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MicroRNA-375 inhibits colorectal cancer growth by targeting PIK3CA

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ABSTRACT

Colorectal cancer (CRC) is the second most common cause of death from cancer. MicroRNAs (miRNAs) represent a class of small non-coding RNAs that control gene expression by triggering RNA degradation or interfering with translation. Aberrant miRNA expression is involved in human disease including cancer. Herein, we showed that miR-375 was frequently down-regulated in human colorectal cancer cell lines and tissues when compared to normal human colon tissues. PIK3CA was identified as a potential miR-375 target by bioinformatics. Overexpression of miR-375 in SW480 and HCT15 cells reduced PIK3CA protein expression. Subsequently, using reporter constructs, we showed that the PIK3CA untranslated region (3′-UTR) carries the directly binding site of miR-375. Additionally, miR-375 suppressed CRC cell proliferation and colony formation and led to cell cycle arrest. Furthermore, miR-375 overexpression resulted in inhibition of phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. SiRNA-mediated silencing of PIK3CA blocked the inhibitory effect of miR-375 on CRC cell growth. Lastly, we found overexpressed miR-375 effectively repressed tumor growth in xenograft animal experiments. Taken together, we propose that overexpression of miR-375 may provide a selective growth inhibition for CRC cells by targeting PI3K/Akt signaling pathway.

1. Introduction

Colorectal cancer (CRC) is the leading cause of cancer deaths in both men and women, accounting $50,830 deaths per year in the United States [1]. Despite significant advances in the management of CRC, survival of patients with advanced CRC has changed little over the past 10 years. Therefore, further elucidation of the molecular mechanisms underlying CRC tumorigenesis and development of therapeutic targets are crucial for this deadly disease.

MicroRNAs (miRNAs) are endogenous short noncoding RNAs that regulate gene expression through binding to complementary sequences, preferentially 3′-UTR regions of mRNAs. miRNAs are significantly involved in the regulation of cellular functions, such as metabolism, differentiation, proliferation and death [2–4], and have been recognized as important regulators in human cancers including CRC [5,6]. miRNAs, functioning as either tumor-suppressors or oncogenes, are useful as both biomarkers for the diagnosis and as therapeutic targets for the cancer treatment.

Deregulated miR-375 expression has been indicated in various cancer types including oral carcinoma [7], breast cancer [8], esophageal adenocarcinoma [8], lung cancer [9], glioma [10], gastric cancer [11] and hepatocellular carcinoma [12] Chang et al. Interestingly, miR-375 can function as tumor suppressors in some cancer types such as oral cancer [13], ovarian cancer [14], cervical cancer [15] and gastric cancer [11]. In contrast, miR-375 may also function as an oncogene in breast cancer [16], prostate cancer [17], and other cancer types. Recent prolife studies have showed that the expression of miR-375 is significantly decreased in CRC tumor tissues [18]. In this study, we found that miR-375 was downregulated in CRC cell lines and tissues. We identified PIK3CA as a novel target of miR-375 in CRC cells. Furthermore, ectopic expression of miR-375 in CRC cells suppressed cell growth. miR-375 functions as a tumor suppressor by downregulating phosphatidylinositol 3-kinase (PI3K)/Akt pathway. In an *in vivo* xenograft model, we showed miR-375 significantly decreased the tumor volumes and weights. Our data provided a potential diagnostic and therapeutic target for CRC treatment.

2. Materials and methods

2.1. Samples

Paired human normal and malignant colorectal tissues were obtained after review and approval by Third Affiliated Hospital of Harbin Medical University between January 2011 and November 2012. None of the patients in the study received any chemotherapy or radiation therapy before surgery. Written informed consent was

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obtained from all patients. Clinicopathologic information was available and two pathologists independently determined diagnoses.

2.2. Cell lines

The human colorectal cancer cell lines Caco2, SW480, HT29, HCT15 and SW620 were obtained from American Type Culture Collection (ATCC). The cell lines were cultivated in DMEM/F12 1:1 modified medium supplemented with 10% FBS (Invitrogen, Carlsbad, CA) under recommended conditions, and the cells were detached with trypsin–EDTA (Invitrogen).

2.3. Plasmids, transfections, and luciferase assay

miR-375 was purchased from Shanghai Gene-Pharma Co (Shanghai, China), along with the negative control (NC). 3′-UTR of *PIK3CA* was PCR-amplified from SW480 genomic DNA and cloned downstream of luciferase gene in pGL vector (Promega, Madison, WI). Mutant vectors containing five mutated bases on the separately predicted miR-375 binding sites were constructed using the site-directed mutagenesis kit (Stratagene, La Jolla, CA). PIK3CA gene with full length wild-type 3′-UTR (PIK3CA-WtUTR) and PIK3CA gene with mutant 3′-UTR on 1–5 miRNA-375 binding sites (PIK3CA-MutUTR) were constructed into pcDNA3.1 vector. miRNA was transfected with wild-type or mutant *PIK3CA* 3′-UTR plasmids into SW480 cells, using X-tremeGENE (Roche, Penzberg, Germany). Cell lysates were harvested 48 h after transfection. Luciferase activity was measured using dual-luciferase reporter system (Promega). The Renilla activity was used as an internal control. Each transfection was performed in triplicate.

