MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 4431-4437
DOI: 10.12659/MSM.898452

Received: 2016.03.11
Accepted: 2016.03.31
Published: 2016.11.18

MicroRNA-455-3p Inhibits Tumor Cell Proliferation and Induces Apoptosis in HCT116 Human Colon Cancer Cells

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1 Jiantao Zheng
BCDF 2 Zhenlv Lin
BCF 1 Lin Zhang
AEG 1 Hui Chen

1 Department of Gastrointestinal Surgery, The First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, P.R. China
2 Department of Emergency Surgery, The First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, P.R. China

Corresponding Author: Hui Chen, e-mail: chenhui1714@126.com
Source of support: Fujian Young and Middle-Aged Backbone Talents Training Project of Health Systems (NO: 2015-ZQN-JC-23)

Background: MicroRNAs have been reported to play significant roles in pathogenesis of colorectal cancer (CRC). In the present study, we aimed to investigate the functional role of microRNA-455-3p (miR-455-3p) in CRC, as well as its underlying mechanisms.

Material/Methods: Human colon cancer cell line HCT116 cells were transfected with miR-455-3p mimics, inhibitors, or controls. After transfection, the effects of miR-455-3p mimics or inhibitors on cell proliferation were analyzed by 3-(4, 5-dimethyl-2- thiazolyl)-2, 5-diphenyl -2-H-tetrazolium bromide (MTT) assay and BrdU assay, and the effects of miR-455-3p mimics or inhibitors on cell apoptosis were determined. In addition, the underlying mechanisms of cell proliferation and apoptosis were explored by assessing the protein levels of cell cycle regulators and apoptosis-related protein.

Results: The results showed that overexpression of miR-455-3p significantly inhibited the cell proliferation ($P<0.05$ or <0.01) in HCT116 cells compared with the control group, but significantly increased the apoptosis ($P<0.01$). On the contrary, suppression of miR-455-3p significantly increased the cell proliferation but decreased the apoptosis. Moreover, we found that overexpression of miR-455-3p significantly elevated the protein levels of p27 kinase inhibition protein (KIP) 1, Bax, pro-caspase-3, and active caspase-3, and markedly downregulated the levels of B-cell lymphoma-2 (Bcl-2). Contrary results were found by suppression of miR-455-3p. However, there were no significant differences in p21 expression.

Conclusions: MiRNA-455-3p functions as an anti-oncogene in HCT116 cells by inhibiting cell proliferation and inducing of apoptosis.

MeSH Keywords: Apoptosis • Cell Proliferation • Colorectal Neoplasms • MicroRNAs

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/898452

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Colorectal cancer (CRC) is the third most common type of cancer and the fourth most common cause of cancer deaths worldwide [1]. It has been estimated that the overall incidence of CRC is ~5%, meaning more than 1 million people are diagnosed with CRC each year [2,3]. The 5-year survival rate of CRC is 40–60% [4], imposing tremendous financial burden and reduction of quality of life [5]. Tremendous advances have been made in recent years in diagnosis and treatment of CRC, but the pathological mechanisms remain unclear. CRC is a complex genetic disease involving abnormalities in gene structure and/or expression of both coding and non-coding genes [6]. Deregulated expression of microRNAs (miRNAs) has been reported in CRC [7–9]. The abnormal expression of certain miRNAs has been reported to be related with poor prognosis and therapeutic outcome in patients with CRC [10,11].

MiRNAs are a family of short, single-stranded, conserved, non-coding RNAs that negatively regulate gene expression by binding to the 3'-untranslated region (3'-UTR) of target mRNAs at the post-transcriptional level [12,13]. MiRNAs are enriched in certain cells and tissues and are implicated in the control of diverse biological processes, including regulation of cell proliferation and apoptosis [14]. Particular miRNAs function either as tumor suppressors or oncogenes, which leads to abnormal activity of miRNA target genes [15,16]. The functional role of miR-455-3p in diseases has rarely been studied. Guled et al. found that miR-455-3p was elevated in smokers with malignant mesothelioma patients compared with non-smoker group [17]. Ujifuku et al. suggested that miR-455-3p is implicated in acquired temozolomide resistance in glioblastoma multiforme cells [18]. However, its function on CRC remains unclear.

