Acta Biochim Biophys Sin, 2015, 47(8), 630–638
doi: 10.1093/abbs/gmv054
Advance Access Publication Date: 29 June 2015
Original Article

Original Article

MiR-181a-5p inhibits cell proliferation and migration by targeting Kras in non-small cell lung cancer A549 cells

Zhongliang Ma[1,†], Xiang Qiu[1,†], Detao Wang[1], Yanli Li[1], Bingjie Zhang[1], Tianwei Yuan[1], Jiali Wei[1], Botao Zhao[1], Xintai Zhao[4], Jiatao Lou[3,*], Yan Jin[2,*], and Youxin Jin[1,*]

[1]School of Life Sciences, Shanghai University, Shanghai 200444, China, [2]Institute of Biomedicine and Biotechnology, Shenzhen Institutes of Advanced Technology Chinese Academy of Sciences, Shenzhen 518055, China, [3]Department of Laboratory Medicine, Shanghai Chest Hospital Affiliated to Shanghai Jiaotong University, Shanghai 200030, China, and [4]Shanghai Shines Pharmaceuticals Co., Ltd., Shanghai 200032, China

[†]These authors contributed equally to this work.
*Correspondence address. Tel/Fax: +86-21-22200000 (Ext. 1503); E-mail: loujiatao@126.com (J.L.)/Tel/Fax: +86-755-86585216; E-mail: yan.jin@siat.ac.cn (Y.J.)/Tel: +86-21-66137016; Fax: +86-21-66132177; E-mail: jinyouxin@shu.edu.cn (Y.J.)

Received 16 March 2015; Accepted 25 April 2015

Abstract

MicroRNAs play important roles in carcinogenesis and tumor progress. Lung cancer is the leading cause of cancer mortality worldwide. In this study, the function of miR-181a-5p was investigated in non-small-cell lung cancer (NSCLC). Results showed that miR-181a-5p was significantly decreased in NSCLC tissues and cell lines. The proliferation and migration of A549 cells transfected with miR-181a-5p mimic was significantly inhibited. Luciferase activity assay results demonstrated that two binding sites of Kras could be directly targeted by miR-181a-5p. Furthermore, Kras was down-regulated by miR-181a-5p at both transcriptional and translational levels. SiRNA-mediated Kras down-regulation could mimic the effects of miR-181a-5p mimic in A549 cells. Our findings suggest that miR-181a-5p plays a potential role in tumor suppression by partially targeting Kras and has the potential therapeutic application in NSCLC patients.

Key words: miR-181a-5p, proliferation, migration, non-small cell lung cancer

Introduction

Lung cancer is the leading cause of cancer-related death and the most commonly diagnosed cancer in the world. Non-small-cell lung cancer (NSCLC), including squamous cell carcinoma and adenocarcinoma, is the most common type of lung cancer, accounting for 80% of lung cancer clinical cases [1]. Despite intensive researches and resources being dedicated to elucidate the molecular mechanisms of NSCLC, the precise processes of initiation and progression remain largely unclear. Recently, accumulating evidence has shown that non-coding small RNAs may be involved in NSCLC pathogenesis, providing new insights into lung tumor biology [2–5].

MicroRNAs (miRNAs) are a class of short, endogenous non-coding RNAs that have been identified as post-transcriptional regulators of gene expression. MiRNAs perform their functions through imperfect base-pairing with the 3′ untranslated region (3′UTR) of target mRNAs [6]. In recent years, the importance of genetic background in lung tumor has been well highlighted and miRNAs have been studied to characterize various types of tumors [7–11]. In human cancers, miRNAs can act as tumor suppressor genes or oncogenes during tumourigenesis. In general, several hundred genes can be regulated by one miRNA, and as a result, miRNA profiling could serve as a better classifier than gene expression profiling [12]. Some studies have shown that miR-181a plays important roles in cell proliferation, migration,

invasion, and apoptosis via targeting specific genes [13–15], and it may be a biomarker for cancer diagnosis [2,16–18].

Kras oncogene encodes small guanosine triphosphatase that functions as a critical molecular switch for various biological processes, including cell proliferation, survival, differentiation, and death [19]. It was reported that there is a strong association between Kras mutation and survival in NSCLC patients [20]. Some miRNAs have also been shown to target Kras in different types of cancers, including let-7i, miR-96, miR-126, miR-217, miR-30c, and miR-143 [9,10,21,22]. Kras has become a focal point for potential miRNA-targeted therapies as previously described. Currently, the role of Kras in NSCLC has not been well established.

In this study, the biological functions of miR-181a-5p were investigated in NSCLC cell line A549. The relationship between miR-181a-5p and Kras was also investigated. Results indicate that miR-181a-5p functions as a tumor suppressor in A549 cells and that miR-181a-5p may provide a potential treatment approach for NSCLC patients.

Materials and Methods

Cell lines and clinical samples

NSCLC cell lines A549, SPCA-1, 95-D, HCC827, non-tumorigenic bronchial epithelium cell line BEAS-2B, and human embryonic kidney cells (HEK293T) were purchased from the Cell Bank, China Academy of Sciences (Shanghai, China). H1299 and H23 cells were purchased from the American Type Culture Collection (ATCC, Manassas, USA). BEAS-2B cells were isolated from normal human bronchial epithelium. 95-D cells were highly metastatic human lung cancer cells. BEAS-2B cells were cultured in LHC-9 medium (Gibco, Gaithersburg, USA) including epidermal growth factor, hydrocortisone, and adrenaline. A549 cells were cultured in F12K medium (Gibco). All the other lung cancer cells were cultured in RPMI-1640 medium (Gibco). HEK293T cells were cultured in Dulbecco's modified Eagle's medium (Gibco). All the media were supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories, Logan, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco). Cells were maintained in a humidified 37°C incubator with an atmosphere of 5% CO_2.

NSCLC and corresponding paracancerous lung tissues were obtained from Department of Laboratory Medicine, Shanghai Chest Hospital affiliated to Shanghai Jiaotong University under ethical assessment.

Animal models and Solexa sequencing

The normal mouse and NSCLC mouse model were kindly provided by Prof Hongbing Ji (Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Shanghai, China), and Solexa sequencing was determined by BGI (Shenzhen, China). L822T1/$Kras^{+/+}$, L903T1/$Kras^{+/+}$+$LKB1^{-/-}$, and L703T2/$Kras^{+/+}$+$p53^{-/-}$ were three types of NSCLC mouse model.