2.4. Lentivirus production and transduction

The miR-375 sequences were cloned into the pGCSIL-GFP lentiviral vector. Before transduction, lentivirus was filtered through a 0.45 μm low protein binding-polysulfonic filter (Millipore, Bedford, MA) and concentrated with Optima™L-100 XP ultracentrifuge (Beckman Coulter, Miami, FL). SW480 or HCT15 cells were infected with 20 MOI of miR-375-expressing lentivirus (Lv-miR-375) or control lentivirus (Lv-NC) by spin infection for 2 h, followed by incubation at 37 °C for 2 h.

2.5. Protein isolation and Western blot

Total proteins were extracted with RIPA lysis buffer with proteinase/phosphotase inhibitors (Thermo Scientific, Hudson, NH). Lysate was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the gel was blotted onto PVDF membrane (Millipore). The membrane was blocked in 5% non-fat milk, and then incubated with one of the following antibodies: anti-PIK3CA, anti-p-Akt, anti-Akt, anti-p-mTOR and anti-mTOR or anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA). Subsequent to being incubated with anti-rabbit or anti-mouse secondary antibodies (Santa Cruz Biotechnology) for 1 h, the immune complexes were detected using the enhanced chemiluminescence (ECL) method.

2.6. RNA extraction and real time PCR (RT-PCR)

Total miRNA was extracted using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Complementary DNA was synthesized from 5 ng of total RNA, using the Taqman miRNA reverse transcription kit (Applied Biosystems, Foster City, CA). The expression levels of miR-375 were quantified using the miRNA-specific TaqMan miRNA assay kit (Applied Biosystems) and calculated using the $2^{-\Delta\Delta Ct}$ method, with U6 snRNA as endogenous control.

2.7. Cell growth assay

Lv-miR-375 or Lv-NC infected cells (2000/well) were seeded into 96-well culture plates and cell growth was measured at 0–5 days following addition of 0.5 mg/ml MTT solution (Sigma, St. Louis, MO). Absorbance was then recorded at 490 nm using a microplate reader (Bio-Rad, Hercules, CA). In addition, total cell counts were determined by trypan blue exclusion method using a hemocytometer. The data presented are from three independent experiments.

2.8. Colony formation assay

Lv-miR-375 or Lv-NC infected cells were placed in a fresh six-well plate and maintained in DMEM containing 10% FBS. In 24 h, the medium was replaced with new medium. After 14 days, cells were fixed with methanol and stained with 0.1% crystal violet. Visible colonies were manually counted.

2.9. Cell cycle analysis

Cells were trypsinized and fixed with 70% ethanol at 4 °C overnight before being stained with propidium iodide (PI). DNA contents were detected by LSRII flow cytometer (BD Biosciences, San Jose, CA). Data were analyzed by Flow Jo (Tree Star, Ashland, OR).

2.10. Animals and subcutaneous tumor growth

Male athymic BALB/c nude mice (12 weeks old, 18–20 g) were purchased from Beijing Wei-tong Li-hua Laboratory Animals and Technology Ltd. All surgical and care procedures administered to the animals were in accordance with Harbin Medical University institutional guidelines. The animals were bred in specific pathogen-free condition in mesh cages under controlled conditions of temperature (23 °C ± 3 °C) and relative humidity (50% ± 20%), with 10–15 air changes per hour and light illumination for 12 h a day. The animals were allowed access to food and tap water ad libitum throughout the acclimatization and experimental periods. Lv-miR-375-SW480 cells or Lv-NC-SW480 cells suspension (1×10^6) in 100 μl were injected s.c. into the right scapular region of nude mice. Tumor size was determined every 5 days by caliper measurement of two perpendicular diameters of the implants. The tumor volume (mm^3) was calculated according to the formula: volume ($mm^3 = 1/2 \times \text{length} \times \text{width}^2$). The tumorbearing mice were sacrificed on day 25.

2.11. Statistics

All values are expressed as mean ± SEM. Differences between groups were analyzed by one way ANOVA followed by Bonferroni post hoc analyses as appropriate. A P value less than 0.05 was considered significant.

