Int J Clin Exp Pathol 2016;9(6):5937-5943
www.ijcep.com /ISSN:1936-2625/IJCEP0023331

Original Article

MiR195 downregulating Siah-1S level to inhibit cell growth and promote cell apoptosis in colorectal cancer cells SW620

Gang Yu[1*], Tao Wang[1*], Zhengwei Cheng[2], Wenjia Lv[2]

Departments of [1]Gastrointestinal Surgery, [2]GI Medicine, Xianning Central Hospital (The First Affiliated Hospital, Hubei University of Science and Technology), Xianning 437100, Hubei, China. [*]Equal contributors.

Received January 5, 2016; Accepted April 27, 2016; Epub June 1, 2016; Published June 15, 2016

Abstract: MicroRNAs regulate cell growth, proliferation, apoptosis, and cell cycle. The role of miR195 in colorectal cancer cells SW620 remains to be further studied. MiR195 and control miRNA were synthetized and transfected to SW620 cells using liposome. Cell growth was tested by MTT assay. Cell apoptosis was determined by flow cytometry and western blot. Siah-1 level was detected by western blot. Siah-1S plasmid was transfected to overexpress Siah-1S, RNA interference was used to downregulate Siah-1S, and miR195 and control miRNA were further transfected to test cell apoptosis. MiR195 transfection inhibited SW620 cell growth, promoted SW629 cell apoptosis, and reduced Siah-1S level. Siah-1S overexpression inhibited miR195 induced cell apoptosis. Siah-1S downregulation enhanced miR195 induced cell apoptosis. MiR195 promotes colorectal cancer cells SW620 apoptosis through downregulating Siah-1S.

Keywords: MiR195, colorectal cancer cell SW620, Siah-1S, cell apoptosis

Introduction

Colorectal cancer is a common malignant tumor in digestive system. Following the living tempo acceleration and eating habits changes, colorectal cancer mortality and morbidity keep on rising year by year, which seriously threatened to people's life and health [1]. At present, clinical cancer treatment mainly includes radiotherapy, chemotherapy, and surgery, etc. [2]. Radiotherapy and chemotherapy play critical roles in the treatment of colorectal cancer. However, they often are accompanied by a variety of clinical complications after treatment. Furthermore, radiotherapy and chemotherapy cannot fundamentally provide a radical cure. In addition, since the pathogenic site of rectal cancer often extends to the pelvic cavity, surgery is difficult and has many complications. Thus, it is urgently needed in clinic to search for more effective approaches for rectal cancer treatment.

With the molecular biology technology progress and genetic engineering emergence, targeted therapy has become an important direction of cancer, especially rectal cancer treatment. The emphasis and difficulty of targeted therapy in treating rectal cancer is to choose an important molecule as target that has critical effect in rectal cancer occurrence and development [3]. Therefore, clarifying the molecular mechanism of rectal cancer at gene and protein levels not only has important theoretical research significance, but also has potential clinical application value. This study intends to explore the regulatory mechanism of miRNA in colorectal cancer cells and to find the targeted therapy molecules.

MicroRNA is a type of small noncoding RNA with multiple regulatory functions. It is involved in cell proliferation, survival, apoptosis, autophagy, and signal transduction [4]. MiR195 is a recently discovered new miRNA. Previous studies suggested that miR195 can regulate cell growth and proliferation. However, the role of miR195 in regulating colorectal cancer cells remains to be further studied [5]. This study investigated the effect of miR195 on colorectal

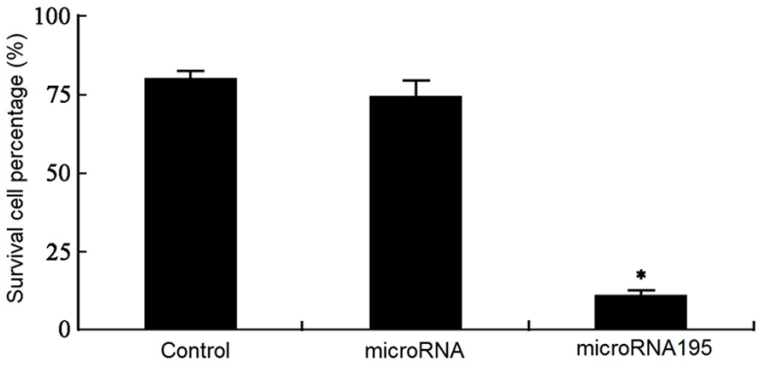

Figure 1. MiR195 transfection inhibited SW620 cell proliferation. *P < 0.05, compared with blank control.

cancer cell growth and proliferation, and the related molecular mechanism.

