Decreased expression of miR-133a correlates with poor prognosis in colorectal cancer patients

Li-Li Wang, Lu-Tao Du, Juan Li, Yi-Min Liu, Ai-Lin Qu, Yong-Mei Yang, Xin Zhang, Gui-Xi Zheng, Chuan-Xin Wang

Abstract

AIM: To investigate microRNA-133a (miR-133a) expression in colorectal cancer (CRC) and its relationship with tumorigenesis and disease prognosis.

METHODS: Quantitative real-time polymerase chain reaction was used to measure levels of miR-133a in tumor samples and adjacent non-cancerous tissues from 169 patients undergoing radical resection for CRC. The associations between miR-133a expression and patient age, sex, as well as clinicopathologic parameters, such as tumor size, differentiation, location, invasion depth, metastasis, tumor-node-metastasis (TNM) stage and overall patient survival, were analyzed by Mann-Whitney U and Kruskal-Wallis tests. The Kaplan-Meier method and Cox proportional hazards regression analyses were performed to estimate the prognostic factors for patient survival prediction.

RESULTS: The expression of miR-133a was significantly downregulated in CRC tissues compared with adjacent non-cancerous tissues ($P < 0.05$). This reduction was associated with the depth of the local invasion, poor differentiation, lymph node metastasis and advanced disease ($P < 0.05$). Moreover, Kaplan-Meier analysis demonstrated that patients with low miR-133a expression had poorer overall survival (OS) than those with high miR-133a expression ($P < 0.001$). Univariate analysis revealed statistically significant correlations between OS and miR-133a level, tumor local invasion, lymph node metastasis and TNM stage ($P < 0.001$). Furthermore, miR-133a levels and TNM stage were independently associated with OS (HR = 0.590, 95%CI: 0.350-0.995, $P < 0.05$; and HR = 6.111, 95%CI: 1.029-36.278, $P < 0.05$, respectively).

CONCLUSION: The downregulation of miR-133a may play an important role in the progression of CRC and can be used as an independent factor to determine CRC prognosis.

Core tip: In the present study, the level of microRNA-133a (miR-133a) was found to be downregulated in colorectal cancer (CRC) tissues. The altered expression of miR-133a was significantly associated with malignant behavior, including tumor cell differentiation, local invasion, lymph node metastasis and tumor-node-metastasis stage. Multivariate analysis suggested that low expression of miR-133a is an independent prognostic factor for CRC. Furthermore, the data suggest that miR-133a may play a critical role in CRC progression, and thus may serve as a potential therapeutic target.
Wang LL, Du LT, Li J, Liu YM, Qu AL, Yang YM, Zhang X, Zheng GX, Wang CX. Decreased expression of miR-133a correlates with poor prognosis in colorectal cancer patients. World J Gastroenterol 2014; 20(32): 11340-11346 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i32/11340.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i32.11340

INTRODUCTION
Colorectal cancer (CRC) is the second most common malignancy in females, and third most common in males worldwide, with over 1.2 million new cases and an estimated 608700 deaths in 2008 alone[1]. The five-year survival rate of CRC ranges from 90% for stage I patients to 8% for metastatic cases, and nearly 25% of patients with stage II may relapse or develop metastases[2]. Biomarkers such as carcinoembryonic antigen have been recommended for the prediction of CRC prognosis and postoperative surveillance in advanced disease[3], however, they show a limited sensitivity of only 30%-40% for early diagnosis and prognosis. Therefore, novel and reliable prognostic CRC biomarkers are urgently needed.

