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Long noncoding RNA SNHG14 accelerates cell proliferation, migration, invasion and suppresses apoptosis in colorectal cancer cells by targeting miR-944/KRAS axis through PI3K/AKT pathway

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Abstract. – OBJECTIVE: Colorectal cancer (CRC) is a gastrointestinal tract cancer, which threatens the well-being of million of patients due to high metastasis. Recently, numerous studies have recognized nuclear RNA host gene 14 (SNHG14) as a remarkable oncogene in different cancers. However, the regulatory mechanism of SNHG14 in CRC development is mostly unclear.

PATIENTS AND METHODS: The expression of SNHG14, miR-944 and Kirsten rat sarcoma (KRAS) in tissues and cells was measured by quantitative Real-time polymerase chain reaction (qRT-PCR). Cell viability and apoptosis were evaluated by cell counting kit-8 (CCK-8) and flow cytometry assay, respectively. Cell migration and invasion were assessed using transwell assay. Protein expression of KRAS, AKT, phosphorylated AKT (p-AKT), phosphatidylinositol-3-kinase (PI3K) and phosphorylated PI3K (p-PI3K) was detected by Western blot. Animal models were constructed by subcutaneously injecting SW620 cells stably transfected with sh-SNHG14 and sh-NC. The interaction among SNHG14, miR-944 and KRAS was determined by luciferase reporter assay and RIP assay.

RESULTS: The expression of SNHG14 and KRAS was up-regulated whereas miR-944 was down-regulated in CRC tumors and cells compared with normal tissues and cells. In addition, SNHG14 silencing attenuated cell proliferation, migration and invasion, while accelerated apoptosis in CRC cells by suppressing PI3K/AKT pathway. Consistently, SNHG14 knockdown hindered tumor growth *in vivo*. MiR-944 was a target of SNHG14 and directly targeted KRAS. Moreover, miR-944 inhibitor abrogated silenced SNHG14-mediated inhibition on proliferation, migration and invasion, as well as promotion on apoptosis in CRC cells. Similarly, miR-944 regulated CRC cell progression by targeting KRAS through PI3K/AKT pathway.

CONCLUSIONS: SNHG14 contributed to cell proliferation, migration and invasion, while suppressed apoptosis in CRC cells by targeting miR-944/KRAS axis through PI3K/AKT pathway, representing novel biomarkers for CRC therapy.

Key Words:
SNHG14, MiR-944, KRAS, PI3K/AKT pathway, CRC.

Abbreviations

CRC: Colorectal cancer, lncRNAs: Long non-coding RNAs, SNHG14: Small nucleolar RNA host gene 14, EMT: epithelial-mesenchymal transition, miRNAs: MicroRNAs, KRAS: Kirsten rat sarcoma, mRNA: messenger RNA, CCK-8: cell counting kit-8, p-AKT: phosphorylated AKT, PI3K: phosphatidylinositol-3-kinase, p-PI3K: phosphorylated PI3K.

Introduction

Colorectal cancer (CRC) is a malignant cancer derived from the gastrointestinal tract, which threatens the health of million of patients globally[1,2]. Distant metastasis of CRC to the downstream organs, such as liver and bone, is the major cause of CRC-related death[3]. Despite optimized strategies that have improved the therapeutic outcomes, the 5-year survival rate for CRC patients at stage IV was only approximately 10% due to drug resistance, delayed diagnosis, distant metastasis and side effects[4-6]. Therefore, a better understanding of the pathogenesis of CRC might be pivotal in order to provide more effective CRC therapy.

Long non-coding RNAs (lncRNAs) are fundamental modulators for cell cycle, survival, differentiation, inflammation, autophagy and apoptosis in a variety of diseases[7-9]. Small nucleolar RNA

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host gene 14 (SNHG14), mapped on chromosome 15q11.2, has been validated as a carcinogenic gene[10]. Hence, the dysregulation of SNHG14 was implicated in various cancers. For instance, overexpression of SNHG14 contributed to cell viability, invasion, metastasis in breast cancer and ovarian cancer by interacting with miR-193a-3p and miR-219a-5p, respectively[11,12]. Consistently, SNHG14 was reported to accelerate cell survival, epithelial-mesenchymal transition (EMT) and invasion in gastric cancer by up-regulating SOX9 via absorbing miR-145[13]. By contrast, knockdown of SNHG14 enhanced cisplatin sensitivity and led to cell migration, invasion and repression in non-small cell lung cancer[14]. However, the function of SNHG14 during CRC tumorigenesis and development is unclear.

MicroRNAs (miRNAs) are evolutionary conserved transcripts with limited protein-encoding capacity[15]. They participate in cell growth, infiltration, inflammation, autophagy and death by binding to the specific messenger RNA (mRNA), negatively regulating gene expression and leading to mRNA degradation and protein translation blockage[16-18]. As tumor promoters or suppressors, they are frequently diagnosed in many cancers[19]. For example, miR-944, which located in the tumor protein p63 gene, functioned as a suppressor in breast cancer to restrict cell migration by targeting SIAH1/PTP4A1 axis[20]. Conversely, miR-944 acted as oncogene in cervical cancer to expedite proliferation, migration and invasion by directly targeting HECW2 or S100PBP[21]. Whether miR-944 serves as oncogene or suppressor in CRC is poorly understood.

We aimed to investigate the function of SNHG14 and reveal the underlying biological mechanism of SNHG14 for CRC tumorigenesis and progression. Up-regulation of SNHG14 and down-regulation of miR-944 suggested that SNHG14 acted as oncogene in CRC. We discovered that SNHG14 contributed to CRC cell progression by absorbing miR-944 and up-regulating KRAS through activation of PI3K/AKT pathway.

Patients and Methods

Tissue Samples

32 CRC patients were recruited from the Department of Gastroenterology, People's Hospital of Xinjiang Uygur Autonomous Region. The participants signed the informed consent and the protocols were approved by the Ethics Committee of the Department of Gastroenterology, People's Hospital of Xinjiang Uygur Autonomous Region. Fresh CRC tumors and normal tissues were collected by surgery from those patients and subjected to biological analysis.

