MicroRNA-198 Inhibits Proliferation and Induces Apoptosis of Lung Cancer Cells Via Targeting FGFR1

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ABSTRACT

Lung cancer is the most common cause of death from cancer worldwide and recent studies have revealed that microRNAs play critical roles to regulate lung carcinogenesis. Here we present evidence to show the role of miR-198 in lung cancer development. Our results showed that ectopic expression of miR-198 inhibits the viability and induces the apoptosis of human non-small cell lung cancer cells A549 and NCI-H460, while miR-198 inhibition resulted in opposite changes. In nude mice miR-198 inhibits A549 growth of tumor graft. We further demonstrated that miR-198 directly targets fibroblast growth factor receptor 1 (*FGFR1*) in lung cancer cells. Restoring FGFR1 expression blocked the inhibitory function of miR-198, while FGFR1 inhibition achieved the similar phenotypes of miR-198 overexpression. Hence, our data delineates the molecular pathway by which miR-198 inhibits lung cancer cellular proliferation and induces apoptosis, and may have important implication for the treatment of lung carcinogenesis. J. Cell. Biochem. 115: 987–995, 2014.   © 2013 Wiley Periodicals, Inc.

KEY WORDS: miR-198; FGFR1; VIABILITY; APOPTOSIS; LUNG CARCINOGENESIS

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death worldwide, accounting for 26–29% of estimated cancer deaths [Ihsan et al., 2011]. With the advanced development of the society, lung cancer has become a global health problem with a poor clinical outcome. More than 1 million deaths annually attributed to lung cancer and <10% of people with this disease live longer than 5 years after diagnosis [Marshall and Christiani, 2013]. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers, and the majority (60–80%) of patients with NSCLC possess unresectable advanced or metastatic disease at diagnosis for which the prognosis of NSCLC patients remains very poor with a 5-year survival of 15%. While recent developments in diagnostic technique for NSCLC may lead to detection of tumors at earlier stages, currently over 70% of lung cancers are loco-regionally advanced or metastatic at the time of diagnosis and despite advances in chemotherapy, radiotherapy, and surgery the death rate from lung cancer has remained largely unchanged [Rothschild and Gautschi, 2013]. Therefore, it is of great significance to investigate the molecular mechanisms involved in lung carcinogenesis, and to identify diagnostic and prognostic markers for early detection and targeted treatment of lung cancer.

MicroRNAs (miRNAs) are a group of small, noncoding, endogenous single-stranded RNAs that regulate the expression of ∼60% human genes. They reduce gene expression by binding to complementary regions of mRNA and either blocking translation or degrading mRNA through the RNA-induced silencing complex [Iorio and Croce, 2009]. Recent studies have demonstrated that miRNAs are involved in tuning of many important signal pathways, including developmental, and oncogenic pathways. Thus, the alteration of miRNA regulation may play a critical role in development and differentiation processes of tissues and organs, and their aberrant expression may be implicated in carcinogenesis and disease progression [Farazi et al., 2011]. miRNA expression profiles have been seen not only as a new class of diagnostic and prognostic tools but also as candidates for pharmacological targeting [Schee et al., 2013]. Most importantly, miRNA expression signatures in both tumor cells and peripheral blood cells from cancer patients can predict outcome [Díaz-García et al., 2013].

Although little information is available concerning potential functions and mRNA targets for miR-198, recent researchers have indicated miR-198 as a miRNA that is strongly down-regulated in NSCLC [Yanaihara et al., 2006]. Emerging evidence also showed that miR-198 is down-regulated in hepatocellular carcinoma compared with normal liver [Varnholt, 2008] and miR-198 plays a tumor suppressor role by negatively regulating the HGF/c-MET pathway [Han et al., 2013]. However, its involvement in clinical NSCLC has not been fully elucidated. The aim of this study was to investigate the clinical significance and molecular mechanism of miR-198 expression in NSCLC.

MATERIALS AND METHODS

CELL CULTURE AND DETECTION

Hela cells and human NSCLC cell lines including NCI-H460 and A549 were purchased from American Type Culture Collection and cultured in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco BRL, Carlsbad, CA), 100 U/ml penicillin and 100 U/ml streptomycin. The cultures were incubated at 37°C in a humidified incubator containing 5% CO_2.

Cell viability was measured using the CellTiter-Glo luminescent cell viability assay kit (Promega, USA) according to the manufacturer's protocol. Briefly, A549, HeLa, and NCI-H460 cells were plated onto 96-well plates. Forty-eight hours after transfection, the CellTiter-Glo reagent was added and luminescent intensity was read by a Microplate Luminometer (Turner Biosystems, USA) using the respective Promega protocols.

Apoptotic cell death and necrotic cell death were analyzed following double staining with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI).

PLASMIDS AND REAGENTS

To generate miR-198 expression vector, a ~600 bp fragment carrying pre-miR-198 was amplified from A549 genomic DNA by the high fidelity polymerase Phusion enzyme (New England Biolabs, Ipswich, MA, USA) using PCR primers miR-198-5, 5′-GAATTC CAG TAC TCG GTA GTT GTC TGG-3′ and miR-198-3, 5′-GGTACC GCG GTG CTT TTT CCA ATC TGC-3′. The amplified fragment was first cloned into pCR8 (Invitrogen) and then subcloned into a modified pCMV vector carrying hygromycin resistance gene. To construct miR-198 lentiviral expression vector, the same miR-198 insert was released from pCMV vector and then subcloned into pCDH-CMV-MCS-EF1-copGFP (System Biosciences, Mountain View, CA).

For miR-198 knockdown, we constructed the sponge plasmid as previously described [Ebert et al., 2007]. Briefly, we constructed PolII sponges by inserting eight tandemly arrayed miR-198 binding sites into the 3′ UTR of Dsred which has been previously cloned into pMSCVhygro. The sequence of miR-198 binding site was "AGTCTAGGTGTCGTGAGTGTATACA."

