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Original Article

MicroRNA-301a promotes growth and migration by repressing TGFBR2 in non-small cell lung cancer

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Abstract: A number of recent studies reported that miR-301a was significantly up-regulated and implicated in several types of human cancers. However, the functional involvement of miR-301a in non-small cell lung cancer (NSCLC) remains largely unknown. Herein, miR-301a mimics and inhibitors were used to manipulate miR-301a expression in NSCLC cells, respectively. Our findings revealed that miR-301a expression was significantly up-regulated in non-small cell lung cancer (NSCLC) tissues compared with normal lung tissues. Overexpression of miR-301a significantly enhanced proliferation and migration in lung cancer cells. Conversely, inhibition of miR-301a remarkably suppressed cell growth and motility. Systemic target gene analysis demonstrated that TGFBR2 is a critical downstream target negatively regulated by miR-301a in lung cancer cells. Notably, TGFBR2 expression was remarkably down-regulated and inversely correlated with miR-301 expression in NSCLC tissues. Consistently, the expression levels of TGF-β signaling genes were modulated by miR-301a, and negatively correlated with that of miR-301a in NSCLC tissues. Moreover, low TGFBR2 expression was significantly associated with poorer survival in NSCLC patients. Out data indicated that miR-301a promotes cell growth and migration by targeting TGFBR2 to modulate TGF-β signaling pathway in lung cancer.

Keywords: MicroRNA-301a, lung cancer, TGFBR2, TGF-β signaling, growth, migration

Introduction

Lung cancer is the most common cause of cancer-related deaths worldwide [1]. Of lung cancer, non-small cell lung cancer (NSCLC) is the most common type of primary lung cancer, accounting for 80%-85% of all lung cancer cases [2], which is classified intolung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and large-cell carcinoma [3]. Although significant advances have taken place in understanding of the disease process during the past few decades [4, 5], lung cancer treatment has not been significantly improved. Thus, effective treatments for the disease demand rapid development for therapeutic strategies. Recent evidences have indicated that therapeutic potentiality based on modulation the expression of endogenous microRNA (miRNA) in cancer is promising, as miRNA have the ability to affect cellular behavior [6-8].

miRNAs, which are one class of small non-coding RNAs (18-22 nucleotides in length), negatively regulate the expression of its target genes by binding to their mRNAs, usually in the 3' untranslatedregions (3'-UTR) [9]. Increasing evidences reveal that miRNAs have played pivotal roles in the modulation of cell proliferation, apoptosis, migration, and tumorigenesis [10, 11]. Recent studies demonstrated that dysregulation of miRNAs remarkably contributes to the development of various types of cancers, including breast cancer, colorectal cancer, gastric cancer [12-15], and lung cancer [16].

Our recent study found that spindle and kinetochore associated 2 (SKA2) and its adjacent gene, proline-rich protein 11 (PRR11), constitutes a classic bidirectional transcription unit, and play an important role in lung cancer development [17]. Intriguingly, SKA2 harbors a small non-coding RNA of microRNA-301a (miR-301a)

in its first intron. Growing body of evidences have shown that miR-301a is overexpressed in several types of human cancers, and mediates proliferation and invasion in breast, pancreatic and hepatocellular cancers through repression of several downstream target genes. Of note, one preliminary evidence showed that inhibition of miR-301a resulted into a decreased proliferation in lung cancer cell line A549, suggesting the potential role of miR-301a in lung cancer [18]. However, the expression profile of miR-301a and its potential functional impact in lung cancer have not been determined and remain unclear.

In the present study, we have investigated the role of miR-301a as well as its underlying molecular mechanisms in lung cancer, and for the first time demonstrated that miR-301a is aberrantly overexpressed and promotes proliferation and migration in lung cancer. Target gene analysis revealed that miR-301a negatively regulates the expression of transforming growth factor β type II receptor (TGFBR2) gene. Our findings thus provide a novel insight into the functional involvement of miR-301a in lung cancer.

Materials and methods

Cell culture

The lung cancer cell lines, A549 and H1299, were maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone) and RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, respectively. All the cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C.

Transfection with miRNA mimics and inhibitors

MiR-301a mimics, miR-301a inhibitors (anti-miR-301a), and their corresponding negative controls (miR-NC, anti-miR-NC) were designed and chemically synthesized by GenePharma (Shanghai, China). The sequences of the mimics and inhibitors were listed in Supplementary Table 2. A549 and H1299 cells were plated in 6-well plates and cultured for 24 hours before transfection. Then, miRNAs were transfected into cells at a final concentration of 50 nmol/L using Lipofectamine RNAiMAX reagent (Invitrogen, USA) according to the manufacturer's instructions. Forty-eight hours after transfection, cells were collected and subjected to analysis.

Cell proliferation assays

A549 or H1299 Cells were seeded at a density of 2×10^3 cells per well in triplicates in 96-well plates 24 hours after transfection with miR-301a mimics and inhibitors. Cell proliferation was assessed at the indicated time points using the Cell Counting Kit-8 (Dojindo). The number of viable cells was assessed by measurement of absorbance at 450 nm.

Wound-healing assays

Wound-healing assays were conducted to assess migration activity. Cells were cultured in complete medium to a confluent monolayer and then were scraped with a 200 μl pipette tips to create an artificial wound after which the wound was monitored. Floating cells were then washed away with PBS, and fresh medium with 1% FBS were added. Cell migration wasobserved under microscope at the indicated time points.

Quantitative RT-PCR analysis

Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instruction. Total RNA (500 ng) was reverse transcribed to obtain cDNA using PrimeScript® RT reagent Kit (TaKaRa) according to the manufacturer's protocol. Quantitative PCR was performed using SYBR Green PCR Mix kit (TaKaRa). The primers were listed in Supplementary Table 3. PCR reactions were performed in a 10 μL qPCR reaction under the following conditions: 95°C for 30 s, then 40 cycles at 95°C for 3 s, 60°C for 30 s. The housekeeping gene of GAPDH was used as an internal control gene, the relative abundances of mRNAs were calculated according to the method 2-ΔΔCt. Each experiment was performed in triplicates.

Western blot analysis

Total proteins were isolated from A549 at 48 h post-transfection using protein extraction reagent RIAP (Beyotime, China). Then, proteins were separated on 10% sodium dodecyl sulfate polyacrylamide gel and transferred to PVDF membranes. The target proteins were detected by the primary antibodies (anti-TGFBR2, 1:1000; anti-SMAD4 1:1000, anti-GAP-

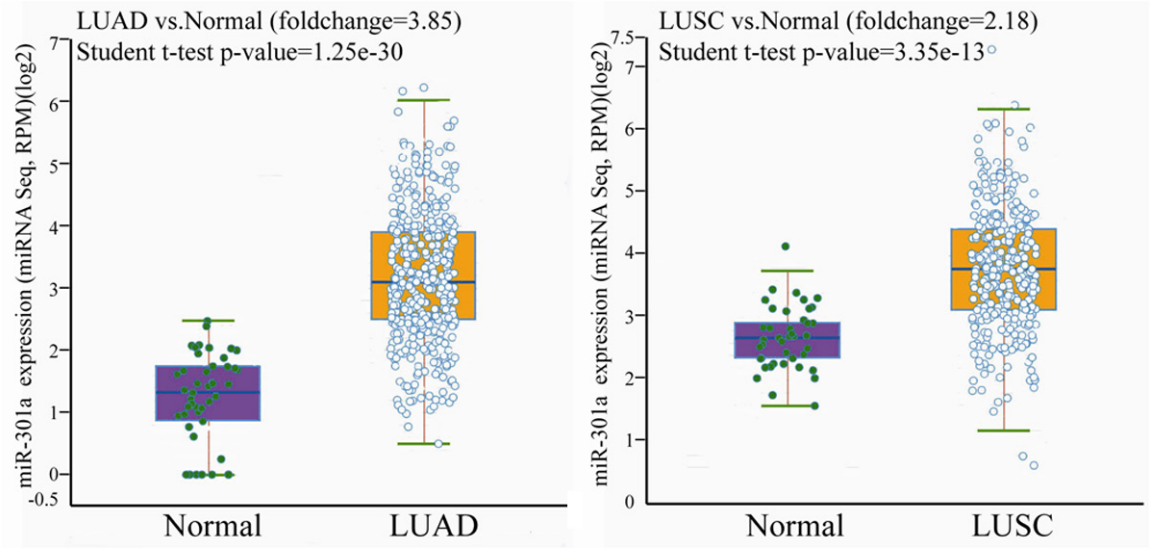


Figure 1. Expression level of miR-301a is significantly up-regulated in non-small cell lung cancer (NSCLC) tissues. The miR-301a expression was analyzed in lung adenocarcinoma (LUAD) and squamouscell carcinoma (LUSC) samples using starBase v2.0 platform (http://starbase.sysu.edu.cn/) [19]. The numbers of LUAD samples were 430, LUSC samples were 332, and the corresponding normal samples were 46 and 45, respectively. The student's t-test was used to compare thedata between normal groupand carcinoma group.

DH, 1:2000) followed by incubating with a secondary antibody (Horseradish peroxidase-conjugated goat anti-rabbit, 1:5000). The signals were detected by ECL advance Western Blotting Detection Kits (GE Healthcare).

Expression analysis of miR-301a and its potential target genes in lung cancer

The expression levelsof miR-301a and its target genes including TGFBR2, SMAD4, CTGF, ZEB1 and SMAD7, and the correlation between themin lung cancer were analyzed using datasets from starBase v2.0 platform (http://starbase.sysu.edu.cn/), which systematically identify the RNA-RNA and protein-RNA interaction networks from 108 CLIP-Seq data sets, and provide the most comprehensive CLIP-Seq experimentally supported miRNA-mRNA network [19].

Prognostic analysis of TGFBR2 in NSCLC patients

The prognostic value of TGFBR2 expression was evaluated using published lung cancer microarray data from the Nagoya University cohort containing 117 lung cancer patients [48]. Microarray and patient survival data were downloaded from the GEO database (GSE-13213). The microarray raw data were routinely processed according to previous study [49] and further used for Kaplan-Meier analyses. Receiver operating characteristic curve analysis was performed to identify a rational cut-off point. Briefly, patients who lived longer than 5 years after diagnosis were considered to have good prognoses, and TGFBR2 expression values that reached optimal sensitivity and specificity were chosen.

Statistical analyses

All statistical analyses were carried out using the SPSS 18.0 statistical software package (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to analyze the data of cell proliferation assay. Overall survival curve was plotted using the Kaplan-Meier method and compared with the log-rank (Mantel-Cox) test. Univariate regression analyses were performed with the Cox proportional hazards regression model to calculate the hazard ratios and their 95% confidence intervals for each variable and to analyze independent factors affecting prognosis. *P*-value < 0.05 was considered statistically significant.

