Biochemical and Biophysical Research Communications 457 (2015) 370–377

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

miR-342-3p affects hepatocellular carcinoma cell proliferation via regulating NF-κB pathway

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ARTICLE INFO

Article history:
Received 19 December 2014
Available online 9 January 2015

Keywords:
Cell proliferation
Hepatocellular carcinoma
miR-342-3p
NF-κB signaling

ABSTRACT

Recent research indicates that non-coding microRNAs (miRNAs) help regulate basic cellular processes in many types of cancer cells. We hypothesized that overexpression of miR-342-3p might affect proliferation of hepatocellular carcinoma (HCC) cells. After confirming overexpression of miR-342-3p with qRT-PCR, MTT assay showed that HCC cell proliferation was significantly inhibited by miR-342-3p, and that it significantly decreased BrdU-positive cell proliferation by nearly sixfold. Searching for targets using three algorithms we found that miR-342-3p is related to the NF-κB pathway and luciferase assay found that IKK-γ, TAB2 and TAB3 are miR-342-3p target genes. Results of western blot on extracted nuclear proteins of HepG2 and HCT-116 cells showed that miR-342-3p reduced and miR-342-3p-in increased p65 nuclear levels and qRT-PCR found that NF-κB pathway downstream genes were downregulated by miR-342-3p and upregulated by miR-342-3p-in, confirming that miR-342 targets NF-κB pathway. Overexpression of Ikk-γ, TAB2 and TAB3 partially rescued HCC cells proliferation inhibited by miR-342-3p. Using the GSE54751 database we evaluated expression from 10 HCC samples, which strongly suggested down-regulation of miR-342-3p and we also found inverse expression between miR-342-3p and its targets IKK-γ, TAB2 and TAB3 from 71 HCC samples. Our results show that miR-342-3p has a significant role in HCC cell proliferation and is suitable for investigation of therapeutic targets.

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1. Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and is estimated to be the third leading cause of cancer death worldwide [1]. As HCC is seldom detected in the early stages, survival of HCC patients is as short as a few months [2]. Approximately 90% of HCC cases start with cirrhosis, which can be caused by a wide range of factors including hepatitis B and C infections, alcohol abuse, non-alcoholic fatty liver disease, autoimmune-mediated hepatitis, primary biliary cirrhosis (PBC) and exposure to carcinogens [3]. Considerable progresses on unraveling molecular mechanisms of HCC have been achieved recently, paving the way to the early detection and treatment of HCC.

MicroRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally regulate gene expression by binding to complementary sequence in the 3′-UTR of target mRNAs. Such binding results in either degradation of the target mRNAs or inhibits their translation into proteins [4,5]. miRNAs have been implicated in biological processes including metabolism, cell proliferation, developmental timing, apoptosis, morphogenesis and response to stress [6–8]. Regulation by miRNAs have been found to correlate with cancers, with roles as both oncogenes and tumor suppressors [9,10] and miRNAs are considered potential diagnostic and prognostic biomarkers [11–14] and perhaps therapeutic targets [15,16].

Cittelly et al., published results suggesting that the miRNA miR-342 regulates tamoxifen response in breast cancer cells [17]. Their clinical data showed a trend toward reduced miR-342 expression and tamoxifen resistance, and their study results suggest miR-342 regulates expression of genes involved in tamoxifen-mediated tumor-cell apoptosis and cell cycle progression. They identified miR-342 as an important mediator of tamoxifen response in breast cancer tumor cell lines and breast cancer patients [17].

In an analysis of differential expression of miRNAs in the brains of bovine spongiform encephalopathy-infected cynomolgus macaques as a model for Creutsfeldt-Jakob disease, Montag and colleagues [18] hypothesized that miRNAs are also regulated in response to human prion disease, based on recent evidence that the lack of miRNA processing promotes neurodegeneration, and that deregulation of several miRNAs was reported as associated with

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http://dx.doi.org/10.1016/j.bbrc.2014.12.119
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Scrapie in mice. They found significant upregulation of hsa-miR-342-3p and hsa-miR-494 in the brains of BSE-infected macaques compared with uninfected controls.

FOXM1 is a well-established oncogenic factor shown to be involved in multiple biological processes including cell proliferation, growth, angiogenesis, migration and invasion and can be regulated by miRNAs [19,20]. Li et al. reported that FOXM1 is directly targeted by miR-342-3p, which is down-regulated along with its host gene, EVL, in human cervical cancer tissue, compared to the adjacent normal tissues [21]. Functional studies suggested that the overexpression of miR-342-3p inhibits cell proliferation, migration and invasion in cervical cancer cell lines. FOXM1 was upregulated and negatively correlated with miR-342-3p in cervical cancer tissues, and the overexpression of FOXM1 rescued the phenotype changes induced by the overexpression of miR-342-3p.

In the present study, we sought to determine whether overexpression of miR-342-3p similarly suppresses proliferation of HCC cells. Using the cell lines HepG2 and HCT-116, as well as clinical samples and publically available algorithms, we found that miR-342-3p regulates the NF-κB pathway and thereby suppresses cell proliferation in HCC.