For FGFR1 overexpression, the human FGFR1 cDNA with 3′UTR was cloned into the pMSCV-hygro vector. The primers corresponded to NCBI Reference Sequence (BC018128.1) forward, 5′-CAG TCG ACA ATC ACT CCT GGG AAG ATC TCA TTG-3′ and reverse, 5′-GG TGG ATC CAG GGC CAC AAG GTG GAC AAT CGG-3′. The FGFR1 cDNA was packed into a pMD®19-T Simple Vector (Takara, Otsu, Japan) to form the pMD®19-T-FGFR1 vector. Following the sequencing, the recombinant segment of the correct clone was incised by BamHI and SalI (Takara). The recombinant segment was packed into pMSCV-hygro, which was incised by the same two restriction endonucleases. The pMSCV-hygro-FGFR1 clones were sequenced and the correct clones were amplified and identified by restriction enzyme digestion. The day before transfection, about 1×10^6 A549 cells were seeded in the media onto a 60 mm dish and incubated for 24 h. The next day, cells were transfected with Sofast gene transfection reagent kit (Sunma Corp., Xiamen, China) according to the manufacturer's instruction. The transfected cells were selected using G418 for 3–4 weeks for the following experiments. Monoclonal cells were then cloned and screened for FGFR1 expression.

For FGFR1 knockdown, the sense and antisense shRNA oligonucleotides were annealed and cloned into pSUPER.retro.puro (OligoEngine). All the cells with stable overexpression or knockdown of miR-198 and/or FGFR1 were polyclonal derivatives with puromycin or hygromycin selection to avoid clonal variations in functional assays.

For 3′UTR reporter assays, a ~2.3 kb fragment of FGFR1 3′UTR was cloned into pMIR-REPORT (Ambion). Sites 1–3 were further mutated to 5′-GAAT-3′ from 5′-TGGA-3′.

miRNA DETECTION

Total RNA was extracted using TRIzol (Invitrogen). Mature miRNAs were reverse-transcribed and quantitated with the TaqMan microRNA Assays (Applied Biosystems). The data was normalized to U6 expression.

miR-198 TARGET PREDICTION

Two independent online databases, TargetScan and miRDB, were used to predict miR-198 targets. The genes predicted by TargetScan with no less than two miR-198 binding sites and at least one evolutionarily conserved site, and those predicted by miRDB with the scores higher than the average score of all miR-198 targets were selected as the miR-198 candidate targets.

LUCIFERASE DUAL–REPORTER ASSAYS

A549 cells were co-transfected with the control or miR-198-expressing plasmid, the indicated firefly luciferase reporter plasmid and a renilla luciferase plasmid with a ratio of 2:2:1. Lysates were collected 72 h after transfection. Firefly and renilla luciferase activities were measured with a Dual-Luciferase Reporter System (Promega).

PROTEIN EXTRACTION AND WESTERN BLOT

Forty-eight hours after transfection, the cells were lysed using cell lysis buffer (Cell Signaling). Protein concentration was determined with the BCA Protein Assay kit (Pierce) and equal amounts of total proteins were separated in 10% SDS–polyacrylamide gels, transferred to polyvinylidene difluoride membranes (Bio-Rad). Membranes were blocked for 1 h with 1% BSA in Tris-buffered saline containing 0.05% Tween-20, incubated overnight with primary antibody, washed and incubated with secondary antibody, and visualized by chemiluminescence. The antibodies used were as follows: anti-Caspase-3 and anti-PARP antibody (Santa Cruz), anti-GAPDH antibody (Abcam), anti-CCNT1, and anti-FGFR1 antibody (Cell Signaling).

TUMOR FORMATION ASSAY IN A NUDE MOUSE MODEL

Athymic BALB/c nude mice were maintained under specific pathogen-free conditions. Mice were manipulated and housed according to protocols approved by the Shanghai Medical Experimental Animal Care Commission. A549 cells stably expressing miR-198, FGFR1, miR-198 + FGFR1, or vector control, were harvested from subconfluent cell culture plates, washed with PBS, and resuspended at a concentration of 10^7 cells/ml of PBS. Of the suspending cells, 0.1 ml was subcutaneously injected into either side of the flank of the same female nude mouse at 5 weeks of age. Tumor growth was examined every 3 days for 5 weeks and its volume (V) was monitored by measuring the length (L) and width (W) of the tumor with calipers and calculated with the formula $V = 1/2(L \times W^2)$. After 6 weeks, these mice were sacrificed and examined for the growth of subcutaneous tumors.

HISTOLOGIC EVALUATION

IHC staining for Ki-67 antigens was performed on 5 μm sections obtained from formalin-fixed, paraffin embedded blocks, using avidin-biotin peroxidase complex method. The primary antibodies were monoclonal mouse anti Ki-67 antigen. The sections stained by IHC were examined alongside H&E stained specimens, to identify the precise locations of the lesions.

STATISTICAL ANALYSIS

The Kaplan–Meier method was used to estimate the survival curves for the mice, and Cox proportional hazard regression was used to compare the survival. Two-sided independent student's t-test without assumption of equal variance was performed to analyze the results of in vitro assays. All the qPCR and luciferase assays were repeated at least three times.

