**Original Article**

**miRNA-34a suppresses colon carcinoma proliferation and induces cell apoptosis by targeting SYT1**

Haichao Lu, Liang Hao, Hongfan Yang, Jianshe Chen, Jinxin Liu

**Department of Gastrointestinal Surgery, The First People’s Hospital of Nanning, Nanning, Guangxi Zhuang Autonomous Region, China**

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**Abstract:** Background: MicroRNAs are emerging as the important regulators in cancer-related processes. This research were performed to find the function and mechanism of miR-34a effect on colon cancer. Methods: In this study, we examined the expression of miR-34a in colon cancer tissues and cell lines by qRT-PCR. In vitro cell functional assays studies were built to define miR-34a and SYT1 function involved in cell growth, migration, and invasion and apoptosis. EGFP reporter assay was used to determine the relationship of SYT1 and miR-181a. To confirmed the relationship between SYT1 and miR-34a, the SYT1 restoration rescued miR-34a mediated growth and inhibited cell apoptosis were detect. Result: Our studies show that microRNA-34a (miR-34a) is downregulated in human colon cancer relative to normal colon mucosal epithelial cells, and downexpression of miR-34a promotes cell proliferation, migration, and invasion, nevertheless overexpression of miR-34 facilitates cell apoptosis in vitro. Furthermore, SYT1 3′-UTR is found to be down-regulated directly by miR-34a, demonstrating that SYT1 is a important target of miR-34a in colon cancer. The knockdown of SYT1 markedly inhibits colon cancer cell proliferation, migration, and invasion, and induces cell apoptosis, indicating that SYT1 may function as an oncogene in colon cancer. The restoration of SYT1 expression can counteract the effect of miR-34a on cell proliferation, and induces cell apoptosis, of colon cancer cells. Conclusion: Together, these results indicate that miR-34a is a new regulator of SYT1, and both miR-34a and SYT1 play the important roles in the pathogenesis of colon cancer.

**Keywords:** miR-34a, SYT1, colon cancer

**Introduction**

Colon cancer is a major cause of cancer-related morbidity and mortality, and is a public health problem worldwide [1]. For many economically developing countries, the incidence of colon cancer has increased over the past 20 years; most likely attributed to changes in the environment, individual lifestyle and eating habits [2]. Although advances in the detecting and treatment of colorectal cancer, which have increased the life expectancy of patients, the prognosis of colon cancer patients remains poor. Therefore, studying the mechanisms underlying colorectal cancer progression is important for the human being.

MicroRNAs (miRNAs) are a class of non-coding RNAs, which specifically modulate generous gene expression posttranscriptionally by binding to partly complementary sites within 3′-untranslated regions (3′-UTR) of target gene mRNA [3]. They are implicated in a numerous cellular processes including cell proliferation, apoptosis and so on. Previous studies have discovered the important roles of miRNAs in cancers and suggested that abnormal expression of miRNAs may be associated with tumor occurrence and progression by regulating the related genes and pathways. miR-34a, which resides on chromosome 1p36, has been identified in some kinds of cancers, containing hepatocellular carcinoma [4, 5], breast adenocarcinoma [6, 7], gastric cancer [8], osteosarcoma [9, 10], colorectal cancer [11, 12], acute myeloid leukemia [13], myeloma [14, 15], and lung cancer [16]. Recently, using RT-PCR assay, we found that miR-34a was down-regulated in colon cancer and may due to the metastatic activity of colon cancer. These facts led us to further study whether miR-34a also contributed to the tumor suppressor of colon carcinoma.

In this research, we found that miR-34a dued to the cell proliferative activity and apoptosis of human colon cancer cell line SW620 and
miR-34a suppresses colon carcinoma

SW480. Moreover, the SYT1 was confirmed to be a direct functional target of miR-34a in colon carcinoma. Given that SYT1 gene plays an important role in synovial sarcomas [17] and may also be participated in some other cellular pathways, we demonstrated that SYT1 also affects colon cancer cell proliferation in SW620 and SW480 cells. Understanding mechanisms underlying the modulation pathway of miR-34a in colon cancer cells might be helpful to study the colon cancer progression.

Materials and methods

We collected a number of 30 matched colon cancer tissue and adjacent normal tissue from patients who underwent colon cancer surgery at Affiliated Hospital of Chifeng University between Dec 2016 and July 2017. The exclusion criteria contained chemotherapy or radiotherapy. Tissue specimens were immediately frozen rapidly for PCR using. All subjects were approved by the Institute Research Ethics Committee of Affiliated Hospital of Chifeng University.

