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Original Article

Inhibition of miR-224 Repressed Cell Growth, Migration and Invasiveness in Gastric Cancer

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ABSTRACT

Aim/Background: MiR-224 has been reported to be associated with many cancers, however, the role of miR-224 in gastric cancer was unclear. **Materials and Methods:** In the present study, we first used RT-PCR to detect miR-224 level in gastric cancer fresh tissue. Then we performed miR-224 inhibitor transfection in gastric cancer cells, and MTT, cell invasion and migration and colony formation were performed to explore the role of miR-224 in gastric cancer *in vitro*. **Results:** The results showed that the relative level of miR-224 in human gastric cancer tissue was significantly higher than that in the adjacent non-tumor gastric mucosa. After transfected by miR-224 inhibitor, the gastric cancer cell proliferation rate was lower compared with the control group, and the migration and invasion are also inhibited by miR-224 inhibitors. The colony number of gastric cancer cells transfected with miR-224 inhibitors was significantly lower than that of the control group. **Conclusion:** Our results demonstrated that miR-224 is upregulated in gastric cancer. miR-224 inhibitors repressed the proliferation, migration, invasion and colony formation capacity of gastric cancer cells, which indicated that inhibition of miR-224 might be a promising therapeutic option for human gastric cancer.

Keywords: miR-224, Growth, Migration, Invasiveness, Gastric Cancer.

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INTRODUCTION

Gastric cancer is one of the most common malignancies in the world. Although the worldwide incidence has declined in recent few decades, the gastric cancer is still the second leading cause of cancer death, following only lung cancer in overall mortality.[1] In recent years, many studies have shown that the occurrence and development of gastric cancer is the result of multi-gene and multi-site mutations in genome, but the related mechanism is still unclear. MicroRNAs (microRNAs, miRNAs) are endogenous non-coding RNAs with a length of approximately 22 nucleotides. Recent studies have identified more than 500 miRNAs in human, which regulate the expression of more than 5000 genes and 30% of the proteins.[2,3] Recently, miRNA has been found to play a role in the progression of gastric cancer, including invasion and metastasis..[4] This indicated that miRNAs may play a vital role in tumorigenesis and cancer development.

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MiR-224 has been associated with numerous cancers. Zheng *et al.*,[5] showed that miR-224 has impact on cell migration and invasion, which suggested that it could be a prospective therapeutic target for early-stage non-metastatic colorectal cancer. Analysis performed by TCGA datasets revealed that the expressions of miR-224 were upregulated in lymph node metastasis samples compared with non-metastasis samples.[6] miRNA-seq data analysis also identified that miR-224 were upregulated in lung adenocarcinoma tissues from patients with lymph node metastases compared to those without lymph node metastases.[7] In gastric cancer, Xia *et al.*,[8] found that exogenous miR-224 expression promoted the proliferation and migration of gastric cells. However, there are still few studies on the role and mechanism of miR-224 in gastric cancer, and the role and mechanism are not clear. Here we study the roles and its related mechanisms of miR-224 in gastric cancer.

MATERIALS AND METHODS

Cell Culture

Human gastric cancer cell lines SGC-7901, AGS, BGC-823, MKN-45, MKN-28, HCG-27 and human normal gastric epithelial cells GES-1 were generated from the Key Laboratory of Gastroenterology in our hospital.

Take out the previously frozen cells from liquid nitrogen and quickly put them into a 37°C water bath. After thawing, transfer the cell suspension into the centrifuge tubes containing 10 mL 10% Fetal Bovine Serum (FBS) in RPMI 1640 medium. Centrifuge at 1200 rpm for 5 min. After discarding the supernatant, resuspend and transfer the cells to a culture flask containing 10% FBS in RPMI 1640 medium, and place them into a 37°C, 5% CO_2 incubator for static culture.