RESULTS

miR-198 INHIBITS LUNG CANCER CELLS PROLIFERATION

To study the role of miR-198 in lung carcinogenesis, we first assessed its effect on cell viabilities of A549 and Hela cells by Celltiter assay. A549 or Hela cells were transfected with 50 nM of the human miR-198, miR-192, miR-214, or miR-21 FGFR1ics or scramble control in 96-well plate. Cell viability assays were then performed 48 h after transfection. The results showed that the overexpression of miR-198
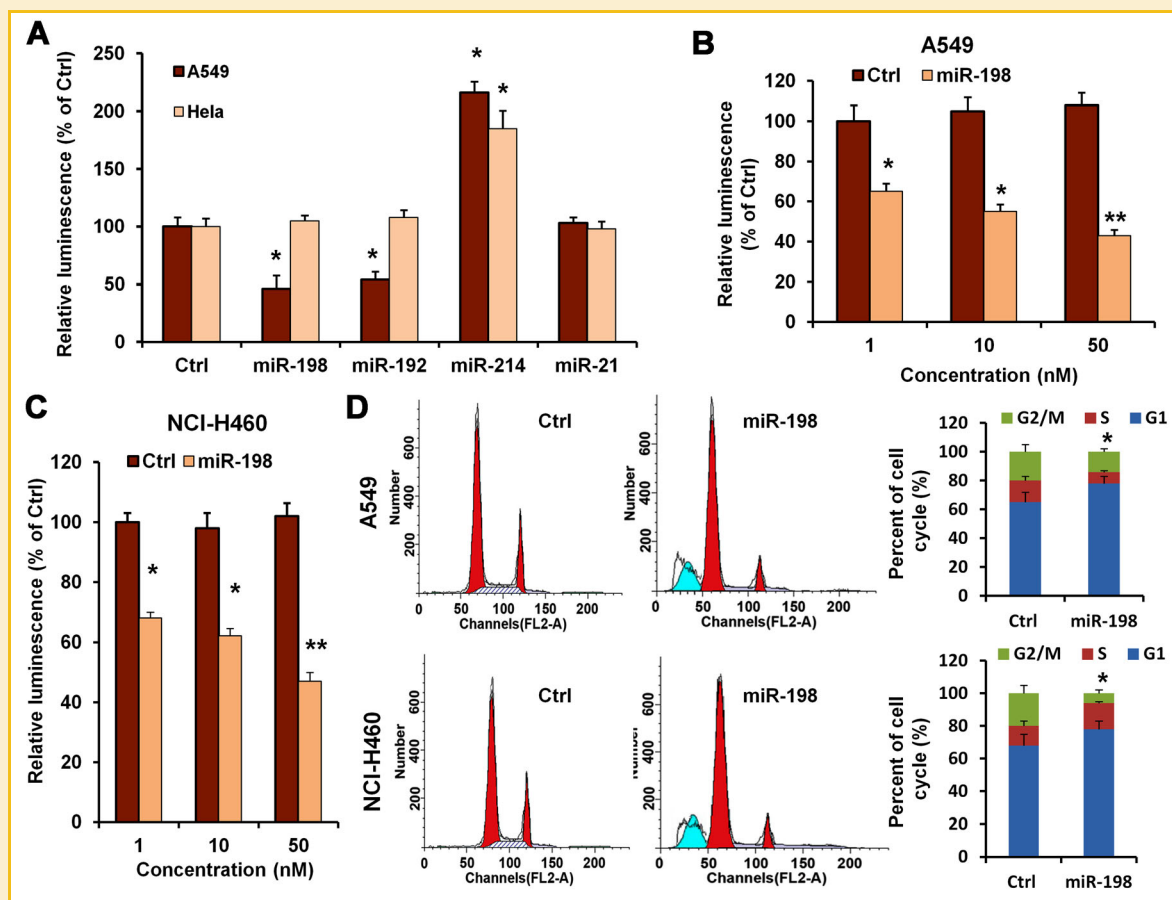

Fig. 1. miR-198 inhibits lung cancer cells proliferation. A: Cell viabilities of A549 and Hela by Celltiter assay. Cells were transfected with 50 nM of miRNA mimics or scramble control (Ctrl) in 96-well plate. Cell viability assays were performed 48 h after transfection, CellTiter–Glo reagent was added and luminescent intensity was measured by a Microplate Luminometer. B: A549 and (C) NCI-H460 cells viabilities with different concentrations of miRNA mimics. A549 cells were transfected with 1, 10, and 50 nM miRNA mimics and scramble control in 96-well plate. Relative luminescence was calculated with luminescence miRNA/luminescence scramble. D: A549 or NCI-H460 cell apoptosis assays by flow cytometry were performed 48 h after transfection. Data were the average of three independent experiment runs; bars, SEM. *P < 0.05; **P < 0.01, compared with scramble control.

or miR-192 significantly inhibited cell viabilities in A549 cell lines and overexpression of miR-214 had a profound increase in cell viabilities in both of cell lines (Fig. 1A).

To further confirm the effects of miR-198 on lung cancer cells viabilities, we analyzed the cell viability of A549 or NCI-H460 cells treated with different doses of miR-198. As shown in Figure 1B,C, miR-198 suppressed A549 or NCI-H460 cells viabilities in a dose-dependent manner. At doses of 1 and 10 nM, treatment with miR-198 significantly lowered cell viability ($P < 0.05$) when compared with the scramble control miRNA. The difference was extraordinary significant ($P < 0.01$) after treatment of 50 nM of miR-198 in both of cell lines.

A comparison of treatments with miR-198 and with the scramble control miRNA (Fig. 1D) indicated that the proportion of A549 or NCI-H460 cells in G2/M phase was decreased ($P < 0.05$).

miR-198 ENHANCES CELL APOPTOSIS IN LUNG CANCER CELLS

Moreover, we examined cell apoptosis in A549 or NCI-H460 cells using an Annexin V-PE apoptosis detection kit and found that levels of cell apoptosis were elevated in cells exposed to miR-198 versus the mock cells or scramble control cells (Fig. 2A,B). We also used a miR198 inhibitor (ant-miR198, fully 2′ O-methyl modified RNA oligo) and assessed the phenotype by miR-198 silencing. The miR-198 inhibitor treatment of A549 or NCI-H460 lung cancer cells resulted in a marked reduction of cell apoptosis (Fig. 2A,B). Further experiments revealed that miR-198 induced the expression of poly (ADP-ribose) polymerase (PARP) and of cleaved Caspase-3 (Fig. 2C), a member of a well-known apoptosis pathway, in both of lung cancer cells.

miR-198 INHIBITS A549 GROWTH OF TUMOR GRAFT IN THE NUDE MOUSE

Next we assessed the in vivo functions of miR-198 in lung cancer tumor growth. First we constructed A549 cells stable overexpressed the miR-198 or scramble control and confirmed the miR-198 expression in A549 cells with scramble control (Ctrl) or A549 cells with miR-198 overexpression (miR-198) group using qPCR analyses (Fig. 3A). We found that miR-198 suppressed A549 cells viabilities (Fig. 3B) and cell cycles (Fig. 3C).

