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MicroRNA-144 inhibits hepatocellular carcinoma cell proliferation, invasion and migration by targeting ZFX

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MicroRNA 144 (miR-144), a small non-coding RNA, is frequently dysregulated in human several tumour progression, but its role and the underlying mechanisms in hepatocellular carcinoma (HCC) is poorly investigated. In the present study, the expression of miR-144 was firstly analysed in datasets derived from GSE21362 and TCGA, and then detected in HCC tissues and cell lines by quantitative RT-PCR (qRT-PCR) analysis. MiR-144 was shown to be significantly down-regulated in HCC tissues and cell lines. Subsequently, overexpression of miR-144 was transfected into HCC cell lines so as to investigate its biological function, including MTT, colony formation, and transwell assays. Gain of function assay revealed miR-144 remarkably inhibited cell proliferation, migration and invasion. In addition, bioinformatical analysis and luciferase reporter assay identified ZFX as a novel target of miR-144 in HCC cells, as confirmed by qRT-PCR and Western blot. Furthermore, ZFX was found to be significantly up-regulated using Oncomine database analysis. Loss of function assay further indicated knockdown of ZFX had similar effects of miR-144-mediated HCC cell proliferation and invasion. Therefore, miR-144 has been demonstrated to act as a tumour suppressor in HCC cell growth and motility by directly targeting ZFX, which implicates its potential applications in the development of HCC treatment.

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide (Tang *et al.* 2013). It is characterized as low detection rate, poor prognosis, metastatic tendency and high recurrence rate (Dhanasekaran *et al.* 2012). Despite significant progresses in the diagnosis and treatment for HCC, the morbidity and mortality rates are expected to increase in coming years. Thus, there is an urgent need to elucidate the molecular mechanisms underlying the progression and metastasis of HCC, which might helpful to find new molecular targets for early detection and effective treatment of these patients.

MicroRNAs (miRs) are identified as small noncoding single stranded RNAs containing 21–25 nucleotides that play important regulatory roles in the post-transcriptional level of gene expression through binding to the 3′-untranslated region (3′-UTR) of target mRNAs (Bartel 2009; Paranjape *et al.* 2009; Shukla *et al.* 2011). MiRs have frequently been demonstrated to be involved in multiple biological activities, including cell proliferation, migration, invasion and angiogenesis (Song *et al.* 2015; Yang *et al.* 2014). In addition, accumulating evidences have indicated that miRs function as oncogenes or tumour suppressor and contribute to various human cancers, including HCC (Song *et al.* 2010; Voorhoeve 2010; Zhang *et al.* 2007). For instance, miR-9-3p suppressed tumour growth by targeting TAZ expression in HCC (Higashi *et al.* 2015). Zhou *et al.* found that miR-503 inhibited cell proliferation, migration

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and invasion in HCC (Zhou *et al.* 2011). Furthermore, miR-765, as an oncogene, promotes cell proliferation by down-regulating INPP4B expression in HCC (Xie *et al.* 2016). Recent reports revealed that miR-144 suppressed colon cancer cell proliferation and migration by targeting GSPT1 (Xiao *et al.* 2015). Additionally, miR-144 was shown to be associated with lung cancer (Liu *et al.* 2016), nasopharyngeal carcinoma (Zhang *et al.* 2012) and renal cell carcinoma (Xiang *et al.* 2016). However, the role of miR-144 in regulating HCC cell biological function remains largely unclear.

Therefore, the expression of miR-144 was firstly investigated in HCC through analysing the data from two public datasets (Gene Expression Omnibus and Cancer Genome Atlas Project) and fresh clinical tissues, as well as HCC cell lines. Furthermore, we explored the biological function of miR-144 in HCC cell and its downstream target gene. Our findings suggest that miR-144 acts as a tumour suppressor in HCC cells by inhibiting cell proliferation, migration and invasion, which directly targeting ZFX gene.

2. Materials and methods

2.1 *Expression datasets*

To investigate the expression level of miR-144 in HCC, one set of mRNA expression data was downloaded from the Cancer Genome Atlas project (TCGA dataset, *https://gdc-portal.nci.nih.gov/*) dataset. Total 424 specimens were available including 374 HCC and 50 normal tissues. In addition, the other set of microarray data was downloaded from GeneExpression Omnibus (GEO) under the accession number GSE21362 (*http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE21362*, Platform information: GPL10312, 3D-Gene Human miRNA Oligo chip v12-1.00), which contained 146 specimens including 73 HCC and 73 normal tissues. The *t*-test method was used to compare the expression ofmiR-144between HCC and adjacent normal tissues in these two datasets.

2.2 *Tissue specimens and cell lines*

Total 20 pairs of primary HCC tissues and their corresponding adjacent normal liver tissues were collected from patients who underwent hepatic resection in Harrison International Peace Hospital of Hengshui, Hebei in China. Fresh specimens were snap-frozen andstored in liquid nitrogen tanks immediately after resection. Sample acquisition was approved by the Institutional Ethics Committee of China Harrison International Peace Hospital.

Human HCC cell lines, HepG2, SMMC-7721, HCC-LM3, Bel-7402, Huh7 and normal liver cell line LO2 were obtained from Shanghai Institute for Cell Biological Science (Shanghai, China). HepG2, SMMC-7721, HCC-LM3 and Hub7 were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA). Bel-7402 and LO2 were maintained in RPMI 1640 medium containing 10% FBS. All cells were incubated in a humidified incubator with 5% CO_2 at 37°C.

