Original Article
MicroRNA-128 suppresses colorectal cancer cell proliferation through targeting LRP6

Dedong He[1], Yu Bai[2], Dezhong Zhang[3], Xiangjie Fang[3], Qinghui Yang[4]

[1]Department of General Surgery Ward 4, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan, China; [2]Department of Pathology, Xinxiang Medical University, Xinxiang 453003, Henan, China; [3]Department of General Surgery Ward 2, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan, China; [4]Department of Oncology, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan, China

Abstract: Background: Colorectal cancer (CRC) is one of the most commonly cancer worldwide and many studies demonstrated that microRNAs (miRNAs) play important roles in the tumorigenesis and development of CRC. The purpose of this study was to investigate the expression level and biological functions of miR-128 in CRC. Methods: The expression level of miR-128 in 30 CRC specimens and 5 CRC cell lines was detected by quantitative real-time PCR (qRT-PCR). Cell proliferation was assessed by CCK-8 assay. Flow cytometry was performed to analyze the cell apoptosis and cell cycle distribution. Moreover, luciferase reporter assay was conducted to explore the potential target of miR-128 in CRC cells. Results: MiR-128 was significantly down-regulated in CRC specimens as well as in CRC cell lines. Overexpression of miR-128 in SW480 cells transfected with miR-128 mimics could suppress CRC cell proliferation and induces cell cycle arrest and cell apoptosis. In addition, we found that LRP6 was a direct and functional target of miR-128. In keeping with the effect induced by overexpression of miR-128, knockdown of LRP6 inhibited CRC cell proliferation. Conclusion: Our study indicated that miR-128 could suppress CRC cell proliferation through targeting LRP6. MiR-128 might act as a potential therapeutic target for CRC treatment in the future.

Keywords: miR-128, colorectal cancer, proliferation, LRP6

Introduction

Colorectal cancer (CRC) is one of the most commonly cancer worldwide [1], and the incidence of CRC has increased annually in China [2]. Although treatment strategies for patients with CRC have significantly improved within the past decade, the treatment results are still unsatisfactory, particularly for metastatic tumors. The pathological process and underlying mechanism of CRC remain poorly understood. Therefore, it is necessary to develop novel and effective therapeutic strategies.

MicroRNAs (miRNAs) are a large family of small non-coding RNA molecules that play important roles in variety of cellular processes such as proliferation, differentiation, and apoptosis [3, 4]. MiRNAs regulate gene expression by binding to the 3'-untranslated region (UTR) of target mRNAs, resulting in translation repression or mRNA degradation [5-7]. An accumulating body of evidence have shown that the deregulation of miRNAs involve in the initiation and progression of cancer including CRC [8-11]. Some miRNAs function as tumor-suppressor genes and are downregulated in cancer tissue compared with normal tissue [12-14], whereas other miRNAs act as oncogenes and are over-expressed [15, 16]. Multiple reports indicated that miR-128 has been shown to be downregulated in several types of cancer including hepatocellular carcinoma [17], prostate cancer [18], head and neck squamous cell carcinoma [19], non-small cell lung cancer [20], gastric cancer [21], and to function as a tumor-suppressor gene. However, the mechanism and function of miR-128 in CRC have not been fully determined.

Thus, in this study, we investigated the expression and function of miR-128 in CRC. Furthermore, we explored the mechanism underlying CRC progression of miR-128 by identifying possible target genes. This study may be useful for the development of novel therapeutic and diagnostic strategies for CRC.

Materials and methods

Human tissue specimens

A total of 30 CRC tissue specimens and their adjacent non-tumor tissues were obtained from The First Affiliated Hospital of Xinxiang Medical University after surgical resection. All tissue specimens were immediately snapped frozen in liquid nitrogen until use. The study was approved by the Institute Research Ethics Committee of Shanghai Tenth's People Hospital of Tongji University Chongming Branch. All patients wrote informed consent for the use of the tissues for research purposes.

Cell lines and culture

Human CRC cell lines HCT116, SW403, HT29, SW480, SW620 and normal colonic cell line FHC were purchased from the American Type Culture Collection (ATCC, USA). All CRC cell lines were cultured in Dulbecco's modified Eagle medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Sigma, USA) and 100 units/ml of penicillin-streptomycin (Invitrogen, Carlsbad, CA). FHC cells were cultured in DMEM/F-12 medium with 10% FBS and 100 units/ml of penicillin-streptomycin. Cell lines were cultured in a humidified atmosphere of 95% air and 5% CO_2 incubator at 37°C.

RNA isolation and quantitative real-time PCR

Total RNA was isolated from tissues and cell lines by using the Trizol extraction kit (Invitrogen, USA) according to the manufacturer's instructions. Both miRNA and mRNA were reverse transcribed to cDNA by using reverse transcription kit (Takara, Japan). Quantitative real-time PCR (qRT-PCR) were performed by using SYBR Green PCR Kit (Takara, Japan) on ABI 7500 Fast Real-Time PCR system according to the manufacturer's instructions. U6 small nuclear RNA and GAPDH were used as an internal control for normalization and quantification of miR-128 and LRP6 expression. All experiments were repeated independently in three times. The relative expression of genes was calculated by using the $2^{-\Delta\Delta Ct}$ method.

