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ORIGINAL ARTICLE

The Role of MicroRNA-1207-5p in Colorectal Cancer

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SUMMARY

Background: **MicroRNA-1207-5p (MiR-1207-5p) has been identified as a tumor suppressor in many types of cancer. However, less research has been done with respect to the role of miR-1207-5p in colorectal cancer (CRC). The aim of our study was to analyze the role of miR-1207-5p in CRC.**
Methods: **Total RNA was extracted from clinical specimens and CRC cell line, and the expression of miR-1207-5p was examined by real-time PCR (q-PCR). The human CRC cell lines were transfected with the miR-1207-5p mimic, negative control (NC). Cell Counting Kit-8 (CCK-8) assays were performed to measure cell viability. The colony formation assay was carried out to analyze the clone forming ability. The cell cycle was analyzed by flow cytometer. The significance of the data was calculated by the SPSS software.**
Results: **The relative expression of miR-1207-5p in CRC tissues and adjacent noncancerous tissues (normalized to U6 expression) were 0.0546 ± 0.0131 and 0.1928 ± 0.0317, ($p < 0.01$), with obvious significant difference between the two groups. The expression of miR-1207-5p was significantly lower in CRC patients with advanced TNM stage ($p = 0.018$) and positive lymph node metastasis ($p = 0.033$) and shorter overall survival ($p = 0.049$). In addition, it enhances the expression of miR-1207-5p suppressed cell proliferation ability, clone forming ability, and promoted cell apoptosis *in vitro*.**
Conclusions: **The lower expression of miR-1207-5p was correlated with advanced TNM stage and lymph node metastasis and shorter patient overall survival. These results indicated that miR-1207-5p may function as tumor suppressor in CRC.**
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KEY WORDS

microRNA-1207-5p, colorectal cancer, clinical pathology, tumor suppressor

INTRODUCTION

Colorectal cancer (CRC), the third most common cancer worldwide, the fourth most common cause of cancer-related death, caused 1,000,000 new patients and 600,000 patents deaths every year [1,2]. Despite the advanced method of diagnosis and treatment, the death rate remained at about 9% [3]. In recent years, even with the application of molecular targeted therapy, CRC still has a high mortality rate, mainly because of the frequent recurrence and early metastasis [4,5]. Despite the hard work of scientists, the mechanisms underlying the process of colorectal tumorigenesis remain unclear. Early diagnosis and prognostic evaluation of CRC is of great

importance.

MicroRNA is a large class of 19 - 24nt short, noncoding RNA, which regulate target gene expression by inhibiting the mRNA expression at the translation level or interfering with protein translation at the post transcriptional level. MicroRNA plays this regulatory role by direct interactions with the 3'-untranslated region (3'UTR) or 5'-untranslated region (5'UTR) of the target genes. Then microRNA plays an important role in multiple physiological and pathological processes, including cell proliferation, invasion, migration and apoptosis [6-9]. It has been 15 years since the first study confirmed the close relationship between the abnormal microRNA expression and the development of cancers [10]. In recent years, there have been many studies which proved the tumor-related role of microRNA in many types of cancer, such as lung cancer, breast cancer, hepatocellular carcinoma, colorectal cancer, lymphocytic leukemia, gastric cancer, bladder cancer, etc. [10-15].

MicroRNA-1207-5p played an important role as a tumor suppressor gene in many types of cancer, such as gastric cancer, hepatocellular cancer, lung cancer and breast cancer [14,16-18]. However, rarely research reports the expression level and the functional role of miR-1207-5p in CRC. In this study, we confirmed that miR-1207-5p is down-regulated in both CRC tissues and CRC cell lines. The lower expression of miR-1207-5p was correlated with shorter patient overall survival. When exogenous miR-1207-5p mimic-synthetic RNA that mimics endogenous miR-1207-5p were transiently transfected to SW480 colorectal cancer cells, the cell proliferation, colony formatting ability and cell cycle were all suffered a certain impact. Thus, we came to the conclusion that miR-1207-5p functioned as a tumor suppressor in CRC.

MATERIALS AND METHODS

Clinical specimens

The clinical specimens were obtained from 40 colorectal cancer patients at the China-Japan Union Hospital of Jilin University from 2013-2014. None of the 40 patients had received radiotherapy and chemotherapy or other treatment for the cancers before the surgical operation. All tissue samples were immediately frozen in liquid nitrogen and stored at -80°C in the hospital tissue bank until use. The patients' clinical pathological information was available for all patients. Our study was approved by the Medical Ethics Committee of the China-Japan Union Hospital, and all participations gave informed consent.