3. Results

3.1. Downregulation of miR-375 in CRC cell lines and tissues

To investigate the role of miR-375 in human CRC, we first examined the expression levels of miR-375 in CRC cell lines. RT-PCR analysis showed that the expression of miR-375 was markedly downregulated in all five CRC cell lines, as compared with the

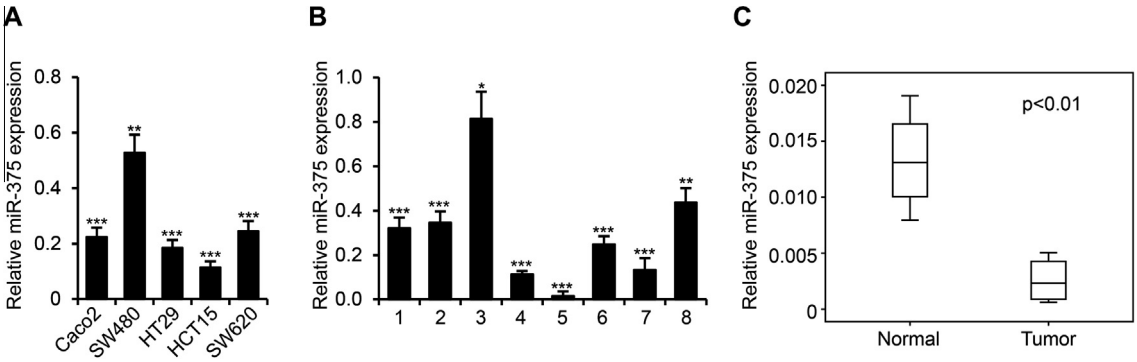

Fig. 1. Downregulation of miR-375 expression in human colorectal cancer (CRC) cell lines and tissues. (A) Real time PCR analysis of relative miR-375 expression in five CRC cell lines. Transcript levels were normalized to *U6* expression and data are shown as the relative levels compared with eight normal colon specimens. (B) The expression levels of miR-375 were examined in primary CRC tissues with paired adjacent normal tissues of eight individual patients. Transcript levels were normalized to *U6* expression and data are shown as the relative levels compared with corresponding normal colon specimen. (C) miR-375 was downregulated in CRC tissues compared with that in matched normal tissues (n = four normal samples, n = four CRC samples; NCBI/GEO/GSE45349, $P < 0.05$). Data (A and B) are shown as mean ± SEM of three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$.

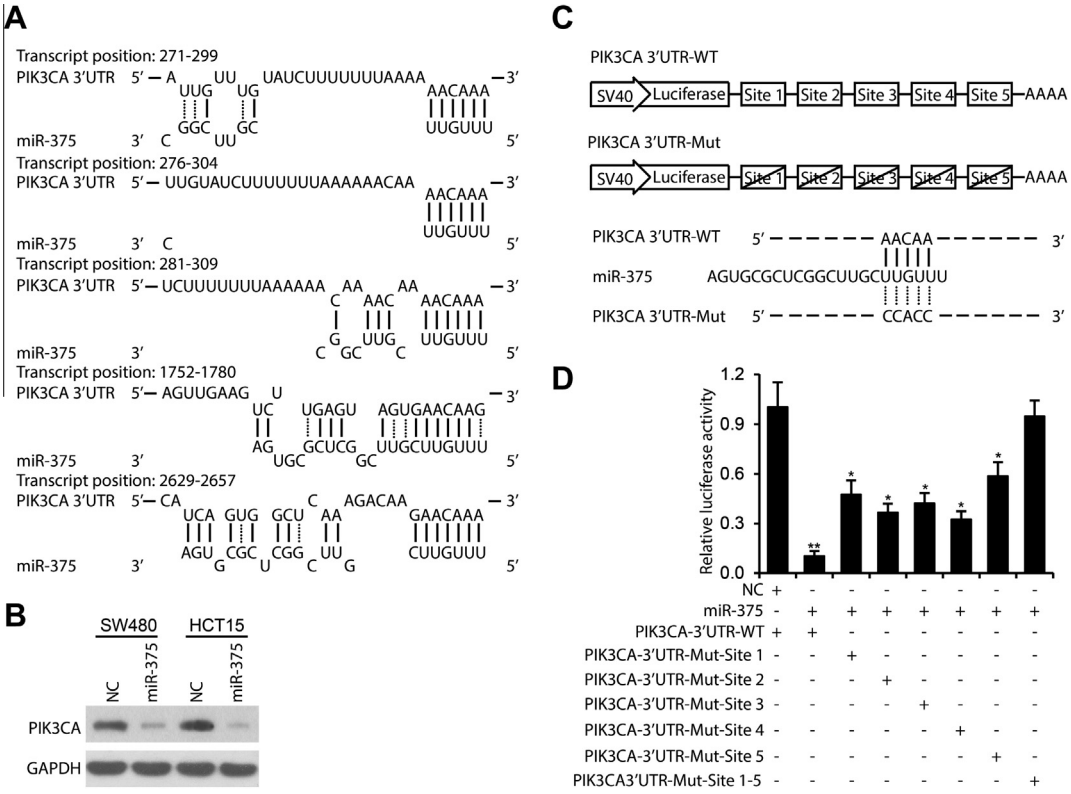

Fig. 2. PIK3CA is direct target of miR-375. (A) Schematic representation of five miR-375 putative target sites in *PIK3CA* 3′-UTR. (B) Western blot analysis of PIK3CA protein levels in response to miR-375 overexpression in SW480 (left) and HCT15 (right) cells. GAPDH was used as loading control. (C) Schematic representations of the luciferase reporter constructs containing wild-type or mutated *PIK3CA* 3′-UTR. (D) Relative luciferase activity of SW480 cells transfected with plasmids carrying wild-type or mutant 3′-UTR of *PIK3CA* gene and miR-375. $n = 4$. NC: negative control. Data are shown as mean ± SEM of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with NC.