When the cells reach 70-80% confluent, discard the culture medium, wash 3 times with PBS, add 0.25% trypsin (including EDTA) solution and digest for 3-5 min at 37°C. Then add 2 mL RPMI-1640 culture medium with 10% FBS to stop the digestion, transfer the cell suspension to a 10 mL centrifuge tube, centrifuge at 1200 rpm for 5 min, discard the supernatant, and resuspend the cells. Inoculate the cells into a culture flask containing RPMI-1640 culture medium with 10% FBS. The cells were cultured in 5% CO_2 incubator at 37°C. When necessary, freeze and store the cells at -80°C or in liquid nitrogen tank.

Fresh tissue specimen

Fresh gastric cancer tissue specimens and adjacent non-tumor gastric mucosal tissues were selected from 44 cases of radical gastric cancer resection in our hospital during 2010-01-2010-08. None of the cases had received treatment before surgery and were confirmed through pathology. The clinicopathological diagnosis of HE sections were verified by two experienced pathologists. Among the 44 gastric cancer specimens, 32 were males and 12 were females, with a median age of approximately 61 (41-78) years. Among the 44 gastric cancer specimens, 7 tumors were located in the fundus of the cardia, 20 tumors were in gastric body, and 17 tumors were located in gastric antrum; The size of tumors were smaller than 5 cm in 18 cases and larger than 5 cm in 26 cases. According to the classification standards for gastric cancer in American Joint Cancer Council (AJCC) 2010, 16 cases were moderately differentiated adenocarcinoma, 27 cases were poorly differentiated adenocarcinoma, and 1 case was signet ring cell carcinoma; 9 cases had no lymph node metastasis, 35 cases had lymph node metastasis; There were 2 cases of TNM stage I gastric cancer, 6 cases of stage II gastric cancer, 31 cases of stage III gastric cancer, 5 cases of stage IV gastric cancer, and 7 cases had distant metastasis (liver and peritoneum, etc.,). Each control specimen was collected from the edge of the tumor more than 5 cm without proliferation or paired non-tumor gastric mucosal tissue with dysplasia.

Quantitative Real-time RT-PCR

Cells were thoroughly lysed by TRIZOL reagent. Shake the test tube for 15 sec after adding chloroform and incubate it at room temperature for 2-3 min; then centrifuge at 10,000 g for 15 min at 4°C. Carefully aspirate the supernatant, add isopropanol and mix well, incubate at room temperature for 10 min, centrifuge at 10,000 g for 15 min at 4°C. Remove the upper suspension, wash the RNA pellet once with 75% ethanol, centrifuge at 7,500 g for 5 min at 4°C. Air-dry RNA pellet for 5 min. Dissolve RNA in DEPC-treated water by passing solution a few times through a pipette tip. The first strand of cDNA was synthesized according to the instructions, the reaction solution included 1 μg total RNA, 4 μL miScript RT Buffer and 1 μL miScript reverse transcriptase mix, add water to 20 μL. The reaction mix was incubated at 37°C for 60 min. The miRNA-224 PCR reaction system included 1 μL of universal primer, 1 μL of target primer, 1 μL of template cDNA, QuantiTect SYBR Green PCR Master Mix 10 μL, RNase free H2O 7 μL. The PCR reaction conditions are 95°C 15 min; 95°C, 30 sec, 56°C, 20 sec, 72°C 20 sec, 40 cycles; 95°C, 30 sec, 60°C, 30 sec, 90°C, 30 sec. U6 is used as an internal reference, and the data is analyzed by $2^{-\triangle CT}$ method, $^{\Delta Ct}$=Ct target gene-Ct internal reference gene.

miR-224 inhibitor transfection in gastric cancer cells

When the confluence of adherent cells reached 70% or more, then was transfected by miR-224 inhibitor and negative control with the siPORT™ Amine Transfection Agent. Briefly, miR-224 inhibitor and negative control were diluted and mixed in Opti-MEM I serum-free medium. After incubating it at room temperature for 10 min, the diluted siPORT™ Amine Transfection Agent was added. Then the mixture and RPMI-1640 were added to the culture plate. The culture medium serves as a normal control. Then put it into a 37°C, 5% CO_2 cell incubator for culture. 6 hr later, replace it with 2 mL RPMI-1640 (10% FBS) culture medium to continue the culture, and the cells were used for subsequent experiments after 24 hr.

