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MicroRNA-301a (miR-301a) is induced in hepatocellular carcinoma (HCC) and down- regulates the expression of interferon regulatory factor-1

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Abstract

Hepatocellular carcinoma (HCC) tumors evade death in part by downregulating expression of the tumor suppressor gene Interferon regulatory factor-1 (IRF-1). However, the molecular mechanisms accounting for IRF-1 suppression in HCC have not been well described. In this study, we identified a novel microRNA-301a (miR-301a) binding site in the 3′-untranslated region (3′-UTR) of the human IRF-1 gene and hypothesized a functional role for miR-301a in regulating HCC growth. We show that miR-301a is markedly upregulated in primary HCC tumors and HCC cell lines, while IRF-1 is down-regulated in a post-transcriptional manner. MiR-301a regulates basal and inducible IRF-1 expression in HCC cells with an inverse relationship between miR-301a and IRF-1 expression in HCC cells. Chronic hypoxia induces miR-301a in HCC in vitro and decreases IRF-1 expression. Finally, miR-301a inhibition increases apoptosis and decreases HCC cell proliferation. These findings suggest that targeting of IRF-1 by miR-301a contributes to the molecular basis for IRF-1 downregulation in HCC and provides new insight into the regulation of HCC by miRNAs.

Keywords

IRF-1; miR-301a; HCC; Apoptosis; Proliferation; Hypoxia

1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Hepatitis B and C viral infection, fatty liver disease, and alcohol are risk factors for cirrhosis and development of HCC. The majority of patients are not surgical or transplant candidates at

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Declaration of competing interest

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diagnosis, and over 700,000 deaths have been reported globally per year. Therefore, identifying biomarkers, signal transduction pathways, and better treatment options are vitally important.

Interferon regulatory factor-1 (IRF-1) was shown to be an IFN-inducible nuclear transcription factor that plays important roles in immunity and oncogenesis [1]. IRF-1 functions as a tumor-suppressor gene by regulating the cell cycle and apoptosis along with exerting immuno- modulatory and antiviral responses [2–7]. Previously we found that interferon-γ (IFNγ) induced autophagy in HCC cells through IRF-1 [8]. Additionally, aberrant expression of IRF-1 has been found in many malignant tumors including melanoma, leukemia, gastric and breast cancer, and esophageal squamous cell carcinoma [9]. Carcinogenesis signaling in HCV-related HCC was associated with suppression of IRF-1, and downregulation of IRF-1 was found to predict a poor prognosis in HCC [10,11]. However, the molecular mechanisms of IRF-1-mediated suppression of HCC growth are not well defined.

MicroRNAs (miRNAs) are small non-coding 20–30 nucleotide RNA molecules that can suppress mRNA expression at the post-transcriptional level by exerting a translational blockade or causing mRNA degradation [12]. MiRNAs have important roles in regulating gene expression during cell cycle and differentiation, and their expression has been observed in different types of cancers [12,13]. Functionally, miRNAs have been shown to contribute to tumor progression and are found to have regulatory effects in HCC [14–17]. In HCC, upregulation of miR-23a was shown to decrease TGFβ-induced tumor-suppressive activity [18]. Recently we showed that miR-23a downregulates the expression of IRF-1 in HCC by binding to a specific site in the IRF-1 3′-untranslated region [19]. During that study, we also noticed a potential binding site for miR-301 in the 3′UTR of the IRF-1 gene. However, nothing is known about the role of miRNA-301 in regulating IRF-1 expression.

In the present study, we investigated the role of miR-301a in HCC. We found that miR-301a expression was significantly increased in human HCC tumors. We identified a novel binding site for miR-301a in the 3′-UTR of the IRF-1 gene. MiR-301 mimics decreased post-transcriptional expression of IRF-1, while miR-301a inhibition increased IRF-1 protein in HCC cells. Moreover, miR-301a is inversely correlated to IRF-1 in HCC. These findings suggest that the targeting of IRF-1 by miR-301a contributes to the molecular basis for IRF-1 downregulation in HCC and provide new insight into the regulation of HCC by miRNAs.

2. Materials and methods

2.1. Cell lines and HCC tissue specimens

HCC cell lines Huh7, Hep3B, HepG2 and Hepa1- 6(mouse) were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). Routinely mycoplasma-free cells have been verified. They were grown with 10% fetal- borne bovine serum (FBS) (Clontech, Mountain View, USA), 100 U/ml of penicillin and 200 mmol/l L-glutamine, in Dulbecco's modified Eagle medium (DMEM) (Lonza). At a humidified incubator containing 5% CO_2, all cells were incubated at 37 C. Human HCC tumors (n = 20) and

adjacent background liver were obtained from the University of Pittsburgh human liver tumor bank under an IRB-approval protocol.

2.2. RNA isolation and quantitative PCR (qRT-PCR)

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The IRF-1 mRNA expression was quantified as previously described [20]. The IRF-1 primers were: 5′- ACCCTGGCTAGAGATGCAGA-3'(forward), and 5′-GCTTTGTATCGGCCTGT GTG-3' (reverse); GAPDH primers were: 5′-GGGAAGCTTGT CATCAATGG-3' (forward), and 5′-CATCGCCCCACTTGA TTTTG-3' (reverse). Quantitative RT - PCR expression of miR-301a was determined through TaqMan miRNA protocol assays. Reverse transcription reaction was prepared by using TaqManTM Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, Foster City, CA, USA) and amplified by TaqMan® Universal Master Mix II (Applied Biosystems). U6 snRNA was used for normalization. miR-301a and U6 snRNA primers were purchased from Applied Biosystems. The relative gene expression levels were calculated using the $2^{-\Delta\Delta ct}$ method.