Cell transfections

The miR-128 mimics and corresponding miRNA negative control were purchased from RiboBio

Company (Guangzhou, China). Small interfering RNA (siRNA) for SOX4 and corresponding negative control were synthesized and purified by GenePharma Company (Shanghai, China). Transfection was performed by using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instruction. After transfected and cultured for 48 h, cells were collected for further assays.

Cell proliferation analysis

Cell growth viability was measured by the Cell Counting Kit-8 (CCK-8, Japan) assay by following the manufacturer's protocol. Cells were plated in a 96-well plate and incubated for 24, 48, 72 h and 96 h at 37°C in a humidified atmosphere with 5% CO_2. The absorbance was recorded at 450 nm, and all experiments were performed in triplicate.

Cell cycle analysis

Cells were harvested at 48 h after transfection, centrifuged and washed three times with cold PBS. Cells were fixed in 70% ethanol at 4°C overnight. After a 30-min digestion in RNase A (50 μg/ml), propidium iodide (PI, 50 μg/mL) was added to the cells and further incubated at room temperature for 30 min in the dark. The cell cycle was then analyzed by using the flow cytometry (FACSCanto™ II, BD, USA).

Cell apoptosis analysis

Cell apoptosis analysis was performed by using Annexin V-FITC/PI apoptosis detection kit (BD, USA) according to the manufacturer's protocol. After adding 5 μL Annexin V-FITC reagent, the cells were incubated in the dark for 15 min at room temperature. Subsequently, 5 μL propidium iodide (PI) was added and the cells were incubated in the dark for 5 min at room temperature. The cell apoptosis was then analyzed by using the flow cytometry.

Western blot analysis

Total proteins of cells were extracted with RIPA buffer containing protease inhibitors. The concentrations of the proteins were detected by using BCA Protein Assay Kit (Beyotime, Shanghai, China). Proteins were fractionated by using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto nitrocellulose mem-
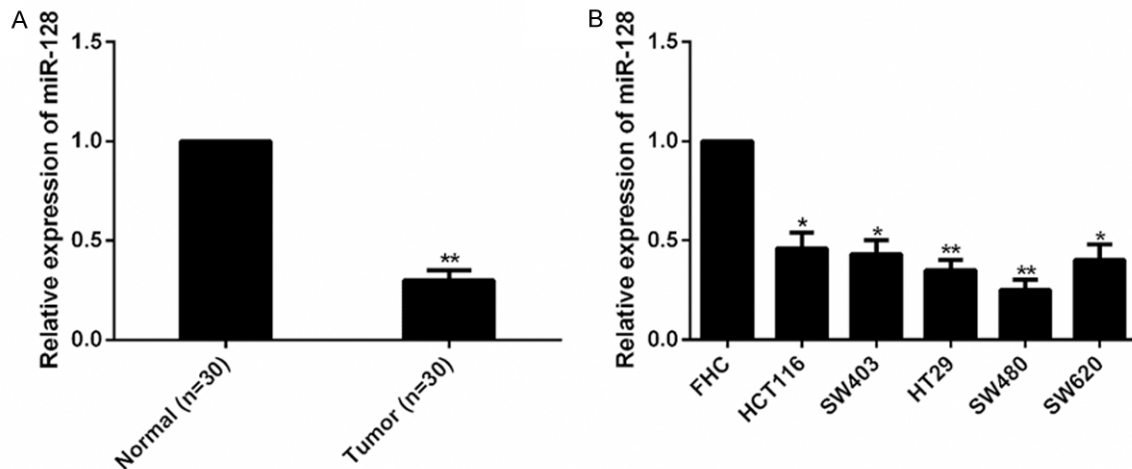
Figure 1. MicroRNA-128 (miR-128) is downregulated in colorectal cancer (CRC) tissues and cell lines. A. The expression of miR-128 in 30 paired tumor tissues and adjacent non-tumor tissues was examined by using quantitative real-time PCR. B. The expression of miR-128 in CRC cell lines (HCT116, SW403, HT29, SW480, SW620) and normal colonic cell line FHC. *P<0.05 and **P<0.01.

branes, which were blocked with 5% non-fat milk at room temperature for 1 hour and incubated with primary antibodies against human LRP6 or GAPDH (CST, USA). GAPDH was used as a control. After being washed, the membranes were incubated with secondary antibodies at room temperature for 1 h. After three washes with PBST, results were detected by using the Odyssey Scanning system (Li-Cor, Lincoln, USA).

Luciferase reporter analysis

The 3'-UTR sequence of human LRP6 containing the wild-type (Wt) or mutant (Mut) miR-128 binding site were cloned into the pGL3 vector (Invitrogen, USA). Cells were seeded in 24-well plates and co-transfected with WT or Mut 3'-UTR vector and miR-128 mimics using Lipofectamine 2000. Cells were collected 48 hours after transfection. Luciferase activities were analyzed by using the Dual Luciferase Reporter Assay (Promega, USA) following the manufacturer's instructions. All experiments were performed in triplicate.