Seven in absentia homolog (Siah-1S) is a recently discovered antiapoptotic protein [6]. It was showed that Siah-1S expression was significantly higher in multiple cancers compared with para-carcinoma tissues, suggesting that Siah-1 can promote cell growth and proliferation. Moreover, Siah-1 can inhibit cell apoptosis. Siah-1 impact on rectal cancer remains to be further discussed [7]. We hypothesized that if Siah-1 also has the effect of promoting cell growth and inhibiting cell apoptosis, downregulating Siah-1 may enhance rectal cell sensitivity to chemotherapy. Thus, Siah-1 may be treated as a potential target in anti-rectal cancer cells.

The function and related molecular mechanism of miR195 on SW620 is still unclear. This study aimed to investigate the role and molecular mechanism of miR195 on SW620.

Materials and methods

Reagents and cells

Human colorectal cancer cell line SW620 was bought from ATCC. Cell viability detection reagent MTT was purchased from Beyotime. Apoptosis detection reagent caspase-3 activity kit and FITC-Annexin-V were got from Santa Cruz. Cell culture medium DMEM and fetal bovine serum were purchased from Beijing Huamei biological technology co., LTD. Mouse antihuman Siah-1 polyclonal antibody and actin antibody were from Sigma. MiR195 and control miRNA were synthetized by Genepharma. The sequence of Siah-1 and control siRNA were (5'-3') TACGACTTTACCGTTGGTT/TGCCTGGTTGATACAGCTA, and (5'-3') TACTGTACGTCTAGTTG/CTGTTGTGCACTTGACTAAGC. Siah-1 overexpression plasmid was constructed and preserved by our lab [8].

Cell culture

SW620 cells were routinely cultured at 5% CO_2 and 37°C [8].

MTT assay

Cell proliferation and viability were tested by MTT [8]. Specially, SW620 cells were diluted by medium containing 10% FBS, and then seeded into the 96-well plate at 10,000, 2,000, 400, 80, 16 cells/well. After cultured for 72 h, SW620 cells were treated by MTT solution for 6 h at 5% CO_2 and 37°C. DMSO was then added to each well for 15 min to stop the reaction. The plate was read on microplate reader at 492 nm to draw the proliferation curve.

Siah-1 siRNA transfection

Siah-1 and control siRNA were transfected to SW620 cells using liposome [9]. Siah-1 and control siRNA sequence were (5'-3') TACGACTTTACCGTTGGTT/TGCCTGGTTGATACAGCTA, and (5'-3') TACTGTACGTCTAGTTG/CTGTTGTGCACTTGACTAAGC, respectively. Specially, SW620 cells were seeded into 6-well plate at 1×10^6 cells/well and cultured at 5% CO_2 and 37°C for 24 h. Transfection reagent mixture was prepared in test tube at room temperature for 10 min. After washing the cells, transfection mixture was added to the well at 5% CO_2 and 37°C for 24 h. Then the cells were further cultured in normal medium for 48 h.