Cell Culture and Transfection

SW620, HCT116 cells were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA) and human normal epithelial colonic cells NCM460 were obtained from INCELL (San Antonio, TX, USA). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 0.05% penicillin/streptomycin.

Small interfering RNA (siRNA) targeting SNHG14 (si-SNHG14#1, si-SNHG14#2, si-SNHG14#3), small harboring RNA (shRNA) targeting SNHG14 (sh-SNHG14), negative control (si-NC, sh-NC), pcDNA, SNHG14 overexpression vector (SNHG14), KRAS overexpression vector (KRAS) were synthesized by Genepharma (Shanghai, China). MiR-944 mimics, miR-944 inhibitor (in-miR-944) and negative control (miR-NC) were purchased from RiboBio (Guangzhou, China). The vectors were transfected in SW620 and HCT116 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

CRC tissues and cells were resuspended with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) to obtain total RNA. The cDNA for SNHG14, miR-944 and KRAS was synthesized by All-in-One™ First-Strand cDNA Synthesis Kit (FulenGen, Guangzhou, China). Then, qRT-PCR was performed using SYBR green (Applied Biosystems, Foster City, CA, USA). GAPDH and U6 were exploited as internal reference. The primers for SNHG14, miR-944 and KRAS were listed: SNHG14, (Forward, 5'-GGGTGTTTACGTAGACCAGAACC-3'; Reverse, 5'-CTTCCAAAAGCCTTCTGCCTTAG-3'); miR-944, (Forward, 5'-GCGGCGGAAATTATTGTACATC-3'; Reverse, 5'- ATCCAGTGCAGGGTCCGAGG-3'); KRAS (Forward, 5'-AGGTGCGGGAGAGAGGCCTG-3'; Reverse, 5'-ACTGTACTCCTCTTGACCTGCTGTG-3'); GAPDH, (Forward, 5'-AGGTCGGTGTGAACGGATTTG-3'; Reverse, 5'-GGGGTCGTTGATGGCAACA-3'); U6, (Forward, 5'-ACCCTGAGAAATACCCTCACAT-3'; Reverse, 5'-GACGACTGAGCCCCTGATG-3').

Cell Counting Kit-8 (CCK-8) Assay

Transfected SW620 and HCT116 cells were placed on 96-well plates for 24 h, 48 h and 72 h. Then, 10 μL CCK-8 reagent (Beyotime, Shanghai, China) was added to each well for 2 h. Finally cell viability was determined by measuring OD value (450 nm) by a spectrophotometer.

Flow Cytometry

Transfected SW620 and HCT116 cells were placed on 24-well plates for 48 h. After collection, the cells were co-strained with Annexin V-FITC and propidium iodide (PI) (Vazyme, Nanjing, China). Then, the apoptosis rate was counted by the flow cytometer.

Transwell Assay

The upper chamber of transwell was pre-coated with Matrigel for 4 h (for invasion assay; without Matrigel treatment for migration assay). After that, transfected SW620 and HCT116 cells were placed on the upper chamber for 48 h. Next, the migrated and invaded cells at the lower chamber were stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) and counted by a microscope.

Western Blot Assay

Western blot was conducted following the standard procedure. The primary antibodies against KRAS, AKT, p-AKT, PI3K and p-PI3K were purchased from Abcam (Cambridge, MA, USA) and HRP-conjugated secondary antibody was obtained from Sangon (Shanghai, China).

Murine Xenograft Assay

Male nude mice (5 weeks old, n=6) were purchased from the Department of Gastroenterology, People's Hospital of Xinjiang Uygur Autonomous Region company. We subcutaneously injected SW620 cells stably transfected with sh-SNHG14 and sh-NC to construct the xenograft mice. After 28 d, tumor volume was measured by caliper, tumors were collected and tumor weight was measured. All the animal experiment protocols were approved by the Department of Gastroenterology, People's Hospital of Xinjiang Uygur Autonomous Region.

Luciferase Reporter Assay

Wild type (SNHG14 WT, KRAS 3'UTR WT) and mutant type (SNHG14 MUT, KRAS 3'UTR MUT) luciferase vectors were constructed. Those vectors were co-transfected with miR-944 or miR-NC in SW620 and HCT116 cells. Luciferase activities were examined by dual-luciferase assay.

RNA Immunoprecipitation (RIP) Assay

SW620 and HCT116 cells transfected with miR-944 or miR-NC were lysed by RIP buffer, and the cell lysis was incubated with magnetic beads coated with anti-Ago2 or IgG antibody (Millipore, Billerica, MA, USA). The enrichment of SNHG14 and KRAS was analyzed by qRT-PCR.

Statistical Analysis

Data were presented as means ± standard deviation (SD). Statistical analysis was performed by SPSS 18 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7 (San Diego, CA, USA). The correlation among SNHG14, miR-944 and KRAS was analyzed by Pearson's correlation coefficient. A p-value less than 0.05 ($p<0.05$) was considered as statistically significant.

Results

Up-Regulation of SNHG14 While Down-Regulation of miR-944 in CRC

The expression of SNHG14 and miR-944 was analyzed by qRT-PCR to investigate the potential role of SNHG14 and miR-944 in CRC. As illustrated in Figure 1A-B, SNHG14 expression was evidently higher in CRC tumors and cell lines (SW620, HCT116) than that of the corresponding normal tissues and cells (NCM460). Conversely, the expression of miR-944 was down-regulated in CRC tumors and cells compared with normal tissues and cells (Figure 1D-E). Moreover, the high level of SNHG14 led to low survival rate whereas the low level of SNHG14 resulted in high survival rate (Figure 1C). On the contrary, the survival rate was sharply reduced in patients with low level of miR-944. However, the survival rate exhibited no significant change in high miR-944 group (Figure 1F). Taking together, SNHG14 was overexpressed while miR-944 was low expressed in CRC.