Therefore, in the present study we aimed to investigate the functional role of miR-455-3p in CRC. We focused on the effect of miR-455-3p on cell proliferation and apoptosis and its underlying mechanisms. Understanding the function of miRNAs and their regulatory mechanisms in CRC may provide new insights into the pathogenesis of CRC and new targets for CRC therapy, as well as helping to estimate prognosis and treatment modalities.

Material and Methods

Cell culture

Human colon cancer cell line HCT116 was obtained from the American Type Culture Collection (ATCC). HCT116 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) at 37°C in a humidified atmosphere with 5% CO_2. DMEM was supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) (Sigma Chemical Co., St. Louis, MO), 2 mM glutamine (Invitrogen), 50 U/ml penicillin (Invitrogen), and 50 µg/ml streptomycin (Invitrogen). The study was performed in compliance with the Helsinki Declaration and was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University.

Mimics and inhibitor transfection assays

MiR-455-3p mimics, inhibitors, and controls were designed and produced by Gene Pharma (Shanghai, China). The HCT116 cells were seeded in a 6-well plate with DMEM/F12 supplemented with 10% FBS and allowed to grow to 70–80% confluence. The cells were then transfected with miR-455-3p mimics, inhibitors, or controls using Lipofectamine 2000 Transfection Reagent (Invitrogen, USA) according to the manufacturer's instructions. The cells were incubated at 37°C for 48 h.

Cell viability

After 6 h of transfection with miR-455-3p mimics or inhibitors, HCT116 cell viability was determined by 3-(4, 5-dimethyl-2- thiazolyl)-2, 5-diphenyl -2-H-tetrazolium bromide (MTT) assay. Briefly, the cells were seeded in a 96-well plate (5×10^3 cells/well). MTT solution (20 µl, 10 mg/mL, Sigma Chemical Co., St. Louis, MO) was added to each plate at different time points (1 d, 2 d, 3 d, 4 d, and 5 d), followed by incubation for 4 h at 37°C. Dimethylsulfoxide (DMSO, Sigma Chemical Co., St. Louis, MO) was added to dissolve the reduced the formazan crystals, after which absorbance at 590 nm was measured with a Multiskan EX spectrophotometer (LabSystem, Helsinki, Finland). Error bars represent 3 independent assays.

BrdU assay

To determine the effects of miR-455-3p mimics or inhibitors on cell proliferation, we performed BrdU assay according to the manufacturer's instructions. Briefly, the cells were seeded in 96-well plates (2×10^3 cells/well) and were transfected with miR-455-3p mimics or inhibitors. After 48 h of transfection, 10 µM BrdU solution (Sigma-Aldrich, St. Louis, MO) was added to each plate and incubated at 37°C for an additional 2 h. The cells were washed 3 times, resuspended in a mixture of washing buffer, and incubated with 4 M hydrogen chloride (HCl) for 30 min at room temperature. Thereafter, the cells were resuspended in Borax buffer, washed, re-suspended in washing buffer, and then labeled with anti-BrdU antibody (Santa Cruz Biotechnology) for 1 h at 4°C in the dark. The cells were then incubated with secondary green-fluorescence dye conjugated antibody (Santa Cruz Biotechnology) for 45 min at room temperature. Finally, the cells were subjected to flow cytometric (FCM) analysis. Absorbance at 488 nm was recorded on a FACS Aria II device (BD Biosciences).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