Results

MiR-301a expression is significantly up-regulated in NSCLC tissues

To understand the potential role of miR-301a in NSCLC development, we firstly examined the

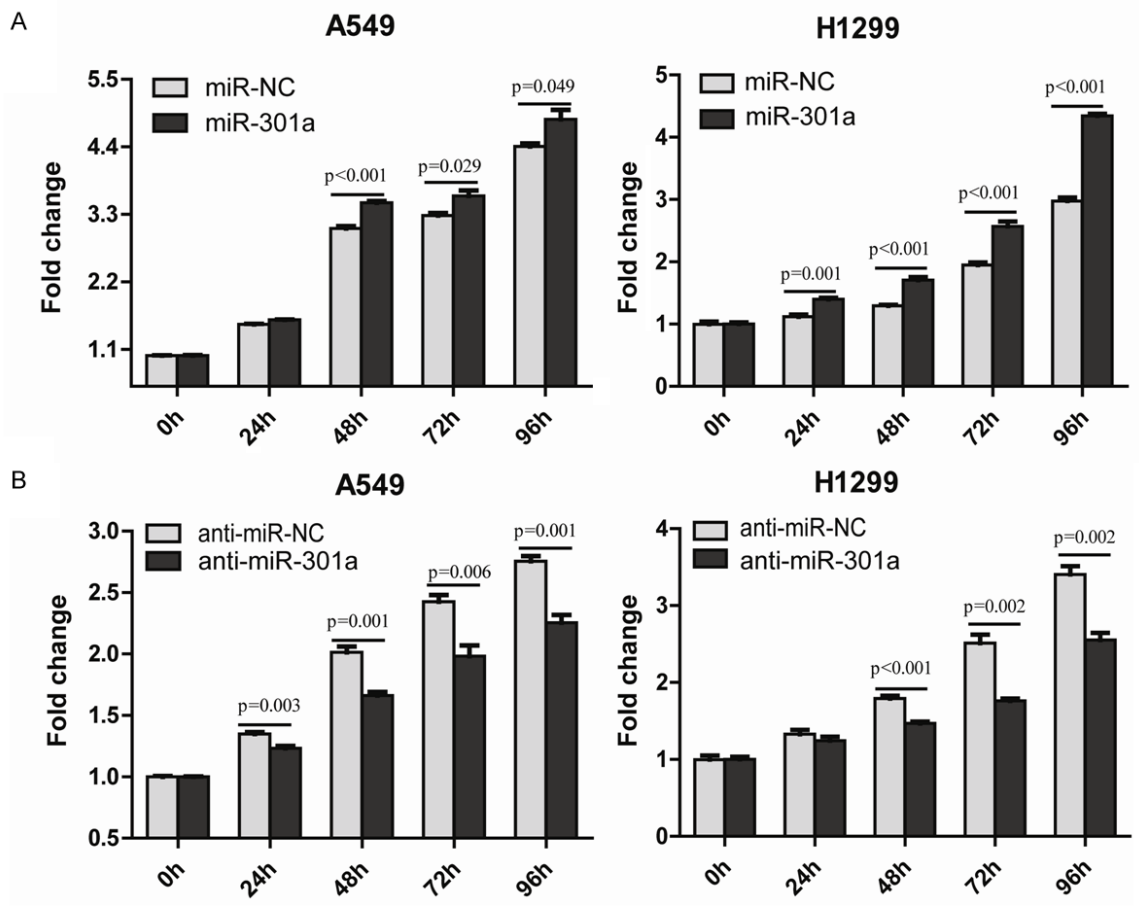


Figure 2. MiR-301a enhances NSCLC cells growth. Overexpression of miR-301a promotesproliferation (A), inhibition of miR-301a suppresses proliferation in both two NSCLC cells A549 and H1299 (B). The NSCLC cells, A549 and H1299, were transiently transfected with miR-301a mimics and anti-miR-301a, respectively. Twenty-four hours after transfection, cells were subjected to the proliferation assay using CCK-8 kit.

expression of miR-301a in lung cancer tissues using the starBase v2.0 pan-cancer platform. The platform was designed for deciphering pan-cancer networks of non-codingRNAs by mining clinical and expression profiles of14 cancer types (>6000 samples) from the cancer genome atlas (TCGA) data portal, including LUAD and LUSC [19]. According to the starBase pan-cancer platform, the data of miR-301a expression were available 430 LUAD and 332 LUSC samples. The results revealed that miR-301a expression was significantly up-regulated in NSCLC tissues compared with corresponding normal tissues. The fold-changes of LUAD vs. normal and LUSC vs. normal were approximately 3.85 and 2.18, respectively, and the *p*-value was 1.25e-30 and 3.35e-15, respectively (**Figure 1**). These findings suggested that up-regulation of miR-301a may be implicated in NSCLC development.

MiR-301a promotes NSCLC cell growth and migration

Given that the expression of miR-301a was significantly up-regulated in NSCLC tissues, we further examined whether miR-301a could affect the proliferation of NSCLC cells. To this end, we utilized the gain-of-function and loss-of-function strategies by transfecting the cells with miR-301a mimics, miR-301a inhibitors (anti-miR-301a) as well as the corresponding negative control (miR-NC, anti-miR-NC), respectively. CCK-8 assays showed that miR-301a mimics were able to significantly promote proliferation in both A549 and H1299 cells (**Figure 2A**). On the contrary, miR-301a inhibitors (anti-miR-301a) caused NSCLC cells to proliferate more slowly than the negative control (**Figure 2B**).

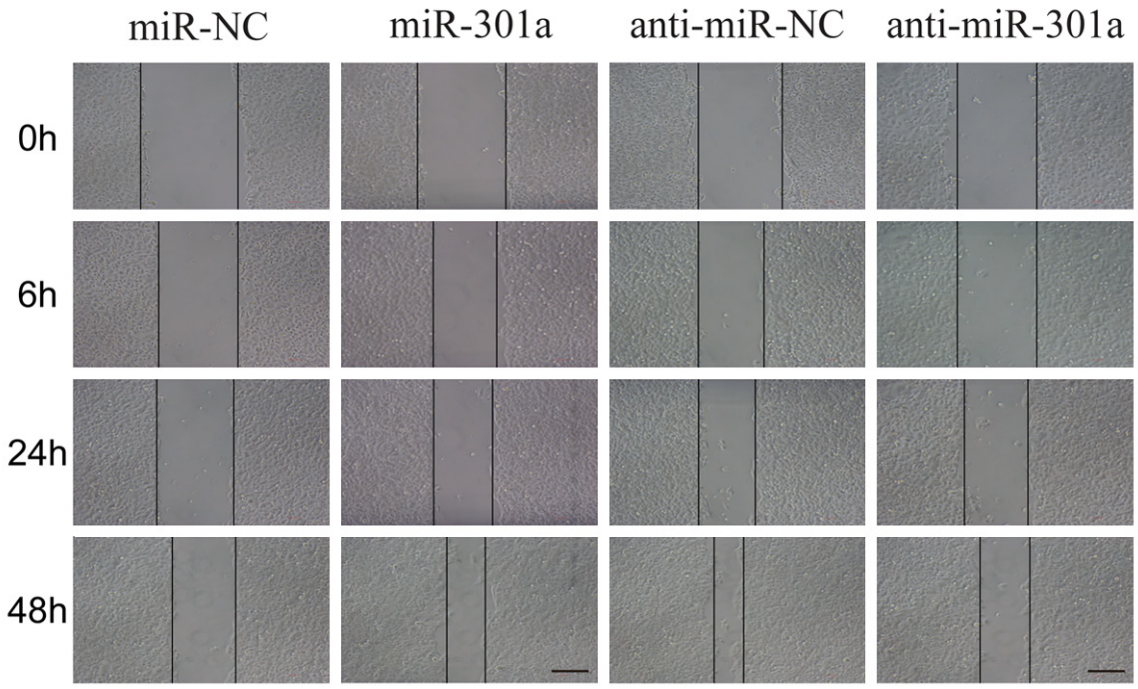


Figure 3. MiR-301a promotes NSCLC cell migration. A549 cells were transfected with miR-301a mimics and anti-miR-301a, respectively. A scratch was drawn on the monolayer cells. Wound was observed under microscope at 0 h, 6 h, 24 h, 48 h, respectively.

To further determine whether miR-301a could affect the migration of NSCLC cells, wound-healing assays were performed after transiently transfecting A549 cells with miR-301a mimics and miR-301a inhibitors, respectively. As shown in **Figure 3**, overexpression of miR-301a significantly enhanced the migration activity of A549 cells compared with the negative control, whereas inhibition of miR-301a with anti-miR-301a reduced the migration ability. Taken together, these data demonstrated that miR-301a promotes the proliferation and migration of NSCLC cells.

MiR-301a down-regulates the expression of TGFBR2

To deeply explore how miR-301a functions, we firstly searched for its potential target genes using three online in silico prediction algorithms (TargetScan, miRanda and Pictar) and one databases (StarBase). The potential target genes were listed in Supplementary Table 1. Simultaneously, the experimentally reported target genes of miR-301a have been also thoroughly retrieved and summarized in **Table 1**. By combining the algorithm prediction and literature retrieval data, seven miR-301a target genes with potential roles in NSCLC were selected for further verification. As shown in **Figure 4A**, qRT-PCR analysis revealed that transient transfection of miR-301a mimics significantly decreased the mRNA levels of six genes (TGFBR2, SMAD4, RUNX3, PIAS3, NKRF, and FOXF2) whereas transfection with anti-miR-301a caused significant increases in the mRNA levels of four genes (TGFBR2, SMAD4, PIAS3, and NKRF). TGFBR2 and SMAD4 are critical regulators of the transforming growth factor-β (TGF-β) signaling pathway. On binding TGF-β, TGFBR2 phosphorylates and activates the TGFβR1 that then propagate the signal by phosphorylating Smad transcription factors. Once activated, the receptor substrate Smads (RSmads) form a complex with SMAD4, which is a binding partner common to RSmads [20]. Given that the mis-regulation of TGF-β signaling pathway can result in tumor development. Therefore, the two genes TGFBR2 and SMAD4 attracted our more attentions.

We next examined whether miR-301a could affect the protein levels of TGFBR2 and SMAD4. As shown in **Figure 4B**, the results demonstrated that TGFBR2 proteins were markedly

Table 1. The experimentally reported target genes of miR-301a

Target genes	Validation methods	Cell lines	Cancer Type	Pathway	miR-301a expression	Function	Ref
TGFBR2	Luciferase reporter assay	SW480 SW620	Colorectal cancer		Up-regulation	Promoted the metastatic and invasive ability in human colorectal cancers	[21]
	qRT-PCR Western blot						
SOCS6	Luciferase reporter assay	SW480 SW620	Colorectal cancer		Up-regulation	Increased cell proliferation, migration and invasion as well as tumor growth	[37]
	Luciferase reporter assay	HCT116, SW480	Colorectal Cancer	TGF-β/Smad	Up-regulation	Enhances cell proliferation and migration	[20]
	qRT-PCR						
SMAD4	Luciferase reporter assay	Hep-2, Tu-177	Laryngeal squamous cell carcinoma		Up-regulation	Functioned as an oncogene in LSCC and participated in the epithelial–mesenchymal transition (EMT) process	[50]
	Luciferase reporter assay	SW1990	Pancreatic cancer		Up-regulation	Enhanced PDAC cells colony, invasion and migration abilities in vitro as well as tumorigenicity in vivo	[33]
	qRT-PCR Western blot						
BIM	Luciferase reporter assay	BxPC-3 Hs766T	Pancreatic cancer		Up-regulation	Promoted PC cell proliferation	[34]
	Western blot						
NKRF	Luciferase reporter assay Microarray qRT-PCR Western blot	293T PANC-1 Hs-766T *et al.*	Pancreatic cancer	NK-κB	Up-regulation	NK-κB activation, miR-301a inhibition reduces xenograft tumour growth	[35]
MnSOD	qRT-PCR	CRL-1469	Pancreatic cancer		Up-regulation	Lower miR-301a levels are associated with increased MnSOD expression and inhibition of PDAC growth	[51]
RUNX3	Luciferase reporter assay	KATO-III SNU-1 SNU-16	Gastric cancer		Up-regulation	Promoted cell growth, migration, invasion, and decreased cell apoptosis in vitro, enhanced the subcutaneous tumorigenesis in vivo	[52]
	qRT-PCR Western blot						
PTEN	Luciferase reporter assay	5637, UM-UC-2	Bladder cancer	p-FAK, p-AKT	Up-regulation	Promoted invasion and migration of bladder cancer cells	[53]
	qRT-PCR Western blot						
	qRT-PCR Western blot	five ES cell lines	Ewing's sarcoma	AKT	Up-regulation	Anti-miR-301a inhibited ES cell proliferation and cell cycle progression, and significantly suppressed tumor growth in vivo	[54]
	Luciferase reporter assay Western blot	NCTC1469	Hepatocytes	AKT/GSK	Down-regulation in mouse	Diminished the effect of IL-6 on the AKT/GSK pathway and hepatic glycogenesis	[55]
	Luciferase reporter assay	MCF7 MDA-MB-231	Breast cancer	Wnt/β-catenin	Up-regulation	Promoted breast cancer cell migration, invasion and metastasis both in vitro and in vivo	[32]
	qRT-PCR Western blot						
FOXF2, BBC3, PTEN, COL2A1	Luciferase reporter assay	MCF7 T47D	Breast cancer		Up-regulation	Promoted cell proliferation, migration and invasion	[31]
	Western blot						
MEOX2	Luciferase reporter assay	A549	Lung cancer	ERK/CREB	No experimentally examined	Increase of the mitotic index and a decrease in colony Formation after inhibition of miR-301a	[18]