Depletion of SNHG14 Repressed Cell Proliferation, Migration, Invasion and Induced Apoptosis in CRC Cells by Blockage of PI3K/AKT Pathway

Loss-of-function experiments were performed by silencing SNHG14 to further explore the regulatory effect of SNHG14 on CRC cell progression. A significant decline of SNHG14 expression was noticed in SW620 and HCT116 cells transfected with si-SNHG14#1, si-SNHG14#2 and si-SNHG14#3, indicating high transfection efficiency (Figure 2A). SW620 and HCT116 cells transfected with si-SN-

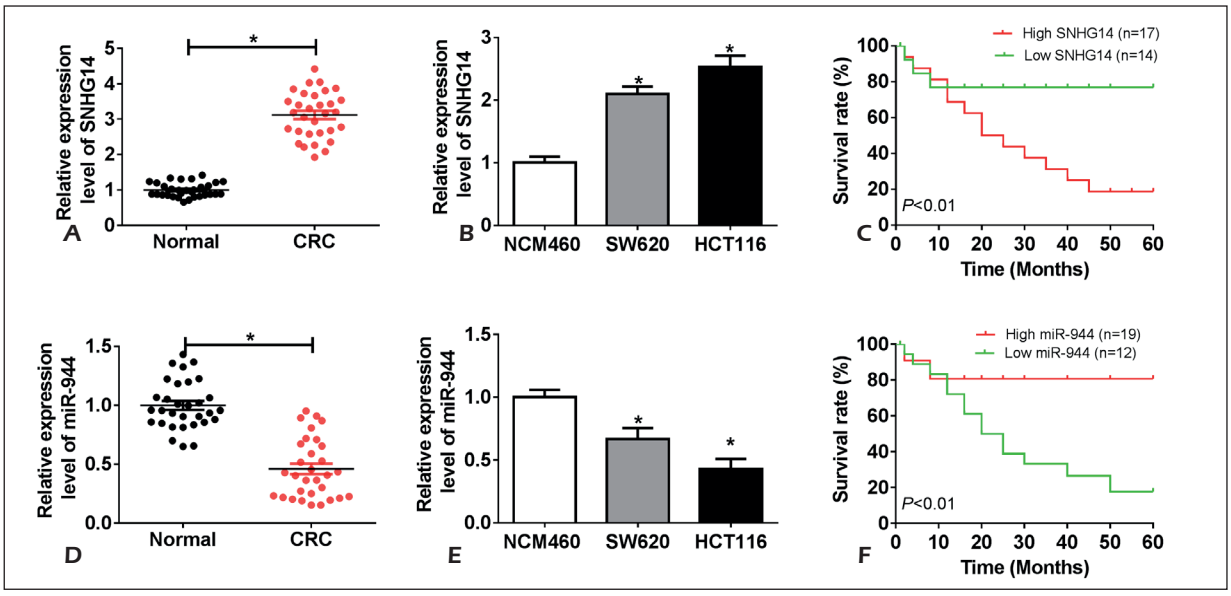

Figure 1. SNHG14 was up-regulated while miR-944 was down-regulated in CRC. **A**-**B**, SNHG14 expression in CRC tumors and cells (SW620, HCT116), as well as in normal tissues and NCM460 cells was examined by qRT-PCR. **C**, Survival rate of CRC patients with high and low level of SNHG14 was analyzed. **D**-**E**, The expression of miR-944 in CRC tumors and cells, as well as in normal tissues and NCM460 cells, was measured by qRT-PCR. **F**, Survival rate of CRC patients with high and low level of miR-944 was determined. **p*<0.05.

