSPAN6 TULP4 UBE2D3 UBE2F UBE2G1
UBE2K UBN2 UBR7 UGGT1 UGT2B4 UHMK1 UNC79 UNKL USP47 VAPB VPS13C VPS53
WDR36 WDR72 WNK3 WNT9B XPO4 YAP1 ZBTB33 ZBTB34 ZC3H11A ZC3H13 ZC3HAV1 ZEB1
ZFHX4 ZMYM2 ZMYND12 ZNF148 ZNF207 ZNF24 ZNF260 ZNF33A ZNF33B ZNF451 ZNF510 ZNF557
ZNF645 ZNF746 ZNF770 ZNHIT6 ZNRF2 ZSWIM6

GO enrichment analysis for predicted target genes of miR-101-3p

GO enrichment analysis was conducted for the target genes of miR-101-3p predicted by all four databases. As shown in Fig. 2 and Table 2, GO analysis results showed that these target genes were particularly enriched in biological processes, including positive regulation of transcription, intracellular signal transduction, protein autophosphorylation, activation of MAPKK activity, and so on. For cell component, they were enriched within both nucleoplasm and cytoplasm. In addition, GO molecular function also displayed that miR-101-3p target genes were enriched in protein binding, transcription coactivator activity, transcription factor activity and sequence-specific DNA binding.

Table 2
Gene ontology (GO) analysis for predicted target genes of miR-101-3p

Term	Count	P-value	Genes
Molecular functions			
GO: 0005515 ~ protein binding	317	2.78E-07	MECP2 TMED5 PTGER4 KLF6 POMP SLC7A11 TAF1 MAGI2. . .
GO: 0003713 ~ transcription coactivator activity	22	1.68E-05	YAP1 MED13 SMARCA4 MYSM1 RXRB KLF7 ARL2BP TAF5L. . .
GO: 0003700 ~ transcription factor activity sequence-specific DNA binding	49	2.88E-04	TEAD1 ZNF24 TEAD3 MECP2 TRPS1 RFX3 TRERF1 ZNF746. . .
GO: 0017137 ~ Rab GTPase binding	13	6.50E-04	RAB3GAP1 RAB11FIP4 DNM1L TBC1D12 C9orf72 MYRIP BICD2 CHM. . .
GO: 0003682 ~ chromatin binding	25	6.52E-04	EZH2 YAP1 NONO MECP2 TRPS1 EGFR DNA RFX4. . .
GO: 0000978 ~ RNA polymerase II core promoter proximal region sequence-specific DNA binding	23	9.69E-04	TEAD1 PGR SMARCA4 ZNF148 NACC2 EBF2 RFX4 RFX3. . .
GO: 0046872 ~ metal ion binding	85	1.65E-03	GDE1 NEK7 ZNF148 KLF6 ZC3HAV1 PPP6C TRERF1 CLEC7A. . .
GO: 0070577 ~ lysine-acetylated histone binding	5	1.68E-03	TRIM24 SMARCA4 BRD3 TAF1 KMT2A
GO: 0004674 ~ protein serine/threonine kinase activity	23	2.01E-03	DYRK1A WNK3 NLK SIK3 NEK7 CAMK2D GSK3B PDPK1. . .
GO: 0030165 ~ PDZ domain binding	9	3.98E-03	SRR ATP2B2 ADRB1 CXADR SDC2 PLEKHA1 PSEN1 TGFB3 KIDINS220
Cellular components			
GO: 0005654 ~ nucleoplasm	117	1.19E-05	EZH2 NEK7 POMP TAF1 NR6A1 NR4A3 MPP5 CAPN14. . .
GO: 0005667 ~ transcription factor complex	18	4.13E-05	YAP1 TRPS1 RFX3 TAF1 TRERF1 NR6A1 PBX3 NR4A3. . .