MicroRNAs (miRNAs) are a novel class of small endogenous non-coding RNAs (17-28 nucleotides) that are involved in the initiation and progression of tumors by the dysregulation of oncogenes and tumor suppressor genes. A growing amount of evidence demonstrates the valuable role of miRNAs in tumor diagnosis, progression and therapy response, which has led both researchers and clinicians to focus on the identification of novel miRNAs. Ma et al[4] carried out a comprehensive systematic review of miRNA expression in CRC and identified several up- and downregulated miRNAs as candidate biomarkers. Among those identified, the ectopic expression of miR-133a was associated with various human malignancies, including lung squamous cell carcinoma, breast cancer, renal cell carcinoma, prostate cancer and bladder urothelial carcinoma[5-9]. In addition, a correlation between miR-133a expression and the carcinogenesis of CRC has also been reported[10,11]. Recently, Wang et al[12] demonstrated that miR-133a affects CRC cell motility and represses tumor growth and metastasis by targeting Lin11, Isl-1 and Mec-3 and SRC homology 3 protein 1, and inhibiting the mitogen-activated protein kinase (MAPK) pathway. However, another study showed that miR-133a served as a gene promoter for brain metastasis[13]. Thus, further analyses are needed to clarify the role of miR-133a in CRC prognosis based on clinicopathologic stage. In the present study, miR-133a expression levels in CRC were analyzed, and the clinicopathologic significance and potential prognostic value for CRC were assessed.

MATERIALS AND METHODS

Patients and sample collection
A total of 182 CRC patients who underwent radical resection for CRC in the Department of General Surgery, Qilu Hospital of Shandong University between June 2005 and December 2007 were recruited for this study. Of these subjects, seven were excluded because of incomplete follow-up data and six with distant metastases were excluded for the reason of non-statistical significance. The remaining 169 patients had not received preoperative adjuvant therapy and were deemed eligible for the study. All patient data were obtained from clinical and pathologic records, including age, sex, tumor size and depth, lymph node metastasis and distant metastasis. The postoperative pathologic staging of each subject was determined according to the 7th edition of the Union for International Cancer Control tumor-node-metastasis (TNM) staging system for CRC. The resected tumor tissues and paired adjacent non-cancerous tissues (at least 5 cm away from the tumor margin) were immediately collected, frozen in liquid nitrogen and stored at -80 °C. This study was approved by the ethics committee of the Qilu Hospital of Shandong University and written informed consent was obtained from each patient or legal representative.

Follow-up
The patients were followed every 3 to 6 mo after the operation. The clinical end point of this study was death or the end of the study period (January 2013) with a median follow-up period of 63 mo (range: 10-77 mo). Overall survival (OS) was defined as the period from surgery to death. All data including physical examination, laboratory results and computed tomography findings were collected from hospital records or by patient interviews.

Cell culture
The human colon cancer cell line Caco-2 was kindly provided by Shuo Chen (Department of Gastroenterology, Qilu Hospital, Shandong University, China). Cells were cultured in Dulbecco’s modified Eagle’s medium (HyClone, Logan, UT, United States), supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, United States) and maintained at 37 °C with 5% CO2.

RNA preparation and quantitative real-time polymerase chain reaction
Total RNA was isolated from tissue or cells using TRIzol® reagent (Invitrogen, Carlsbad, CA, United States) following the manufacturer’s instructions and the concentration was determined using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, United States). The cDNA was synthesized as follows: (1) 1 μg of isolated RNA was incubated at 65 °C for 5 min with 1 μL specific reverse transcription primer (RiboBio, Guangzhou, China), 1 μL U6 reverse transcription primer, and 1 μL dNTP in a 12 μL total reaction volume; (2) 4 μL of 5 × first-strand buffer, 2 μL of DTT, and 1 μL of RNase inhibitor were added and the reaction was incubated at 37 °C for 2 min; (3) 1 μL of MMLV reverse transcriptase was added and
the final reaction was incubated at 37 °C for 50 min, followed by 70 °C for 5 min. For quantitative real-time polymerase chain reaction (qRT-PCR), the amplification protocol was carried out in the ABI PRISM 7500 Sequence Detection System (Applied Biosystems Inc., Waltham, MA, United States) as follows: initiation at 95 °C for 1 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 45 s, followed by a dissociation protocol. The U6 small nuclear RNA was used as a reference gene to normalize RNA concentrations, and the relative expression of miR-133a in Caco-2 cells was used as a calibrator by the comparative threshold cycle (Ct) method ($2^{-\Delta\Delta C_t}$). Triplicate quantification tests were performed and the average was calculated for each sample.