2. Materials and methods

2.1. Data base search and miR-342-3p potential target prediction

The GSE54751 database was used to determine whether miR-342-3p expression is altered in 10 HCC tissues compared to adjacent normal liver tissues, and three algorithms (miRanda, DIANA and TargetScan) were subsequently used to predict potential miR-342-3p targets.

2.2. Cell lines and cultures

HepG2 and HCT-116 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in Earle's minimal essential medium (MEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 pg/ml streptomycin and 4 mM glutamine (Invitrogen). All cells were cultured in a 5% carbon dioxide humidified incubator at 37 °C.

2.3. miRNA and transfection

The miR-342-3p, miR-342-3p-in and control mimics were purchased from RiboBio (Guangzhou, Guangdong, China). Cells were transfected with miR-342-3p, miR-342-3p-in or control mimics using Lipofectamine LTX (Invitrogen) according to manufacturer's instructions. Experiments on cells were performed 2 days after transfection.

2.4. MTT assay

Twenty-five hundred HepG2 and HCT-116 cells per well were seeded in 96-well plates. Cell viability was determined by MTT assay after transfection with miR-342-3p or control mimics.

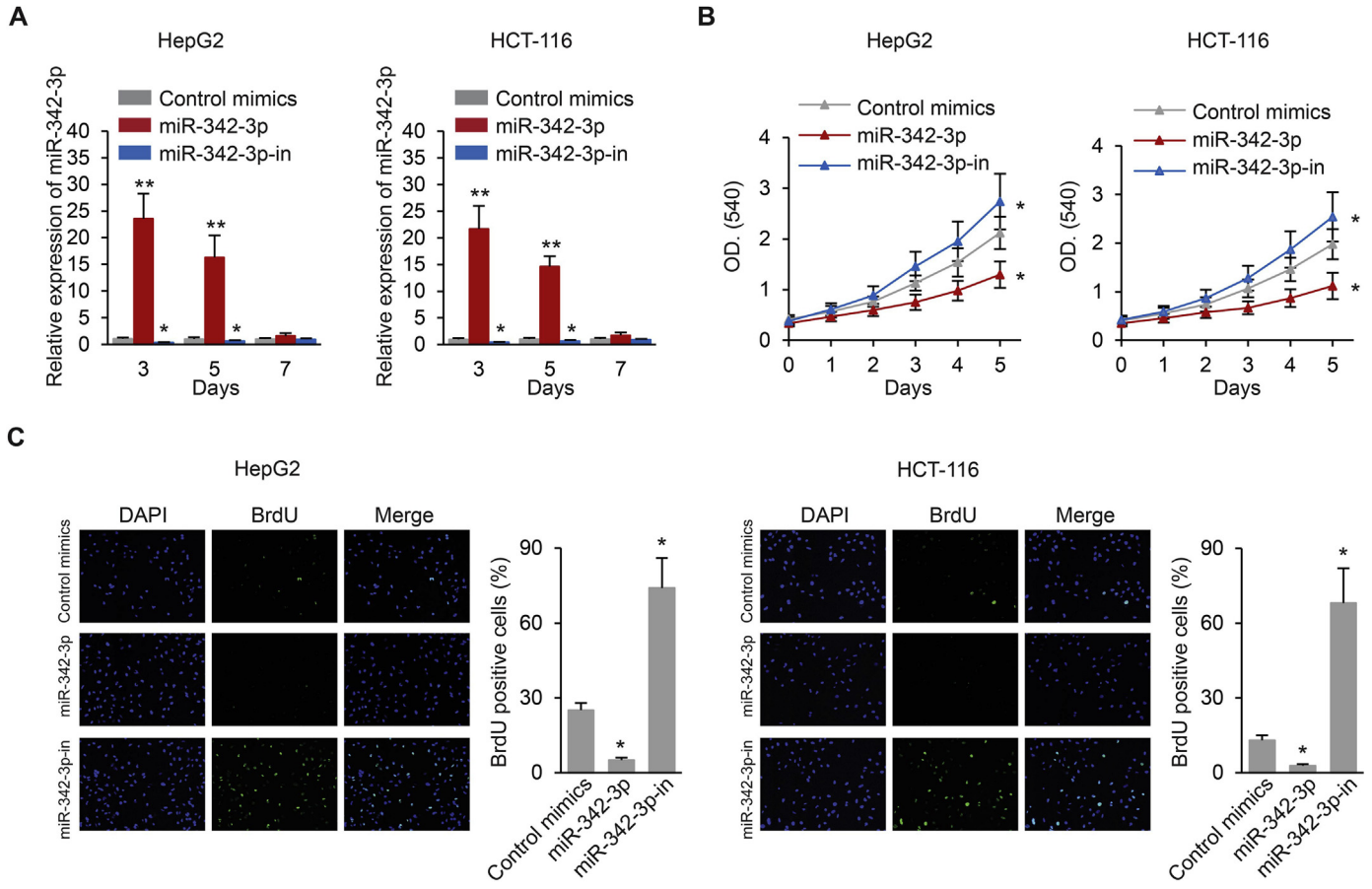
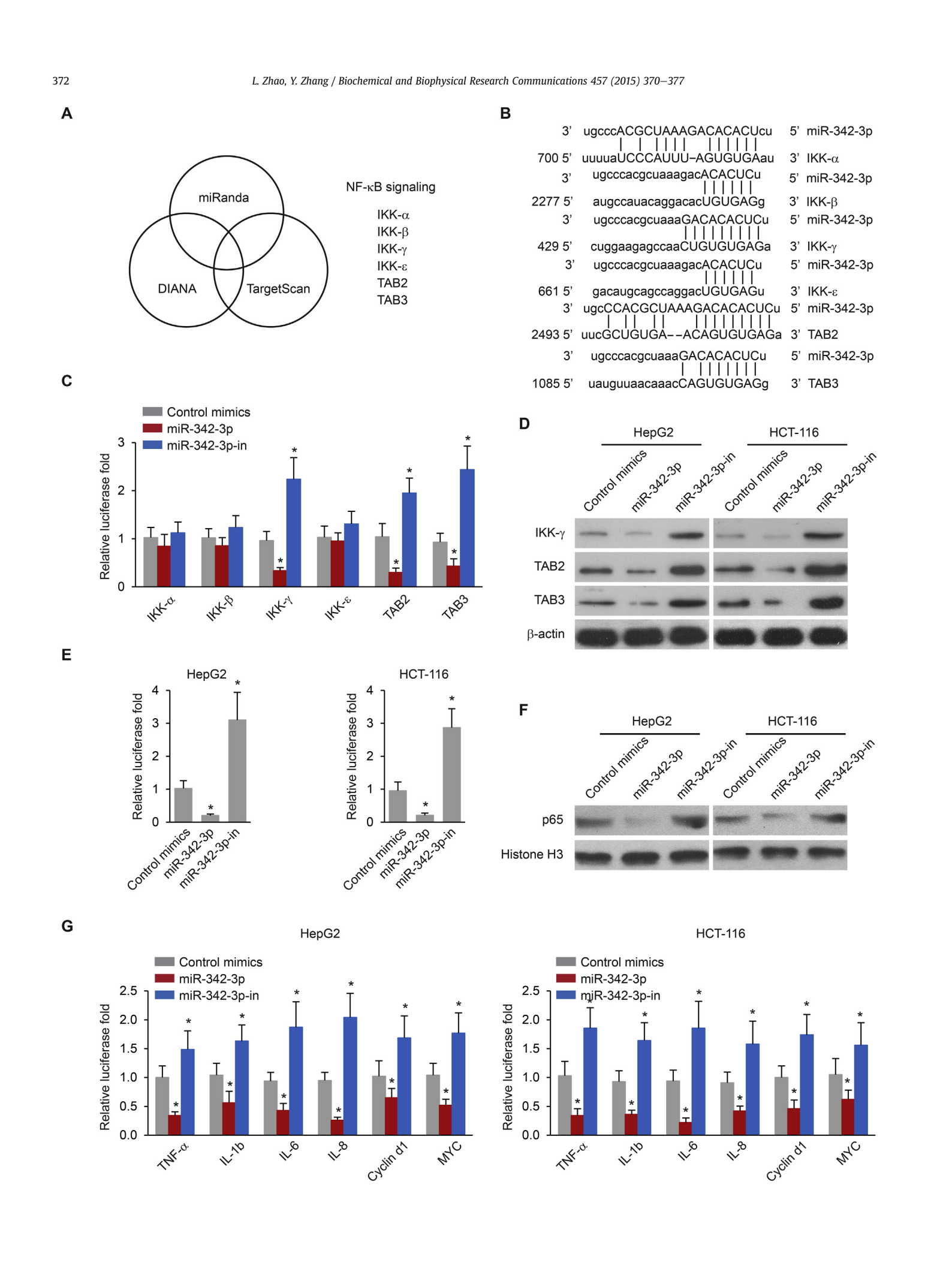


