Oncology Research, Vol. 25, pp. 1207–1214
Printed in the USA.

0965-0407/17 $90.00 + .00
DOI: https://doi.org/10.3727/096504017X14886679715637
E-ISSN 1555-3906
www.cognizantcommunication.com

Long Noncoding RNA CRNDE Promotes Multiple Myeloma Cell Growth by Suppressing miR-451

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It has been determined that long noncoding RNAs (lncRNAs) are identified as a potential regulatory factor in multiple tumors as well as multiple myeloma (MM). However, the role of colorectal neoplasia differentially expressed (CRNDE) in the pathogenesis of MM remains unclear. In this study, we found that the CRNDE expression level, in MM samples and cell lines, is higher than that in the control detected by real-time qPCR, which is also closely related to tumor progression and poor survival in MM patients. Knockdown of CRNDE significantly inhibits the proliferative vitality of MM cells (U266 and RPMI-8226), induces cell cycle arrest in the G_0/G_1 phase, and promotes apoptosis. After being transfected with siRNA, miR-451 expression observably increases. Bioinformatics analysis and luciferase assay reveal the interaction by complementary bonding between CRNDE and miR-451. Pearson's correlation shows that CRNDE is negatively correlated to miR-451 expression in human MM samples. Subsequently, miR-451 inhibitor rescues the inhibited tumorigenesis induced by CRNDE knockdown. Our study illustrates that lncRNA CRNDE induces the proliferation and antiapoptosis capability of MM by acting as a ceRNA or molecular sponge via negatively targeting miR-451, which could act as a novel diagnostic marker and therapeutic target for MM.

Key words: Colorectal neoplasia differentially expressed (CRNDE); miR-451; Long noncoding RNAs (lncRNAs); Multiple myeloma (MM)

INTRODUCTION

Multiple myeloma (MM), a form of hematologic cancer originating from the malignant transformation of plasma cells, is one of the most common blood cancers[1]. It is characterized by various clinical and pathophysiologic heterogeneities and is always accompanied by a very poor prognosis[1]. The 5-year survival rate of MM is about 40%[2]. Because MM is characterized by a very complex pathogenesis, aberrations in molecular expression have become essential regulators of cellular function, such as proliferation, differentiation, and apoptosis[3,4]. The identification of abnormally expressed molecules, including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), has undoubtedly contributed to the diagnosis of MM[5]. Recently, research has confirmed that lncRNA and miRNA could regulate the biological activities of MM cells and play the role of tumor suppressor or oncogene in tumorigenesis and metastasis. It may even have the effect of bidirectional regulation[6]. The exploration of a novel functional molecule could provide a more effective therapeutic target for MM.

lncRNAs are a new sort of noncoding RNA longer than 200 nucleotides without protein-coding capacity[7]. The literature has reported that there are 6,736 lncRNA-coding genes in humans; however, only 127 human lncRNAs have been functionally annotated on the lncRNAdb.org database[8,9]. Many studies have revealed that lncRNAs adjust gene expression through the processes of transcription regulation, posttranscription regulation, genomic imprinting, and chromatin modification[10,11]. Results have suggested that lncRNAs play an essential role in the osteogenic differentiation of bone marrow MSCs, including MEG3 and MALAT1, which regulate the mechanism of promoter-specific transcriptional activation. Colorectal neoplasia differentially expressed (CRNDE), formerly known as the LOC388279 or LOC643911 gene, is localized to the 16q12.2 on the human chromosome. CRNDE has been tested to be upregulated in colorectal carcinomas, gliomas, and other solid tumors, as well as in leukemia and MM.

In our preceding study, we found that lncRNA CRNDE is upregulated in the serum of MM patients. Based on this phenomenon, we assumed that CRNDE is closely related to the pathogenesis of MM. To expand this research, we assessed the corresponding miRNA expression changes in MM cell lines. Our study focuses on the effect of CRNDE

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on pathological progression and the combined effect of lncRNA and miRNAs to biological features.

MATERIALS AND METHODS

Patients and Sample Collection

Seventy-seven MM and 19 healthy control samples were collected from April 2011 to August 2016 at Hong-Hui Hospital, Xi'an Jiaotong University. The study protocol was approved by the ethics committee of Xi'an Jiaotong University. Informed written consent was obtained from all patients and healthy controls.

