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Doctor of Philosophy

The role and mechanism of miR-342-3p in pancreatic cancer

The Graduate School

of the University of Ulsan

Department of Medicine

Bong Jun Kwak

The role and mechanism of miR-342-3p in pancreatic cancer

Supervisor : Song Cheol Kim

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Doctor of Philosophy

By

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Department of Medicine
Ulsan, Korea
February 2020

Synergistic anticancer therapeutic effect of miR-342-3p inhibitor and gemcitabine in pancreatic cancer

This certifies that the dissertation

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February 2020

Abstract

Pancreatic cancer (PC) is a lethal malignancy with aggressive behavior. The median survival rate remains below 10 %, indicating that the identification of pathogenesis and novel therapeutic target is urgently needed. MicroRNA (miRNA) play a central part in cell biology and it was dysregulated in numerous diseases, including cancer. These miRNAs can be potential candidate for therapeutics for PC. This study aimed to investigate the role of microRNA-342-3p (miR-342-3p) in PC development. MiR-342-3p was upregulated in PC cell lines. Among eight target gene candidate of miR-342-3p found using web-database, FosB, AP-1 transcription factor subunit was confirmed as a direct target gene of miR-342-3p. MiR-342-3p inhibitor transfection in PC cells decreased PC cell survival. Combination treatment with miR-342-3p inhibitor and Gemcitabine showed definite synergistic antitumor effect in PC. In conclusion, miR-342-3p was upregulated in PC cell. The direct target of miR-342-3p was FosB. Inhibition of miR-342-3p decreased cell survival in PC. Combination treatment with miR-342-3p inhibitor and Gemcitabine showed synergistic antitumor effect in PC. Therefore, miR-342-3p may provide a new molecular target for therapeutic strategy against PC.

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1. Introduction

Pancreatic cancer (PC) is well known as a lethal disease with very poor prognosis. Five year survival rate of PC was only 8 % from 2006 to 2012 in united states (1). Due to lack of effective strategies for early detection and invasive characteristics during the early stages of carcinogenesis, up to 80 % patients have locally advanced or metastatic disease at diagnosis (1, 2). Total deaths from PC have increased dramatically, and PC is predicted to become the second leading cause of cancer-related deaths by 2030 (3). Therefore, new insight into the carcinogenic mechanism of PC is urgently needed to advance the development of early detection modalities and effective therapeutics.

MicroRNAs (miRNAs) are a class of small, endogenous, noncoding RNA molecules that lead to mRNA translation inhibition or degradation by binging with complementary sequences in the 3'-untranslated region (3'-UTR) of their target mRNAs (4). It has been reported that miRNAs play a central part in cell differentiation, proliferation and survival and it was dysregulated in numerous diseases, including cancer (4, 5). Recently, some studies reported miR-21, miR-182, and miR-144-3p are related with PC carcinogenesis (6-8). These miRNAs can be potential candidate for therapeutics for PC.

The present study aimed to investigate the role and mechanism of miR-342-3p in PC carcinogenesis. We detected the expression of miR-342-3p on PC cell lines, and identified

FosB proto-oncogene, AP-1 transcription factor subunit (FosB) as a direct target of miR-342-3p. We investigated the effects of its expression on proliferation by miRNA modulation. And also investigated synergistic anticancer effect by combination with gemcitabine. The result showed that miR-342-3p promotes tumor cell proliferation by regulating the expression of its target FosB. In addition, combination with miR-342-3p inhibitor and gemcitabine showed synergistic anticancer effect in PC cell.

2. Materials and Methods

2.1. Cell culture and hypoxic conditions

We purchased human PC cell lines, including MIA PaCa-2 (ATCC® CRL-1420™), and PANC-1 (ATCC® CRL-1469™) cell lines from American Type Culture Collection (ATCC) Manassas, VA, USA. MIA PaCa-2 and PANC-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) high-glucose (Hyclone, Thermo Fisher Scientific, Rockford, IL, USA), supplemented with 10 % fetal bovine serum (FBS; Mediatech, Manassas, VA, USA) and 100 U/ml penicillin/streptomycin (HyClone).

We cultured all the cells in a standard humidified incubator at 37 ℃ in an atmosphere of 5.0 % CO_2. To induce hypoxic conditions, we placed cells in a hypoxia incubator (MCO-18M; Sanyo, Tokyo, Japan) filled with a mixture of 5.0 % CO_2, 94.0 % N_2, and 1.0 % O_2 gas.

2.2. Cell Transfection

The synthetic, chemically modified short single-or double-stranded RNA oligonucleotides including anti-miR-342-3p and its appropriate negative control (anti-miR-NC), as well as miR-342-3p mimics and its appropriate negative control (miR-NC), were purchased from Qiagen (Germany). For transfection, MIA PaCa-2 cells were seeded on a 24-well plate with 1.0×10^5 cells per well and incubated overnight. Lipofectamine 2000 (11668-2019, Thermo Fisher Scientific, Scotts Valley, CA, USA) was used for transfection according to the manufacturer's instructions. The expression levels were quantified after 24h and 48h of transfection.

2.3. RNA extraction and RT-PCR

We isolated total RNA from PC cells and patient derived cells using QIAzol reagent (Cat no. 79306, Qiagen, Germany). We homogenized PC cell and patient derived cell suspensions in QIAzol reagent. The homogenates were mixed thoroughly after adding chloroform and were centrifuged at 16,000 × g for 15 min. The aqueous phase was removed, and the RNA was precipitated with isopropyl alcohol. RNA was pelleted, washed with 70 % ethanol, dried, and eluted using DEPC-treated water. We used a spectrophotometer Nanodrop 2000 (Nanodrop, Wilmington, DE, USA) to quantify the final concentration.

We quantified transcription of relevant genes using real-time reverse transcription-polymerase chain reaction (RT-PCR) in a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA) with 5× HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Tartu, Estonia), according to the manufacturer's instructions. In brief, the samples were first denatured at 95 ℃ for 15 min, followed by 40 cycles of denaturation at 95 ℃ for 15 s, annealing at 55 ℃–60 ℃ for 15 s, and elongation at 72 ℃ for 20 s. The data are expressed as the fold changes in the treatment groups relative to the control level and were normalized to *GAPDH* levels using the delta-delta Ct methods (9).