2.3 *RNA preparation and quantitative real-time PCR*

Total RNA was extracted from tissues and cell lines using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For miR-144 detection, miRNA expression levels were normalized against the endogenous U6 small nuclear RNA (U6 snRNA) control using ABI PRISM® 7900HT Sequence Detection System. ZFX expression was determined by SYBR green qPCR assay and β-actin was used as an endogenous control. The comparative delta CT method was used to calculate the mean of triplicate CT value for genes. The primer sequences used for the indicated genes analysis were as follows: MiR-144 (forward): 5′-CTCAACTGGTGTCTTCAGTTGAGGGTGGAGTCGGCC-3′ and MiR-144 (reverse): 5′-ACACTCCAGGGCGCCGCGCTGGGAGGCGG-3′; U6 (forward):5′-CTCGCTTCGGCAGCACA-3′ and U6 (reverse): 5′-AACGCTTCACGAATTTGCGT-3′; ZFX (forward): 5′-GGCAGTCCACAGCAAGAAC3′ and ZFX (reverse): 5′-TTGGTATCCGAGAAAGTCAGAAG-3′; β-actin (forward): 5′-GTGGACATCCGCAAAGAC-3′ and β-actin (reverse): 5′-AAAGGGTGTAACGCAACTA-3′.

2.4 *Cell transfection*

The miR-144 agomiR (agomiR-144), small interfering RNA for ZFX (siZFX) and their corresponding negative control vectors were purchased from Shanghai GenePharma Co. Ltd. Cells were transfected with vectors mentioned above using Lipofectamine 2000Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.5 *MTT assay*

HepG2 and Huh7 cells (5000 cells per well) were cultured in 96-well plate after transfection and incubated at 37°C for 1, 2, 3, 4 and 5 days. On the indicated days, 100 μL fresh serum-free medium containing 0.5 g/L MTT (KyeGENBioTECH, Nanjing, China) was added to each well. After incubation for 4 h, the medium was removed and 150 μL dimethyl sulfoxide (Sigma-Aldrich Co.) was added to each well to stop the reaction. The absorbance of each well was measured at 595 nm using a plate reader. This experiment was repeated three times.

2.6 *Colony formation assay*

After transfection, approximately 500 HCC cells per well were reseeded in 6-well plates and incubated for 10 days. Then the cells were fixed by 4% paraformaldehyde and stained using 0.1% crystal violet. The number of colonies, each included 50 cells at least, was manually counted. Triplicate independent experiments were performed and all the visible colonies were calculated manually.

2.7 *Migration and invasion assays*

After transfection, HCC cells (2×10^5 cells per mL) were in serum-free DMEM. About 200 µL cell suspension was added in the upper chamber of Transwell chamber (Corning, NY, USA). The lower chamber was filled with 500 µL medium containing 10% FBS. For invasion assay, the upper chamber was coated with 25µL of Matrigel (BD Biosciences, Bedford, MA, USA) without FBS. After 24 h (migration assay) or 48 h (invasion assay) of incubation, the cells that migrated or invaded through the upper surface to the lower surface were fixed and stained 0.1% crystal violet for 30 min. Cell numbers in five random microscopic fields in each replicate were counted and imaged using an Olympus light microscope.

2.8 *Bioinformatic prediction and Luciferase reporter assay*

TargetScan (*www.targetscan.org*) was used to predict the putative target genes of miR-144. Then the wild-typeZFX-3′UTR (WT) or mutantZFX-3′UTR (MUT) with predicted binding site of miR-144 were constructed and cloned in the pGL3 control vector (Promega, Madison, WI, USA). For the luciferase reporter assay, HCC cells were seeded into 24-well plates and transfected with ZFX-3′-UTR-WT and ZFX-3′-UTR-MUT and subsequently transduced with corresponding miRNA vectors using Lipofectamine 2000 (Invitrogen). After 48 h, the luciferase and renilla signals were detected using Dual Luciferase Reporter Assay Kit (Promega, Madison, WI) according to the manufacturer's protocols.

2.9 *Western blot*

Total proteins were extracted with RIPA lysis buffer containing protease inhibitors (Beyotime, China). Cell protein lysates were separated by SDS-PAGE and then transferred onto the polyvinylidene fluoride membrane (Millipore, USA). The membrane was blocked with TBST buffers containing 5% skimmed milk and incubated with primary antibody for ZFX (ProteinTech Group, USA) and GAPDH (Cell Signaling Technology, USA). Subsequently, the membranes were washed in TBST and incubated with horseradish peroxidase conjugated secondary antibodies (ProteinTech Group, USA).The signals of membranes were detected and visualized using ECL kit (Pierce).

2.10 *Statistical analysis*

Quantitative data are presented as mean ± standard deviation (SD) of triplicate experiments. Comparison between two groups was performed using a two-tailed Student's *t*-test. Difference were considered significant when $p<0.05$.