Cell Culture and Transfection

Human MM cell lines (MM1.S, NCIH929, U266, and RPMI-8226) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, P.R. China). MM cell lines and normal plasma cells (nPCs) were cultured in RPMI-1640 (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin at 37°C with a 5% CO_2 atmosphere. miR-451 inhibitor and siRNAs to CRNDE were purchased from Genechem (Shanghai, P.R. China). The sequences were listed as follows: miR-451 inhibitor, 5′-AGUGACGGACGUGUUGGGCCAU-3′; si-CRNDE-1, 5′-CCATTCCATTCATTTCTCTTTCCTA-3′; si-CRNDE-2, 5′-CCTCTCATTATTCATTCCTTTCCTA-3′. Cells were transfected with the indicated nucleotides or plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions.

Real-Time PCR

Total RNA was extracted from tissues or cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Expression levels of CRNDE and miR-451 were determined by SYBR Premix Ex Taq (Takara, Dalian, P.R. China) according to the manufacturer's instructions, as well as the other operation sequence. The following primers were used: CRNDE, 5′-CTAAGGTGGTCA AGAGTAACAG-3′ (forward) and 5′-AAGACACCATC GACTCTCGTAC-3′ (reverse); miR-451, 5′-GCCGCTA ATA CTGCCTGGTAATG-3′ (forward) and 5′-GTGCA GGGTTCCGAGGT-3′ (reverse); U6, 5′-CGCTAGCAC ATATCGGCTA-3′ (forward) and 5′-TTCTGCGACGAA TTTGTCAT-3′ (reverse). The level of gene expression was expressed relative to β-actin and calculated using the $2^{-\Delta\Delta}$Ct method.

Cell Proliferation Assay

Cell proliferation assay was operated with the cell counting kit-8 (CCK-8; Beyotime, Jiangsu, P.R. China). The transfected cells were seeded into 96-well plates with a density of 2,000 per well. After 48 h, CCK-8 (20 μl) was added into each well and incubated at 37°C for 2 h. The absorbance was detected at 450 nm.

Colony Formation Assay

Cells were plated into six-well plates at a density of 600 per well and cultured for 14 days. The cells were fixed with methanol and stained with 0.2% crystal violet. After being washed mildly with PBS and air dried, the visible colonies consisting of >50 cells were manually counted.

Dual-Luciferase Reporter Assay

The 3′-UTR of CRNDE was amplified using PCR with primers. The amplified PCR products were then cloned into the pMD-18T vector (TaKaRa, Shiga, Japan) and confirmed by DNA sequencing. Afterward, these sequences were released from pMD-18T vectors and subcloned into pGL3 vectors (Promega, Madison, WI, USA) and confirmed with DNA sequencing. The pGL3-CRNDE-3′UTR vectors were cotransfected with control plasmid or miR-451 plasmid into U266 cells using Lipofectamine 2000. Cells were seeded into 24-well plates overnight, and the luciferase activity was measured at 48 h after transfection by Dual-Luciferase Reporter Assay Kit (Promega). Firefly luciferase activity was normalized to that of *Renilla* luciferase.

Cell Cycle Analysis

Cell cycle analysis was operated by Cell Cycle Analysis Kit (Lianke, P.R. China). Cells (4×10^5 per well) were seeded into six-well plates for 24 h and starved in FBS-free medium for 12 h. The transfected cells were then harvested and fixed with 500 μl of 70% cold ethanol for 2 h. After centrifugation at 2,000 rpm for 10 min, DNA staining was carried out with 1 ml of propidium iodide (PI) at room temperature for 30 min. Cell cycle distribution was analyzed by measuring DNA content using flow cytometry, and profiles were generated using flow cytometry with Modifit software (BD Biosciences, San Jose, CA, USA).

Apoptosis Analysis

Apoptotic cells were performed by Annexin-V/Dead Cell Apoptosis Kit (Invitrogen). Briefly, cells (1×10^6 per well) were seeded into six-well plates and resuspended in binding buffer. Cells were starved in FBS-free medium for 12 h before being treated with the indicated agents for 48 h. Cells were resuspended in double staining with annexin V–FITC and PI. Finally, cells were analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with cell quest software (Becton Dickinson).

Statistical Analysis

All experimental data were presented as mean±standard deviation (SD). Differences were analyzed by SPSS 19.0 and GraphPad software with Student's *t*-test or one-way

ANOVA. A value of $p<0.05$ was considered as a significant difference.