Fig. 1. miR-342-3p inhibits HCC cells proliferation. (A) miR-342-3p expression in HepG2 and HCT-116 by qRT-PCR after miR-342-3p or miR-342-3p-in transfected for 2 days. (B) MTT assay of HepG2 and HCT-116 cells transfected with miR-342-3p or miR-342-3p-in for 2 days. (C) BrdU assay in HepG2 and HCT-116 cells 2 days post miR-342-3p or miR-342-3p-in transfection, and quantitation of BrdU positive cells. $^{*}p < 0.05$ $^{**}p < 0.01$ compared to control mimics transfection.


A
miRanda
DIANA
TargetScan
NF-κB signaling
IKK-α
IKK-β
IKK-γ
IKK-ε
TAB2
TAB3
B
3' ugcccACGCUAAAGACACACUcu 5' miR-342-3p
700 5' uuuuaUCCCAUUU–AGUGUGAau 3' IKK-α
3' ugcccacgcuaaagacACACUCu 5' miR-342-3p
2277 5' augccauacaggacacUGUGAGg 3' IKK-β
3' ugcccacgcuaaaGACACACUCu 5' miR-342-3p
429 5' cuggaagagccaaCUGUGUGAGa 3' IKK-γ
3' ugcccacgcuaaagacACACUCu 5' miR-342-3p
661 5' gacaugcagccaggacUGUGAGu 3' IKK-ε
3' ugcCCACGCUAAAGACACACUCu 5' miR-342-3p
2493 5' uucGCUGUGA––ACAGUGUGAGa 3' TAB2
3' ugcccacgcuaaaGACACACUCu 5' miR-342-3p
1085 5' uauguuaacaaacCAGUGUGAGg 3' TAB3
C
Control mimics
miR-342-3p
miR-342-3p-in
Relative luciferase fold
IKK-α
IKK-β
IKK-γ
IKK-ε
TAB2
TAB3
D
HepG2
HCT-116
Control mimics
miR-342-3p
miR-342-3p-in
IKK-γ
TAB2
TAB3
β-actin
E
HepG2
HCT-116
Relative luciferase fold
Control mimics
miR-342-3p
miR-342-3p-in
F
HepG2
HCT-116
Control mimics
miR-342-3p
miR-342-3p-in
p65
Histone H3
G
HepG2
HCT-116
Control mimics
miR-342-3p
miR-342-3p-in
Relative luciferase fold
TNF-α
IL-1b
IL-6
IL-8
Cyclin d1
MYC