Statistical analysis

All statistical analyses were performed by using SPSS 18.0 (USA). Data were presented as mean ± SD. Differences between groups were analyzed by using student's t-test or one-way ANOVA analysis. A value of *P*<0.05 was considered statistically significant.

Results

MiR-128 is downregulated in CRC tissues and cell lines

To investigate the expression level of miR-128 in CRC tissues, we detected its expression in 30 paired tumor tissues and adjacent non-tumor tissues by using quantitative real-time PCR. MiR-128 was significantly downregulated in tumor tissues compared with the adjacent normal tissues (**Figure 1A**). Furthermore, the expression of miR-128 in all five CRC cell lines (HCT116, SW403, HT29, SW480, SW620) were significantly decreased compared to the normal colonic cell line FHC (**Figure 1B**). These results suggested that miR-128 was abnormally downregulated both in human CRC tissues and cell lines.

MiR-128 suppresses CRC cell proliferation and induces cell cycle arrest and cell apoptosis

To study the potential role of miR-128 in the pathogenesis of CRC, we then transfected miR-128 mimics and negative control into SW480 cells. As expected, miR-128 expression was obviously upregulated in SW480 cells transfected with miR-128 mimics compared to cells transfected with the negative control (**Figure 2A**). We then investigated the effect of miR-128 on cell proliferation, cell viability assay was performed at 24, 48, 72 and 96 h after transfection. Compared with the negative control group,
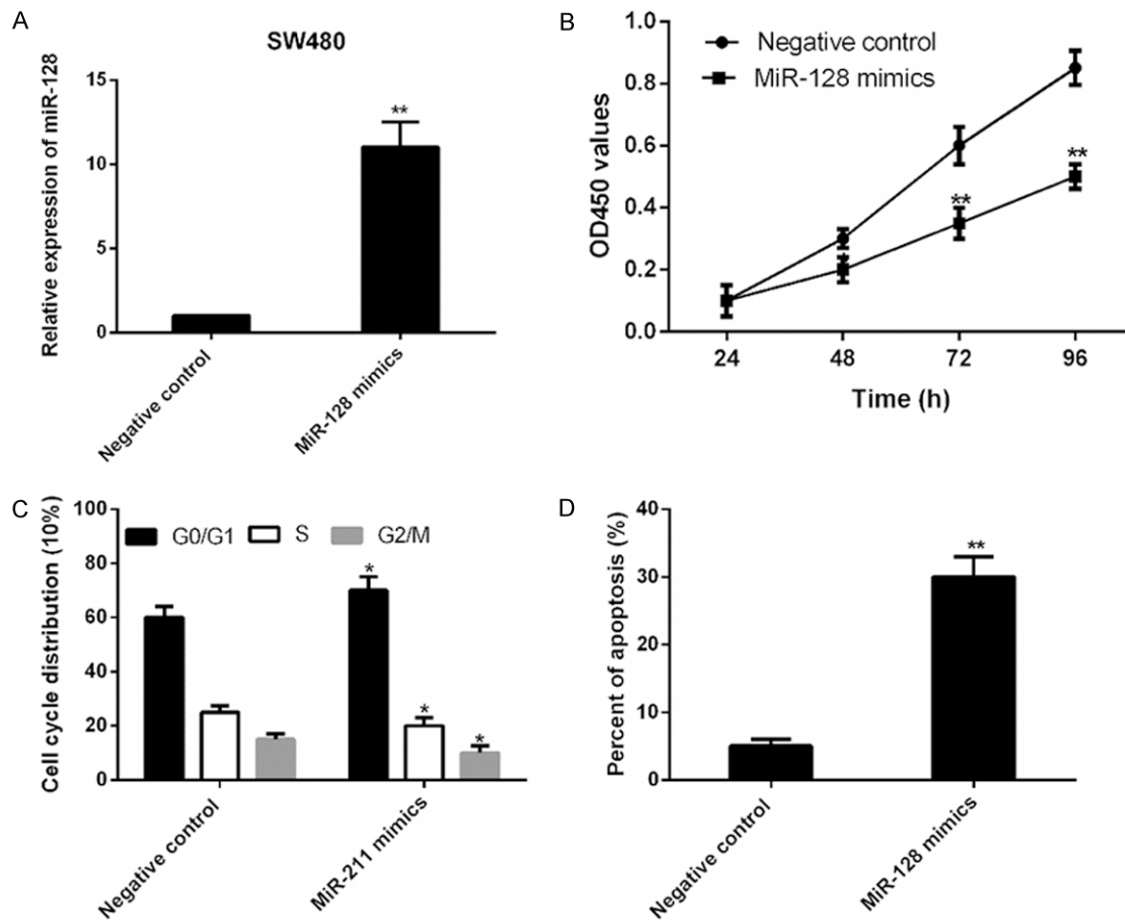
Figure 2. MiR-128 suppresses CRC cell proliferation, induces cell cycle arrest and cell apoptosis. A. MiR-128 mimics up-regulated the expression level of miR-128 compared to negative control in SW480 cells. B. CCK-8 proliferation assay showed that overexpression of miR-128 significantly inhibited the proliferation of the SW480 cells compared to the negative control. C. Effect of miR-128 mimics on the cell cycle distribution of SW480 cells. Results were analyzed by flow cytometry 48 h after transfection. MiR-128-treated SW480 cells showed obvious cell cycle arrest compared to the negative control. D. Effect of miR-128 mimics on the cell apoptosis of SW480 cells. Results revealed that the proportion of apoptosis cells was significantly higher in cells transfected with miR-128 mimics than that in negative control group. *P<0.05 and **P<0.01.

overexpression of miR-128 could significantly inhibit SW480 cell proliferation (**Figure 2B**).