**Statistical analysis**

All statistical analyses were performed using SPSS 17.0 statistical software (IBM Corporation, Chicago, IL, United States). The median concentrations of miR-133a were compared among different groups using a Mann-Whitney U test or Kruskal-Wallis test. OS curves were calculated using the Kaplan-Meier method and the statistical differences between subgroups were compared by the log-rank test. The Cox proportional hazards regression model was employed for univariate and multivariate analyses to estimate the prognostic factors for survival prediction. Data are expressed as median and interquartile range. $P < 0.05$ was considered statistically significant.

**RESULTS**

**Decreased expression of miR-133a in CRC**

To reveal the role of miR-133a in CRC, qRT-PCR was performed to measure miR-133a levels in 169 pairs of CRC tissues and adjacent non-cancerous tissues. Median miR-133a levels were significantly lower in CRC tissues compared with matched non-cancerous tissues [133.6 (33.7-508.2) vs 804.8 (298.6-1727.5), $P < 0.05$] (Figure 1A). In addition, the miR-133a expression was found to be decreased at least 2-fold compared with adjacent non-cancerous tissue in 55.6% (94/169) of cases (Figure 1B).

**Correlations between miR-133a expression and CRC clinicopathologic characteristics**

The relationship between miR-133a expression and clinicopathologic parameters was evaluated. As shown in Table 1, the level of miR-133a in CRC was strongly correlated with tumor differentiation, local invasion, lymph...
A Cox proportional hazards regression model analysis was performed to determine the independent prognostic indicators for patients with CRC. The results of univariate analyses revealed statistically significant correlations between OS and miR-133a level (HR = 0.385, 95%CI: 0.232-0.638, \( P < 0.001 \)), tumor local invasion (HR = 4.328, 95%CI: 1.780-10.020, \( P < 0.05 \)), lymph node metastasis (HR = 2.416, 95%CI: 1.488-3.923, \( P < 0.001 \)) and TNM stage (HR = 2.336, 95%CI: 1.609-3.393, \( P < 0.001 \)) (Table 2). A multivariate analysis of these factors showed that miR-133a level and TNM stage maintained their significance as independent prognostic factors for OS (HR = 0.590, 95%CI: 0.350-0.995, \( P < 0.05 \); and HR = 6.111, 95%CI: 1.029-36.278, \( P < 0.05 \), respectively).

**DISCUSSION**

There is mounting evidence demonstrating the tissue-specificity and stability of miRNA expression patterns in various tumors, which has provided insights into the molecular mechanisms involved\(^{14}\). Although the precise mechanisms are unknown, many functional studies suggest that miRNA dysregulation is involved in the initiation and progression of cancer\(^{15-17}\). miR-21 and miR-92a, two highly investigated miRNAs that function as promoters for cell proliferation, invasion and metastasis, are significantly overexpressed in CRC\(^{18}\). Recently, the

![Kaplan-Meier curves for overall survival in 169 colorectal cancer patients.](image)

**Figure 2** Kaplan-Meier curves for overall-survival in 169 colorectal cancer patients. A: MiR-133a level; B: Local invasion; C: Lymph node metastasis; D: Tumor-node-metastasis stage (TNM).
Wang LL et al. Downregulation of miR-133a in CRC

**Table 2** Univariate and multivariate analyses for overall survival in colorectal cancer patients

| Variable                        | Univariate analysis | Multivariate analysis |
|---------------------------------|---------------------|-----------------------|
|                                 | HR                  | 95%CI                 | p value | HR            | 95%CI             | p value |
| Age (yr)                        | 1.107               | 0.685-1.789           | 0.679   | 1.196         | 0.424-3.370       | 0.725   |
| Sex (male vs female)            | 1.154               | 0.714-1.863           | 0.559   | 0.238         | 0.033-1.717       | 0.154   |
| Tumor location (rectum vs colon) | 1.006               | 0.623-1.625           | 0.981   | 6.111         | 1.029-36.278      | < 0.05  |
| Tumor size                      | 1.318               | 0.814-2.134           | 0.261   | 0.590         | 0.350-0.995       | < 0.05  |
| Differentiation                 | 0.788               | 0.490-1.268           | 0.326   | 0.385         | 0.232-0.638       | < 0.001 |
| Local invasion (T3-4 vs T1-2)   | 4.328               | 1.780-10.020          | 0.001   | 1.196         | 0.424-3.370       | 0.725   |
| Lymph node metastasis           | 2.416               | 1.488-5.923           | < 0.001 | 0.238         | 0.033-1.717       | 0.154   |
| TNM stage                       | 2.336               | 1.609-3.393           | < 0.001 | 6.111         | 1.029-36.278      | < 0.05  |
| miR-133a expression (high vs low)| 0.385               | 0.232-0.638           | < 0.001 | 0.590         | 0.350-0.995       | < 0.05  |