2.3. Cell transfection

The miR-301a mimic and inhibitor were transfected in cells in 6-well plates using Lipofectamine 2000 (Invitrogen) for 48 h according to the manufacturer's protocol. miR-301a mimics and inhibitors were purchased from Ambion (Life Technologies, Grand Island, NY, USA). The procedure was followed according to the manufacturer's protocol. MiRNA mimics and inhibitors have been transfected either at concentrations 50 nmol/L. For the luciferase assay, the pMIR-IRF-1–3′UTR plasmid was transfected into cells in 12-well plates using Lipofectamine 2000 (Invitrogen) for 48 h.

2.4. Luciferase reporter assay

The pMIR-REPORTTM miRNA expression reporter vector system (Applied Biosystems) was used to evaluate miRNA regulation and the β-gal reporter control plasmid was used to normalize the transfection efficiency [19]. Huh7 and Hep3B cells were cultured in a 12-well plate and transfected with 200 ng β-gal combined with 500 ng of the pMIR-REPORT empty vector or pMIR-IRF-1–3′UTR plasmid. Furthermore, the cells were co- transfected with the pMIRIRF-1–3′UTR plasmid and 50 pmol of the miR-301a mimic, inhibitor and its NC (Ambion), respectively. miRNA NC was used to normalize the total volume for transfection. Serum-free medium was replaced with growth medium after 6 h. Relative luciferase and β-galactosidase activities were measured with the reporter lysis buffer and luciferase substrate (Promega, Madison, WI, USA). The cells were lysed 48 h after transfection. The relative luciferase unit (RLU) was measured using the Dual-Luciferase Report Assay (Bio-Tek, Winooski, VT, USA).

2.5. Protein extraction

The Huh7 and Hep3B cells have been washed and scraped for a microfuge at 3500 rpm, in the ice-cold phosphate buffered solution (PBS). The pelleted cells were suspended in buffer A with protease inhibitors 40 min on ice. Nuclei were recovered by micro-centrifugation at 12,000 rpm for 15 min. Then buffer A suspending and spinning steps run twice. Nuclear

proteins were resuspended in buffer C with protease inhibitors for 40 min on ice. Buffer A, C and protease inhibitors were prepared as previously described [21]. Protein concentrations are measured by the Bio-Rad protein assay, using BSA as a standard.

Western blot—Western blot analysis was performed as previously described [19]. The membranes were developed onto Odyssey Infrared Imager. Immunofluorescent staining. Immunofluorescence staining was done as described previously.

2.6. Cell proliferation analysis

The Cell Counting Kit-8 (CCK-8) solution (MedChem Express, 10 μL/well) was added to each well at 0, 24, 48, and 72 h. After incubation for 30 min, the optical density (OD) values were measured with a microplate reader (Bio-Rad) at 450 nm.

Statistical analysis.—Statistical analysis was performed using SPSS for Windows version 19.0 (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± SD. Student's t-test was used for raw data analysis and a value of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. MiR-301a is induced in HCC tumors and HCC cell lines

We evaluated the expression in miR-301a in primary human HCC tumors and adjacent background liver from the same resected specimens. The results demonstrated that miR-301a was significantly up-regulated in HCC tumors compared to adjacent liver tissues using qRT-PCR (Fig. 1A). In contrast, IRF-1 expression was down-regulated in HCC tumor samples compared to background liver (Fig. 1B). Next, we examined MiR-301a expression in three human HCC cell lines (Huh7, Hep3B, HepG2), one murine hepatoma cell line (Hepa 1–6), and in primary human hepatocytes (HC). Differential expression of constitutive miR-301a was seen, with high expression in Hep3B and HepG2, low expression in Huh7, and intermediate expression in Hepa 1–6 cells, while no expression was detected in normal primary hepatocytes (Fig. 1C). Since IFNγ is known to be a strong inducer of IRF-1 and can also be present in the inflammatory tumor microenvironment, we also stimulated the HCC cells with IFNγ. IFNγ did not increase miR- 301a expression above basal levels in either the high (Hep3B) or low (Huh7) MiR-301a expressing cell lines (Fig. 1D). We then tested for the ability of exogenous miR-301a mimics and inhibitors to modify intracellular levels of miR-301a. Transfection of the low miR-301a expressing Huh7 cells with miR-301a mimic significantly increased miR-301a levels (Fig. 1E), while transfection of the high miR-301a expressing Hep3B cells with miR-301a inhibitor decreased miR-301a levels (Fig. 1F).

3.2. miR-301a targets a specific binding site in the IRF-1 mRNA 3′UTR

MiRNAs are known to modulate gene expression by binding to specific miRNA sequences in the 3′-UTR of target genes. Therefore, we used Target scan Human (www.targetscan.org) software to identify putative miR-301a binding sequence(s) in the 3′-UTR of the human IRF-1 mRNA. A miR-301a binding sequence match was identified at nucleotide 395–401 in the 528-base pair (bp) IRF-1 3′UTR (Fig. 2A). To show a functional role for this binding

site, the 528-bp human IRF-1 3′UTR was cloned into a luciferase reporter plasmid pMIR-IRF-1 3′-UTR and transfected into human Hep3B cells. Addition of the miR-301a mimic significantly decreased basal luciferase reporter activity, while addition of the miR-301a inhibitor significantly increased basal reporter activity (Fig. 2B). Transfection with the β-galactosidase expression plasmid was used to control for transfection efficiency (data not shown). These results suggest that miR-301a binds to the human IRF-1 3′-UTR region and suppresses post-transcriptional activity.

