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MicroRNA-124 suppresses growth of human hepatocellular carcinoma by targeting STAT3

Yanxin Lu [a,b,1], Xupeng Yue [b,1], Yuanyuan Cui [a], Jufeng Zhang [b,*], KeWei Wang [a,b,c,*]

[a] Department of Neurobiology, Neuroscience Research Institute, Peking University Health Science Center, Beijing 100191, China
[b] Biomedical Research Institute, Shenzhen-PKU-HKUST Medical Center, Guangdong Province, Shenzhen 518036, China
[c] Department of Molecular and Cellular Pharmacology, State Key Laboratory of Natural and Biomimetic Drugs, Peking University School of Pharmaceutical Sciences, Beijing 100191, China

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ABSTRACT

The aberrant expression of microRNAs is associated with development and progression of cancers. Down-regulation of miR-124 has been demonstrated in the hepatocellular carcinoma (HCC), but the underlying mechanism by which miR-124 suppresses tumorigenesis in HCC remains elusive. In this study, we found that miR-124 suppresses the tumor growth of HCC through targeting the signal transducers and activators of transcription 3 (STAT3). Overexpression of miR-124 suppressed proliferation and induced apoptosis in HepG-2 cells. Luciferase assay confirmed that miR-124 binding to the 3′-UTR region of STAT3 inhibited the expression of STAT3 and phosphorylated STAT3 proteins in HepG-2 cells. Knockdown of STAT3 by siRNA in HepG-2 cells mimicked the effect induced by miR-124. Overexpression of STAT3 in miR-124-transfected HepG-2 cells effectively rescued the inhibition of cell proliferation caused by miR-124. Furthermore, miR-124 suppressed xenograft tumor growth in nude mice implanted with HepG-2 cells by reducing STAT3 expression. Taken together, our findings show that miR-124 functions as tumor suppressor in HCC by targeting STAT3, and miR-124 may therefore serve as a biomarker for diagnosis and therapeutics in HCC.

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1. Introduction

MicroRNAs (miRNAs) are endogenous short non-coding RNAs that suppress gene expression by either degrading mRNA or repressing mRNA translation through imperfect complementary binding to their target mRNAs in the 3′-UTR [1]. MiRNAs contribute to most basic biological processes, such as cell proliferation, differentiation and apoptosis [2]. Studies have shown that miRNAs are differentially expressed in tumors and can function as oncogenes (oncomirs) or tumor suppressors during tumor development and progression, indicating their potential as biomarkers for diagnosis and therapy [3–5].

Hepatocellular carcinoma (HCC) is the fifth malignant carcinoma worldwide and the third common cause of death from cancer [6,7]. The pathogenesis of HCC is complicated and poorly understood, and current therapeutic strategies for HCC are less effective. Despite great advances have been made in uncovering cellular signaling events and pathways, there is lack of ideal biomarkers for HCC diagnosis and therapy. Therefore, identification of biomarkers for early diagnosis and therapy can help control progression and mortality of HCC. In recent years, a number of reports have described multiple microRNAs that are dysregulated in HCC development and progression [3], such as let-7 [8], miR-145 [9], miR-140-5p [10], miR-372 [11], miR-490-3p [12], and miR-657 [13]. Among the microRNAs that are implicated in HCC, miR-124 has recently been found to be down-regulated in metastatic HCC tissues using microRNA array [14]. MiR-124 also suppresses cell proliferation in HCC by targeting phosphoinositide 3-kinase catalytic subunit alpha [15], indicating a tumor suppressive function of miR-124 in tumorigenesis. Because one miRNA may be involved in regulation of many different mRNAs, we sought to identify other potential targets of miR-124 in heptocarcinogenesis.

In this study, we found that the signal transducers and activators of transcription 3 (STAT3) is a novel target of miR-124 in HCC HepG-2 cells. Knockdown of STAT3 by siRNA in HepG-2 cells mimicked the effect induced by miR-124. Over-expression of miR-124 suppressed proliferation and induced apoptosis in HepG-2 cells and it can be rescued by re-introduction of STAT3. Furthermore, miR-124 suppressed xenograft tumor growth in vivo by reducing STAT3 expression. Taken together, our findings show that miR-124 suppresses tumor growth in HCC, and may serve as a potential biomarker and therapeutic target for treatment of HCC.

* Corresponding authors. Address: Biomedical Research Institute, Shenzhen-PKU-HKUST Medical Center, Guangdong Province, Shenzhen 518036, China. Fax: +86 10 82805065 (K. Wang).
E-mail addresses: jfzhang111@163.com (J. Zhang), wangkw@bjmu.edu.cn (K. Wang).
[1] These authors contributed equally to this work.