Cell culture and transfection

SW620 and SW480 cells were cultured in α-MEM medium (Sigma, St. Louis, USA) containing 10% fetal bovine serum, supplement with 100 IU/ml penicillin, and 100 mg/ml streptomycin at 37°C in the humidified atmosphere containing 5% CO₂. All miRNA controls, miR-34a inhibitor (inhibitor), miR-34a mimic (mimic) were provided from Ribobio Company (Guangzhou, China). Transfection the vectors and siRNA was performed using the Lipofectamine 2000 reagent (Invitrogen), according to the manufacturer’s protocol.

Cell viability assay

MTT assay was used for colon cancer cells viability analysis. Briefly, 5 × 10⁵ SW620 and SW480 cells were seeded into each well of a 96-well plate, and cultured at 37°C overnight for attachment. Then the cell were transferred into 100 µL medium containing 10% fetal bovine serum and evodiamine at indicated concentrations and incubated for 30 h. After that, 10 µL MTT reagent was added into each well, mixed with the medium, and incubated at 37°C for 4 h. Finally, a microplate reader was used to measure the absorbance at 450 nm.

Colony formation assay

After transfection, SW620 and SW480 cells were seeded in 12-well plates at 100 cells per well, respectively. Culture medium was changed every 3 days. Colonies were counted only if the number of cells was more than 50 cells, and the number of colonies was counted at the sixth day after cell inoculation. The rate of colony formation was calculated: colony formation rate (%) = (the number of colonies/the number of seeded cells) · 100%.

Cell apoptosis assay

After transfection, SW620 and SW480 cells (5 × 10⁵ per well) were seeded into 6-well plates and then cultured for 3 days respectively. The apoptosis was tested using an AnnexinV-FITC and PI kit (BD Biosciences, Franklin Lakes, NJ, USA) for 15 min at room temperature in the dark. All the stained cells were analyzed using a FACSCalibur (BD Bioscience) and the results were analyzed with FlowJo software.

Real-time reverse transcriptase PCR

All RNA was extracted from tissue and cultured cells by using Trizol reagent (Invitrogen, Carlsbad, USA) and miRNAs were purified by using the mirVana miRNA isolation kit (Ambion, Austin, USA), according to the manufacturer’s instructions. cDNA was synthesized using SuperScript First-stand Synthesis system and PCR was performed on AB7300 thermo-recycler (Applied Biosystems, Carlsbad, CA, USA). The sequence of the primer as follows: miR-34a, forward, 5’-TGGCAGTGTCTTAGCTGGTT-3’, reverse, 5’-AAGCTCCATTTCGCAACCTTAC-3’; SYT1, forward, 5’-CGCTTCGGCAGCACATATACTAAAATTGGAAC-3’, reverse, 5’-TTGGTCAGCACAGATCATCG-3’. Sequence of the primer for PCR methods were as follows: initial denaturation 4 min at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 50°C, 40 s at 72°C. U6 was used as an endogenous control for miRs and GAPDH was used as a housekeeping gene for mRNAs. Three independent experiments were performed, and the relative expression levels were presented using histogram.

Transwell invasion and migration assay

For the colon cancer cell migration studies in vitro, a Matrigel-based invasion assay was per-
miR-34a suppresses colon carcinoma

**Figure 1.** Decreased expression of miR-34a in colorectal cancer (CRC) cell lines. A. RT-PCR was performed to test miR-34a expression in adjacent normal tissues and colon cancer tissues; B. The expression of miR-34a in five CRC cell lines compared with the normal human colon mucosal epithelial cells, was determined using qRT-PCR. Data are shown as the mean ± SD (three replicates). *P < 0.05, and **P < 0.01 vs control.

formed using 8-μm pore-size culture inserts (BD Biosciences, Bedford, MA) coated with Matrigel (BD Biosciences) according to the manufacturer’s instruction. After 48 hours of transfection, cells were harvested and suspended in serum-free DMEM at density of 5 × 10^4 cells. After 24 hours of incubation, the cells that had migrated through the Matrigel and adhered to the bottom of the insert membrane were fixed and stained with 0.1% crystal violet in 20% methanol and then conducted under a microscope. For the invasion assay, the membranes of each chamber were pre-coated with a layer of Matrigel (BD Biosciences) for 6 h in a homothermal incubator at 37°C.