MTT assay for cell proliferation analysis

After 24 hr of transfection, digest SGC-7901 cells with trypsin for 5 min, add 1 mL of 1640 complete culture medium to make a cell suspension, and inoculate it into a 96-well plate at a density of 3000 cells/well. The experiment set up 6 parallel wells for each group. The cell proliferation inhibition rate was measured by the MTT method after culturing for 24 hr, 48 hr and 72 hr. Add 20 μL MTT to each well and place at 37°C for 4 hr; discard the supernatant, add 150 μL DMSO into each well, shake for 10 min, adjust to zero by the blank control method, and measure the absorbance value (A value) of each well at 570 nm and 630 nm wave lengths, calculate the value of proliferation inhibition rate according to the following formula. Repeat the experiment three times.

$$\text{Proliferation inhibition rate (\%)} = \left(1 - \frac{A_{experiment}\text{-}A_{blank}}{A_{control}\text{-}A_{blank}}\right) \times 100\%$$

Cell invasion and migration experiments

Resuspend the SGC-7901 cell line with serum-free RPMI-1640 medium at 24 hr after transfection. Add 2×10^5 cells in 250 μL to the upper chamber of the QCMTM cell migration or invasion

kit. The bottom chamber was filled with cell culture medium. The cells were cultured in a 37°C and 5% CO_2 cell incubator for 48 hr.

Then the chamber cells were fixed with pre-cooled 95% ethanol for 15 min and wiped with a cotton swab. After washing with PBS for 5 min × 3 times, the cells were stained with hematoxylin for 10 min, rinsed with water for 5 min, hydrochloric acid alcohol 2 Sec, eosin for 10-20 sec and water for 5 min. After dehydration with gradient alcohol, the chambers were mounted with neutral gum and counted under light microscope at × 400 magnification, across 10 high-power fields.

Colony formation experiment

The SGC-7901 cells were digested into single cells with 0.25% trypsin at 24 hr after transfection and resuspended in 10% FBS/DMEM medium. Then 200 cells were inoculated into each well of 6-well plates. The cells were cultured in a 37°C, 5% CO_2 cell incubator for 2 weeks.

When the colony clusters appear in the plate, the supernatant was removed, and the cells were fixed with pure methanol for 15 min. Then the cells were stained with GIMSA solution and rinsed with distilled water. Count the wells with more than 50 colonies, and calculate the colony formation rate according to the formula:

$$\text{Colony formation rate} = \frac{\text{Colony Number}}{\text{Number of Inoculated Cells}} \times 100\%$$

Statistical analysis

The statistical analyses were performed by Statistic Package for Social Science (SPSS) 16.0 Statistics Software Package (SPSS, Inc., Chicago, IL, USA). The expressions of miR-224 from fresh tissue were analyzed by paired *t* test. The data of cell analysis were obtained from three independent experiments and showed as Mean±Standard Deviation (SD), and the Student's t-test were used to analyze experimental data. The value of $p<0.05$ was considered statistically significant.

RESULTS

The expression level of miR-224 was high in human gastric cancer tissues

The expression of miR-224 was detected in the gastric cancer tissues and adjacent non-tumor gastric mucosa tissues via qRT-PCR. According to the $2^{-\Delta CT}$ method, the relative expression of miR-224 in the 65.9% (29/44) of human gastric cancer tissue was significantly higher than that in the adjacent non-tumor gastric mucosa. The difference was statistically significant (Figure 1. $t = 3.203$, $p=0.003$).