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2. Materials and methods

2.1. Cell culture and transfection

The human HCC cell lines, HepG-2 and SMMC-7721, and normal human hepatocyte, HL-7702, were obtained from the Cell Bank of Chinese Academy of Science, Shanghai, China. Cells were transfected with miRNA (100 nM) or siRNA (50 nM) by siPORT NeoFX (Ambion). MiR-124 precursor (pre-miR-124), miRNA precursor control (pre-miR-Ctrl), CY3 dye-labled miR-Scramble, siRNA against STAT3 (siR-STAT3), and scrambled siRNA-oligonucleotide (siRNA-NC) were purchased from Ambion (Austin, TX, USA).

2.2. RNA extraction and quantitative real-time PCR

Total RNA was extracted from cells using TRIzol reagent. The expression of mature miR-124 was quantified using TaqMan MicroRNA assays kit (Ambion) with specific primers for hsa-miR-124. The TaqMan real-time RT-PCR was performed according to manufacturer's instruction. For STAT3 mRNA analysis, the total RNA was reversely transcribed into cDNA using M-MLV kit (TaKaRa, Dalian, China). Real-time PCR was performed using SYBR Green chemistry (Applied Biosystems, Foster City, CA). The primers used for real-time PCR are: STAT3 Forward primer: 5′-ATCACGCCTTCTACAGACTGC-3′, Reverse primer: 5′-CATCCTGGAGATTCTCTACCACT-3′. GAPDH Forward primer: 5′-CCACTCCTCCACCTTTGAC-3′, Reverse primer: 5′-ACCCTGTTGCTGTAGCCA-3′. The expression level of GAPDH was used as control. Relative expression was calculated using the comparative C_T method. Specificity of the PCR products was confirmed by melting curve analysis.

2.3. Plasmid construction and luciferase assay

The 3′-UTR segment of STAT3 containing wild type or mutant miR-124 target sites was amplified by PCR or overlap-PCR, and was inserted into the SpeI/HindIII sites downstream of the luciferase gene in pMIR-REPORT Luciferase miRNA Expression Reporter Vector (Ambion). The primers for wild type 3′-UTR of STAT3 were: Forward primer, 5′-CGGACTAGTAAATGAGTGAATGTGGGTG-3′, and Reverse primer, 5′-CCAAGCTT TGTTGCTGGAGAAGTAAGAG-3′ (the nucleotides underlined are enzyme sites). Primers for miR-124 target sites mutant 3′-UTR were: 3′-UTR-STAT3-M-Reverse, 5′-CCAG CCCTGAGGACTACACCACAGAAACAACCTAGCC-3′, 3′-UTR-STAT3-M-Forward, 5′-GTTTCTGTGGTGTAGTCCTCAGGGCTGGGATACTTCT G-3′ (the nucleotides underlined are mutant sites). All constructs were confirmed by restriction digestions and DNA sequencing.

HepG-2 and SMMC-7721 cells were co-transfected with luciferase constructs containing 3′-UTR of STAT3 (with wild type or mutant miR-124 binding sites) and pre-miR-124 or pre-miR-Ctrl. The luciferase activities were measured at 48 h post-transfection using a luciferase assay kit (Promega, Madison, WI, USA) according to manufacturer's protocol. pMIR-REPORT-β-gal was used for normalization.

2.4. Western blotting

Equal amounts of total cellular proteins were separated by 10% SDS–PAGE and transferred to PVDF membranes. The membranes were blocked in 5% non-fat milk and incubated with antibodies against STAT3, phosphorylated-STAT3 (p-STAT3) and caspase 3 (Cell Signaling Technology, BSN, USA). Specific proteins were detected with the enhanced chemi-luminescence system.

2.5. Immunofluorescence staining for STAT3

HepG-2 cells were cultured on coverslips and transfected with pre-miR-124 or pre-miR-Ctrl. Rabbit antibodies against STAT3 were used as primary antibody, and FITC-conjugated goat anti-rabbit IgG (Chemicon International, Temecula, CA, USA) used as secondary antibody. Cell nuclei were stained with DAPI, and images were photographed with a Zeiss Axio Imager Z1 (Zeiss, Germany).

2.6. Cell proliferation assay

For analysis of cell viability and proliferation, transfected cells were assessed by WST-1 (Cell Proliferation and Cytotoxicity Assay Kit; Beyotime) at 0 h, 24 h, 48 h, and 72 h post-transfection. All experiments were performed in triplicates. For colony formation assay, about 200 cells from each treatment group were seeded into 12-well plate with DMEM containing 10% FBS for 14 days. The colonies were fixed and stained with 0.5% crystal violet for 2 min, and then washed 3 times.

2.7. Apoptosis analysis

HepG-2 cells were photographed under microscope, and stained with Hoechst 33258 as described at 48 h post-transfection [16]. Cell nuclei stained with Hoechst were examined by fluorescence microscopy. Apoptotic cells were identified by the presence of highly condensed or fragmented nuclei. Equal amounts of total cellular proteins were analyzed by western blotting using caspase 3 antibodies.