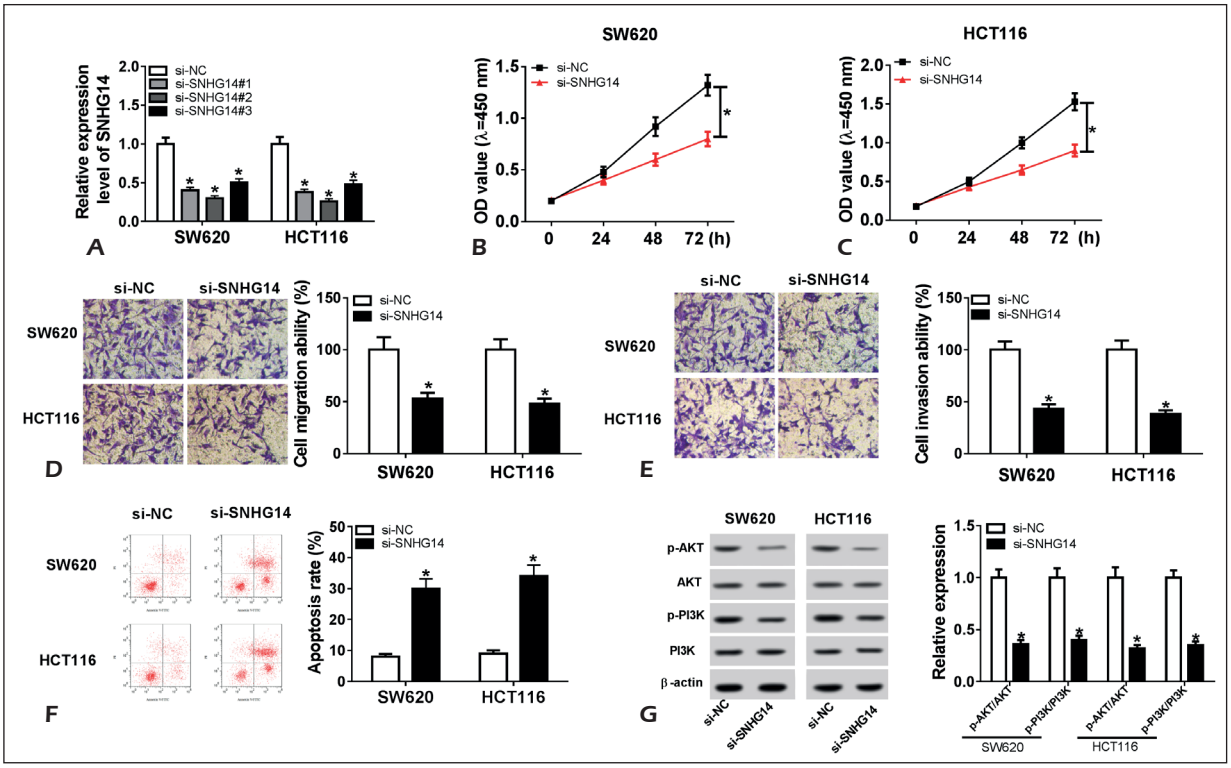

Figure 2. SNHG14 knockdown inhibited proliferation, migration and invasion, whereas induced apoptosis in CRC cells by blockage of PI3K/AKT pathway. SW620 and HCT116 cells were transfected with si-SNHG14 (si-SNHG14#1, si-SNHG14#2 and si-SNHG14#3) and si-NC. **A**, SNHG14 expression in transfected SW620 and HCT116 cells was monitored by qRT-PCR. **B**-**C**, Cell viability of transfected SW620 and HCT116 cells was measured by CCK-8 assay. **D**-**E**, Cell migration and invasion of transfected SW620 and HCT116 cells were determined by transwell assay (magnifications: ×300). **F**, Cell apoptosis of transfected SW620 and HCT116 cells was analyzed by flow cytometry. **G**, Protein expression of p-AKT, AKT, p-PI3K and PI3K in transfected SW620 and HCT116 cells was evaluated by Western blot. β-actin was used as internal reference. **p*<0.05.

HG14#2 were employed for the following experiments due to the optimal transfection efficiency. More importantly, CCK-8 results demonstrated that SNHG14 knockdown apparently retarded cell proliferation (Figure 2B-C). As expected, cell migration and invasion were hampered after SNHG14 silencing (Figure 2D-E). Oppositely, cell apoptosis was enhanced by SNHG14 silencing (Figure 2F). In addition, decreased protein expression of p-AKT and p-PI3K in cells transfected with si-SNHG14 revealed that SNHG14 silencing suppressed the activation of PI3K/AKT pathway (Figure 2G). All the data demonstrated that SNHG14 knockdown inhibited CRC cell proliferation, migration, invasion and facilitated apoptosis by blocking PI3K/AKT pathway.

Interference of SNHG14 Attenuated Tumor Growth In Vivo

Xenograft mice models were established by subcutaneously injecting SW620 cells stably transfected with sh-SNHG14 and sh-NC to determine the effect of SNHG14 on tumor growth *in vivo*. As displayed in Figure 3A, tumor growth was dramatically suppressed in sh-SNHG14 xenograft mice compared with sh-NC group. Synchronously, tumor weight was much lower in sh-SNHG14 xenograft mice than that of sh-NC group (Figure 3B). Biological analysis results by qRT-PCR revealed that SNHG14 was decreased in sh-SNHG14 xenograft mice (Figure 3C). Altogether, SNHG14 depletion hindered tumor growth *in vivo*.

SNHG14 was a Sponger of miR-944

By searching from online database DianaTools, we observed that there were potential binding sites between SNHG14 and miR-944 (Figure 4A). Decreased luciferase activity in SW620 and HCT116 cells co-transfected with SNHG14 WT and miR-944 validated the interaction between SNHG14 and miR-944 (Figure 4B-C). In addition, SNHG14 enrichment was boosted in CRC cells transfected with miR-944 compared with cells transfected with miR-NC (Figure 4D). As analyzed by Pearson's correlation coefficient, we found there was a negative linear relationship between SNHG14 and miR-944 ($r=-0.8999$, $p<0.0001$) (Figure 4E). As expected, SNHG14 expression was elevated in CRC cells transfected with SNHG14 (Figure 4F). Besides, the expression of miR-944 was reduced by SNHG14 and enhanced by SNHG14 silencing (Figure 4G). Therefore, we suggested that SNHG14 acted as a sponger of miR-944 in CRC.

SNHG14 Modulated Cell Proliferation, Migration, Invasion and Apoptosis in CRC Cells by Targeting MiR-944 via PI3K/AKT Pathway

After the interaction between SNHG14 and miR-944 was validated, we further explored the influence of SNHG14/miR-944 axis on CRC cell development. SW620 and HCT116 cells were transfected with si-NC, si-SNHG14, si-SNHG14+in-miR-NC and si-SNHG14+in-miR-944. The expression of miR-944 was increased by SNHG14 silencing and decreased by miR-944 inhibitor (Figure 5A). Interestingly, cell proliferation was restricted by SNHG14 silencing, and the effect was reversed by miR-944 inhibitor (Figure 5B-C). Similarly, the abundance of miR-944 repressed cell migration and invasion, while deficiency of miR-944 accelerated cell migration and invasion in CRC cells (Figure 5D-E). Moreover, miR-944 inhibitor abolished SNHG14 silencing inducing a promotive effect on cell apoptosis in CRC (Figure 5F). Overexpression of miR-944 suppressed whereas low

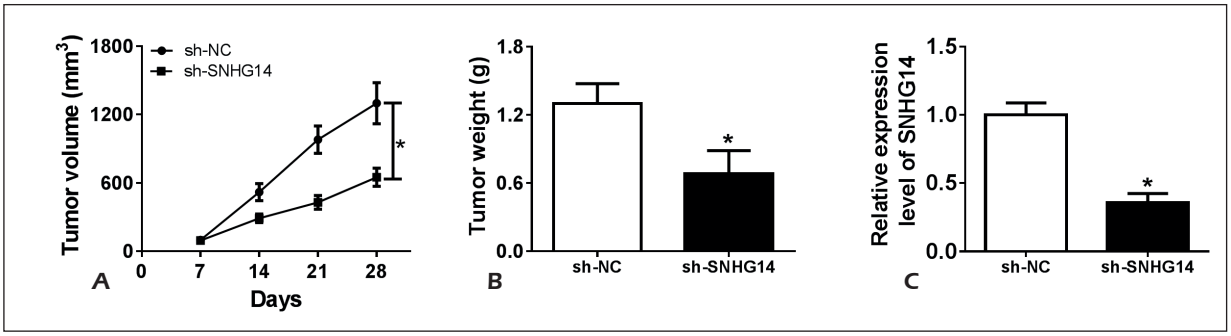

Figure 3. SNHG14 depletion impeded tumor growth *in vivo*. **A**, Tumor volume of SW620 xenograft mice was measured every 7 d. **B**, Tumor weight was measured at day 28. **C**, SNHG14 expression in tumors collected from the xenograft mice was examined by qRT-PCR. *$p<0.05$.