2.4. Western blot analysis

MIA PaCa-2 cells and patient derived pancreatic cancer cells (1.0 x 10^5 cells) were obtained for further processing. Cytoplasmic and nuclear extracts were collected separately using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific) including a protease inhibitor (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions. The protein concentration was measured using the BCA method with 2 mg/mL bovine serum albumin (BSA) as the standard (Thermo Fisher Scientific). Equal amounts of protein were separated by 10 % sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). The separated proteins were transferred onto nitrocellulose membranes. The membranes were blocked with 5 % BSA in Tris-buffered saline with 0.1 %

Tween-20 (TBST, pH 7.6) for 1 h at room temperature (22–24 °C),followed by incubation with the appropriate primary antibody overnight at 4 °C. Membranes were treated with the chemiluminescence reagent SuperSignal West Femto Substrate (Thermo Fisher Scientific) and detected using the Image Quant LAS-4000 imaging system (GE Healthcare Life Sciences, Marlborough, MA, USA). Western blot image was quantified using Image Studio Lite 5.0 (LICOR Biosciences, Lincoln, NE). Protein levels were normalized to Actin. The levels of other compounds were normalized to matching control group.

2.5. Cell growth and viability test

Cell growth and viability were determined by performing a 3- (4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in 24 well cell culture plates (NEST Biotechnology, Wuxi, China). MIA PaCa-2 cells were seeded at a density of 1×10^5 per well for 48 h and cultured in DMEM containing 10 % FBS. The cells were then treated with miR-342-3p inhibitor or gemcitabine. After incubation for 24 and 48h, 20 µl of MTT solution (5 mg/ml in phosphate buffer saline) was added to each well, and the plates were incubated in the dark for 4 h at 37 ℃. Cell growth and viability were expressed as the percentage of absorbance of MTT-treated cells relative to that of untreated cells at a wavelength of 540 nm. Measurements for all treatment groups were taken four times.

2.6. Luciferase reporter gene assay

MIA PaCa-2 cells were seeded on a 24-well plate with 1.0×10^5 cells per well and transfected with FosB 3'-UTR containing wild-type (WT) or mutated (Mut) predictive miR-342-3p binding site was subcloned into pmiRGlo Dual-luciferase miRNA Target Expression Vector (Promega Corporation, USA) located 5' to the firefly luciferase. The MIA PaCa-2 cells were seeded on a 48-well plate with 1.0×10^5 cells per well and co-transfected with a 1 ug of pRL-SV40, 1.5 μg of pmirGlo-FosB 3'-UTR construct, 60 nM of NC. Lipofectamine 2000 (11668-2019, Thermo Fisher Scientific, Scotts Valley, CA, USA) was used for transfection. Luciferase assay was conducted using the Promega dual-luciferase reporter assay system (E1910, Promega, Madison, WI, USA) following the manufacturer's instructions. The GloMax 96 microplate luminometer (Promega) was used to detect the activity.

2.7. Statistical analysis

We performed all statistical analyses using the GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA). All other data are presented as the mean ± SD. For western blot analyses, we repeated each experiment three times. We used a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test and considered all $p < 0.05$ as statistically significant.

3. Results

3.1. MiR-342-3p was upregulated in PC cell lines

The miR-342-3p expression in MIA PaCa-2 and PANC-1 cell lines under 6hr hypoxia condition (1% O2 condition) was examined by qRT-PCR. As shown in Figure 1A, the expression of miR-342-3p in MIA-PaCa-2 cell was significantly higher in hypoxia condition than in normoxia condition. ($p<0.0001$). The expression of miR-342-3p in PANC-1 cell was also significantly higher in hypoxia condition than in normoxia condition. ($p<0.0001$) (Figure 1B). These results suggested that miR-342-3p may be associated with PC progression.

3.2. FosB was downregulated in PC cell lines and Patient derived PC cells (PDC)

MiR-342-3p target gene candidates were found using web-database; miRDB, miRmap, and Target scan Huma 7.2. Selected eight candidates are commonly predicted in three databases. (Figure 2A). Relative target gene expression level under 6h hypoxia condition in MIA PaCa-2 and PANC-1 cell line was exmed by qRT-PCR.. Primer sequence for qRT-PCR for target gene analysis were shown in Table 1.The expression of FosB ($p=0.0000$) in MIA-PaCa-2 cell and the expression of FAM208A ($p<0.0001$) and NUBPL ($p=0.0014$) in PANC-1 cell were significantly lower in hypoxia condition than in normoxia condition. We also examed FosB proteins by western blot analysis and densitometry. The result showed FosB protein was

significantly decreased in hypoxia condition than in normoxia condition (Figure 3B, 3C)

FosB expression in patient derived cell under 2, 6hr hypoxia condition (1% O2 condition) was examined by qRT-PCR. As shown in Figure 4A, the expression of FosB in patient derived cell (PDC) was time dependently decreased in hypoxia condition. (2hr, p=0.0378; 6hr, p=0.0079). Western blot analysis and densitometry (2hr, p=0.0403; 6hr, p=0.0057) showed time dependent significant decrease of FosB proteins under hypoxia condition (Figure 4B,4C). FosB expression in 3 pairs of PDC and normal pancreatic cells was examined by qRT-PCR. The result showed the expression of FosB was significantly lower in PDC than in normal pancreatic cell (p=0.0016) (Figure 4D). Western blot analysis and densitometry (p=0.0008) showed that FosB proteins significantly decreased in PDC than in normal pancreatic cell (Figure 4E,4F).

3.3. FosB was a target of miR-342-3p in PC cell line

We search and found predicted miR-342-3p sequence and binding sites of miR-342-3p in the 3'UTR of FosB. Sites. Some part of the binding site was mutated and deleted as controls for the luciferase assay. The area are shown in bold black (Figure 5A). The Vector map transfected in MIA PaCa-2 cell was shown in Figure 5B. Luciferase reporter assay for the direct and specific interaction of miR-342-3p with predicted binding sites in the 3'UTR of

FosB was performed. Mutation of the binding site in the FosB 3'UTR abolishes the inhibition of luciferase activity by miR-342-3p (Figure 5C). These results suggested that miR-342-3p directly targets FosB in MIA PaCa-2 cells.

3.4. Synergistic cytotoxicity by combination with miR-342-3p inhibitor and gemcitabine* in *the MIA PaCa-2 cell line study

The MTT assay for cell survival assessment demonstrated a concentration-dependent decrease in cell survival after 24, 48-hour treatment with 100-250 nmol/mL of miR-342-3p inhibitor. The greatest decrease was observed after 100 nM/mL treatment for 48 hour of miR-342-3p inhibitor. (Figure6A, 6B). The MTT assay by gemcitabine showed a concentration-dependent decrease in cell survival after 24, 48-hour treatment with 100 nM/mL-100 µmol/mL of gemcitabine. The greatest decrease was observed after 50 µmol/mL treatment for 48 hour of gemcitabine (Figure6C, 6D). Thus, the treatment concentration of 100 nM/mL of miR-342-3p inhibitor + 50 µmol/mL of gemcitabine for 48 hour were used for further in vitro studies.