RNA preparation and quantitative real-time PCR analysis

Total RNA was isolated from the cell cultures using Trizol reagent (Bio Basic Inc., Toronto, Canada). Each RNA sample reverse transcription was performed using M-MLV RTase cDNA Synthesis Kit according to the manufacturer's instructions (TaKaRa, Dalian, China). A cDNA pool of miRNAs was synthesized using QuantiMir cDNA Kit (TaKaRa). U6 small nuclear RNA (snRNA) and 18S RNA were used as the endogenous control for miRNA and mRNA, respectively. The $2^{-\Delta\Delta Ct}$ method for relative quantification of gene expression was used to determine mRNAs and miRNAs expression levels. The miRNA mimic, miRNA mimic negative control (NC), and miRNA inhibitor for miR-181a-5p (anti-miR-181a-5p) were purchased from RiboBio (Guangzhou, China). Forty-eight hours after transfection, cells were harvested for quantitative real-time PCR (qRT-PCR) analysis. All experiments were performed with three replicates and three technical repetitions.

Cell proliferation assay

Cell proliferation was monitored using Cell Counting Kit-8 (CCK8; Dojindo, Kumamoto, Japan). In brief, 2×10^3 cells were seeded in 96-well culture plates 6 h post-transfection, then 10 μl of CCK8 was added to each well at 24, 48, 72, and 96 h. Cells were incubated with CCK8 reagent in the dark for 2 h at 37°C and light absorbance was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, USA). The experiments were independently performed in triplicates.

Colony formation assay

In colony formation assay, cells were seeded into 6-well plates at a density of 1000 cells/well at 6 h post-transfection. Colonies are fixed with methanol, stained with crystal violet (0.5%, w/v), and counted using a stereomicroscope.

Cell motility assays

For wound healing assay, cells transfected with miR-181a-5p, NC, or anti-miR-181a-5p were cultured in 6-well plates to 100% confluence. A yellow pipette tip was used to generate a wound area across the center of the well. Cell debris was removed by washing with phosphate-buffered saline, and cells were allowed to grow in the serum-free medium. The wound was assessed by a microscope (Nikon, Tokyo, Japan). Three wounds were made for each sample, and the migration distance was photographed and measured.

For the migration assays, 5×10^4 cells in serum-free medium were placed into the upper chamber of an insert (8 μm pore size; Millipore, Billerica, USA). The bottom of the insert was incubated in medium containing 15% FBS. The A549 cells were allowed to migrate for 12 h. The cells in the upper chamber were removed by cotton swab and the cells that had reached the underside of the membrane were fixed with 4% paraformaldehyde and stained with 10% Giemsa for 10 min. The cells that located on the underside of the filter (5 fields/filter) were counted. Experiments were independently repeated three times.

Bioinformatics methods

Given the limitations of any single prediction program, two separated prediction programs Targetscan (http://www.targetscan.org) and Pictar (http:// pictar.mdc-berlin.de/cgi-bin/new_PicTar_vertebrate.cgi.) were used to identify common predicted targets for miR-181a-5p.

Plasmid construction and luciferase activity assay

Two binding sites of miR-181a-5p were found in the 3′UTR of human Kras. In order to study which binding site is more important, three different 3′UTR fragments of Kras were cloned into the pGL3 vector. The first binding site of 3′UTR was named as Kras-1 (from 919 to 1198 bp). The second binding site of 3′UTR was named as Kras-2 (from 1191 to 1311 bp). In addition, another fragment Kras-(1 + 2) including the binding sites 1 and 2 (from 924 to 1318 bp) was cloned. The 3′UTR of Kras mRNA was generated by reverse transcription-PCR

using total RNA isolated from A549 cells. Mutant vectors (pGL3-Kras-3′UTR-mut) were generated using designed mutagenic oligonucleotide primers. Two oligonucleotides were used for the mutation of binding site 1. The sequences of primers were as follows: sense: 5′-TTTCTTACCAATTGCGGACGCTGGTGTGAAACAAATTAATGAAGCTTTT-3′; and antisense: 5′-TGTTTCACACCAGCGTCCGCAATTGGTAAGAAAAATAAGAAGTAATCA-3′. Another two oligonucleotides were used for binding site 2. Sense: 5′-GTTTGTCATCCCTGACGGACGCAAAGTTACACTGTTCACAAAGGTTTTGTCTCC-3′; and antisense: 5′-ACAGTGTAACTTTGCGTCCGTCAGGGATGACAAACTATAGGACATGAT-3′. The recognition sequence was underlined, and the seed sequence of miR-181a complementary sites, TGAATGT, was substituted by CGGACGC.

Luciferase reporter vectors, either wild-type (wt) or mutant (200 ng), together with pRL-SV40 (40 ng) (Promega, Madison, USA) were co-transfected with miRNA mimic at the final concentration of 150 nM using Lipofectamine 2000 (Invitrogen, Carlsbad, USA). Reporter assays were performed at 36 h post-transfection using the Dual-luciferase reporter assay system as described by the manufacturer (Promega). Relative luciferase activity was normalized with *Renilla* luciferase activity for each sample. Each experiment was performed in triplicates.

Western blot analysis

Total cellular proteins were isolated from cultured cells using lysis buffer and assayed by Western blot analysis according to the procedures as previously described [4,23]. In brief, protein determination was followed as the manufacturer's instruction. Equal amounts of protein samples were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinyl difluoride (PVDF) membranes (Millipore). Then, the PVDF membrane was incubated with primary antibody overnight, washed using TBST, and then incubated with a second antibody. The final chemiluminescence ECL (Millipore) was detected. The primary antibodies used were mouse anti-Kras (1:1000) and mouse anti-GAPDH (1:1000). The secondary antibody was horseradish peroxidase-conjugated rabbit anti-mouse IgG (1:10,000). All antibodies were purchased from Cell Signaling Technology (Danvers, USA).

Statistical analysis

Results were shown as the mean ± SEM and analyzed by using a Student's *t*-test for two-group comparisons and one-way ANOVA for multiple-group comparisons. Differences were considered statistically significant when $P < 0.05$.