Flow cytometry

SW620 apoptosis was tested using routine method by FITC-Annexin-V [10]. Specially, the cells were resuspended in PBS at 1×10^5 cells/mL. Cell suspension, Annexin-V buffer, and FITC-Annexin-V were mixed at 250:50:1 (v/v/v) at room temperature for 30 min avoid of light. Then the total volume was adjusted to 300 μL and detected on flow cytometry (emission wavelength 488 nm, absorption wavelength

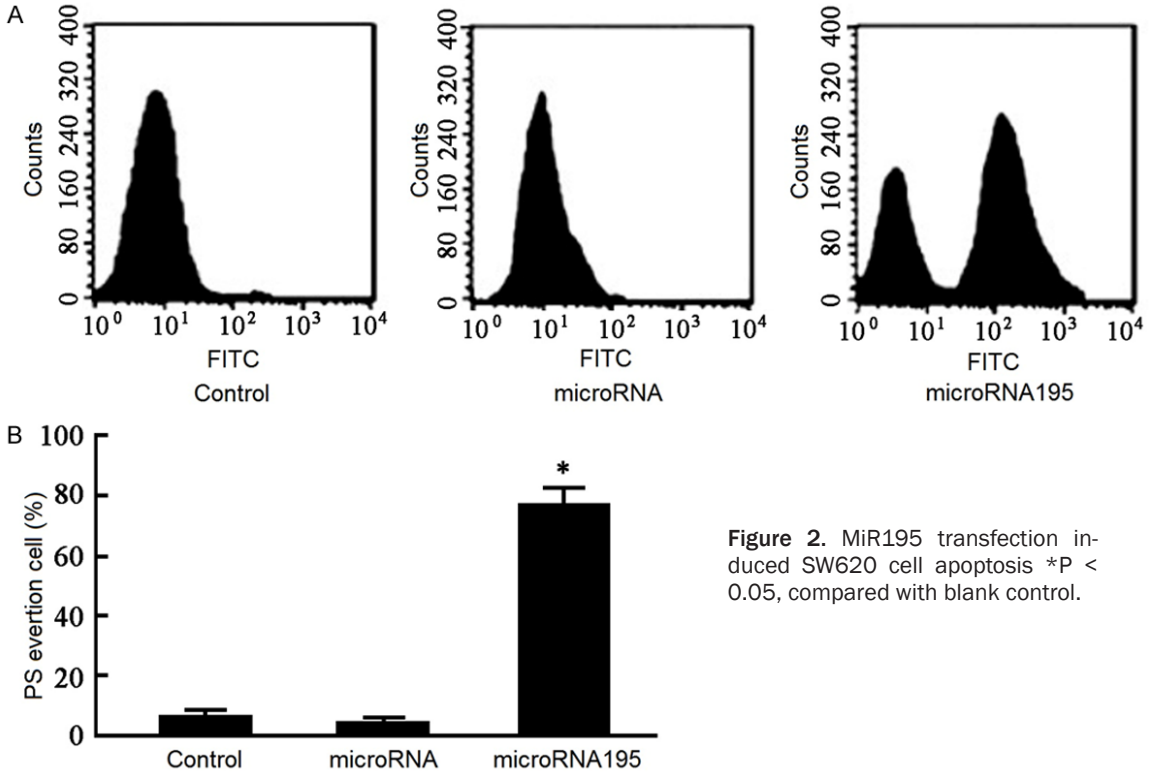

Figure 2. MiR195 transfection induced SW620 cell apoptosis *P < 0.05, compared with blank control.

625 nm). The percentage of positive FITC-Annexin-V cells was detected.

Western blot

Siah-1 protein expression level in SW620 cells were routinely tested by Western blot [8]. SW620 cells were collected and cracked on ice for 30 min to extract the total protein. The protein was separated by SDS-PAGE and transferred to PVDF membrane. After blocked by 5% skim milk at room temperature for 2 h, the membrane was incubated in primary antibody (1:1,000) at room temperature for 3 h. After washed, the membrane was further incubated in secondary antibody (1:1,000) at room temperature for 3 h. At last, the membrane was developed to analyze Siah-1 protein level.

Caspase-3 activity detection

Caspase-3 activity in SW620 cells were tested by detection kit produced by Beyotime [8]. SW620 cells were cracked by lysis provided by the kit and added with Ac-DEVD-pNA at 37°C for 45 min. Then the cells were read on microplate reader at 492 nm to calculate caspase-3 activity.