Then we implanted the miR-198-overexpressing and control A549 cells into eight nude mice subcutaneously and found significant differences of tumor growth between these two groups, a phenomenon
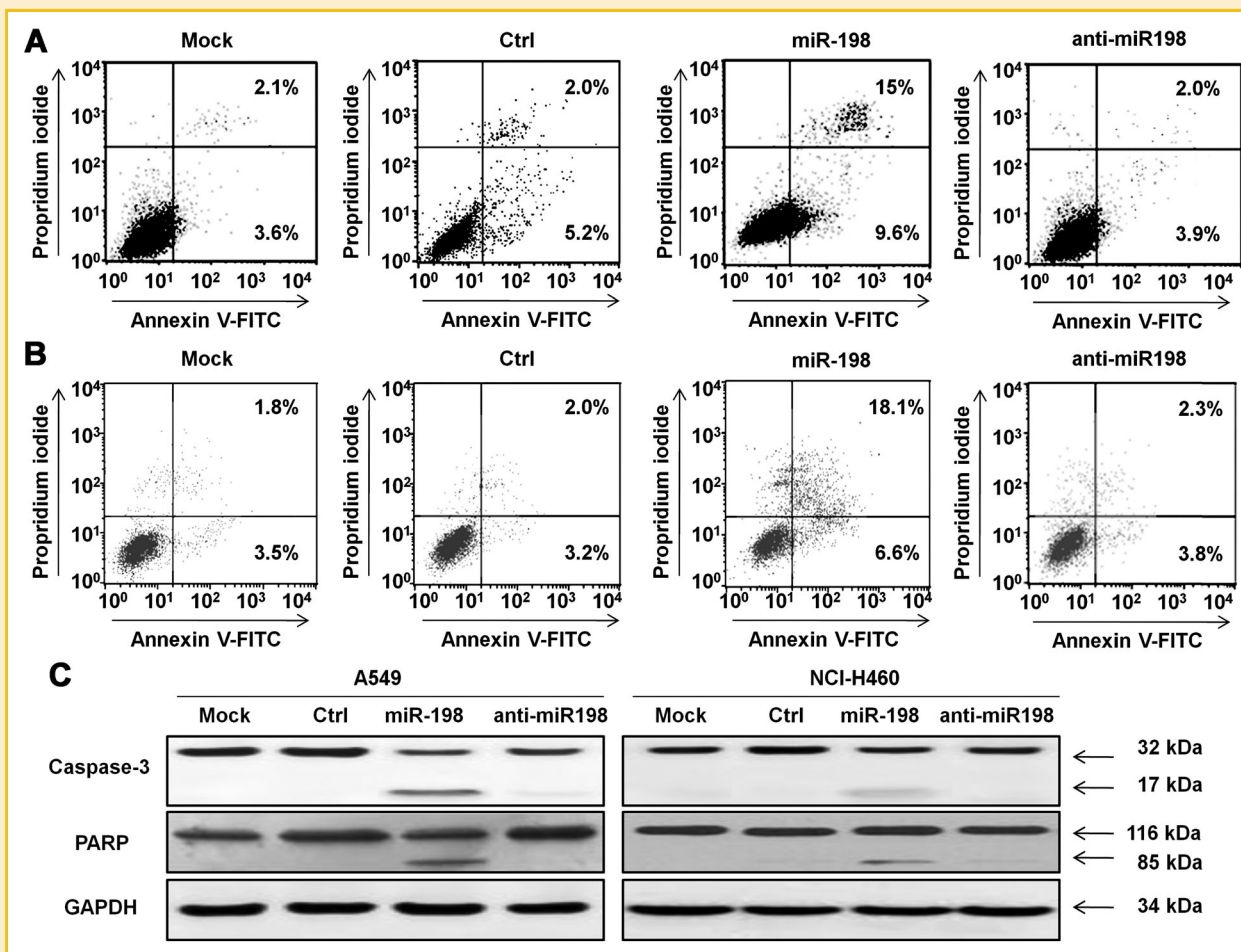

Fig. 2. Effect of miR-198 on lung cancer cells apoptosis. Apoptotic A549 (A) or NCI-H460 (B) cells in mock, scramble control (Ctrl), 50 nM of miRNA mimics or anti-miR198 were analyzed using double staining with Annexin-V-FITC and PI. C: Equal amounts of protein (20 μg) from A549 or NCI-H460 lysates were separated by SDS–PAGE and immunoblotted. PARP cleavage and caspase activation were analyzed. GAPDH is shown as an internal standard. Cell apoptosis assays were performed 48 h after transfection.
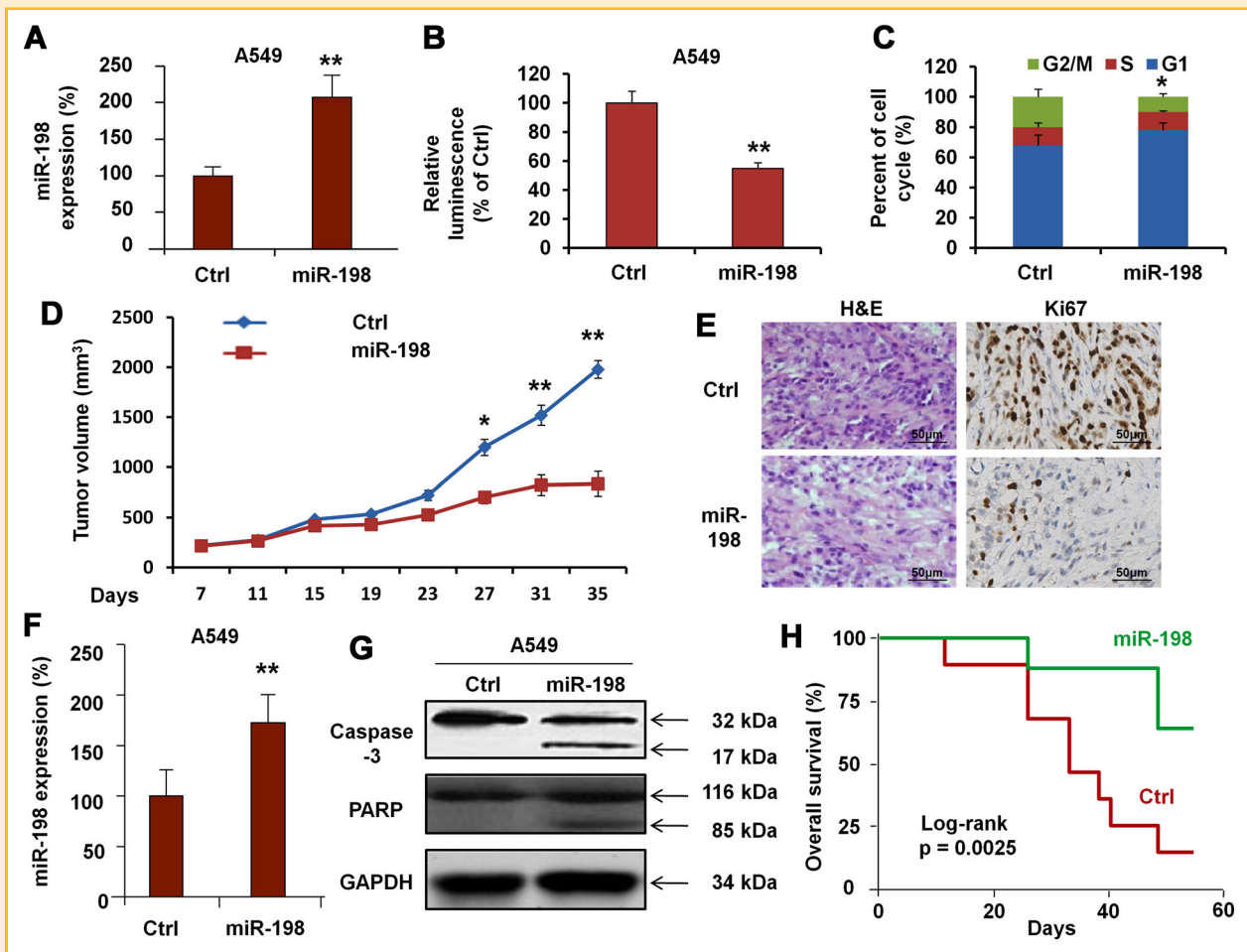
Fig. 3. miR-198 inhibits A549 growth of tumor graft in the nude mouse. qPCR analyses of miR-198 expression (A), cell viability (B) and cell cycle assays (C) were performed in A549 cells with scramble control (Ctrl) or A549 cells with miR-198 stably overexpression (miR-198) group. D: A549 cells with scramble control (Ctrl) or A549 cells with miR-198 stably overexpression (miR-198) were injected subcutaneously in eight nude mice. The tumor volume was measured every 4 days with calipers after tumor appeared. E: Representative H&E and Ki67 staining of A549 cells in nude mice in Crtl (upper) and miR-198 groups (lower). F: qPCR analyses of miR-198 expression in the subcutaneous implanted tumor in A549 cells with scramble control (Ctrl) or A549 cells with miR-198 stably overexpression (miR-198) group. G: PARP cleavage and caspase activation were analyzed in the subcutaneous implanted tumor. H: Overall survival analysis of the nude mice. Numbers present mean $\pm$ s.e. Scale bars, 100 μm. $^*P < 0.05$, $^{**}P < 0.01$.