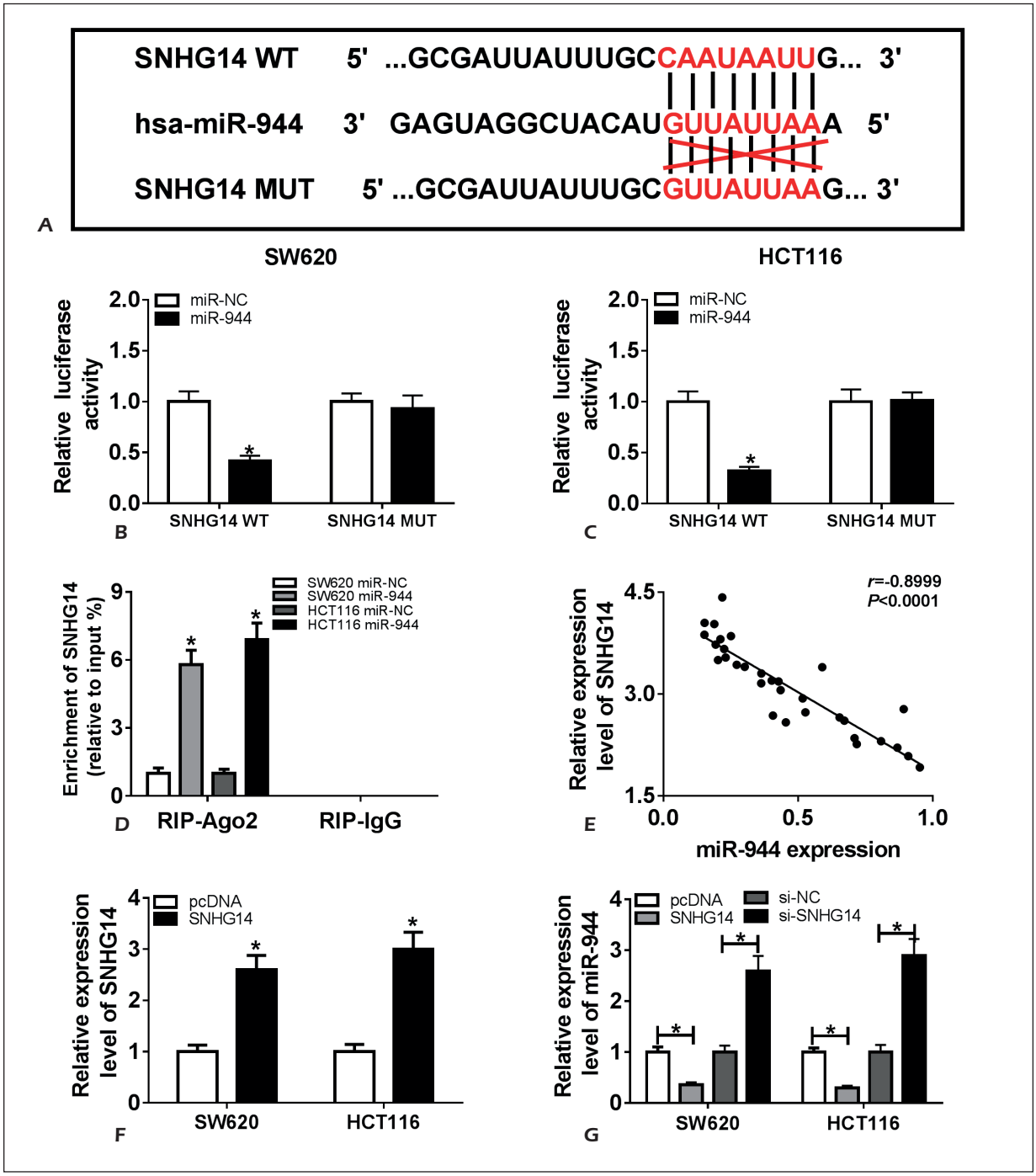

Figure 4. SNHG14 directly interacted with miR-944. **A**, The putative binding sites between SNHG14 and miR-944 were shown. **B-C**, Luciferase activity of SW620 and HCT116 cells co-transfected with SNHG14 WT or SNHG14 MUT and miR-944 or miR-NC was measured. **D**, The enrichment of SNHG14 in SW620 and HCT116 cells transfected with miR-944 or miR-NC was evaluated by RIP assay. **E**, The correlation between SNHG14 and miR-944 (r=-0.8999, $p<0.0001$) was analyzed by Pearson's correlation coefficient. **F**, SNHG14 expression in SW620 and HCT116 cells transfected with pcDNA and SNHG14 was tested by qRT-PCR. **G**, The expression of miR-944 in SW620 and HCT116 cells transfected with pcDNA, SNHG14, si-NC and si-SNHG14 was analyzed by qRT-PCR. *$p<0.05$.

expression of miR-944 accelerated p-AKT and p-PI3K protein production (Figure 5G). Collectively, silenced SNHG14 cell inhibited proliferation, migration, invasion and facilitated apoptosis in CRC cells by targeting miR-944 through PI3K/AKT pathway.