Cell cultures and transfection

The human CRC cell lines (SW480 and HCT116) were purchased from the Cell Bank of The Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in DMEM (GIBCO, Grand Island, NY, USA) supplemented with 10% FBS in an atmosphere of 5% CO_2 at 37°C. When the cells reached 60% to 70% confluence, they were transfected with the miR-1207-5p mimic, miR-1207-5p inhibitors, negative control, and microRNA inhibitor N.C. using lipofectamine 2000 (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions. The transfected cells were incubated in an atmosphere of 5% CO_2 at 37°C for 24 to 48 hours before they were collected for experimental analysis.

RNA extracted and real-time quantitative reverse transcriptase-PCR (RT-qPCR)

The total RNAs of both tissues and colorectal cancer cell lines were extracted using RNAzol® RT RNA Isolation Reagent (Gene Copoeia, Guangzhou, China) according to the manufacturer's instructions. RNA concentration was measured using NanoDrop2000 (Thermo Fisher Scientific). The cDNAs were reverse-translated from 2.0 μg of total RNA and RT-qPCR was performed using the All-in-One™ miRNA RT-qPCR Detection Kit (GeneCopoeia, Guangzhou, China) by Mastercycler Ep Realplex real-time PCR System (Eppendorf). The endogenous control was U6 small nuclear RNA (snRNA U6). The miRNA q-PCR primers were purchased from GeneCopoeia, Inc. The catalog numbers of the All-in-OneTM miRNA q-PCR Primers were as follows: hsa-miR-1207-5p, HmiRQP0053; hsnRNA U6, HmiRQP-9001; Positive Control cDNA Mix, HmiRQT0001. The PCR program for detecting miR-1207-5p was as follows: 95°C for 10 minutes, 40 cycles of 95°C for 10 seconds, 60°C for 20 seconds, and 72°C for 10 seconds. Each sample was performed 3 times. The relative expression levels of miR-1207-5p to U6 were measured using the equation $2^{-\Delta\Delta CT}$, and $\Delta\Delta CT = (\Delta CT_{miR\text{-}1207\text{-}5p} - \Delta CT_{U6})$.

Synthesis of miR-1207-5p mimic and inhibitor

The precursor sequence of miR-1207-5p was obtained from miRBase (www.mirbase.org), and the miR-1207-5p mimic, inhibitor, negative control, and microRNA inhibitor N.C. were synthesized by Gene Pharma (Shanghai, China). Sequences are as follows: miR-1207-5p: 5'-UGGCAGGGAGGCUGGGAGGGG-3', miR-1207-5p mimic: 5'-UGGCAGGGAGGCUGGGAGGGGCCUCCCAGCCUCCCUGCCAUU-3', miR-1207-5p inhibitor: 5'-CCCCUCCCAGCCUCCCUGCCA-3', negative control: sense 5'-UUCUCCGAACGUGUCACGUTT-3' antisense: 5'-ACGUGACACGUUCGGAGAATT-3', and microRNA inhibitor N.C.: 5'-CAGUACUUUUGUGUAGUACAA-3'.

Cell viability assay

CCK-8 assay was performed to measure the cell viability. The transfected cells were seeded at a density of 4000 cells/well/100 μL into 96-well plates and cultured for 96 hours. At the indicated time, 10 μL of CCK-8 (Beyotime, Shanghai China) was added to each well and incubated for another 2 hours at 37°C protected

from light. The absorbance was measured at the wavelength of 450 nm.

Colony formation assay
SW480 cells were transfected with miR-1207-5p mimic and negative control (NC), and then grown in 6-well plates for 24 hours. For each group, 500 cells were seeded into 6-well plates and the cells were cultured in medium with 10% FBS at 37°C for 14 days, during this period the medium was not changed. Then the cells were fixed with 4% formaldehyde and stained with crystal violet for 10 minutes and washed with phosphate-buffered saline (PBS) at least 3 times.

Flow cytometry analysis
The transfected cells were harvested by trypsinization and fixed in acoolingice bath in 70% ethanol in PBS. Cell Cycle and Apoptosis Analysis Kits (Beyotime, Shanghai, China) were prepared according to manufacturer's instructions. To each tube of cell samples, 0.5 mL Dyeing buffer, 25 μL propidium iodide, and 10 μL RNAse staining, slow and full cell suspension precipitation, 37°C water bath for 30 minutes. Propidium iodide-stained cells (> 10,000 cells) were analyzed using a FACSCalibur flow cytometer (BD Biosciences, USA). All the experiments were performed in triplicate. The FACSCalibur flow cytometer was used to detect the red fluorescence at the wavelength of excitation, 488 nm, using the appropriate analysis software to carry out the cell cycle data.