Term	Count	P-value	Genes
GO: 0005634 ~ nucleus	192	3.22E-04	EZH2 MECP2 HMGB3 KLF6 POMP TAF1 MAGI2 NR6A1...
GO: 0005901 ~ caveola	9	5.10E-04	BMPR2 EFNA5 EMP2 PTGS2 NOS1 PTCH1 MAPK1 JAK2...
GO: 0005643 ~ nuclear pore	9	1.02E-03	XPO4 KPNA1 EIF5A2 NUPL2 NUP62CL BICD2 ENY2 IPO5...
GO: 0005794 ~ Golgi apparatus	42	1.04E-03	GCC2 RAB27A JAKMIP3 TMED5 RAB3GAP1 ZNF148 GOLGA8B STX6...
GO: 0005737 ~ cytoplasm	181	1.73E-03	EZH2 ZMYM2 NEK7 HMGB3 GOLGA8B RNF213 POMP MAGI2...
GO: 0002116 ~ semaphorin receptor complex	4	3.23E-03	NRP2 NRP1 PLXNA1 PLXNB1
GO: 0005829 ~ cytosol	120	3.83E-03	ZMYM2 GCC2 MECP2 RNF213 POMP PPP6C ANK3 BTBD3...
GO: 0031092 ~ platelet alpha granule membrane	4	5.36E-03	TMX3 PCDH7 PHACTR2 SPARC
Biological processes			
GO: 0009791 ~ post-embryonic development	15	2.52E-08	BCL2 MECP2 TAPT1 HEG1 PLEKHA1 PSEN1 CHST11 RC3H2...
GO: 0045893 ~ positive regulation of transcription DNA-templated	41	2.89E-08	AFAP1L2 SMARCA1 MED13 PLCB1 MECP2 SMARCA4 KLF6 PSEN1...
GO: 0035556 ~ intracellular signal transduction	30	1.08E-05	PLCB1 TNS3 GSK3B PSEN1 ICK ARHGEF3 DUSP1 SOCS2...
GO: 0018107 ~ peptidyl-threonine phosphorylation	9	1.26E-05	DYRK1A BCL2 CAMK2D GSK3B PDPK1 TAF1 TGFB1 MAPK1...
GO: 0045944 ~ positive regulation of transcription from RNA polymerase II promoter	53	3.44E-05	ADCYAP1 MED13 SMARCA4 ZNF148 RFX4 TAF1 RFX3 NR6A1...

Term	Count	P-value	Genes
GO: 0046777 ~ protein autophosphorylation	17	5.47E-05	DYRK1A ERBB4 WNK3 PEAK1 NLK CAMK2D EGFR GSK3B. . .
GO: 0000186 ~ activation of MAPKK activity	9	5.47E-05	MAP3K13 PLCG1 EGFR PSEN1 MAP3K7 TGFBR1 MAP3K2. . .
GO: 0006351 ~ transcription DNA-templated	87	1.07E-04	ZMYM2 EZH2 SMARCA1 MECP2 HMGB3 SMARCA4 ING3 KLF6
GO: 0045892 ~ negative regulation of transcription DNA-templated	31	2.07E-04	EZH2 ZNF24 PLCB1 MECP2 SMARCA4 ZNF148 TNFSF4 NACC2. . .
GO: 0001822 ~ kidney development	11	2.26E-04	ARID5B PROX1 ROBO2 BCL2L11 TGFBR1 SOX11 TET2 BMP6. . .

KEGG pathway analysis for predicted target genes of miR-101-3p

As shown Table 3, the top rankings were related to axon guidance, MAPK signaling pathway, non-small cell lung cancer, colorectal cancer, neurotrophin signaling pathway, pathways in cancer, proteoglycans in cancer, inositol phosphate metabolism and adherens junction (all $P < 0.05$). Among them, axon guidance, MAPK signaling pathway, pathways in cancer and adherens junction were well known to be associated with the pathogenesis of PCa. Figure 3 shows the rich factor, Q value, and gene number corresponding to each pathway term.

