Oncology Research, Vol. 27, pp. 311–316
Printed in the USA.

0965-0407/19 $90.00 + .00
DOI: https://doi.org/10.3727/096504018X15220579006875
E-ISSN 1555-3906
www.cognizantcommunication.com

miR-455 Functions as a Tumor Suppressor Through Targeting GATA6 in Colorectal Cancer

Hua Yunqi,*[1] Yin Fangrui,†[1] Yang Yongyan,* Jin Yunjian,* Zhang Wenhui,* Cao Kun,* Li Min,* Liu Xianfeng,* and Ba Caixia*

*Cancer Biotherapy Center and Oncology Department, Baotou Tumor Hospital, Clinical Oncology of Baotou Medical College, Baotou, Inner Mongolia, P.R. China
†Department of Central Laboratory, The First Affiliated Hospital of Baotou Medical College, Baotou, Inner Mongolia, P.R. China

Emerging evidence indicates that microRNAs (miRNAs) are often aberrantly expressed in human cancers. Meanwhile, the importance of miRNAs in regulating multiple cellular biological processes has been appreciated. The aim of this study was to investigate the significance of miR-455 and identify its possible mechanism in regulating colorectal cancer (CRC) progression. We found that the expression of miR-455 was sharply reduced in CRC tissues and cell lines. Importantly, the low expression of miR-455 was associated with poor overall survival of CRC patients. Overexpression of miR-455 in CRC cell lines significantly inhibited cell proliferation and migration in vitro. Moreover, GATA-binding protein 6 (GATA6), whose expression can be inversely regulated by miR-455 in CRC cell lines, was validated as a direct target of miR-455. Overall, our results revealed that miR-455 functions as a tumor suppressor, and its downregulation may contribute to CRC progression. Our study may provide a novel therapeutic target for CRC in the future.

Key words: miR-455; Colorectal cancer (CRC); GATA6; Proliferation; Migration

INTRODUCTION

Colorectal cancer (CRC) ranks as the third most commonly diagnosed cancer in the US[1]. In the US, the estimated number of diagnosed CRC cases was 150,250 with 50,630 deaths in 2018, which was similar to the estimated numbers in 2017[1,2]. The overall survival rate of CRC patients remains poor mainly due to late diagnosis or metastasis[2–4]. Therefore, understanding and investigating the mechanism underlying CRC progression or metastasis will be helpful in identifying novel biomarkers for new antitumor strategy development.

MicroRNAs (miRNAs) are small noncoding RNAs that regulate the expression of many protein-coding genes or noncoding genes by complementary binding[5]. Intensive studies have revealed that miRNAs play important roles in cellular biological processes including cell proliferation, migration, invasion, or apoptosis[6–9]. Several miRNAs were revealed to be associated with the progression of CRC including miR-495, miR-411, miR-215-5p, miR-19a, and miR-455[10–14]. Among these miRNAs, miR-455 was widely recognized as a tumor suppressor as it was downregulated in multiple tumors including prostate cancer, esophageal squamous cell carcinoma, gastric cancer, and non-small cell lung cancer[15–18]. However, the exact role of miR-455 in the progression of CRC remains to be fully elucidated.

GATA-binding protein 6 (GATA6), a member of the C2-type zinc finger protein, was previously reported to be highly expressed in CRC and predicts the poor prognosis and lung metastasis of CRC[19]. Importantly, studies revealed that REG4 and BMP were the downstream targets of GATA6 and were key players in the CRC tumorigenesis process mediated by GATA6[20,21]. Unfortunately, the upstream molecules that can regulate the expression of GATA6 in CRC are poorly understood.