consistent with the in vitro observations (Fig. 3D). At week 4, the growth burden was significant lower in the animal group of miR-198 overexpression (Fig. 3E). When the mice were sacrificed, nearly 1.7-fold more miR-198 expression was observed in tumor nodules inoculated by miR-198-expressing cancer cells (Fig. 3F). The difference was also confirmed by histological observation of the organs (Fig. 3E). Further experiments revealed that miR-198 induced the expression of poly (ADP-ribose) polymerase (PARP) and of cleaved Caspase-3 (Fig. 3G), members of a well-known apoptosis pathway. Kaplan–Meier analysis showed that miR-198 overexpression led to longer overall survival of the animals (Fig. 3H), which demonstrated an inhibitory role of miR-198 in lung cancer via inhibition of proliferation and enhancement of apoptosis.

FGFR1 IS A DIRECT TARGET OF miR-198

Next we aimed to find the target genes of miR-198 to explain its inhibitory function. We first performed a bioinformatic screen for its target gene candidates. We analyzed the 3′UTR binding site prediction by two independent online databases, TargetScan and miRDB. A total of 221 genes were predicted to be miR-198 targets by TargetScan, and 218 genes by miRDB. Overlap analysis revealed 12 genes appearing in all the three gene sets. Among these 12 genes, *FGFR1* was of particular interest since previous studies have reported the correlation of its overexpressing with carcinogenesis.

The 3′UTR of *FGFR1* contains three predicted binding sites of miR-198 at the positions of 302, 341, and 359 bp, respectively (Fig. 4A). To determine whether *FGFR1* is a bona fide target of miR-198, the 3′UTR DNA fragment containing these three sites was cloned for luciferase reporter assays. The 3′UTR of *CCNT1*, a known target gene of miR-198, was used as a positive control. The luciferase activities of both *FGFR1* and *CCNT1* 3′UTRs were significantly reduced upon miR-198 transfection in HeLa (Fig. 4B) and A549 cells (Fig. 4C). *FGFR1* RNA levels in A549 or NCI-H460 cells transfected with the miR-198-expressing vector or miR-198 inhibitor (Fig. 4D).
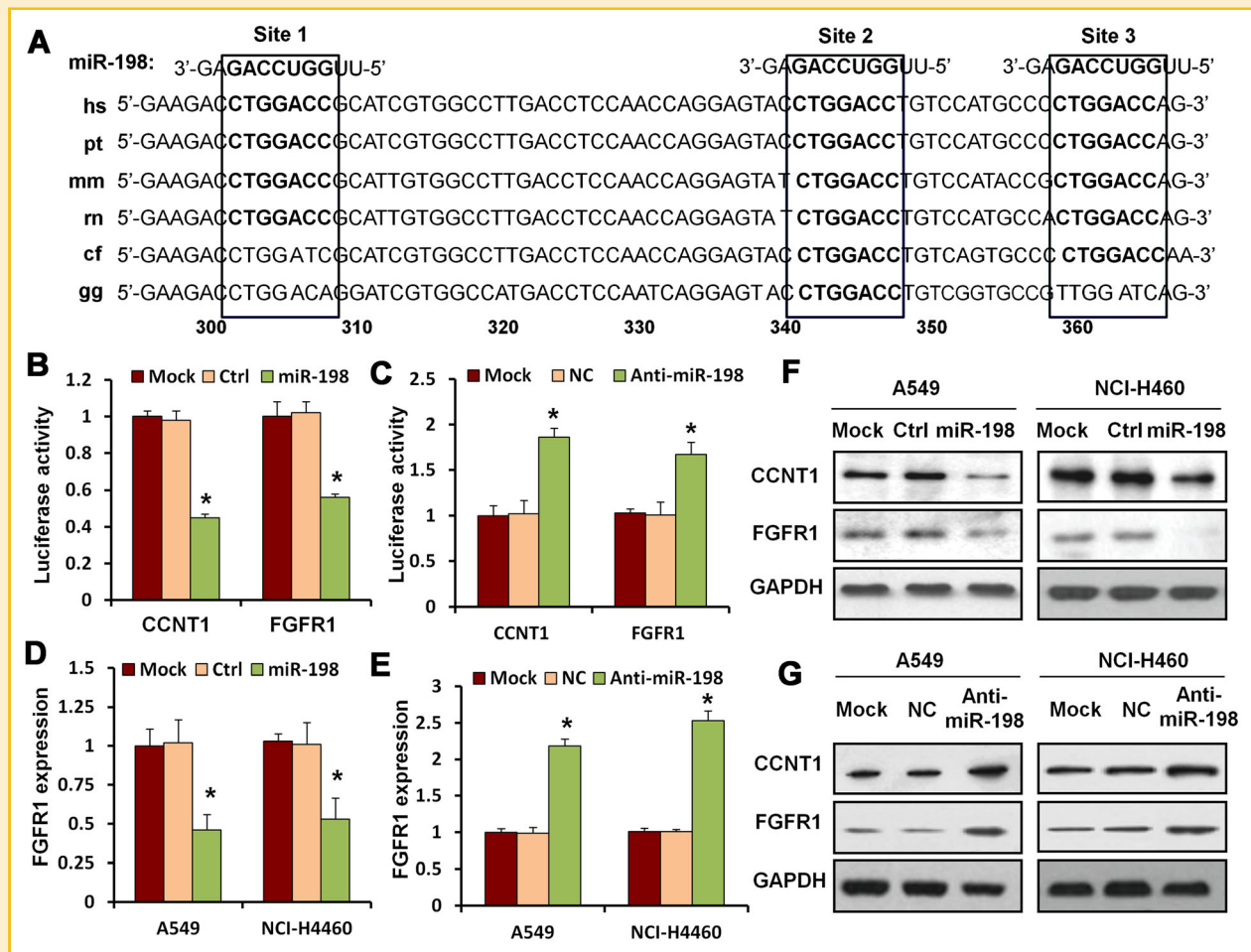
Fig. 4. FGFR1 is regulated by miR-198 in human lung cancer cell lines. A: The binding site of miR-198 in the predicted target sequences. hs, Homo sapiens; pt, Pan troglodytes; mm, Mus musculus; rn, Rattus norvegicus; cf, Canis familiaris; gg, Gorilla gorilla. B: Luciferase reporter assays of *CCNT1*, *FGFR1* 3'UTR or vector in A549 cells (Mock), A549 cells transfected with empty vector (Ctrl) or the miR-198-expressing (miR-198). C: Luciferase reporter assays of *CCNT1*, *FGFR1* 3'UTR or vector in A549 cells (Mock), A549 cells transfected with No-targeted sponge plasmid (NC) or the miR-198 sponge plasmid (Anti-miR-198). Lysates were collected 72 h after transfection. D: *FGFR1* RNA levels in A549 or NCI-H460 cells (Mock), cells after 48 h transfection with empty (Ctrl) or the miR-198-expressing (miR-198) vector. E: *FGFR1* RNA levels in A549 or NCI-H460 cells (Mock), cells after 48 h of treatment with No-targeted sponge plasmid (NC) or the miR-198 sponge plasmid (Anti-miR-198). F: CCNT1 and FGFR1 protein levels in A549 or NCI-H460 cells (Mock), cells after 48 h transfection with empty (Ctrl) or the miR-198-expressing (miR-198) vector. G: CCNT1 and FGFR1 protein levels in A549 or NCI-H460 cells (Mock), cells after 48 h of treatment with No-targeted sponge plasmid (NC) or the miR-198 sponge plasmid (Anti-miR-198).