normal colon tissue samples (Fig. 1A). Next, to explore whether downregulation of miR-375 in CRC cell lines is clinical relevant, we compared the expression levels of miR-375 between clinical CRC and paired adjacent non-neoplastic tissue specimens from eight patients. As shown in Fig. 1B, miR-375 expression was lower in all eight CRC tumor samples, compared with those of adjacent normal tissues. Consistently with our results, a published database (NCBI/GEO/GSE45349) showed similar downregulation of miR-375 in CRC tumor tissues (Fig. 1C; $P < 0.01$). These results indicate that the reduced miR-375 expression is a frequent event in human CRC cells and tissues, which may be involved in CRC progression.

3.2. PIK3CA is a direct target of miR-375 in CRC cells

Using DIANA-microT web server v5.0 [19,20], we identified PIK3CA as a potential target of miR-375. There are five putative binding sites of miR-375 broadly conserved in vertebrates *PIK3CA* 3′-UTR (Fig. 2A). In order to prove that miR-375 targets *PIK3CA* 3′-UTR, we transiently transfected SW480 cells with miR-375 or control miRNA. Notably, the expression of PIK3CA substantially decreased 48 h after miR-375 was transfected (Fig. 2B). Furthermore, we performed luciferase reporter assay. Wild-type full length 3′-UTR of *PIK3CA* was cloned into the downstream of luciferase

gene in pGL3 vector. In addition, mutant vectors containing six mutated bases on the predicted binding sites were constructed and as shown in Fig. 2C. As expected, miR-375, rather than NC significantly suppressed the luciferase activity of reporter genes containing wild-type *PIK3CA* 3′-UTR. Moreover, the inhibition was partially rescued when one of the binding sites was mutated or almost fully relieved with all sites mutated (Fig. 2D). These results indicate that miR-375 directly targets PIK3CA in CRC cells.

3.3. Overexpression of miR-375 inhibits CRC cell growth

To investigate the biological significance of miR-375 in CRC tumorigenesis, we established miR-375 stably expressing cell lines of SW480 and HCT15 by lentivirus transduction. Increased expression of miR-375 by lentiviral transduction was confirmed by RT-PCR (Fig. 3A). Interestingly, overexpression of miR-375 significantly decreased the proliferation of SW480 and HCT15 cells compared with negative control by both MTT assay and cell count methods (Fig. 3B and C). Next, we conducted the colony formation assay to evaluate the tumorigenicity of a single cell. As shown in Fig. 3D, the forced overexpression of miR-375 reduced the number of surviving colonies from the two CRC cell lines to about 50% compared with the NC vector-infected cells. Cell cycles analysis further confirmed that miR-375 overexpression led to cell cycle arrests in both cell types. Our results suggest that miR-375 inhibits CRC cell growth.

3.4. miR-375 regulates the PI3K/Akt pathway in CRC cells

Given that PIK3CA is an important member of PI3K family, we examined the expression of key components of the PI3K/Akt pathway in CRC cells with or without miR-375 overexpression. As shown in Fig. 4A, the levels of Akt and mTOR, the two major components of PI3K/Akt pathway, were dramatically down-regulated by miR-375. Additionally, total and phosphorylated protein levels of both molecules showed the same results, indicating that miR-375 may be an important regulator of PI3K/Akt signaling pathway. To investigate whether miR-375 affects the CRC cell growth through regulating PIK3CA, we co-transfected PIK3CA siRNA together with miR-375 or NC into SW480 and HCT15 cells. Results from both cell lines showed that PIK3CA siRNA and NC co-transfected cells proliferated at a slower rate than did control cells, whereas PIK3CA siRNA and miR-375 co-transfected cells did not exhibit any greater reduction in the proliferative rate ($P < 0.01$; Fig. 4B). To further determine if the anti-tumor formation effect of miR-375 is mediated by downregulating PIK3CA, we cloned PIK3CA gene with wild-type full length or mutant 3′-UTR of *PIK3CA* into pcDNA3.1 vector and co-transfected these plasmids with miR-375 in SW480 and HCT15 cells (Fig. 4C). Western blot results showed that, in the presence of miR-375, PIK3CA protein level was significantly increased in cells transfected with PIK3CA-MutUTR plasmids, but not with PIK3CA-WtUTR plasmids (Fig. 4D). Consistently, the inhibitory effect of miR-375 on CRC cell proliferation