3.3. miR-301a regulates basal and inducible IRF-1 expression in HCC cells

The effects of miR-301a mimic and inhibitor on IRF-1 expression were examined on HCC cells using immunofluorescent staining and Western blot for IRF-1. Low level IRF-1 expression was detected in resting cells, while IRF-1 staining (Fig. 2C) and protein expression (Fig. 2D and 2E) were markedly increased after IFNγ exposure. Addition of miR-301a mimic had no effect on basal IRF-1 levels, but decreased IFNγ-induced IRF-1 expression. In contrast, addition of miR- 301a inhibitor mildly increased basal and IFNγ-induced IRF-1 expression. Taken together, these data show an inverse relationship between miR-301a and IRF-1 expression in HCC tumors and HCC cell lines.

3.4. Hypoxia induces MiR-301a in HCC in vitro and decreases IRF-1 expression

To simulate the hypoxic tumor microenvironment, human HCC cells lines were exposed to hypoxic conditions. Hypoxia significantly increased basal miR-301a expression in low (Huh7) and high (Hep3B) miR-301a expressing human HCC cells in a time-dependent manner (Fig. 3A and 3B). IRF-1 protein levels were gradually decreased by chronic hypoxia exposure of HCC cells from 12 to 48 h (Fig. 3C). IFNγ stimulation of IRF-1 protein in the Huh7 cells served as positive control. Next, to see if the inhibitory effect of hypoxia on IRF-1 expression was occurring at the transcriptional level, we performed qRT-PCR for IRF-1 mRNA. Chronic hypoxia exposure for 48 h did not decrease IRF-1 mRNA in the low (Huh7) or high (Hep3B) miR-301a expressing human HCC cells (Fig. 3D). These results are consistent with a post-transcriptional blockade of IRF-1 expression where hypoxia increases endogenous miR-301a expression in HCC which then binds to the IRF-1 3′-UTR to exert a translational blockade.

3.5. MiR-301a regulates HCC cell proliferation and apoptosis

Next, we sought to determine the functional role for miR-301a on human HCC cell growth. Using a tumor cell proliferation assay, transfection of miR-301a inhibitor significantly decreased HCC cell proliferation at 48 h in both the Huh7 (Fig. 4A) and Hep3B (Fig. 4B) cells, while mimic had no significant effect compared to HCC cells alone. Likewise, the miR-301a inhibitor increase apoptosis by increasing cleaved caspase 3 in both HCC cell lines (Fig. 4C and 4D).

4. Discussion

IRF-1 functions as a tumor-suppressor gene to inhibit the progression of malignant tumors through regulation of apoptosis and the cell cycle. IRF-1 has been shown to cooperate with the tumor suppressor p53 in response to DNA damage [22]. IRF-1 also transcriptionally

upregulates PUMA to activate apoptosis in cancer cells [5]. In a previous study, we identified that IFNγ induced autophagy in HCC cells through IRF-1 [8], and recently we showed that miR-23a downregulates the expression of IRF-1 in HCC [19]. In the present study, we investigated the role of miR-301a in HCC. The major and novel findings of the current study are: 1) Endogenous miR-301a is up-regulated in human HCC tumors and HCC cell lines and has an inverse relationship with IRF-1 expression; 2) MiR-301a binds to a specific site in the 3′-UTR to down- regulate IRF-1 expression with a translational blockade; 3) Chronic hypoxia induces miR-301a in HCC in vitro and decreases IRF-1 expression; 4) MiR-301a inhibition increases apoptosis and decreases HCC cell proliferation.

Recently, we reported that miR-23a also downregulates the expression of IRF-1 in HCC [19]. The current study identifying a second microRNA (miR-301a) that regulates IRF-1 post-transcriptional expression indicates the complex regulation of this gene. Since IRF-1 serves as a master transcriptional factor regulating many target genes during inflammation, teleologically it makes sense to have overlapping pathways regulating its expression. The findings also highlight the great degree to which HCC tumors have developed means of evading immune regulation by upregulating specific microRNA such as miR-301a to inhibit expression of the tumor suppressor gene IRF-1.

A noteworthy observation was that miR-301a was differentially upregulated in human HCC tumors, and not all tumors exhibited increased miR-301a expression. A similar finding was seen for expression of miR-301a in HCC tumor cell lines where low (Huh7) and high (Hep3B and HepG2) constitutive expression of miR-301a was demonstrated. In an attempt to correlate miR- 301a expression with clinical features, He et al. compared miR-301a levels with tumor differentiation and found high miR-301a expression in tumors was associated with poor differentiation, while low miR-301a expression in tumors was associated with moderate to well- differentiation [23]. In our study, we could not identify any consistent pattern of differentiation with degree of miR-301a expression in a set of 20 patients (data not shown). Interestingly, we compared survival in 20 patients with HCC. Low miR-301a expressing HCC tumors (n = 9) showed a trend towards improved patient 2-year overall survival (OS) of 80% compared to high miR-301a expressing tumors (n = 11) with 2-year OS of 71%, but this was not significantly different (data not shown) and may be explained in part by small sample size.

Recent studies have shown that miRNA-301 expression was increased in prostate and pancreatic cancer cells [22,24], and decreased in cholangiocarcinoma cells [25]. In contrast, miRNA-301 was reported to be increased in HCC [23] and has been suggested to be a potential diagnostic serum biomarker for HCC [26]. A limitation of these studies is the descriptive nature of miRNA-301a expression without identifying a mechanistic role for miR-301a. Our current study provides mechanistic insight into the role of miR-301a in targeting the tumor suppressor gene IRF-1 in HCC to augment the malignant phenotype by down-regulating IRF-1 expression.