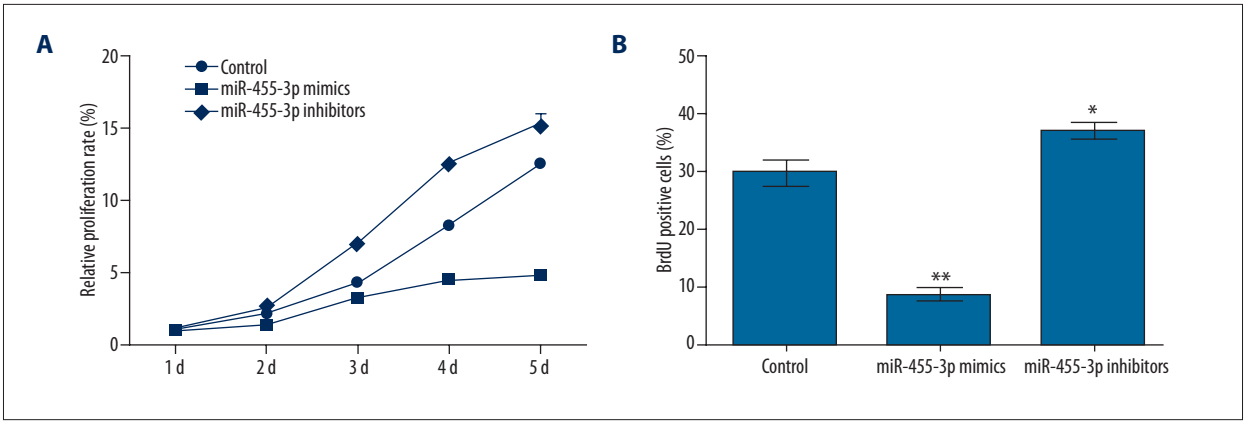

Figure 1. Overexpression of miR-455-3p reduces cell proliferation in HCT116 cells. (**A**) Overexpression of miR-455-3p significantly inhibits the cell viability of HCT116 cells at 3 d, 4 d, and 5 d. (**B**) Overexpression of miR-455-3p significantly decreases positive cells. * $P<0.05$ compared with the control group; ** $P<0.01$ compared with the control group.

Apoptosis assay

We performed apoptosis assay to determine the effects of miR-455-3p mimics or inhibitors on apoptosis of HCT116 cells. Briefly, after 48 h of transfection with miR-455-3p mimics or inhibitors, the cells were harvested, washed with phosphate-buffered saline (PBS), and fixed with cold methanol overnight. Then the cells were incubated with 1× binding buffer supplemented with Annexin V-FITC (10 µl) and propidium iodide (PI) (5 µl) for 30 min in the dark at room temperature. Early apoptotic cells were positive for Annexin V and negative for PI, and while late apoptotic cells were both Annexin V- and PI-positive. Apoptotic cells were recognized by a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and the data were analyzed using CellQuest® software (Becton Dickinson, Heidelberg, Germany). Each condition was repeated 3 times.

Western blot analysis

After 48 h of transfection with miR-455-3p mimics or inhibitors, the cells were lysed in lysis solution and prepared for protein extraction. The protein concentration was measured by a BCA protein assay kit (Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions. Soluble protein (the same concentration per lane) was resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to nitrocellulose membrane. Then the membranes were blocked with 5% non-fat dried milk and incubated with following primary antibodies overnight at 4°C: anti-p27 kinase inhibition protein (KIP) 1 antibody (P2092, Sigma-Aldrich), anti-p21 antibody (SAB4500065; Sigma-Aldrich), anti- B-cell lymphoma (Bcl)-2 antibody (AB1722, Sigma-Aldrich), anti-Bax antibody (B8429, Sigma-Aldrich), anti-pro-caspase 3 antibody (9662, Cell Signaling Technology), and anti-active caspase-3 antibody (ab2302, Abcam, Cambridge, UK). The membranes were then incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibody. GAPDH was used as a loading control. Reactions were visualized with an enhanced chemiluminescence (ECL) system (Santa Cruz, CA).

Statistical analysis

The data are shown as the mean ± standard deviation (SD). All the data were statistically assessed by using the Statistic Package for Social Science (SPSS, version 16.0, SPSS Inc, Chicago, IL) statistical software. A *t* test was performed for comparison of paired data. Difference was considered statistically significant at $P<0.05$.

Results

Overexpression of miR-455-3p reduced cell proliferation in HCT116 cells

To determine the functional role of miR-455-3p in colon cancer, we first measured the effects of miR-455-3p on cell proliferation by MTT assay and BrdU assay. As indicated in Figure 1A, after transfection with miR-455-3p mimics or inhibitors, the cell viability of HCT116 cells was significantly inhibited by overexpression of miR-455-3p at 3 d, 4 d, and 5 d compared with the control group ($P<0.05$). However, the cell viability of HCT116 cells was significantly increased by suppression of miR-455-3p at 3 d, 4 d, and 5 d compared with the control group ($P<0.05$). The results of BrdU assay showed that the positive cells were significantly decreased by overexpression of miR-455-3p ($P<0.01$), but were markedly increased by suppression of miR-455-3p ($P<0.05$) (Figure 1B). These results demonstrate that miR-455-3p may be a key tumor-suppressor in colon cancer.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