3. Results

3.1 *MiR-144 was down-regulated in HCC tissues and cell lines*

To explore the potential role of miR-144 in HCC, the expression data of miR-144 was downloaded from TCGA and GEO, and then analysed the differential expression between HCC tissues and non-tumour tissues. As shown in figure 1A and B, the expression of miR-144 was significantly down-regulated in HCC ($p<0.001$) in comparison with normal tissues. To further validate the expression of miR-144 in HCC, 20 pairs of HCC tissues and adjacent non-tumour liver tissues were detected by qRT-PCR. The result showed that down-regulation of miR-144 was observed in most cases of HCC tissues, which was remarkably lower than that in matched non-tumour tissues (figure 1C; $p<0.001$). In addition, miR-144 expression was downregulated in HCC cell lines (HepG2, SMMC-7721, HCC-LM3, Bel-7402 and Huh7) than that in the normal liver cell lines LO2 (figure 1D; $p<0.05$; $p<0.01$; $p<0.001$). Taken together, these data indicated that miR-144 was down-regulated in HCC. Notably, HepG2 and Huh7, with the most significant decrease in miR-144 expression, were chosen for the following experiments.

3.2 *Up-regulation of miR-144 inhibits HCC cell proliferation*

To investigate the function roles of miR-144 in HCC, the expression of miR-144 was up-regulated in HepG2 and Huh7 cells by transfecting with agomiR-144 and the control vector. As measured by qRT-PCR, the expression of miR-144 was confirmed to be overexpressed in the two HCC cell lines (figure 2A and B; $p<0.001$). Then the proliferation ability of two HCC cell lines was determined using MTT and colony formation assays. We found that up-regulation of miR-144 led to a significant reduction in cell proliferation rate compared with the negative control groups (figure 2C and D; $p<0.001$). Consistent with MTT assay, colony

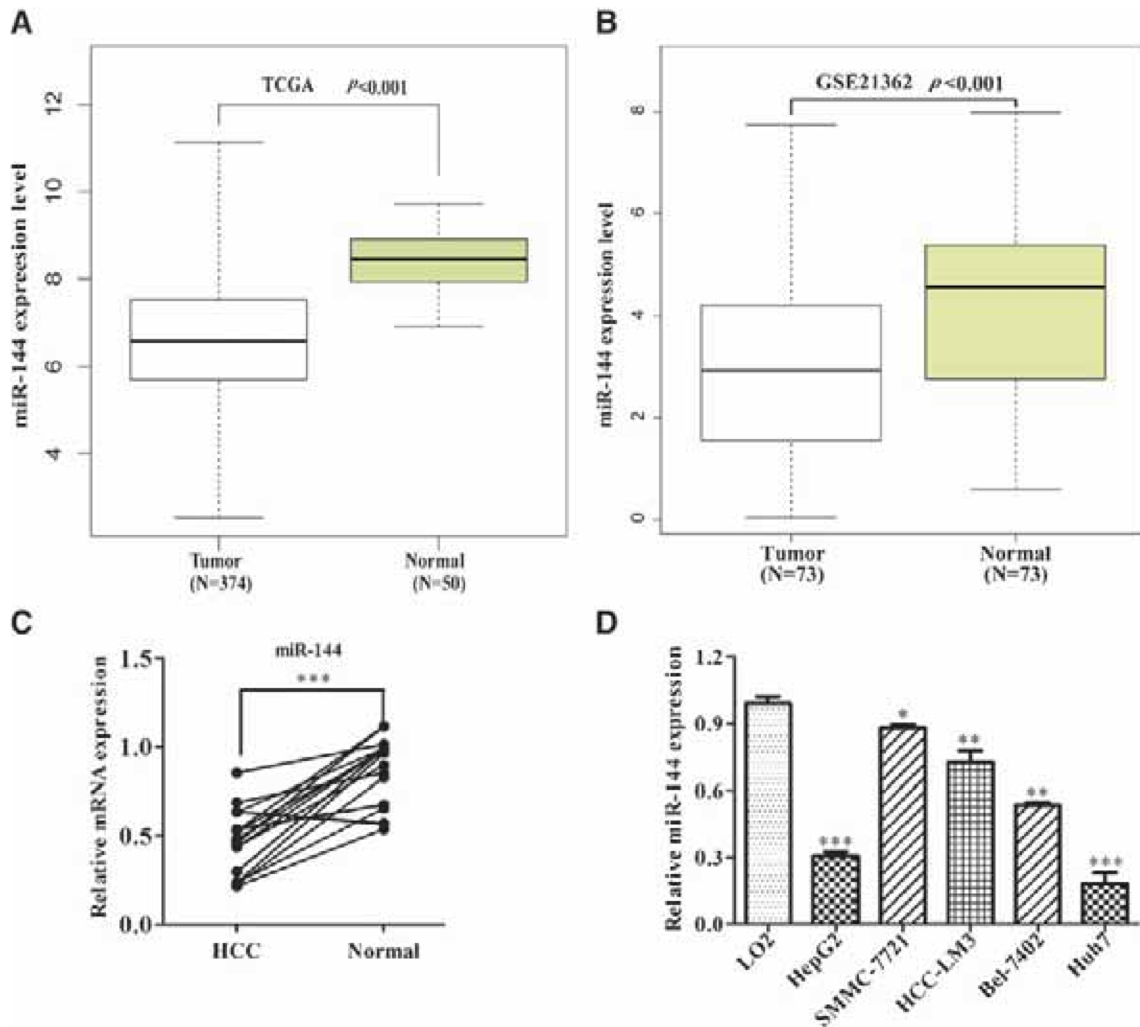

Figure 1. miR-144 was down-regulated in HCC tissues and cell lines. (**A**) The expression levels of miR-144 in HCC tissues from TCGA dataset (n = 73, $p< 0.001$). (**B**) Microarray data analysis of miR-144 from the Gene Expression Omnibus (GEO) database (accession number: GSE21362). (**C**) Quantitative real-time PCR analysis of miR-144 expression in 20 pairs of primary HCC tissues and their corresponding adjacent normal liver tissues. (**D**) Quantitative real-time PCR analysis ofmiR-144 expression in HCC cell lines (HepG2, SMMC-7721, HCC-LM3, Bel-7402 and Huh7) and normal liver cell line LO2. The relative expression of miR-144 was normalized to U6. *$p<0.05$; **$p<0.01$; ***$p<0.001$ *vs* normal tissues or cells.

formation assay also indicated that up-regulation of miR-144 could significantly inhibit colony formation ability, as shown in reduction of colony number and size in HCC cells (figure 2E and F; $p<0.001$). Thus, miR-144 could play a positive role in cell proliferation in HCC.