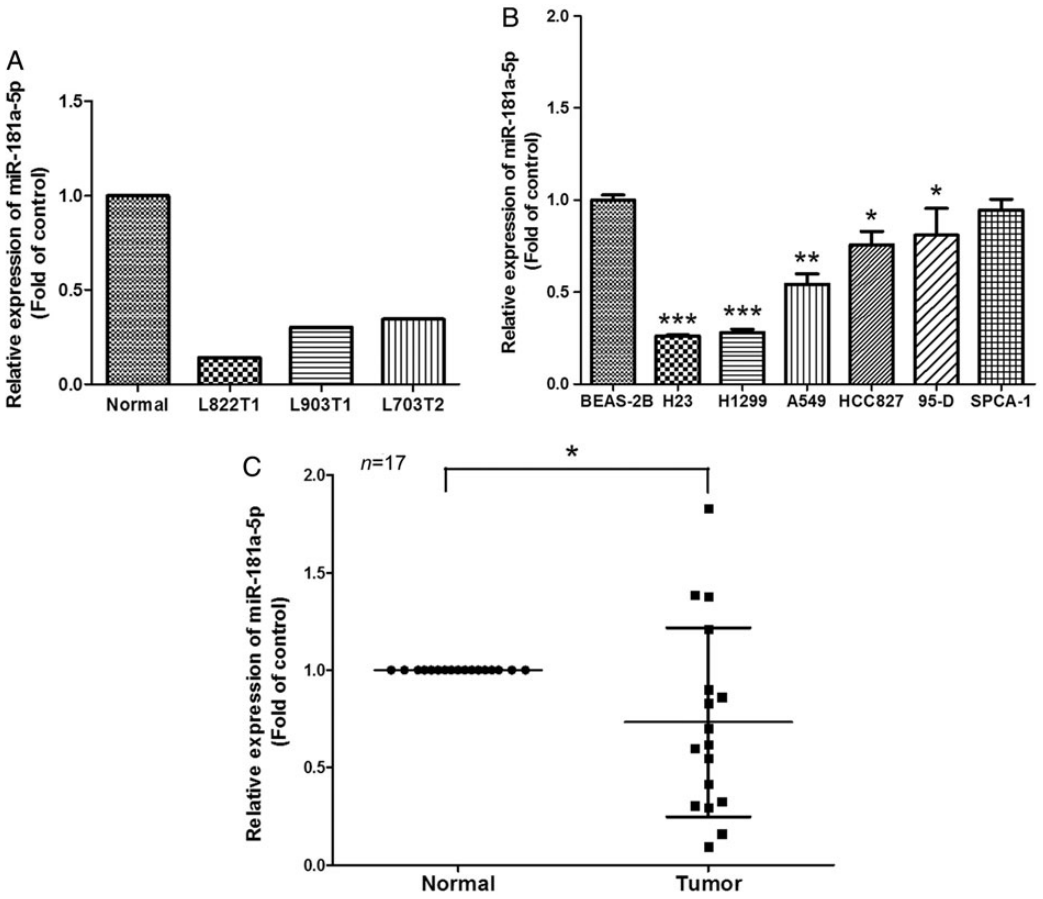

Figure 1. The expression level of miR-181a-5p in NSCLC tissues and cell lines (A) The expression of miR-181a-5p was determined by Solexa sequencing in lung tissues of the normal mouse and NSCLC mouse models. L822T1 ($Kras^{+/+}$), L903T1 ($Kras^{+/+}$ + $LKB1^{-/-}$), and L703T2 ($Kras^{+/+}$ + $p53^{-/-}$) were mouse NSCLC tissues. (B) The expression of miR-181a-5p in human cell lines, H23, H1299, A549, HCC827, 95-D, and SPCA-1 compared with the non-tumorigenic bronchial epithelial cell line (BEAS-2B).U6 was used as an internal control. (C) Relative expression of miR-181a-5p in NSCLC and corresponding paracancerous lung tissues ($n = 17$). Each assay was performed in triplicate. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Results

MiR-181a-5p expression is down-regulated in NSCLC tissues and human cell lines

The level of miR-181a-5p in lung tissues of the normal mouse and NSCLC models (L822T1/Kras$^{+/+}$, L903T1/Kras$^{+/+}$ + LKB1$^{-/-}$, and L703T2/Kras$^{+/+}$ + p53$^{-/-}$) has been analyzed by Solexa sequencing, and results show that miR-181a-5p is significantly down-regulated. The results showed that the expression level of miR-181a in all NSCLC mouse tissues was much lower than that in normal tissues (**Supplementary Table S1**). Then, miR-181a-5p expression was also detected in six human NSCLC cell lines by qRT-PCR. Our results showed that the expression level of miR-181a-5p was significantly down-regulated in five of the six NSCLC cell lines compared with BEAS-2B, especially in A549, H23, and H1299 cells (**Fig. 1B**). qRT-PCR analysis was further performed to examine the expression of miR-181a-5p in 17 human NSCLC samples and corresponding paracancerous lung tissues. MiR-181a-5p was significantly down-regulated in cancerous tissues compared with corresponding normal tissues (**Fig. 1C**). These results indicated that down-regulation of miR-181a-5p plays an important role in NSCLC progression and development.