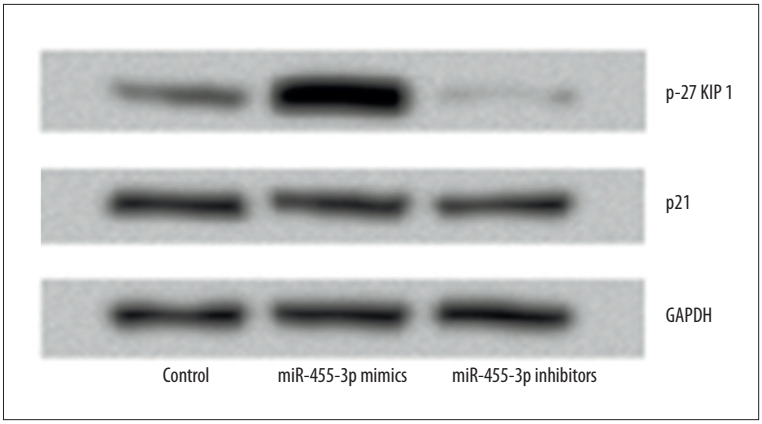

Figure 2. Overexpression of miR-455-3p reduces cell proliferation by regulating p27 KIP1 expression. The image shows that overexpression of miR-455-3p significantly increases the protein levels of p27 KIP1 but there was no significant difference in p21 expression. KIP, kinase inhibition protein.