The transfection effect of miR-224 inhibitor in gastric cancer cell lines

The expression level of miR-224 in human gastric cancer cell line SGC-7901, MKN-45, BGC-823 and AGS is higher than that in normal human gastric mucosal epithelial cell line GES-1. However, its expression in gastric cancer cell line MKN28 and HCG-27 is relatively lower (Figure 2A).

The expression of miR-224 in human gastric cancer line SGC-7901 cell line was significantly reduced than that of the control group at 24 hr after the miR-224 inhibitor transfection. The difference was statistically significant ($p<0.05$) (Figure 2B).

The proliferation of human gastric cancer cells was repressed by miR-224 inhibitor

At 24 hr after miR-224 inhibitor transfection, the MTT assay showed that the cell proliferation rate (31%±6.2) of human gastric cancer cells was significantly lower than the control group (46%±5.7). At 48 hr, the proliferation rate of gastric cancer cells (56 %±6.1) was also significantly lower than the control group (75%±5.3), and the difference was statistically significant ($p<0.05$); At 72 hr, the gastric cancer proliferation rate (75%±7.6) was still lower compared with the control group (97%±5.6). The difference is statistically significant ($p<0.01$) (Figure 2C).

miR-224 inhibitor suppress the migration of gastric cancer cells

The gastric cancer cell line SGC-7901 was transfected with miR-224 inhibitor for 24 hr and the cell migration experiment

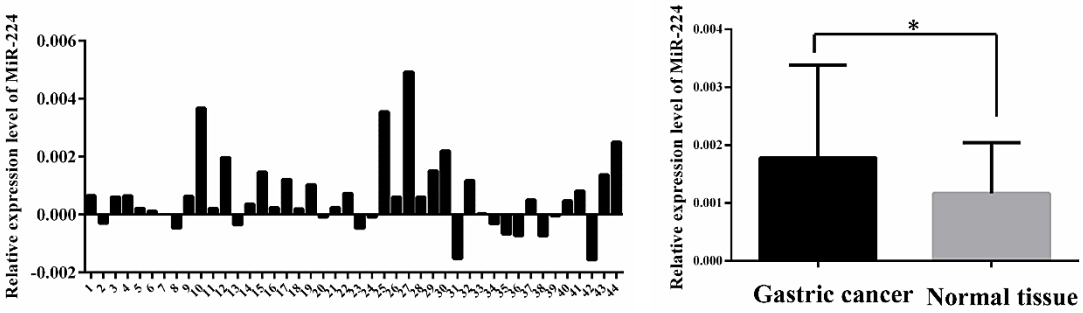

Figure 1: Mir-224 level was high in gastric cancer than in adjacent non tumor tissues. *$p<0.05$.