To investigate the mechanism mediating this anti-proliferative effect, a cell cycle analysis was performed in SW480 cells transfected with miR-128 mimics by using flow cytometry. MiR-128-treated SW480 cells showed obvious cell cycle arrest in G0/G1 phase at 48 h after transfection compared to the negative control, characterized by nearly 70% of cells in the G1 phase of the cell cycle (**Figure 2C**). Moreover, we explored the level of apoptosis in SW480 cells by using flow cytometry analysis, the proportion of apoptosis cells was significantly higher in

cells transfected with miR-128 mimics than that in negative control group (**Figure 2D**). These results showed that miR-128 inhibited CRC cell proliferation, together with inducing cell cycle arrest and cell apoptosis in CRC cell lines.

LRP6 is a direct target of miR-128

Next, we tried to predict the potential target gene involved in miR-128 regulation on CRC. Through bioinformatics analyses using Target-Scan, we found that the 3'-UTR of LRP6 mRNA contained a target site for miR-128 (**Figure 3A**). To further determine whether LRP6 is a direct
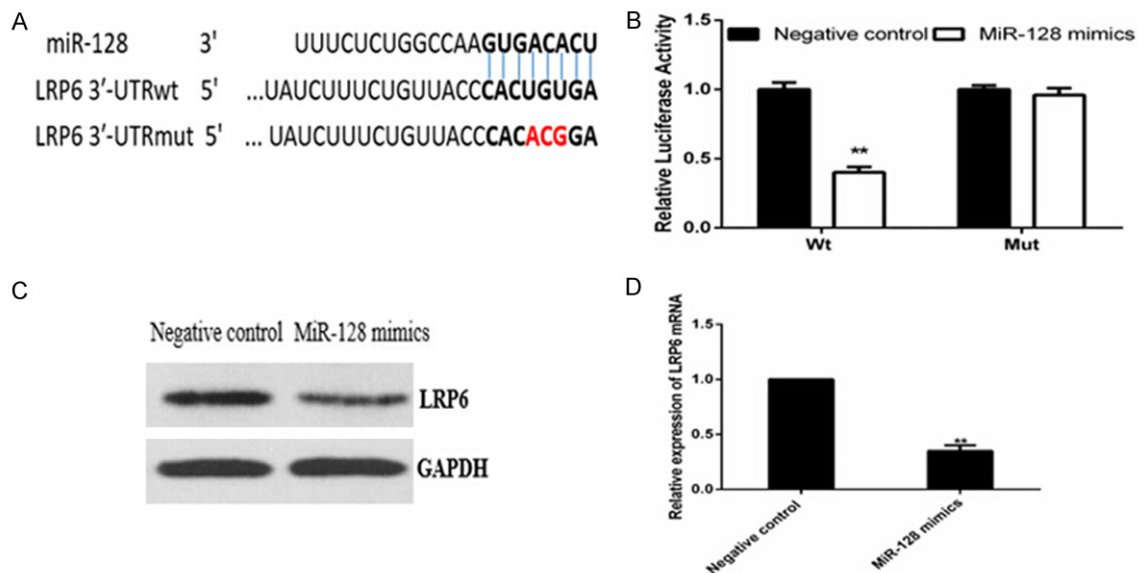
Figure 3. LRP6 is a direct target of miR-128 in CRC. A. The 3'-UTR of LRP6 mRNA contained a target site for miR-128. B. Luciferase activity assay were performed with co-transfection of wild-type or mutant LRP6 and miR-128 mimics or negative control in SW480 cells. C. The effect of miR-128 mimics on the expression level of LRP6 protein by using western blot analysis. D. The effect of miR-128 mimics on the expression level of LRP6 mRNA by using qRT-PCR analysis. *P<0.05 and **P<0.01.

target of miR-128, we performed luciferase reporter assay in SW480 cells. The 3'-UTR of the human LRP6 gene containing the miR-128 binding site or the mutant site was cloned into the pGL-3 vector. These luciferase reporter vectors were co-transfected into SW480 cells with miR-128 mimics or negative control, and the luciferase activities were measured at 48 h after transfection. As shown in **Figure 3B**, miR-128 could act on the 3'-UTR of wild-type LRP6 and lead to a significant decrease in luciferase activity compared to the negative control, however, miR-128 mimics did not have the effect on luciferase activity of the mutant LRP6 site. Moreover, the levels of LRP6 mRNA and protein were consistently and substantially downregulated in SW480 cells transfected with miR-128 mimics by using western blot and qRT-PCR analyses (**Figure 3C**, **3D**). Taken together, these results suggested that miR-128 could directly target LRP6 in CRC cells.