A microscopic assessment of the cell morphology revealed abundant apoptosis after treatment with gemcitabine, miR-342-3p inhibitor, and their combination (Figure 7A). Fluorescence microscopy with DAPI-Hoechst staining revealed apoptosis after treatment with gemcitabine or miR-342-3p inhibitor, and the highest apoptosis levels were observed after

combination therapy (Figure 7B). MTT assay in MIA PaCa-2 showed that the decrease of cell viability was observed after either gemcitabine or miR-342-3p inhibitor treatment and the highest decrease was observed after combination therapy (Figure 7C).

4. Discussion

In the present study, we investigated the role of miR-342-3p in PC and explored the mechanisms. Our results showed that miR-342-3p was overexpressed in PC cell lines. FosB, AP-1 transcription factor subunit was identified as a direct target of miR-342-3p. In vitro experiments showed that miR-342-3p overexpression and FosB silencing promoted tumor growth and confirmed that FosB counteracts the oncogenic effect of miR-342-3p. And combination with miR-342-3p inhibitor and gemcitabine showed synergistic antitumor effect in PC cell lines.

An increasing number of studies have confirmed that miRNA dysregulation is casual in many cancers. MiRNAs acting as oncogenes and tumor suppressor genes has been studied for molecular targets of anticancer therapy (5, 10, 11). Some reports have indicated that miR-342-3p has been involved and down-regulated in pathogenesis of gastric cancer, breast cancer, and lung cancer (12-14). However, our data showed that the expression of miR-342-3p was markedly increased in PC cell lines, suggesting its antitumor effect. The definite role of miR-

342-3p in human cancer pathogenesis is still unclear. Further studies are needed.

The predominant function of miRNAs is to regulate protein translation by binding to complementary sequences in the 3' untranslated region (UTR) of target mRNAs, and thereby negatively regulate mRNA translation (15). Therefore, miRNA target prediction is important to decipher the role of miRNAs in disease. In this study, we revealed that FosB was a target gene of miR-342-3p. FosB is a member of the FOS gene family, which can dimerize with proteins of the JUN family, forming the transcription factor complex activator protein 1 (AP-1) (16). The AP-1 complexes are important regulators in wide range of cellular processes, including cell growth, differentiation, and death. AP-1 activity is often regulated DNA binding dimer composition, interaction with various binding partners, and post-translational modifications (17, 18). Interestingly, each types of subunits of AP-1 might have different functions according to tumor type. C-Jun is a positive regulator of cell proliferation, whereas JunB has the converse effect in epidermal neoplasia. Fra-1, an oncogenic transcription factor of the bZIP family, Fos subfamily overexpression enhances the motility and invasion of breast and colorectal cancer cells, but inhibits the tumor growth in cervical cancer cell lines(18, 19).

FosB gene has been reported to be a proto-oncogene. Recently, FosB was reported to be associated with increased tumor growth in PC and colorectal cancer (8, 20). In contrast of these results, other studies showed FosB have tumor suppressive function in gastric cancer and

breast cancer. Tang, C., et al suggested that overexpression of FosB significantly suppressed cell proliferation, clone formation and migration in gastric cancer cell lines (21). Ting, C.H., et al. showed that FosB overexpression results in triple-negatie breast cancer cell death (22). In agreement with the findigns above, our results showed that FosB was down regulated in PC cell lines. And FosB silencing promoted proliferation of PC cells. However. the definite role of FosB in human cancer pathogenesis is still not clarified. Therefore, further studies are needed.

Gemcitabine is generally used first-line agent for PC therapy. However, primary or secondary drug resistance and metastasis during Gemcitabine therapy is still a major problem that remains to be solved (23). Previous studies reported that miR-429, miR-210, and miR-218 can promotes gemcitabine sensitivity in gemcitabine resistant PC cells (24-26). And Li, Y., et al.showed that combination with antisense oligonucleotides (ASO)-miR-21 and Gem had synergistic antitumor effect in vitro and in vivo (27). Similarly in this study, combination treatment with miR-342-3p inhibitor and Gem showed definite synergistic antitumor effect in PC cell line.

5. Conclusion

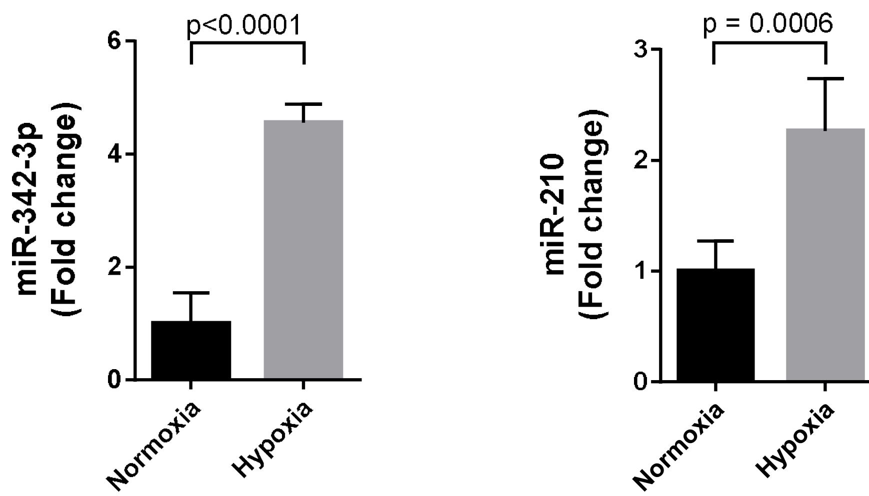
In conclusion, our study revealed that miR-342-3p was upregulated in PC. The direct target of miR-342-3p was FosB. Inhibition of miR-342-3p decreased cell survival in PC. Combination treatment with miR-342-3p inhibitor and Gemcitabine showed synergistic antitumor effect in PC. Therefore, miR-342-3p may provide a new molecular target for therapeutic strategy against PC.