	Luciferase reporter assay qRT-PCR Western blot	Hep G2	Hepatocellular carcinoma	NK-κB	Up-regulation	Increase proliferation, migration and invasion of HCC cells	[36]
TIMP2	Luciferase reporter assay Western blot	U266	Multiple myeloma		Up-regulation	Promoted cell proliferation and inhibited apoptosis	[22]
AMPKα1	Luciferase reporter assay	U2OS	Osteosarcoma		Up-regulation	Promoted resistance of osteosarcoma cells to doxorubicin	[56]
PIAS3	Luciferase reporter assay qRT-PCR Western blot	Myelin-specific $CD4^+$T cells	Autoimmune demyelination	IL-6/ 23-STAT3	Up-regulation	Control autoimmune demyellination via regulating T-helper 17 immune response	[47]
SEPT7	Luciferase reporter assay		Glioma	Wnt/ β-catenin	Up-regulation	Promoted the invasion of glioma cells	[57]
	qRT-PCR						
AR	Luciferase reporter assay	C4-2, 22Rv1	Prostate cancer	TGF-β1/ Smad/MMP9	Up-regulation	Increased prostate cancer metastasis	[58]
	qRT-PCR Western blot						
NDRG2	Luciferase reporter assay	LNCaP, PC-3, DU145	Prostate cancer		Up-regulation	Increased autophagy and cell viability and reduced cell apoptosis	[59]
	qRT-PCR Western blot						
p63	Luciferase reporter assay	PC3, LNCaP	Prostate cancer	E-cadherin	Up-regulation	Increased growth both in vitro and in xenografted tumors, increased risk of biochemical recurrence, and induced EMT	[60]
	qRT-PCR Western blot						

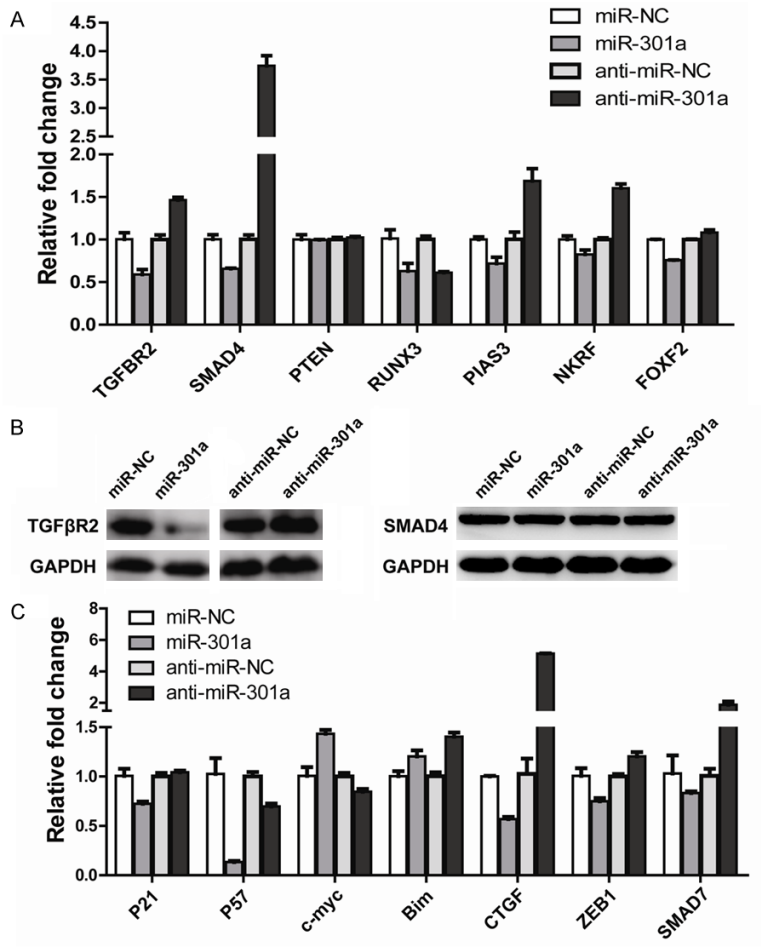


Figure 4. Identification and validation of miR-301 target genes. Seven miR-301a target genes were selected based on bioinformatic analysis and previously reported references. The expression levels of the seven genes were detected after transfection with miR-301a mimics and anti-miR-301a in A549 cells using qRT-PCR (A). Protein levels of TGFBR2 and SMAD4 were measured after transfection with miR-301a mimics and anti-miR-301a in A549 cell using westernblotting (B). mRNA levels of TGF-β signaling downstream target genes were determined using qRT-PCR in A549 cells (C).

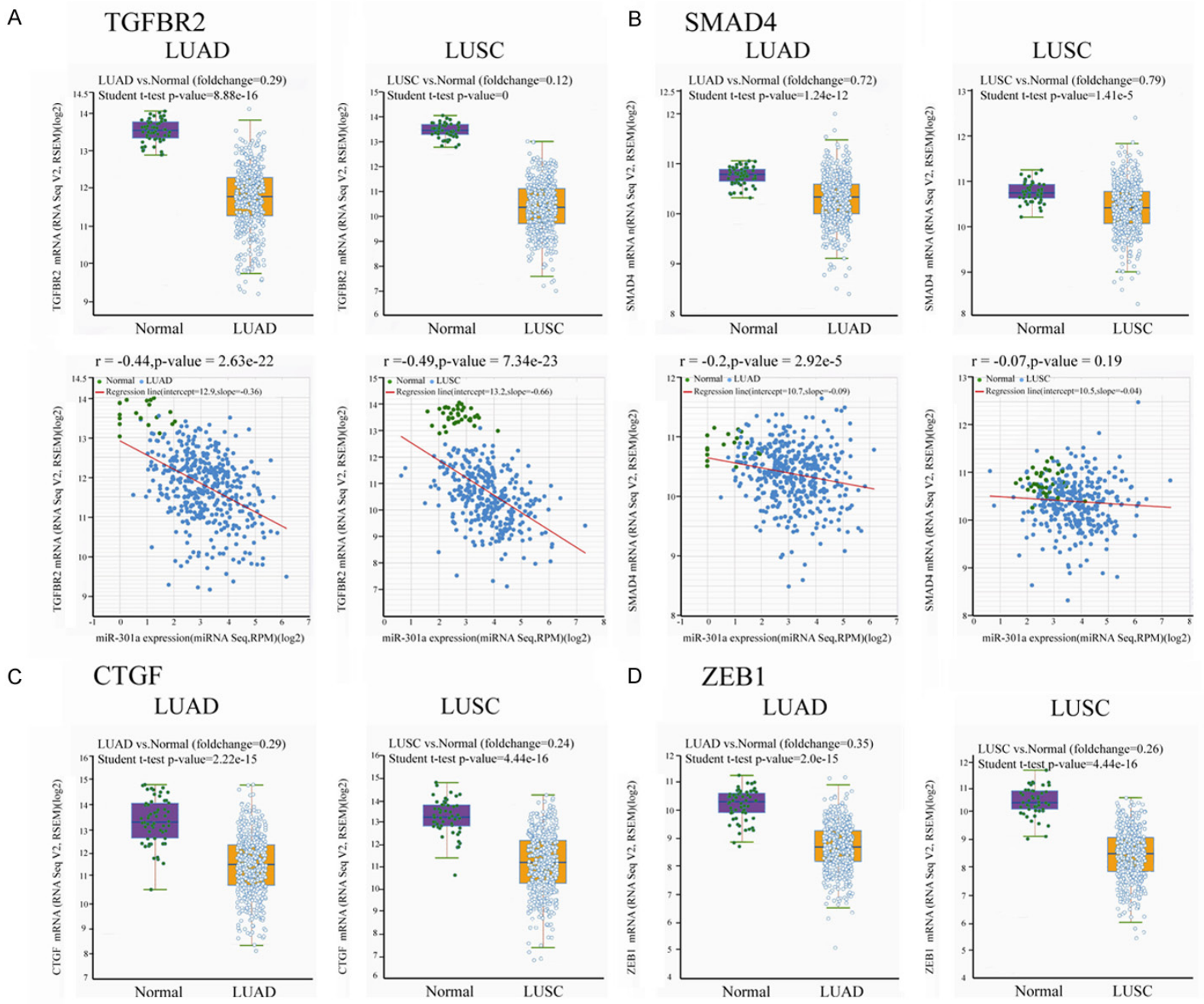
reduced in cells transfected with miR-301a mimics and significantly increased in anti-miR-301a transfected cells compared with their corresponding negative control. However, transfection of either miR-301a mimics or anti-miR-301a did not significantly alter the Smad4 expression at protein levels (**Figure 4B**). Our online algorithm analysis and a previous study revealed that the 3'-UTR of TGFBR2 harbors a highly conserved miR-301a binding region [21]. Moreover, decreased expression of TGFBR2 occurs frequently in lung cancer [22-24]. Therefore, these data suggested that TGFBR2 was a bona fide target gene of miR-301a in lung cancer.

MiR-301a modulates the expressions of TGF-β signaling target genes

We next tested whether the expression of TGF-β signaling target genes were regulated by miR-301a. TGF-β is an important cytostatic regulator owing to its ability to activate the transcription of cell-cycle inhibitors and/or apoptotic regulators. Inhibition of the cell cycle occurs through up-regulation of cyclin-dependent kinases (p21Cip1, p15Ink4b and p57Kip2) and down-regulation of proproliferative genes such as c-myc [25]. TGF-βinduces expression of Bcl2 family members via transcription of the proapoptotic factor Bim to control apoptosis [26]. In addition, expression levels of CTGF, ZEB1, and SMAD7 have been also shown to be up-regulated at least two-fold induced by TGF-β [27, 28]. As shown in **Figure 4C**, qRT-PCR analysis revealed that transfection of miR-301a mimics caused down-regulation of p21, p57, CTGF, and ZEB1, and up-regulation of c-myc. On the other hand, transfection with anti-miR-301a resulted in the up-regulation of Bim, CTGF, ZEB1 and SMAD7 as compared with the negative control. Taken together, these findings suggested that miR-301a could regulate the expression of TGF-β signaling downstream genes through targeting TGFBR2.