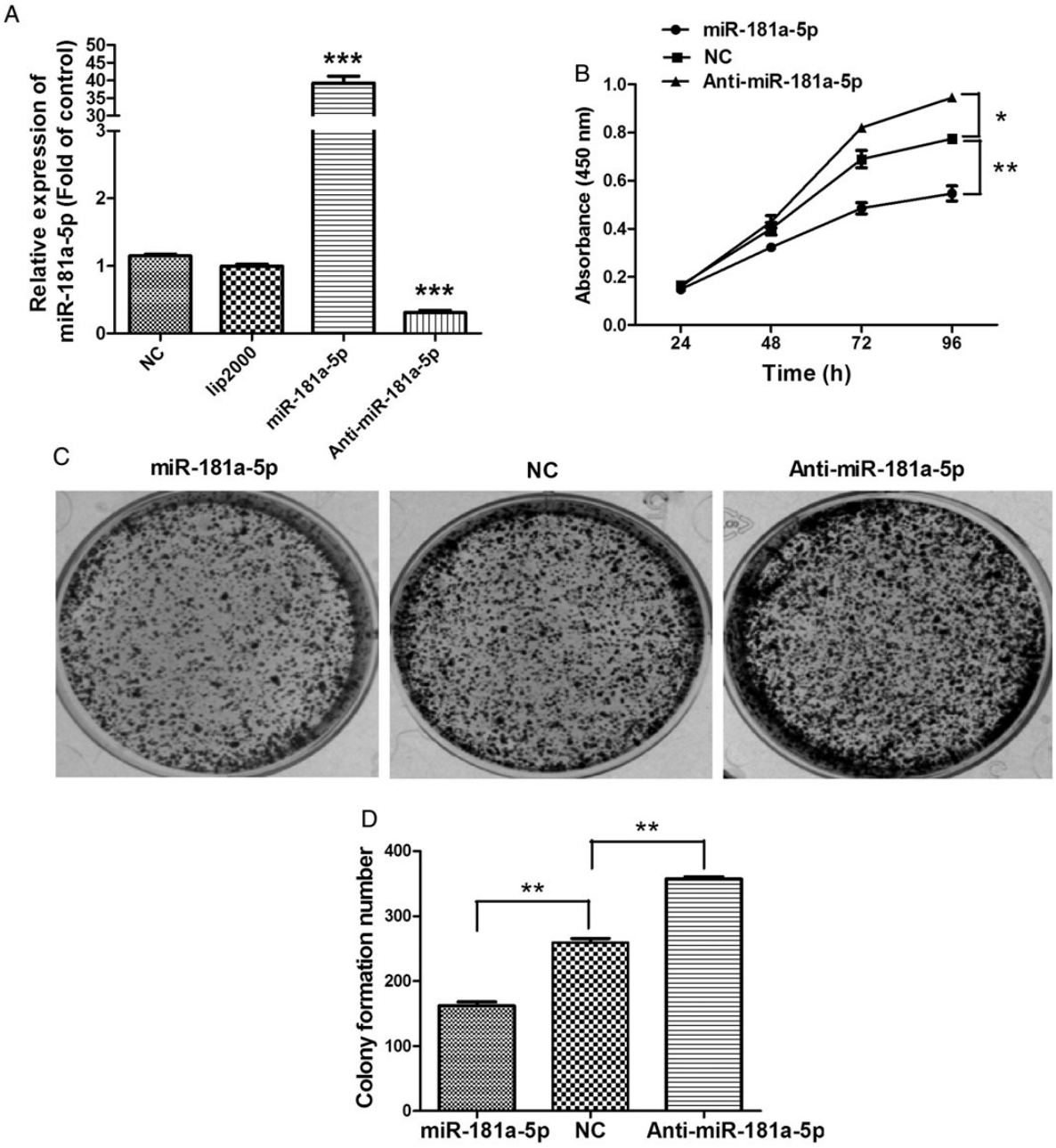

Figure 2. MiR-181a-5p inhibits cell proliferation and colony formation of A549 cells (A) MiR-181-5p mimic could significantly up-regulate miR-181-5p level, whereas miR-181-5p inhibitor could significantly down-regulate the miR-181-5p level in A549 cells. (B) The proliferation of A549 cell line was determined by CCK-8 assay after transfection with miR-181-5p mimic, anti-miR-181-5p, or NC within 96 h. (C) Colony formation assay of A549 cells transfected with miR-181-5p mimic, anti-miR-181-5p, or NC. (D) Schematic diagram for colony formation. *P < 0.05, **P < 0.01, and ***P < 0.001. NC, control.

MiR-181a-5p inhibits the cell proliferation of A549 cells

MiR-181a-5p mimic or miR-181a-5p inhibitor was transfected into A549 cell line. Forty-eight hours later, the cells were collected for detecting miR-181a-5p levels by qRT-PCR analysis. Results showed that miR-181a-5p mimic could significantly up-regulate miR-181a-5p level in A549 cells, whereas miR-181a-5p inhibitor down-regulated miR-181a-5p level (**Fig. 2A**). Then, the effects of miR-181a-5p mimic or inhibitor on the proliferation of A549 cells were determined *in vitro*. Results showed that A549 cells transfected with miR-181a-5p mimic grew more slowly than those cells transfected with NC (**Fig. 2B**).

To determine the tumor suppressive effect *in vitro*, the effect of miR-181a-5p on colony formation was examined. For colony formation assay, 7 days after seeding equal cell number, cells were stained with crystal violet. Results showed that miR-181a-5p could significantly decrease the colony formation in A549 cells (**Fig. 2C,D**).

MiR-181a-5p reduces the migration of A549 cells

Cell migration is a significant aspect of cancer progression. The effect of miR-181a-5p on the migration ability of A549 cell was assessed by wound healing assay and transwell method. In wound healing assay, cells transfected with miR-181a-5p migrated toward the wound at a much slower rate than the NC group cells, with cell migration index of 18 and 30%, respectively. In contrast, cells transfected with anti-miR-181a-5p has a higher mobility than these two groups of cells, with cell migration index ~45% after 48 h (**Fig. 3A,C**). In