Twenty microliter of MTT (Promega, Madison, WI) were added to each well and incubated for 4 h. Absorbance was measured at 540 nm wavelength to measure cell viability.

2.5. (5-Bromo-2′-deoxyuridine) BrdU assay

Transfected cells were seeded on coverslips in 24-well plates and cultured overnight. BrdU (10 μg/ml) was added to the culture medium and cells further incubated for 1 h. Cells were immediately fixed in 4% paraformaldehyde for 10 min and stained with an anti-BrdU antibody (Biocompare, South San Francisco, CA) per manufacturer's instructions. The coverslips were counterstained with DAPI and imaged acquired with fluorescence microscopy (Olympus, Tokyo, Japan). Results were expressed as the percentage of BrdU + cells in DAPI + cells.

2.6. Plasmid constructions, transfections and luciferase activity assay

IKK-α, IKK-β, IKK-γ, IKK-ε, TAB2 and TAB3 3′UTR were generated by PCR amplification and inserted into the pGL3-basic luciferase reporter plasmid (Promega). We purchased NF-κB luciferase reporter plasmid from QIAGEN, Inc. (Valencia, CA). IKK-γ, TAB2 and TAB3 cDNA was amplified by RT-PCR using RNA from 293 cells as template. PCR primers were based on GenBank accession number NM_001099856, NM_001292034 and NM_152787. The entire open reading frame of IKK-γ, TAB2 or TAB3 was cloned into NheI and NotI sites of the mammalian expression vector pcDNA3.1. HCC cells were seeded into 24-well plates and cotransfected with each reporter plasmid and miRNA encoding plasmid using Lipofectamine LTX. Luciferase and renilla signals were measured 2 days after transfection using a Dual Luciferase Reporter Assay Kit (Promega).

2.7. RNA extraction and quantitative reverse transcriptase PCR (qRT-PCR) analysis

HepG2 and HCT-116 cells were transiently transfected with miR-342 control mimics or miR-342-3p for 2 days. Cell were then lysed with TRIzol reagent (Invitrogen) for dissociation of any RNA-protein complexes. Chloroform was added for phase separation, followed by aqueous precipitation of total RNA using isopropyl alcohol. After centrifugation the RNA pellet was washed in 75% ethanol and suspended in nuclease-free TE buffer. Two micrograms of total RNA were reverse transcribed into cDNA using a High-Capacity RNA-to-cDNA kit (Invitrogen). qRT-PCR was performed with primers for miR-342-3p, IKK-γ, TAB2, TAB3 TNF-α, IL-1b, IL-6, IL-8, cyclin d1 and MYC with SYBR in the 7500 HT real-time PCR System (Applied Biosystems, Carlsbad, California). β-actin and U6 were used as endogenous controls for mRNA and miRNA measurements. Results were expressed using the $2^{\Delta\Delta Ct}$ method. The primers used for qRT-PCR measurement were *IKK-γ* forward 5′-CTT TTGGGGTAGATGCG-3′ and reverse 5′-GGTTAAATACACATCGGTCTG-3′; *TAB2* forward 5′-CTCGAAGGCGCCTGAAAAGA-3′ and reverse 5′- GGGTTTTGGTG-GCACAGGAC-3′; *TAB3* forward 5′-CAGCCCACAGCTTGATATTC-3′ and reverse 5′-CATGACTTTGCCCGAGTTAG-3′; *TNF-α* forward 5′-GCCGCATCGCCGTCTCCTAC-3′ and reverse 5′- CCTCAGCCC-CCTCTGGGGTC-3′; *IL-1b* forward 5′-AATCTGTACCTGTCCTGCGTGTT-3′ and reverse 5′-TGGGTAATTTTTGGGATCTACACTCT-3′; *IL-6* forward 5′-TTCTCCACAAGCGCCTTCGGTC-3′ and reverse 5′-TCTGTGTGGGGCGGCTACATCT-3′; *IL-8* forward 5′-GTGCAGTTTTGC-CAAGGAGT-3′ and reverse 5′-CTCTGCACCCAGTTTTCCTT-3′; *cyclin d1* forward 5′-CCGTCCATGCGGAAGATC-3′ and reverse 5′-GAA-GACCTCCTCCTCGCACT-3′; *MYC* forward 5′- GCCACGTCTCCACA-CATCAG-3′ and reverse 5′- TCTTGGCAGCAGGATAGTCCTT-3′; β-actin forward 5′-ATGGGTCAGAA GGATTCCTATGTG-3′ and reverse 5′-CTTCATGAGGTAGTCAGTCAGGTC-3′.