Inverse relationship between LRP6 and miR-128 expression

Furthermore, we examined the expression levels of LRP6 in CRC tissues and cell lines by using qRT-PCR. The results showed that the expression level of LRP6 mRNA was markedly increased in CRC tissues (**Figure 4A**) and cell lines (**Figure 4B**) compared to the normal controls. Furthermore, the expression of LRP6 in CRC tissues was inversely associated with the expression of miR-128 (**Figure 4C**).

MiR-128 suppresses CRC cell proliferation by targeting LRP6

Our results demonstrated that overexpression of miR-128 could suppress the proliferation of CRC cells, and LRP6 was a direct target of miR-128. To further investigate the role of LRP6 reduction in miR-128-induced CRC proliferation, SW480 cells were transfected with negative control, miR-128 mimics, LRP6 siRNA and co-transfected with miR-128 mimics and LRP6 siRNA. Results revealed that the mRNA and protein levels of LRP6 were markedly decreased in LRP6 siRNA group compared to negative control (**Figure 5A**, **5B**). In keeping with the effect induced by overexpression of miR-128, knockdown of LRP6 significantly suppressed the cell viability, whereas co-transfected with LRP6 siRNA and miR-128 mimics did not have further suppressive effect on cell growth in CRC cells (**Figure 5C**). These results indicated that miR-128 could suppress CRC cell proliferation through targeting LRP6.
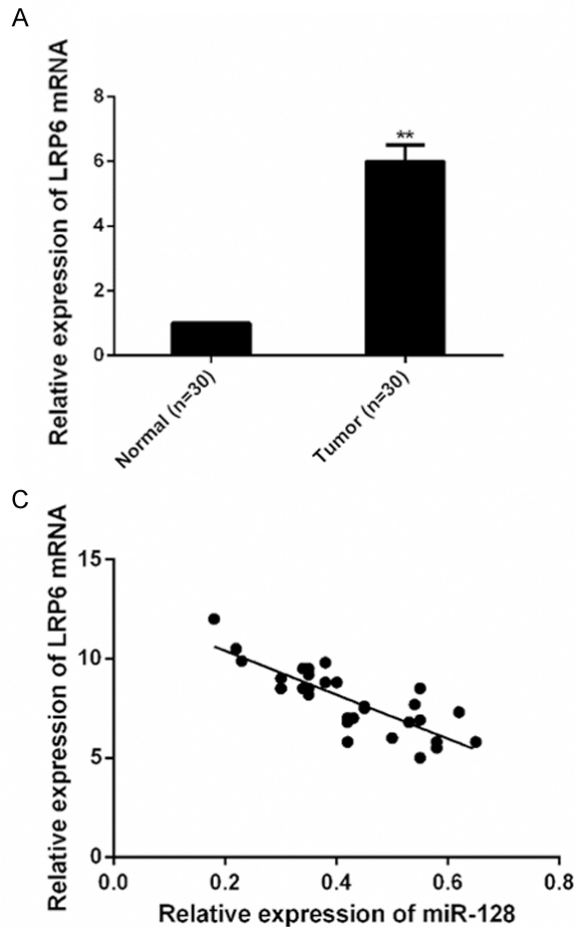
Figure 4. Inverse relationship between LRP6 and miR-128 expression in CRC tissues. The expression of LRP6 is up-regulated in 30 paired CRC tissues (A) and 5 CRC cell lines (B), the expression of LRP6 in CRC tissues was inversely associated with the expression of miR-128 (C, r=-0.48, P<0.01). *P<0.05 and **P<0.01.

Discussion

In this study, we examined the expression level of miR-128 in colorectal cancer. Our results showed that miR-128 was significantly down-regulated in CRC tissues as well as in CRC cell lines. Overexpression of miR-128 in SW480 cells transfected with miR-128 mimics could suppress CRC cell proliferation and induces cell cycle arrest and cell apoptosis. In addition, we found that LRP6 was a direct and functional target of miR-128 in CRC cells. The expression of LRP6 was markedly increased in CRC tissues and cell lines compared with normal controls, while knockdown of LRP6 by using siRNA inhibited CRC cell proliferation. These results demonstrate that miR-128 plays an essential role in CRC development, miR-128 suppresses CRC cell proliferation by targeting LRP6.

Mounting evidence have shown that the deregulation of miRNAs involve in development of CRC. For example, Li et al. [22] reported that miR-766 promoted cell proliferation of human colorectal cancer by targeting regulation of SOX6. Ren et al. [23] found that miR-206 functioned as a tumor suppressor in the progression of CRC by targeting FMNL2 and c-MET. Furthermore, Sumbul et al. [24] revealed that miR-211 expression was upregulated and associated with poor prognosis in colorectal cancer. However, different miRNAs might play different roles in CRC, so we still need to clarify the clinical significance and function of certain specific miRNA. Many studies indicated that miR-128 was downregulated in several types of cancer including hepatocellular carcinoma, prostate cancer, and so on. However, the expression and function of miR-128 in CRC have not been fully understood. Our study revealed that miR-128 is significantly down-regulated in CRC and inhibits CRC cell proliferation in vitro, miR-128 might play a tumor suppresser role in the pathogenesis of CRC.