3.3 *Up-regulation of miR-144 inhibits HCC cell migration and invasion*

Furthermore, transwell chamber assay was used to analyse the function of miR-144 in cell migration and invasion in HCC cells. As shown in figure 3A, the number of migrated and invasive cells in agomiR-144 group (65±12 and 38±6, respectively) was significantly decreased, compared with the negative control group (112±8 and 73±7, respectively) in HepG2 cells. The similar results (figure 3B) were also observed in Huh7 cells (56±8 and 41±7 *vs* 85±6 and 70±7, respectively). Based on these results, we concluded that up-regulation of miR-144 decreased the migration and invasion of HCC cells.

3.4 *ZFX is the target of miR-144 in HCC cells*

To further discover the molecular mechanisms by which miR-144 inhibits HCC cell proliferation and invasion, TargetScan

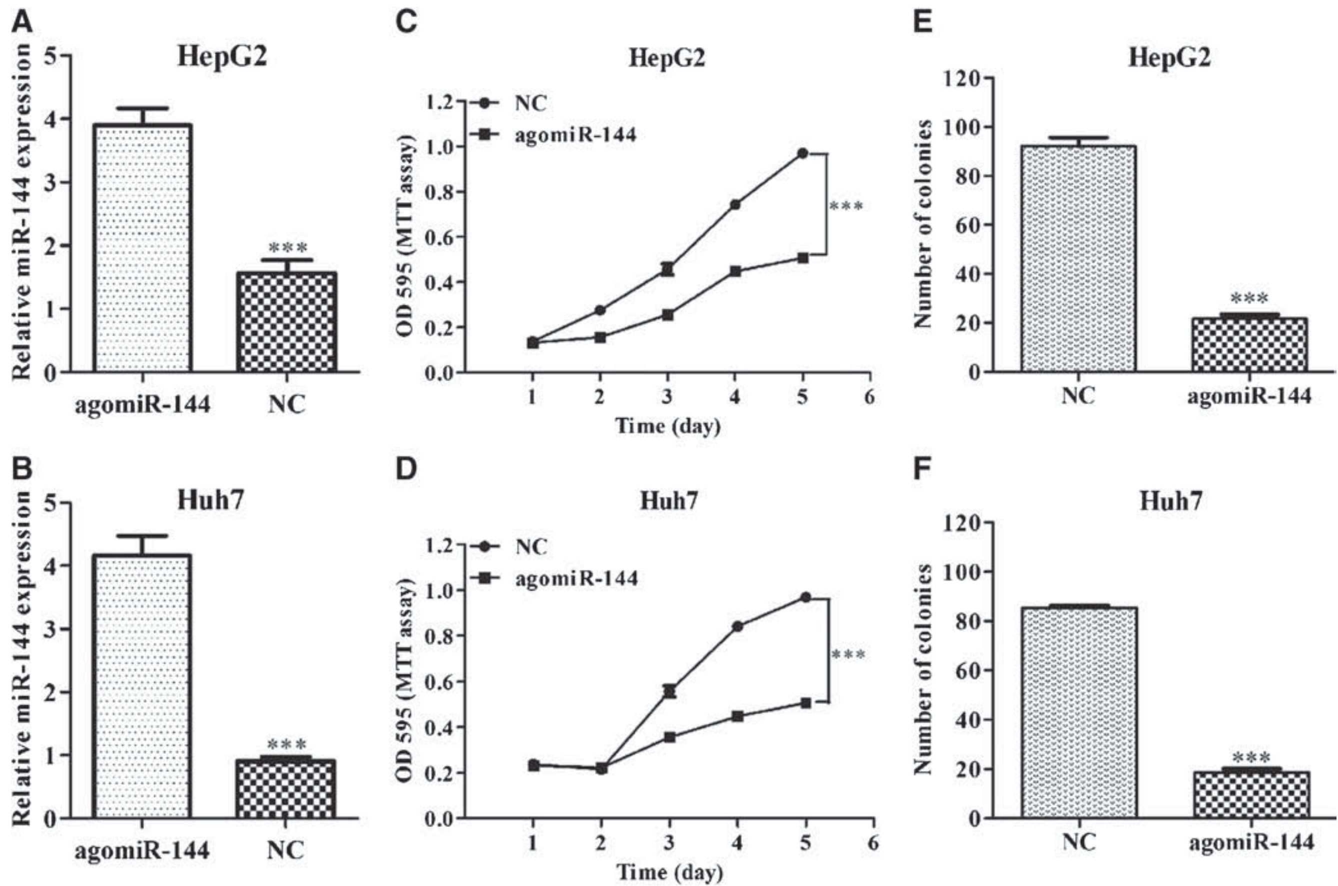

Figure 2. miR-144 suppressed HCC cell proliferation and colony formation ability. QRT-PCR analysis of miR-144 transfection efficiency after agomiR-144 transfection in HepG2 (**A**) and Huh7 (**B**) cells; (**C** and **D**) The proliferation ability of HCC cells was evaluated using MTT assay after transfection with agomiR-144. (**E** and **F**) Colony formation analysis of HCC cells after treatment with agomiR-144 or controls. Data are expressed as mean ± SD of triplicate experiments, ****p*<0.001 *vs* controls.