RESULTS

Expression of CRNDE Was Upregulated in MM Patients and Cell Lines

In the initial stage of our research project, we detected the relative expression level of CRNDE in MM patients ($n=77$) and normal healthy controls ($n=19$). The expression of CRNDE in MM patients was observably higher than in normal healthy controls (Fig. 1A). We assessed CRNDE expression in MM cell lines, including MM.1S, NCI-H929, U266, and RPMI-8226, and compared them to normal healthy bone marrow-derived plasma cells (nPCs) using quantitative RT-PCR (qRT-PCR). The results showed that the expression of CRNDE in the four cell lines was markedly increased compared with that of nPCs (Fig. 1B). Thus, existing evidence showed that CRNDE expression was upregulated in both tissues and cell lines.

To evaluate the interrelation within CRNDE expression and MM patients' prognosis, we applied Kaplan–Meier analysis and log-rank test. The results showed that the overall survival of patients with a high CRNDE expression level had a poorer prognosis than those with a low expression (Fig. 1C). The above results indicate that the upregulated expression of CRNDE might play an important tumor-promoting role in the development of MM.

Knockdown of CRNDE Inhibited Proliferation and Induced Cell Cycle Arrest and Apoptosis

On the basis of our previous study, we found that lncRNA CRNDE was significantly overexpressed in MM samples and cell lines compared to the control. CRNDE siRNA was transfected into U266 and RPMI-8226 cell lines (Fig. 2A). CCK-8 and colony formation assays showed that CRNDE knockdown observably repressed proliferation and colony formation vitality (Fig. 2B–D). Furthermore, CRNDE knockdown significantly induced cell cycle arrest in the G_0/G_1 phase and promoted apoptosis of U266 and RPMI-8226 cells. Our data convincingly indicate that CRNDE knockdown inhibits the tumorigenicity of MM cells, which also illustrates its potential tumor-promoting effect.