2.8. In vivo tumor growth assay

Specific pathogen-free (SPF) female athymic BALB/c nude mice, 4–6 weeks old (20–30 g), were obtained from the Guangdong medical laboratory animal center. All animal feeding and experiment procedures were performed according to National Institutes of Health (NIH) Guidelines. HepG-2 cells transfected with pre-miR-124 or pre-miR-Ctrl about 5×10^6 cells in 100 μl PBS suspension were injected subcutaneously into the flanks of nude mice ($n = 10$ mice/group). 40 days later, the nude mice were executed and tumor masses were dissected, weighed and then fixed in 10% formalin for immunohistological examination.

2.9. Immunohistochemical analysis

Tumor specimens were taken from the sacrificed nude mice, fixed in 10% formalin, paraffin embedded, and cut into 4 μm thick sections. Sections were deparaffinized in xylene before being rehydrated in graded alcohols and microwave antigen retrieved within 10 mM citrate buffer solution (pH 6.0 for 15 min). After inactivation by exposure to 3% H_2O_2 for 10 min, sections were blocked with 5% goat serum for 30 min at room temperature and incubated with STAT3 antibody in 5% goat serum at 4 °C overnight. The sections were washed and incubated with secondary antibody labeled with peroxidase for 30 min at room temperature. Sections were then incubated with a solution of 3% diamino-benzidine (DAB) as the chromogen for 2–8 min and examined under microscope.

2.10. Statistical analysis

All data are presented as the mean ± S.E.M. The differences between groups were assessed by the t test. The difference was considered to be statistically significant when $P < 0.05$.

3. Results

3.1. MiR-124 is down-regulated in human HCC cells

To confirm the expression level of miR-124, we performed real-time RT-PCR in two HCC cell lines, HepG-2 and SMMC-7721, and one normal human hepatocyte HL-7702 cell line using TaqMan assay. The results showed that the expression of miR-124 in both HepG-2 and SMMC-7721 was significantly lower than that of normal human hepatocyte HL-7702 cells ($P < 0.001$) (Fig. 1A), confirming that miR-124 was down-regulated in HCC.

3.2. miR-124 inhibits cell proliferation and induces apoptosis

To explore the role of miR-124 in HCC, we transfected the pre-miR-124 into both HepG-2 and SMMC-7721 cells and examined the effect of miR-124 overexpression on the HCC cells. 24 and 48 h after transfection real-time PCR was used to determine the expression level of miR-124. As shown in Fig. 1B, transfection of pre-miR-124 resulted in a significant increase of miR-124 expression about 250- to 1200-fold, as compared with control transfected with pre-miR-Ctrl ($P < 0.001$). Using the WST-1 assay, we measured the rate of proliferation of HepG-2 cells transfected with pre-miR-124. Compared with transfection by pre-miR-Ctrl, cells transfected with pre-miR-124 showed significantly less viable at time points 24, 48 and 72 h post-transfection (Fig. 1C). Consistent with the result from WST-1 assay, the colony formation assay showed much less and smaller colonies formed in pre-miR-124 transfected HepG-2 cells, as compared with the control (Fig. 1D). These results indicated that miR-124 suppressed the growth of HepG-2 cells.

To examine the apoptotic effect of miR-124 on HCC cells, we transfected HepG-2 cells with pre-miR-124. After 48 h overexpression of miR-124, cells started to round up and shrink in cell size with the membrane blebbing (Fig. 1E), indicating that the cells were under programmed cell death. Hoechst staining results showed the nuclei condensed or fragmented in cells transfected with pre-miR-124 (Fig. 1F). In contrast, the cell nuclei in pre-miR-Ctrl transfected control group were observed as rounded contours (Fig. 1F). Caspase 3 is activated and cleaved after apoptotic signaling events occur [17]. To further verify the apoptotic effect