Expression of miR-301a inversely correlates with that of TGFBR2 and TGF-β signaling target genes in NSCLC

Furthermore, we determined whether expression of miR-301a was correlated with that of TGFBR2 and TGF-β signaling target genes. To this end, correlations between them were investigated via starBase v2.0 platform. As shown in **Figure 5A**, expression level of TGFBR2


A
TGFBR2
LUAD
LUAD vs.Normal (foldchange=0.29)
Student t-test p-value=8.88e-16
TGFBR2 mRNA (RNA Seq V2, RSEM)(log2)
Normal
LUAD
LUSC
LUSC vs.Normal (foldchange=0.12)
Student t-test p-value=0
r = -0.44,p-value = 2.63e-22
Regression line(intercept=12.9,slope=-0.36)
miR-301a expression(miRNA Seq,RPM)(log2)
r =-0.49,p-value = 7.34e-23
Regression line(intercept=13.2,slope=-0.66)
B
SMAD4
LUAD vs.Normal (foldchange=0.72)
Student t-test p-value=1.24e-12
SMAD4 mRNA (RNA Seq V2, RSEM)(log2)
LUSC vs.Normal (foldchange=0.79)
Student t-test p-value=1.41e-5
r = -0.2,p-value = 2.92e-5
Regression line(intercept=10.7,slope=-0.09)
r = -0.07,p-value = 0.19
Regression line(intercept=10.5,slope=-0.04)
C
CTGF
LUAD vs.Normal (foldchange=0.29)
Student t-test p-value=2.22e-15
CTGF mRNA (RNA Seq V2, RSEM)(log2)
LUSC vs.Normal (foldchange=0.24)
Student t-test p-value=4.44e-16
D
ZEB1
LUAD vs.Normal (foldchange=0.35)
Student t-test p-value=2.0e-15
ZEB1 mRNA (RNA Seq V2, RSEM)(log2)
LUSC vs.Normal (foldchange=0.26)
Student t-test p-value=4.44e-16

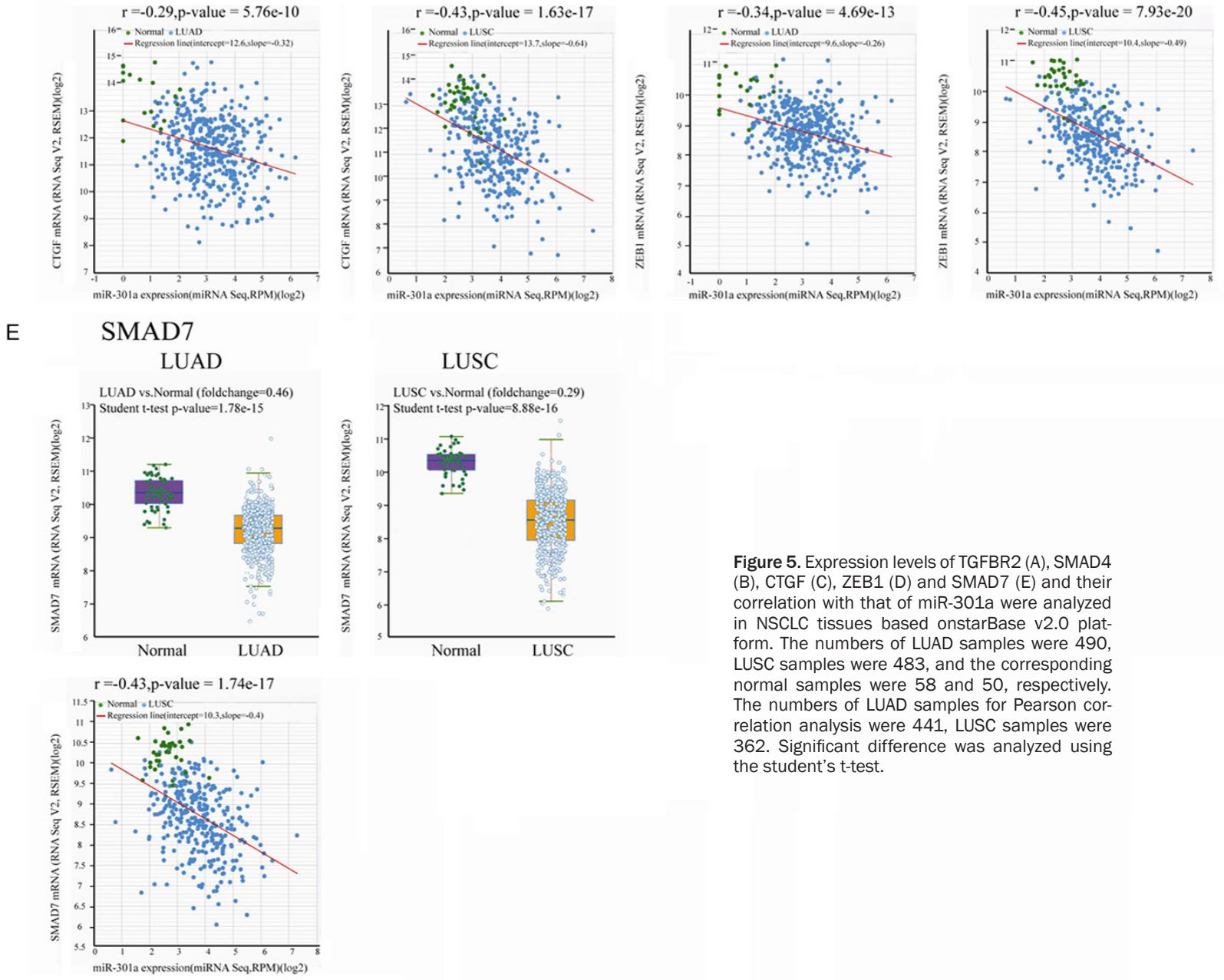


Figure 5. Expression levels of TGFBR2 (A), SMAD4 (B), CTGF (C), ZEB1 (D) and SMAD7 (E) and their correlation with that of miR-301a were analyzed in NSCLC tissues based onstarBase v2.0 platform. The numbers of LUAD samples were 490, LUSC samples were 483, and the corresponding normal samples were 58 and 50, respectively. The numbers of LUAD samples for Pearson correlation analysis were 441, LUSC samples were 362. Significant difference was analyzed using the student's t-test.

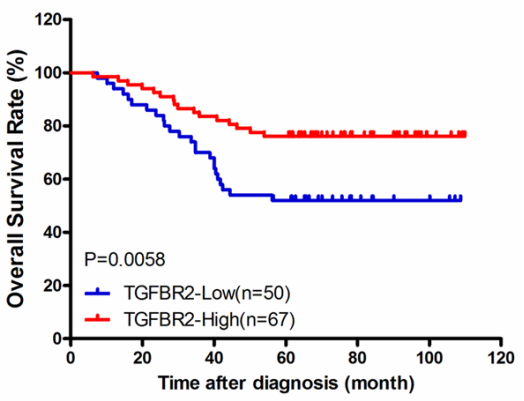


Figure 6. Univariate survival analysis was performed based on TGFBR2 expression in NSCLC patients. Kaplan-Meier method was used to plot of overall-survival of NSCLC patients in the Nagoya University cohorts.

was significantly decreased in NSCLC tissues compared with that of normal tissues (fold-change = 0.29 in LUAD, 0.12 in LUSC). Of note, TGFBR2 expression value was highly negatively correlated with that of miR-301a, with Pearson correlation r = -0.44/-0.49; *P*-value = 2.63×10^{-22}/7.34×10^{-23} in LUAD/LUSC. The expression level of SMAD4 was neither significantly decreased (fold-change = 0.72 in LUAD, 0.79 in LUSC), nor significantly correlated with that of miR-301a in NSCLC tissues (**Figure 5B**).

In addition, we also found that the expression levels of three TGF-β signaling target genes were significantly down-regulated in NSCLC tissues compared with that of normal tissues, including ZEB1 (fold-change = 0.35 in LUAD, 0.26 in LUSC), CTGF (fold-change = 0.27 in LUAD, 0.24 in LUSC) and SMAD7 (fold-change = 0.46 in LUAD, 0.29 in LUSC). Notably, regression analysis indicated that expression values of these three genes were inversely correlated with that of miR-301a, with r ranging from -0.29 to -0.45 (**Figure 5C-E**).

The clinical significance of TGFBR2 expression in NSCLC patients

Finally, we evaluated the prognostic value of TGFBR2 expression using the Nagoya University lung cancer cohort. Survival analysis demonstrated that low TGFBR2 expression (n = 50) was significantly associated with poorer survival, and high expression level of TGFBR2 (n = 67) was closely associated with better clinical outcome in the Nagoya University lung cancer cohort (**Figure 6**).

Discussion

Lung cancer is the leading cause of cancer deaths worldwide. However, no better strategy has been developed for treatment of the disease during thepast two decades. In this study, our results showed that miR-301a expression is markedly up-regulated in NSCLC tissues, and miR-301a promotes NSCLC cell growth and migration, suggesting that miR-301a might promote NSCLC development. These findings might provide a potential target in the diagnosis and/or therapy of lung cancer.

Up-regulation of oncogenic miRNAs or down-regulation of tumor suppressor miRNAs plays important roles in tumorigenesis [29, 30]. MiR-301a has been discovered to be up-regulated in several types of human cancers, and functional analysis revealed that miR-301a promotes proliferation and/or invasion in breast cancer [31, 32] pancreatic cancer [33-35], hepatocellular carcinoma [36] and colorectal cancer [21, 22, 37]. Lu et al. reported that miR-301a down-regulates NF-κB-repressing factor (NKRF) and leads to NF-κB activation in pancreatic cancer [35]. MiR-301a has been also shown to inhibit distinctive target genes in different cancer cell lines (**Table 1**). Of note, a systemic study in breast cancer revealed that miR-301a acts through multiple pathways by repressing the expression of target genes such as FOXF2, PTEN, and COL2A1 [31]. In this study, we systemically analyzed the effects of miR-301a on sever experimentally reported target genes with potential roles in NSCLC, and found that miR-301a could down-regulate the expression of TGFBR2, SMAD4, PIAS3, and NKRF, but not PTEN, a frequently reported target gene of miR-301a. This suggests that miR-301a might regulate distinctive target genes in a cell-type or cancer-type dependent manner.

TGFBR2, a serine-threonine kinase, is a major transmembrane receptor of TGF-β signaling. TGF-β acts as a tumor suppressor in epithelial cells, where it inhibits proliferation, induces apoptosis, and mediates differentiation. Previous studies reported that TGF-β signaling is impeded by the loss of TGFBR2 [38, 39]. Targeted deletion of TGFBR2 causes increased tumor progression and metastases in both colonic epithelium [40] and breast carcinoma [41]. Decreased of TGFBR2 expression was associated with increased risk for the develop-

ment of invasive breast cancer [42]. TGFBR2 expression was also decreased in multiple lung cancer cell lines [43, 44] and lung tumor specimens [45]. In the present study, our data showed that ectopic over-expression of miR-301a mimics suppressed the TGFBR2 expression while knockdown of miR-301a by anti-miR-301a up-regulated its expression. The expression of TGF-β signaling target genes was also altered in response to miR-301a mimics and inhibitors. Moreover, we also observed that the TGFBR2 expression as well as several TGF-β signaling target genes was significantly down-regulation, and inversely correlated with the expression miR-301a in NSCLC tissues. More importantly, the low expression of TGFBR2 was associated with poorer survival. Recently, Zhang et al. also reported that miR-301a promotes migration by targeting TGFBR2 in human colorectal cancer [21]. Taken together, we thus can define a pathway in which miR-301a attenuates the TGF-β signaling via targeting TGFBR2 to alter a series of downstream target genes expression, thereby stimulating lung cancer development.

The TGF-β signaling cascade is initiated when active TGF-β binds to its receptors TGFβR1 and TGFBR2. TGFβR1 is then activated by TGFBR2 through phosphorylation. After that, activated TGFβR1 propagates the signal through either a SMAD dependent pathway or SMAD independent pathways [24, 46]. Of note, our results showed that manipulation of miR-301a expression only caused a significant change in the mRNA levels of SMAD4 expression, and the protein levels of SMAD4 expression remained unchanged. Indirect immunofluorescence assay also revealed that SMAD4 did not translocated into nucleus upon manipulation of miR-301a expression (data not shown). Moreover, we also found that the SMAD4 expression was not remarkably reduced in NSCLC tissues, and no significantly correlation between the expression levels of SMAD4 and miR-301a was observed. Taken together, we thus deduced that miR-301a might regulate the TGF-β signaling in a SMAD4-independent manner, and the underlying molecular mechanisms needs further study.

We also found that miR-301a could down-regulate the expression of PIAS3, and NKRF. NKRF is a potent inhibitor of the STAT3. Mycko et al. reported that miR-301a regulates the STAT3 pathway through down-regulation of PIAS3 [47]. However, our data revealed that manipulation of miR-301a levels by miR-301a mimics and anti-miR-301a did not alter the expression of PIAS3 at protein levels (data not shown), suggesting that miR-301a does not regulate the PIAS3 expression and STAT3 pathway in lung cancer. As mentioned above, NKRF is a NF-κB-repressing factor, and has been shown be down-regulated and mediates the oncogenic effect of miR-301a in pancreatic cancer [35]. Therefore, further study is required to investigate whether miR-301a regulates NF-κB pathway through down-regulating NKRF in lung cancer.