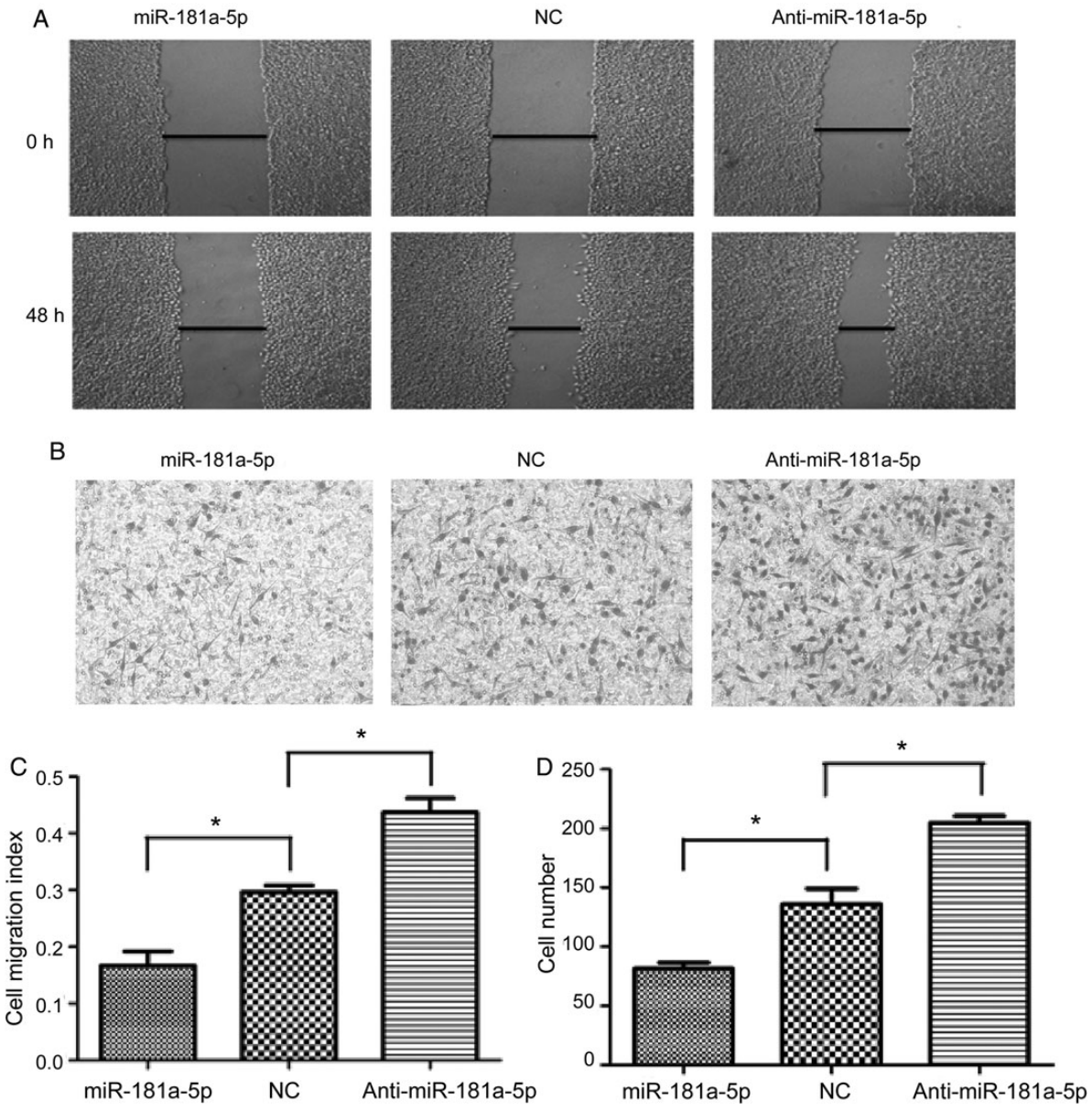

Figure 3. MiR-181-5p negatively regulates cell migration of A549 cells *in vitro* (A) A549 cells transfected with miR-181a-5p or anti-miR-181a were subject to wound healing assay and images were taken at 0 and 48 h. (B) Transwell migration assays of A549 cells transfected with miR-181a-5p or anti-miR-181a-5p 48 h later. The migrated cells were stained with crystal violet for 30 min and photographed. (C) Cells that migrated into the wound areas were counted and analyzed. (D) The migrated cells were counted and analyzed. *$P < 0.05$. NC, control.

transwell assay, cells were seeded in serum-free medium on the top chamber of a two-chamber trans-well cell culture plate, and the cells migrated to the lower chamber after 48 h were photographed and analyzed. Results showed that miR-181a-5p could inhibit A549 cell migration and anti-miR-181a-5p could promote A549 cell migration (**Fig. 3B,D**).

These results, taken together, clearly demonstrated that miR-181a-5p could significantly reduce the migration of A549 cells.

MiR-181a-5p directly targets Kras gene

To explore the molecular mechanism by which miR-181a-5p contributes to the proliferation of NSCLC cells, potential targets were predicted by TargetScan and PicTar. First, eight candidate genes were chosen according to the prediction score. Five of them were down-regulated (**Supplementary Fig. S1**). Kras was selected for further analysis, owing to its relatively high prediction score and as an important oncogene in cancer. To determine whether Kras mRNA is a direct