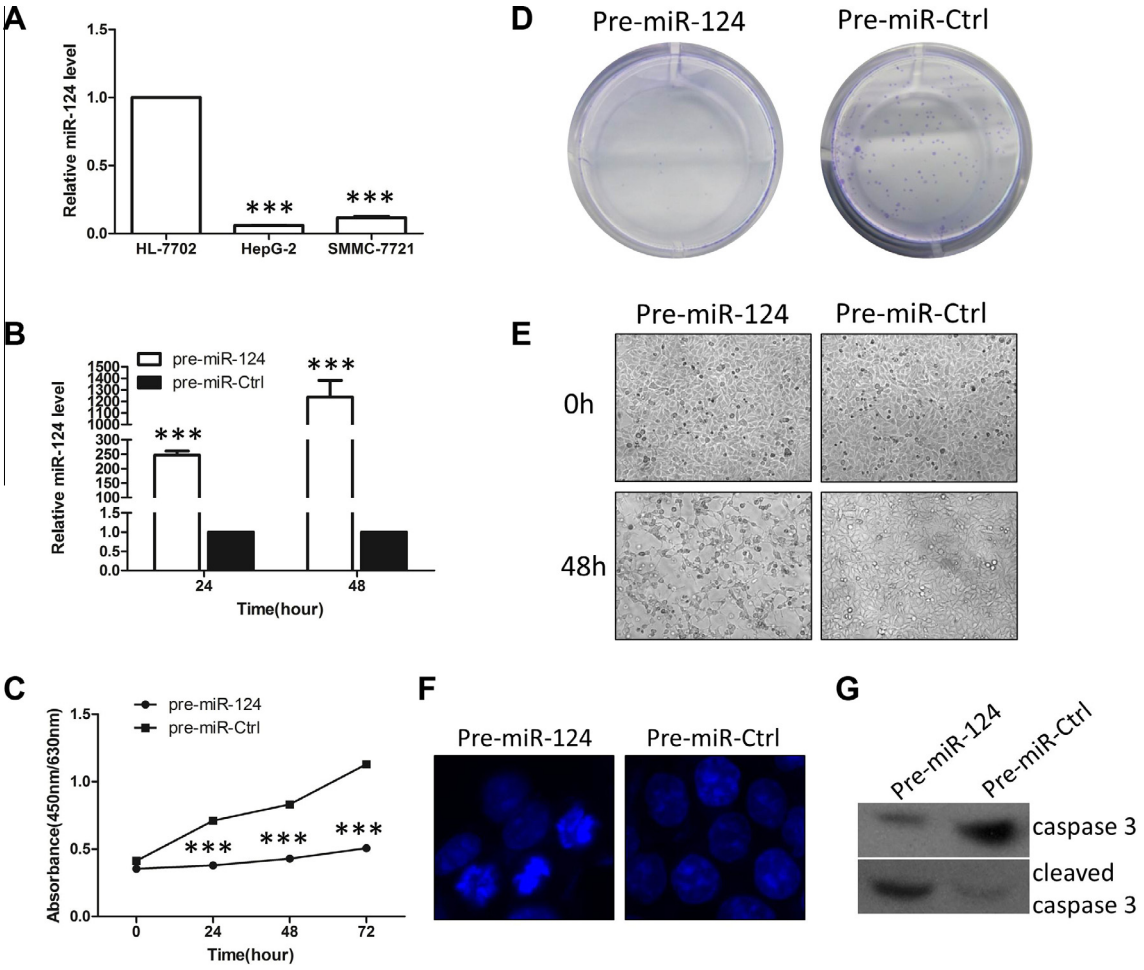

Fig. 1. MiR-124 expression in hepatocellular carcinoma cells and its effects on cell viability, proliferation and apoptosis. (A) MiR-124 expression was detected by TaqMan real-time RT-PCR, showing significant decrease of miR-124 in HepG-2 and SMMC-7721 cells, as compared with HL-7702 cells ($n = 4$; $^{***}P < 0.001$). (B) Relative expression level of miR-124 in HepG-2 cells was significantly increased in time-dependent manner after transfection with pre-miR-124 by TaqMan real-time RT-PCR ($n = 4$; $^{***}P < 0.001$). (C) Effect of pre-miR-124 on cell viability of HepG-2 cells was measured by WST-1 assay. Over expression of miR-124 resulted in significant inhibition of HepG-2 cells viability in time-dependent manner as compared with control. ($n = 3$; $^{***}P < 0.001$). (D) Effect of pre-miR-124 on the proliferation rate of HepG-2 cells was measured by colony formation assay. Over expression of miR-124 inhibited clonogenicity of HepG-2 cells. (E) Pre-miR-124 induced morphological changes typical of apoptosis in HepG-2 cells. Cells displaying round up, shrink and membrane blebbing were significant in pre-miR-124 transfected cells (left panels), as compared with control (right panels). (F) Hoechst 33258 staining of cells treated with pre-miR-124, showing nuclei condensed or fragmented, typical for apoptotic nucleus morphology (left panel), and no typical apoptotic cells were observed in control groups (right panel). (G) Western blot analysis of total and cleaved caspase 3. Cleaved caspase3 increased and caspase3 decreased in cells treated with pre-miR-124. Data are expressed as mean ± S.E.M.

induced by miR-124, we detected total caspase 3 and cleaved caspase 3 proteins in pre-miR-124 or pre-miR-Ctrl transfected HepG-2 cells. Compared to pre-miR-Ctrl, pre-miR-124 transfected cells showed a decrease of total caspase 3 and an increase of cleaved caspase 3 proteins (Fig. 1G). These results showed that miR-124 suppressed cell proliferation and promoted apoptosis of HepG-2 cells.