Statistical analysis

All data analysis was performed on SPSS 16.0 software [8]. All data was presented as mean ± standard deviation. One-way ANOVA was applied for data comparison. $P < 0.05$ was considered as statistical significance.

Results

MiR195 transfection inhibited SW620 cell proliferation

MTT assay was used to test SW620 cell proliferation (**Figure 1**). It was showed that control miRNA (1 μg) transfection did not affect SW620 cell proliferation, while miR195 transfection (1 μg) obviously suppressed SW620 proliferation ($P = 0.0068$).

MiR195 transfection induced SW620 cell apoptosis

As shown in **Figure 2**, flow cytometry revealed that control miRNA (1 μg) transfection did not impact SW620 cells phosphatidylserine eversion, whereas miR195 transfection (1 μg) significantly upregulated SW620 cells phosphati-

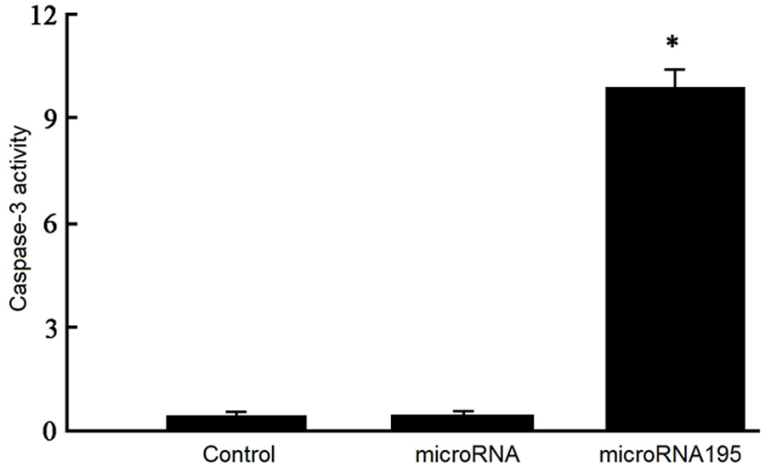

Figure 3. MiR195 transfection activated caspase-3 in SW620 cells. *P < 0.05, compared with blank control.

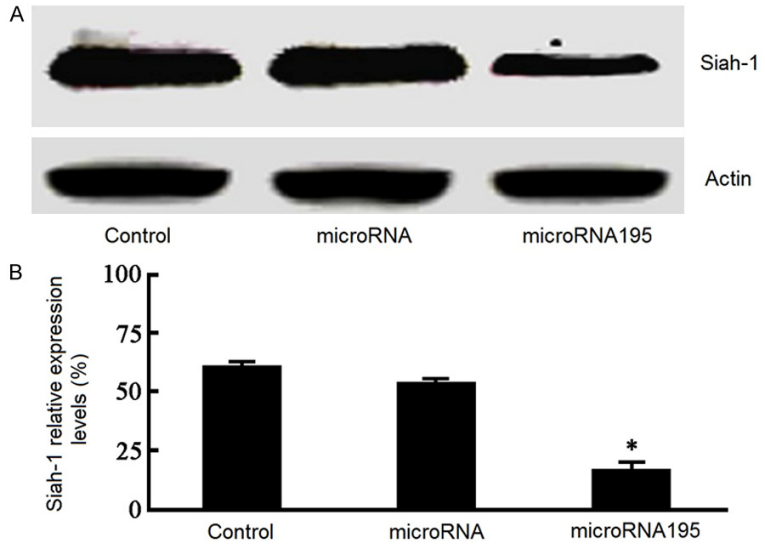

Figure 4. MiR195 transfection downregulated Siah-1 protein level. *P < 0.05, compared with blank control.

dylserine eversion compared with control (P = 0.018).

As shown in **Figure 3**, caspase-3 activity detection demonstrated that control miRNA (1 μg) transfection did not influence caspase-3 activity in SW620 cells, while miR195 transfection (1 μg) markedly activated caspase-3 level compared with control (P = 0.027).