In this study, we explored the expression of miR-455 in CRC tissues to stratify the enrolled patients into a high or low miR-455 expression group. Following this, we investigated the effect of miR-455 expression on the 5-year overall survival rate and found that low expression of

[1]These authors provided equal contribution to this work.
Address correspondence to Liu Xianfeng, Cancer Biotherapy Center and Oncology Department, Baotou Tumor Hospital, Clinical Oncology of Baotou Medical College, No. 18 United Street, Qingshan District, Baotou, Inner Mongolia 014030, P.R. China. E-mail: xianfeng_bth@163.com *or* Ba Caixia, Cancer Biotherapy Center and Oncology Department, Baotou Tumor Hospital, Clinical Oncology of Baotou Medical College, No. 18 United Street, Qingshan District, Baotou, Inner Mongolia 014030, P.R. China. E-mail: caixia_ba@163.com

miR-455 predicts poor overall survival of CRC patients. In vitro functional assays revealed that CRC cell proliferation and migration were repressed when miR-455 was overexpressed. Also, we identified that GATA6 was a direct target of miR-455 in CRC and was negatively related to the miR-455 level.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the Ethics Committee at Baotou Tumor Hospital (Inner Mongolia, P.R. China). Written informed consent was obtained from all of the enrolled patients.

Human Tissue Samples

A total of 82 CRC tissues and adjacent noncancerous tissues were obtained from patients at Baotou Tumor Hospital, from 2010 to 2012. The fresh tissues were snap frozen in liquid nitrogen and then stored at −80°C for further usage. None of these patients received any antitumor treatments before surgery.

Cell Lines and Cell Culture

Human CRC cell lines (HCT116 and HT29) and a normal human colon cell line (NCM460) were obtained from the American Type Culture Collection (Manassas, VA, USA). These cells were cultured in complete Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc.). Cells were incubated in a humidified incubator with 5% CO_2 at 37°C.

The miR-455 mimic, miR-455 inhibitor, negative control (NC) miRNA, siRNA of GATA6, and NC siRNA were purchased from GenePharma (Shanghai, P.R. China). Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Inc.) was used to transfer the aforementioned miRNAs or siRNAs into the cell lines according to the supplier's instructions.

RNA Extraction and Quantitative Real-Time PCR (RT-qPCR)

Total miRNA and RNA were extracted from the tissues and cultured cell lines using TRIzol reagent (Beyotime, Jiangsu, P.R. China) per the manufacturer's recommendations. miRNA expression level determination was performed using SYBR PrimeScript miRNA RT PCR Kit (TaKaRa, Dalian, P.R. China). To determine the expression level of mRNA, the PrimeScript RT Reagent Kit (TaKaRa) was first used to reverse transcribe the mRNA into cDNA. The SYBR Green Master Mix (Applied Biosystems, Thermo Fisher Scientific, Inc.) was used to quantify the expression level of mRNA. RT-qPCR was performed at the ABI 7500 Sequence Detection System (Applied Biosystems, Thermo Fisher Scientific, Inc.). The primers used in this study were as follows: miR-455-F 5 -ACACTCCAGCTGGGTATGTGCCTT-3 and miR-455-R 5 -GTGCAGGGTCCGAGGT-3 ; U6 snRNA-F 5 -CTCGCTTCGGCAGCACA-3 and U6 snRNA-R 5 -AACGCTTCACGAATTTGCGT-3 ; GATA6-F 5 -CACACGCTGACAGTGCTGG-3 and GATA6-R 5 -TACAGGGCGATACAAAGCAGGAGAA-3 ; GAPDH-F 5 -ACGGGAAGCTCACTGGCATGG-3 and GAPDH-R 5 -GGTCCACCACCCTGTTGCTGTA-3 .

Western Blot

Total protein was extracted from the tissues, and cell lines were cultured using RIPA lysis buffer (Beyotime). The protein concentration was measured by BCA Assay Kit (Beyotime). The same quantity of protein sample was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. The membranes were blocked at room temperature for 1 h with 5% skim milk. The membranes were then incubated with primary antibodies against GATA6 (ab106066) or GAPDH (ab8245) (both from Abcam, Cambridge, MA, USA) at 4°C overnight. Then the membranes were incubated with horseradish peroxidase-conjugated rabbit anti-mouse IgG antibody (ab6728) at room temperature for 1 h. Proteins were detected by ECL Plus Western Blotting Detection Kit (Beyotime). The bands were analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Luciferase Reporter Assay

According to the prediction results of TargetScan (www.targetscan.org), we found that the 3 -UTR of GATA6 contains a putative binding site for miR-455. Then the wild-type (WT) and mutant (Mut) 3 -UTR of GATA6 were cloned and inserted into a pmirGLO vector (Promega, Madison, WI, USA). The vector containing WT or Mut 3 -UTR of GATA6 was cotransfected with miR-455 inhibitor or NC miRNA. The luciferase activity was assayed at 48 h posttransfection using the Dual-Luciferase Reporter Assay System (Promega).