3.3. MiR-124 directly targets STAT3 by posttranscriptional repression

To identify the downstream target of miR-124, we searched for theoretical targets of miR-124 using target prediction software (TargetScan) and identified STAT3 as a potential target. To test this hypothesis, we used the luciferase assay in which the 3′-UTR region of STAT3 harboring either miR-124 or mutant miR-124 response element was inserted into the downstream of the luciferase reporter gene in pMIR-REPORT vector (Fig. 2A). This assay allowed us to investigate whether miR-124 can bind to the 3′-UTR of STAT3 and regulate the luciferase expression through STAT3 binding sites. After co-transfected with luciferase vector carrying wild-type STAT3 3′-UTR and pre-miR-124 in HepG-2 cells, the luciferase activity was significantly reduced to 50%, as compared with pre-miR-Ctrl (Fig. 2B). In contrast, co-transfection of mutant STAT3 3′-UTR, pre-miR-124 had no effect on the luciferase activity, as compared with pre-miR-Ctrl (Fig. 2B). These results showed that miR-124 binding to the 3′-UTR resulted in inhibition of STAT3 expression in HepG2 cells.

MiRNAs are known to down-regulate their target genes by degradation of target mRNA or inhibition of mRNA translation. To investigate the effect of miR-124 overexpression on endogenous STAT3 expression, we used SMMC-7721 and HepG-2 cells that robustly expressed STAT3, and tested the effect of pre-miR-124 on STAT3 mRNA stability. Real-time PCR assay showed that the STAT3 mRNA level between cells transfected with pre-miR-124 and pre-miR-Ctrl was not significantly different (Fig. 2C). In contrast, Western blotting showed that the expression of both STAT3 and phosphorylated STAT3 (p-STAT3) was significantly reduced in both SMMC-7721 and HepG2 cells transfected with pre-miR-124 as compared to the cells transfected with pre-miR-Ctrl (Fig. 2D and E). Immunofluorescent staining analysis further confirmed that the expression level of STAT3 in HepG-2 cells was reduced in the pre-miR-124 transfected cells, as compared with the control (Fig. 2F). These results demonstrated that miR-124 down-regulated STAT3 expression by inhibiting its translation, indicating that STAT3 is a direct target for miR-124 in HCC cells.

3.4. MiR-124 suppresses HCC cell growth by targeting STAT3

To confirm the effect of STAT3 on cell proliferation and apoptosis, we knocked down the STAT3 in HepG-2 cells by siRNA silencing. Transfection of STAT3 siRNA (siR-STAT3) in HepG-2 resulted in a significant reduction in STAT3 expression (Fig. 3A and B). Knockdown of endogenous STAT3 by silencing resulted in a time-dependent reduction of cell viability and proliferation measured by WST-1 and colony formation assay (Fig. 3C and D), consistent with the suppressed cell proliferation mediated by miR-124 over-expression. Hoechst staining and detection of caspase 3 proteins by Western blot further confirmed that silencing STAT3 exhibited an increased apoptosis in HepG-2 cells (Fig. 3E and F). These results showed that down-regulation of STAT3 by silencing suppressed the proliferation and induced the apoptosis in HepG-2 cells, consistent with the effect of miR-124 over-expression on inhibition of cell proliferation and promotion of apoptosis.

To further verify *STAT3* involved in *miR-124*–mediated antitumor properties in HepG-2 cells, we then performed rescue experiments. The transient transfection of pcDNA3.1-STAT3 was used to restore STAT3 expression in HepG-2 cells and pcDNA3.1 vector was used as control. Using the colony formation (Fig. 3G and H) and WST-1 assay (Fig. 3I), inhibition of cell viability and proliferation by *miR-124* was significantly attenuated by re-introduction of *STAT3,* as compared to control groups. These and previously described experiments show that *STAT3* serves as a functional target of *miR-124* and mediates the inhibition of tumor growth in HCC.