Table 1. Primer sequence for qRT-PCR

Gene		Sequence
AGPAT4	F	5'-CAC GGA ATG CAC CAT CTT CA-3'
	R	5'-GAA CCA CGA TGG CAT TTT CCT-3'
DTNBP1	F	5'-TCC CAG CTT TAA TCG CAG AC-3'
	R	5'-CAG ATG CAG CAG GTT GTT CT-3'
FAM208A	F	5'-TGA AGA CAT TGC AGG TTT CAT TC-3'
	R	5'-CAT CCA GGC TAT CAA CAC CAG-3'
FosB	F	5'-ATG GCT AAT TGC GTC ACA GG-3'
	R	5'-GCA CTG TCC AGC AAG AGG TC-3'
HDAC7	F	5'-GTC CAG GTG ATC AAG AGG TCA-3'
	R	5'-AAC ACC TGA GGG TGC TGC T-3'
NUBPL	F	5'-GTT GTA GAC ATG CCA CCA GGA A-3'
	R	5'-CAC GTG GAC TCT GCG AAA CA-3'
RGS4	F	5'-ACA TCG GCT AGG TTT CCT GC-3'
	R	5'-GTT GTG GGA AGA ATT GTG TTC AC-3'
RNF144B	F	5'-GAG CAC CGA GCC CTC TTT GGG A-3'
	R	5'-GAG CGC AGC CTT CAT TGC GT-3'

Figure 1. miR-342-3p expression increased in MIA PaCa-2 and PANC-1 cell line under 6h hypoxia condition. (A) MIA PaCa-2 (B) PANC-1 cell line Data are presented as the mean± SEM. n=6 per group.

A



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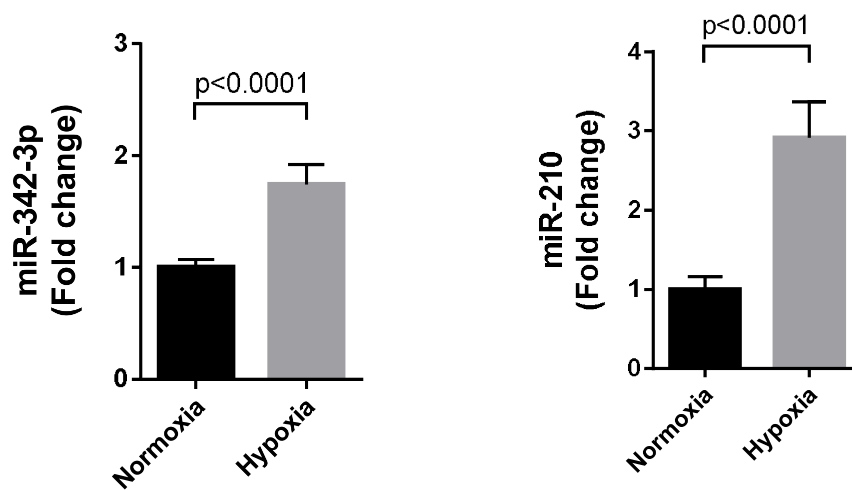
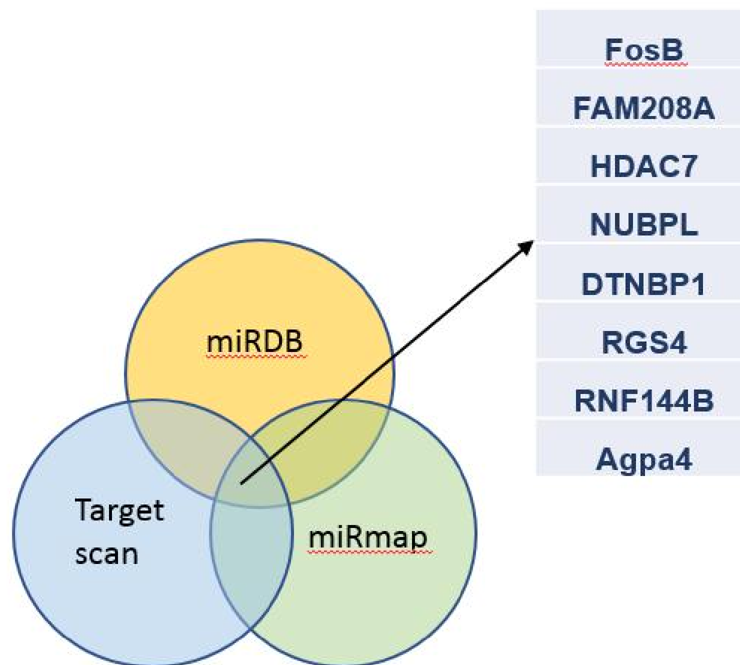


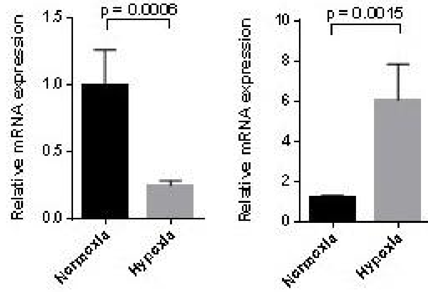
Figure 2. target gene prediction of miR-342-3p. (A) MiR-342-3p target gene candidates were found using web-database; miRDB, miRmap, and Target scan Huma 7.2. Selected eight candidates are commonly predicted in three databases. (B) Relative target gene expression level under 6h hypoxia condition in MIA PaCa-2 and PANC-1 cell line.



B

MIA PaCa-2 PANC-1 | MIA PaCa-2 PANC-1

FosB



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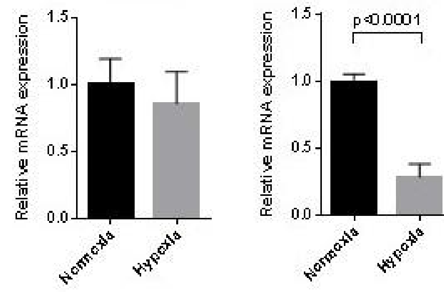

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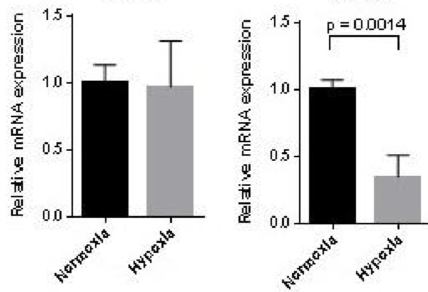

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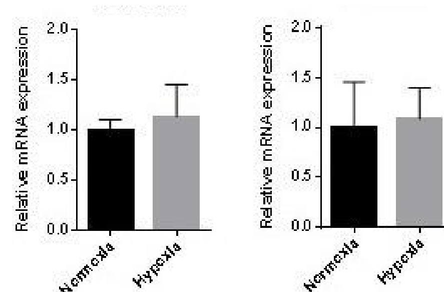