Table 3

Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis for predicted target genes of miR-101-3p

Term	ID	Count	P-value	Genes
Axon guidance	hsa04360	14	3.14E-05	EFNA5 EPHA7 ROBO2 PLXNA1 GSK3B SRGAP1 PLXNB1 SEMA3G. . .
MAPK pathway	hsa04010	18	4.50E-04	CACNB2 RAP1B CACNB4 NLK EGFR RASGRF2 MAP3K7 CASP3. . .
Non-small cell lung cancer	hsa05223	8	6.73E-04	CDK6 PLCG1 STK4 TGFA EGFR RXRB PDPK1 MAPK1
Colorectal cancer	hsa05210	8	1.25E-03	BCL2 GSK3B FOS TGFB1 RAC1 CASP3 MAPK1 SMAD2
Neurotrophin pathway	hsa04722	11	1.34E-03	BCL2 RAP1B PLCG1 CAMK2D GSK3B PSEN1 PDPK1 KIDINS220. . .
Pathways in cancer	hsa05200	22	1.80E-03	WNT9B ITGA2 PLCB1 BCL2 TGFA PTGS2 PTGER4 EGFR. . .
Proteoglycans in cancer	hsa05205	14	2.65E-03	ERBB4 ITGA2 WNT9B CAMK2D EGFR PDPK1 PTCH1 CASP3. . .
Prolactin signaling pathway	hsa04917	8	2.76E-03	SOCS2 TNFRSF11A GSK3B FOS SOCS5 ELF5 MAPK1 JAK2
Inositol phosphate metabolism	hsa00562	8	2.76E-03	PLCB1 IPMK PLCG1 MTMR1 PIP5K1C INPP4B PIK3C2B PIKFYVE
Adherens junction	hsa04520	8	2.76E-03	SSX2IP NLK EGFR MAP3K7 TGFB1 RAC1 MAPK1 SMAD2

Hub genes and module screening from PPI network

STRING database and Cytoscape software were used construct the PPI network based on the predicted miR-101-3p targets, nodes with top ten degrees were defined as hub genes, including MAPK1 (n = 47), PIKFYVE (n = 47), EGFR (n = 43), SMARCA4 (n = 41), TOP2B (n = 41), GSK3B (n = 34), FOS (n = 30), RAC1(n = 30), BCL2 (n = 27) and TAF1 (n = 27). Then, we made the PPI network of these top 10 hub genes with higher degree of connectivity (Fig. 4A). In addition, we also used MCODE plug-in and selected top two modules in order to detect significant modules in PPI network of miR-101-3p target genes (Fig. 4b and 4c). KEGG pathway enrichment analysis showed that these two modules were mainly associated with some proliferation-related pathways, such as PI3K-Akt signaling pathway, ErbB signaling pathway and Ras signaling pathway.

Screening target genes and signaling pathways inhibited by miR-101-3p on PCa in published studies

A comprehensive electronic search of PubMed and Web of Science databases was performed until November 1, 2019, to obtain target genes and signaling pathways modulated by miR-101-3p in published studies. Finally, 13 papers including ten target genes and six signaling pathways inhibited by miR-101-3p were obtained; most of them focus on the functions of miR-101-3p suppressing tumor growth, migration, and invasion in PCa tissues and cells. The details were shown in Table 4.

Table 4

Target genes and signaling pathways modulated by miR-101 on prostate cancer in published studies.

Author (Year)	Target gene	Inhibited pathways	Associated functions	Cell or tissue types
Qiu X (2019)	EZH2	NA	Overcome docetaxel resistant	PCa cell lines
Lo (2019)	ZEB1 and Slug	NA	Inhibit epithelial-to-mesenchymal transition	PCa cell lines
Antognelli (2018)	Glyoxalase 1	TGF- β 1/Smad	Suppress cell metastasis	PCa cell lines
Martino (2018)	SOD1	ROS	Suppress cell proliferation	PCa cell lines
Chakravarthi (2016)	SUB1	NA	Reduce cell proliferation and migration	PCa cell lines and human PCa tissues
Huang (2017)	TIGAR	Pentose phosphate	Inhibit viability and induced apoptosis	PCa cell lines
Lin (2016)	NR2F2	NR2F2-FOXN1-CENPF	Inhibit metastasis and drug resistance	PCa cell lines and human PCa tissues
Yang (2015)	RLIP76	PI3K-Akt	Promote cell apoptosis	PCa cell lines
Li (2013)	EZH2	NA	Inhibit proliferation and induce apoptosis	Prostate cancer stem cells
Keller (2012)	EZH2	Histone modification	Suppress cell progression and metastasis	PCa cell lines
Hao (2011)	COX-2	NA	Suppresses cell proliferation	PCa cell lines and mice PCa model
Cao (2010)	EZH2	NA	Suppress cell proliferation and migration	PCa cell lines
Varambally (2008)	EZH2	NA	Suppress tumor growth and metastasis	PCa cell lines and human PCa tissues
PCa: prostate cancer; EZH2: enhancer of zeste homolog 2; SOD1: superoxide dismutase 1; SUB1: SUB1 homolog; TIGAR: tp53-induced glycolysis and apoptosis regulator; NR2F2: also COUP-TF2: chicken ovalbumin upstream promoter-transcription factor II; FOXN1: forkhead box M1; CENPF: centromere protein F; RLIP76: rala binding protein 1; COX-2: cyclooxygenase-2; NA: not available.				