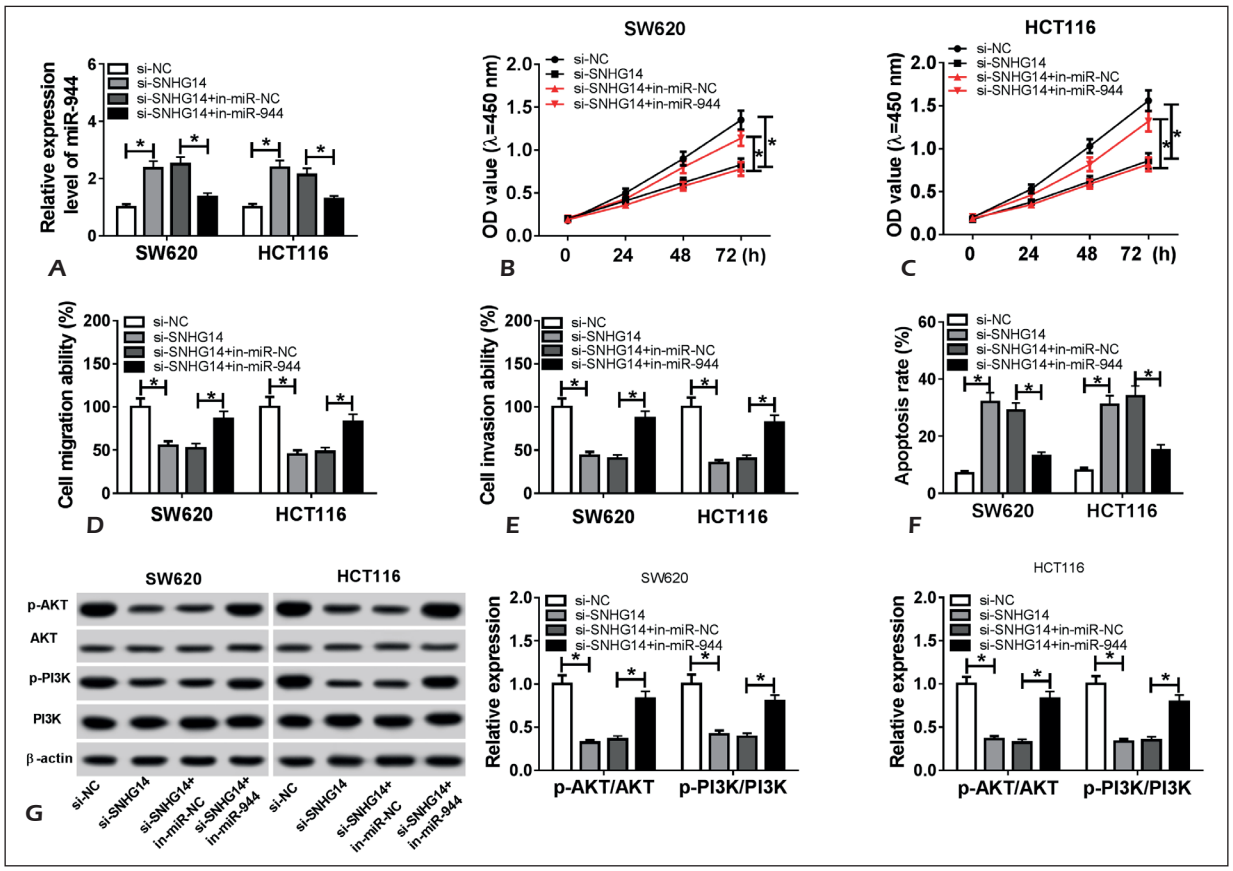

Figure 5. MiR-944 inhibitor attenuated SNHG14 silencing mediated inhibition on proliferation, migration, invasion and promotion on apoptosis in CRC *via* PI3K/AKT pathway. SW620 and HCT116 cells were transfected with si-NC, si-SNHG14, si-SNHG14+in-miR-NC and si-SNHG14+in-miR-944. **A**, QRT-PCR was employed to test the expression of miR-944 in transfected cells. **B**-**C**, Cell viability of transfected SW620 and HCT116 cells was determined by CCK-8 assay. **D**-**E**, Cell migration and invasion of transfected SW620 and HCT116 cells were examined *via* transwell assay. **F**, Cell apoptosis of transfected SW620 and HCT116 cells was detected by flow cytometry. **G**, Protein expression of p-AKT, AKT, p-PI3K and PI3K in transfected SW620 and HCT116 cells was analyzed by Western blot. β-actin was used as internal reference. *p<0.05.

KRAS was a Target of miR-944

Bioinformatics analysis by DianaTools predicted that miR-944 was capable of binding to 3' untranslated regions (3'UTR) of KRAS (Figure 6A). Luciferase activity reduced remarkably in SW620 and HCT116 cells co-transfected with KRAS 3'UTR WT and miR-944, manifesting the interaction between KRAS and miR-944 (Figure 6B-C). Also, the enrichment of KRAS distinctly increased in CRC cells transfected with miR-944 (Figure 5D). The level of KRAS in CRC tumors and cells was further assessed by qRT-PCR. As shown in Figure 6E-F, KRAS expression was relatively higher in CRC tumors and cells in comparison with normal tissues and cells. By calculation, we discovered that KRAS was inversely correlated with miR-944 (r=-0.9122, p<0.0001) (Figure 6G). However, KRAS was correlated with SNHG14 positively (r=-0.8867, p<0.0001) (Figure 6H). Furthermore, KRAS protein expression was blocked by miR-944 and boosted by SNHG14 (Figure 6I). These results revealed that KRAS was a target of miR-944.

KRAS Neutralized miR-944 Induced Inhibition on Proliferation, Migration, Invasion and Promotion on Apoptosis in CRC Cells by Regulating PI3K/AKT Pathway

Rescue experiments were conducted to clarify the effects of miR-944/KRAS axis on CRC cell proliferation, migration, invasion and apoptosis by transfecting miR-NC, miR-944, miR-944+pcDNA and miR-944+KRAS in SW620 and HCT116 cells. The inhibition on KRAS protein expression initiated by miR-944 was recovered by KRAS (Figure