In another study, miR-301a inhibition causes increased proliferation of breast cancer cells by enhancing the PI3K-Akt pathway leading to PTEN nuclear accumulation [27]. Moreover,

miRNA-301a induced apoptosis of CML cells by directly targeting TIMP2/ERK1/2 and AKT pathways [28].

In summary, these results demonstrate that miR-301a is markedly upregulated in primary HCC tumors and HCC cell lines, while IRF-1 is down-regulated. IRF-1 is a target gene for miR-301a binding to the 3′-UTR, thereby creating an inverse relationship in HCC. These data are consistent with the notion that targeting of IRF-1 by endogenous miR-301a may contribute to the molecular basis for IRF-1 downregulation in HCC. These findings also provide new insight into the regulation of HCC by miRNAs during inflammatory conditions.

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References

[1]. Tamura T, Yanai H, Savitsky D, Taniguchi T, The IRF family transcription factors in immunity and oncogenesis, Annu. Rev. Immunol 26 (2008) 535–584. [PubMed: 18303999]

[2]. Yim JH, Ro SH, Lowney JK, Wu SJ, Connett J, Doherty GM, The role of interferon regulatory factor-1 and interferon regulatory factor-2 in IFN-gamma growth inhibition of human breast carcinoma cell lines, J. Interferon Cytokine Res 23 (2003) 501–511. [PubMed: 14565859]

[3]. Connett JM, Hunt SR, Hickerson SM, Wu SJ, Doherty GM, Localization of IFN-gamma- activated Stat1 and IFN regulatory factors 1 and 2 in breast cancer cells, J. Interferon Cytokine Res 23 (2003) 621–630. [PubMed: 14651776]

[4]. Armstrong MJ, Stang MT, Liu Y, Yan J, Pizzoferrato E, Yim JH, IRF-1 inhibits NF-κB activity, suppresses TRAF2 and cIAP1 and induces breast cancer cell specific growth inhibition, Cancer Biol. Ther 16 (2015) 1029–1041. [PubMed: 26011589]

[5]. Gao J, Senthil M, Ren B, Yan J, Xing Q, Yu J, Zhang L, Yim JH, IRF-1 transcriptionally upregulates PUMA, which mediates the mitochondrial apoptotic pathway in IRF-1induced apoptosis in cancer cells, Cell Death Differ 17 (2010) 699–709. [PubMed: 19851330]

[6]. Kim PK, Armstrong M, Liu Y, Yan P, Bucher B, Zuckerbraun BS, Gambotto A, Billiar TR, Yim JH, IRF-1 expression induces apoptosis and inhibits tumor growth in mouse mammary cancer cells in vitro and in vivo, Oncogene 23 (2004) 1125–1135. [PubMed: 14762441]

[7]. Stang MT, Armstrong MJ, Watson GA, Sung KY, Liu Y, Ren B, Yim JH, Interferon regulatory factor-1-induced apoptosis mediated by a ligand-independent fas-associated death domain pathway in breast cancer cells, Oncogene 26 (2007) 6420–6430. [PubMed: 17452973]

[8]. Li P, Du Q, Cao Z, Guo Z, Evankovich J, Yan W, Chang Y, Shao L, Stolz DB, Tsung A, et al., Interferon-γ induces autophagy with growth inhibition and cell death in human hepatocellular carcinoma (HCC) cells through interferon-regulatory factor-1 (IRF-1), Cancer Lett 314 (2012).

[9]. Yanai H, Negishi H, Taniguchi T, The IRF family of transcription factors: inception, impact and implications in oncogenesis, OncoImmunology 1 (2012) 1376–1386. [PubMed: 23243601]

[10]. Esmat G, El-Bendary M, Zakarya S, Ela MA, Zalata K, Role of *Helicobacter pylori* in patients with HCV-related chronic hepatitis and cirrhosis with or without hepatocellular carcinoma: possible association with disease progression, J. Viral Hepat 19 (2012) 473–479. [PubMed: 22676359]

[11]. Yi Y, Wu H, Gao Q, He HW, Li YW, Cai XY, Wang JX, Zhou J, Cheng YF, Jin JJ, et al., Interferon regulatory factor (IRF)-1 and IRF-2 are associated with prognosis and tumor invasion n HCC, Ann. Surg. Oncol 20 (2013) 267–276. [PubMed: 22805863]

[12]. Carthew RW, Sontheimer EJ, Origins and mechanisms of miRNAs and siRNAs, Cell 136 (2009) 642–655. [PubMed: 19239886]

[13]. Nazarov PV, Reinsbach SE, Muller A, Nicot N, Philippidou D, Vallar L, Kreis S, Interplay of microRNAs, transcription factors and target genes: linking dynamic expression changes to function, Nucleic Acids Res 41 (2013) 2817–2831. [PubMed: 23335783]

[14]. He XX, Kuang SZ, Liao JZ, Xu CR, Chang Y, Wu YL, Gong J, Tian DA, Guo AY, Lin JS, The regulation of microRNA expression by DNA methylation in hepatocellular carcinoma, Mol. Biosyst 11 (2015) 532–539. [PubMed: 25424171]

[15]. D'Anzeo M, Faloppi L, Scartozzi M, Giampieri R, Bianconi M, Del Prete M, Silvestris N, Cascinu S, The role of micro-RNAs in hepatocellular carcinoma: from molecular biology to treatment, Molecules 19 (2014) 6393–6406. [PubMed: 24853455]

[16]. Otsuka M, Kishikawa T, Yoshikawa T, Ohno M, Takata A, Shibata C, Koike K, The role of microRNAs in hepatocarcinogenesis: current knowledge and future prospects, J. Gastroenterol 49 (2014) 173–184. [PubMed: 24258409]

[17]. Bai Y, Xue Y, Xie X, Yu T, Zhu Y, Ge Q, Lu Z, The RNA expression signature of the HepG2 cell line as determined by the integrated analysis of miRNA and mRNA expression profiles, Gene 548 (2004) 91–100.