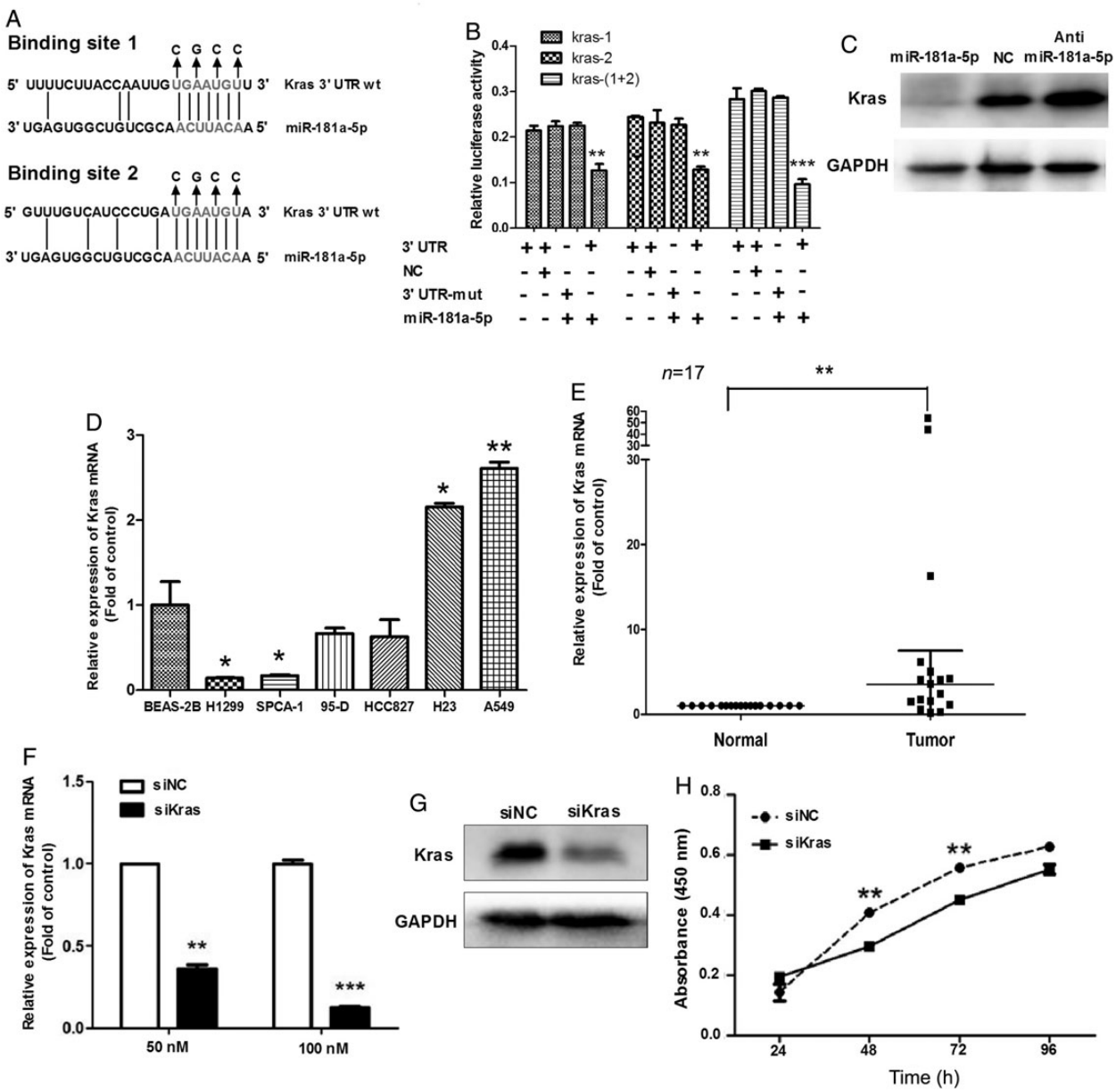

Figure 4. MiR-181a-5p down-regulates Kras expression by directly targeting its 3′UTR (A) Kras 3′UTR and corresponding fragments were inserted into the region immediately downstream of the luciferase gene in pGL3 vector and validated by DNA sequencing. The sequences of predicted miR-181a-5p binding sites within the Kras 3′UTR, including wild-type UTR or UTR segments containing mutant (shown as arrows) binding site are shown. (B) Relative luciferase activity was analyzed after co-transfection of miR-181a-5p or NC with the above reported plasmids along with an endogenous control Renilla luciferase pRL-TK vector in HEK293T cells. (C) Kras protein levels were examined in A549 cells transfected with miR-181a-5p mimic, NC, or anti-miR-181a-5p by western blot using GAPDH as a loading control. (D) The expression level of Kras mRNA in human NSCLC cell lines, H23, H1299, A549, HCC827, 95-D, and SPCA-1. BEAS-2B was the non-tumorigenic control. Data were performed through qRT-PCR. 18S RNA was used as an internal control. (E) Kras mRNA expression levels in NSCLC tissues. (F) qRT-PCR analysis of Kras mRNA in A549 cells transfected with Kras siRNA or siNC. (G) Western blot analysis of Kras protein expression in A549 cells transfected with Kras siRNA or siNC. (H) CCK-8 analysis of the proliferation in A549 cells at different time points (24, 48, 72, and 96 h) after transfection with Kras siRNA or siNC. Each experiment was performed at least in triplicate.*$P<0.05$, **$P<0.01$, and ***$P<0.001$. NC, control.

target of miR-181a-5p, 3′UTR of Kras mRNA was subcloned to downstream of the luciferase gene in pGL3 reporter plasmid. To confirm that miR-181a-5p targets Kras mRNA through one or two pairing sites in 3′UTR, we introduce TGAATGT → CGGACGC mutation in these regions in order to disrupt miRNA/mRNA interaction (**Fig. 4A**). Results of luciferase activity indicated that two mutated 3′UTR affect the binding of miRNA. Co-transfection experiments showed that miR-181a-5p mimic, but not the NC, inhibited luciferase activity in A549 cells (**Fig. 4B**). Then, whether miR-181a-5p affects the expression of endogenous Kras at translational levels was determined. As shown in **Fig. 4C**, western blot analysis showed that the level of Kras protein expression in miR-181a-5p mimic transfected A549 cells was significantly inhibited compared with that in NC-transfected cells. Furthermore, correlation analysis revealed that low expression of Kras was more likely correlated with high levels of miR-181a-5p. The expression of Kras was also examined in lung cancer cell lines. Results showed that Kras mRNA level in A549 cells was much higher than that in BEAS-2B cells (**Fig. 4D**), and miR-181a-5p was lower in A549 cells (**Fig. 1B**). However, Kras had lower expression in human NSCLC H1299 and SPCA-1 than in BEAS-2B cells (**Fig. 4D**).

As miR-181a-5p is under-expressed in NSCLC and targets Kras by binding to its 3′UTR, whether Kras expression was negatively associated with miR-181a-5p was investigated in primary NSCLC patient tissues. Analysis of Kras expression level in 17 NSCLC tissues and corresponding normal tissues by qRT-PCR revealed that Kras was significantly up-regulated in NSCLC (**Fig. 4E**).

SiRNA targeting Kras was used to down-regulate the expression and investigated its effects on NSCLC cells. The expression levels of Kras mRNA and protein were significantly down-regulated in A549 cells transfected with Kras siRNA compared with that in control siRNA transfected A549 cells, respectively (**Fig. 4F,G**). CCK-8 assay results indicated that the proliferation rate of A549 cells transfected with Kras siRNA was significantly lower than that of control cells (**Fig. 4H**). These data showed that siRNA-mediated Kras down-regulation could mimic the effects of miR-181a-5p up-regulation in NSCLC cells. All these results demonstrated that miR-181a-5p could directly target Kras by interaction with the 3′UTR of Kras gene in A549 cells.

Discussion

Since miRNAs commonly have multiple target genes, the frequent aberrant expression of miRNAs implies that they have tumor suppressor or oncogene function. A single miRNA can affect multiple cellular processes in tumorigenesis [24,25]. As summarized in **Fig. 5**, miR-181a-5p has been shown to target multiple cancer-related genes and processes. The aberrant expression of miR-181a-5p is a frequent event in various cancers, suggesting that miR-181a-5p may play an important role in tumorigenesis and tumor progression. Indeed, several key suppressor functions have been attributed to miR-181a-5p in the context of tumorigenesis (**Fig. 5**). In breast cancer and gastric cancer, miR-181a over-expression suppressed cell migration and invasion by directly regulating UPA, BCL2L11, and KLF6 [26–28]. Alteration of miR-181a expression promoted apoptosis in colorectal cancer [6]. Conversely, in cervical cancer and chronic myelogenous leukemia, high levels of miR-181a were found to inhibit cancer cell apoptosis [29,30]. Moreover, miR-181a was down-regulated in oral squamous cell carcinoma, and its over-expression suppressed the cell proliferation [31]. In other human cancers, miR-181a was also found to inhibit the growth of tumor cells during the process of cancer development and progression, including malignant glioma [32] and T-cell acute lymphoblastic leukemia [33].