software was used to predict the putative target genes of miR-144. As shown in figure 4A, ZFX, which is known to be an important transcriptional regulator in HCC progression (Lai *et al.* 2014), was predicted as one of the target genes of miR-144. Then we investigated whether the miR-144 directly interacted with the 3′UTR of ZFX mRNA using luciferase reporter assay. As predicted, overexpression of miR-144 significantly suppressed the luciferase activity of the ZFX 3′–UTR (WT), but didn't inhibit activity of ZFX with 3′–UTR (MUT) in both HepG2 ($p<0.01$; figure 4 B) and Huh7 cells ($p<0.05$; figure 4C). Accordingly, our data suggested that miR-144 can directly bind to the 3′UTR of ZFX.

Furthermore, we investigated the effect of miR-144 on the mRNA and protein expression of ZFX in HCC cells. As shown in Figure 4D and F, overexpression of miR-144 obviously down-regulated the expression of ZFX at both mRNA and protein levels in HepG2 cells ($p<0.001$). Consistent results were also observed in Huh7 cells (figure 4E and G, $p<0.001$). Collectively, we demonstrated that miR-144 negatively regulated the expression of ZFX via directly bind to the 3′UTR of ZFX.

3.5 *Knockdown of ZFX inhibited HCC cell proliferation, migration and invasion*

As ZFX was confirmed as a direct target of miR-144, but its functional role in HCC remains unclear. Therefore, we firstly investigated the differential expression of ZFX between HCC and normal tissues by conducting meta-analysis on online Oncomine Expression Array database (*www.oncomine.org*) under the key terms, including 'ZFX', 'liver cancer', 'mRNA' and 'Cancer *vs* Normal Analysis'. As shown in figure 5A, meta-analysis of total five datasets revealed that ZFX mRNA expression was significantly higher in HCC than the normal tissues with a median rank of 3303.0 and P-value of 0.010. The further results revealed that siZFX mediated down-