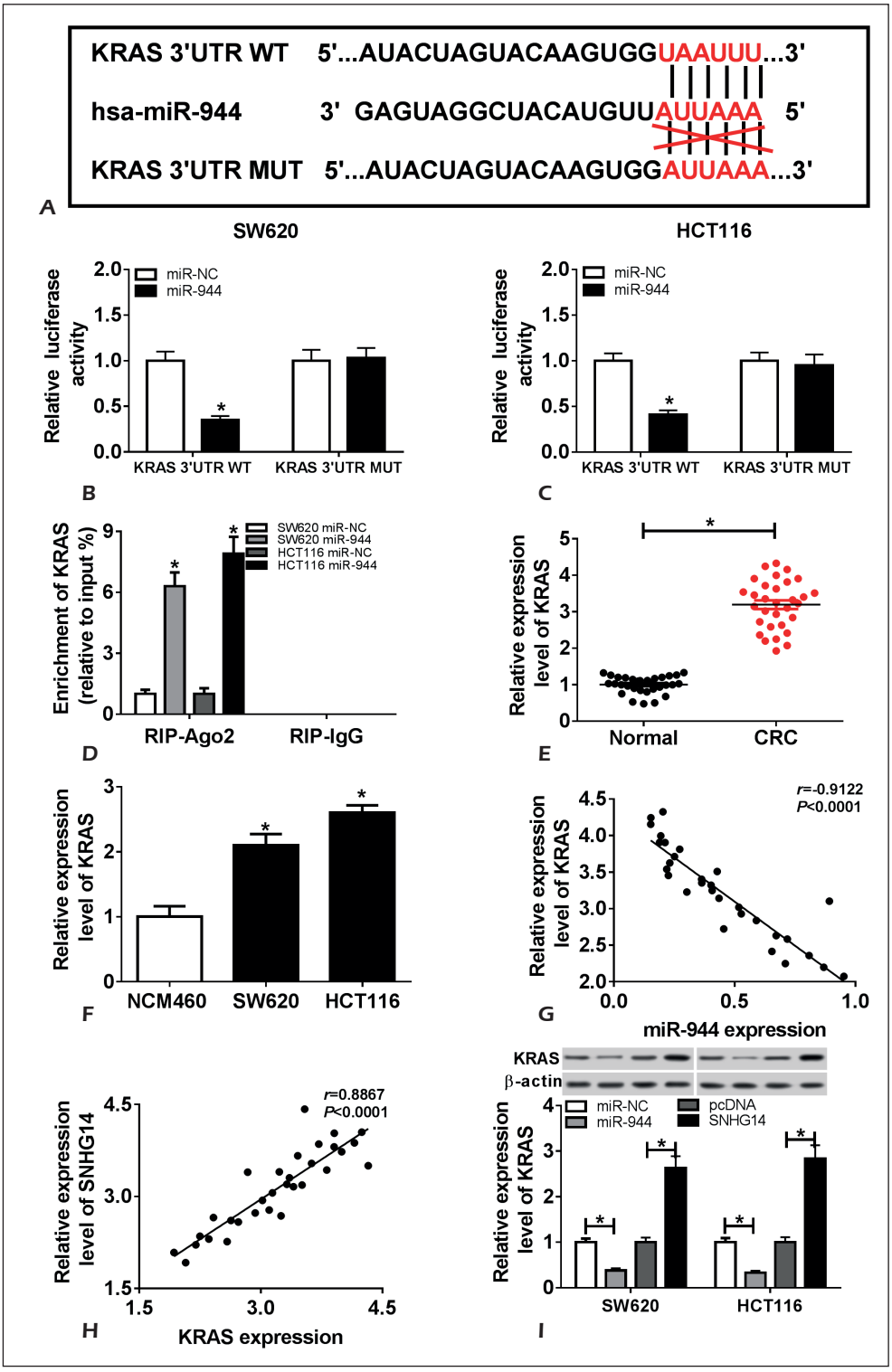

Figure 6. KRAS was a target of miR-944. **A**, The putative binding sites between KRAS and miR-944 were exhibited. **B-C**, Luciferase activity of SW620 and HCT116 cells co-transfected with KRAS 3'UTR WT or KRAS 3'UTR MUT and miR-944 or miR-NC was measured. **D**, The enrichment of KRAS in SW620 and HCT116 cells transfected with miR-944 or miR-NC was analyzed. **E-F**, KRAS expression in CRC tumors and cells compared with normal tissues and cells was determined by qRT-PCR. **G**, The correlation between KRAS and miR-944 was validated through Pearson's correlation coefficient (r=-0.9122, $p<0.0001$). **H**, The correlation between SNHG14 and KRAS was analyzed (r=-0.8867, p<0.0001). (I) KRAS protein expression in SW620 and HCT116 cells transfected with miR-NC, miR-944, pcDNA and SNHG14 was measured by Western blot. *$p<0.05$.