MiR195 transfection downregulated Siah-1 protein level

Western blot showed that control miRNA (1 μg) transfection did not affect Siah-1 protein level in SW620 cells, whereas miR-195 transfection (1 μg) significantly downregulated Siah-1 level compared with control (P = 0.039) (**Figure 4**).

Siah-1 knockdown enhanced miR195 induced SW620 cell apoptosis

Western blot revealed that Siah-1 siRNA transfection obviously declined Siah-1 protein level in SW620 cells (P = 0.0041), while control miRNA transfection failed to impact Siah-1 level in SW620 cells (**Figure 5A** and **5B**).

Caspase-3 activity detection showed that both Siah-1 siRNA and control miRNA transfection did not activate caspase-3, further miR195 transfection markedly elevated caspase-3 activity in SW-620 cells (P = 0.0017). It suggested that knockdown Siah-1 enhanced miR195 induced SW620 cell apoptosis (**Figure 5C**).

Siah-1 overexpression restrained miR195 induced SW620 cell apoptosis

As shown in **Figure 6A** and **6B**, it was found that Siah-1 plasmid significantly upregulated Siah-1 protein level in SW620 cells (P = 0.0023). Control plasmid transfection did not affect Siah-1 level in SW620 cells.

Caspase-3 activity detection showed that Siah-1 and control plasmid transfection failed to change caspase-3 activation, whereas further miR195 transfection obviously decreased caspase-3 activity (P = 0.0023). It indicated that Siah-1 overexpression inhibited miR195 induced SW620 cell apoptosis.

Discussion

Rectal cancer is a common gastrointestinal tumor in clinic [8]. Following life rhythm accel-