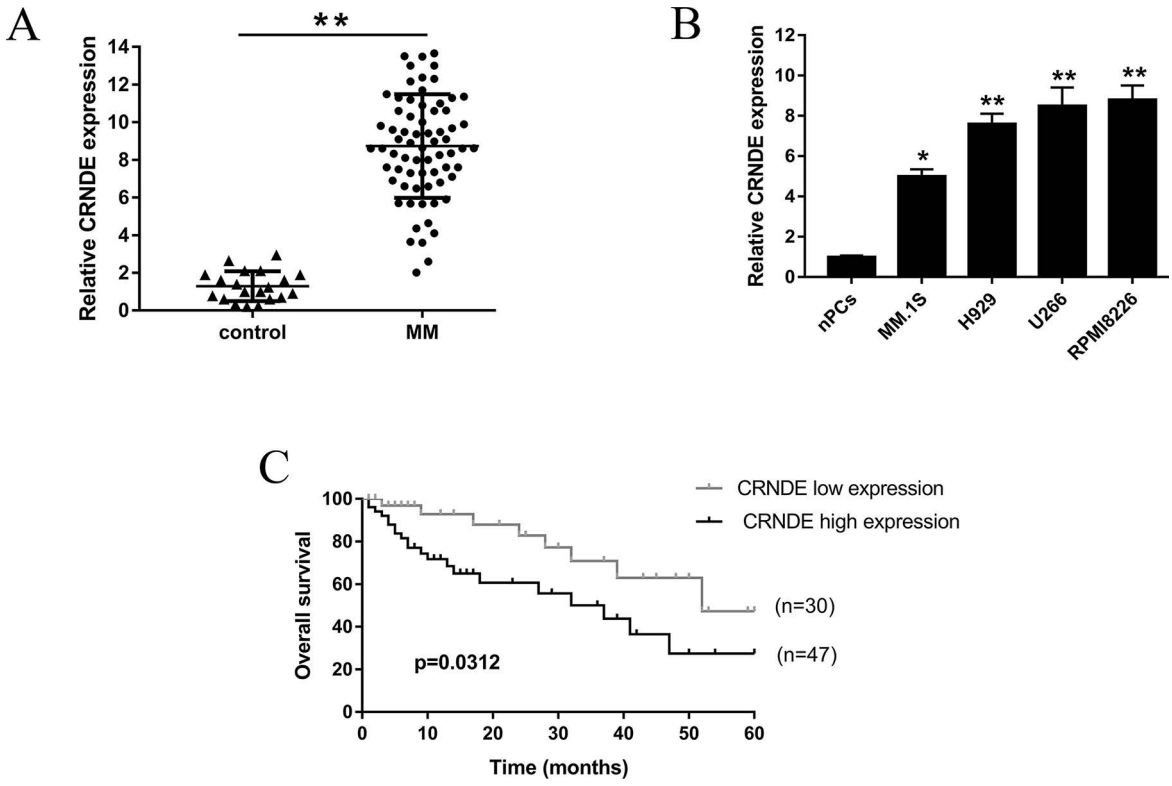

Figure 1. Expression of CRNDE was increased in multiple myeloma (MM) patients and cell lines and predicts a poor prognosis. (A) Relative expressions of colorectal neoplasia differentially expressed (CRNDE) in MM patients and healthy controls were examined using quantitative real-time (qRT)-PCR analysis. Statistical analysis was performed with Student's *t*-test. (B) CRNDE expression was significantly higher in MM cell lines (MM.1S, H929, U266, and RPMI-8226) than in normal, healthy bone marrow-derived plasma cells (nPCs). (C) Difference of overall survival between high and low CRNDE expression demonstrated by Kaplan–Meier analysis. Data are presented as the mean ± standard deviation (SD). *$p<0.05$, **$p<0.01$ compared to the control.