2.8. Western blot

Proteins in HepG2 and HCT-116 cells 2 days after miR-342-3p transfection were extracted with RIPA lysis buffer (Applygen, Beijing, China) mixed with a proteinase inhibitor cocktail (Santa Cruz Biotechnology, Santa Cruz, CA). The total protein concentrations were determined with a commercial BCA kit (Bio-Rad, Hercules, CA). Samples of 20–30 μg were loaded into each well and further separated on a 10% SDS-PAGE. Protein bands were transferred to PVDF membranes (Bio-Rad) and blocked with 5% non-fat milk for 1 h at room temperature. The membranes were further incubated with specific primary antibodies against p65, IKK-γ, TAB2 and TAB3 (Santa Cruz Biotechnology), then with secondary HRP-labeled antibodies and finally visualized using ECL.

2.9. Patient samples

This study was approved by the Institutional Review Board of Harbin Medical University Cancer Hospital. Fresh specimens from 71 patients that had undergone surgery for HCC were obtained from the Harbin Medical University Cancer Hospital Department of Hepatopancreatobiliary Surgery. We also obtained samples of healthy tissues adjacent to the carcinoma samples. Tissue samples were immediately frozen at −80 °C. All patients signed informed consent forms before their operations and none had any other cancer history. All cases were reviewed and confirmed by two pathologists.

2.10. Statistical analysis

The statistical analysis was performed using SPSS17 software. A two-tailed Student's *t* test was used to compare two groups. One-way analysis of variance was used to analyze data with more than two groups using the Tukey–Kramer *post-hoc* test. Results were expressed as mean ± standard error of the mean (SEM) of three independent experiments and a *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. miR-342-3p inhibits HCC cell proliferation

To investigate the function of miR-342-3p in HCC, we hypothesized that overexpression of miR-342-3p might affect proliferation of HCC cells. We first transiently transfected HepG2 and HCT-116 cells with miR-342 control mimics, miR-342-3p and miR-342-3p-in for 2 days, then used qRT-PCR to confirm miRNA overexpression (Fig. 1A). MTT assay was used to determine how miR-342-3p affected cell proliferation and showed that proliferation of

Fig. 2. miR-342-3p regulates NF-κB pathway via directly targeting Ikk-γ, TAB2 and TAB3. (A) A schematic diagram of miR-342-3p predicted targets (IKK-α, IKK-β, IKK-γ, IKK-ε, TAB2 and TAB3 3′UTR) using three algorithms (miRanda, DIANA and TargetScan). (B) Compared sequence of miR-342-3p and its predicted targets. (C) Luciferase assay of miR-342-3p predicted target genes with miR-342-3p or miR-342-3p-in in HepG2 cells. (D) Western blot in HepG2 and HCT-116 cells transfected with miR-342-3p or miR-342-3p-in for 2 days. (E) NF-κB luciferase assay in HepG2 and HCT-116 cells transfected with miR-342-3p or miR-342-3p-in and NF-κB reporter plasmid for 2 days. (F) Western blot of p65 in nucleus of HepG2 and HCT-116 cells transfected with miR-342-3p or miR-342-3p-in for 2 days. (G) qRT-PCR of NF-κB downstream genes (TNF-α, IL-1b, IL-6, IL-8, cyclin d1 and MYC) in HepG2 and HCT-116 cells transfected with miR-342-3p or miR-342-3p-in for 2 days. $^{*}p < 0.05$ compared to control mimics transfection.

HCC cells was significantly inhibited by miR-342-3p and stimulated by miR-342-3p-in (Fig. 1B). We performed a BrdU assay on HepG2 and HCT-116 cells (Fig. 1C) and found that miR-342-3p overexpression significantly decreased proliferation by five-to six fold compared to control cells. In contrast, miR-342-3p increased cell proliferation more than seven fold compared to control cells.

3.2. miR-342-3p directly targets Ikk-γ, TAB2 and TAB3

Searching for miR-342-3p targets using three algorithms, we found that miR-342-3p is closely related to the NF-κB pathway, in which six 3′UTRs of NF-κB pathway genes; IKK-α, IKK-β, IKK-γ, IKK-ε, TAB2 and TAB3, were predicted (Fig. 2A). Since the NF-κB pathway is active in HCC development [22], we focused on the correlation between miR-342-3p and the NF-κB pathway. We compared the sequence of miR-342-3p with its putative targeted genes (Fig. 2B), then performed a luciferase assay by transfecting miR-342-3p with the luciferase reporter plasmid of 3′UTR of each predicted gene. The results showed that miR-342-3p inhibited and miR-342-3p-in increased luciferase activity of IKK-γ, TAB2 and TAB3 3′UTR (Fig. 2C), indicating them as miR-342-3p target genes. Furthermore, western blot analysis showed deregulation of IKK-γ, TAB2 and TAB3 expression by miR-342-3p and miR-342-3p-in (Fig. 2D). These cumulative results suggest that miR-342-3p directly targets IKK-γ, TAB2 and TAB3.