TNM: Tumor node metastasis; HR: Hazard ratio.

downregulation of other miRNAs, such as miR-126 and miR-218, has also been implicated in CRC. Con- 
dictory data concerning the role of miR-133a in the de- 
velopment and progression of CRC have emerged, with 
evidence that it can behave both as a promoter and a 
suppressor. To reconcile this discrepancy, the expression 
patterns of miR-133a in CRC tissues were examined, 
along with their association to CRC development. 

The expression of miR-133 has been characterized in 
multiple species and found to play roles in the devel- 
opment of different types of malignant tumors. Results 
of this study indicated that miR-133a is significantly 
downregulated in CRC tissues compared with the adja- 
cent normal tissues, which is consistent with previous 
studies showing a reduction in miR-133a in CRC patients compared to healthy controls. Furthermore, the levels of 
miR-133a were significantly lower in tumors with poor 
differentiation, greater depth of local invasion, positive 
lymph node metastasis and advanced disease. Hamara 
et al. reported that miR-133a is homologous to the 
3’-UTRs of iron-related genes and consistently reduced 
in CRC patients in comparison to healthy colon mucosa. 

The present study confirms the repression of miR-133a in CRC tissues, and provides evidence that it can behave both as a promoter and a suppressor. To reconcile this discrepancy, the expression patterns of miR-133a in CRC tissues were examined, along with their association to CRC development.

**Background**

Colorectal cancer (CRC) is one of the most common malignancies worldwide, with over 1.2 million new cases and an estimated 608,700 deaths occurring in 2018 alone. Although recent advances have been achieved in comprehensive therapeutic strategies, CRC outcome remains poor. Biomarkers such as carcinoembryonic antigen have been recommended for CRC prognosis and post-operative surveillance, despite limited sensitivity. Therefore, the identification of novel and reliable biomarkers is urgently needed for CRC prognosis.

**Research frontiers**

There is increasing evidence for microRNA (miRNA) dysregulation in tumor development, which can be utilized as valuable biomarkers. Previous data have indicated that the ectopic expression of miR-133a is associated with various human malignancies. However, one study showed that miR-133a repressed tumor growth and metastasis, while another study indicated that miR-133a serves as a gene promoter for brain metastasis. The present study confirms the repression of miR-133a in CRC tissues, and provides evidence that miR-133a expression can serve as a reliable prognostic indicator for the progression of CRC.

**COMMENTS**

**Background**

Colorectal cancer (CRC) is one of the most common malignancies worldwide, with over 1.2 million new cases and an estimated 608,700 deaths occurring in 2018 alone. Although recent advances have been achieved in comprehensive therapeutic strategies, CRC outcome remains poor. Biomarkers such as carcinoembryonic antigen have been recommended for CRC prognosis and post-operative surveillance, despite limited sensitivity. Therefore, the identification of novel and reliable biomarkers is urgently needed for CRC prognosis.

**Research frontiers**

There is increasing evidence for microRNA (miRNA) dysregulation in tumor development, which can be utilized as valuable biomarkers. Previous data have indicated that the ectopic expression of miR-133a is associated with various human malignancies. However, one study showed that miR-133a repressed tumor growth and metastasis, while another study indicated that miR-133a serves as a gene promoter for brain metastasis. The present study confirms the repression of miR-133a in CRC tissues, and provides evidence that miR-133a expression can serve as a reliable prognostic indicator for the progression of CRC.
In the present study, miR-133a levels were measured in CRC tissues by quantitative real-time PCR, and the clinical significance of miR-133a expression was investigated. The data indicate that the expression of miR-133a was reduced in CRC tissues and was significantly associated with tumor differentiation, local invasion, regional lymph node metastasis and TNM stage. Moreover, CRC patients with low miR-133a expression had poorer overall survival than those with high miR-133a expression. Multivariate analysis suggested that miR-133a is an independent factor for CRC prognosis.