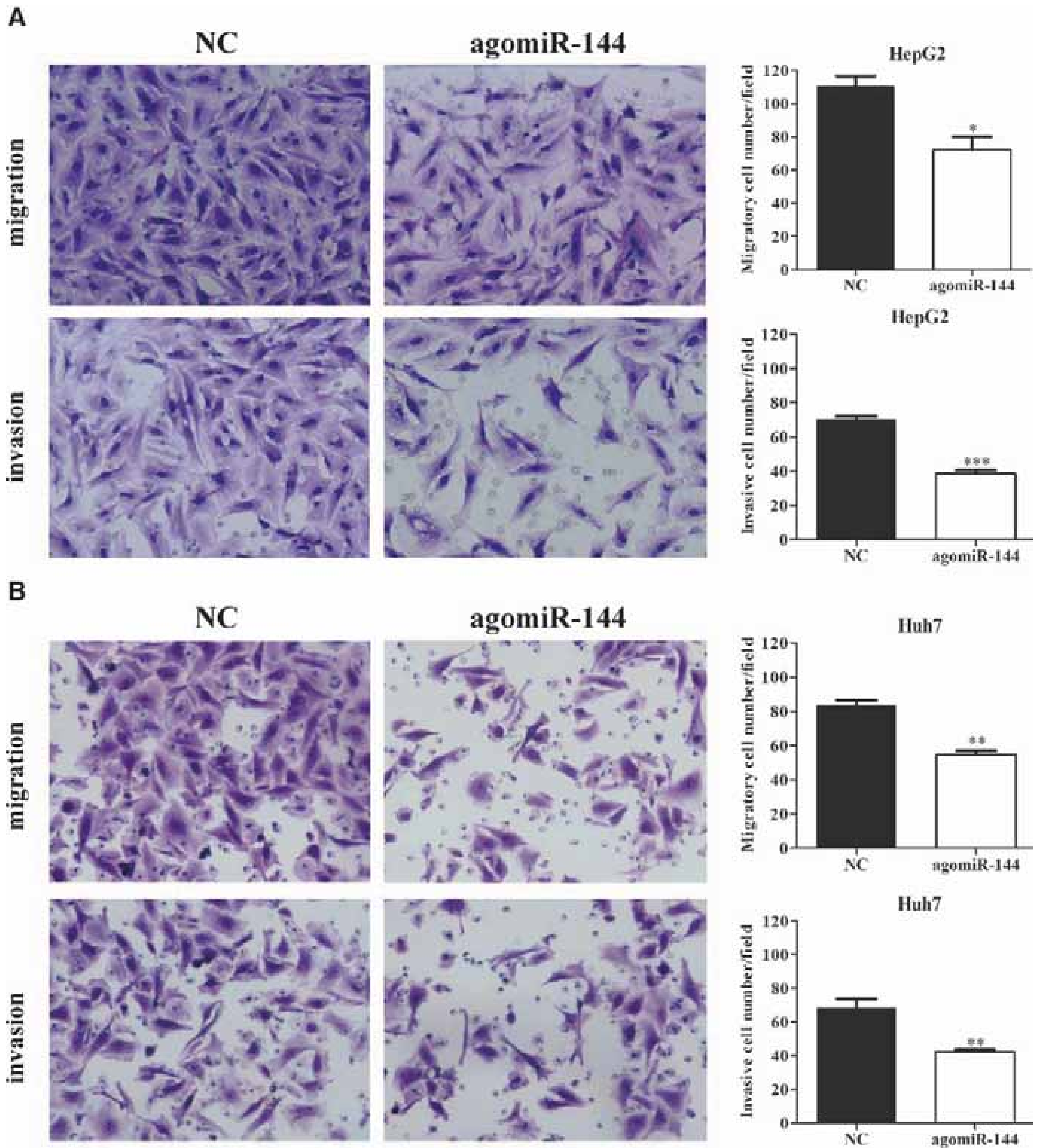

Figure 3. Enhancement of miR-144 inhibited migration and invasion ability of HCC cell lines. Transwell analysis was used to determine effect of miR-144 on migration and invasion in HepG2 (**A**) and Huh7 (**B**) cells. Data are expressed as mean ± SD of triplicate experiments, *p<0.05; **p<0.01; ***p<0.001 *vs* controls.

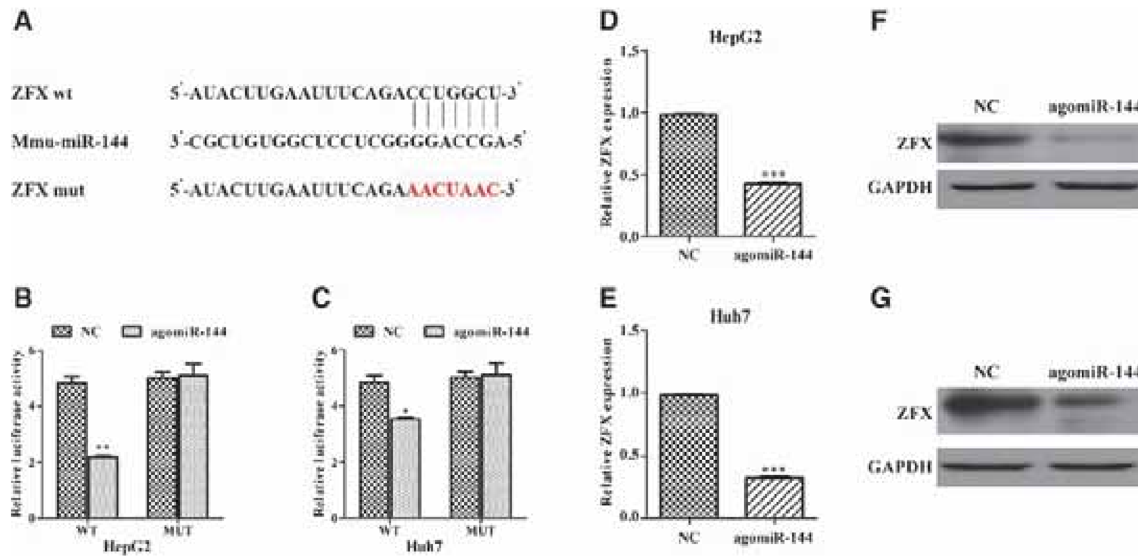

Figure 4. miR-144 negatively regulated the expression of ZFX via directly bind to the 3′ UTR of ZFX. (**A**) The predicted sequence alignment of miR-144 seed sequence with ZFX wild-type 3′ UTR and its mutated sequence in the matched binding sites. (**B**-**C**) The luciferase reporter constructs was created to detect luciferase activity in HepG2 and Huh7 cells transfected with agomiR-144 and wild-type or mutated 3′UTR of ZFX. (**D**-**E**) qRT-PCR analysis of the expression of ZFX mRNA in HepG2 and Huh7 cells transfected with agomiR-144. (**F**-**G**) Western blot analysis the expression of ZFX protein in HepG2 and Huh7 cells transfected with agomiR-144. Data are expressed as mean ± SD of triplicate experiments, *p<0.05; **p<0.01; ***p<0.001 *vs* controls.

regulated of ZFX expression inhibited the proliferation, migration and invasion in HepG2 cells (figure 5B, C and D; p<0.001). The effect of ZFX silencing was consistent with the effect of miR-144 overexpression on proliferation, migration and invasion of HepG2 cells. Thus, these data further suggested that miR-144 inhibited the proliferation, migration and invasion of HCC cells by targeting ZFX.

4. Discussion

Emerging evidences have proved that miRNAs play a crucial role in regulating proliferation and metastasis in cancer occurrence. The potential role of miR-144 has been investigated in human cancer malignancy, such as lung cancer, colon cancer and renal cell carcinoma. Herein we first demonstrated that miR-144 was down-regulated in HCC through analysing the expression data derived from GEO and TCGA datasets. To verify this result, qRT-PCR further confirmed miR-144 was down-regulated in HCC tissues and cell lines. Consistent with our findings, miRNA-144 has been shown to widely down-regulated in about 30 studies of various cancer types, including colorectal cancer (Iwaya *et al.* 2012), nasopharyngeal carcinoma (Zhang *et al.* 2012) and follicular thyroid carcinoma (Rossing *et al.* 2012). These evidences suggested that miR-144 might play an important role in malignancies.