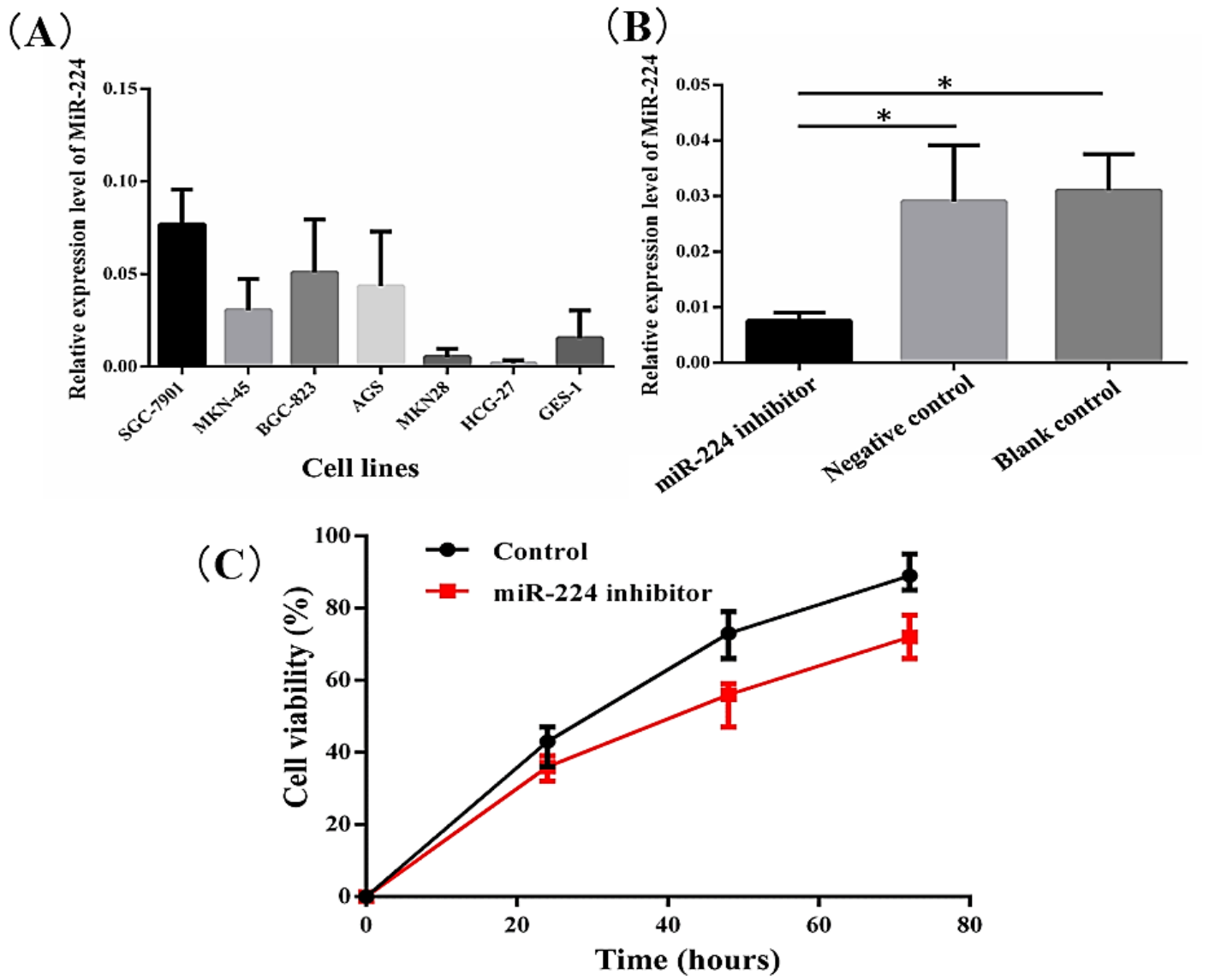

Figure 2: (A) Mir-224 level was high in gastric cancer cell line than normal gastric epithelial cells. (B) After transfection with mir-224 inhibitor, SGC-7901 cells showed significantly downregulated expression level of mir-224. (C) MTT proliferation curve showed that mir-224 inhibitor significantly reduced the proliferation of SGC-7901 cells. *$p<0.05$.

was carried out. After cultured for 24 hr in an incubator at 37°C and 5% CO_2, the cells on the inner membrane were wiped off with a wet cotton swab and the results of migration were observed after HE staining. The number of migrating cells in the miR-224 inhibitor group (14.8±1.92) was significantly less than that in the control group (39.4±2.30), and the difference was statistically significant (t = 23.894, $p<0.001$) (Figure 3A).

The gastric cancer cell invasion was inhibited by miR-224 inhibitor transfection

The cell invasion experiment was performed with gastric cancer cell line SGC-7901 after the miR-224 inhibitor transfection for 24 hr. After the static culture for 48 hr, the results of HE staining were observed and counted. After miR-224 inhibitor transfection, the number of SGC-7901 cells that penetrated the membrane (4.4±1.34) was significantly less than that of the control group (18.8±3.03), and the difference was statistically significant (t=13.987, $p<0.001$) (Figure 3B).