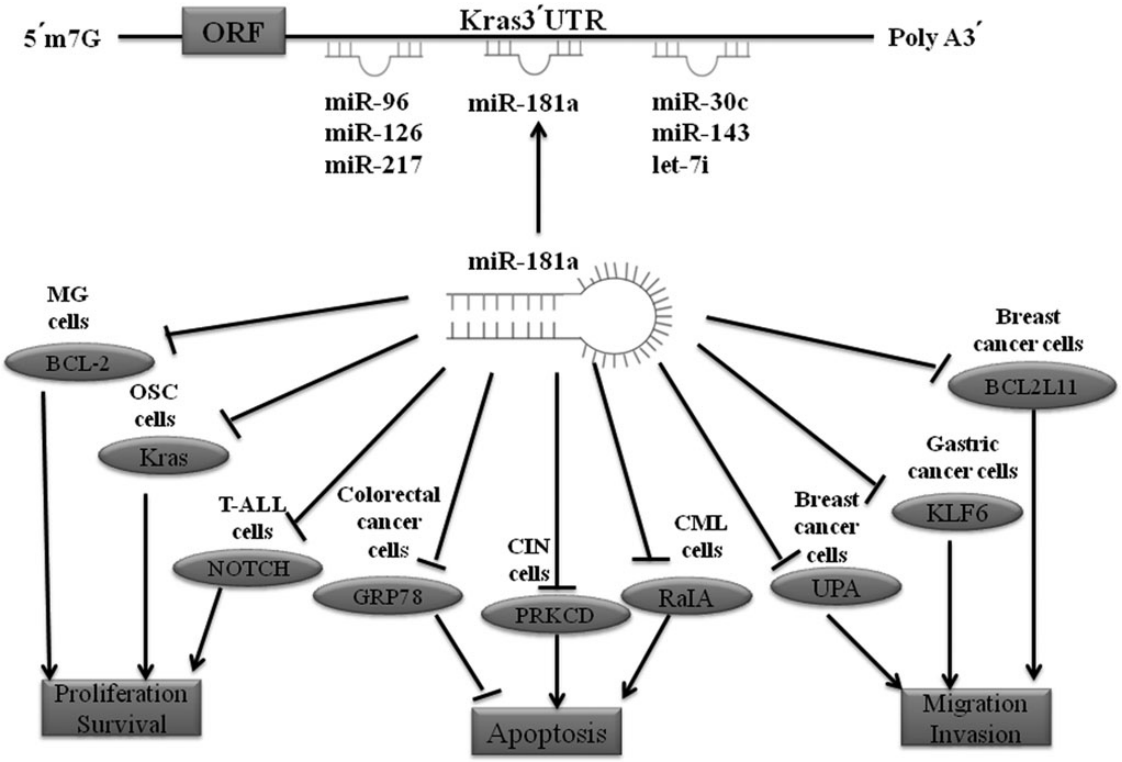

Figure 5. The multiple roles of miR-181a in hallmarks of human cancer

In this study, we analyzed miRNA expression profiles in mouse NSCLC tissues by Solexa sequencing, and identified miR-181a-5p as a significantly down-regulated miRNA compared with the normal mice lung tissues. The expression of miR-181a-5p was further examined in tissues from NSCLC patients. Consistent with the Solexa analysis, miR-181a-5p was dramatically down-regulated in NSCLC tissues. Our results also demonstrated that miR-181a-5p significantly inhibited the cell proliferation and migration of A549 cells, which is consistent with results obtained in oral squamous cell carcinoma [31]. Kras has been verified as a direct target of miR-181a-5p, and it has been demonstrated that miR-181a-5p significantly reduces Kras expression at both mRNA and protein levels in NSCLC cells. Kras is associated with increased cell growth and differentiation in many cancers [34,35]. In addition, Yoon *et al*. [20] showed that mutation of Kras plays an important role in signal transduction in NSCLC cancer.

MiR-181a-5p mediates the inhibition of NSCLC by targeting of multiple targets, including Kras; however, its mechanism should be investigated in the future. Further studies of miR-181a-5p will undoubtedly enhance the understanding of how miR-181a-5p functions in regulating NSCLC cell growth and metastasis.

In conclusion, we found that miR-181a-5p was down-regulated in NSCLC, and that miR-181a-5p could significantly inhibit NSCLC by targeting oncogene Kras. These results indicated a suppressive function of miR-181a in the progression of NSCLC, and implicated its potential application in NSCLC therapy.

Supplementary Data

Supplementary data is available at *ABBS* online.