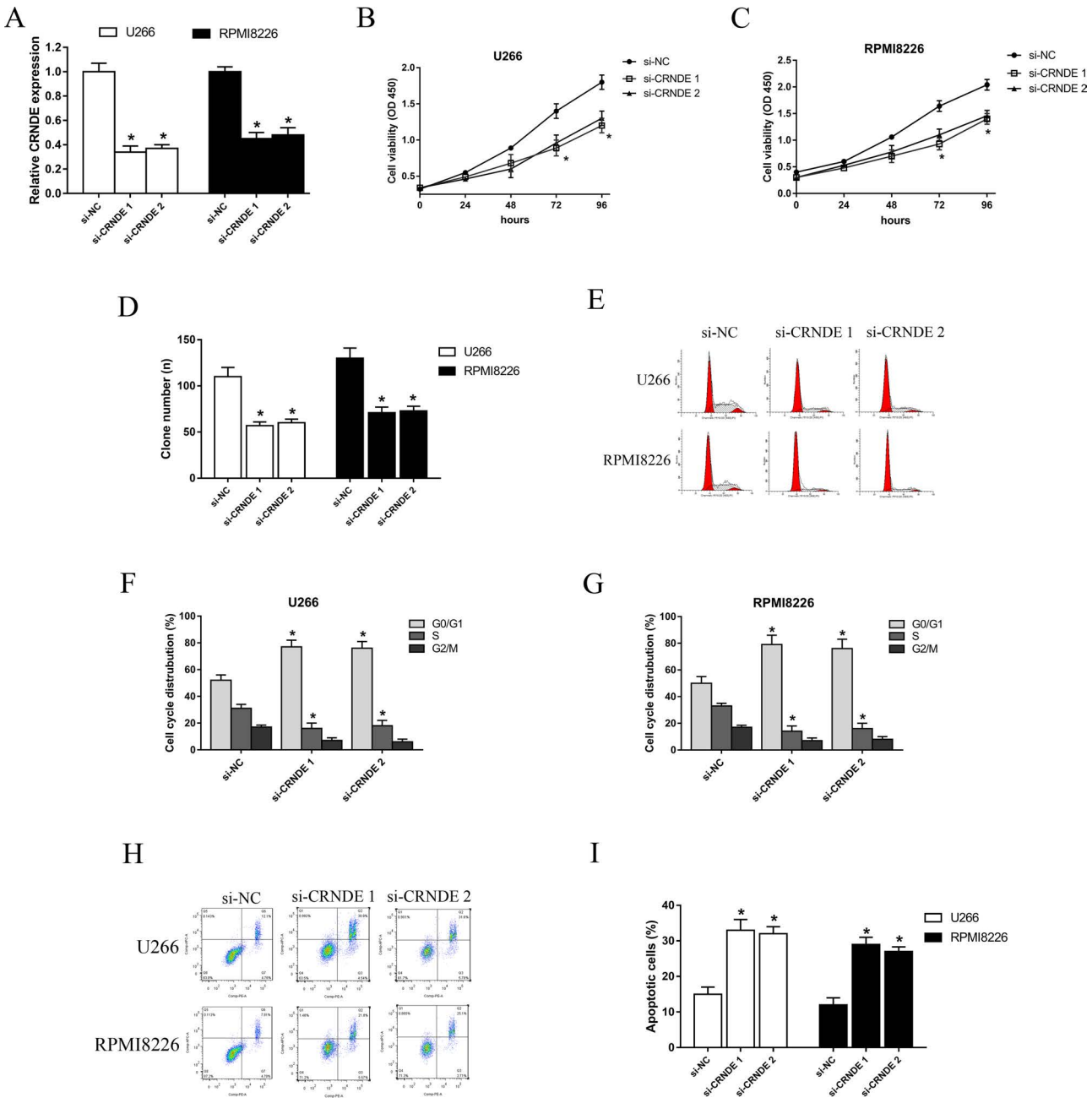

Figure 2. Knockdown of CRNDE inhibited proliferation and induced cell cycle arrest and apoptosis in U266 and RPMI-8226 cells. (A) Knockdown of CRNDE with siRNAs in U266 and RPMI-8226 cell lines. (B, C) Cell viability was determined at the indicated time points after seeding into 96-well plates using the cell counting kit-8 (CCK-8) assay. (D) Clone number of colony formation assay. (E–G) Cell cycle arrest in the G_0/G_1 phase. (H, I) Apoptosis of MM cells detected by flow cytometry. Data are presented as the mean ± SD. $^*p < 0.05$ compared to the control.

CRNDE Acts as Target of miR-451

To investigate the related miRNA in MM cell lines, we transfected si-NC and si-CRNDE into U266 cells and then detected the miRNA expression by real-time PCR. A series of miRNAs that expressed differently in MM were detected by qRT-PCR, and miR-451 expression was markedly increased compared to the others (Fig. 3A). In addition, miR-451 was downregulated in the MM samples (Fig. 3B). Bioinformatics analysis (Starbase v2.0) for the feasible interaction predicted a putative complementary region at the 3′-UTR of CRNDE and miR-451 (Fig. 3C). Afterward, the alignment of complementary binding of miR-451 and CRNDE 3′-UTR was verified by dual-luciferase reporter assay (Fig. 3D). Pearson's correlation showed that CRNDE was