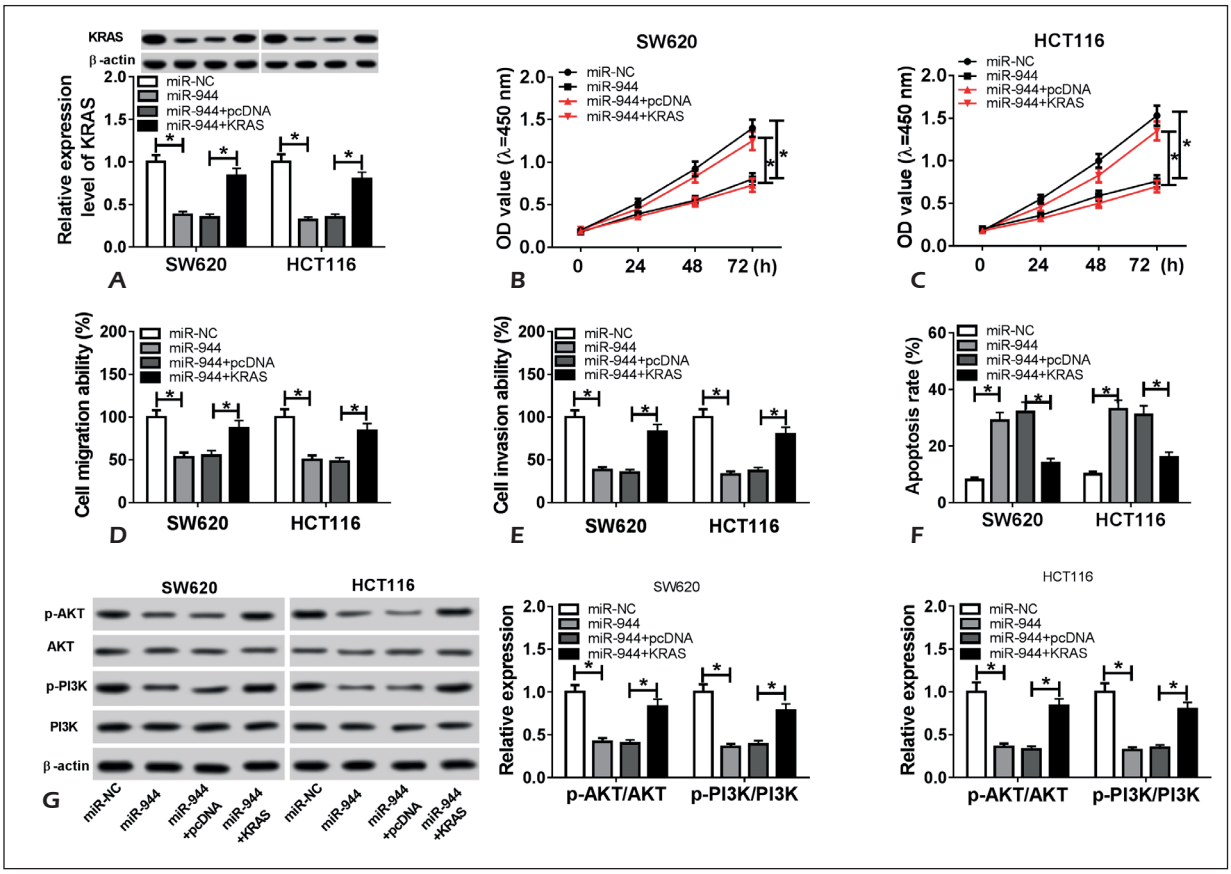

Figure 7. Restoration of KRAS reversed miR-944-induced suppression on proliferation, migration, invasion and acceleration on apoptosis in CRC cells by regulating PI3K/AKT pathway. SW620 and HCT116 cells were transfected with miR-NC, miR-944, miR-944+pcDNA and miR-944+KRAS. **A**, KRAS expression in transfected cells was tested by qRT-PCR. **B**-**C**, Cell viability of transfected SW620 and HCT116 cells was examined by CCK-8 assay. **D**-**E**, Cell migration and invasion of transfected SW620 and HCT116 cells were determined by Transwell assay. **F**, Cell apoptosis of transfected SW620 and HCT116 cells was detected by flow cytometry. **G**, Protein expression of p-AKT, AKT, p-PI3K and PI3K in transfected SW620 and HCT116 cells was analyzed by Western blot. β-actin was used as internal reference. *$p<0.05$.

7A). Restoration of KRAS abrogated the suppressive effects of miR-944 on cell proliferation (Figure 7B-C). Consistently, cell migration and invasion were repressed by miR-944 and the effects were inversed by KRAS (Figure 7D-E). By comparison, miR-944 enhanced apoptosis whereas KRAS reduced apoptosis (Figure 7F). Importantly, the abundance of KRAS expedited p-AKT and p-PI3K protein expression. However, the deficiency of KRAS represented the opposite effects (Figure 7G). Hence, we concluded that miR-944 could modulate CRC cell progression by targeting KRAS and regulating PI3K/AKT pathway.

Discussion

Growing evidence clarified that SNHG14 was a critical competing endogenous RNA (ceRNA) in many diseases, such as cerebral ischemia injury and cancers[22,23]. For example, SNHG14 functioned as competing endogenous RNA (ceRNA) to sponge miR-340 and further promote cell progression *in vitro* and *in vivo* in non-small cell lung cancer[24]. Consistently, SNHG14 induced by SP1 served as ceRNA in clear cell renal cell carcinoma to accelerate cell metastasis by interacting with N-WASP[25]. In addition, SNHG14 was overexpressed in cervical cancer and abundance of SNHG14 expedited cell proliferation, migration and invasion as well as inhibited apoptosis by sponging miR-206 to enhance YWHAZ level[26]. Similarly, in addition, SNHG14 had close relation with drug resistance against cancers. For example, SNHG14 improved drug resistance of gemcitabine by targeting miR-101 and enhancing cell proliferation in pancreatic cancer[27]. Likewise, trastuzumab resistance was also strengthened by the efficiency of SNHG14 to alter PABPC1 generation through H3K27 acetyla-

tion in breast cancer[28]. Whether SNHG14 contributed to CRC cell progression and the underlying mechanism require in-depth exploration.

According to bioinformatics analysis using DianaTools, we discovered that miR-944 could bind to SNHG14. Therefore, we assumed that SNHG14 might regulate CRC cell behavior by interacting with miR-944. In recent years, miR-944 has been identified as significant therapeutic and prognostic biomarker of different cancers, such as lung adenocarcinoma, gastric and breast cancer[29-31]. However, the role of miR-944 in CRC progression is still controversial. He et al[32] claimed that miR-944 served as a promoter to facilitate cell cycle and growth in endometrial cancer. On the contrary, Tang et al[33] demonstrated that miR-944 repressed cell proliferation and invasion by targeting GATA binding protein 6 in CRC, which was in line with our study. Consistently, miR-944 attenuated cell development and improved the therapeutic efficiency of hepatocellular carcinoma through interacting with IGF-1R and inducing the suppression of PI3K/Akt pathway[34]. Therefore, it is essential to disclose the exact role of miR-944 in CRC.

We proved that SNHG14 could regulate cell progression in CRC by targeting miR-944. Firstly, we discovered that the expression of SNHG14 and KRAS was up-regulated, whereas miR-944 was down-regulated in CRC tumors and cells compared with normal tissues and cells. Also, high SNHG14 caused low survival rate in CRC patients, suggesting that SNHG14 might function as oncogene in CRC. Silencing of SNHG14 restricted cell proliferation, migration, invasion and induced apoptosis in CRC cells. As expected, SNHG14 knockdown impeded tumor growth *in vivo*, further confirming the oncogenic role of SNHG14. The biological mechanism was investigated, and the results indicated that SNHG14 silencing exerted the inhibition effects by blocking PI3K/AKT pathway. The interaction between miR-944 and SNHG14 or KRAS was validated by luciferase reporter system and RIP assay. The rescue experiments displayed that miR-944 inhibitor counteracted SNHG14 silencing-mediated inhibition on proliferation, migration, invasion and promotion on apoptosis in CRC cells via PI3K/AKT pathway. Similarly, miR-944 regulated CRC cell progression by targeting KRAS through PI3K/AKT pathway.

Conclusions

In this report the regulatory mechanism of oncogene SNHG14 in CRC development has been clarified. We demonstrated that SNHG14 promoted cell proliferation, migration and invasion as well as initiated apoptosis by sponging miR-944 to enhance KRAS level via activation of PI3K/AKT pathway.

Funding

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of People's Hospital of Xinjiang Uygur Autonomous Region. The methods used in this study were performed in accordance with relevant guidelines and regulations. Written consent was obtained from the participants or guardians of participants under 16 years old.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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