The formation colonies were repressed by miR-224 inhibitor in human gastric cancer cells

At 24 hr after miR-224 inhibitor transfection, the colony formation experiment were performed with SGC-7901 cells and the results were observed 2 weeks later. The number of colonies transfected with miR-224 inhibitors in SGC-7901 cells (63±11.67) was significantly lower than that of the control group (109±16.82), which was statistically significant (t=3.863, p=0.018) (Figure 4).

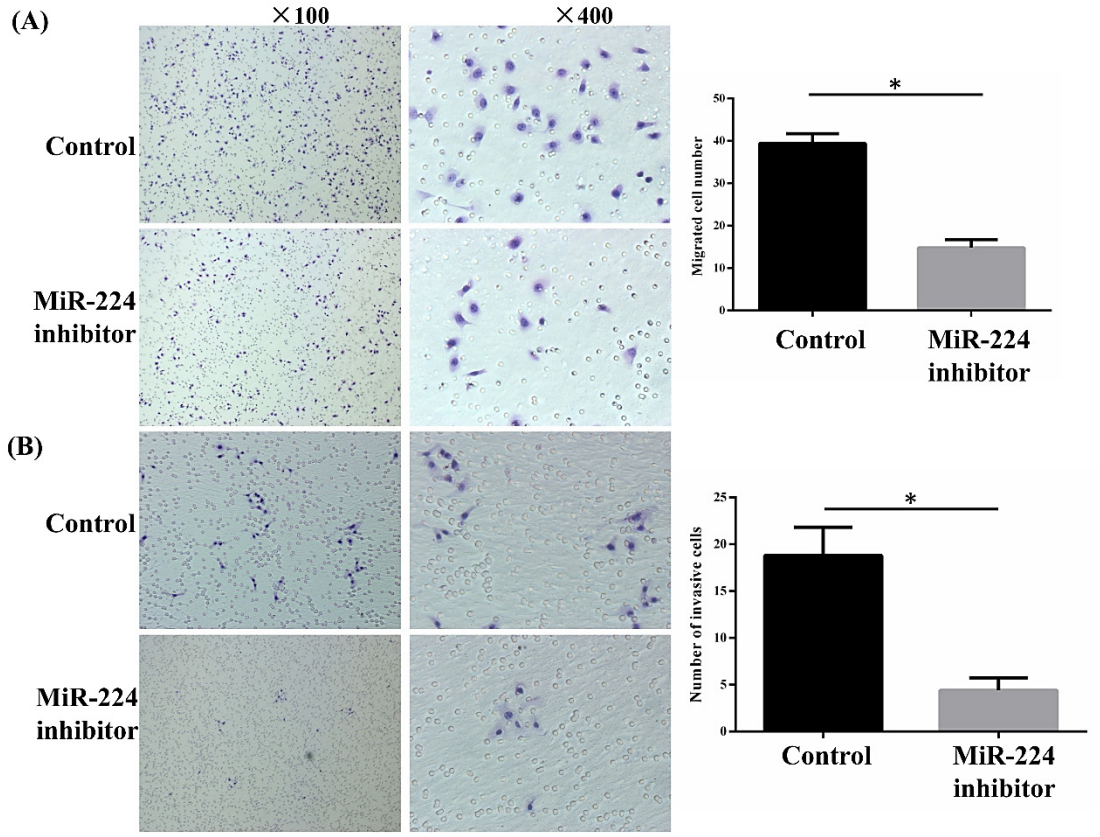

Figure3: The effect of mir-224 on migration and invasion was detected by traswell analysis. (A) Migration analysis showed that mir-224 inhibitor significantly reduced migrated gastric cancer cells. (B) Invasion analysis showed that mir-224 inhibitor significantly reduced invasive gastric cancer cells. *$p<0.05$.