Cell Proliferation Assay

For cell proliferation analysis, the cell counting kit-8 (CCK-8) was employed in line with the manufacturer's instructions. The cells were seeded in 96-well plates at a density of 2 10^3 cells/well. Then 10 μl of CCK-8 reagent (Beyotime) was added into each well at 0, 24, and 48 h. After further cultivation for 2 h, the optical density value was measured at 450 nm using Multiskan EX spectrophotometer (LabSystem, Helsinki, Finland).

Cell Migration Assay

For cell migration analysis, the wound healing assay was conducted. Briefly, a wound in the cell surface was created using a plastic pipette tip. The cells were washed with PBS to remove the nonadherent cells. After cultivation for 0 and 24 h, the cells were visualized using an inverted microscope (Olympus, Tokyo, Japan). The cell migration distance was measured using ImageJ software (National Institutes of Health).

Statistical Analysis

Data were presented as mean ± SD of three independent experiments. Data analysis was performed using GraphPad Prism 5 software (GraphPad, La Jolla, CA, USA). The Student's *t*-test was performed for statistical analysis of data from two groups. The ANOVA test was conducted to compare the statistic difference among three or more groups. Correlation analysis was performed using Pearson's correlation analysis. Kaplan–Meier curve and log-rank test were conducted to evaluate the effect of miR-455 expression on the overall survival of CRC patients. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

miR-455 Was Downregulated in CRC Tissues and Cell Lines

RT-qPCR was used to analyze the expression of miR-455 in CRC tissues and cell lines. As shown in Figure 1A, the expression of miR-455 was sharply reduced in CRC tissues compared with the adjacent noncancerous tissues. We also measured miR-455 expression in CRC cell lines and found that miR-455 expression was also reduced in CRC cell lines in comparison with the normal colon cell line (Fig. 1B). These data imply that miR-455 might contribute to CRC progression.

miR-455 Correlates With Poor Overall Survival of CRC Patients

According to the expression level of miR-455 determined in the CRC tissues, we stratified the enrolled CRC patients into high or low miR-455 group (cutoff value: 0.52). We then investigated the effect of miR-455 expression on the overall survival of CRC patients. A shown in Figure 1C, the patients with a high expression of miR-455 had a clearly better 5-year overall survival than those with a low expression of miR-455 ($p = 0.039$).

Overexpression of miR-455 Inhibits Cell Proliferation and Migration

To determine the functional role of miR-455 in CRC, we measured the effect of miR-455 on cell proliferation and migration. Since the expression of miR-455 in HCT116 was lower than that in HT29, we thus selected the HCT116 cell line for the in vitro functional assay. First, miR-455 expression was manipulated by transfection of the synthesized miRNAs into the HCT116 cell line. As shown in Figure 2A, the miR-455 mimic transfection could elevate the expression level of miR-455, while the expression of miR-455 was further reduced by miR-455 inhibitor transfection. The CCK-8 assay showed that the cell proliferation rate was reduced by miR-455 overexpression but enhanced by miR-455 downregulation (Fig. 2B). The wound healing assay revealed that cell migration showed a similar trend, which was reduced by miR-455 overexpression but enhanced by miR-455 downregulation (Fig. 2C). Collectively, these data demonstrated that miR-455 plays a very important role in CRC cell proliferation and migration.