[18]. Huang S, He X, Ding J, Liang L, Zhao Y, Zhang Z, Yao X, Pan Z, Zhang P, Li J, et al., Upregulation of miR-23a decreases transforming growth factor-beta-induced tumor-suppressive activities in human hepatocellular carcinoma cells, Int. J. Cancer 123 (2008) 972–978. [PubMed: 18508316]

[19]. Yan Y, Liang Z, Du Q, Yang M, Geller DA, MicroRNA-23a downregulates the expression of interferon regulatory factor-1 in hepatocellular carcinoma cells, Oncol. Rep 36 (2016) 633–640. [PubMed: 27279136]

[20]. Guo Z, Shao L, Zheng L, Du Q, Li P, John B, Geller DA, miRNA-939 regulates human inducible nitric oxide synthase posttranscriptional gene expression in human hepatocytes, Proc. Natl. Acad. Sci. U. S. A 109 (2012) 5826–5831. [PubMed: 22451906]

[21]. Shao L, Guo Z, Geller DA, Transcriptional suppression of cytokine-induced iNOS gene expression by IL-13 through IRF-1/ISRE signaling, Biochem. Biophys. Res. Commun 362 (2007) 582–586. [PubMed: 17723228]

[22]. Tanaka N, Ishihara M, Lamphier MS, Nozawa H, Matsuyama T, Mak TW, Aizawa S, Tokino T, Oren M, Taniguchi T, Cooperation of the tumour suppressors IRF-1 and p53 in response to DNA damage, Nature 382 (1996) 816–818. [PubMed: 8752276]

[23]. Jiang J, Gusev Y, Aderca I, Mettler TA, Nagorney DM, Brackett DJ, Roberts LR, Schmittgen TD, Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival, Clin. Cancer Res 14 (2008) 419–427. [PubMed: 18223217]

[24]. Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ, Schmittgen TD, Expression profiling identifies microRNA signature in pancreatic cancer, Int. J. Cancer 120 (2007) 1046–1054. [PubMed: 17149698]

[25]. Braconi C, Huang N, Patel T, MicroRNA-dependent regulation of DNA methyltransferase- 1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes, Hepatology 51 (2010) 881–890. [PubMed: 20146264]

[26]. He K, Hu Z, Ruan J, Ma Q, Zhong F, Cheng X, Sun S, Zhou J, MicroRNA301 is a potential diagnostic biomarker for hepatocellular cancer, Int. J. Clin. Exp. Pathol 8 (2015) 5603–5608. [PubMed: 26191271]

[27]. Jain MV, Shareef A, Likus W, Cieslar-Pobuda A, Ghavami A, Los MJ, Inhibition of miR301 enhances Akt-mediated cell proliferation by accumulation of PTEN in nucleus and its effects on cell-cycle regulatory proteins, Oncotarget 7 (2016) 20953–20965. [PubMed: 26967567]

[28]. Huang X, Qi L, Lu W, Yang G, Chen Y, Zhang R, Rao J, Ji D, Huang R, Chen G, miRNA-301a induces apoptosis of chronic myelogenous leukemia cells by directly targeting TIMP2/ERK1/2 and AKT pathways, Oncol. Rep 37 (2017) 945–952. [PubMed: 28035415]