Conclusion

In summary, our present study demonstrated that miR-301a promotes NSCLC cell proliferation and migration by targeting TGFBR2 to modulate TGF-β signaling. Our findings suggested that miR-301a might serve as a novel potential diagnostic and/or therapeutic target for human lung cancer.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. The potential target genes of miR-301a

Name	Gene Name	Position	TargetScan Sites	MiRanda Sites	PicTar Sites
hsa-miR-301a-3p	TGFBR2	chr3:30733357-30733363[+]	10785[19]	10785[19]	10785[19]
hsa-miR-301a-3p	SMAD4	chr18:48606192-48606198[+]	423[13]	423[13]	423[13]
hsa-miR-301a-3p	RUNX3	chr1:25226132-25226139[-]	25[4]	50[5]	25[4]
hsa-miR-301a-3p	PTEN	chr10:89725641-89725647[+]	8[2]	8[2]	8[2]
hsa-miR-301a-3p	FOXF2	chr6:1395757-1395763[+]	1294[12]	1309[12]	1294[12]
hsa-miR-301a-3p	PRKAA1	chr5:40760396-40760402[-]	2786[17]	2788[17]	2786[17]
hsa-miR-301a-3p	ULK2	chr17:19679064-19679071[-]	108[6]	108[6]	108[6]
hsa-miR-301a-3p	TET3	chr2:74335293-74335299[+]	21[1]	61[2]	21[1]
hsa-miR-301a-3p	HOXA3	chr7:27147117-27147123[-]	30[4]	30[4]	30[4]
hsa-miR-301a-3p	NAA50	chr3:113440579-113440585[-]	125[8]	137[9]	125[8]
hsa-miR-301a-3p	NCKAP5	chr2:133430346-133430352[-]	130[4]	130[4]	130[4]
hsa-miR-301a-3p	ANKRD28	chr3:15710177-15710183[-]	325[7]	325[7]	325[7]
hsa-miR-301a-3p	LRIG1	chr3:66429615-66429621[-]	4[3]	4[3]	4[3]
hsa-miR-301a-3p	NPTN	chr15:73852668-73852674[-]	3731[22]	3839[23]	3731[22]
hsa-miR-301a-3p	DCBLD2	chr3:98515494-98515500[-]	290[8]	290[8]	290[8]
hsa-miR-301a-3p	PHF20	chr20:34538149-34538155[+]	371[3]	371[3]	371[3]
hsa-miR-301a-3p	IKZF4	chr12:56429700-56429707[+]	9[1]	9[1]	9[1]
hsa-miR-301a-3p	WDFY3	chr4:85591227-85591233[-]	110[4]	110[4]	110[4]
hsa-miR-301a-3p	ZFP91	chr11:58385980-58385986[+]	361[11]	361[11]	361[11]
hsa-miR-301a-3p	PPP2R1B	chr11:111597635-111597641[-]	18[2]	18[2]	18[2]
hsa-miR-301a-3p	SHANK2	chr11:70315539-70315546[-]	30[3]	30[3]	30[3]
hsa-miR-301a-3p	SHANK2	chr11:70315610-70315616[-]	18[2]	18[2]	18[2]
hsa-miR-301a-3p	NFIB	chr9:14083381-14083387[-]	5286[19]	10571[19]	5286[19]
hsa-miR-301a-3p	FAM179B	chr14:45543039-45543046[+]	277[8]	570[8]	277[8]
hsa-miR-301a-3p	DICER1	chr14:95553931-95553937[-]	910[4]	910[4]	910[4]
hsa-miR-301a-3p	DICER1	chr14:95555382-95555388[-]	976[11]	488[11]	482[11]
hsa-miR-301a-3p	DGKE	chr17:54943205-54943212[+]	142[11]	284[11]	142[11]
hsa-miR-301a-3p	HOXA5	chr7:27181092-27181098[-]	299[14]	302[15]	299[14]
hsa-miR-301a-3p	KLF3	chr4:38701927-38701934[+]	314[7]	407[10]	314[7]
hsa-miR-301a-3p	EDA	chrX:69255772-69255779[+]	5[1]	5[1]	5[1]
hsa-miR-301a-3p	ANKIB1	chr7:92030561-92030567[+]	698[16]	698[16]	698[16]
hsa-miR-301a-3p	PHF3	chr6:64424016-64424022[+]	0[2]	0[2]	0[2]
hsa-miR-301a-3p	WNK3	chrX:54223277-54223283[-]	21[4]	21[4]	21[4]
hsa-miR-301a-3p	HIVEP2	chr6:143073830-143073837[-]	117[11]	117[11]	117[11]
hsa-miR-301a-3p	PSAP	chr10:73576094-73576101[-]	2031[22]	2031[22]	2031[22]
hsa-miR-301a-3p	CSNK1G1	chr15:64463379-64463385[-]	60[7]	60[7]	60[7]
hsa-miR-301a-3p	RASD1	chr17:17397887-17397893[-]	732[8]	732[8]	732[8]
hsa-miR-301a-3p	HECW2	chr2:197065029-197065035[-]	28[1]	28[1]	28[1]
hsa-miR-301a-3p	MAP3K12	chr12:53874550-53874556[-]	73[8]	73[8]	73[8]
hsa-miR-301a-3p	TMEM159	chr16:21191492-21191498[+]	314[11]	628[11]	314[11]
hsa-miR-301a-3p	MBNL1	chr3:152182600-152182606[+]	2927[21]	5853[21]	2927[21]
hsa-miR-301a-3p	MBNL1	chr3:152183341-152183347[+]	328[8]	2993[8]	328[8]
hsa-miR-301a-3p	BCL2L11	chr2:111925901-111925907[+]	940[16]	19177[19]	940[16]
hsa-miR-301a-3p	ACSL4	chrX:108884599-108884605[-]	217[2]	255[3]	217[2]
hsa-miR-301a-3p	ACSL4	chrX:108885860-108885866[-]	904[18]	904[20]	892[17]
hsa-miR-301a-3p	BAHD1	chr15:40759300-40759307[+]	26[3]	26[3]	26[3]

hsa-miR-301a-3p	TOB2	chr22:41830128-41830134[-]	265[11]	283[11]	265[11]
hsa-miR-301a-3p	MDFIC	chr7:114656091-114656097[+]	268[3]	268[3]	268[3]
hsa-miR-301a-3p	LONRF1	chr8:12580359-12580366[-]	1302[13]	1302[13]	1302[13]
hsa-miR-301a-3p	NPNT	chr4:106892682-106892689[+]	743[4]	1496[5]	743[4]
hsa-miR-301a-3p	ZCCHC14	chr16:87443387-87443394[-]	526[7]	583[10]	526[7]
hsa-miR-301a-3p	SNX27	chr1:151667243-151667249[+]	113[7]	113[7]	113[7]
hsa-miR-301a-3p	ASXL2	chr2:25964663-25964669[-]	54[3]	54[3]	54[3]
hsa-miR-301a-3p	C10orf140	chr10:21802457-21802463[-]	5[1]	5[1]	5[1]
hsa-miR-301a-3p	C10orf140	chr10:21802573-21802579[-]	720[10]	720[12]	720[10]
hsa-miR-301a-3p	C10orf140	chr10:21803630-21803636[-]	263[1]	286[4]	263[1]
hsa-miR-301a-3p	AKAP7	chr6:131604408-131604414[+]	65[1]	65[1]	65[1]
hsa-miR-301a-3p	FAM45A	chr10:120896665-120896671[+]	47[4]	94[4]	47[4]
hsa-miR-301a-3p	SPTY2D1	chr11:18630367-18630373[-]	166[9]	166[9]	166[9]
hsa-miR-301a-3p	FAM73A	chr1:78343407-78343414[+]	731[15]	731[15]	708[14]
hsa-miR-301a-3p	STC1	chr8:23699736-23699742[-]	1[6]	1[6]	1[6]
hsa-miR-301a-3p	HBS1L	chr6:135357548-135357554[-]	367[7]	367[7]	367[7]
hsa-miR-301a-3p	SOCS5	chr2:46988493-46988500[+]	7[1]	7[1]	7[1]
hsa-miR-301a-3p	WDR20	chr14:102689600-102689606[+]	121[5]	121[5]	121[5]
hsa-miR-301a-3p	WDR20	chr14:102689731-102689737[+]	824[14]	824[14]	824[14]
hsa-miR-301a-3p	IRF1	chr5:131819226-131819233[-]	291[8]	301[9]	291[8]
hsa-miR-301a-3p	MIER1	chr1:67452213-67452219[+]	14[1]	7[1]	7[1]
hsa-miR-301a-3p	MIER1	chr1:67452939-67452946[+]	62[5]	186[8]	31[5]
hsa-miR-301a-3p	MIER1	chr1:67453270-67453276[+]	42[4]	92[4]	42[4]
hsa-miR-301a-3p	MYBL1	chr8:67475677-67475683[-]	18[2]	26[3]	18[2]
hsa-miR-301a-3p	MYBL1	chr8:67476009-67476016[-]	769[12]	769[12]	769[12]
hsa-miR-301a-3p	AKIRIN2	chr6:88384937-88384943[-]	1723[18]	1739[19]	1723[18]
hsa-miR-301a-3p	DLL1	chr6:170591735-170591742[-]	7[5]	7[5]	7[5]
hsa-miR-301a-3p	HABP4	chr9:99252446-99252452[+]	297[4]	297[4]	297[4]
hsa-miR-301a-3p	M6PR	chr12:9094406-9094412[-]	1224[10]	1224[10]	1224[10]
hsa-miR-301a-3p	RALBP1	chr18:9537237-9537243[+]	8[1]	8[1]	8[1]
hsa-miR-301a-3p	ARRDC3	chr5:90666326-90666332[-]	223[7]	502[7]	223[7]
hsa-miR-301a-3p	ATXN1	chr6:16299624-16299631[-]	84[5]	113[6]	84[5]
hsa-miR-301a-3p	C12orf34	chr12:110208251-110208257[+]	10[1]	10[1]	10[1]
hsa-miR-301a-3p	EXOC5	chr14:57670630-57670637[-]	260[8]	383[9]	260[8]
hsa-miR-301a-3p	FMR1	chrX:147031119-147031125[+]	70[5]	70[5]	35[5]
hsa-miR-301a-3p	CNOT6	chr5:180001816-180001822[+]	653[14]	658[15]	653[14]
hsa-miR-301a-3p	ACSL1	chr4:185676950-185676957[-]	93[4]	106[7]	93[4]
hsa-miR-301a-3p	PTPRG	chr3:62279202-62279209[+]	210[7]	210[7]	210[7]
hsa-miR-301a-3p	ARFIP1	chr4:153831663-153831669[+]	104[2]	104[2]	104[2]
hsa-miR-301a-3p	C7orf60	chr7:112460482-112460488[-]	103[5]	153[9]	103[5]
hsa-miR-301a-3p	C7orf60	chr7:112460942-112460948[-]	12[3]	38[5]	12[3]
hsa-miR-301a-3p	C7orf60	chr7:112461412-112461418[-]	129[10]	129[10]	129[10]
hsa-miR-301a-3p	STX7	chr6:132780836-132780842[-]	8[1]	28[5]	8[1]
hsa-miR-301a-3p	COL19A1	chr6:70920477-70920483[+]	17[2]	17[2]	17[2]
hsa-miR-301a-3p	MAP3K14	chr17:43340984-43340990[-]	524[6]	525[8]	524[6]
hsa-miR-301a-3p	USP8	chr15:50791874-50791880[+]	86[4]	110[5]	86[4]
hsa-miR-301a-3p	NEK9	chr14:75549174-75549180[-]	5[1]	5[1]	5[1]
hsa-miR-301a-3p	LCORL	chr4:17847323-17847330[-]	8[1]	8[1]	8[1]
hsa-miR-301a-3p	CALM2	chr2:47387504-47387511[-]	7396[23]	7442[25]	7396[23]