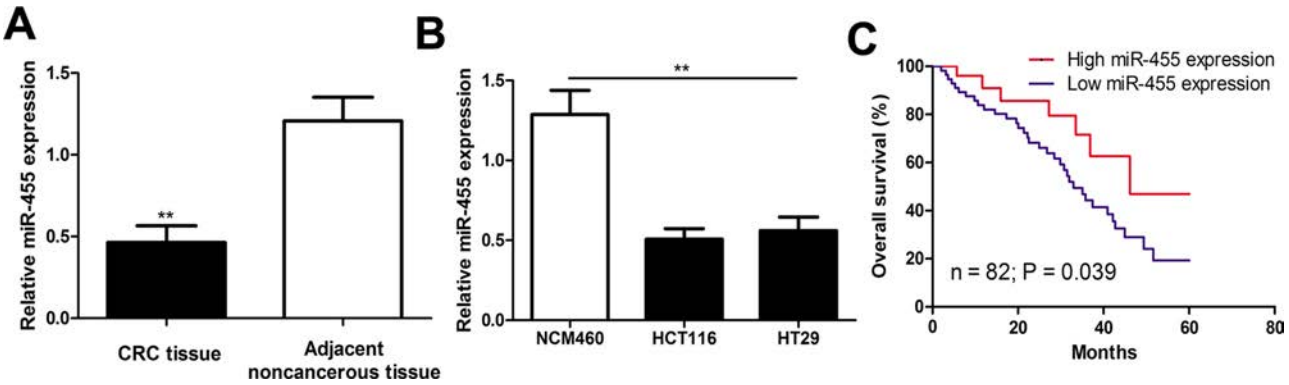

Figure 1. MicroRNA-455 (miR-455) was downregulated in colorectal cancer (CRC). (A) Expression of miR-455 in human CRC tissues was lower than that in adjacent noncancerous tissues. (B) Expression of miR-455 in human CRC cell lines was lower than that in normal colon cell line. (C) Low expression of miR-455 was correlated with poor prognosis of CRC patients. $^{**}p < 0.01$.

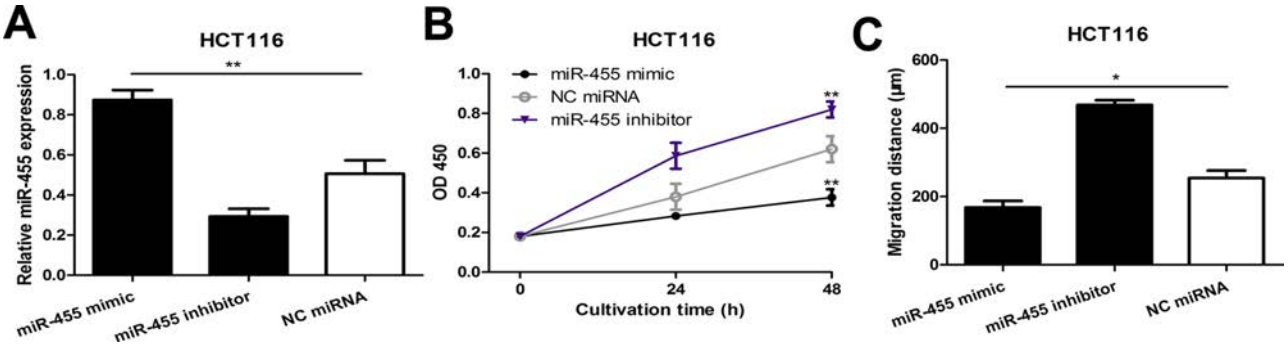

Figure 2. miR-455 reduced CRC cell proliferation and migration. (A) Expression of miR-455, (B) cell proliferation rate, and (C) cell migration in the HCT116 cell line with miR-455 mimic, miR-455 inhibitor, or NC miRNA transfection. *$p<0.05$, **$p<0.01$.