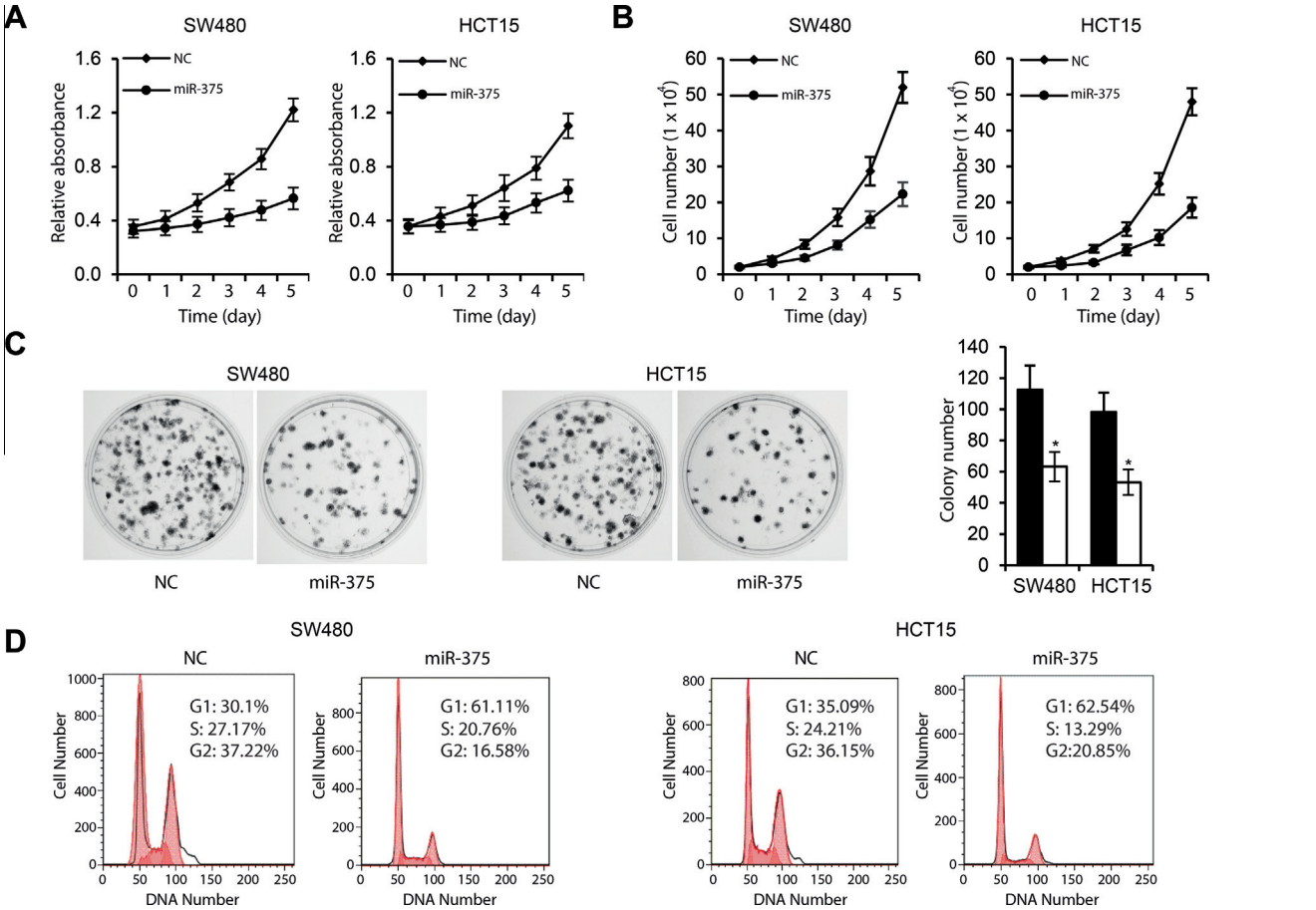

Fig. 3. miR-375 overexpression inhibits CRC cell growth. (A) Real time PCR analysis of relative miR-375 expression in Lv-NC and Lv-miR375-transduced SW480 or HCT15 cells at 2 days after transduction. Transcript levels were normalized to *U6* expression and data are shown as the relative levels compared with Lv-NC transduced cells. (B and C) SW480 and HCT15 cell proliferation was determined by the MTT assays (B) and cell counting method (C). $n = 4$. (D) Representative micrographs (left) and quantification (right) of crystal violet-stained cell colonies in Lv-miR-375- or Lv-NC-transduced SW480 and HCT15 cells. $n = 4$. (E) Cell cycle profiles of Lv-miR-375- or Lv-NC-transduced SW480 and HCT15 cells. $n = 3$. Data are shown as mean ± SEM of three independent experiments. $^*P < 0.05$ compared with NC.