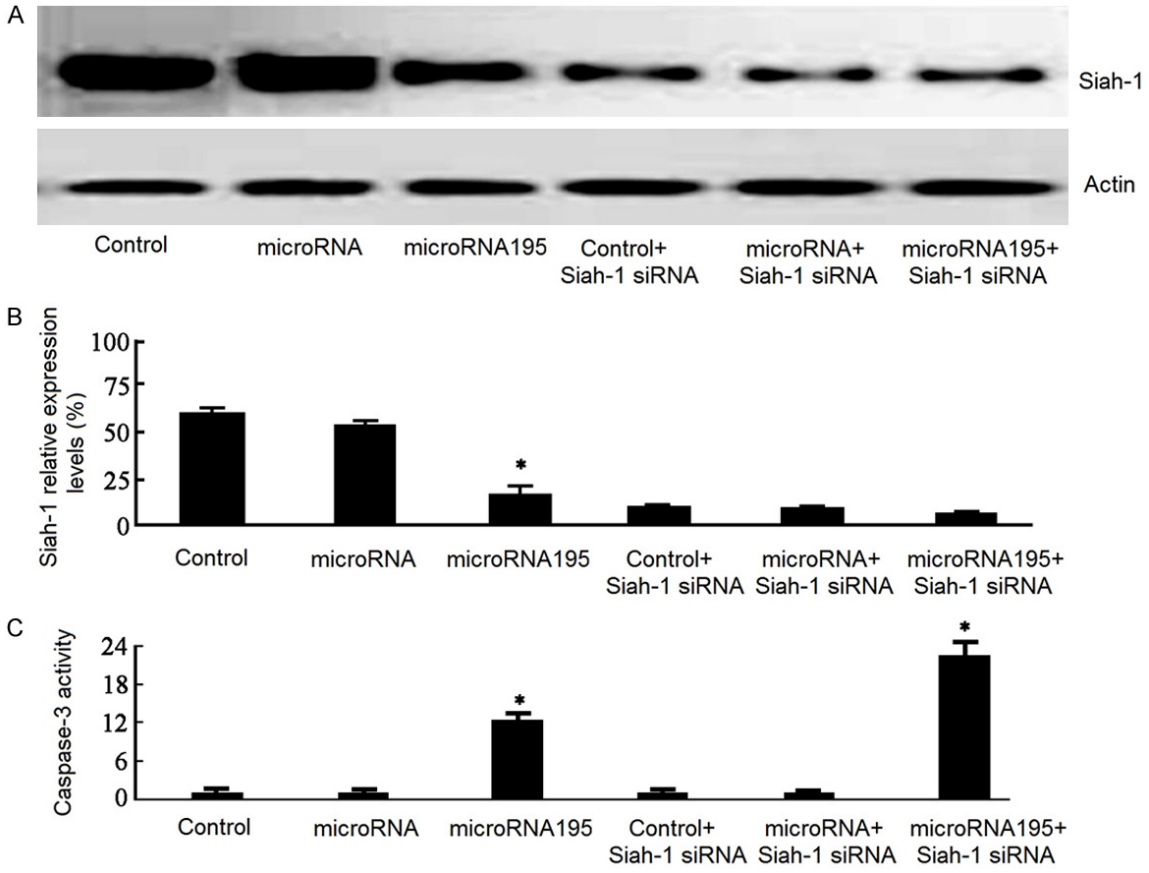

Figure 5. Siah-1 knockdown enhanced miR195 induced SW620 cell apoptosis. *P < 0.05, compared with blank control.

eration and eating habit changes around the world, rectal cancer incidence and mortality rates keep on rising [8]. Thus, it is of significance both in theory and clinic to investigate rectal cancer cell SW620 proliferation and survival.

In clinic, the main treatment methods for rectal cancer include radiotherapy, chemotherapy, and surgery [8]. Though the aforementioned methods are of great significance in rectal cancer treatment, there are a variety of clinical complications [8]. More effective method is urgently needed for clinical treatment. This study discussed rectal cancer oncogenesis from molecular level, aiming to provide theoretical basis for molecular targeted therapy.

MiR195 is a currently new discovered microRNA, and its regulating role in rectal cancer still needs further investigation [11]. We explored miR195 impact on human rectal cancer cells SW620 and related mechanism. The results showed that miR195 transfection inhibited SW620 cell proliferation, promoted SW620 cell apoptosis, and downregulated Siah-1S level. Siah-1S overexpression suppressed miR195 induced cell apoptosis, while Siah-1S downregulation enhanced miR195 induced cell apoptosis.

Siah-1 is a type of anti-apoptotic protein [8]. Our results also demonstrated that Siah-1 played a regulating role in miR195 induced SW620 cell apoptosis, which was in accordance with previous investigation [8]. Interestingly, Siah-1S overexpression inhibited miR195 induced apoptosis [8]. Siah-1S overexpression enhanced miR195 induced cell apoptosis. These results suggested that miR195 may cause SW620 cell apoptosis through regulating Siah-1S level directly or indirectly.

The relationship between miR195 and Siah-1S still needs further discussion. From our results, Siah-1 may be a direct target gene of miR195.