In addition, overexpression of miR-198 in A549 or NCI-H460 cells caused modest but significant attenuation of protein levels of FGFR1 and CCNT1 (Fig. 4E), indicating that the suppression of FGFR1 by miR-198 was manifest at both the mRNA and the protein level. Furthermore, the treatment of anti-miR-198 oligonucleotide restored their activities (Fig. 4E,G). Therefore, the *FGFR1* 3'UTR was targeted by miR-198.

To investigate which binding sites at the FGFR1 3'UTR contribute to the regulation by miR-198, we cloned each of the binding sites individually into the reporter vector and found that only sites 2 (341 bp) and 3 (359 bp), which are the evolutionarily conserved regions among the three (Fig. 4A), showed decreased luciferase activities upon miR-198 transfection (Fig. 5A,B). In addition, mutating the sequences of sites 2 and 3 in the seed regions reversed the miR-198 suppression (Fig. 5A,C), thus establishing these two regions as the direct target sites of miR-198.

FGFR1 IS A LUNG CANCER ONCOGENE

Next, we overexpressed or RNA interfered of *FGFR1* in A549 or NCI-H460 cells (Fig. 6A,B), and observed obvious repressions of cell viabilities after *FGFR1* RNAi (Fig. 6C,D). Meanwhile, overexpression of *FGFR1* significantly increased cell viabilities in A549 or NCI-H460 cells (Fig. 6C,D).

Then we examined whether *miR-198 depletion* is sufficient to promote the malignant traits of lung cancer cells using overexpression approach in *FGFR1* overexpression A549 cells (Fig. 6A,B). The results showed that overexpression of miR-198 rescued these activities.

To further assess the function of FGFR1 in lung carcinogenesis, we analyzed the in vivo property of *FGFR1* overexpression A549 cells with or without miR-198 overexpression. Our results showed that A549 cells with *FGFR1* overexpression developed subcutaneous implanted tumor rapidly in nude mice, while miR-198 overexpression suppressed the growth of A549 cells in the animal (Fig. 6E). The
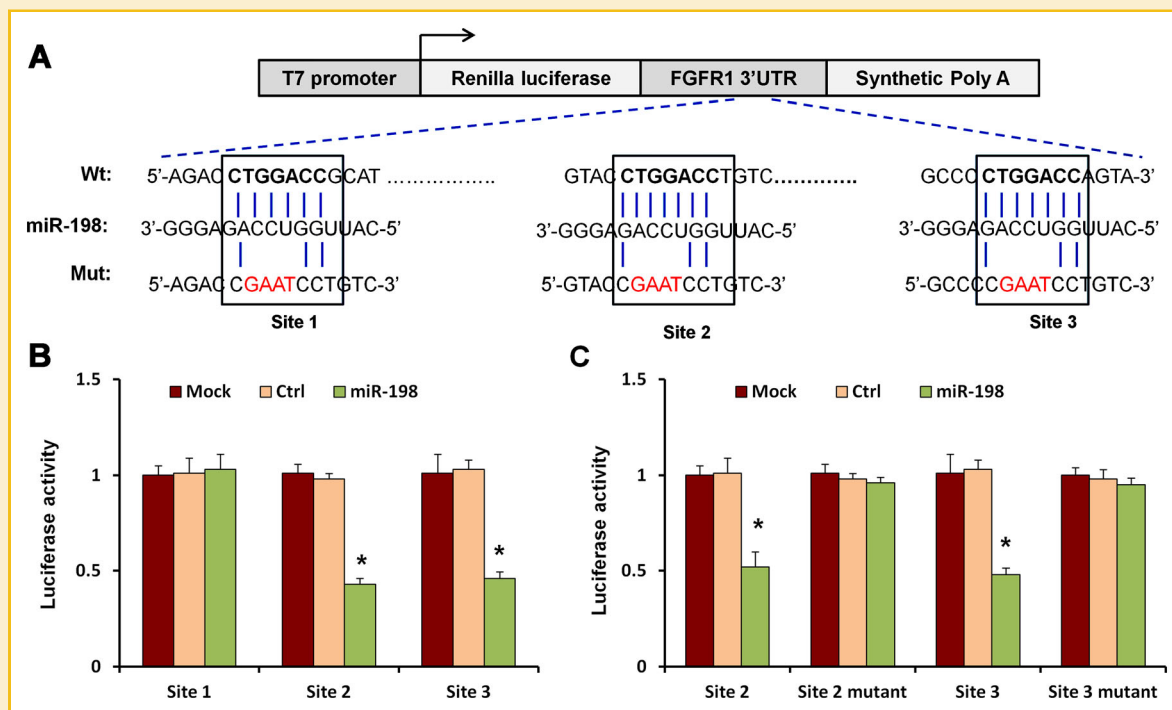
Fig. 5. miR-198 directly targets *FGFR1*. A: The sketch of wild-type and 4 nt mutations of the *FGFR1* 3′UTR. B: Luciferase activities of wild-type and three individual binding sites of *FGFR1* 3′UTR in HeLa cells (Mock), HeLa cells transfected with empty vector (Ctrl) or the miR-198-expressing vector (miR-198). C: Luciferase activities of wild-type and two mutated individual binding sites of *FGFR1* 3′UTR in HeLa cells (Mock), HeLa cells transfected with empty vector (Ctrl) or the miR-198-expressing vector (miR-198). Lysates were collected 72 h after transfection.