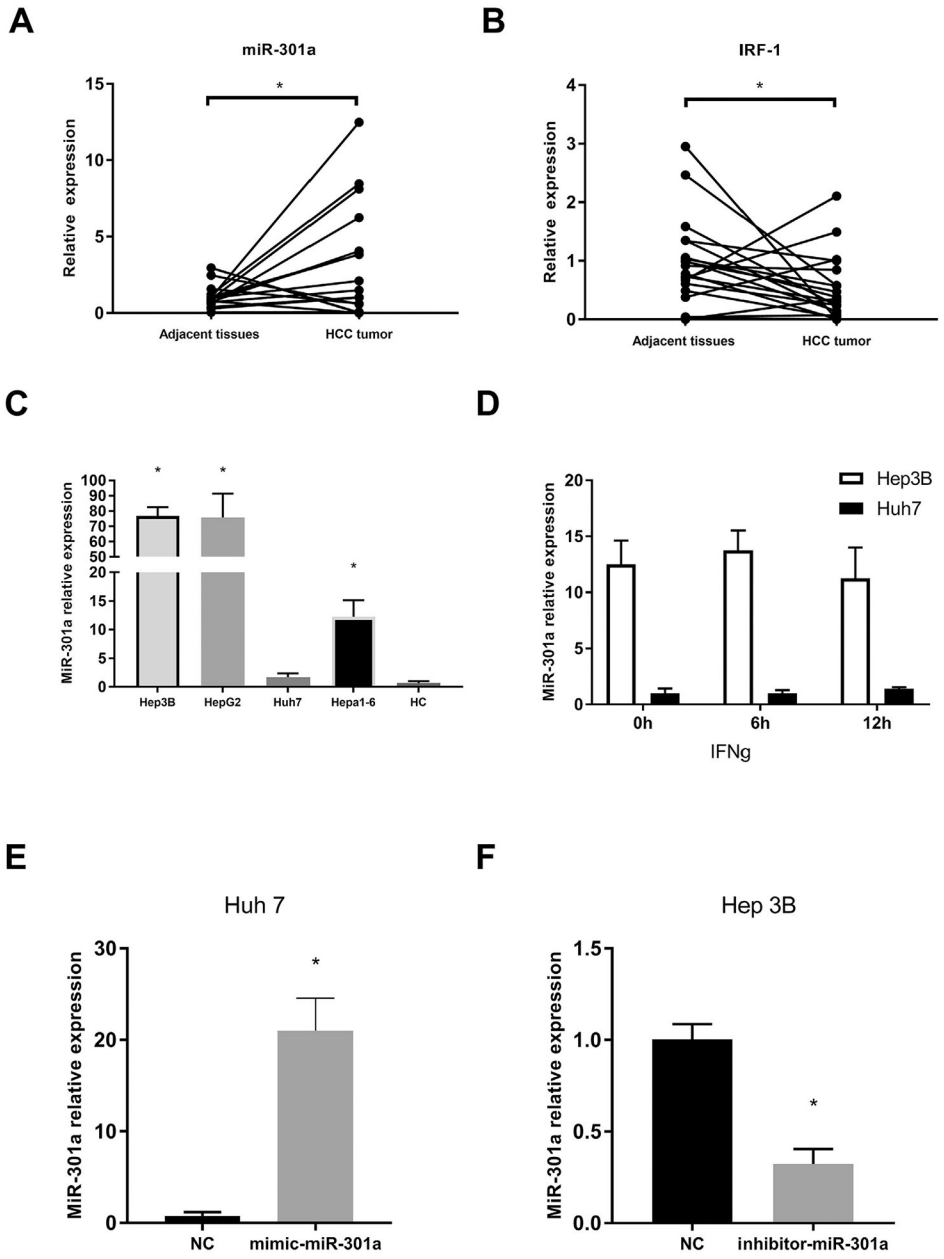

Fig. 1.

IRF-1 and miR-301a expression in HCC tumor and HCC cell lines. A. PCR analysis of miR-301a expression in human HCC tumor and adjacent background liver tissue. * indicates $P < 0.05$ HCC tumor vs. adjacent tissues. B. PCR analysis of IRF-1 expression in HCC tumor and adjacent background liver tissue. * indicates $P < 0.05$ HCC tumor vs. adjacent tissues. C. MiR- 301a expression in HCC cell lines and primary hepatocytes. * indicates $P < 0.05$ vs. primary hepatocytes (HC). D. MiR-301a expression in HCC cell lines induced by IFNγ E. MiR-301a expression in Huh7 cells after transfection with miR-301a mimic. * indicates $P < 0.0001$ vs. negative mimic control (NC). F. MiR-301a expression in Hep3B cells after transfection with miR-301a inhibitor. * indicates $P < 0.0001$ vs. negative inhibitor control (NC).