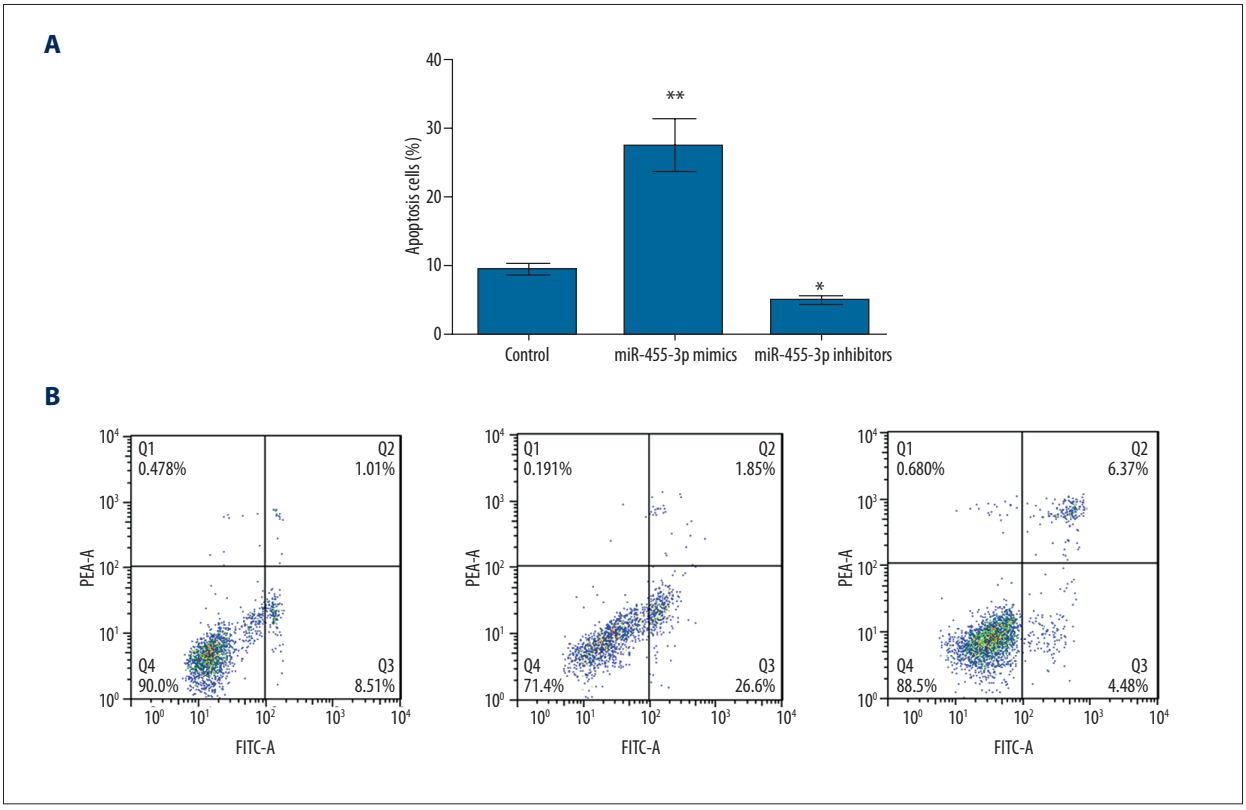

Figure 3. (**A, B**) Overexpression of miR-455-3p induces apoptosis in HCT116 cells. Overexpression of miR-455-3p significantly upregulates the apoptotic cells. * *P*<0.05 compared with the control group; * *P*<0.01 compared with the control group.

Overexpression of miR-455-3p reduced cell proliferation by increasing p27 KIP1

We then explored the underlying mechanism of the effects of miR-455-3p on cell proliferation by determining the protein levels of cell cycle regulators p27 KIP1 and p21 in HCT116 cells. As shown in Figure 2, overexpression of miR-455-3p significantly increased the protein expression of p27 KIP1 compared to the control group, and while suppression of miR-455-3p statistically decreased the protein expression of p27 KIP1 compared to the control group. However, there were no significant differences in the protein expression of p21 by overexpression or suppression of miR-455-3p. These results indicate that overexpression of miR-455-3p induced reduction of cell proliferation in HCT116 cells by regulation of p27 KIP1.

Overexpression of miR-455-3p induced apoptosis in HCT116 cells

After transfection with miR-455-3p mimics or inhibitors, we determined the effects of miR-455-3p on cell apoptosis. As demonstrated in Figure 3A, 3B, the apoptotic cells were significantly

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

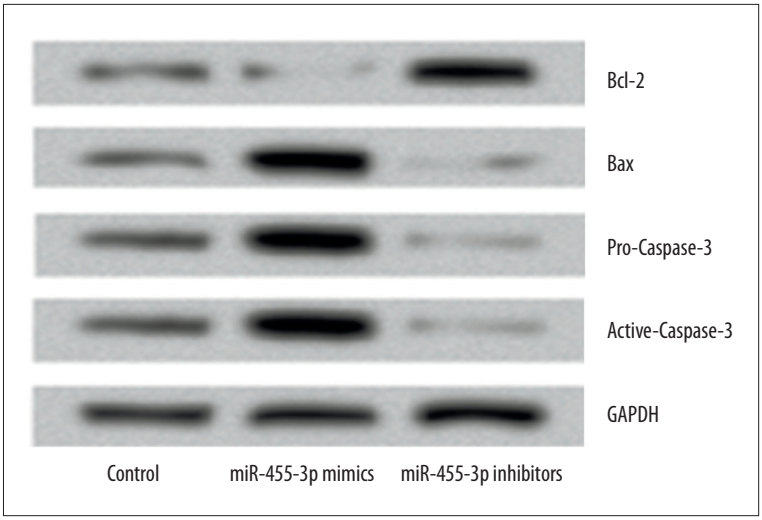

Figure 4. Overexpression of miR-455-3p induces apoptosis by regulating the expression of Bcl-2, Bax, and caspase-3. The picture shows that overexpression of miR-455-3p significantly upregulates the protein levels of Bax, pro-caspase-3, and active caspase-3, but statistically downregulates the levels of Bcl-2. Bcl-2, B-cell lymphoma-2.

higher in the overexpression of miR-455-3p group than the cells in the control group ($P<0.01$), but were obviously lower in the suppression of miR-455-3p group than the cells in the control group ($P<0.05$). These results indicate that overexpression of miR-455-3p induced apoptosis in HCT116 cells.