**Applications**

The confirmation of miR-133a dysregulation in CRC and its correlation with disease progression suggest that miR-133a is a potential novel target for therapeutic strategies. Moreover, results of this study may help to develop the use of miR-133a levels as a biomarker to independently predict clinical outcome.

**Terminology**

MicroRNAs are a class of short non-coding ribonucleic acids that have been shown to regulate gene expression. Multiple miRNAs have been implicated in the initiation and progression of tumors through dysregulation of oncogenes and tumor suppressor genes, including miR-133a, which may be involved in the development of different types of malignant tumors. The three known genes of miR-133 include miR-133a-1, miR-133a-2 and miR-133b, located on chromosomes 18, 20 and 6, respectively.

**Peer review**

The authors present a study concerning the clinical implication of miR-133a expression in colorectal cancer. The results demonstrate that miR-133a is reduced in CRC tissues, and low expression is associated with poorer overall survival of CRC patients. These data indicate that miR-133a levels may serve as a useful prognostic biomarker for clinical assessment of patient outcome, and implicate miR-133a as a potential therapeutic target for CRC.

## REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2010; 61: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
2. Jemal A, Siegel R, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2011; 60: 277-300 [PMID: 20610543 DOI: 10.3322/caac.20073]
3. Dufy MJ, Lamerz R, Haglund C, Nicolini A, Kalousová M, Holubec L, Sturgeon C. Tumor markers in colorectal cancer, gastric cancer and gastrointestinal stromal cancers: European group on tumor markers 2014 guidelines update. Int J Cancer 2014; 134: 2513-2522 [PMID: 23852704 DOI: 10.1002/ijc.28384]
4. Ma Y, Zhang P, Yang J, Liu Z, Yang Z, Qin H. Candidate microRNA biomarkers in human colorectal cancer: systematic review profiling studies and experimental validation. Int J Cancer 2012; 130: 2077-2087 [PMID: 21671476 DOI: 10.1002/ijc.26232]
5. Moriya Y, Nohata N, Kinoshita T, Mutallip M, Okamoto T, Yoshida S, Suzuki M, Yoshino I, Seki N. Tumor suppressive microRNA-133a regulates novel molecular networks in lung squamous cell carcinoma. J Hum Genet 2012; 57: 38-45 [PMID: 22089643 DOI: 10.1038/jhg.2011.126]
6. Wu ZS, Wang CQ, Xiang R, Liu X, Ye S, Yang XQ, Zhang GH, Xu XC, Zhu T, Wu Q. Loss of miR-133a expression associated with poor survival of breast cancer and restoration of miR-133a expression inhibited breast cancer cell growth and invasion. BMC Cancer 2012; 12: 51 [PMID: 22292894 DOI: 10.1186/1471-2407-12-51]
7. Kawakami K, Enokida H, Chiyomaru T, Tatarano S, Ho, MR, Tsai KW. Silencing of miR-1 and miR-133a-2 cluster expression by DNA hypermethylation in colorectal cancer. Oncol Rep 2012; 28: 1069-1076 [PMID: 22766605 DOI: 10.3892/or.2012.1899]
8. Song Y, Zhao J, Wu CW, Zhang L, Liu X, Kang W, Leung WW, Zhang N, Chan FK, Sung JJ, Ng SS, Yu J. Tumor suppressor functions of miR-133a in colorectal cancer. Mol Cancer Res 2013; 11: 1051-1060 [PMID: 23723074 DOI: 10.1158/1541-7786.MCR-13-0061]