Statistical analysis
Statistical analyses were performed using SPSS version 22 software (Chicago, IL, USA) and Graph Pad Prism 5 (Graph Pad Software Inc., La Jolla, CA, USA). The statistical differences of miR-1207-5p expression levels between CRC cell lines and normal tissues were analyzed using Student's *t*-test. The statistical differences of miR-1207-5p expression levels between cancer tissues and paired normal tissues were calculated using the Wilcoxon Matched Pairs Tests. The relationships between the miR-1207-5p expression levels and patients' clinical pathologic characteristics were evaluated with the X^2 test. Kaplan-Meier curves were calculated to analyze the overall survival time, and the difference in survival rates were assessed with log-rank test. The p-value < 0.05 was considered statistically significant and the p-values were two-sided.

RESULTS

MiR-1207-5p is down-regulated in CRC tissues and CRC cell lines
RT-qPCR tests was conducted to study the expression pattern of miR-1207-5p in CRC and CRC cell lines in 40 CRC clinical samples and the pair-matched adjacent normal tissue and in two CRC cell lines. After normalization to U6 expression levels, miR-1207-5p is significantly down-regulated in CRC tissues (mean ± SD: 0.0546 ± 0.0131) compared to the pair-matched adjacent normal tissues (mean ± SD: 0.1928 ± 0.0317, ** p < 0.01) (Figure 1A), and the relative expression levels (ΔΔCT) of miR-1207-5p in CRC cell lines (HCT116 (mean ΔΔCT ± SD: 5.407 ± 0.1968) and SW480 (mean ΔΔCT ± SD: 6.995 ± 0.1608)) were significantly lower compared to normal colorectal tissues (mean ΔΔCT ± SD: 3.062 ± 0.2359, ** p < 0.01, ** p < 0.01) (Figure 1B).

The association between miR-1207-5p expression levels and various clinical pathological parameters
The patients were divided into two groups according to the levels of miR-1207-5p the high group and the low group, using the mean level of their miR-1207-5p expression level as the cutoff. The results demonstrated that the lower the expression of miR-1207-5p,the stronger the association with advanced TNM stage and positive lymph node metastasis compared with the high expression group (p = 0.018, p = 0.033). However, there was no significant difference between the miR-1207-5p expression levels and other clinical pathological parameters (Table 1).

Low miR-1207-5p expression confers poor prognosis in CRC patients
All 40 patients were divided into two groups according to the levels of miR-1207-5p: the high group and the low group, using the mean level of their miR-1207-5p expression level as the cutoff. The low expression group was correlated with poorer prognosis than the high expression group (p = 0.049) (Figure 2).

Role of miR-1207-5p on colon cancer cells proliferation ability, colony formation ability and cell cycle distribution
After transfection with miR-1207-5p mimic and NC, the expression level of miR-1207-5p in the transfected group was about 40 times higher than that in the NC group (normalized to U6) (Figure 3A). The ability of cell proliferation was measured at 24 hour intervals between the transfected group and NC group. The proliferative capacity of the transfected group was significantly lower than that in the NC group at 72 hours and 96 hours (72 hours, * p < 0.05), (96 hours, ** p < 0.01) (Figure 3B). The transfected group has a significantly reduced clone forming ability compared to the NC group (** p < 0.01) (Figure 3C). Meanwhile, the percentage of cells in S and G2 phase in transfected group was significantly lower than that of the NC group (* p < 0.05) (Figure 3D).

DISCUSSION

In this study, we observed that miR-1207-5p was down-regulated in CRC tissues and cell lines which has never been reported before, and the lower miR-1207-5p ex-

Table 1. The association between miR-1207-5p expression levels and various clinical pathological parameters.