To further investigate the biological function of miR-144 in HCC, the expression of miR-144 was overexpressed in HCC cell lines, HepG2 and Huh7. Overexpression of miR-144 significantly reduced cell growth, colony formation, migration and invasion of HCC cells, which indicated that miR-144 acted as a tumour suppressor in HCC. Then we demonstrated that miR-144 directly down-regulated ZFX by binding its 3′-UTR using TargetScan software and luciferase report assay. Furthermore, the endogenous ZFX mRNA and protein was down-regulated in overexpressed miR-144 HCC cells, suggesting significant inverse correlations between miR144 and ZFX expression in vitro. ZFX is reported as a highly conserved zinc finger protein of Zfy family involved in human tumour proliferation and metastasis, such as lung cancer (Li *et al.* 2013), gallbladder cancer (Bao *et al.* 2016; Hao *et al.* 2015). Recently, overexpression of ZFX has been shown to be associated with poor prognosis for several malignant neoplasms, including colon cancer (Jiang *et al.* 2015) and renal cell carcinoma (Li *et al.* 2014). Similar results were also observed in our study, meta-analysis on online Oncomine datasets indicated that ZFX was remarkably up-regulated in HCC tissues compared with normal tissues. Based on this result, we knocked down its

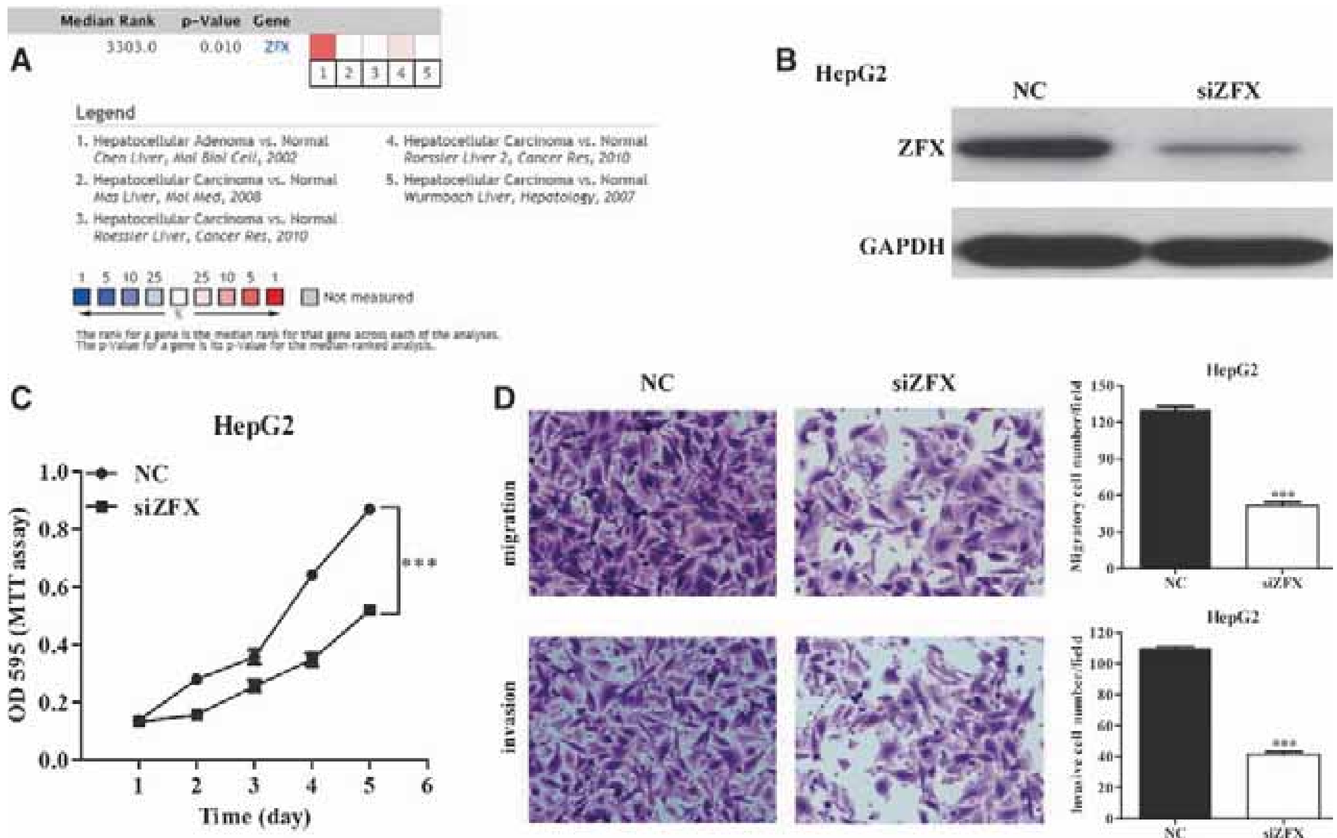

Figure 5. Knockdown of ZFX-suppressed HCC cell proliferation and metastasis. (**A**) Oncomine cancer microarray database screened five microarray datasets about ZFX mRNA expression between HCC and normal tissues, which were included in meta-analysis. (**B**) The expression of ZFX protein levels was detected by Western blot analysis in ZFX-knockdown group in HepG2 cells. (**C**) The proliferation rate was measured after down-regulation of ZFX in HepG2 cells by MTT assay. (**D**) Transwell analysis was used to determine effect of ZFX knockdown on migration and invasion in HepG2. Data are expressed as mean ± SD of triplicate experiments. ***$p<0.001$ *vs* controls.

expression in HepG2 cells and confirmed it was efficiently silenced using Western blot assay. Moreover, we found knockdown of ZFX also inhibited HCC cell proliferation, migration and invasion, suggesting that ZFX is involved in miR-114-mediated proliferation and invasion of HCC cells.