9. Chen WS, Leung CM, Pan HW, Hu LY, Li SC, Ho MR, Tsai KW. Silencing of miR-1 and miR-133a-2 cluster expression by DNA hypermethylation in colorectal cancer. Oncol Rep 2012; 28: 1069-1076 [PMID: 22766605 DOI: 10.3892/or.2012.1899]
10. Dong Y, Zhong C, Li F, Wang C, Liang Y, Cui W, Li H. Differential miRNA expression profiles in bladder urothelial carcinomas. Asian Pac J Cancer Prev 2010; 11: 905-911 [PMID: 21135599]
11. Wang H, An H, Wang B, Liao Q, Li W, Jin X, Cui S, Zhang Y, Ding Y, Zhao L. miR-133a represses tumour growth and metastasis in colorectal cancer by targeting LIM and SH3 protein 1 and inhibiting the MAPK pathway. Eur J Cancer 2013; 49: 3924-3935 [PMID: 23968734 DOI: 10.1016/j.ejca.2013.07]
12. Li Z, Gu X, Fang Y, Xiang J, Chen Z. microRNA expression profiles in human colorectal cancers with brain metastases. Oncol Lett 2012; 3: 346-350 [PMID: 22740910]
13. Nohata N, Hanazawa T, Enokida H, Seki N. microRNA-1/133a and microRNA-206/133b clusters: dysregulation and functional roles in human cancers. Oncotarget 2012; 3: 9-21 [PMID: 22308266]
14. Nelson KM, Weiss GJ. MicroRNAs and cancer: past, present and potential future. Mol Cancer Ther 2008; 7: 3655-3660 [PMID: 19074842 DOI: 10.1158/1535-7163.MCT-08-0586]
15. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010; 11: 597-600 [PMID: 20661255 DOI: 10.1038/nrg2843]
16. Davis-Dusenbery BN, Hata A. MicroRNA in Cancer: The Involvement of Aberrant MicroRNA Biogenesis Regulatory Pathways. Cancer Gene Ther 2010; 1: 1100-1114 [PMID: 21533017 DOI: 10.1177/1947610109362123]
17. Liu GH, Zhou ZG, Chen R, Wang MJ, Zhou B, Li Y, Sun XF. Serum miR-21 and miR-92a as biomarkers in the diagnosis and prognosis of colorectal cancer. Tumour Biol 2013; 34: 2175-2181 [PMID: 23625654 DOI: 10.1007/s13277-013-0753-8]
18. Yu H, Gao G, Jiang L, Guo L, Lin M, Jiao X, Jia W, Huang J. Decreased expression of miR-218 is associated with poor prognosis in patients with colorectal cancer. Int J Clin Exp Pathol 2013; 6: 2904-2911 [PMID: 24294377]
19. Zhou Y, Feng X, Liu YL, Ye SC, Wang H, Tan WK, Tian T, Qiu YM, Luo HS. Down-regulation of miR-126 is associated with colorectal cancer cells proliferation, migration and invasion by targeting IRS-1 via the AKT and ERK1/2 signaling pathways. PLoS One 2013; 8: e58120 [PMID: 24312276 DOI: 10.1371/journal.pone.0081203]
20. Hamara K, Bielecka-Kowalska A, Przybylowska-Sygut K, Sygut A, Dziki A, Szemraj J. Alterations in expression profile of iron-related genes in colorectal cancer. Mol Biol Rep 2013; 40: 5573-5585 [PMID: 24078156 DOI: 10.1007/s11033-013-2699-3]
Wang LL et al. Downregulation of miR-133a in CRC

24 Uchida Y, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Kawahara K, Nishiyama K, Seki N, Nakagawa M. MiR-133a induces apoptosis through direct regulation of GSTP1 in bladder cancer cell lines. Urol Oncol 2013; 31: 115-123 [PMID: 21396852 DOI: 10.1016/j.urolonc.2010.09.017]

25 Yoshino H, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Nishiyama K, Nohata N, Seki N, Nakagawa M. The tumour-suppressive function of miR-1 and miR-133a targeting TAGLN2 in bladder cancer. Br J Cancer 2011; 104: 808-818 [PMID: 21304530 DOI: 10.1038/bjc.2011.23]

P-Reviewer: Haier J, II Kim T, Seow-Choen F S-Editor: Ma YJ L-Editor: Wang TQ E-Editor: Wang CH