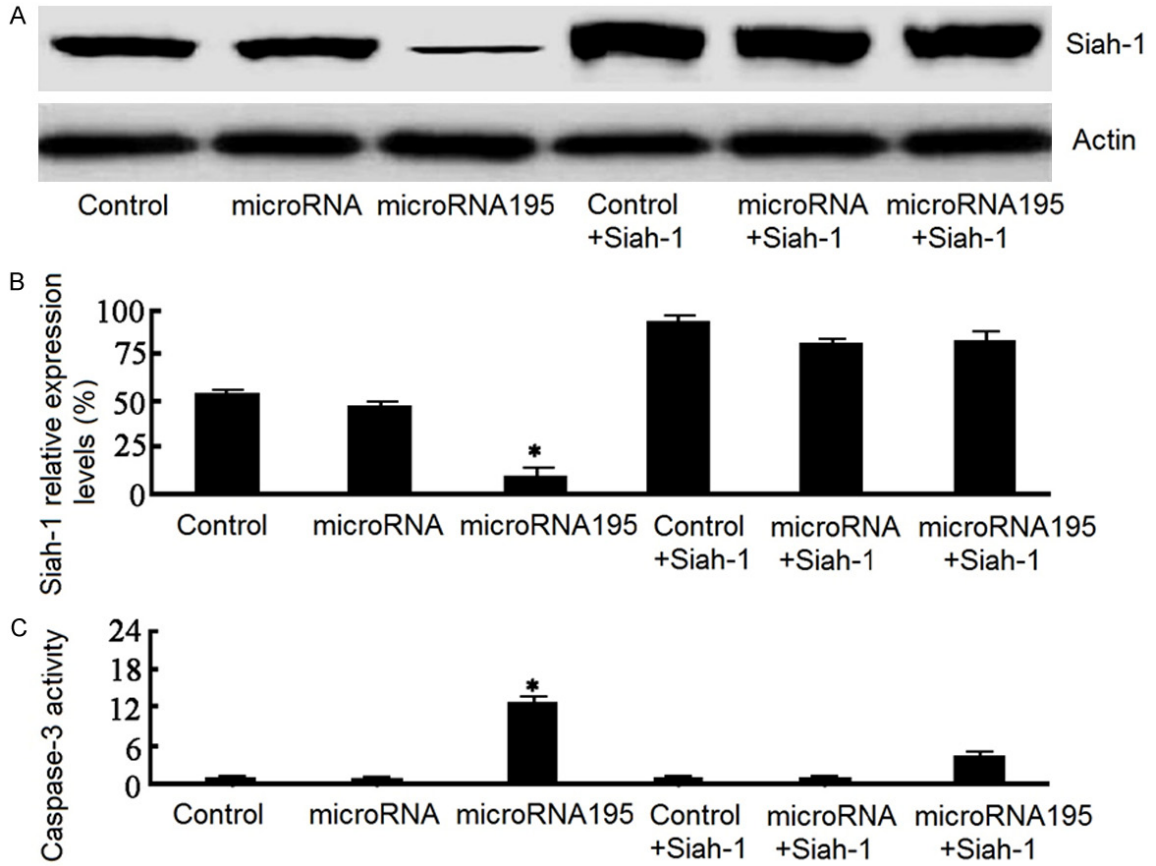

Figure 6. Siah-1 overexpression restrained miR195 induced SW620 cell apoptosis. *P < 0.05, compared with blank control.

In addition, it is also possible that Siah-1 is an indirect target of miR195, namely miR195 may regulate Siah-1 level indirectly through other molecules. Siah-1 can regulate cell apoptosis directly as an anti-apoptotic protein. Limited to our laboratory condition, we did not verified abovementioned hypothesis. However, we could provide the possible solution. Firstly, using bioinformatics to predict whether Siah-1 has miR195 binding domain, and combining luciferase reporter gene to analyze Siah-1 promoter. Secondly, using immune precipitation technique to detect the candidate direct interaction protein, and then analyzing whether the candidate protein is regulated by miR195. On the other side, using immune precipitation to test whether miR195 exists in the coprecipitate of Siah-1. More investigation is needed to test the aforementioned possibility.

In this study, there are three evidences to confirm that miR195 induced SW620 cell apoptosis has a close relationship with Siah-1 protein level. (1) Western blot showed that Siah-1 protein level was significantly decreased in SW620 cells after miR195 transfection. (2) Siah-1 siRNA obviously enhanced SW620 cell apoptosis induced by miR195. (3) Siah-1 overexpression plasmid suppressed miR195 induced SW620 cell apoptosis. All these data suggested that Siah-1 protein played a regulation role in miR195 induced SW620 cell apoptosis, indicating that Siah-1 may be a potential new target for rectal cancer treatment [8].

This study also has three shortcomings and insufficiency. (1) This study did not collect rectal cancer patients specimens in different stages and detect Siah-1 protein level in cancer tissue and para-carcinoma tissue, which was facilitate to investigate the relationship between Siah-1 level and rectal cancer. (2) This study failed to collect rectal cancer specimens that received chemotherapy or radiotherapy and detect Siah-1 protein expression in cancer tissue and para-carcinoma tissue, which was

useful to explore whether Siah-1 could be treated as a rectal cancer marker. (3) This study did not establish animal model [8] and change Siah-1 protein and miR195 levels through siRNA, which was helpful to test the curative effect of treating rectal cancer through regulating Siah-1 protein and miR195 levels.