Variables	Expression of miR-1207-5p		p-value
	Low (n = 27)	High (n = 13)	
Age			
≤ 60	11	3	0.273
> 60	16	10	
Gender			
Male	15	10	0.191
Female	12	3	
Location			
Colon	10	7	0.314
Rectum	17	6	
Dukes			
A, B	14	5	0.427
C, D	13	8	
Stage			
I, II	10	10	0.018 *
III, IV	17	3	
Lymph node metastasis			
Negative	9	9	0.033 *
Positive	18	4	
Venous invasion			
Negative	15	8	0.720
Positive	12	5	
Nerve invasion			
Negative	20	9	0.748
Positive	7	4	

Note: Lower expression of miR-1207-5p was strongly associated with advanced TNM stage and positive lymph node metastasis compared with the high expression group (p = 0.018, p = 0.033 *, p < 0.05). P-value was calculated by X^2 test.

pression was correlated with poorer prognosis than the high expression group. In addition, up-regulation of the expression of miR-1207-5p suppresses CRC SW480 cell line proliferation, colony forming ability, and promotion of cell apoptosis *in vitro*.

There have been many reports about ectopic miR-1207-5p expression in many types of cancer. Ribas G et al. found the ectopic expression of miR-1207 in very young women with breast cancer [19]. miR-1207-5p, miR-1228, and miR-939 are the most connected microRNAs that regulate a large number of genes, such as ST8SIA2, MED1, and HDAC4, and SPN, which are in the center of the network involved in non-small-cell lung cancer [20]. Dang W et al. confirmed that miR-1207-5p suppresses lung cancer growth and metastasis by targeting colony stimulating factor 1 (CSF1). The experiment indicated that miR-1207-5p could modulate macrophage function of the tumor micro-environment [17]. Another study carried out by Zhao G et al. showed the role of miR-1207-5p in hepatocellular carcinoma and the regulation between miR-1207-5p and fatty acid synthase, a unique oncologic target for the treatment of cancers, including hepatocellular carcinoma. Experiments showed that the enhanced expression of miR-1207-5p inhibited the Akt/mTOR signal pathway, and restoration of FASN expression partially reversed the inhibitory effect of miR-207-5p on Akt phosphorylation [21]. Similar studies have shown that the expression of miR-1207-5p was an independent risk factor of LNM [22]. Earlier study indicated that miR-1207-5p suppressed gastric cancer growth and invasion by suppressing human telomerase reverse transcriptase (hTERT). Another study showed that BC032469, a novel lncRNA, could combine with miR-1207-5p and then worked as a sponge for miR-1207-5p to modulate the depression of hTERT [23,24]. A recent study showed that CRC pa-

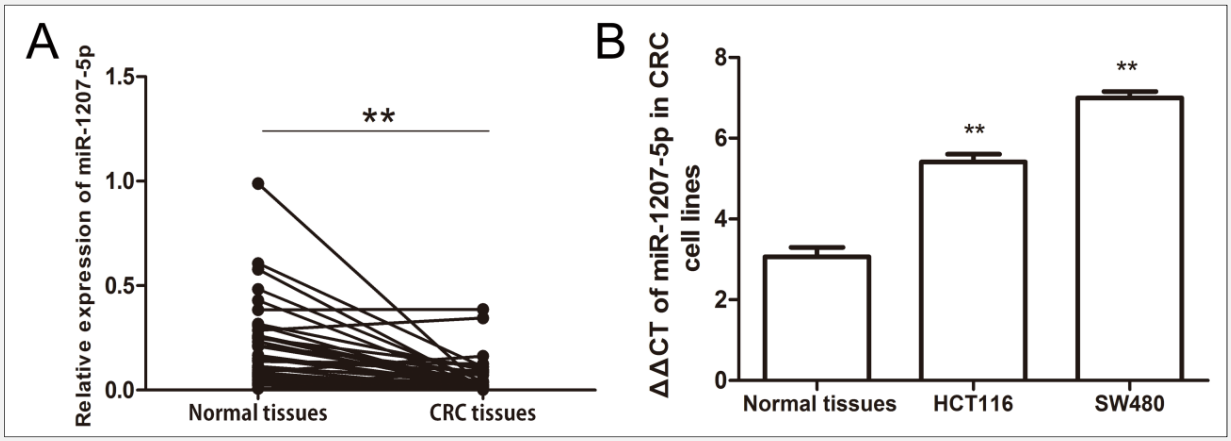

Figure 1. The relative expression levels of miR-1207-5p in CRC tissues and cell lines.