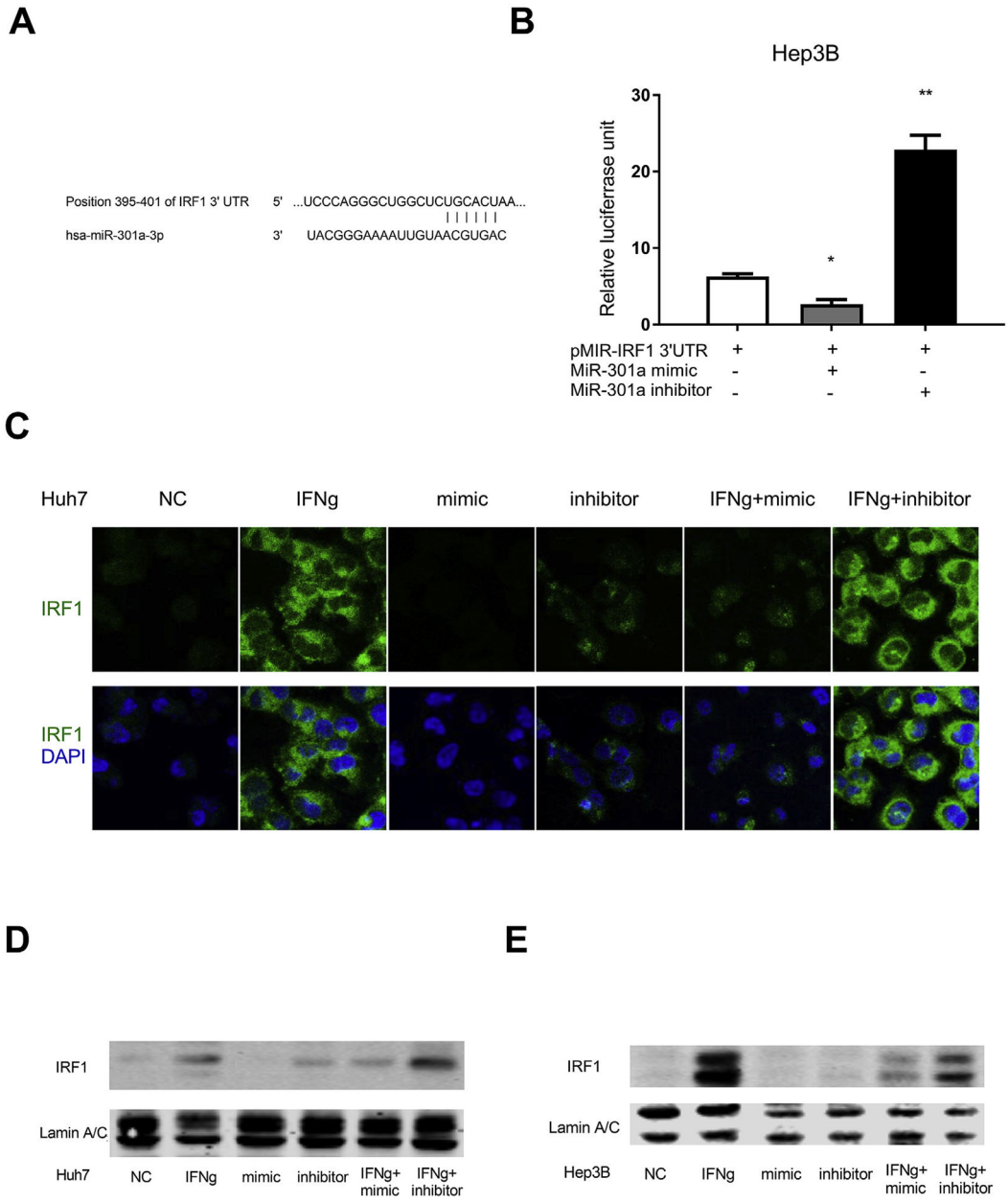

Fig. 2.

MiR-301a regulates IRF-1 expression via binding to the 3′-UTR of IRF-1. A. The 3′-untranslated region binding site of miR-301a in the IRF-1 gene. B. IRF-1 3′UTR reporter assay after transfection with miR-3–1a mimic and inhibitor. * indicates $P < 0.01$ vs. pMIR-IRF-1 3′UTR. ** indicates $P = 0.001$ vs. pMIR-IRF-1 3′UTR. C. Immunofluorescence staining for IRF-1 in Huh7 tumor cells after IFNγ stimulation ± miR-301a mimic and inhibitor. D. Western blot assay for IRF-1 in Huh7 cells after IFNγ stimulation ± miR-301a mimic and inhibitor.Blot shown is representative of two similar experiments. E. Western blot assay for IRF-1 in Hep3B cells after IFN γ stimulation ± miR-301a mimic and inhibitor. Blot shown is representative of two similar experiments.