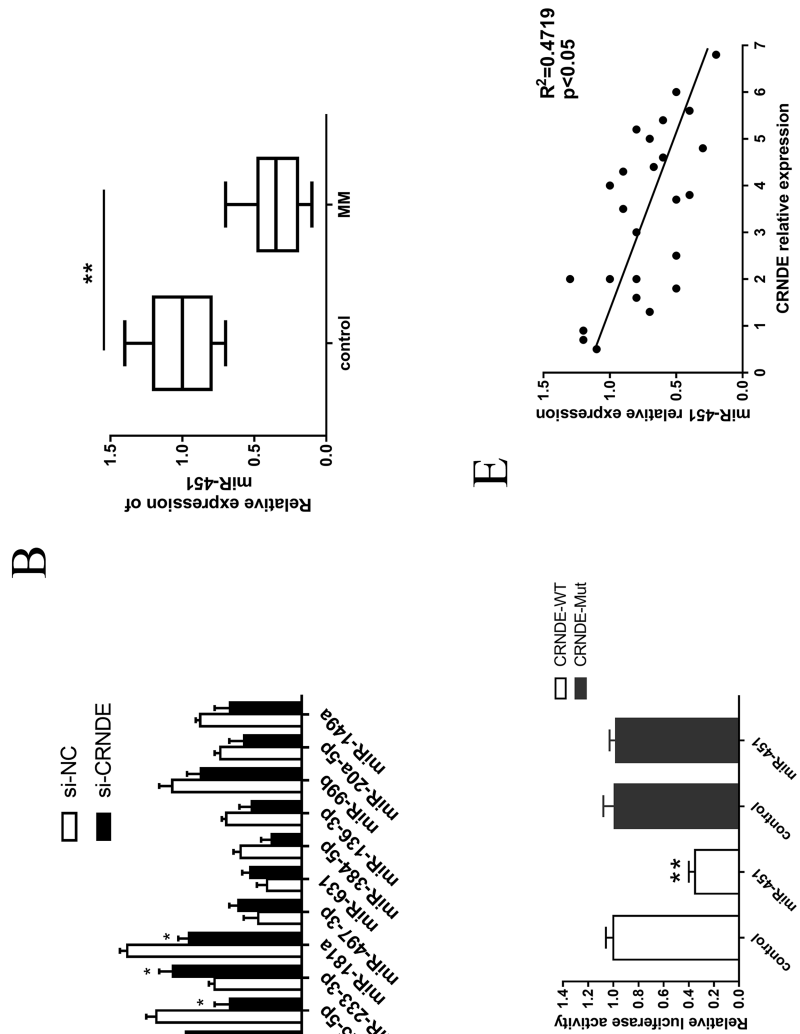

Figure 3. CRNDE acted as a direct target of miR-451. (A) MicroRNA (miRNA) expression in U266 cells transfected with si-NC and si-CRNDE was assessed by qRT-PCR. miR-451 was highly expressed in the si-CRNDE group. (B) miR-451 relative expression was assessed by qRT-PCR in MM patients compared to the normal control. (C) Putative complementary sites within miR-451 and CRNDE 3′-UTR. (D) Dual-luciferase reporter assay shows the putative complementary sites within miR-451 with the 3′-UTR of CRNDE. (E) The correlation between CRNDE and miR-451 expression is shown by Pearson's correlation (R^2=0.4719, p<0.05) in MM samples. All data are expressed as mean±SD. *p<0.05, **p<0.01.

negatively correlated to miR-451 expression in human MM samples. The above results effectively indicate that CRNDE directly targeted miR-451 and negatively mediates it.

miR-451 Inhibitor Rescued the Inhibition of CRNDE Knockdown on Tumorigenesis of MM

Rescue experiments were applied to assess whether CRNDE knockdown mediated the inhibition of tumorigenesis on MM through targeting miR-451. In U244 cells stably cotransfected with si-CRNDE, the miR-451 expression was observably higher than that in the si-NC group, which was reversed by the miR-451 inhibitor (Fig. 4A). The cotransfection of the miR-451 inhibitor rescued inhibition of si-CRNDE on cell proliferation and colony formation (Fig. 4B and C). Similarly, the miR-451 inhibitor cotransfection significantly reversed cell cycle arrest in the G_0/G_1 phase and the promotion of apoptosis induced by si-CRNDE (Fig. 4D–G). The results indicated that CRNDE knockdown exerted tumor inhibition partially through regulating miR-451.