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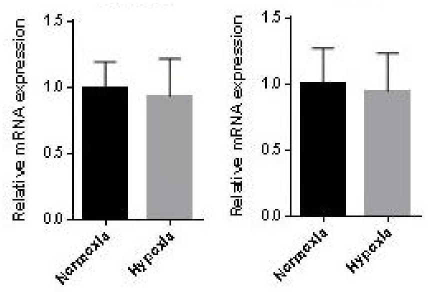

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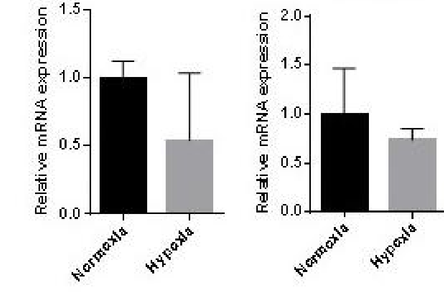

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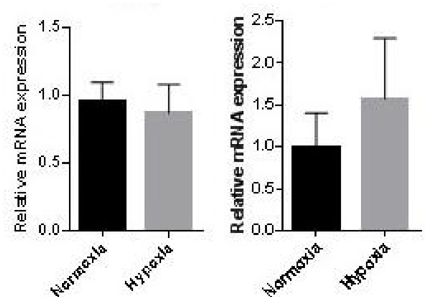

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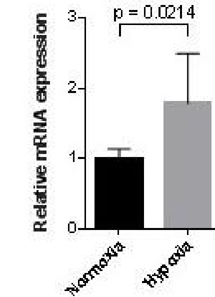


Figure 3. FosB expression decreased in MIA PaCa-2 cell line under 6h hypoxia condition. (A) Quantification of FosB expression level by qRT-PCR (B) FosB proteins as assessed by western blot analysis. (C) Densitometry of western blot analysis for FosB. Data are shown as the mean± S.D. Images shown are representative of three independent experiments. N normoxia, H hypoxia.. n=4 per group

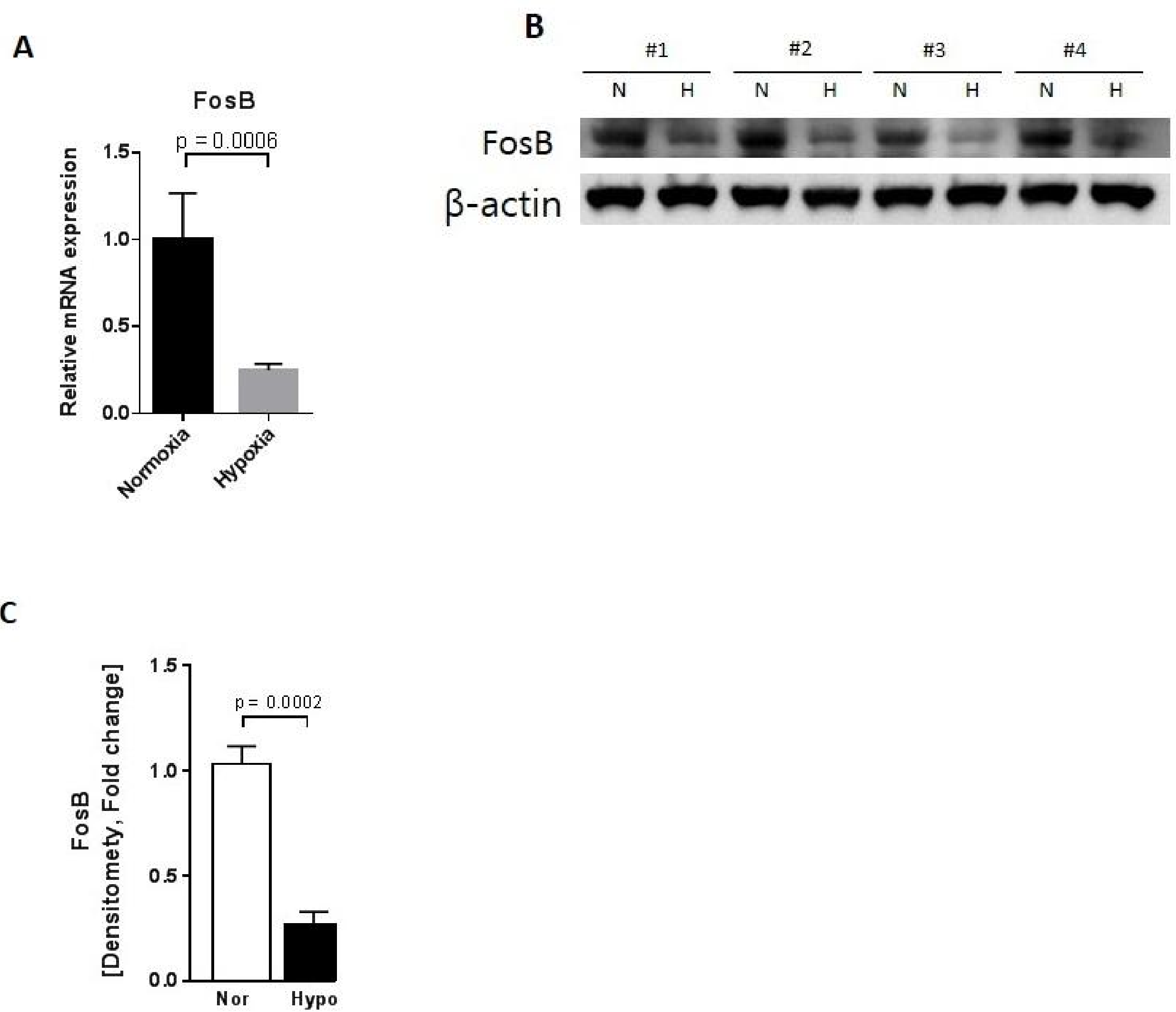
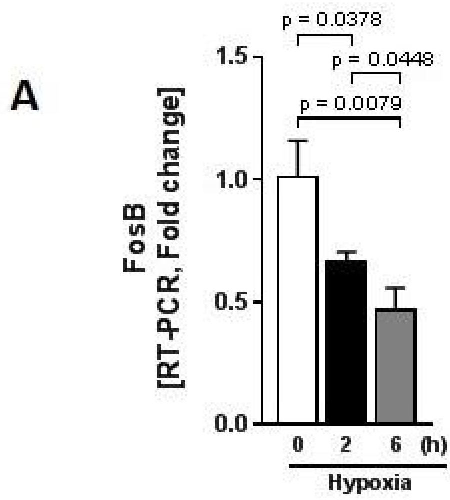
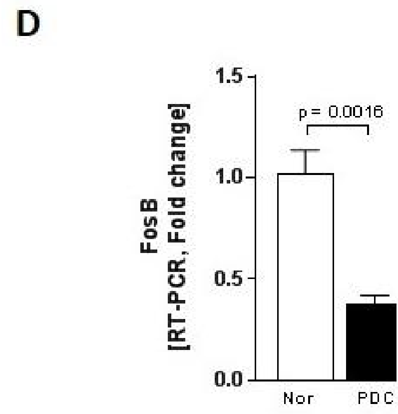
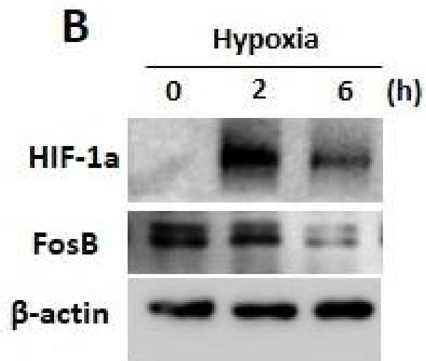
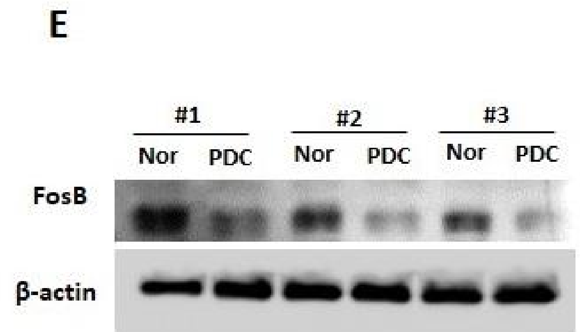