Funding

This work was supported in part by the grants from the National Natural Science Foundation of China (Nos. 31170750 and 31100570), the National Basic Research Program of China (No. 2011CBA01105), and the State Key Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011, 61: 69–90.
2. Zandberga E, Kozirovskis V, Abols A, Andrejeva D, Purkalne G, Line A. Cell-free microRNAs as diagnostic, prognostic, and predictive biomarkers for lung cancer. *Genes Chromosomes Cancer* 2013, 52: 356–369.
3. Vannini I, Fanini F, Fabbri M. MicroRNAs as lung cancer biomarkers and key players in lung carcinogenesis. *Clin Biochem* 2013, 46: 918–925.
4. Wang DT, Ma ZL, Li YL, Wang YQ, Zhao BT, Wei JL, Qiu X, *et al*. miR-150, p53 protein and relevant miRNAs consist of a regulatory network in NSCLC tumorigenesis. *Oncol Rep* 2013, 30: 492–498.
5. Xing L, Su J, Guarnera MA, Zhang H, Cai L, Zhou R, Stass SA, *et al*. Sputum microRNA biomarkers for identifying lung cancer in indeterminate solitary pulmonary nodules. *Clin Cancer Res* 2015, 21: 484–489.
6. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005, 120: 15–20.
7. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, *et al*. MicroRNA expression profiles classify human cancers. *Nature* 2005, 435: 834–838.
8. Zhang WC, Liu J, Xu X, Wang G. The role of microRNAs in lung cancer progression. *Med Oncol* 2013, 30: 675.
9. Frampton AE, Krell J, Jacob J, Stebbing J, Castellano L, Jiao LR. Loss of miR-126 is crucial to pancreatic cancer progression. *Expert Rev Anticancer Ther* 2012, 12: 881–884.
10. Chen X, Guo X, Zhang H, Xiang Y, Chen J, Yin Y, Cai X, *et al*. Role of miR-143 targeting KRAS in colorectal tumorigenesis. *Oncogene* 2009, 28: 1385–1392.
11. Bhatt K, Mi QS, Dong Z. microRNAs in kidneys: biogenesis, regulation, and pathophysiological roles. *Am J Physiol Renal Physiol* 2011, 300: F602–F610.
12. Liu CG, Calin GA, Volinia S, Croce CM. MicroRNA expression profiling using microarrays. *Nat Protocols* 2008, 3: 563–578.
13. Ji DB, Chen ZG, Li M, Zhan TC, Yao YF, Zhang ZQ, Xi JZ, *et al*. MicroRNA-181a promotes tumor growth and liver metastasis in colorectal cancer by targeting the tumor suppressor WIF-1. *Mol Cancer* 2014, 13: 86.
14. Korhan P, Erdal E, Atabey N. miR-181a-5p is downregulated in hepatocellular carcinoma and suppresses motility, invasion and branching-morphogenesis by directly targeting c-Met. *Biochem Biophys Res Commun* 2014, 450: 1304–1312.
15. Liu M, Wang JG, Huang HZ, Hou JS, Zhang B, Wang AX. miR-181a-Twist1 pathway in the chemoresistance of tongue squamous cell carcinoma. *Biochem Biophys Res Commun* 2013, 441: 364–370.
16. Xiang Z, Dong X, Sun Q, Li X, Yan B. Clinical significance of up-regulated miR-181a in prognosis and progression of esophageal cancer. *Acta Biochim Biophys Sin* 2014, 46: 1007–1010.
17. Jeon MJ, Kim EJ, Lee M, Kim H, Choi JR, Chae HD, Moon YJ, *et al*. MicroRNA-30d and microRNA-181a regulate HOXA11 expression in the uterosacral ligaments and are overexpressed in pelvic organ prolapse. *J Cell Mol Med* 2015, 19: 501–509.
18. Won YS, Jeong JS, Kim SJ, Ju MH, Lee SW. Targeted anticancer effect through microRNA-181a regulated tumor-specific hTERT replacement. *Cancer Lett* 2015, 356: 918–928.
19. Rajalingam K, Schreck R, Rapp UR, Albert S. Ras oncogenes and their downstream targets. *Biochim Biophys Acta* 2007, 1773: 1177–1195.
20. Yoon YK, Kim HP, Han SW, Oh do Y, Im SA, Bang YJ, Kim TY. KRAS mutant lung cancer cells are differentially responsive to MEK inhibitor due to AKT or STAT3 activation: implication for combinatorial approach. *Mol Carcinog* 2010, 49: 353–362.
21. Ali S, Ahmad A, Aboukameel A, Bao B, Padhye S, Philip PA, Sarkar FH. Increased Ras GTPase activity is regulated by miRNAs that can be attenuated by CDF treatment in pancreatic cancer cells. *Cancer Lett* 2012, 319: 173–181.
22. Tanic M, Yanowsky K, Rodriguez-Antona C, Andres R, Marquez-Rodas I, Osorio A, Benitez J, *et al*. Deregulated miRNAs in hereditary breast cancer revealed a role for miR-30c in regulating KRAS oncogene. *PLoS ONE* 2012, 7: e38847.
23. Ma ZL, Hou PP, Li YL, Wang DT, Yuan TW, Wei JL, Zhao BT, *et al*. MicroRNA-34a inhibits the proliferation and promotes the apoptosis of non-small cell lung cancer H1299 cell line by targeting TGFbetaR2. *Tumour Biol* 2014, 36: 2481–2490.
24. Sharma M, Juvvuna PK, Kukreti H, McFarlane C. Mega roles of microRNAs in regulation of skeletal muscle health and disease. *Front Physiol* 2014, 5: 239.
25. Valastyan S, Weinberg RA. Roles for microRNAs in the regulation of cell adhesion molecules. *J Cell Sci* 2011, 124: 999–1006.
26. Noh H, Hong S, Dong Z, Pan ZK, Jing Q, Huang S. Impaired microRNA processing facilitates breast cancer cell invasion by upregulating urokinase-type plasminogen activator expression. *Genes Cancer* 2011, 2: 140–150.
27. Taylor MA, Sossey-Alaoui K, Thompson CL, Danielpour D, Schiemann WP. TGF-beta upregulates miR-181a expression to promote breast cancer metastasis. *J Clin Investigat* 2013, 123: 150–163.
28. Zhang X, Nie Y, Du Y, Cao J, Shen B, Li Y. MicroRNA-181a promotes gastric cancer by negatively regulating tumor suppressor KLF6. *Tumour Biol* 2012, 33: 1589–1597.
29. Fei J, Li Y, Zhu X, Luo X. miR-181a post-transcriptionally downregulates oncogenic RalA and contributes to growth inhibition and apoptosis in chronic myelogenous leukemia (CML). *PLoS ONE* 2012, 7: e32834.

30. Ke G, Liang L, Yang JM, Huang X, Han D, Huang S, Zhao Y, *et al*. MiR-181a confers resistance of cervical cancer to radiation therapy through targeting the pro-apoptotic PRKCD gene. *Oncogene* 2013, 32: 3019–3027.
31. Shin KH, Bae SD, Hong HS, Kim RH, Kang MK, Park NH. miR-181a shows tumor suppressive effect against oral squamous cell carcinoma cells by down-regulating K-ras. *Biochem Biophys Res Commun* 2011, 404: 896–902.
32. Chen G, Zhu W, Shi D, Lv L, Zhang C, Liu P, Hu W. MicroRNA-181a sensitizes human malignant glioma U87MG cells to radiation by targeting Bcl-2. *Oncol Rep* 2010, 23: 997–1003.
33. Fragoso R, Mao T, Wang S, Schaffert S, Gong X, Yue S, Luong R, *et al*. Modulating the strength and threshold of NOTCH oncogenic signals by mir-181a-1/b-1. *PLoS Genet* 2012, 8: e1002855.
34. Friday BB, Adjei AA. K-ras as a target for cancer therapy. *Biochim Biophys Acta* 2005, 1756: 127–144.
35. Arvanitakis M, Van Laethem JL, Parma J, De Maertelaer V, Delhaye M, Deviere J. Predictive factors for pancreatic cancer in patients with chronic pancreatitis in association with K-ras gene mutation. *Endoscopy* 2004, 36: 535–542.