**EGFP reporter assay**

The wild type (WT) and mutant type (Mut) of SYT1 3'UTR were independently amplified with PCR. Genomic DNA was extracted from the HeLa cells, and the WT of SYT1 3'UTR containing miR-34a binding site was amplified. The overlap PCR was employed for the mutation of seed region of 3’UTR of SYT1, and then the mutant sequence of 3’UTR of WT SYT1 was obtained. The PCR products were inserted into pmirGLO vector to acquire recombinant SYT1-WT and SYT1-Mut. SW480 cells and SW620 cells were seeded, and the vectors (SYT1-WT and SYT1-Mut) were independently transfected into colon cancer cells with miR-34a mimics or scramble miRNA by using LipofectamineTM 2000 kit (Invitrogen, Carlsbad, CA, USA). After 24 h, dual luciferase reporter gene assay kit (Promega) was used to detect the dual luciferase signals.

**Western blot analysis**

Total cellular extracts were prepared at 4°C by incubation in RIPA buffer for 30 min, and then centrifugation at 10,000 g for 10 min. Total protein extracts of the colon cells were collected from the supernatant and the proteins concentrations were quantified using the Pierce BCA Protein Assay kit (Thermo Scientific, Rockford, IL) according to the manufacturer’s instructions. All proteins were separated on the 10% SDS-PAGE gel. The membrane was incubated with antibody anti-SYT1 (dilution 1:500) and anti-GAPDH (dilution 1:2000) overnight. Anti-SYT1 and anti-GAPDH antibody were purchased from Abcam.

**Statistical analysis**

The statistics analysis of the data was performed using Student’s t-test on the SPSS (version 21.0, Chicago, IL) software, expressed as mean ± standard deviation (SD). The statistics difference was considered to be significant when P < 0.05.

**Result**

**Decrease expression of miR-34a in colon carcinoma tissues and cells**

It has been shown that expressions of miR-34a between normal tissues and cancer tissues were significantly different (P < 0.05), and miR-34a expression within colon carcinoma tissues was remarkably beyond that within adjacent normal tissues (Figure 1A). In order to investigate the role of miR-34a in colon carcinoma
miR-34a suppresses colon carcinoma

cells, RT-PCR was used to detect the level of miR-34a in 6 different human colon carcinoma cells (i.e. SW480, SW620, HCT116, LoVo, HT29, and normal human colon mucosal epithelial cells). The results showed that the expression level of miR-34a was significantly decreased in 5 colon carcinoma cell lines compared with normal human colon mucosal epithelial cells (Figure 2B).

**miR-34a suppressed cell proliferation, invasion and migration, promoted apoptosis in colon carcinoma cells**

Given that miR-34a acts as a tumor suppressor in many kinds of cancers, we further studied here whether miR-34a has an effect on cell malignant phenotypes, i.e. growth and migration activity in colon cancer cells. After confirming miR-181a inhibitor could get a good activity for inhibiting miR-181a by PCR assays (Figure 2A), the cell proliferation of colon carcinoma cells transfected with miR-34a inhibitor was detected by using MTT and colony formation assay respectively. Our data indicated that proliferation of SW480 and SW620 cells were obviously increased when miR-34a knockdown detected from MTT test (Figure 2B and 2C) and colony formation assay (Figure 2D and 2E). Furthermore, the potential impact of miR-34a in colon carcinoma cells migration and invasion were explored by using transwell assay. As shown in Figure 2F and 2G, the migration was dramatically increased in SW480 and SW620 cells transfected with miR-34a inhibitor. Counting the invaded cells further demonstrated that miR-34a acts as a suppressor miRNA and contributes to inhibition of migration and invasion in colon carcinoma cells (Figure 2H and 2I). In addition, SW480 and SW620 cells transfected with miR-34a mimic displayed significant induction of apoptosis activity compared with the control cells (Figure 2J and 2K). Based on the above results, we found that miR-34a was involved in cell proliferation, invasion, migration and apoptosis activities in SW480 and SW620 cells.

**SYT1 is a direct target of miR-34a**

The computerized algorithms (TargetScan, Pictar and miRBase Targets) were used for predicting, and the result showed that miR-34a targets putatively was SYT1 (Figure 3A). Next, we employed the EGFP reporter assay to confirm that miR-34a directly regulates the SYT1 expression. The alignment of miR-34a-SYT1 sequence is illustrated (Figure 3B). First, the 3'UTR of SYT1 was cloned into vector on the downstream of EGFP codon region. In addition, we constructed another vector muted three bases in the seed sequence of SYT1 3'UTR. The EGFP reporter assay showed that with wild-type 3'UTR, the EGFP value was distinctly lower than mutants (Figure 3C), demonstrating the role of mature miR-34a on SYT1 mRNA. Based on the above data, we found that miR-34a could down-regulate SYT1 expression by directly binding to the specific sequence of SYT1 3'UTR.