Until now, hundreds of miRNAs have been demonstrated to participate in the development of cancer though the regulation of oncogenes or
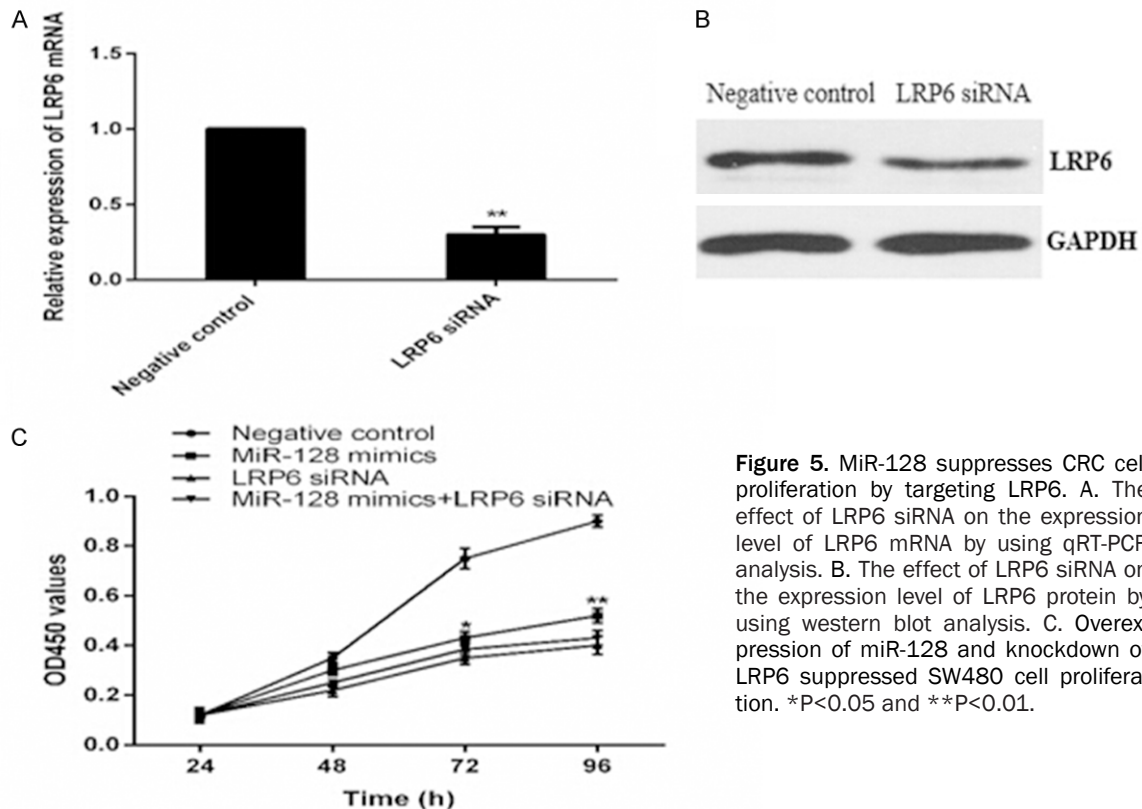
Figure 5. MiR-128 suppresses CRC cell proliferation by targeting LRP6. A. The effect of LRP6 siRNA on the expression level of LRP6 mRNA by using qRT-PCR analysis. B. The effect of LRP6 siRNA on the expression level of LRP6 protein by using western blot analysis. C. Overexpression of miR-128 and knockdown of LRP6 suppressed SW480 cell proliferation. *$P<0.05$ and **$P<0.01$.

suppressors. It is still necessary to explore the molecular mechanism underlying CRC progression of miR-128 by identifying the possible target gene. By using bioinformatics analyses and luciferase reporter assay, we demonstrated that low-density lipoprotein receptor-related protein 6 (LRP6) was a target of miR-128. LRP6 is one of the co-receptors of Wnt/b-catenin pathway which form a signaling complex to activate downstream signaling, and is essential for the activation of Wnt/b-catenin pathway [25]. However, recent studies demonstrated that the expression of LRP6 was upregulated in many types of human cancers including breast cancer [26], prostate cancer [27], glioblastoma [28], hepatocellular carcinoma [29], and the potential role of LRP6 in tumorigenesis was regarded as an oncogenic protein. Tung et al. [25] found that LRP6 was frequently overexpressed in human hepatocellular carcinoma, overexpression of LRP6 contributed to the hyperactivation of the Wnt/β-catenin signaling pathway and it may play a role in hepatocarcinogenesis. Zhang et al. [30] reported that miR-202 suppressed cell proliferation in human hepatocellular carcinoma by downregulating LRP6. Furthermore, Lemieux et al. [31]. indicat-

ed that oncogenic activation of KRAS/BRAF/MEK signaling stimulated the Wnt/β-catenin pathway through LRP6 in colorectal cancer, which in turn promoted tumor growth and invasion.