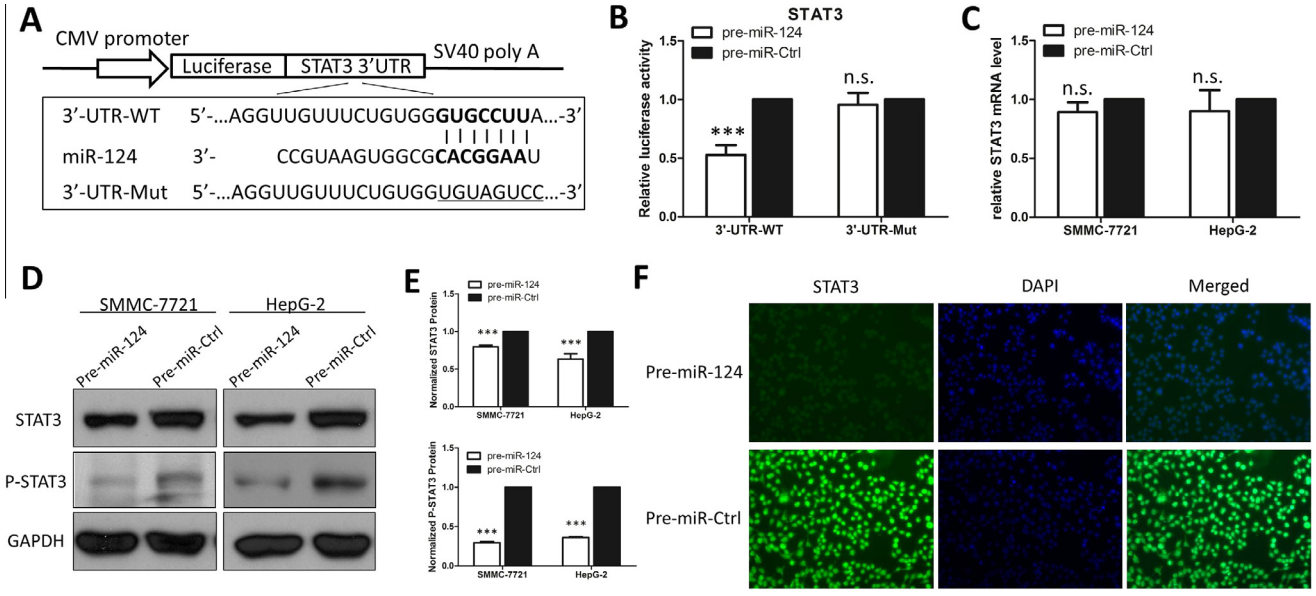

Fig. 2. Suppression of STAT3 by miR-124 in HCC HepG-2 cells. (A) Schematic construction of wild-type or mutant miR-124 binding sequences in STAT3 3′-UTR vector. The mutant binding sequences are underlined. (B) Suppressed luciferase activity of wild-type 3′-UTR of STAT3 by pre-miR-124 ($n = 3$; $^{***}P < 0.001$). (C) Comparison of STAT3 mRNA expression level in HepG-2 and SMMC-7721 cells transfected with pre-miR-124 or pre-miR-Ctrl by real-time PCR analysis ($n = 3$; n.s. $P > 0.05$). (D) & (E) Western blot analysis of STAT3 and phosphorylated STAT3 protein expression levels and STAT3 proteins were depressed by pre-miR-124 in HepG-2 and SMMC-7721 cells. GAPDH was used as an internal control ($n = 4$; $^{***}P < 0.001$). (F) The expression level of STAT3 in HepG-2 cells was analyzed by immunofluorescence. STAT3 proteins were depressed in the pre-miR-124 transfected cells as compared with control. Nuclei were stained with DAPI. Data are expressed as the mean ± S.E.M.