hsa-miR-301a-3p	ARHGAP1	chr11:46698662-46698668[-]	898[16]	898[16]	898[16]
hsa-miR-301a-3p	ZFPM2	chr8:106816284-106816290[+]	11[2]	16[3]	11[2]
hsa-miR-301a-3p	MIER3	chr5:56216966-56216972[-]	246[9]	288[10]	246[9]
hsa-miR-301a-3p	CHIC1	chrX:72901034-72901040[+]	333[10]	724[11]	333[10]
hsa-miR-301a-3p	FAM59A	chr18:29847744-29847751[-]	46[6]	105[7]	34[5]
hsa-miR-301a-3p	SPOPL	chr2:139326947-139326954[+]	115[8]	115[8]	115[8]
hsa-miR-301a-3p	DDX6	chr11:118622579-118622586[-]	1385[6]	1428[11]	1385[6]
hsa-miR-301a-3p	DDX6	chr11:118622607-118622613[-]	1408[8]	1426[10]	1408[8]
hsa-miR-301a-3p	MID1IP1	chrX:38665243-38665250[+]	559[14]	559[14]	559[14]
hsa-miR-301a-3p	MLL	chr11:118395373-118395379[+]	153[12]	153[12]	148[11]
hsa-miR-301a-3p	TBL1XR1	chr3:176741006-176741012[-]	281[7]	511[8]	281[7]
hsa-miR-301a-3p	TBL1XR1	chr3:176741985-176741992[-]	761[28]	761[28]	761[28]
hsa-miR-301a-3p	LRCH1	chr13:47325801-47325807[+]	7[1]	7[1]	7[1]
hsa-miR-301a-3p	GOLT1B	chr12:21670551-21670557[+]	325[8]	1328[8]	325[8]
hsa-miR-301a-3p	CNOT7	chr8:17087799-17087805[-]	134[6]	134[6]	134[6]
hsa-miR-301a-3p	ACBD5	chr10:27486196-27486203[-]	6284[19]	6284[19]	6284[19]
hsa-miR-301a-3p	ITPRIPL2	chr16:19132873-19132879[+]	11[4]	156[5]	11[4]
hsa-miR-301a-3p	CLOCK	chr4:56300664-56300670[-]	5[1]	17[2]	5[1]
hsa-miR-301a-3p	NIPA1	chr15:23043627-23043633[-]	333[6]	351[8]	333[6]
hsa-miR-301a-3p	ITPK1	chr14:93406245-93406251[-]	124[8]	124[8]	124[8]
hsa-miR-301a-3p	JMY	chr5:78621286-78621293[+]	345[11]	345[11]	345[11]
hsa-miR-301a-3p	EREG	chr4:75254158-75254164[+]	230[5]	230[5]	230[5]
hsa-miR-301a-3p	TRERF1	chr6:42193778-42193784[-]	5[1]	25[2]	5[1]
hsa-miR-301a-3p	MAML1	chr5:179203000-179203006[+]	1[1]	10[2]	1[1]
hsa-miR-301a-3p	WASL	chr7:123322047-123322053[-]	111[4]	118[5]	111[4]
hsa-miR-301a-3p	ARID4B	chr1:235331811-235331817[-]	708[17]	708[17]	708[17]
hsa-miR-301a-3p	CCNY	chr10:35860450-35860457[+]	24[1]	24[1]	24[1]
hsa-miR-301a-3p	ZIC5	chr13:100615478-100615485[-]	244[9]	509[14]	244[9]
hsa-miR-301a-3p	ATP11A	chr13:113538265-113538271[+]	227[9]	681[9]	227[9]
hsa-miR-301a-3p	NCOA1	chr2:24993317-24993323[+]	8[1]	24[1]	8[1]
hsa-miR-301a-3p	CDS1	chr4:85572054-85572060[+]	0[2]	0[2]	0[2]
hsa-miR-301a-3p	WHSC1L1	chr8:38174371-38174378[-]	509[5]	509[5]	509[5]
hsa-miR-301a-3p	PRR5L	chr11:36485232-36485238[+]	88[1]	719[4]	44[1]
hsa-miR-301a-3p	DLC1	chr8:12941452-12941458[-]	405[10]	405[10]	405[10]
hsa-miR-301a-3p	MTMR12	chr5:32228074-32228080[-]	55[3]	55[3]	55[3]
hsa-miR-301a-3p	SMOC1	chr14:70497100-70497106[+]	2030[9]	2030[9]	2030[9]
hsa-miR-301a-3p	NFIA	chr1:61923853-61923859[+]	204[8]	686[9]	204[8]
hsa-miR-301a-3p	TMEM50B	chr21:34821756-34821762[-]	56[4]	56[4]	56[4]
hsa-miR-301a-3p	PFKFB3	chr10:6275711-6275717[+]	70[3]	420[3]	70[3]
hsa-miR-301a-3p	ZNF800	chr7:127010539-127010546[-]	4615[29]	4615[29]	4615[29]
hsa-miR-301a-3p	RAPGEF4	chr2:173917381-173917387[+]	85[3]	85[3]	85[3]
hsa-miR-301a-3p	PRKD3	chr2:37478132-37478138[-]	21[2]	29[3]	21[2]
hsa-miR-301a-3p	CD69	chr12:9905691-9905698[-]	7[1]	7[1]	7[1]
hsa-miR-301a-3p	CANX	chr5:179157188-179157194[+]	2592[14]	2596[15]	2592[14]
hsa-miR-301a-3p	KBTBD8	chr3:67059744-67059751[+]	360[11]	360[11]	360[11]
hsa-miR-301a-3p	MAP4	chr3:47893101-47893107[-]	69[6]	298[9]	69[6]
hsa-miR-301a-3p	B4GALT5	chr20:48249622-48249628[-]	675[11]	675[11]	675[11]
hsa-miR-301a-3p	BMPR1B	chr4:96076277-96076283[+]	22[2]	22[2]	22[2]
hsa-miR-301a-3p	BIRC6	chr2:32843556-32843562[+]	26[1]	26[1]	26[1]

hsa-miR-301a-3p	ARHGAP12	chr10:32096474-32096480[-]	678[13]	696[14]	678[13]
hsa-miR-301a-3p	BMPR2	chr2:203424873-203424879[+]	205[7]	210[8]	205[7]
hsa-miR-301a-3p	SLAIN1	chr13:78338265-78338272[+]	780[17]	780[17]	780[17]
hsa-miR-301a-3p	BTBD7	chr14:93708320-93708326[-]	551[9]	804[15]	551[9]
hsa-miR-301a-3p	RAB5A	chr3:20026477-20026484[+]	67[4]	134[4]	67[4]
hsa-miR-301a-3p	FERMT2	chr14:53324944-53324950[-]	35[3]	43[4]	35[3]
hsa-miR-301a-3p	CREB5	chr7:28863571-28863577[+]	144[7]	144[7]	144[7]
hsa-miR-301a-3p	PITPNM2	chr12:123470104-123470110[-]	5[1]	5[1]	5[1]
hsa-miR-301a-3p	EFNB2	chr13:107142397-107142403[-]	160[6]	160[6]	160[6]
hsa-miR-301a-3p	NPAT	chr11:108028848-108028855[-]	44[4]	51[4]	44[4]
hsa-miR-301a-3p	ZEB1	chr10:31816692-31816698[+]	667[7]	667[7]	667[7]
hsa-miR-301a-3p	QKI	chr6:163987113-163987119[+]	2687[16]	1791[16]	896[16]
hsa-miR-301a-3p	AKAP1	chr17:55197715-55197721[+]	61[4]	61[4]	61[4]
hsa-miR-301a-3p	PPARG	chr3:12475686-12475693[+]	67[4]	134[4]	67[4]
hsa-miR-301a-3p	SUV420H1	chr11:67924285-67924291[-]	25[2]	25[2]	25[2]
hsa-miR-301a-3p	SOX21	chr13:95363180-95363186[-]	10[1]	10[1]	10[1]
hsa-miR-301a-3p	S1PR1	chr1:101705948-101705954[+]	17[2]	17[2]	17[2]
hsa-miR-301a-3p	SH3D19	chr4:152043164-152043171[-]	269[3]	271[4]	269[3]
hsa-miR-301a-3p	CMPK1	chr1:47843409-47843415[+]	1446[14]	2891[14]	1446[14]
hsa-miR-301a-3p	ACBD3	chr1:226333393-226333400[-]	123[1]	123[1]	123[1]
hsa-miR-301a-3p	WDR47	chr1:109513108-109513114[-]	29[3]	218[3]	29[3]
hsa-miR-301a-3p	ZNF711	chrX:84528359-84528365[+]	482[10]	482[10]	482[10]
hsa-miR-301a-3p	C3orf64	chr3:69026554-69026560[-]	141[10]	838[12]	141[10]
hsa-miR-301a-3p	ARFGEF1	chr8:68110521-68110527[-]	0[2]	20[3]	0[2]
hsa-miR-301a-3p	ATG16L1	chr2:234204030-234204036[+]	827[21]	827[21]	827[21]
hsa-miR-301a-3p	SOX4	chr6:21598287-21598293[+]	5919[18]	5919[18]	5919[18]
hsa-miR-301a-3p	APPL1	chr3:57304797-57304804[+]	100[6]	144[7]	100[6]
hsa-miR-301a-3p	SMAD5	chr5:135514014-135514020[+]	5817[28]	5817[28]	5817[28]
hsa-miR-301a-3p	SPEN	chr1:16266274-16266281[+]	99[6]	99[6]	99[6]
hsa-miR-301a-3p	WNK1	chr12:1020503-1020510[+]	1905[14]	1912[15]	1905[14]
hsa-miR-301a-3p	VGLL4	chr3:11599545-11599552[-]	460[13]	460[15]	460[13]
hsa-miR-301a-3p	ACVR1	chr2:158593589-158593595[-]	151[6]	176[10]	151[6]
hsa-miR-301a-3p	ACVR1	chr2:158593617-158593624[-]	270[13]	270[13]	270[13]
hsa-miR-301a-3p	C5orf30	chr5:102612758-102612764[+]	82[7]	82[7]	82[7]
hsa-miR-301a-3p	DIP2A	chr21:47987858-47987864[+]	25[2]	50[2]	25[2]
hsa-miR-301a-3p	CCRN4L	chr4:139967083-139967089[+]	60[1]	60[1]	60[1]
hsa-miR-301a-3p	PIGA	chrX:15338250-15338256[-]	3[1]	3[1]	3[1]
hsa-miR-301a-3p	PIGA	chrX:15339432-15339438[-]	226[9]	226[9]	226[9]
hsa-miR-301a-3p	PMEPA1	chr20:56226490-56226497[-]	827[5]	827[5]	827[5]
hsa-miR-301a-3p	ENPP5	chr6:46128736-46128742[-]	152[13]	3454[14]	152[13]
hsa-miR-301a-3p	ENPP5	chr6:46128761-46128768[-]	2206[17]	4412[17]	2206[17]
hsa-miR-301a-3p	MAFB	chr20:39314895-39314901[-]	14[2]	14[2]	14[2]
hsa-miR-301a-3p	GDA	chr9:74863827-74863833[+]	86[1]	86[1]	86[1]
hsa-miR-301a-3p	SIK1	chr21:44836458-44836464[-]	7105[10]	7105[10]	7105[10]
hsa-miR-301a-3p	ZNF3	chr7:99667621-99667627[-]	50[7]	60[8]	50[7]
hsa-miR-301a-3p	ZNF3	chr7:99668509-99668516[-]	1[1]	15[2]	1[1]
hsa-miR-301a-3p	NPTX1	chr17:78441873-78441879[-]	230[12]	247[13]	230[12]
hsa-miR-301a-3p	IMPDH1	chr7:128032961-128032968[-]	49[2]	49[2]	49[2]
hsa-miR-301a-3p	ZEB2	chr2:145146712-145146718[-]	26[2]	26[2]	26[2]