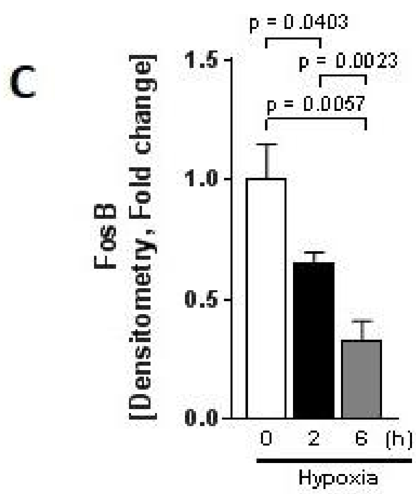
Figure 4. FosB expression decreased in patient derived pancreatic cancer cell line under hypoxia condition. FosB expression decreased in patient derived pancreatic cancer cell line than in normal pancreatic cell line (A) Quantification of FosB expression level in patient derived cell under hypoxia condition (B) FosB proteins in patient derived cell under hypoxia condition. (C) Densitometry of western blot analysis for FosB in patient derived cell under hypoxia condition. (D) Quantification of FosB expression level in patient derived pancreatic cancer cell line and normal pancreatic cell line (E) FosB proteins in patient derived pancreatic cancer cell line and normal pancreatic cell line (F) Densitometry of western blot analysis for FosB in patient derived pancreatic cancer cell line and normal pancreatic cell line Data are shown as the mean± S.D. Images shown are representative of three independent experiments. HIF-1a, Hypoxia-inducible factor 1-alpha; Nor, normal pancreas tissue; PDC, patient derived cell;. n=4 per group


A
p = 0.0378
p = 0.0448
p = 0.0079
FosB
[RT-PCR, Fold change]
1.5
1.0
0.5
0.0
0
2
6
(h)
Hypoxia


D
p = 0.0016
FosB
[RT-PCR, Fold change]
1.5
1.0
0.5
0.0
Nor
PDC


B
Hypoxia
0
2
6
(h)
HIF-1a
FosB
β-actin


E
#1
#2
#3
Nor
PDC
Nor
PDC
Nor
PDC
FosB
β-actin


C
p = 0.0403
p = 0.0023
p = 0.0057
FosB
[Densitometry, Fold change]
1.5
1.0
0.5
0.0
0
2
6
(h)
Hypoxia

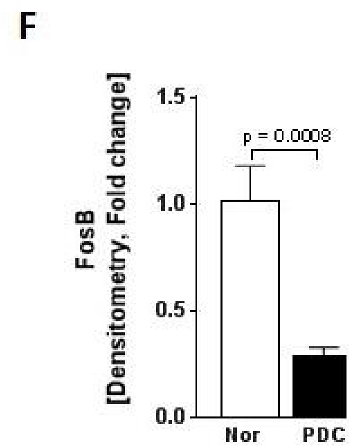
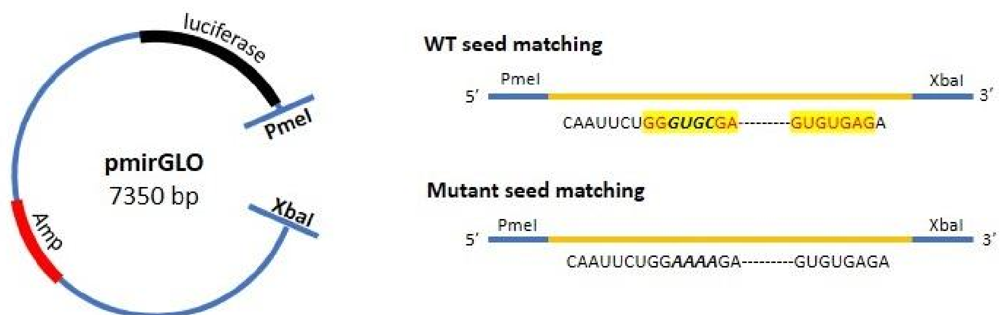

F
p = 0.0008
FosB
[Densitometry, Fold change]
1.5
1.0
0.5
0.0
Nor
PDC

Figure 5. miR-342-3p directly targets FosB in MIA PaCa-2 cell line. (A) Predicted binding sites of miR-342-3p in the 3'UTR of FosB. Sites mutated and deleted as controls for the luciferase assay are shown in bold black. (B)Vector map. (C) Luciferase reporter assay for the direct and specific interaction of miR-342-3p with predicted binding sites in the 3'UTR of FosB. Mutation of the binding site in the FosB 3'UTR abolishes the inhibition of luciferase activity by miR-342-3p. Data are shown as the mean± S.D. n=6 per group.