To further examine whether miR-34a depresses endogenous SYT1 expression, we transfected SW620 cells with miR-34a mimic or miR-34a inhibitor, and detected the expression level of SYT1 mRNA and protein. The above data showed that overexpression of miR-34a reduced SYT1 mRNA (Figure 3D) and protein expression (Figure 3E and 3F). Conversely, the expression level of endogenous SYT1 mRNA in SW620 cells, which transfected with miR-34a inhibitor, was significantly increased (Figure 3D) and the SYT1 protein level was also elevated (Figure 3E and 3F). All of these data showed that miR-34a directly inhibits the SYT1 expression.

**Knockdown of SYT1 inhibited growth, invasion, migration and induced apoptosis of colorectal cancer cells**

To understand the effects of whether SYT1 plays a functional role in colorectal cancer cells proliferation, invasion and apoptosis, we designed a series of experiments by using MTT assay, transwell assay, cell apoptosis assay and colony formation assay. Above all, SW480 and SW620 cells were transfected with specific siRNA for targeting SYT1, which detected by using both PCR and western blot to verify SYT1 knockdown (Figure 4A-C). Then, we detected the cell viability and proliferation by using MTT assay, which showed downexpression of SYT1 significantly suppressed proliferative rate, as compared with the controls from day 3 (Figure 4D and 4E). The similarly observed that cell proliferative rates were significantly decreased by using colony formation assay (Figure 4F and 4H). At the same time, we were also aware that colon carcinoma cell migration was dramatically decreased when transfected with si-SYT1 (Figure 4H and 4I). As shown in Figure 4J and
miR-34a suppresses colon carcinoma

Figure 2. miR-34a suppressed cell proliferation, migration and invasion, promoted apoptosis in colon carcinoma cells. (A) miR-34a inhibitor was used to achieve miR-34a inhibition which verified by RT-PCR; (B, C) MTT assay and (D, E) Colony formation assay was performed to determine the role of miR-34a on the proliferation activity in SW620 and SW480 cells; (F, G) The effects of miR-34a knockdown on the migration on SW620 and SW480 cells; (H, I) Matrigel invasion assay was performed to determine the effects of miR-34a knockdown on colon cancer cell invasion. (J, K) Cell apoptosis of SW620 and SW480 cells transfected with miR-34a-mimic was detected. All data were analyzed by three independent experiments. *P < 0.05, and **P < 0.01 vs control.
miR-34a suppresses colon carcinoma

4K, a similar result also appears in invasion ability SW480 and SW620 cells transfected with si-SYT1. expression could distinctly promote apoptosis rate in colorectal cancer cells (Figure 4L and 4M). What was certain from the above research results was that SYT1 acts as a cancer-promoting gene and SYT1 knockdown contributes to inhibition of proliferation, migration and invasion in colon carcinoma cells and induced apoptosis, which are consistent with the role of miR-34a down-expression in both SW480 and SW620 cells.

SYT1 restoration rescued miR-34a mediated growth and inhibited cell apoptosis

To confirm that the role of miR-34a on the cell proliferation, invasion, and apoptosis of colon carcinoma cells is regulated through SYT1, we constructed a pcDNA6.2/SYT1 vector containing the SYT1 ORF in the pcDNA6.2 vector without the terminal 3’UTR to avoid the influence of miR-34a. Transfection in colon cancer cells with pcDNA6.2/SYT1 vector rescued the negative effects of miR-34a on SYT1 mRNA (Figure 5A) and protein (Figure 5B and 5C). To further understand the effects of miR-34a further ascertain whether it play a function role in colon carcinoma cells proliferation, the MTT assay and colony formation assay were performed. The data suggest that upexpression of SYT1 promote colon carcinoma cells proliferation (Figure 5D-F) and inhibition of cell apoptosis (Figure 5G), which could rescued by co-transfected miR-34a with the pcDNA6.2/SYT1. The overexpression of SYT1 rescued the effect...
miR-34a suppresses colon carcinoma

Figure 4. Knockdown of SYT1 inhibited growth, migration and invasion, induced apoptosis of colorectal cancer cells. (A) si-SYT1 was used to achieve SYT1 inhibition which verified by RT-PCR; (B, C) si-SYT1 was used to achieve SYT1 inhibition which verified by western blot; (D, E) MTT assay and (F, G) Colony formation assay was performed to determine the role of SYT1 in the proliferation activity in SW620 and SW480 cells; (H, I) The effects of SYT1 knockdown on the migration on colon cancer cell; (J, K) Matrigel invasion assay was performed to determine the effects of SYT1 knockdown on colon cancer cell invasion; (L, M) Cell apoptosis of SW620 and SW480 cells transfected with si-SYT1 was detected. *P < 0.05, and **P < 0.01 vs control.
miR-34a suppresses colon carcinoma