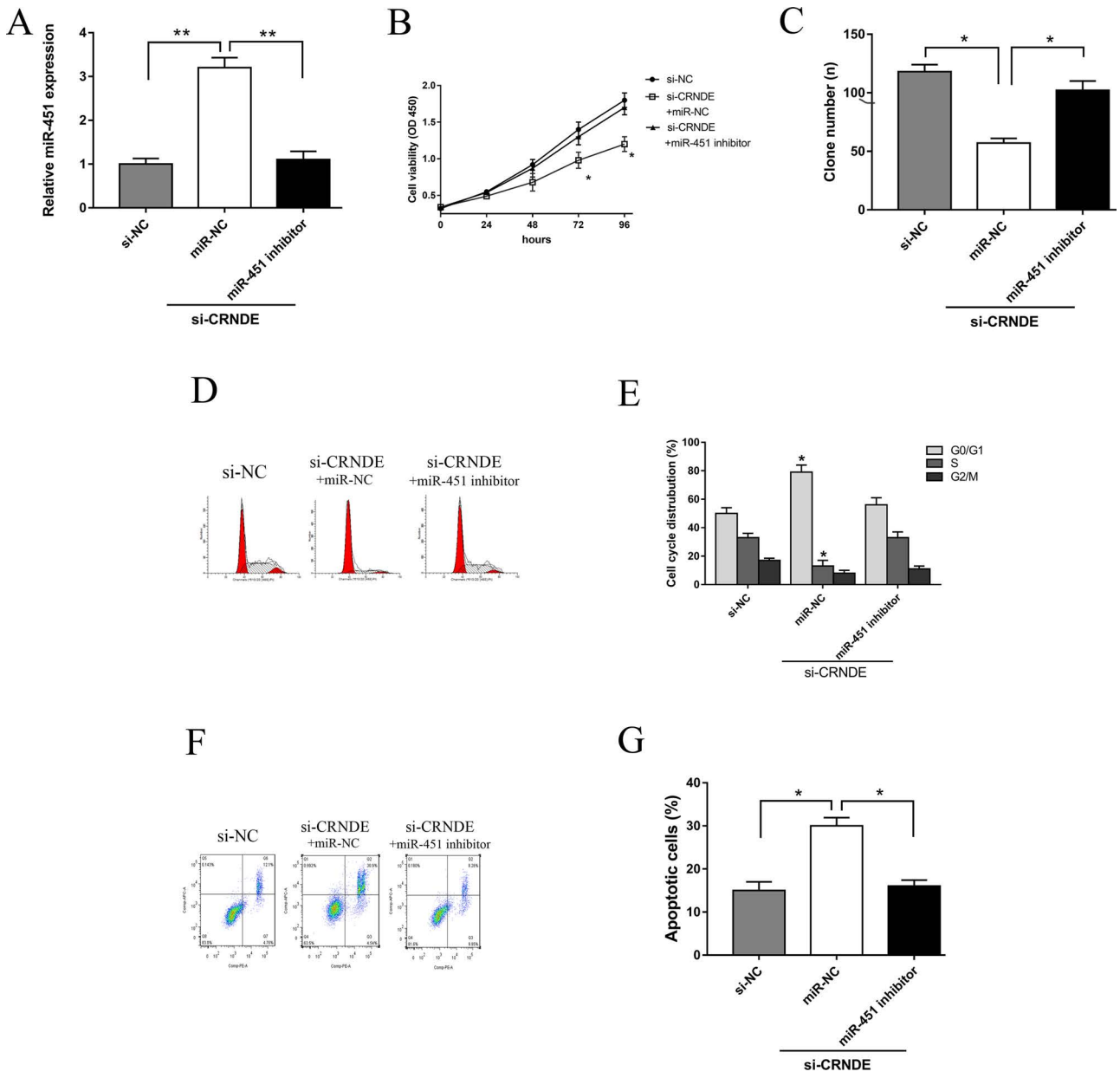

Figure 4. miR-451 inhibitor rescued the inhibition of CRNDE knockdown on tumorigenesis of MM in U266 cells. (A) U266 cells were respectively cotransfected with the miR-451 inhibitor and si-CRNDE. Expression of miR-451 is shown. (B) Cell viability was detected by the CCK-8 assay at the indicated time. (C) Clone number of colony formation assay. (D, E) Cell cycle arrest detected by flow cytometry. (F, G) Apoptosis was detected by flow cytometry. Data are presented as the mean±SD. *$p<0.05$, **$p<0.01$ compared to the control.

DISCUSSION

On the foundation of our existing research results, we observed that the lncRNA CRNDE expression level is closely related to the clinicopathologic feature of MM patients. High expression of CRNDE indicates a poorer prognosis and lower overall survival of patients than those with low expression, which has been verified with Kaplan–Meier analysis and log-rank test. In addition, the CRNDE expression in MM cell lines, including MM.1S, NCI-H929, U266, and RPMI-8226, is markedly higher than in nPCs. Thus, existing evidence indicates that the upregulated expression of CRNDE might play an important tumor-promoting role in the development of MM.

To explore the regulating effect of CRNDE on MM cancer progression, we structured CRNDE knockdown by targeting with siRNA. With the CRNDE being obviously decreased, proliferation is inhibited and apoptosis is increased. In addition, CRNDE knockdown induces cell cycle arrest in the G_0/G_1 phase. lncRNA CRNDE has been identified as a tumor oncogene in glioma, which is closely related to glioma tumor progression and could be an independent prognostic factor[12]. In renal cell carcinoma, CRNDE overexpression promotes cell proliferation via activating the Wnt/β-catenin pathway. CRNDE regulates the RCC cell cycle transition from the G_0/G_1 stage to the S stage and promotes cell proliferation and growth in vitro and in vivo[13]. Therefore, our results powerfully reveal the participation of CRNDE in the tumor physiological metabolism of MM, which is worth further study.

Systematic analysis showed that the lncRNA-participated competing endogenous RNA (ceRNA) regulation network played a more significant role in various types of tumorigenesis, including glioblastoma[14], lung cancer[15], gastric cancer[16], and breast cancer[17]. By means of computational prediction, the targeting relationships within lncRNAs and miRNAs have been continually reported in phymatology. Emerging evidence and studies have revealed that the ceRNA activity of lncRNAs binding to miRNAs functions as a sponge in pathophysiological conditions. lncRNAs together with miRNAs mediate a series of biological and pathological processes, including cell cycle, gene transcription, and posttranscriptional regulation[18]. In addition, the cross-function of lncRNAs and miRNAs has been tested in multiple tumor types[19].