GATA6 Is a Direct Target of miR-455 in CRC

To further elucidate the underlying mechanism of miR-455 in CRC progression, we predicted the potential targets of miR-455 using TargetScan. The results showed that GATA6 contains a possible binding sequence for miR-455 in its 3 -UTR (Fig. 3A). To validate that, the Dual-Luciferase Reporter Assay was performed by cotransfection of the WT or Mut 3 -UTR GATA6 construct and miR-455 mimic or NC miRNA into the HCT116 cell line. The results showed that luciferase activity can be reduced by miR-455 mimic in the WT 3 -UTR GATA6 construct-transfected group but did not have much impact on the Mut 3 -UTR GATA6 construct-transfected group (Fig. 3B). Additionally, we analyzed the expression level of GATA6 and its correlation with miR-455 in CRC tissues. We found that the expression of GATA6 was enhanced in CRC tissues and negatively correlated with the expression of miR-455 (Fig. 3C and D).

Ectopic Expression of miR-455 Reduced the Expression of GATA6

We examined whether miR-455 can regulate the expression of GATA6 in the HCT116 cell line. We examined the protein expression level of GATA6 in the miR-455 mimic- or NC miRNA-transfected HCT116 cell line and found the protein expression level of GATA6 was obviously reduced

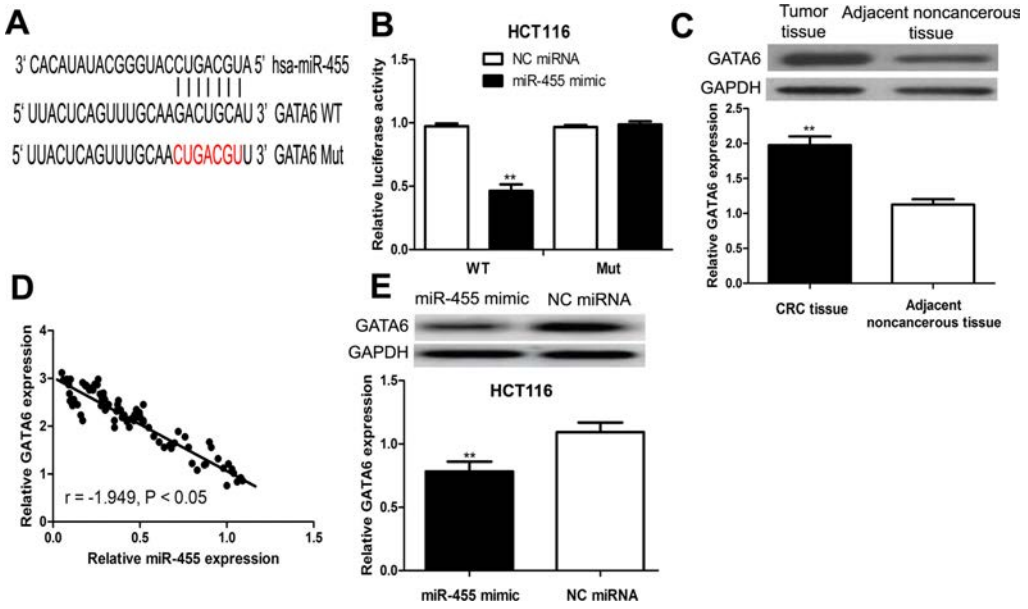

Figure 3. miR-455 directly targeted GATA-binding protein 6 (GATA6) in CRC. (A) Predicted binding region between wild-type (WT) GATA6 3 -UTR or mutant (Mut) GATA6 3 -UTR and miR-455. (B) Luciferase activity of WT GATA6 3 -UTR or Mut GATA6 3 -UTR in HCT116 cell line transfected with the miR-455 mimic or NC miRNA. (C) Expression of GATA6 in human CRC tissues was higher than that in adjacent noncancerous tissues. (D) Expression of GATA6 in human CRC tissues was negatively correlated with expression of miR-455. (E) Expression of GATA6 in the HCT116 cell line with miR-455 mimic or NC miRNA transfection. **$p<0.01$.

by miR-455 mimic when compared with those transfected with NC miRNA (Fig. 3E), which strengthens the conclusion that GATA6 is a direct target of miR-455 in CRC.

Knockdown of GATA6 Inhibits Cell Proliferation and Migration

To explore the functional significance of GATA6 expression, we studied the effect of GATA6 depletion using siRNA. As presented in Figure 4A, the siRNA of GATA6 transfection could effectively reduce the protein expression of GATA6 when compared with NC siRNA transfection. We found that the silencing of GATA6 could reverse the effect of miR-455 downregulation on CRC cell proliferation and migration when cotransfected with the miR-455 inhibitor and siRNA of GATA6 (Fig. 4B and C).