In our study, LRP6 was upregulated in CRC tissues and cell lines, and the expression of LRP6 in CRC tissues was inversely correlated with the expression of miR-128. Moreover, knockdown of LRP6 by siRNA in CRC cells led to inhibition of cellular proliferation. Our results demonstrated that LRP6 act as a target of miR-128 and showed that overexpression of miR-128 was correlated with knockdown of LRP6 leading to the inhibition of cell proliferation, whereas co-transfected with LRP6 siRNA and miR-128 mimics did not have further suppressive effect on cell growth in CRC cells. Taken together, miR-128 plays a tumor suppressor role in colorectal cancer by down-regulating LRP6.

In conclusion, our study revealed that miR-128 was downregulated in CRC tissues and cell lines, while LRP6 was upregulated. The expression of LRP6 in CRC tissues was inversely correlated with the expression of miR-128. We

also demonstrated that LRP6 was a direct target of miR-128. Overexpression of miR-128 could suppress CRC cell proliferation through targeting LRP6. These results suggested that miR-128 may act as a potential therapeutic target for CRC.

Disclosure of conflict of interest

None.

Address correspondence to: Dedong He, Department of General Surgery Ward 4, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan, China. E-mail: Dedongh337@163.com

References

[1] Chen XJ, Shi KQ, Wang YQ, Song M, Zhou W, Tu HX and Lin Z. Clinical value of integrated-signature miRNAs in colorectal cancer: miRNA expression profiling analysis and experimental validation. Oncotarget 2015; 6: 37544-56.

[2] Chen WQ, Zheng RS, Zhang SW, Zeng HM and Zou XN. The incidences and mortalities of major cancers in China, 2010. Chin J Cancer 2014; 33: 402-5.

[3] Cui F, Li X, Zhu X, Huang L, Huang Y, Mao C, Yan Q, Zhu J, Zhao W and Shi H. MiR-125b inhibits tumor growth and promotes apoptosis of cervical cancer cells by targeting phosphoinositide 3-kinase catalytic subunit delta. Cell Physiol Biochem 2012; 30: 1310-8.

[4] Garzon R, Marcucci G and Croce CM. Targeting microRNAs in cancer: rationale, strategies and challenges. Nat Rev Drug Discov 2010; 9: 775-89.

[5] Valinezhad Orang A, Safaralizadeh R and Kazemzadeh-Bavili M. Mechanisms of miRNA-Mediated Gene Regulation from Common Downregulation to mRNA-Specific Upregulation. Int J Genomics 2014; 2014: 970607.

[6] van Kouwenhove M, Kedde M and Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. Nat Rev Cancer 2011; 11: 644-56.

[7] Su Z, Yang Z, Xu Y, Chen Y and Yu Q. MicroRNAs in apoptosis, autophagy and necroptosis. Oncotarget 2015; 6: 8474-90.

[8] Wang K, Liang Q, Wei L, Zhang W and Zhu P. MicroRNA-608 acts as a prognostic marker and inhibits the cell proliferation in hepatocellular carcinoma by macrophage migration inhibitory factor. Tumour Biol 2015; [Epub ahead of print].

[9] Chen X, Dong C, Law PT, Chan MT, Su Z, Wang S, Wu WK and Xu H. MicroRNA-145 targets TRIM2 and exerts tumor-suppressing functions in epithelial ovarian cancer. Gynecol Oncol 2015; 139: 513-9.

[10] Chen SP, Liu BX, Xu J, Pei XF, Liao YJ, Yuan F and Zheng F. MiR-449a suppresses the epithelial-mesenchymal transition and metastasis of hepatocellular carcinoma by multiple targets. BMC Cancer 2015; 15: 706.

[11] Ghanbari R, Mosakhani N, Asadi J, Nouraee N, Mowla SJ, Yazdani Y, Mohamadkhani A, Poustchi H, Knuutila S and Malekzadeh R. Downregulation of Plasma MiR-142-3p and MiR-26a-5p in Patients With Colorectal Carcinoma. Iran J Cancer Prev 2015; 8: e2329.

[12] Song WH, Feng XJ, Gong SJ, Chen JM, Wang SM, Xing DJ, Zhu MH, Zhang SH and Xu AM. microRNA-622 acts as a tumor suppressor in hepatocellular carcinoma. Cancer Biol Ther 2015; 16: 1754-63.

[13] Ma Y, Bao C, Kong R, Xing X, Zhang Y, Li S, Zhang W, Jiang J, Zhang J, Qiao Z, Zhang D, Ma Z, Sun L and Zhou B. MicroRNA3615p suppresses cancer progression by targeting signal transducer and activator of transcription 6 in nonsmall cell lung cancer. Mol Med Rep 2015; 12: 7367-73.