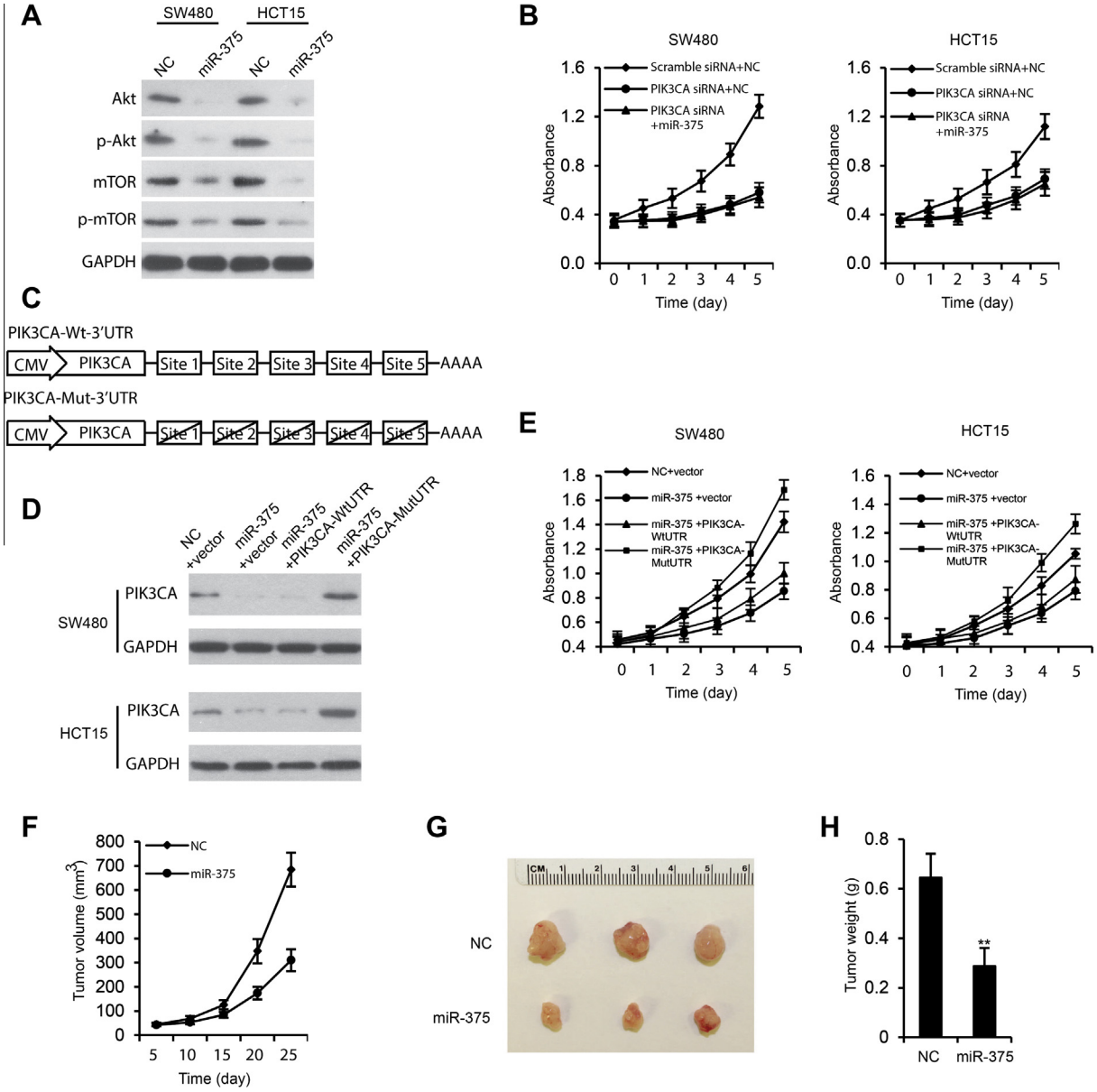

Fig. 4. Overexpression of miR-375 suppresses PI3K/Akt pathway and inhibits *in vivo* tumor formation. (A) Western blot analysis of total and phosphorylated Akt and mTOR protein levels in response to ectopic miR-375 overexpression in SW480 and HCT15 cells. GAPDH was used as loading control. (B) MTT assays on PIK3CA siRNA and/or miR-375 transfected SW480 (left) and HCT15 (right) cells. (C) Schematic representations of the PIK3CA constructs containing wild-type (PIK3CA-WtUTR) or mutated *PIK3CA* 3′-UTR (PIK3CA-MutUTR). (D) Western blot analysis of PIK3CA protein levels in SW480 and HCT15 cells co-transfected with miR-375 and PIK3CA-WtUTR or PIK3CA-MutUTR plasmids. Cells transfected with NC-miRNA and empty vectors were used as control. (E) MTT assay in SW480 (left) and HCT15 (right) cells in response to treatment in (D). $n = 4$. Data are shown as mean ± SEM of three independent experiments. (F) Mean volumes of xenograft tumors in Lv-miR-375 or Lv-NC groups measured every 5 days by caliper measurement up to 25 days. (G) Photography of three representative tumor tissues from each group at 25 days after injection. (H) Average xenograft tumor weights at 25 days. $n = 8$ in each group. $^{**}P < 0.01$ compared with Lv-NC group.

can only been antagonized by PIK3CA-MutUTR plasmids, indicating that miR-375 inhibits CRC cell growth by binding to 3′-UTR of PIK3CA and reducing PIK3CA expression (Fig. 4E). Taken together, our result indicate miR-375 suppresses CRC cell proliferation through inhibiting PI3K/Akt signaling pathway.