mRNA levels of *FGFR1* in the implants were confirmed by qPCR (Fig. 6F). Animal in the *FGFR1*-overexpression group survived shorter when compared with that in *FGFR1* and miR-198 over-expression group (Fig. 6G). These data evidenced the oncogene property of FGFR1 in lung cancer.

DISCUSSION

NSCLC is one of the leading causes of cancer-related deaths in industrialized countries. Despite improvements in early diagnosis made possible by emerging technologies and newly developed chemo-targeted therapies that improve treatment responses, the overall 5-year survival rate for NSCLC patients remains <20% [Vora and Reckamp, 2008]. It is still necessary to further elucidate the mechanisms involved in the tumorigenesis of NSCLC and discover novel therapeutic targets.

The discovery in 1993 of a small endogenous regulatory RNA molecule in *Caenorhabditis elegans* paved the way for description of a large family of short (∼22 nt) single-stranded ribonucleic acids termed microRNAs (miRNAs). miRNAs are a group of endogenous and non-coding small single strain RNRNAs (only 19–22 nucleotides), which can bind with the 3′-untranslated region (3′-UTRs) of messenger RNRNAs of target genes and cleave them post-transcriptively. miRNAs are versatile regulators of gene expression in higher eukaryotes. In order to silence many different mRNAs in a precise manner, miRNA stability, and efficacy is controlled by highly

developed regulatory pathways and fine-tuning mechanisms both affecting miRNA processing and altering mature miRNA target specificity [Yates et al., 2013]. miRNAs play key roles in various biological processes and in the development of human disease by post-transcriptional regulation of gene expression.

In cancer research, patterns of altered miRNA expression in cancer may work as molecular biomarkers for tumor diagnosis, prognosis of disease-specific outcomes, and prediction of therapeutic responses. In addition, miRNAs can serve as specific targets for gene therapies. Many miRNRNAs are also considered as potential oncogenes or tumor-suppressor genes [Farazi et al., 2013]. Increasing bioinformatics evidence and subsequent functional assays of miRNRNAs have indicated that some special miRNRNAs are involved in many steps during cancer progression, such as apoptosis, proliferation, differentiation, migration, and metastasis [Rossi et al., 2013].

Although little information is available concerning potential functions and mRNA targets for miR-198, recent research has indicated miR-198 as a miRNA that is strongly down-regulated in NSCLC [Yanaihara et al., 2006]. In early 2006, Yanaihara et al. [2006] examined miRNA expression profiles for lung cancers to investigate miRNA's involvement in lung carcinogenesis and found that identified statistical unique profiles, including miR-198, which could discriminate lung cancers from noncancerous lung tissues as well as molecular signatures that differ in tumor histology by miRNA microarray analysis.