Figure 5. SYT1 restoration rescued miR-34a mediated growth and inhibited cell apoptosis. (A) The mRNA and (B, C) protein expression of SYT1 in SW620 cell co-transfected with either miR-34a mimic or mimic-NC and pcDNA-SYT1 or pcDNA empty vector; (D) MTT assay, (E, F) Colony formation assay, and (G, H) cell apoptosis assays were used to detect the cell growth capacity, and cell apoptosis in SW620 cells transfected with either miR-34a mimic or mimic-NC and pcDNA-SYT1 or pcDNA empty vector, respectively. *P < 0.05, and **P < 0.01 vs control.
miR-34a suppresses colon carcinoma

Discussion

Over the past few decades, hundreds of human miRNAs have been shown to play significant roles in regulating endogenous gene expression through degradation or repression of mRNA translation. The family of miR-34, containing miR-34a, b and c, has been found that they could regulate several cellular processes, including proliferation, invasion and cell apoptosis [18-21]. Previous studies indicted that miR-34a was involved in diverse biological processes and their dysregulation is a common event in various diseases including cancer, cardiovascular diseases and neuropathology [22-24]. Concurrently, miR-34a is considered to be a tumor suppressor, and its role has been widely reported in many malignancies such as squamous cell carcinoma, hepatocellular carcinoma, osteosarcoma and endometrial cancer [22, 25, 26].

Wu et al showed that miR-34a inhibits proliferation, migration and invasion of colon cancer by targeting Fra-1 [27]. Liu F et al reported that miR-34a play an important role in colorectal cancer pathomechanism by targeting and regulating Notch signaling [28]. Furthermore, it was demonstrated that miR-34a reveals a critical role in tumor suppressor, and its role has been widely reported in many malignancies such as squamous cell carcinoma, hepatocellular carcinoma, osteosarcoma and endometrial cancer [22, 25, 26].

SYT1 (synaptotagmin I) on 12q21.2, which was an integral membrane protein contained structural rearrangements with three different genes and is possibly part of a ring chromosome, played an important role in synovial sarcomas [17]. Nord et al, used a 32K BAC array, and found that SYT1 was a novel oncogene in glioblastoma [32]. Also, Zhu H et al provided important insights into the understanding of the molecular genetic basis for in colon cancer and indicated SYT1 serve as a novel markers in colon cancer [33]. While the precise role of SYT1 in cancer was still unclear, and this gene is prone to breakage was demonstrated by presence of multiple chromosomal rearrangements within it [17]. Therefore, more institutional studies were needed to further elucidate the potential role of SYT1 in cancer.

Herein, our findings reveal a relationship between miRNAs and SYT1 in colon cancer cells. Knockdown of SYT1 suppresses the growth of SW480 and SW620 colon cancer cells, which is consistent with the results of miR-34a overexpression. Overexpression of SYT1 rescues the growth inhibition in SW480 and SW620 cells caused by miR-34a. Thus, the low expression of miR-34a in colorectal adenocarcinoma cells leads to an ectopic high expression of the oncogene SYT1, resulting in cell proliferation, migration, and invasion. In addition, SYT1 is responsible for stabilizing the ER network [34]. We found that abnormal expression of SYT1 inhibited cell apoptosis and overexpression of SYT1 rescues cell apoptosis caused by miR-34a in colon cancer cells. In conclusion, we demonstrated that STY1 functions as an oncogene in colorectal cancer, which is under-regulated by miR-34a.

In brief, we have demonstrated that miR-34a expression is downregulated in colon carcinoma cells, and that miR-34a inhibits colony for-
miR-34a suppresses colon carcinoma

Information, proliferation, migration, invasion and induce cell apoptosis by targeting SYT1. These results suggest that miR-34a could have a significant role in tumorigenesis by inhibition of SYT1. The elucidation of the mechanisms of miR-34a in colon carcinoma helps us to understand the mechanism of colon carcinoma progression.

Disclosure of conflict of interest

None.

Address correspondence to: Jinxin Liu, Department of Gastrointestinal Surgery, The First People’s Hospital of Nanning, No.89, Qixing Road, Qingxiu District, Nanning 530022, Guangxi Zhuang Autonomous Region, China. E-mail: liujinxin_nfph@163.com

References


[19] Bu P, Wang L, Chen KY, Srinivasan T, Murthy PK, Tung KL, Varanko AK, Chen HJ, Al Y, King S, Lipkin SM, Shen X. A miR-34a-Numb feed-forward loop triggered by inflammation regulates asymmetric stem cell division in intestine and...