In conclusion, we presented some evidences that miR-144 was significantly down-regulated in HCC. The functional assays demonstrated that miR-144 acts as tumour suppressor by suppressing cell proliferation, migration and invasion by targeting ZFX, which suggests that it may serve as a novel molecular target for HCC treatment.

References

Bao RF, Shu YJ, Hu YP, Wang XA, Zhang F, Liang HB, Ye YY, Li HF *et al.* 2016 miR-101 targeting ZFX suppresses tumor proliferation and metastasis by regulating the MAPK/Erk and smad pathways in gallbladder carcinoma. *Oncotarget* **19** 22339–22354

Bartel DP 2009 MicroRNAs: target recognition and regulatory functions. *Cell* **136** 215–233

Dhanasekaran R *et al*. 2012 Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis, and therapeutics. *Hepat. Med. Evid. Res.* **4** 19–37

Hao W *et al*. 2015 Zinc finger X-chromosomal protein (ZFX) is a significant prognostic indicator and promotes cellular malignant potential in gallbladder cancer. *Cancer Biol. Ther.* **16** 1462–1470

Higashi T *et al*. 2015 miR-9-3p plays a tumour-suppressor role by targeting TAZ (WWTR1) in hepatocellular carcinoma cells. *Br. J. Cancer* **113** 252–258

Iwaya T *et al*. 2012 Downregulation of miR-144 is associated with colorectal cancer progression via activation of mTOR signaling pathway. *Carcinogenesis* **33** 2391–2397

Jiang J, *et al.* 2015 Zinc finger protein X-linked is overexpressed in colorectal cancer and is associated with poor prognosis. *Oncol. Lett.* **10** 810

Lai KP *et al*. 2014 Overexpression of ZFX confers self-renewal and chemoresistance properties in hepatocellular carcinoma. *Int. J. Cancer* **135** 1790–1799

Li C *et al*. 2014 ZFX is a strong predictor of poor prognosis in renal cell carcinoma. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* **21** 3380–3385

Li K *et al*. 2013 ZFX knockdown inhibits growth and migration of non-small cell lung carcinoma cell line H1299. *Int. J. Clin. Exp. Pathol.* **6** 2460–2467

Liu M *et al*. 2016 Downregulating microRNA-144 mediates a metabolic shift in lung cancer cells by regulating GLUT1 expression. *Oncol. Lett.* **11** 3772–3776

Paranjape T *et al*. 2009 MicroRNAs: tools for cancer diagnostics. *Gut.* **58** 1546–1554

Rossing M *et al*. 2012 Down-regulation of microRNAs controlling tumourigenic factors in follicular thyroid carcinoma. *J. Mol. Endocrinol.* **48** 11–23

Shukla GC *et al*. 2011 MicroRNAs: processing, maturation, target recognition and regulatory functions. *Mol. Cell. Pharmacol.* **3** 83–92

Song G *et al*. 2010 MicroRNAs control hepatocyte proliferation during liver regeneration. *Hepatology* **51** 1735–1743

Song X *et al*. 2015 Loss of miR-532-5p in vitro promotes cell proliferation and metastasis by influencing CXCL2 expression in HCC. *Am. J. Transl. Res.* **7** 2254–2261

Tang B *et al*. 2013 Stable isotope dimethyl labeling combined with LTQ mass spectrometric detection, a quantitative proteomics technology used in liver cancer research. *Biomed. Rep.* **1** 549–554

Voorhoeve PM 2010 MicroRNAs: oncogenes, tumor suppressors or master regulators of cancer heterogeneity? *Biochim. Biophys. Acta.* **1805** 72–86

Xiang C, Cui SP and Ke Y 2016 MiR-144 inhibits cell proliferation of renal cell carcinoma by targeting MTOR. *J. Huazhong Univ. Sci. Technol. Med. Sci.* **36** 186–192

Xiao R *et al*. 2015 miRNA-144 suppresses proliferation and migration of colorectal cancer cells through GSPT1. *Biomed. Pharmacother.* **74** 138–144

Xie BH *et al*. 2016 Mir-765 promotes cell proliferation by downregulating INPP4B expression in human hepatocellular carcinoma. *Cancer Biomark. Sect. A Dis. Markers* **16** 405–413

Yang X *et al*. 2014 MicroRNA-26a suppresses angiogenesis in human hepatocellular carcinoma by targeting hepatocyte growth factor-cMet pathway. *Hepatology* **59** 1874–1885

Zhang B *et al*. 2007 *microRNAs as oncogenes and tumor suppressors* (Inc: John Wiley & Sons)

Zhang LY *et al*. 2012 MicroRNA-144 promotes cell proliferation, migration and invasion in nasopharyngeal carcinoma through repression of PTEN. *Carcinogenesis* **34** 454–463

Zhou J *et al*. 2011 Analysis of microRNA expression profiling identifies microRNA-503 regulates metastatic function in hepatocellular cancer cell. *J. Surg. Oncol.* **104** 278–283

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