Overexpression of miR-455-3p induced apoptosis by regulating Bcl-2, Bax, and caspase-3

We then explored the underlying mechanism of the effects of miR-455-3p on cell apoptosis by confirming the expression levels of apoptosis-related protein Bcl-2, Bax, pro-caspase-3, and active caspase-3 in HCT116 cells. The results show that the protein levels of Bcl-2 were significantly reduced by overexpression of miR-455-3p compared to the control group, but was statistically upregulated by suppression of miR-455-3p. Moreover, all the protein levels of Bax, pro-caspase-3, and active caspase-3 were significantly upregulated by overexpression of miR-455-3p, but were markedly downregulated by suppression of miR-455-3p compared to the control group (Figure 4). Our results reveal that overexpression of miR-455-3p induced apoptosis by regulating the expression of Bcl-2, Bax, and caspase-3 in HCT116 cells.

Discussion

MiRNAs regulate gene expression, and abnormal expression of miRNA has been reported to be responsible for the progression and development of multiple human cancer types, including CRC [19]. However, the function of the deregulated miRNA remains to be further identified. In the present study, we found that overexpression of miR-455-3p significantly restricts cell proliferation of HCT116 cells by regulating the expression of p27 KIP1. In addition, overexpression of miR-455-3p induces apoptosis of HCT116 cells, which is by modulating the expression of Bcl-2, Bax, and caspase 3.

Currently, there are only relatively ineffective clinical treatments for CRC, and CRC thus still remains one of the toughest challenges in oncology. Understanding the molecular mechanisms responsible for carcinogenesis and progression of CRC, especially the identification and characterization of abnormal miRNAs expression in CRC development, has received much attention. Several deregulated miRNAs, such as upregulated miR-196a, miR-21, miR-17-92, and miR-135, and downregulated miR-101, miR-34, miR-365, and miR-195, have been described in CRC carcinogenesis and progression [7,20–24]. However, the underlying mechanisms responsible for the miRNAs deregulation in CRC development have not been clearly identified. Multiply factors, such as genetic and/or epigenetic factors, signaling pathways, and transcriptional factors, may contribute to the deregulation of miRNAs in CRC [9,25,26]. The deregulation of miRNAs could potentially alter various biological processes, including cell growth, cell proliferation, apoptosis, invasion, differentiation, progression, and metastasis [27–29]. In the present study, we focused on the effects of miR-455-3p on cell proliferation and apoptosis in HCT116 cells. The results revealed that the deregulated miR-455-3p altered cell proliferation and cell apoptosis. Overexpression of miR-455-3p decreased cell viability, suppressed cell cycle progression, and promoted cell apoptosis, indicating miR-455-3p functions as a tumor suppressor activity.

Abnormal cell proliferation is a fundamental process integral to carcinogenesis, which is regulated by a multitude of factors [30]. Inhibition of cell proliferation is an initial action of carcinogenic agents and plays an important role in anticancer strategies. In addition, inhibition of cell proliferation is linked to enhanced apoptosis [31]. Several mechanisms have been reported to be involved in the cell proliferation, but it is mainly mediated by induction of cell cycle arrest. Expression of cyclin-dependent kinase (CDK) inhibitor p27 KIP1 has been shown in human tumors. It was demonstrated that there is an inverse

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

correlation between expression of p27 KIP1 and cell proliferation by evaluating its expression in 25 different normal human tissues [32]. P27 KIP1 negatively regulates the cell cycle by inactivation of cyclin-CDK complex and inhibition of the transition from G1 to S phase [33]. Palmqvist et al. suggested that p27 not only controls cell cycle progression, but also might be associated with aggressive tumor behavior in CRC [34], and Tenjo et al. found that p27 might be involved in inhibiting the aggressive growth of CRC by induction of apoptosis [35]. Additionally, Thomas et al. confirmed that down-regulation of p27 is associated with the development of CRC metastases [36]. In our study, we found that p27 KIP1 was significantly increased by overexpression of miR-455-3p. MiR-455-3p overexpression-induced cell growth arrest might occur via regulating the expression of p27 KIP1, but not p21, in HCT116 cells.