In brief, our results suggested that miR195 inhibited SW620 cell proliferation and induced cell apoptosis through downregulating Siah-1S level.

Acknowledgements

Financial support for this study was provided by Health and family planning scientific research project of Hubei Province (No. wj2015mb232).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Zhengwei Cheng and Wenjia Lv, Department of GI Medicine, Xianning Central Hospital (The First Affiliated Hospital, Hubei University of Science and Technology), 228 Jingui Road, Xianning 437100, Hubei, China. Tel: +86-13997502750; E-mail: zhengweichengv@sina.com (ZWC); wenjialvv@163.com (WJL)

References

[1] Yau TO, Wu CW, Tang CM, Chen Y, Fang J, Dong Y, Liang Q, Ng SS, Chan FK, Sung JJ and Yu J. microRNA-20a in human faeces as a non-invasive biomarker for colorectal cancer. Oncotarget 2016; 7: 1559-68.

[2] Cai SD, Chen JS, Xi ZW, Zhang LJ, Niu ML and Gao ZY. MicroRNA144 inhibits migration and proliferation in rectal cancer by downregulating ROCK1. Mol Med Rep 2015; 12: 7396-7402.

[3] Witalison EE, Cui X, Causey CP, Thompson PR and Hofseth LJ. Molecular targeting of protein arginine deiminases to suppress colitis and prevent colon cancer. Oncotarget 2015; 6: 36053-36062.

[4] Huang L, Wang X, Wen C, Yang X, Song M, Chen J, Wang C, Zhang B, Wang L, Iwamoto A, Wang J and Liu H. Hsa-miR-19a is associated with lymph metastasis and mediates the TNF-alpha induced epithelial-to-mesenchymal transition in colorectal cancer. Sci Rep 2015; 5: 13350.

[5] Dai J, Wu W, Zhou J, Gao K, Hu G, Lin C, Zhang Y and Li X. Effect of antisense microRNA targeting survivin on rectal cancer HRC-9698 cells and its mechanism. Int J Clin Exp Pathol 2015; 8: 6057-6063.

[6] Nakao T, Iwata T, Hotchi M, Yoshikawa K, Higashijima J, Nishi M, Takasu C, Eto S, Teraoku H and Shimada M. Prediction of response to preoperative chemoradiotherapy and establishment of individualized therapy in advanced rectal cancer. Oncol Rep 2015; 34: 1961-1967.

[7] Pradhan M, Nagulapalli K, Ledford L, Pandit Y and Palakal M. A system biology approach for understanding the miRNA regulatory network in colon rectal cancer. Int J Data Min Bioinform 2015; 11: 1-30.

[8] Svoboda M, Sana J, Fabian P, Kocakova I, Gombosova J, Nekvindova J, Radova L, Vyzula R, Slaby O. MicroRNA expression profile associated with response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients. Radiat Oncol 2012; 7: 195.

[9] Azizian A, Kramer F, Jo P, Wolff HA, Beissbarth T, Skarupke R, Bernhardt M, Grade M, Ghadimi BM and Gaedcke J. Preoperative Prediction of Lymph Node Status by Circulating Mir-18b and Mir-20a During Chemoradiotherapy in Patients with Rectal Cancer. World J Surg 2015; 39: 2329-2335.

[10] Smith AR, Marquez RT, Tsao WC, Pathak S, Roy A, Ping J, Wilkerson B, Lan L, Meng W, Neufeld KL, Sun XF and Xu L. Tumor suppressive microRNA-137 negatively regulates Musashi-1 and colorectal cancer progression. Oncotarget 2015; 6: 12558-12573.

[11] Yang Y, Peng W, Tang T, Xia L, Wang XD, Duan BF and Shu Y. MicroRNAs as promising biomarkers for tumor-staging: evaluation of MiR21 MiR155 MiR29a and MiR92a in predicting tumor stage of rectal cancer. Asian Pac J Cancer Prev 2014; 15: 5175-5180.