DISCUSSION

The progression of CRC is accompanied with accumulation of aberrant expression of oncogenes or tumor suppressor genes[22]. Decades of research revealed that miRNAs represent a large proportion of the aberrant gene expressions in tumor progression[23]. In this present study, we showed that miR-455 was downregulated in CRC tissues and the cell lines selected, which was in accordance with the previous studies[24,25]. One of the key findings in this study was that for the first time we investigated the effect of miR-455 expression on the 5-year overall survival of CRC patients. We showed that CRC patients with a low expression of miR-455 had a poorer 5-year overall survival than those with a high expression of miR-455.

Previous studies indicated that miR-455 could regulate CRC cell proliferation, apoptosis, and invasion[24,25]. In our study, we also found that the miR-455 overexpression could inhibit CRC cell proliferation and migration in vitro. Meanwhile, two oncogenes, histone deacetylase 2 (HDAC2) and RAF proto-oncogene serine/threonine protein kinase (RAF1), were identified as the direct targets of miR-455 and could be regulated by miR-455 in CRC[24,25]. However, to date, the function and underlying mechanism of miR-455 in CRC progression are still not fully understood. Therefore, studies are still needed to identify the miR-455 regulation networks in tumor progression.

GATA6 has been reported to be aberrantly expressed in diverse cancers and functions as an oncogene or tumor suppressor according to the tumor origin[16,26,27]. A previous study demonstrated that GATA6 expression was elevated in CRC and therefore functions as an oncogene[16]. They also found that overexpression of GATA6 could regulate cell migration and invasion through two possible mechanisms: it can stimulate the expression of BMP4, and it can participate in the activation of BMP4-induced Smad pathway signaling[16]. However, the mechanism by which GATA6 expression was elevated in CRC remains unclear. By using the online target prediction algorithm, we found that the 3 -UTR of GATA6 contains a putative binding site for miR-455. Therefore, it was attractive for us to investigate the association between miR-455 and GATA6. We found that GATA6 expression was inversely correlated with miR-455 in CRC tissues and could be negatively regulated by miR-455. Therefore, we deduced

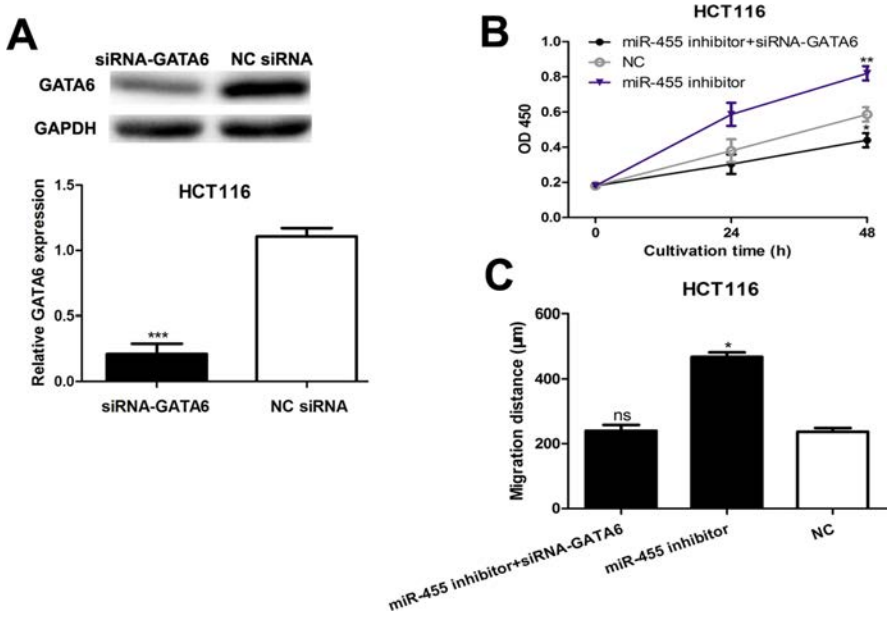

Figure 4. Knockdown of GATA6 inhibits CRC cell proliferation and migration. (A) Expression of GATA6 in the HCT116 cell line with siRNA-GATA6 or NC siRNA transfection. (B) Cell proliferation and (C) cell migration in the HCT116 cell line with siRNA-GATA6 or miR-455 inhibitor transfection. ns, not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