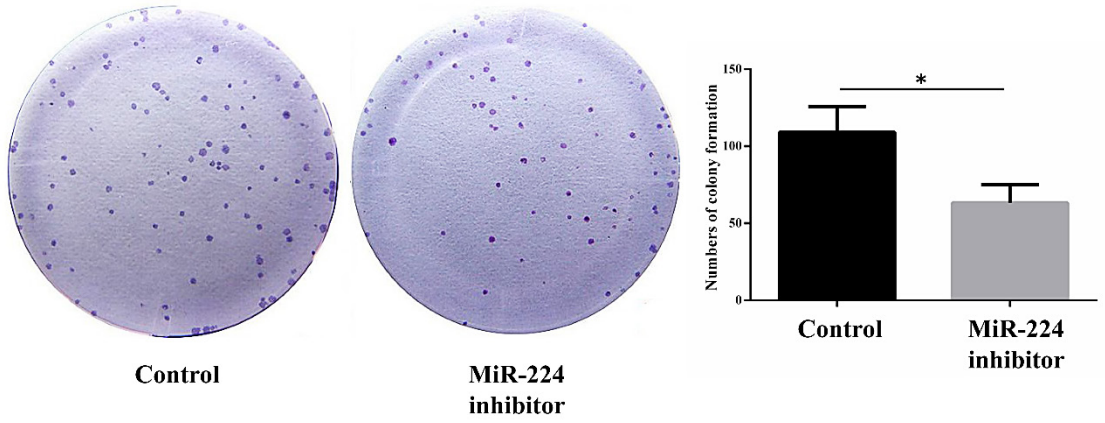

Figure 4: The colony formation of SGC-7901 was repressed by miR-224 inhibitor.

DISCUSSION

Recent advances in the field of epigenetics have shown that human cancer cells harbor global abnormalities of noncoding genes, which play an important role in the formation and development of tumors.[9] Especially a class of RNA molecules called microRNA (MicroRNA, miRNA), which can regulate its target genes by degrading or inhibiting the translation process of mRNA and cause the abnormal expression of target genes. Furthermore, the research on miR-224 has become a hot spot. Motoyama *et al.*,[10] used microarray to analyze the differential expression of miRNAs in 4 cases of colorectal cancer tissues, they found that miR-224 was highly expressed in colorectal cancer tissues. Wang[11] and Arndt *et al.*,[12] also used microarrays to screen the differential expression of miRNAs in colon cancer and surrounding non-tumor colonic mucosa tissues. They found that miR-224 was highly expressed in colon cancer tissues. In this study, we found that the expression of miR-224 was significantly increased in human gastric tumors and gastric cancer tissues, which is consistent with the above studies

and suggest that miR-224 plays important roles in the occurrence and development of human gastric cancer.

In this study, we further transfected the miR-224 inhibitor into a human gastric cancer cell line and observed the effects on the cell proliferation, migration and invasion. We found that miR-224 inhibitor reduced the proliferation, migration, invasion and colony formation ability of human gastric cancer cells, highlighting the crucial roles of miR-224 in human gastric cancer development. Research on liver cancer cells HepG2 revealed that miR-224 promotes cell expansion, migration and invasion by regulating the expression of PAK4 and MMP9.[13] These studies suggest that miR-224 may promote tumor progression by affecting cancer cell proliferation, invasion and migration.

CONCLUSION

Collectively, our results demonstrate that miR-224 is highly expressed in human gastric cancer tissues and cell lines. Inhibitor of miR-224 repressed the proliferation, migration, invasion and colony formation capacity of gastric cancer cells. Importantly, these results suggest that inhibiting miR-224 could be a promising therapeutic approach for human gastric cancer.

ABBREVIATIONS

RT-PCR: Reverse Transcription-Polymerase Chain Reaction; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide; **TCGA:** The Cancer Genome Atlas; **FBS:** fetal bovine serum; **EDTA:** Ethylene Diamine Tetraacetic Acid; **DEPC:** Diethyl Pyrocarbonate; **DMSO:** Dimethyl Sulfoxide; **SPSS:** Statistic Package for Social Science; **PAK4:** p21-activated kinase 4; **MMP9:** Matrix Metalloproteinase-9.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Chang-Ming Shao: Supervision, Investigation and original writing. Qin-Shu Shao: revise and review, Ying-Yu Ma: analyze and review.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was supported by ethics committee of Zhejiang provincial people's hospital.