A. Analysis using the two sample Student's *t*-test shows that the relative expression of miR-1207-5p in CRC tissues are significantly lower than in the adjacent normal colorectal tissues (, p < 0.01).**
B. the relative expression levels (ΔΔCT) of miR-1207-5p in HCT116 and in SW480 were significantly down regulated compared to normal colorectal tissues after normalization to U6 expression (, p < 0.01).**

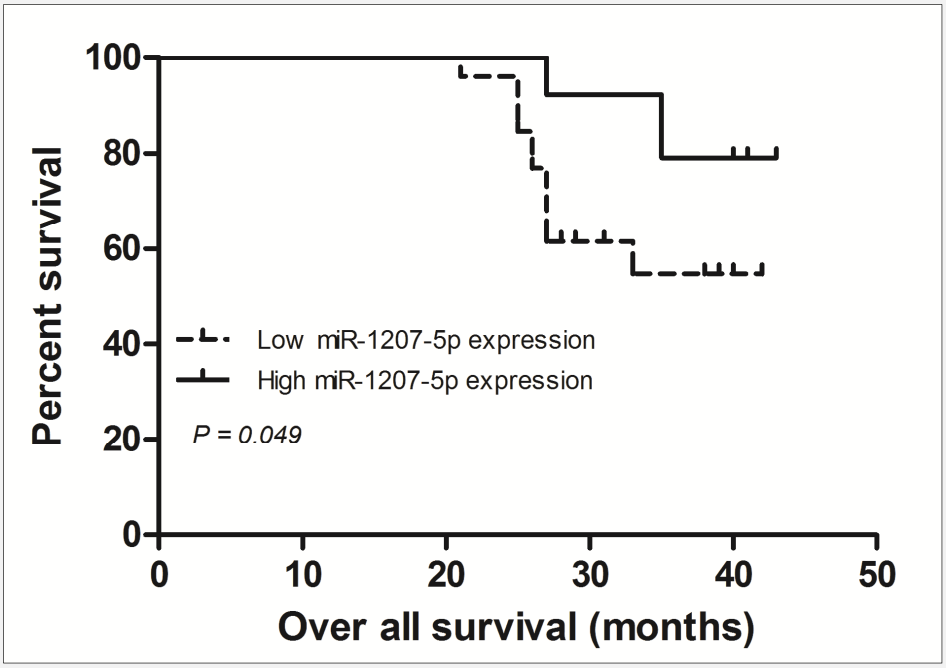

Figure 2. Kaplan-Meier survival curves according to the expression level of miR-1207-5p.

All 40 patients were divided into two groups according to the levels of miR-1207-5p; the high group and the low group, using the mean level of their miR-1207-5p expression level as the cutoff. As shown in Figure 2, the low expression group was correlated with poorer prognosis than the high expression group (p = 0.049).