Discussion

PCa is currently the most commonly diagnosed cancer and the second leading cause of cancer death in men in the United States [10]. Despite recent progress in the identification of genetic and molecular

alterations in PCa, the global management and life quality of PCa patients are still far from ideal. Thus, understanding the molecular mechanism involved in PCa is still extremely important to develop better effective diagnostic and therapeutic strategies. In the present study, we identified 565 target genes of miR-101-3p using bioinformatics analysis. Among these genes, MAPK1, PIKFYVE, EGFR, SMARCA4, TOP2B, GSK3B, FOS, RAC1, BCL2 and TAF1 were defined as hub genes that might provide new ideas for further studies in PCa.

To date, there have been fewer studies concerning the characteristics of miR-101-3p in PCa. Varambally was the first to report that the expression of miR-101-3p was lower in clinically localized PCas and metastatic PCas, and one of miR-101-3p target gene, EZH2, tended to be uniformly elevated in samples with the miR-101-3p genomic loci copy number loss [11]. Later, studies further confirm that miR-101-3p targets EZH2 and suppresses proliferation and migration of PCa cells [12, 13]. After thoroughly reviewing published literature on PCa, we found that ten target genes including SOD1 [14], SUB1 [15], TIGAR [16], NR2F2 [17], RLIP76 [9], COX-2 [18], Glyoxalase 1 [19], ZEB1 [20], Slug [20] as well as EZH2 [11, 13, 21, 22] were truly inhibited by miR-101-3p in PCa tissues or cells. Furthermore, we found that the target genes of miR-101-3p were enriched in many biological processes, such as positive regulation of transcription, intracellular signal transduction, protein autophosphorylation, activation of MAPKK activity. In KEGG pathway analysis, miR-101-3p target genes were mainly located in MAPK signaling pathway, non-small cell lung cancer, colorectal cancer, neurotrophin signaling pathway, pathways in cancer, proteoglycans in cancer, inositol phosphate metabolism and adherens junction.

In the present study, we further identified that candidate target genes for miR-101-3p were involved in the regulation of crucial biological processes in PCa, including MAPK1, PIKFYVE, EGFR, SMARCA4, TOP2B, GSK3B, FOS, RAC1, BCL2 and TAF1. MAPK1 is involved in a number of biochemical signals and cellular processes such as proliferation, differentiation, transcription regulation and development of various cancers [23–25]. Chen demonstrate that miR-378 inhibits PCa cell growth through directly suppresses of MAPK1 in vitro and in vivo [26]. PIKFYVE, a lipid kinase that converts PI(3)P into PI(3,5)P2 in the endocytic pathway [27], has been reported to promote several cancer cells migration and invasion [28, 29]. To our knowledge, no studies have been done to evaluate possible involvement of the PIKFYVE gene in clinical PCa. For EGFR, which is aberrantly expressed in both androgen independent and metastatic PCa, are closely associated with aggressive phenotype, poor clinical prognosis, high Gleason scores, reduced survival rate, then contributing to castrate resistant PCa and progression to metastasis [30, 31]. Shao find that SMARCA4 (also known as BRG1) expression is significantly higher in malignant tissues compared to their benign compartments, especially in high-grade PCa, suggesting increased SMARCA4 expression might promote cell growth and invasion in PCa [32]. TOP2B has been found mediated androgen-induced the double-strand breaks and prostate cancer gene rearrangements [33]. It has been suggested that higher levels of cytoplasmic GSK3B expression are associated with aggressive PCa [34, 35]. Recently, Barrett and colleagues shown that the proto-oncogenes FOS is required for migration and invasion in PCa cells [36]. RAC1, a member of the Rho family GTPases, has been found hyperactivated in the metastatic PCa cells [37] and inhibition of RAC1 activity blocks the migration and invasion of PCa cells [38]. It has been reported an association of high BCL2 expression with higher Gleason scores and