SUMMARY

Our study showed that high expression of miR-224 may play an important role in development of gastric cancer. Inhibition of miR-224 repressed the proliferation, migration, invasion and colony formation capacity of gastric cancer cells, indicating that miR-224 might be a promising therapeutic target for human gastric cancer.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394-424. doi: 10.3322/caac.21492, PMID 30207593.
2. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 2006; 34((Database issue)):D140-4. doi: 10.1093/nar/gkj112, PMID 16381832.
3. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005; 120(1): 15-20. doi: 10.1016/j.cell.2004.12.035, PMID 15652477.
4. Xu X, Dang Z, Zhang J, Feng Y, Wei Z. The miRNA, miR-125b, inhibited invasion and metastasis of gastric-cancer cells by triggering the STAT3 signaling pathway. Cancer Manag Res. 2020; 12: 8569-80. doi: 10.2147/CMAR.S259513, PMID 32982449.
5. Zheng Q, Yu JJ, Li C, Li J, Wang J, Wang S. miR-224 targets BTRC and promotes cell migration and invasion in colorectal cancer. 3 Biotech. 2020; 10(11): 485. doi: 10.1007/s13205-020-02477-x, PMID 33117626.
6. Wang Y, Shang S, Yu K, Sun H, Ma W, Zhao W. miR-224, miR-147b and miR-31 associated with lymph node metastasis and prognosis for lung adenocarcinoma by regulating PRPF4B, WDR82 or NR3C2. PeerJ. 2020; 8: e9704. doi: 10.7717/peerj.9704, PMID 33282547.
7. Meng W, Ye Z, Cui R, Perry J, Dedousi-Huebner V, Huebner A, *et al.* MicroRNA-31 predicts the presence of lymph node metastases and survival in patients with lung adenocarcinoma. Clin Cancer Res. 2013; 19(19): 5423-33. doi: 10.1158/1078-0432.CCR-13-0320, PMID 23946296.
8. Xia M, Wei J, Tong K. MiR-224 promotes proliferation and migration of gastric cancer cells through targeting PAK4. Pharmazie. 2016; 71(8): 460-4. doi: 10.1691/ph.2016.6580, PMID 29442033.
9. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006; 6(11): 857-66. doi: 10.1038/nrc1997, PMID 17060945.
10. Motoyama K, Inoue H, Takatsuno Y, Tanaka F, Mimori K, Uetake H, *et al.* Over- and under-expressed microRNAs in human colorectal cancer. Int J Oncol. 2009; 34(4): 1069-75. doi: 10.3892/ijo_00000233, PMID 19287964.
11. Wang YX, Zhang XY, Zhang BF, Yang CQ, Chen XM, Gao HJ. Initial study of microRNA expression profiles of colonic cancer without lymph node metastasis. J Dig Dis. 2010; 11(1): 50-4. doi: 10.1111/j.1751-2980.2009.00413.x, PMID 20132431.
12. Arndt GM, Dossey L, Cullen LM, Lai A, Druker R, Eisbacher M, *et al.* Characterization of global microRNA expression reveals oncogenic potential of miR-145 in metastatic colorectal cancer. BMC Cancer. 2009; 9: 374. doi: 10.1186/1471-2407-9-374, PMID 19843336.
13. Li Q, Wang G, Shan JL, Yang ZX, Wang HZ, Feng J, *et al.* MicroRNA-224 is upregulated in HepG2 cells and involved in cellular migration and invasion. J Gastroenterol Hepatol. 2010; 25(1): 164-71. doi: 10.1111/j.1440-1746.2009.05971.x, PMID 19793168.

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