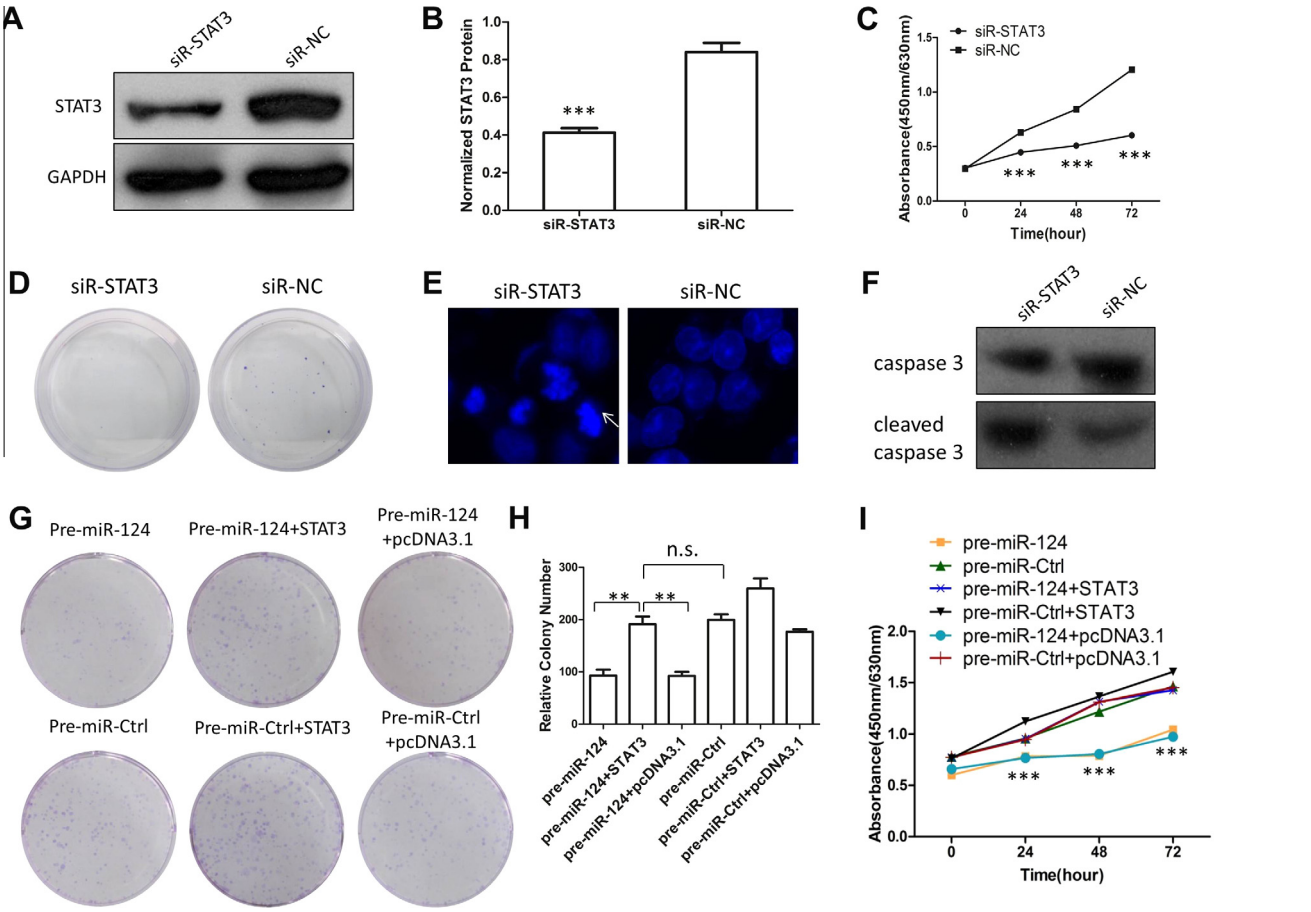

Fig. 3. Effects of knocking down STAT3 on HepG-2 cells viability, proliferation and apoptosis and STAT3 rescue experiments. (A) & (B) Knockdown efficiency of STAT3 by single siRNA (siR-STAT3). SiR-STAT3 was effective in silencing STAT3 expression, as compared with siR-NC. (C) The effect of siR-STAT3 on HepG-2 cell viability was measured by WST-1 assay. Knockdown of STAT3 decreased cell viability ($n = 3$; $^{***}P < 0.001$). (D) The effect of siR-STAT3 on the proliferation rate of HepG-2 cells was measured by colony formation assay. Knockdown of STAT3 depressed proliferation of cells. (E) Knockdown of STAT3 promoted HepG-2 cell apoptosis. HepG-2 cells were stained by Hoechst 33258, 48 h post-transfection with siR-STAT3 or siR-NC. (F) Western bolt analysis of total and cleaved caspase 3 proteins. Knockdown of STAT3 promoted the activation of caspase 3. (G) Colony formation assay shows overexpression of STAT3 can attenuate the reduction in cell proliferation induced by miR-124. (H) The statistics for colony numbers in each group ($n = 3$; $^{**}P < 0.01$; n.s. $P > 0.05$). (I) WST-1 assay shows time-dependent inhibition of cell viability by miR-124, and the rescue by STAT3 over-expression ($n = 3$; $^{***}P < 0.001$). Data are expressed as the mean ± S.E.M.

3.5. MiR-124 suppresses the tumorigenesis in nude mice

To further investigate the effect of miR-124 on tumor growth, HepG-2 cells transfected with pre-miR-124 or pre-miR-Ctrl were injected into nude mice for formation of xenograft tumor. Injection of HepG-2 cells transfected with pre-miR-ctrl into nude mice resulted in a 100% development of measurable tumors after 10 days of injection. The average size of tumors originated from cells treated with pre-miR-124 was significantly smaller (0.87 ± 0.4 cm^3; $n = 10$) than those treated with pre-miR-Ctrl (2.8 ± 0.8 cm^3; $n = 10$), at day 40 after injection (Fig. 4A). The average weight of tumor in the pre-miR-124 group (0.76 ± 0.3 g; $n = 10$) was significantly less than pre-miR-Ctrl group (2.2 ± 0.6 g; $n = 10$) (Fig. 4B). Immunohistochemical analysis further showed that STAT3 expression level was also reduced in pre-miR-124 transfected cell-derived tumors, as compared with the control (Fig. 4C), consistent with the result that suppression of STAT3 by miR-124 inhibited proliferation of HepG-2 cells. These results demonstrated that miR-124 suppresses the tumor growth of HCC by targeting STAT3.

4. Discussion

The goal of this study was to investigate the role of miR-124 in heptocarcinogenesis and the underlying mechanism by which miR-124 exerts its function. We started by searching for theoretical targets of miR-124 using target prediction software (TargetScan), which led us to focus on testing STAT3 as a potential target. In this study, we show that miR-124 is down-regulated in HCC cells. The over-expression of miR-124 promotes apoptosis of HCC cells, and suppresses cell proliferation and tumor growth in nude mice by targeting STAT3. Therefore, miR-124 may have a potential in diagnosis and therapeutic value for human hepatocellular carcinoma.

MiR-124 was initially conformed to be a brain-specific miRNA [18]. It can regulate cocaine-induced neuronal plasticity by inhibiting the expression of BDNF [19]. It was demonstrated that miR-124 is down-regulated mainly in medulloblastoma, suggesting that miR-124 serves as a growth suppressor [20]. Compared to the non-neoplastic brain tissue, miR-124 is significantly down-regulated in anaplastic astrocytomas and glioblastoma multiforme [21]. Recently, miR-124 has been found to be a tumor suppressor, and down-regulation of miR-124 is related to aggressiveness in breast cancer cells [22]. In this study, we show that expression of miR-124 is down-regulated in HCC cells, and over-expression of miR-124 in HCC cells results in increased cell apoptosis and suppressed cell proliferation, and tumor growth in vivo.