Accumulating evidence has indicated that apoptosis is another critical pathological change involved in human cancer [37]. Induction of apoptosis is partially regulated by the Bcl-2 family, which is a potentially therapeutic strategy for cancer treatment. Bcl-2 is an important apoptosis-inhibiting gene, and Bax is a key apoptosis-accelerating gene [38]; both lay significant roles in the process of apoptosis. Moreover, the activation of the caspase family is another central event in apoptosis [39]. Among the caspase family, caspase 3 is especially necessary for apoptosis in cancer [40]. Activation of pro-caspase-3 has been considered as a the final "executing" molecular process leading to apoptosis [41]. Active caspase-3, a critical executioner caspase, proteolytically cleaves and activates other caspases and is responsible for the steps of apoptosis [42]. Our data show that overexpression of miR-455-3p markedly elevated the levels of Bax, pro-caspase 3, and active caspase-3, but significantly reduced the levels of Bcl-2 in HCT116 cells, demonstrating that overexpression of miR-455-3p induced cell apoptosis.

Conclusions

We found that miR-455-3p functions as a tumor suppressor in HCT116 cells by inhibition of cell proliferation and induction of apoptosis. Our results suggest that overexpression of miR-455-3p might be an effective strategy for treatment of CRC.

Conflicts of interest

There are no conflicts of interest.

References:

1. Jemal A, Bray F, Center MM et al: Global cancer statistics. Cancer J Clin, 2011; 61: 69–90
2. Isik A, Peker K, Firat D et al: Importance of metastatic lymph node ratio in non-metastatic, lymph node-invaded colon cancer: A clinical trial. Med Sci Monit, 2014; 20: 1369–75
3. Isik A, Peker K, Gursul C et al: The effect of ozone and naringin on intestinal ischemia/reperfusion injury in an experimental model. Int J Surg, 2015; 21: 38–44
4. Jemal A, Siegel R, Ward E et al: Cancer statistics, 2007. Cancer J Clin, 2007; 57: 43–66
5. Zafar SY, McNeil RB, Thomas CM et al: Population-based assessment of cancer survivors' financial burden and quality of life: A prospective cohort study. J Oncol Pract, 2015; 11: 145–50
6. Dean M: Cancer as a complex developmental disorder – nineteenth Cornelius P. Rhoads Memorial Award Lecture. Cancer Res, 1998; 58: 5633–36
7. Motoyama K, Inoue H, Takatsuno Y et al: Over- and under-expressed microRNAs in human colorectal cancer. Int J Oncol, 2009; 34: 1069–75
8. Hamfjord J, Stangeland AM, Hughes T et al: Differential expression of miRNAs in colorectal cancer: comparison of paired tumor tissue and adjacent normal mucosa using high-throughput sequencing. PLoS One, 2012; 7: e34150
9. Bandres E, Agirre X, Bitarte N et al: Epigenetic regulation of microRNA expression in colorectal cancer. Int J Cancer, 2009; 125: 2737–43
10. Ng EK, Chong WW, Jin H et al: Differential expression of microRNAs in plasma of patients with colorectal cancer: A potential marker for colorectal cancer screening. Gut, 2009; 58: 1375–81
11. Wu CW, Ng SS, Dong YJ et al: Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. Gut, 2012; 61: 739–45
12. He L, Hannon GJ: MicroRNAs: Amall RNAs with a big role in gene regulation. Nat Rev Genet, 2004; 5: 522–31
13. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell, 2004; 116: 281–97
14. Christensen LL, Holm A, Rantala J et al: Functional screening identifies miRNAs influencing apoptosis and proliferation in colorectal cancer. PLoS One, 2014; 9: e96767
15. Zhang B, Pan X, Cobb GP, Anderson TA: microRNAs as oncogenes and tumor suppressors. Dev Biol, 2007; 302: 1–12
16. Chen CZ: MicroRNAs as oncogenes and tumor suppressors. N Engl J Med, 2005; 353: 1768–71
17. Guled M, Lahti L, Lindholm PM et al: CDKN2A, NF2, and JUN are dysregulated among other genes by miRNAs in malignant mesothelioma -A miRNA microarray analysis. Genes Chromosomes Cancer, 2009; 48: 615–23
18. Ujifuku K, Mitsutake N, Takakura S et al: miR-195, miR-455-3p and miR-10a(*) are implicated in acquired temozolomide resistance in glioblastoma multiforme cells. Cancer Lett, 2010; 296: 241–48
19. Lu J, Getz G, Miska EA et al: MicroRNA expression profiles classify human cancers. Nature, 2005; 435: 834–38
20. Nie J, Liu L, Zheng W et al: microRNA-365, down-regulated in colon cancer, inhibits cell cycle progression and promotes apoptosis of colon cancer cells by probably targeting Cyclin D1 and Bcl-2. Carcinogenesis, 2012; 33: 220–25
21. Schimanski CC, Frerichs K, Rahman F et al: High miR-196a levels promote the oncogenic phenotype of colorectal cancer cells. World J Gastroenterol, 2009; 15: 2089–96
22. Schetter AJ, Leung SY, Sohn JJ et al: MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA, 2008; 299: 425–36
23. Monzo M, Navarro A, Bandres E et al: Overlapping expression of microRNAs in human embryonic colon and colorectal cancer. Cell Res, 2008; 18: 823–33
24. Wang X, Wang J, Ma H et al: Downregulation of miR-195 correlates with lymph node metastasis and poor prognosis in colorectal cancer. Med Oncol, 2012; 29: 919–27
25. Graziano F, Canestrari E, Loupakis F et al: Genetic modulation of the Let-7 microRNA binding to KRAS 3'-untranslated region and survival of metastatic colorectal cancer patients treated with salvage cetuximab-irinotecan. Pharmacogenomics J, 2010; 10: 458–64