A

B



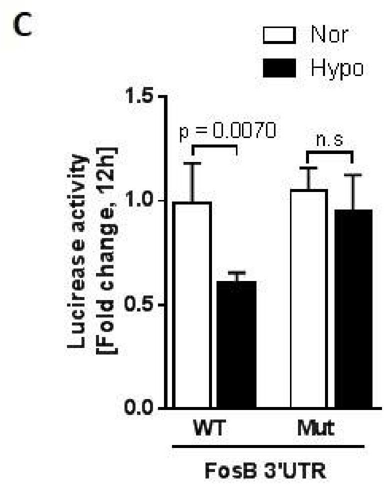
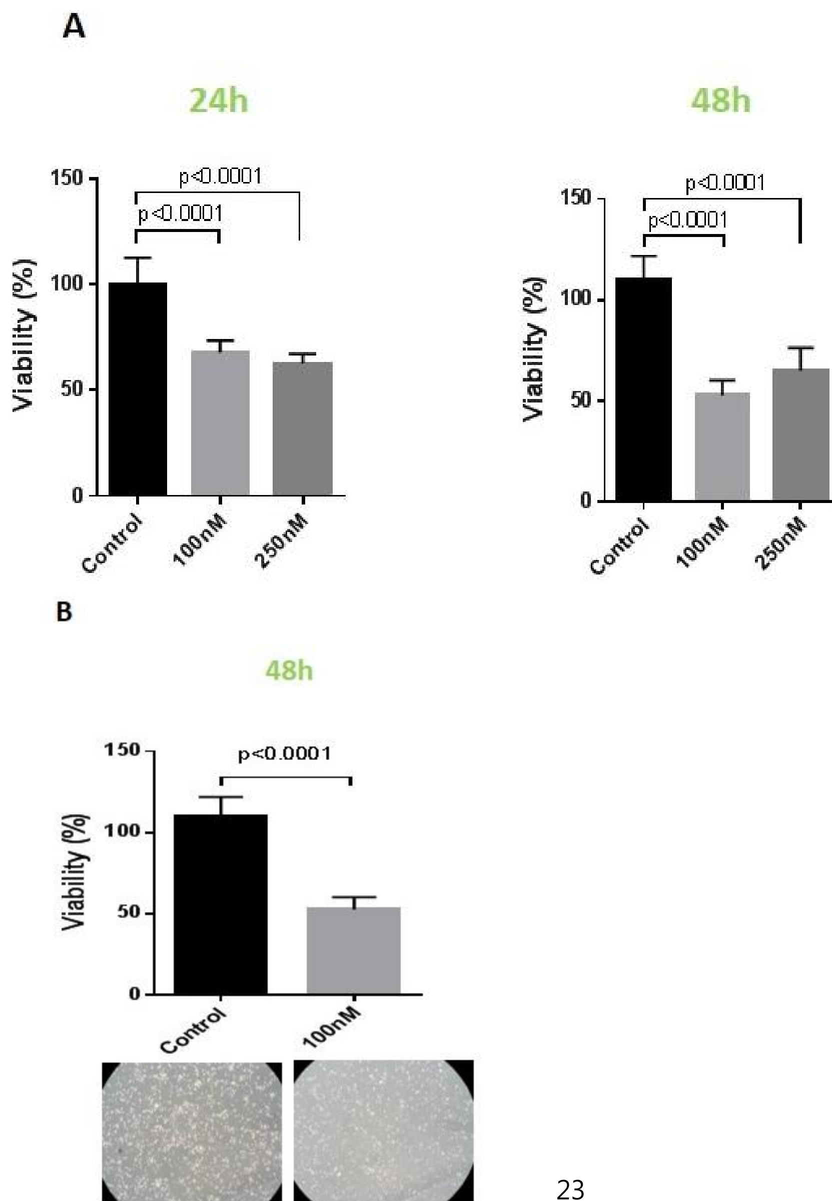
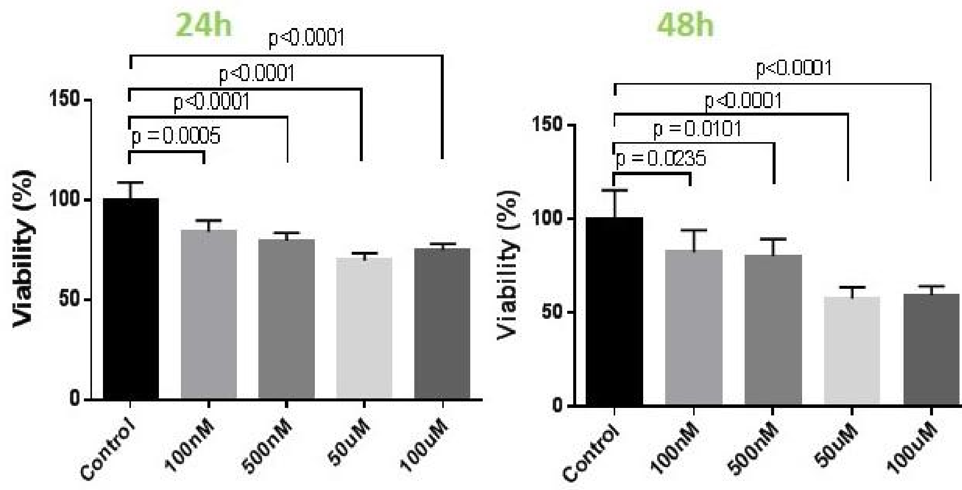

C
Nor
Hypo
Lucirease activity
[Fold change, 12h]
1.5
1.0
0.5
0.0
p = 0.0070
n.s
WT
Mut
FosB 3'UTR

Figure 6. In vitro cytotoxicity in the MIA PaCa-2 cell line treated with miR-342-3p inhibitor and gemcitabine. MTT assay for (A) Cell survival assessment by miR-342-3p inhibitor concentration. (B) The greatest decrease was observed after 100 nM/mL treatment for 48 hour of miR-342-3p inhibitor. (C) Cell survival assessment by Gemcitabine concentration. (D) The greatest decrease was observed after 50 μmol/mL treatment for 48 hour of gemcitabine. Data are shown as the mean± S.D.