hsa-miR-301a-3p	NR3C2	chr4:149000737-149000744[-]	7[1]	7[1]	7[1]
hsa-miR-301a-3p	CSF1	chr1:110472260-110472266[+]	113[5]	113[5]	113[5]
hsa-miR-301a-3p	MED12L	chr3:151150626-151150632[+]	0[6]	0[6]	0[6]
hsa-miR-301a-3p	TIMP2	chr17:76849784-76849790[-]	2936[3]	2940[4]	2936[3]
hsa-miR-301a-3p	RAB34	chr17:27041572-27041578[-]	727[13]	364[13]	364[13]
hsa-miR-301a-3p	PAFAH1B1	chr17:2587964-2587970[+]	317[14]	317[14]	317[14]
hsa-miR-301a-3p	EPHB4	chr7:100400982-100400988[-]	7[3]	15[4]	7[3]
hsa-miR-301a-3p	BTG1	chr12:92537485-92537492[-]	1675[9]	1675[9]	1675[9]
hsa-miR-301a-3p	FAM46B	chr1:27331546-27331552[-]	67[2]	67[2]	67[2]
hsa-miR-301a-3p	STIM2	chr4:27025069-27025076[+]	96[2]	144[2]	48[2]
hsa-miR-301a-3p	FSTL5	chr4:162306849-162306855[-]	545[2]	545[2]	545[2]
hsa-miR-301a-3p	SOS2	chr14:50584800-50584806[-]	3[1]	13[2]	3[1]
hsa-miR-301a-3p	CHD5	chr1:6165205-6165211[-]	14[2]	28[2]	14[2]
hsa-miR-301a-3p	UBE2D1	chr10:60129213-60129219[+]	31[2]	177[9]	31[2]
hsa-miR-301a-3p	DNAJC16	chr1:15898053-15898059[+]	162[5]	186[8]	162[5]
hsa-miR-301a-3p	PTPRM	chr18:8406316-8406322[+]	328[3]	328[3]	328[3]
hsa-miR-301a-3p	GPATCH8	chr17:42474128-42474134[-]	951[24]	951[24]	951[24]
hsa-miR-301a-3p	CCDC126	chr7:23683289-23683296[+]	43[3]	43[3]	43[3]
hsa-miR-301a-3p	SYBU	chr8:110586830-110586836[-]	926[9]	926[9]	926[9]
hsa-miR-301a-3p	SYBU	chr8:110587008-110587015[-]	207[8]	207[8]	207[8]
hsa-miR-301a-3p	SFMBT1	chr3:52939022-52939028[-]	355[9]	355[9]	355[9]
hsa-miR-301a-3p	MEX3D	chr19:1555285-1555291[-]	65[6]	45[7]	33[6]
hsa-miR-301a-3p	MAT2B	chr5:162945503-162945510[+]	449[4]	1076[11]	449[4]
hsa-miR-301a-3p	HCFC2	chr12:104498798-104498805[+]	74[3]	148[3]	74[3]
hsa-miR-301a-3p	RFX7	chr15:56383553-56383560[-]	1492[6]	1492[6]	1492[6]
hsa-miR-301a-3p	TAF4	chr20:60550474-60550480[-]	89[7]	132[9]	89[7]
hsa-miR-301a-3p	DYNLL2	chr17:56166708-56166714[+]	142[9]	142[9]	142[9]
hsa-miR-301a-3p	DYNLL2	chr17:56166889-56166896[+]	301[9]	363[15]	301[9]
hsa-miR-301a-3p	CBFB	chr16:67132840-67132847[+]	492[5]	492[5]	246[5]
hsa-miR-301a-3p	ITFG3	chr16:316079-316085[+]	5[1]	5[1]	5[1]
hsa-miR-301a-3p	DLG5	chr10:79551097-79551103[-]	731[6]	731[6]	731[6]
hsa-miR-301a-3p	FAM178A	chr10:102722646-102722652[+]	29[2]	29[2]	29[2]
hsa-miR-301a-3p	FAM178A	chr10:102724004-102724010[+]	6[5]	6[5]	6[5]
hsa-miR-301a-3p	LRP1B	chr2:140990542-140990548[-]	8[1]	8[1]	8[1]
hsa-miR-301a-3p	KIT	chr4:55606372-55606378[+]	1969[4]	1969[4]	1969[4]
hsa-miR-301a-3p	CYLD	chr16:50830609-50830615[+]	42[5]	51[6]	40[4]
hsa-miR-301a-3p	DENND4C	chr9:19373469-19373475[+]	39[3]	107[5]	39[3]
hsa-miR-301a-3p	ATRX	chrX:76763819-76763826[-]	0[2]	0[2]	0[2]
hsa-miR-301a-3p	NRP1	chr10:33466864-33466870[-]	83[6]	242[8]	83[6]
hsa-miR-301a-3p	MMGT1	chrX:135046838-135046845[-]	2830[19]	2830[19]	2830[19]
hsa-miR-301a-3p	FAM73B	chr9:131834170-131834176[+]	111[11]	223[11]	111[11]
hsa-miR-301a-3p	CLIP1	chr12:122757059-122757066[-]	7218[31]	7218[31]	7218[31]
hsa-miR-301a-3p	MIB1	chr18:19449555-19449561[+]	17[1]	34[2]	17[1]
hsa-miR-301a-3p	VPS37A	chr8:17152686-17152692[+]	662[20]	662[20]	662[20]
hsa-miR-301a-3p	EMX2	chr10:119308241-119308247[+]	20[2]	20[2]	10[2]
hsa-miR-301a-3p	MAPRE3	chr2:27249590-27249596[+]	2732[22]	2732[22]	2732[22]
hsa-miR-301a-3p	CLTC	chr17:57771519-57771525[+]	344[6]	360[8]	344[6]
hsa-miR-301a-3p	SPG20	chr13:36878058-36878065[-]	502[14]	502[14]	502[14]
hsa-miR-301a-3p	YTHDF2	chr1:29095848-29095854[+]	70[5]	86[5]	70[5]

hsa-miR-301a-3p	TROVE2	chr1:193054282-193054288[+]	682[21]	682[21]	682[21]
hsa-miR-301a-3p	ABHD3	chr18:19231278-19231284[-]	1381[2]	1381[2]	1381[2]
hsa-miR-301a-3p	SPHK2	chr19:49133524-49133531[+]	28[5]	81[6]	28[5]
hsa-miR-301a-3p	PAPD4	chr5:78981755-78981761[+]	311[10]	320[11]	311[10]
hsa-miR-301a-3p	TSPYL2	chrX:53117212-53117218[+]	332[5]	332[5]	332[5]
hsa-miR-301a-3p	ITGB8	chr7:20452190-20452197[+]	27[2]	27[2]	27[2]
hsa-miR-301a-3p	SNX5	chr20:17922941-17922947[-]	628[19]	851[21]	628[19]
hsa-miR-301a-3p	THOP1	chr19:2813507-2813514[+]	177[12]	187[12]	177[12]
hsa-miR-301a-3p	C9orf69	chr9:139006687-139006694[-]	456[9]	564[16]	456[9]
hsa-miR-301a-3p	ANKRD12	chr18:9282241-9282247[+]	68[3]	68[3]	68[3]
hsa-miR-301a-3p	MAF	chr16:79630819-79630826[-]	8[4]	8[4]	5[3]
hsa-miR-301a-3p	BPTF	chr17:65979841-65979848[+]	12[2]	12[2]	12[2]
hsa-miR-301a-3p	RAB5B	chr12:56386958-56386964[+]	3816[25]	3816[25]	3816[25]
hsa-miR-301a-3p	PLAA	chr9:26905345-26905351[-]	10[1]	10[1]	10[1]
hsa-miR-301a-3p	MYB	chr6:135539870-135539876[+]	4[1]	4[1]	4[1]
hsa-miR-301a-3p	ARHGEF12	chr11:120356938-120356944[+]	114[4]	114[4]	114[4]
hsa-miR-301a-3p	ZFYVE26	chr14:68213503-68213509[-]	5168[23]	5168[23]	5168[23]
hsa-miR-301a-3p	ATP6V1B2	chr8:20077975-20077981[+]	1690[19]	3380[19]	1690[19]
hsa-miR-301a-3p	DENND1A	chr9:126143434-126143441[-]	1[1]	1[1]	1[1]
hsa-miR-301a-3p	CUL3	chr2:225338401-225338407[-]	283[11]	283[11]	283[11]
hsa-miR-301a-3p	ABCA1	chr9:107543479-107543485[-]	104[2]	153[3]	104[2]
hsa-miR-301a-3p	EPS15	chr1:51820585-51820591[-]	17[3]	17[3]	17[3]
hsa-miR-301a-3p	EPS15	chr1:51820816-51820822[-]	440[5]	440[5]	440[5]
hsa-miR-301a-3p	BLCAP	chr20:36146811-36146817[-]	3377[23]	3377[23]	3377[23]
hsa-miR-301a-3p	TNRC6C	chr17:76101884-76101890[+]	130[8]	157[9]	88[7]
hsa-miR-301a-3p	TOM1L2	chr17:17747089-17747095[-]	232[6]	232[6]	232[6]
hsa-miR-301a-3p	ADAM12	chr10:127705803-127705810[-]	53[1]	53[1]	53[1]
hsa-miR-301a-3p	TSHZ1	chr18:73000626-73000633[+]	92[8]	117[8]	92[8]
hsa-miR-301a-3p	TGFB2	chr1:218615912-218615919[+]	21[1]	63[1]	21[1]
hsa-miR-301a-3p	LRCH2	chrX:114346435-114346441[-]	12[2]	50[3]	12[2]
hsa-miR-301a-3p	TNRC6A	chr16:24835520-24835526[+]	1180[18]	1195[18]	1180[18]
hsa-miR-301a-3p	RNF216	chr7:5659880-5659886[-]	122[6]	122[6]	122[6]
hsa-miR-301a-3p	PGM2L1	chr11:74041438-74041444[-]	158[5]	158[5]	158[5]
hsa-miR-301a-3p	BRWD1	chr21:40568020-40568026[-]	1265[17]	1336[23]	1265[17]
hsa-miR-301a-3p	IER3IP1	chr18:44682375-44682381[-]	544[9]	544[9]	544[9]
hsa-miR-301a-3p	IER3IP1	chr18:44682445-44682451[-]	123[5]	125[5]	123[5]
hsa-miR-301a-3p	DSEL	chr18:65178178-65178185[-]	1624[5]	1624[5]	1624[5]
hsa-miR-301a-3p	HECA	chr6:139501795-139501801[+]	225[12]	225[12]	225[12]
hsa-miR-301a-3p	NRP2	chr2:206641313-206641319[+]	19[3]	19[3]	19[3]
hsa-miR-301a-3p	FASTK	chr7:150773757-150773763[-]	63[11]	125[11]	63[11]
hsa-miR-301a-3p	CNIH	chr14:54894262-54894268[-]	961[17]	1190[18]	961[17]
hsa-miR-301a-3p	CAMTA1	chr1:7826748-7826754[+]	115[11]	229[11]	115[11]
hsa-miR-301a-3p	LMLN	chr3:197768817-197768824[+]	1261[16]	1261[16]	1261[16]
hsa-miR-301a-3p	HOXB3	chr17:46626745-46626751[-]	782[13]	795[17]	782[13]
hsa-miR-301a-3p	GPCPD1	chr20:5527861-5527867[-]	22[1]	33[3]	22[1]
hsa-miR-301a-3p	MLEC	chr12:121136994-121137000[+]	1483[22]	1483[22]	1483[22]
hsa-miR-301a-3p	CDK19	chr6:110934086-110934093[-]	7[2]	15[3]	7[2]
hsa-miR-301a-3p	CDK19	chr6:110934513-110934519[-]	125[10]	125[10]	125[10]
hsa-miR-301a-3p	RNF145	chr5:158584762-158584768[-]	3739[15]	3739[15]	3739[15]