that GATA6 was a direct target of miR-455. To address whether GATA6 participated in the cell proliferation and migration stimulation mediated by miR-455, we cotransfected siRNA of GATA6 and miR-455 inhibitor into the HCT116 cell line. We found that downregulation of GATA6 reversed the stimulatory effect of miR-455 downregulation on CRC cell proliferation and migration.

In conclusion, we showed that the low expression of miR-455 predicts a poor overall survival of CRC patients. miR-455 regulates the proliferation and migration of CRC cell lines, at least in part, via regulating GATA6. These findings provide a theoretical basis for studying miR-455 as an effective targeted therapy biomarker for CRC.

REFERENCES

1. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, Jemal A. Colorectal cancer statistics, 2017. CA Cancer J Clin. 2017;67(3):177–93.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7–30.
3. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin. 2014;64(2):104–17.
4. Shi S, Yang J, Sun D. CT-guided 125I brachytherapy on pulmonary metastases after resection of colorectal cancer: A report of six cases. Oncol Lett. 2015;9(1):375–80.
5. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: MicroRNA biogenesis pathways and their regulation. Nat Cell Biol. 2009;11(3):228–34.
6. Xu FF, Xie WF, Zha GQ, Chen HW, Deng L. MiR-520f promotes cell aggressiveness by regulating fibroblast growth factor 16 in hepatocellular carcinoma. Oncotarget 2017;8(65):109546–58.
7. Sun X, Wang M, Liu H, Wang J. MicroRNA-423 enhances the invasiveness of hepatocellular carcinoma via regulating of BRMS1. Am J Transl Res. 2017;9(12):5576–84.
8. Gang L, Qun L, Liu WD, Li YS, Xu YZ, Yuan DT. MicroRNA-34a promotes cell cycle arrest and apoptosis and suppresses cell adhesion by targeting DUSP1 in osteosarcoma. Am J Transl Res. 2017;9(12):5388–99.
9. Li S, Luo C, Zhou J, Zhang Y. MicroRNA-34a directly targets high-mobility group box 1 and inhibits the cancer cell proliferation, migration and invasion in cutaneous squamous cell carcinoma. Exp Ther Med. 2017;14(6):5611–8.
10. Yan L, Yao J, Qiu J. miRNA-495 suppresses proliferation and migration of colorectal cancer cells by targeting FAM83D. Biomed Pharmacother. 2017;96:974–81.
11. Zhao J, Xu J, Zhang R. MicroRNA-411 inhibits malignant biological behaviours of colorectal cancer by directly targeting PIK3R3. Oncol Rep. 2018;39(2):633–42.
12. Vychytilova-Faltejskova P, Merhautova J, Machackova T, Gutierrez-Garcia I, Garcia-Solano J, Radova L, Brchnelova D, Slaba K, Svoboda M, Halamkova J, Demlova R, Kiss I, Vyzula R, Conesa-Zamora P, Slaby O. MiR-215-5p is a tumor suppressor in colorectal cancer targeting EGFR ligand epiregulin and its transcriptional inducer HOXB9. Oncogenesis 2017;6(11):5.
13. Chen M, Lin M, Wang X. Overexpression of miR-19a inhibits colorectal cancer angiogenesis by suppressing KRAS expression. Oncol Rep. 2018;39(2):619–26.