3.5. MiR-375 overexpression inhibits the CRC cell tumorigenesis in nude mice

To further study the function of miR-375 on inhibition of CRC tumor growth *in vivo*, Lv-miR-375-SW480 or Lv-NC-SW480 were injected subcutaneously into nude mice. As compared with control, the average tumor volume and weight of miR-375-SW480 group was significantly reduced (Fig. 4F–H). These findings further confirm that miR-375 is a tumor suppressor gene in CRC.

4. Discussion

MiRNAs are key regulators of gene expression in diverse cancers and hold promise for the development of novel approaches for cancer diagnosis and therapies. Recent studies indicate the abnormal expression and pathological significance of miR-375 in various human cancers. It appears that miR-375 may act in different types of cancers through different mechanisms. In a study on oral cancer, miR-375 was found to be the most underexpressed miRNA and it

inhibited oral cancer cell growth by regulating CIP2A gene [13]. In cervical cancer, miR-375 was associated with lymph node metastasis and transcription factor SP1 was identified as a direct target of miR-375 [15]. MiR-375 was also shown to be involved in epithelial cell trans-differentiation by inhibiting Wnt/β-catenin pathway [21]. More recently, elevated miR-375 expression was found in gastric cancer and miR-375 can desensitize cells to ionizing radiation and etoposide by targeting p53 [22]. Slaby and colleagues reported the aberrant expression of miR-375 in CRC [18]. However, the detailed mechanisms surrounding the role of miR-375 may play in CRC tumorigenesis needs to be further elucidated.

In this study, we confirmed the downregulation of miR-375 in CRC cell lines and tissues. More importantly, we identified PIK3CA as a target of miR-375. Overexpression of miR-375 led to a significant reduction in PIK3CA protein level and the inhibition of PI3K/Akt signaling pathway. Moreover, we provided the first *in vivo* evidence that miR-375 inhibited CRC tumor formation.

PIK3CA gene, locating at 3q26, encodes the p110α catalytic subunit of PI3K. PIK3CA gene amplification is correlated with PI3K/Akt signaling pathway activity [23]. PI3K is a lipid kinase and generates phosphatidylinositol-3,4,5-trisphosphate (PI(3, 4, 5)P3), which functions as a second messenger for Akt activation [24,25]. Activated Akt protein modulates cell proliferation through numerous downstream targets, such as Bad, procaspase-9, mammalian target of rapamycin (mTOR), glycogen synthase kinase-3 (GSK-3), Cyclin-dependent kinase inhibitors, P21 and P27 [26]. In the present study, we found that miR-375 overexpression regulated the total and phosphorylated protein levels of Akt and mTOR in CRC cells, indicating that miR-375 may be an important regulator of PI3K/Akt signaling pathway. More importantly, we found that the growth inhibition induced by miR-375 can be blocked by knocking down PIK3CA. All these results indicated that miR-375 repressed PIK3CA expression, which in turn, by regulating PI3K/Akt pathway, inhibited CRC cell growth.

In summary, our present study demonstrates miR-375 as a tumor growth suppressor in human colorectal cancer, at least, partially through repression of PI3K/Akt pathway. The ability of miR-375 to inhibit CRC cell growth may provide us a novel perspective on patient treatment.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

None.