[14] Riquelme I, Tapia O, Leal P, Sandoval A, Varga MG, Letelier P, Buchegger K, Bizama C, Espinoza JA, Peek RM, Araya JC and Roa JC. miR-101-2, miR-125b-2 and miR-451a act as potential tumor suppressors in gastric cancer through regulation of the PI3K/AKT/mTOR pathway. Cell Oncol (Dordr) 2016; 39: 23-33.

[15] Yang Z, Wang R, Zhang T and Dong X. MicroRNA-126 regulates migration and invasion of gastric cancer by targeting CADM1. Int J Clin Exp Pathol 2015; 8: 8869-80.

[16] Najafi Z, Sharifi M and Javadi G. Degradation of miR-21 induces apoptosis and inhibits cell proliferation in human hepatocellular carcinoma. Cancer Gene Ther 2015; 22: 530-5.

[17] Huang CY, Huang XP, Zhu JY, Chen ZG, Li XJ, Zhang XH, Huang S, He JB, Lian F, Zhao YN and Wu GB. miR-128-3p suppresses hepatocellular carcinoma proliferation by regulating PIK3R1 and is correlated with the prognosis of HCC patients. Oncol Rep 2015; 33: 2889-98.

[18] Sun X, Yang Z, Zhang Y, He J, Wang F, Su P, Han J, Song Z and Fei Y. Prognostic implications of tissue and serum levels of microRNA-128 in human prostate cancer. Int J Clin Exp Pathol 2015; 8: 8394-401. eCollection 2015.

[19] Hauser B, Zhao Y, Pang X, Ling Z, Myers E, Wang P, Califano J and Gu X. Functions of MiRNA-128 on the regulation of head and neck squamous cell carcinoma growth and apoptosis. PLoS One 2015; 10: e0116321.

[20] Hu J, Cheng Y, Li Y, Jin Z, Pan Y, Liu G, Fu S, Zhang Y, Feng K and Feng Y. microRNA-128 plays a critical role in human non-small cell lung cancer tumourigenesis, angiogenesis and

lymphangiogenesis by directly targeting vascular endothelial growth factor-C. Eur J Cancer 2014; 50: 2336-50.

[21] Liu HT, Xing AY, Chen X, Ma RR, Wang YW, Shi DB, Zhang H, Li P, Chen HF, Li YH and Gao P. MicroRNA-27b, microRNA-101 and microRNA-128 inhibit angiogenesis by down-regulating vascular endothelial growth factor C expression in gastric cancers. Oncotarget 2015; 6: 37458-70.

[22] Li YC, Li CF, Chen LB, Li DD, Yang L, Jin JP and Zhang B. MicroRNA-766 targeting regulation of SOX6 expression promoted cell proliferation of human colorectal cancer. Onco Targets Ther 2015; 8: 2981-8.

[23] Ren XL, He GY, Li XM, Men H, Yi LZ, Lu GF, Xin SN, Wu PX, Li YL, Liao WT, Ding YQ and Liang L. MicroRNA-206 functions as a tumor suppressor in colorectal cancer by targeting FMNL2. J Cancer Res Clin Oncol 2016; 142: 581-92.

[24] Sumbul AT, Gogebakan B, Bayram S, Batmaci CY and Oztuzcu S. MicroRNA 211 expression is upregulated and associated with poor prognosis in colorectal cancer: a case-control study. Tumour Biol 2015; 36: 9703-9.

[25] Tung EK, Wong BY, Yau TO and Ng IO. Upregulation of the Wnt co-receptor LRP6 promotes hepatocarcinogenesis and enhances cell invasion. PLoS One 2012; 7: e36565.

[26] Wang H, He L, Ma F, Regan MM, Balk SP, Richardson AL and Yuan X. SOX9 regulates low density lipoprotein receptor-related protein 6 (LRP6) and T-cell factor 4 (TCF4) expression and Wnt/beta-catenin activation in breast cancer. J Biol Chem 2013; 288: 6478-87.

[27] Tahir SA, Yang G, Goltsov A, Song KD, Ren C, Wang J, Chang W and Thompson TC. Caveolin-1-LRP6 signaling module stimulates aerobic glycolysis in prostate cancer. Cancer Res 2013; 73: 1900-11.

[28] Xu J, Sun T and Hu X. microRNA-513c suppresses the proliferation of human glioblastoma cells by repressing low-density lipoprotein receptor-related protein 6. Mol Med Rep 2015; 12: 4403-9.

[29] Jiang N, Chen WJ, Zhang JW, Xu C, Zeng XC, Zhang T, Li Y and Wang GY. Downregulation of miR-432 activates Wnt/beta-catenin signaling and promotes human hepatocellular carcinoma proliferation. Oncotarget 2015; 6: 7866-79.

[30] Zhang Y, Zheng D, Xiong Y, Xue C, Chen G, Yan B and Ye Q. miR-202 suppresses cell proliferation in human hepatocellular carcinoma by downregulating LRP6 post-transcriptionally. FEBS Lett 2014; 588: 1913-20.

[31] Lemieux E, Cagnol S, Beaudry K, Carrier J and Rivard N. Oncogenic KRAS signalling promotes the Wnt/beta-catenin pathway through LRP6 in colorectal cancer. Oncogene 2015; 34: 4914-27.