3.3. miR-342-3p regulates NF-κB pathway

Given that miR-342-3p directly targets the NF-κB pathway genes IKK-γ, TAB2 and TAB3, we studied the effect of miR-342-3p on the NF-κB pathway. Luciferase assay revealed that miR-342-3p significantly reduced and miR-342-3p-in significantly increased NF-κB pathway activity (Fig. 2E). We extracted nuclear proteins of HepG2 and HCT-116 cells after miR-342-3p transfection and subjected them to western blot for detection of the NF-κB pathway transcription factor p65. As shown in Fig. 2F, miR-342-3p expression reduced and miR-342-3p-in increased p65 nuclear levels. qRT-PCR was then used to detect expression of the NF-κB downstream pathway genes; TNF-α, IL-1b, IL-6, IL-8, cyclin d1 and MYC, and found that they were downregulated by miR-342-3p and upregulated by miR-342-3p-in (Fig. 2G). These cumulative results indicate that miR-342-3p may be an important regulator of the NF-κB pathway.

3.3.1. Overexpression of Ikk-γ, TAB2 and TAB3 partly rescued HCC cells proliferation inhibited by miR-342-3p

To further investigate whether miR-342-3p reduces HCC cell proliferation via Ikk-γ, TAB2 and TAB3, HepG2 cells were first cotransfected with miR-342-3p with Ikk-γ, TAB2 or TAB3 plasmids. After two days, western blot assay was performed to confirm their expression in HepG2 cells (Fig. 3A–C). Next, MTT assay was performed. As showed in Fig. 3D–E, overexpression of Ikk-γ, TAB2 or TAB3 partially rescued cell proliferation inhibited by miR-342-3p. These findings further confirm that miR-342-3p inhibits HCC cell proliferation via downregulating Ikk-γ, TAB2 or TAB3 expression.

3.4. Inverse expression between miR-342-3p and targets

To analyze miR-342-3p expression in HCC tissues, we first searched the GSE54751 [23] database and found that miR-342-3p was significantly downregulated in 10 HCC tissues compared to adjacent normal liver tissues (Fig. 4A). We then evaluated miR-342-3p expression from 71 HCC samples, confirming miR-342-3p downregulation (Fig. 4B). Three miR-342-3p target genes in these samples were detected by qRT-PCR. As expected, Ikk-γ, TAB2 and TAB3 were upregulated in HCC tissues (Fig. 4C–E). These results strongly suggest downregulation of miR-342-3p and inverse relationship between miR-342-3p and its target genes in HCC tissues.