References

[1] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, CA Cancer J. Clin. 63 (2013) (2013) 11–30.
[2] V. Ambros, The functions of animal microRNAs, Nature 431 (2004) 350–355.
[3] A.S. Flynt, E.C. Lai, Biological principles of microRNA-mediated regulation: shared themes amid diversity, Nat. Rev. Genet. 9 (2008) 831–842.
[4] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[5] C. Braicu, G.A. Calin, I. Berindan-Neagoe, MicroRNAs and cancer therapy – from bystanders to major players, Curr. Med. Chem. 20 (2013) 3561–3573.
[6] A.J. Schetter, C.C. Harris, Alterations of microRNAs contribute to colon carcinogenesis, Semin. Oncol. 38 (2011) 734–742.
[7] M. Siow, L. Karen Ng, V. Vincent Chong, M. Jamaludin, M. Abraham, Z. Abdul Rahman, T. Kallarakkal, Y.H. Yang, S. Cheong, R. Zain, Dysregulation of miR-31 and miR-375 expression is associated with clinical outcomes in oral carcinoma, Oral Dis. (2013) [Epub ahead of print].
[8] D. Luo, J.M. Wilson, N. Harvel, J. Liu, L. Pei, S. Huang, L. Hawthorn, H. Shi, A systematic evaluation of miRNA:mRNA interactions involved in the migration and invasion of breast cancer cells, J. Transl. Med. 11 (2013) 57.
[9] X. Wu, J.A. Ajani, J. Gu, D.W. Chang, W. Tan, M.A. Hildebrandt, M. Huang, K.K. Wang, E. Hawk, MicroRNA expression signatures during malignant progression from Barrett's esophagus to esophageal adenocarcinoma, Cancer Prev. Res. (Phila.) 6 (2013) 196–205.
[10] C. Chang, H. Shi, C. Wang, J. Wang, N. Geng, X. Jiang, X. Wang, Correlation of microRNA-375 downregulation with unfavorable clinical outcome of patients with glioma, Neurosci. Lett. 531 (2012) 204–208.
[11] Y. Xu, Y. Deng, X. Yan, T. Zhou, Targeting miR-375 in gastric cancer, Expert Opin. Ther. Targets 15 (2011) 961–972.
[12] Y. Chang, W. Yan, X. He, L. Zhang, C. Li, H. Huang, G. Nace, D.A. Geller, J. Lin, A. Tsung, MiR-375 inhibits autophagy and reduces viability of hepatocellular carcinoma cells under hypoxic conditions, Gastroenterology 143 (2012) 177–187. e178.
[13] H.M. Jung, R.S. Patel, B.L. Phillips, H. Wang, D.M. Cohen, W.C. Reinhold, L.J. Chang, L.J. Yang, E.K. Chan, Tumor suppressor miR-375 regulates MYC expression via repression of CIP2A coding sequence through multiple miRNA–mRNA interactions, Mol. Biol. Cell 24 (1638–1648) (2013) S1631–S1637.
[14] X. Shao, W. Mei, W. Weng, J. Qin, J. Zhou, J. Liu, J. Cheng, miR-375 enhances ruthenium-derived compound Rawq01 induced cell death in human ovarian cancer, Int. J. Clin. Exp. Pathol. 6 (2013) 1095–1102.
[15] F. Wang, Y. Li, J. Zhou, J. Xu, C. Peng, F. Ye, Y. Shen, W. Lu, X. Wan, X. Xie, miR-375 is down-regulated in squamous cervical cancer and inhibits cell migration and invasion via targeting transcription factor SP1, Am. J. Pathol. 179 (2011) 2580–2588.
[16] O. Giricz, P.A. Reynolds, A. Ramnauth, C. Liu, T. Wang, L. Stead, G. Childs, T. Rohan, N. Shapiro, S. Fineberg, P.A. Kenny, O. Loudig, Hsa-miR-375 is differentially expressed during breast lobular neoplasia and promotes loss of mammary acinar polarity, J. Pathol. 226 (2012) 108–119.
[17] J. Szczyrba, E. Nolte, S. Wach, E. Kremmer, R. Stohr, A. Hartmann, W. Wieland, B. Wullich, F.A. Grasser, Downregulation of Sec23A protein by miRNA-375 in prostate carcinoma, Mol. Cancer Res. 9 (2011) 791–800.
[18] P. Faltejskova, M. Svoboda, K. Srutova, J. Mlcochova, A. Besse, J. Nekvindova, L. Radova, P. Fabian, K. Slaba, I. Kiss, R. Vyzula, O. Slaby, Identification and functional screening of microRNAs highly deregulated in colorectal cancer, J. Cell Mol. Med. 16 (2012) 2655–2666.
[19] M. Reczko, M. Maragkakis, P. Alexiou, I. Grosse, A.G. Hatzigeorgiou, Functional microRNA targets in protein coding sequences, Bioinformatics 28 (2012) 771–776.
[20] M.D. Paraskevopoulou, G. Georgakilas, N. Kostoulas, I.S. Vlachos, T. Vergoulis, M. Reczko, C. Filippidis, T. Dalamagas, A.G. Hatzigeorgiou, DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows, Nucleic Acids Res. 41 (2013) W169–173.
[21] Y. Wang, C. Huang, N. Reddy Chintagari, M. Bhaskaran, T. Weng, Y. Guo, X. Xiao, L. Liu, miR-375 regulates rat alveolar epithelial cell trans-differentiation by inhibiting Wnt/beta-catenin pathway, Nucleic Acids Res. 41 (2013) 3833–3844.
[22] Y. Liu, R. Xing, X. Zhang, W. Dong, J. Zhang, Z. Yan, W. Li, J. Cui, Y. Lu, miR-375 targets the p53 gene to regulate cellular response to ionizing radiation and etoposide in gastric cancer cells, DNA Repair (Amst.) 12 (2013) 741–750.
[23] B.I. Bertelsen, S.J. Steine, R. Sandvei, A. Molven, O.D. Laerum, Molecular analysis of the PI3K–AKT pathway in uterine cervical neoplasia: frequent PIK3CA amplification and AKT phosphorylation, Int. J. Cancer 118 (2006) 1877–1883.
[24] F. Chang, J.T. Lee, P.M. Navolanic, L.S. Steelman, J.G. Shelton, W.L. Blalock, R.A. Franklin, J.A. McCubrey, Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy, Leukemia 17 (2003) 590–603.
[25] M. Osaki, M. Oshimura, H. Ito, PI3K–Akt pathway: its functions and alterations in human cancer, Apoptosis 9 (2004) 667–676.
[26] K.M. Nicholson, N.G. Anderson, The protein kinase B/Akt signalling pathway in human malignancy, Cell. Signal. 14 (2002) 381–395.