Theoretically, there are hundreds of potential targets for a single microRNA. It is reported that miR-124 inhibited epithelial-mesenchymal cell transition by suppressing the mRNA and protein expression of ROCK2 and EZH2 [23]. Lang etc. found that miR-124 inhibited cell proliferation by targeting PIK3CA [15]. In our study,

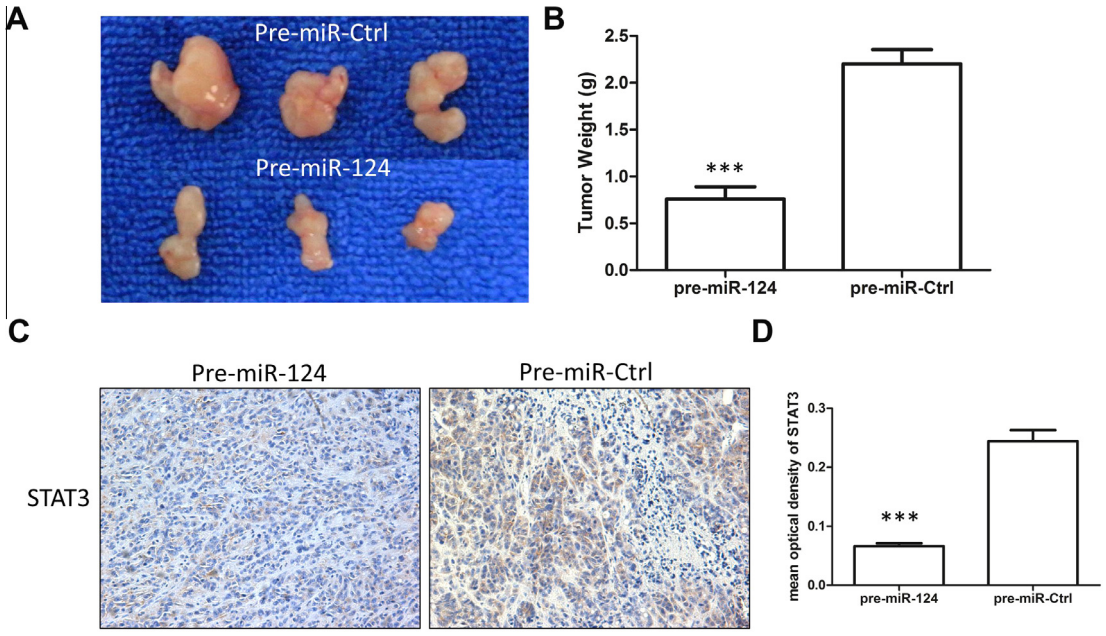

Fig. 4. MiR-124 suppresses STAT3 expression and xenograft tumor growth in nude mice. (A) & (B) Effects of pre-miR-124 or pre-miR-Ctrl on the tumor growth, and suppression of xenograft tumor growth by miR-124 expression. (A) Representative images of the tumors on day 40 post-injection. (B) Measurement of tumor weight on day 40 post-injection ($n = 10$; $^{***}P < 0.001$). (C) The expression level of STAT3 in xenograft tumor implanted with HepG-2 cells transfected with pre-miR-124 or pre-miR-Ctrl, and analyzed by immunohistochemistry. STAT3 expression was significantly depressed by pre-miR-124 as compared with control. (D) Summary of mean optical density values of STAT3 expression ($n = 10$; $^{***}P < 0.001$). Data are expressed as the mean ± S.E.M.

we identified STAT3 as one of direct targets of miR-124 in HCC. Signal transducers and activators of transcription (STATs), originally discovered as cytokine signaling proteins [24], contain seven members such as STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. STATs are known to activate many genes that are involved in malignant progression and have been applied to the cancer clinical therapy as ideal molecular targets [25,26]. STAT3 is activated in multiple human cancers and shown to function as an oncogene [27,28]. STAT3 is found to be activated in the majority of HCCs with poor prognosis but not in normal liver or in surrounding non-tumor tissue [29]. In our study, STAT3 functions as a direct and novel target of miR-124. Silencing STAT3 largely mimicked the function of miR-124 over-expression in HCC cells. However, the mRNA level of STAT3 in pre-miR-124 transfected cells is similar to that of control. This led us to conclude that STAT3 is a functional target of miR-124 by posttranscriptional repression.

In summary, we have shown that miR-124 is expressed in much lower level in HCC cells, as compared with normal human hepatocyte cells. MiR-124 inhibits cell proliferation and induces apoptosis of HCC cells and suppresses growth of HCC in vivo by targeting STAT3. Therefore, miR-124 may serve as a potential diagnosis and therapeutic target for HCC.

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