14. Schepeler T, Reinert JT, Ostenfeld MS, Christensen LL, Silahtaroglu AN, Dyrskjot L, Wiuf C, Sorensen FJ, Kruhoffer M, Laurberg S, Kauppinen S, Qrntoft TF, Andersen CL. Diagnostic and prognostic microRNAs in stage II colon cancer. Cancer Res. 2008;68(15):6416–24.
15. Zhao Y, Yan M, Yun Y, Zhang J, Zhang R, Li Y, Wu X, Liu Q, Miao W, Jiang H. MicroRNA-455-3p functions as a tumor suppressor by targeting eIF4E in prostate cancer. Oncol Rep. 2017;37(4):2449–58.
16. Liu A, Zhu J, Wu G, Cao L, Tan Z, Zhang S, Jiang L, Wu J, Li M, Song L, Li J. Antagonizing miR-455-3p inhibits chemoresistance and aggressiveness in esophageal squamous cell carcinoma. Mol Cancer 2017;16(1):106.
17. Ning T, Peng Z, Li S, Qu Y, Zhang H, Duan J, Wang X, Yang H, Liu R, Deng T, Bai M, Wang Y, Si Y, Zhang L, Wang X, Ge S, Zhou L, Ying G, Ba Y. miR-455 inhibits cell proliferation and migration via negative regulation of EGFR in human gastric cancer. Oncol Rep. 2017;38(1):175–82.
18. Gao X, Zhao H, Diao C, Wang X, Xie Y, Liu Y, Han J, Zhang M. miR-455-3p serves as prognostic factor and regulates the proliferation and migration of non-small cell lung cancer through targeting HOXB5. Biochem Biophys Res Commun. 2018;495(1):1074–80.
19. Shen F, Li J, Cai W, Zhu G, Gu W, Jia L, Xu B. GATA6 predicts prognosis and hepatic metastasis of colorectal cancer. Oncol Rep. 2013;30(3):1355–61.
20. Kawasaki Y, Matsumura K, Miyamoto M, Tsuji S, Okuno M, Suda S, Hiyoshi M, Kitayama J, Akiyama T. REG4 is a transcriptional target of GATA6 and is essential for colorectal tumorigenesis. Sci Rep. 2015;5:14291.
21. Whissell G, Montagni E, Martinelli P, Hernando-Momblona X, Sevillano M, Jung P, Cortina C, Calon A, Abuli A, Castells A, Castellvi-Bel S, Nacht AS, Sancho E, Stephan-Otto Attolini C, Vicent GP, Real FX, Batlle E. The transcription factor GATA6 enables self-renewal of colon adenoma stem cells by repressing BMP gene expression. Nat Cell Biol. 2014;16(7):695–707.
22. Pandurangan AK. Potential targets for prevention of colorectal cancer: A focus on PI3K/Akt/mTOR and Wnt pathways. Asian Pac J Cancer Prev. 2013;14(4):2201–5.
23. Wu Q, Yang Z, Shi Y, Fan D. MiRNAs in human cancers: The diagnostic and therapeutic implications. Curr Pharm Des. 2014;20(33):5336–47.
24. Mao QD, Zhang W, Zhao K, Cao B, Yuan H, Weil LZ, Song MQ, Liu XS. MicroRNA-455 suppresses the oncogenic function of HDAC2 in human colorectal cancer. Braz J Med Biol Res. 2017;50(6):e6103.
25. Chai J, Wang S, Han D, Dong W, Xie C, Guo H. MicroRNA-455 inhibits proliferation and invasion of colorectal cancer by targeting RAF proto-oncogene serine/threonine-protein kinase. Tumour Biol. 2015;36(2):1313–21.
26. Song Y, Tian T, Fu X, Wang W, Li S, Shi T, Suo A, Ruan Z, Guo H, Yao Y. GATA6 is overexpressed in breast cancer and promotes breast cancer cell epithelial-mesenchymal transition by upregulating slug expression. Exp Mol Pathol. 2015;99(3):617–27.
27. Vuorenoja S, Rivero-Muller A, Kiiveri S, Bielinska M, Heikinheimo M, Wilson DB, Huhtaniemi IT, Rahman NA. Adrenocortical tumorigenesis, luteinizing hormone receptor and transcription factors GATA-4 and GATA-6. Mol Cell Endocrinol. 2007;269(1–2):38–45.