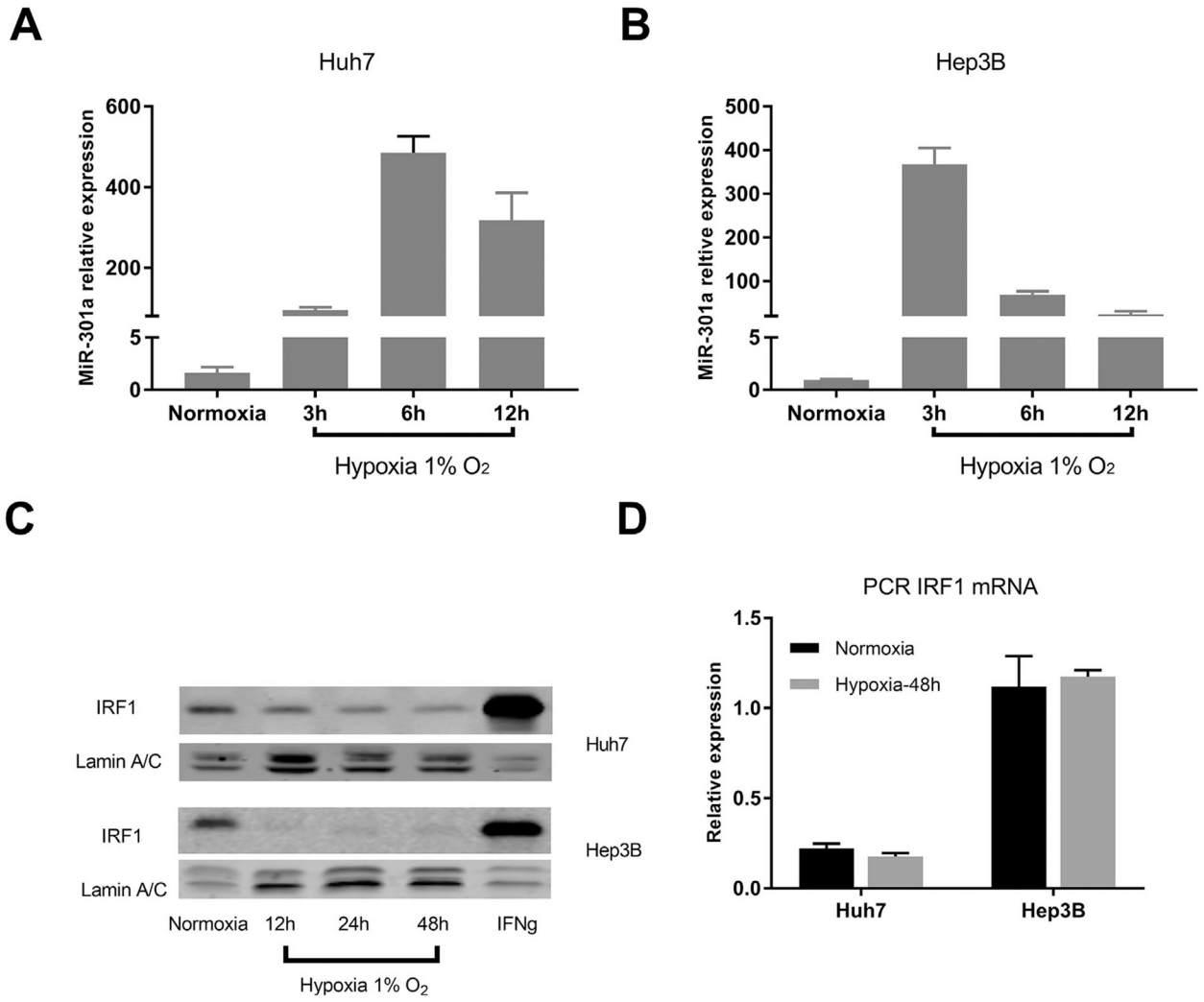

Fig. 3.

Effect of hypoxia on miR-301a and IRF-1 expression in HCC cells. A. Relative expression of mir-301a in Huh7 after 1% hypoxia. B. Relative expression of mir-301a in Hep3B cells after 1% hypoxia. C. IRF-1 protein expression in Huh7 and Hep3B cells after hypoxia. D. qRT-PCR for IRF-1 mRNA in Huh7 and Hep3B cells after hypoxia.

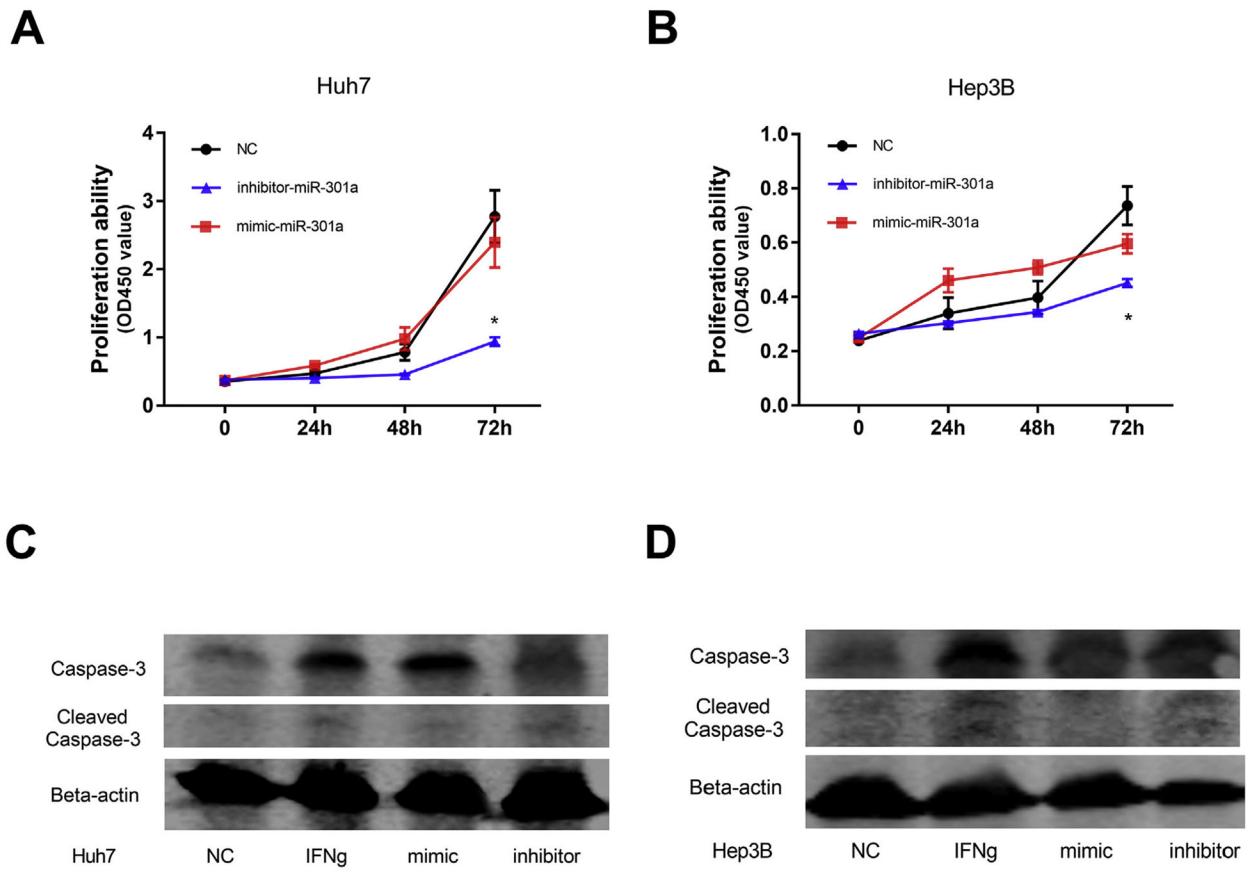

Fig. 4.

HCC cell proliferation and apoptosis regulated by miR-301a. A. Cell proliferation assay in Huh7 cells after transfection with miR-301a mimic or inhibitor. * indicates $P < 0.0001$ vs. negative control (NC). B. Cell proliferation assay in Hep3B cells after transfection with miR-301a mimic or inhibitor. * indicates $P < 0.003$ vs. negative control (NC). C. Western blot for cleaved caspase-3 in Huh7 cells after transfection with miR-301a mimic or inhibitor. D. Western blot for cleaved caspase-3 in Hep3B cells after transfection with miR-301a mimic or inhibitor.