Notably, recent studies have indicated that miR-198 was significantly downregulated in other types of cancers, such as
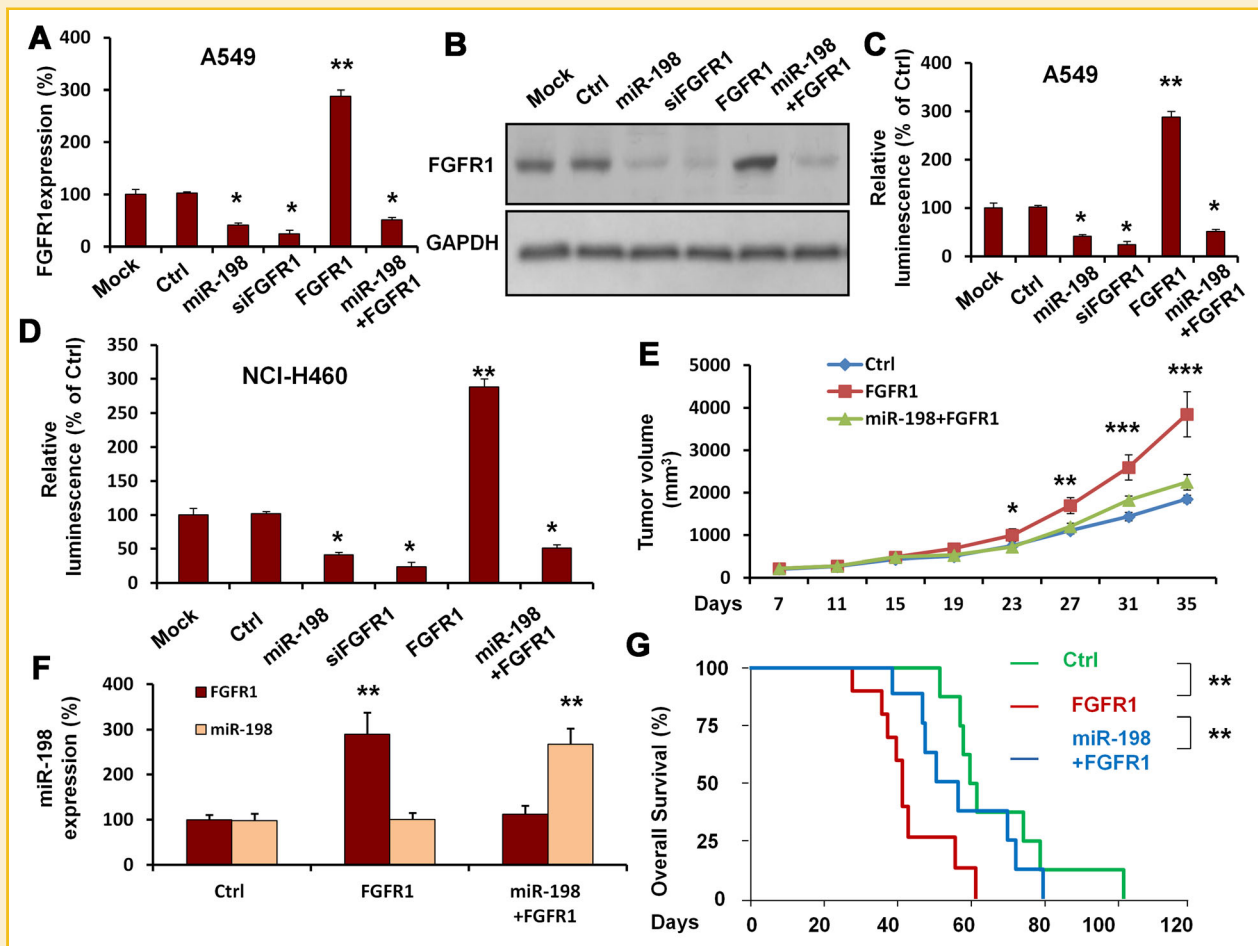
Fig. 6. miR-198 suppresses lung carcinogenesis through targeting *FGFR1*. *FGFR1* RNA levels (A) and protein levels (B) in A549 (Mock), A549 with empty plasmid (Ctrl), A549 with miR-198 overexpression (miR-198), A549 with siFGFR1 (siFGFR1), A549 with *FGFR1* overexpression (FGFR1), A549 with miR-198 and FGFR1 stably overexpression (miR-198 + FGFR1). Cell viabilities of these six groups by Celltiter assay in A549 (C) or NCI-H460 cells (D). E: A549 with empty plasmid (Ctrl), A549 with *FGFR1* overexpression (FGFR1), A549 with miR-198 and FGFR1 overexpression (miR-198 + FGFR1) were injected subcutaneously in eight nude mice. The tumor volume was measured every 4 days with calipers after tumor appeared. F: qPCR analyses of *FGFR1* expression in the subcutaneous implanted tumor. G: Overall survival analysis of the nude mice. Numbers present mean ± s.e. Scale bars, 100 μm. $^*P < 0.05$, $^{**}P < 0.01$.

ovarian cancer [Shen et al., 2010], live cancer [Elfimova et al., 2013], and prostate cancer [Ye et al., 2013].

Various recent different studies have shown that miRNAs are often deregulated in HCC and that several deregulated miRNAs (e.g., miR-198) may possess important roles in hepatic cancer progression and directly contribute to cell proliferation, apoptosis and metastasis of hepatocellular carcinoma (HCC). It is thought that miR-198 plays a tumor suppressor role by negatively regulating the HGF/c-MET pathway. Tan et al. showed that miR-198 is down-regulated in HCC compared to normal liver parenchyma and subsequently found that miR-198 diminishes hepatocyte growth factor (HGF) stimulated migration and invasion of HCC cells negatively by modulates c-MET expression, suggesting miR-198 as a novel suppressor of HCC cell invasion by negative regulation of the HGF/c-MET pathway. Forced expression of miR-198 may therefore provide a therapeutic strategy for patients with HCC [Tan et al., 2011].

Ye et al. [2013] found that miR-198 expression was identified to be negatively correlated with expression level of Livin, an inhibitor of apoptosis, in some prostate cancer cell lines and further study revealed miR-198-mediated repression of Livin expression, indicating that miR-198 is associated with cell apoptosis by targeting in prostate cancer.

The role of miR-198 in the tumorigenesis of NSCLC, however, remains unclear. In the present study, we confirmed that miR-198 is downregulated in clinical tumor tissues of lung adenocarcinoma. Overexpression of miR-198 reduced the proliferation ability of human lung cell line A549 and NCI-H460. The direct targeting of miR-198 to the *FGFR1* mRNA was predicted by bioinformatics analysis and validated by luciferase reporter gene assay. There are two miR-198 binding sites in the 3′UTR of *FGFR1* mRNA. In our luciferase report assay, we showed that Luc-*FGFR1*-wt was specifically responsive to miR-198 overexpression. In contrast, the Luc-*FGFR1*-mut, which contained the mutation of the miR-198 binding sites in the FGFR1 3′UTR successfully abolished the effect of miR-198 on luciferase activity. We therefore concluded that miR-198 can impair human lung cell proliferation via targeting *FGFR1* and then leads to inhibition of lung cancer development.

FGFR1 is a member of the fibroblast growth receptor family, which includes FGFR1, 2, 3, and 4 that serve as receptor tyrosine kinases. FGFR proteins interact with fibroblast growth factors to set in motion a cascade of downstream signaling that ultimately regulates cell proliferation, survival, migration, and differentiation [Heist et al., 2012]. FGFR1 is involved in important processes such as mitogenesis, cell maturation, blood vessel formation, wound healing, embryonic, and nervous system development. The dysregulated activity of FGFR1 in diseases is associated with the gene amplification, mutation, and translocation of FGFR1 [Singh et al., 2012]. Here, we show that FGFR1 expression can also be regulated by a tumor suppressive miRNA, miR-198.

Amplification or activation of FGFR1 has been reported in diverse human cancers including carcinomas [Ishizuka et al., 2002], ovarian cancer [Gorringe et al., 2007], prostate cancer [Edwards et al., 2003], lung cancer [Weir et al., 2007], and so on. In addition, FGFR1 activation promotes the epithelial–mesenchymal transition (EMT) in breast [Xian et al., 2005] and prostate cancers [Acevedo et al., 2007]. The important roles of FGFR1 in a variety of cancers make it a potential therapeutic target for cancer therapy. Several FGFR kinase inhibitors such as brivanib, dovitinib, BIBF112, and SU-6668 have already been shown to inhibit tumor proliferation and are now being tested for application in cancer therapy [Wen et al., 2013].

In this study, we showed that overexpression of miR-198 inhibited lung cancer cell growth and promoted cell apoptosis. Subsequently, we predicted and confirmed that FGFR1 was a direct target of miR-198 in lung cancer cells. FGFR1 was negatively regulated by miR-198, revealing a potential mechanism associated with lung tumorigenesis.

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