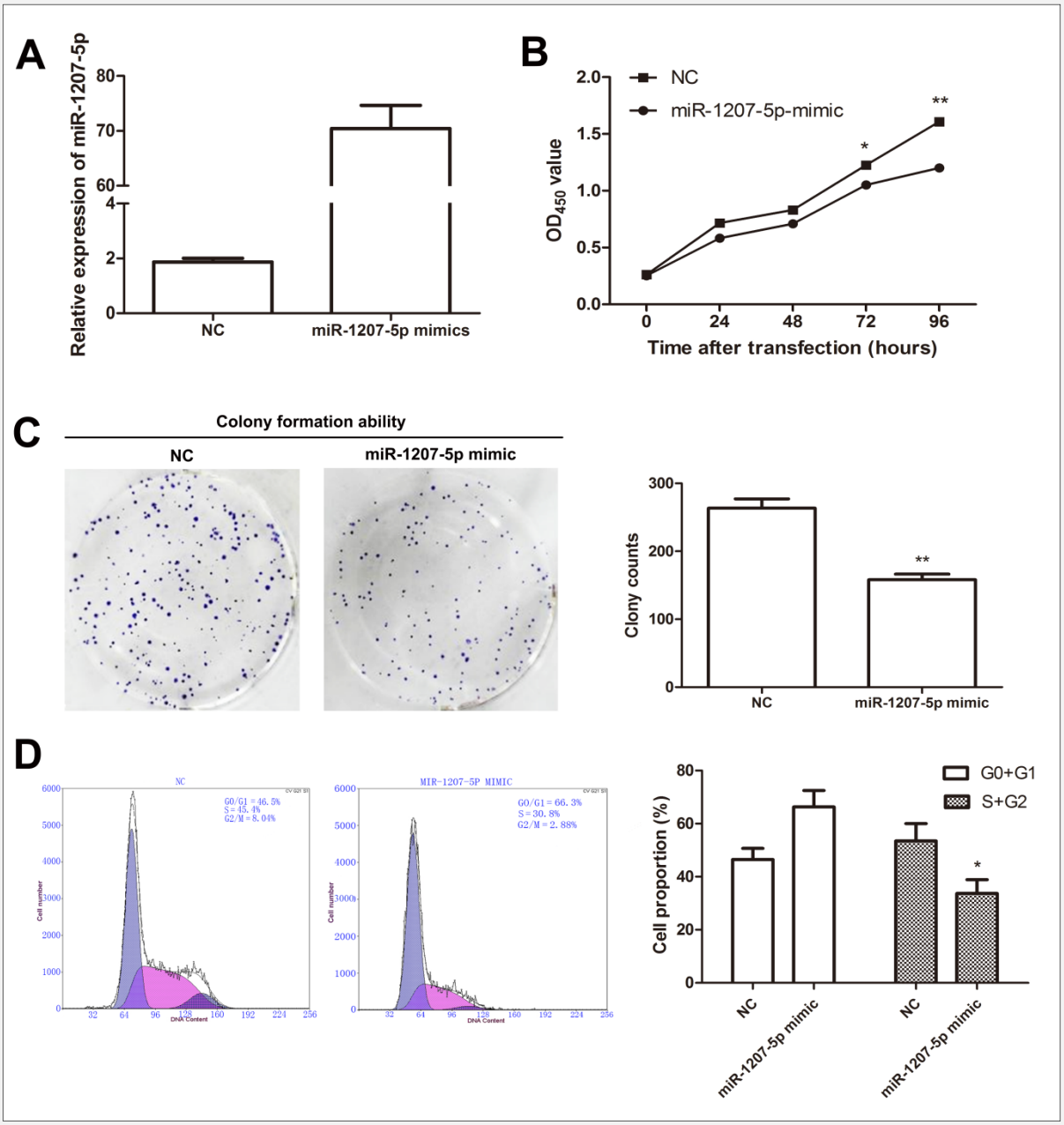

Figure 3. Role of miR-1207-5p on colon cancer cell proliferation ability, colony formation ability, and cell cycle distribution.

A. The expression level of miR-1207-5p in the transfected group was about 40 times higher than that in the NC group (normalize to U6). B. The proliferative capacity of miR-1207-5p mimic group was significantly lower than that in the NC group at 72 hours, 96 hours (72 hours, *, $p < 0.05$), (96 hours, **, $p < 0.01$). C. The transfected group exhibits significantly reduced clone forming ability compared to the NC group (**, $p < 0.01$). D. The percentage of cells in S and G2 phase in the transfected group was significantly lower than the NC group (*, $p < 0.05$).

tients showed an 8-fold increase in miR-1207-5p in African-Americans compared to a 1.2-fold increase of the same in Caucasian people, because of miR-1207-5p induction of stemness in colon epithelial cells in the colon of AAs with adenomas [25]. Altogether, we can conclude that one microRNA perhaps regulates different target genes via different mechanisms in different types of cancers.

Despite the number of clinical specimens we observed in this study, more patients and research are essential for further investigations of miR-1207-5p. Furthermore, there should be more studies on the target and mechanism of how miR-1207-5p takes part in the tumor formation of CRC and how to influence biological characteristics of CRC patients. If miR-1207-5p could be a meaningful biomarker of prognostics and therapeutic target in CRC which need further experiments and research.

Taken together, we observed the significant down-regulation of miR-1207-5p in CRC tissues, and lower miR-1207-5p expression was correlated with poor prognosis. Up-regulated miR-1207-5p inhibited SW480 proliferation, clone forming ability and promoted cell apoptosis *in vitro*. These results indicated the tumor suppressor role of miR-1207-5p in CRC, and miR-1207-5p may function as a potential biomarker and a therapeutic target for CRC.

CONCLUSION

The expressions miR-1207-5p in CRC tissues and cell lines were significantly down-regulated. The deregulation of miR-1207-5p was correlated with lymph node metastasis and advanced TNM stage, and the lower miR-1207-5p expression was correlated with poor prognosis. Additionally, enhancing miR-1207-5p inhibited CRC cell line SW480 proliferation, clone forming ability, and promoted cell apoptosis *in vitro*. These results indicated that miR-1207-5p may function as a tumor suppressor microRNA in CRC, and miR-1207-5p may be a potential biomarker and a novel therapeutic target for CRC.

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Ethics Approval and Consent to Participate:
Our study was approved by the Medical Ethics Committee of the China - Japan Union Hospital.

Declaration of Interest:
The authors declare that they have no competing interests.

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