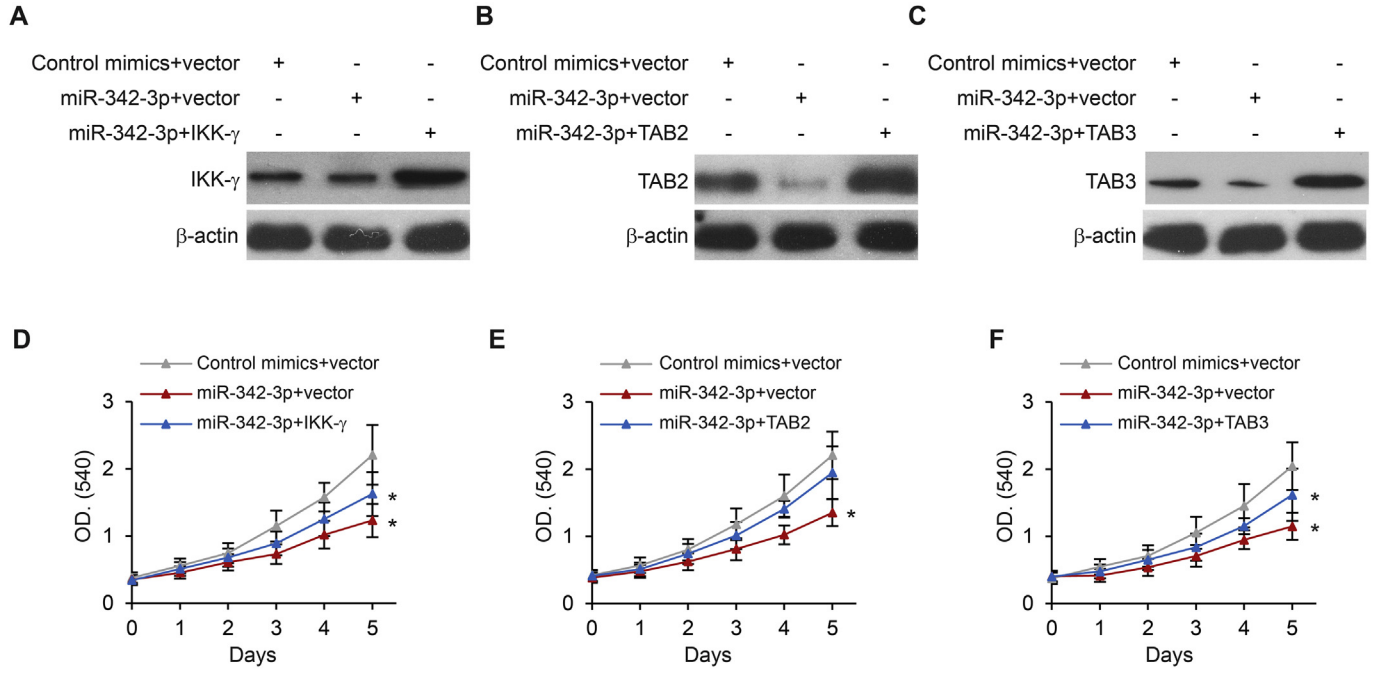


Fig. 3. Overexpression of Ikk-γ, TAB2 and TAB3 partially rescued HCC cells proliferation inhibited by miR-342-3p. Western blot assay in HepG2 cells transfected with miR-342-3p and/or Ikk-γ (A), TAB2 (B) and TAB3 (C) plasmids. MTT assay in HepG2 cells transfected with miR-342-3p and/or Ikk-γ (D), TAB2 (E) and TAB3 (F) plasmids. *$p < 0.05$ compared to control mimics transfection.

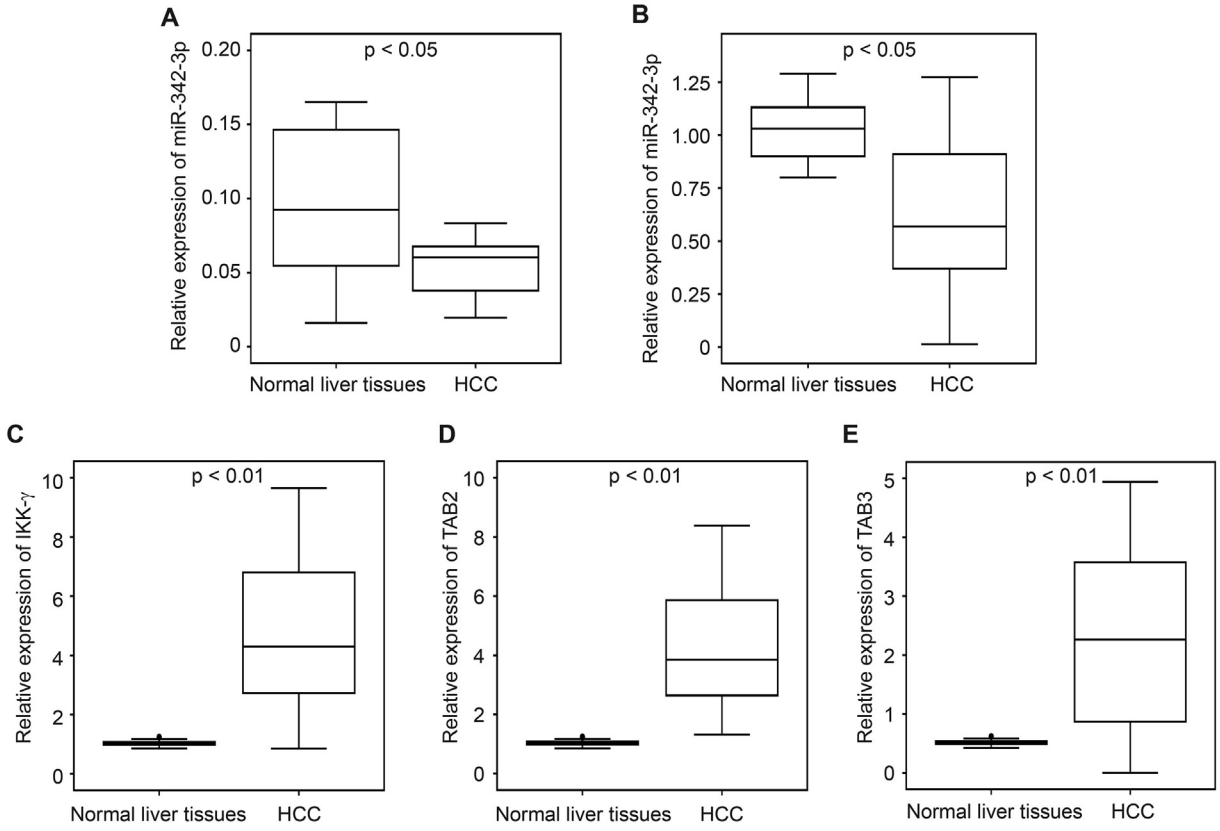


Fig. 4. Inverse expression between miR-342-3p and targets. (A) miR-342-3p expression in 10 HCC samples with paired adjacent normal liver tissues, GSE54751. (B) miR-342-3p expression in 71 HCC samples compared with paired adjacent normal liver tissues. (C–E) IKK-γ (C), TAB2 (D) and TAB3 (E) expression in 71 HCC samples with paired adjacent normal liver tissues. $^{*}p < 0.05$. $^{**}p < 0.01$ compared to adjacent normal liver tissues.

4. Discussion

Utilizing microRNA regulation of basic cellular processes to develop therapeutic targets for new treatments is becoming recognized as a possible key to new drug discoveries. Suppression of tumor cell proliferation by overexpression of miRNAs has been revealed for several types of solid cancers, including cervical, breast, gastric and colon [24].