lower biochemical recurrence-free survival in patients with advanced PCa undergoing androgen deprivation therapy [39]. A previous study proved that TAF1, a coactivator of androgen receptor, increased expression is associated with progression of human PCa to the lethal castration-resistant state [40]. These results indicate that most of miR-101-3p hub genes were involved in the development of PCa.

Conclusions

Our bioinformatics analysis identified miR-101-3p target hub genes in PCa that might play a central role in the occurrence, development and prognosis of PCa. In order to get more accurate correlation results, further experiments are still required to confirm potential functions of these miR-101-3p target genes and pathways in PCa.

Materials And Methods

Analysis of target genes of miR-101-3p

The target genes of miR-101-3p were predicted by the target module of the miRWalk2 (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>) database.[41] To make our predicted target genes more convincible, the predicted miRNA-gene interaction pairs should appear in all four databases, namely miRWalk, miRanda, RNA22, and Targetscan.

GO and KEGG pathway enrichment analysis

Integration Discovery (DAVID) software, version 6.8 (<https://david.ncifcrf.gov/>), was used to perform GO analysis to identify BP, CC, and MF of these target genes of miR-101-3p.[42] Meanwhile, the probable signaling pathways in which these target genes enriched were analyzed by KEGG database (<http://www.genome.jp/kegg/>). The *P*-value < 0.05 was considered significant.

Protein–protein interaction (PPI) network and module analysis

STRING database was used to predict the association between miR-101-3p and the target gene in the regulatory network analysis (<https://string-db.org/>), interactions with a combined score > 0.4 were selected as significant, and the PPI pairs were output to construct the PPI network using Cytoscape software version 3.6.0 (www.cytoscape.org/). Moreover, the Molecular Complex Detection (MCODE) app was utilized to screen modules of PPI network in Cytoscape with degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max. depth = 100. GO and KEGG pathway analysis were also made to explore the potential information.

Screening target genes and signaling pathways inhibited by miR-101-3p on PCa in published studies

An electronic literature search was performed in PubMed and Web of Science (up to November 1, 2019) by using the following terms: (miR-101 or miR-101-3p OR miRNA-101-3p OR microRNA-101-3p) and (prostate cancer OR prostate adenocarcinoma OR prostate tumor OR PCa OR PRAD). Then studies exploring the targets of miR-101-3p were collected in PCa.

Abbreviations

BP: Biological process; CC: Cellular component; CENPF: Centromere protein F; COX-2: Cyclooxygenase-2; EZH2: Enhancer of zeste homolog 2; KEGG: Kyoto Encyclopedia of Genes and Genome; MF: Molecular function; MiRNAs: MicroRNAs; NR2F2: also COUP-TF2, Chicken ovalbumin upstream promoter-transcription factor II; FOXM1: Forkhead box M1; NSCLC: Non-small-cell lung cancer; PCa: Prostate cancer; PPI: Protein–protein interaction; RLIP76: RalA binding protein 1; SOD1: Superoxide dismutase 1; SUB1: SUB1 homolog; TIGAR: Tp53-induced glycolysis and apoptosis regulator; UTR: Untranslated region

Declarations

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Not applicable.

Authors' contributions

Conceptualization: JJZ and HLG. Methodology: KW, HL and RHB. Formal analysis: JJZ. Data curation: GJZ. Software: KW, RHB. Original draft preparation: JJZ. Review and editing: HLG. All authors have read and approved the final article.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

Figure 1

The number of predicted target genes of miR-101-3p.

Figure 2

Gene ontology (GO) enrichment analysis for predicted target genes of miR-101-3p.

Figure 3

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis for predicted target genes of miR-101-3p.

Figure 4

MiR-101-3p target hub genes protein-protein interaction (PPI) network and two significant modules in PPI network of miR-101-3p target genes. (a) MiR-101-3p target hub genes PPI network. (b) Module 1. (c) Module 2.