C



D

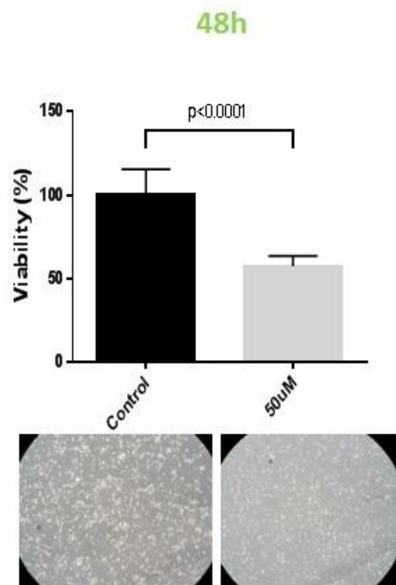
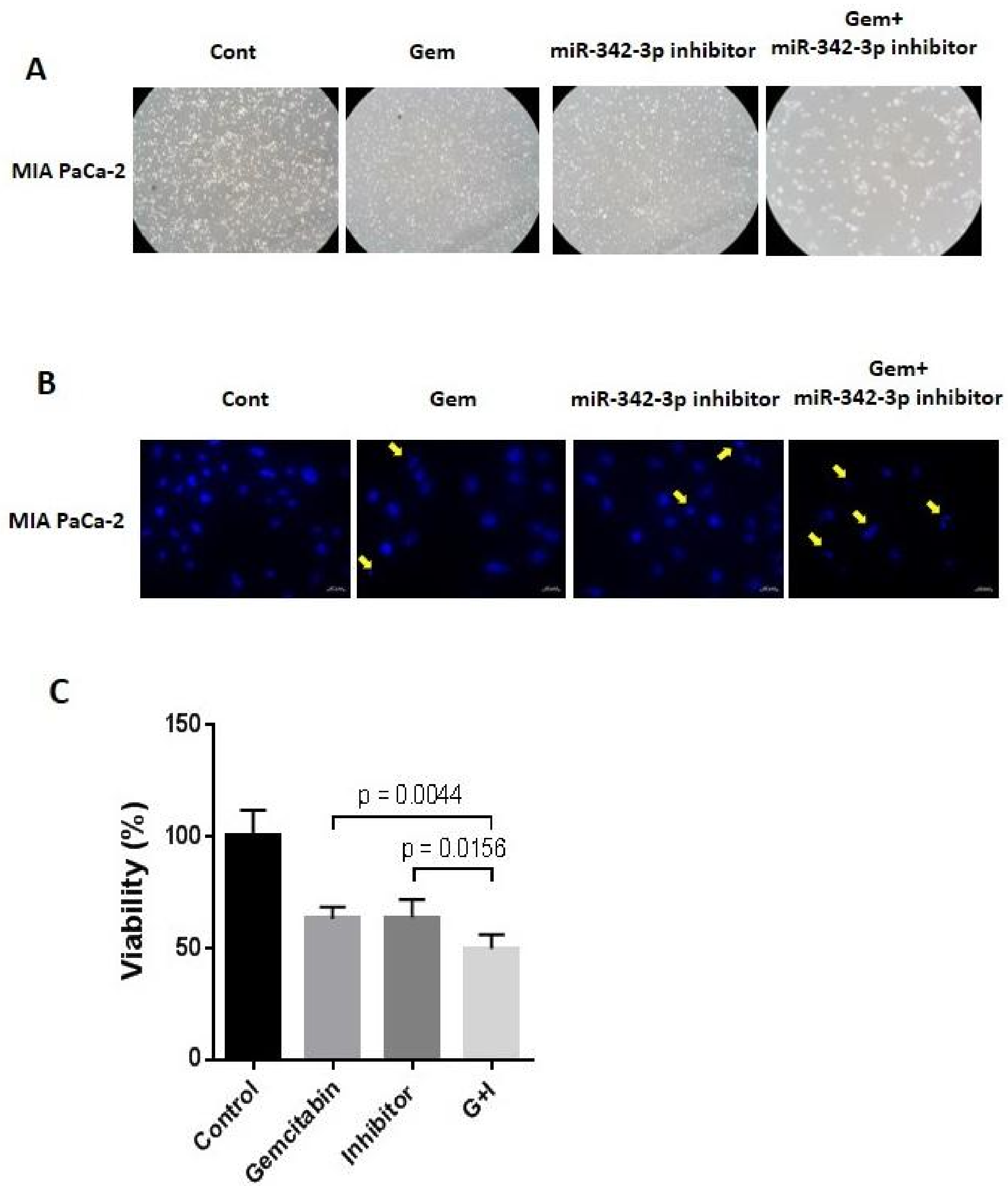


Figure 7. synergistic cytotoxicity by combination with miR-342-3p inhibitor and gemcitabine in MIA PaCa-2 cell line. (A) microscopic assessment of the cell morphology, (B) fluorescence microscopy with DAPI-Hosechst staining (arrows indicate apoptosis), and (C) MTT assay for cell survival assessment. Data are shown as the mean± S.D. n=6 per group.



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국문요약

췌장암은 매우 안좋은 예후를 가지는 치명적인 암이다. 중앙생존률은 여전히 10% 이하이며, 이것은 췌장암의 병리학적 기전 및 효과적인 치료의 표적을 발견하는 것이 시급함을 의미한다. MicroRNA (miRNA)는 세포의 생존에 핵심적인 역할을 하며, 암을 포함한 다양한 질병에서 균형이 깨져있음이 알려져 있다. 이러한 microRNA는 췌장암 치료의 잠재석 대상이 될 수 있다. 본 연구의 목적은 췌장암의 발생에서의 microRNA-342-3p의 역할을 규명하는 것이다. MiRNA-342-3p는 췌장암 세포에서 증가한 소견을 보였다. 웹-데이터베이스 검색을 통해 찾아낸 miR-342-3p의 8개의 표적유전자중에서 AP-1 전사인자를 구성하는 소단위인 FosB 가 miR-342-3p의 직접적인 표적유전자로 밝혀졌다. MiR-342-3p 저해제를 췌장암세포에 투입하였을 때 췌장암의 생존이 감소하였으며, 이것을 항암제 gemcitabine과 같이 투여하였을 때 상승된 항 종양효과를 보였다. 결론적으로, miR-342-3p는 췌장암 세포에서 증가되어 있었으며, 직접적인 표적유전자는 FosB 였다. MiR-342-3p를 억제하였을 때 췌장암 세포의 증식이 억제되었으며 gemcitabine과 함께 치료시 상승된 항 종양효과를 보였다. 따라서 miR-342-3p는 췌장암 치료전략의 새로운 분자생물학적 표적으로 사용될 수 있을 것이다.