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

26. Iwaya T, Yokobori T, Nishida N et al: Downregulation of miR-144 is associated with colorectal cancer progression via activation of mTOR signaling pathway. Carcinogenesis, 2012; 33: 2391–97
27. Kloosterman WP, Plasterk RH: The diverse functions of microRNAs in animal development and disease. Dev Cell, 2006; 11: 441–50
28. Baranwal S, Alahari SK: miRNA control of tumor cell invasion and metastasis. Int J Cancer, 2010; 126: 1283–90
29. Hwang HW, Mendell JT: MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer, 2006; 94: 776–80
30. Sandal T: Molecular aspects of the mammalian cell cycle and cancer. Oncologist, 2002; 7: 73–81
31. de Ruijter AJ, Kemp S, Kramer G et al: The novel histone deacetylase inhibitor BL1521 inhibits proliferation and induces apoptosis in neuroblastoma cells. Biochem Pharmacol, 2004; 68: 1279–88
32. Fredersdorf S, Burns J, Milne AM et al: High level expression of p27(kip1) and cyclin D1 in some human breast cancer cells: Inverse correlation between the expression of p27(kip1) and degree of malignancy in human breast and colorectal cancers. Proc Natl Acad Sci USA, 1997; 94: 6380–85
33. Koff A: How to decrease p27Kip1 levels during tumor development. Cancer Cell, 2006; 9: 75–76
34. Palmqvist R, Stenling R, Oberg A, Landberg G: Prognostic significance of p27(Kip1) expression in colorectal cancer: A clinico-pathological characterization. J Pathol, 1999; 188: 18–23
35. Tenjo T, Toyoda M, Okuda J et al: Prognostic significance of p27(kip1) protein expression and spontaneous apoptosis in patients with colorectal adenocarcinomas. Oncology, 2000; 58: 45–51
36. Thomas GV, Szigeti K, Murphy M et al: Down-regulation of p27 is associated with development of colorectal adenocarcinoma metastases. Am J Pathol, 1998; 153: 681–87
37. Bold RJ, Termuhlen PM, McConkey DJ: Apoptosis, cancer and cancer therapy. Surg Oncol, 1997; 6: 133–42
38. Kelly PN, Strasser A: The role of Bcl-2 and its pro-survival relatives in tumourigenesis and cancer therapy. Cell Death Differ, 2011; 18: 1414–24
39. Fan TJ, Han LH, Cong RS, Liang J: Caspase family proteases and apoptosis. Acta Biochim Biophys Sin (Shanghai), 2005; 37: 719–27
40. Slee EA, Adrain C, Martin SJ: Executioner caspase-3, -6, and -7 perform distinct, non-redundant roles during the demolition phase of apoptosis. J Biol Chem, 2001; 276: 7320–26
41. Cryns V, Yuan J: Proteases to die for. Genes Dev, 1998; 12: 1551–70
42. Samali A, Cai J, Zhivotovsky B et al: Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. EMBO J, 1999; 18: 2040–48

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]