hsa-miR-301a-3p	CASD1	chr7:94186173-94186179[+]	32[3]	32[3]	32[3]
hsa-miR-301a-3p	NHS	chrX:17753515-17753521[+]	7[1]	15[2]	7[1]
hsa-miR-301a-3p	MAP3K9	chr14:71196983-71196989[-]	492[15]	492[15]	492[15]
hsa-miR-301a-3p	TES	chr7:115898586-115898592[+]	945[18]	945[18]	945[18]
hsa-miR-301a-3p	PELI1	chr2:64320542-64320549[-]	108[6]	170[8]	102[5]
hsa-miR-301a-3p	PAN3	chr13:28866713-28866720[+]	349[9]	349[9]	349[9]
hsa-miR-301a-3p	PAN3	chr13:28866783-28866789[+]	2[1]	2[1]	2[1]
hsa-miR-301a-3p	PAN3	chr13:28866945-28866951[+]	39[6]	39[6]	39[6]
hsa-miR-301a-3p	WDR1	chr4:10076028-10076034[-]	500[11]	500[11]	500[11]
hsa-miR-301a-3p	ZNF238	chr1:244218798-244218804[+]	553[16]	576[18]	553[16]
hsa-miR-301a-3p	ZNF238	chr1:244220173-244220179[+]	739[16]	752[18]	739[16]
hsa-miR-301a-3p	FAM13A	chr4:89648679-89648685[-]	8[2]	8[2]	8[2]
hsa-miR-301a-3p	CEP120	chr5:122681843-122681849[-]	662[17]	662[17]	662[17]
hsa-miR-301a-3p	PTGFRN	chr1:117532175-117532181[+]	33[3]	33[3]	33[3]
hsa-miR-301a-3p	SNX2	chr5:122165617-122165624[+]	5[1]	29[2]	5[1]
hsa-miR-301a-3p	SLMAP	chr3:57914842-57914849[+]	229[9]	599[10]	229[9]
hsa-miR-301a-3p	MLL3	chr7:151833336-151833343[-]	2446[27]	4910[27]	2446[27]
hsa-miR-301a-3p	TMEM63B	chr6:44123181-44123187[+]	2[1]	4[1]	2[1]
hsa-miR-301a-3p	DPYSL2	chr8:26515087-26515093[+]	163[9]	325[9]	163[9]
hsa-miR-301a-3p	ELK3	chr12:96661069-96661075[+]	155[6]	155[6]	155[6]
hsa-miR-301a-3p	SNIP1	chr1:38002810-38002816[-]	344[10]	345[11]	344[10]
hsa-miR-301a-3p	NDEL1	chr17:8370575-8370581[+]	151[6]	250[7]	75[6]
hsa-miR-301a-3p	TRPS1	chr8:116424239-116424245[-]	59[4]	59[4]	54[3]
hsa-miR-301a-3p	ERBB2IP	chr5:65376098-65376104[+]	672[16]	672[16]	672[16]
hsa-miR-301a-3p	ZFYVE9	chr1:52811956-52811962[+]	6128[28]	6128[28]	6128[28]
hsa-miR-301a-3p	PEX5L	chr3:179519033-179519040[-]	5[1]	5[1]	5[1]
hsa-miR-301a-3p	ODZ1	chrX:123511432-123511439[-]	32[1]	32[1]	32[1]
hsa-miR-301a-3p	ANKRD13C	chr1:70726444-70726450[-]	17[2]	29[3]	17[2]
hsa-miR-301a-3p	USP13	chr3:179507067-179507074[+]	359[8]	359[8]	359[8]
hsa-miR-301a-3p	ZNF217	chr20:52184174-52184180[-]	3019[17]	3030[17]	3019[17]
hsa-miR-301a-3p	ZNF217	chr20:52184439-52184445[-]	455[7]	455[7]	455[7]
hsa-miR-301a-3p	RAP2C	chrX:131337771-131337777[-]	118[2]	118[2]	118[2]
hsa-miR-301a-3p	RAP2C	chrX:131338609-131338616[-]	31[3]	31[3]	31[3]
hsa-miR-301a-3p	TESK2	chr1:45810009-45810015[-]	36[3]	36[3]	36[3]
hsa-miR-301a-3p	PHF17	chr4:129795525-129795532[+]	37[3]	37[3]	37[3]
hsa-miR-301a-3p	RNF38	chr9:36339618-36339624[-]	88[5]	88[5]	88[5]
hsa-miR-301a-3p	KIAA1468	chr18:59972905-59972911[+]	84[5]	180[13]	84[5]
hsa-miR-301a-3p	NRBF2	chr10:64914508-64914515[+]	315[12]	319[13]	315[12]
hsa-miR-301a-3p	SKP1	chr5:133493368-133493374[-]	22[2]	22[2]	11[2]
hsa-miR-301a-3p	NAA30	chr14:57876383-57876389[+]	255[3]	509[3]	255[3]
hsa-miR-301a-3p	SPATA2	chr20:48520885-48520892[-]	169[6]	169[6]	169[6]
hsa-miR-301a-3p	SNPH	chr20:1287720-1287727[+]	2[3]	2[3]	2[3]
hsa-miR-301a-3p	KLHL15	chrX:24003179-24003186[-]	46[4]	48[4]	46[4]
hsa-miR-301a-3p	EPC2	chr2:149544198-149544204[+]	369[10]	556[10]	369[10]
hsa-miR-301a-3p	EPC2	chr2:149545055-149545061[+]	17[1]	17[1]	17[1]
hsa-miR-301a-3p	KDM2A	chr11:67023214-67023220[+]	26[3]	26[3]	26[3]
hsa-miR-301a-3p	KDM2A	chr11:67023814-67023820[+]	1395[15]	2810[16]	1395[15]
hsa-miR-301a-3p	KDM2A	chr11:67023942-67023948[+]	518[12]	518[12]	518[12]
hsa-miR-301a-3p	C18orf1	chr18:13646235-13646242[+]	44[2]	44[2]	44[2]

hsa-miR-301a-3p	ITPR1	chr3:4888173-4888179[+]	5125[5]	5125[5]	5125[5]
hsa-miR-301a-3p	OTUD3	chr1:20239075-20239082[+]	609[12]	609[12]	609[12]
hsa-miR-301a-3p	SLC44A1	chr9:108152688-108152695[+]	1472[17]	2944[17]	1472[17]
hsa-miR-301a-3p	C16orf70	chr16:67181493-67181500[+]	3356[27]	3356[27]	3356[27]
hsa-miR-301a-3p	KLHL20	chr1:173755494-173755500[+]	2512[23]	2512[23]	2512[23]
hsa-miR-301a-3p	KIAA1211	chr4:57196222-57196229[+]	76[4]	76[4]	76[4]
hsa-miR-301a-3p	DNAL1	chr14:74167951-74167957[+]	0[2]	21[4]	0[2]
hsa-miR-301a-3p	FBXO28	chr1:224346650-224346656[+]	1380[13]	1408[13]	690[13]
hsa-miR-301a-3p	FBXO28	chr1:224348687-224348694[+]	375[15]	1071[22]	375[15]
hsa-miR-301a-3p	INO80	chr15:41271577-41271583[-]	23[2]	33[3]	23[2]
hsa-miR-301a-3p	ZBTB4	chr17:7363543-7363549[-]	1164[24]	1171[24]	1164[24]
hsa-miR-301a-3p	ZBTB4	chr17:7364010-7364017[-]	356[14]	1191[15]	356[14]
hsa-miR-301a-3p	CPEB2	chr4:15070077-15070083[+]	558[7]	558[7]	558[7]
hsa-miR-301a-3p	MARK3	chr14:103969734-103969740[+]	16[3]	16[3]	16[3]
hsa-miR-301a-3p	LDLR	chr19:11242863-11242869[+]	657[9]	1350[11]	657[9]
hsa-miR-301a-3p	LDLR	chr19:11244153-11244159[+]	5975[24]	11951[24]	5975[24]
hsa-miR-301a-3p	USP33	chr1:78162181-78162187[-]	544[9]	1098[9]	544[9]
hsa-miR-301a-3p	TSC1	chr9:135766736-135766743[-]	7[1]	13[2]	7[1]
hsa-miR-301a-3p	TSC1	chr9:135767162-135767169[-]	5[1]	5[1]	5[1]
hsa-miR-301a-3p	CEP170	chr1:243289118-243289125[-]	468[10]	936[10]	468[10]
hsa-miR-301a-3p	G3BP2	chr4:76568734-76568741[-]	49[4]	49[4]	49[4]
hsa-miR-301a-3p	PTEN	chr10:89727482-89727488[+]	686[7]	687[8]	686[7]
hsa-miR-301a-3p	SASH1	chr6:148872899-148872905[+]	2043[16]	2043[16]	2043[16]
hsa-miR-301a-3p	MFSD6	chr2:191366517-191366523[+]	59[5]	59[5]	59[5]
hsa-miR-301a-3p	LNPEP	chr5:96364344-96364350[+]	39[2]	39[2]	39[2]
hsa-miR-301a-3p	PTP4A1	chr6:64290796-64290802[+]	240[9]	259[12]	240[9]
hsa-miR-301a-3p	HBP1	chr7:106842705-106842711[+]	881[3]	881[3]	881[3]
hsa-miR-301a-3p	HBP1	chr7:106842728-106842734[+]	3517[21]	3517[21]	3517[21]
hsa-miR-301a-3p	FAM175B	chr10:126523909-126523916[+]	165[9]	165[9]	165[9]

Supplementary Table 2. Sequences of miRNA mimics and inhibitors

Name	Sequences
miR-301a-3p	5'-CAGUGCAAUAGUAUUGUCAAAGC-'3 UUUGACAAUACUAUUGCACUGUU
miR-NC	5'-UUCUCCGAACGUGUCACGUTT-'3 ACGUGACACGUUCGGAGAATT
Anti-miR-301a-3p	5'-GCUUUGACAAUACUAUUGCACUG-'3
Anti-miR-NC	5'-UUGUACUACACAAAAGUACUG-'3

Supplementary Table 3. Primers for qRT-PCR

Gene name	Forward (5'-3')	Reverse (5'-3')
TGFβR2	AGCACTGTGCCATCATCCT	GACCTCAGCAAAGCGACCT
SMAD4	TCCAGCCTCCCATTTCCAAT	ACCTTGCTCTCTCAATGGCT
PTEN	ACCAGGACCAGAGGAAACCT	GCTAGCCTCTGGATTTGACG
PIAS3	ATGGTGATGAGTTTCCGGGT	GAAGGGAGAGATCAGAGGGC
NKRF	AGCTGCTGAGAAAGATGGGT	AAATCTGTGTGGCTCTCGGA
FOXF2	CCCGTTACCAGCATCACTCT	TGACGCAGGGCTTAATATCC
P21Cip1	ATGAAATTCACCCCCTTTCC	CCCTAGGCTGTGCTCACTT
p57Kip2	GGCGATCAAGAAGCTGTCC	GACTTCTCAGGCGCTGATCT
c-MYC	TTCGGGTAGTGGAAAACCAG	CAGCAGCTCGAATTTCTTCC
Bim	AGCCCAGCACCCATGAGTTGTGAC	CTCTGGGCGCATATCTGCAGG
CTGF	GGAAAAGATTCCCACCCAAT	TGCTCCTAAAGCCACACCTT
SMAD7	TACCGTGCAGATCAGCTTTG	TTTGCATGAAAAGCAAGCAC
ZEB1	TGCACTGAGTGTGGAAAAGC	TGGTGATGCTGAAAGAGACG
GAPDH	ACCTGACCTGCCGTCTAGAA	TCCACCACCCTGTTGCTGTA