We hypothesized that miR-342-3p regulates HCC cell proliferation and investigated this using the cell lines HepG2 and HCT-116. Using MTT assay, we determined that miR-342-3p significantly inhibits HCC cell proliferation. BrdU analysis found that miR-342-3p overexpression decreased proliferation of HepG2 and HCT-116 cells by five or six times. Our results indicate that miR-342-3p directly targets IKK-γ, TAB2 and TAB3 from NF-κB-pathway and that miR-342-3p overexpression results in downregulated expression of NF-κB-pathway downstream genes: TNF-α, IL-1b, IL-6, IL-8, cyclin d1 and MYC. Overexpression of miR-342-3p target genes partially rescued cell proliferation inhibited by miR-342-3p. Then, using the Genomic Spatial Event database to analyze HCC samples, we found strong indications of miR-342-3p downregulation in clinical HCC tissues and an inverse relationship between miR-342-3p and its targets.

NF-κB transcription factors regulate the expression of many genes related to immune and inflammatory responses, apoptosis, cell adhesion, differentiation, and oxidative stress responses (reviewed by Ref. [25]). The NF-κB transcription factor complexes consist of various combinations of homo- and heterodimers formed by the subunits p50, p52, c-Rel, RelA (p65) and RelB (reviewed by Ref. [26]). This transcription factor is found in the cytoplasm in an inactive form, bound to the inhibitor IκBα, which masks the nuclear localization signals of NF-κB proteins and keeps them sequester in the cytoplasm. The IKK complex consists of the kinase subunits IKK-β, IKK-α and IKK-γ. This complex activates NF-κB signaling by phosphorylating IκBα, causing release of the p65/p50 dimer of NF-κB, which then translocates to the nucleus and initiates transcription of downstream genes such as IL-1β, IL-6 and TNF-α [27,28].

Further, NF-κB and IKK complex activation relies on an upstream kinase complex consisting of TAK1 and adaptor proteins TAB1, TAB2 or TAB3. The IKK complex is activated by a plethora of NF-κB stimuli and thereby acts as an amalgamator of signals from several different pathways [29].

Cyclin d1 belongs to the highly conserved cyclin family, which are regulators of CDKs (cyclin dependent kinases). The d1 cyclin forms a complex with, and regulates CDK4 and/or CDK6, which are obligatory for G1/S transition in the cell cycle. Cyclin d1 also interacts with the tumor suppressor protein Rb. Mutations, amplifications and overexpression in the gene encoding cyclin d1 are observed frequently in many tumors and is believed to contribute to tumorigenesis [30,31].

MYC is a multifunctional nuclear phosphoprotein transcription factor with roles in cell cycle progression, apoptosis and transformation, can also function as a transcription repressor and has a direct role in DNA replication. MYC is frequently constitutively expressed in many cancers, leading to the dysregulated expression of many genes involved in proliferation, further resulting in oncogenesis. MYC activation is stimulated by mitotic signals such as Wnt, Shh and EGF, and by regulating the expression of its targets, MYC regulates cell proliferation (by regulating cyclins and p21), cell growth (by regulating ribosomal RNA and proteins), apoptosis (by

regulating Bcl-2), differentiation, and self-renewal of stem cells. As such, MYC is considered a strong proto-oncogene and a promising anti-cancer target [32,33].

Thus, our data suggests that miR-342-3p is involved in the regulation of NF-κB signaling by regulating TAB2, TAB3 and IKK-γ expression, and may consequently regulate CDK-dependent signaling by affecting cyclin d1 expression. MiR-342-3p was also found to regulate MYC activity, which is a central transcription factor in oncogenesis. Our data provides important insight into hepatocellular carcinoma progression and can potentially lead to novel therapeutic interventions for liver cancer.

However, although miRNA-mediated NF-κB signaling pathways are promising cancer treatment targets, miRNAs as cancer therapeutics need to overcome the problems of instability and having many genetic targets, some of which could be protective rather than contributory to the disease being treated [34–36]. As we learn more about the mechanisms of miRNA-mediated cell regulation, and as new technologies emerge in construction of miRNAs, their mimetics, and carrying vehicles, miRNAs will become more viable for many cancer treatments, especially in chemo- and radiotherapy combinations.

The therapeutic potential of miR-342-3p has yet to be explored, but knowing that the molecule greatly affects the NF-κB pathway and suppresses cell proliferation makes it a prime candidate for investigation as a targeted therapy in HCC [37,38]. Fine tuning of overexpression of miR-342-3p in tumor cells might lead to a drug with high tumor suppression activity and very low risk of adverse side effects.

The NF-κB pathway is also relevant to several liver diseases besides HCC, including hepatitis (helicobacter infection, viral, HBV and HCV), fibrosis and cirrhosis [39]. Thus, miR-342-p regulation of the pathway could also have significant therapeutic value in these diseases also. However, the NF-κB pathway also is a potential target for development of hepatoprotective agents [30]. This important function must be considered carefully in development of targeted therapeutics. Attainment of a balance might be possible that reduces the pathway activity enough to inhibit advancement of liver disease, while allowing the hepatoprotective function to remain intact enough to still benefit the patient.

Disclosure statement

The authors declare no competing interests.

Acknowledgment

This work was supported by the Science and Technology Research Project, Department of Education, Heilongjiang Province (No. 12531274).

Transparency document

The transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrc.2014.12.119.

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