After being transfected with siRNA, various miRNA expression changes were compared to the control group. When CRNDE is downregulated, miR-451 expression observably increases, whereas lower expression is originally downregulated in MM patients. Bioinformatics analysis and luciferase assay reveal the interaction by complementary bonding between CRNDE and miR-451. Pearson's correlation shows that CRNDE is negatively correlated to miR-451 expression in human MM tissues. Rescue experiments show that the miR-451 inhibitor could rescue the proliferation capability, cell cycle arrest in the G_0/G_1 phase, and antiapoptosis function. Results show that CRNDE could bind with miR-451 to function as a molecular sponge in mediating miRNAs. In colorectal cancer, lncRNA CRNDE acts as a competing endogenous RNA to promote metastasis and oxaliplatin resistance by sponging miR-181a-5p and miR-136[20,21]. In addition, CRNDE knockdown decreases hepatoblastoma proliferation viability and its angiogenic effect in vitro, and reduces tumor growth and angiogenesis in vivo via regulating mTOR signaling[22]. In MM side population cells, miR-451 targets tuberous sclerosis 1 gene and activates the PI3K/Akt/mTOR signaling[23]. The above results strongly support our hypothesis that CRNDE plays a significant role in tumor biological regulation through inversely regulating miR-451.

In summary, our study illustrates that lncRNA CRNDE is upregulated in MM and negatively correlated with miR-451. CRNDE induces the proliferation and antiapoptosis capability for MM by acting as a ceRNA or molecular sponge via negatively targeting miR-451, which could serve as a promising diagnostic marker and therapeutic target for MM.

ACKNOWLEDGMENTS: This work was supported by the Basic Medical Research Center of Xi'an Jiaotong University. The authors want to thank Hong-Hui Hospital for funding support and Dr. Li for her technical help. The authors declare no conflicts of interest.

REFERENCES

1. Anderson KC. Progress and paradigms in multiple myeloma. Clin Cancer Res. 2016;22(22):5419–27.
2. Abramson HN. Kinase inhibitors as potential agents in the treatment of multiple myeloma. Oncotarget 2016;7(49): 81926–68.
3. Manasanch EE. Recommend maintenance therapy with lenalidomide in multiple myeloma. Semin Oncol. 2016;43(6): 712–3.
4. Szalat R, Avet-Loiseau H, Munshi NC. Gene expression profiles in myeloma: Ready for the real world? Clin Cancer Res. 2016;22(22):5434–42.
5. Malek E, Kim BG, Driscoll JJ. Identification of long noncoding RNAs deregulated in multiple myeloma cells resistant to proteasome inhibitors. Genes (Basel) 2016;7(10).
6. Zhang L, Cao D, Tang L, Sun C, Hu Y. A panel of circulating miRNAs as diagnostic biomarkers for screening multiple myeloma: A systematic review and meta-analysis. Int J Lab Hematol. 2016;38(6):589–99.
7. Zheng C, Hao H, Chen L, Shao J. Long noncoding RNAs as novel serum biomarkers for the diagnosis of hepatocellular carcinoma: A systematic review and meta-analysis. Clin Transl Oncol. 2017. [E-pub ahead of print]
8. Jia H, Osak M, Bogu GK, Stanton LW, Johnson R, Lipovich L. Genome-wide computational identification and manual annotation of human long noncoding RNA genes. RNA 2010;16(8):1478–87.

9. Amaral PP, Clark MB, Gascoigne DK, Dinger ME, Mattick JS. lncRNAdb: A reference database for long noncoding RNAs. Nucleic Acids Res. 2011;39(Database issue): D146–51.
10. Rajan KS, Ramasamy S, George-William JN, Rajendhran J. Emerging cardiac non-coding landscape: The importance of meta-analysis. Biochimie 2017;133:87–94.
11. Vidal AF, Sandoval GT, Magalhaes L, Santos SE, Ribeirodos-Santos A. Circular RNAs as a new field in gene regulation and their implications in translational research. Epigenomics 2016;8(4):551–62.
12. Jing SY, Lu YY, Yang JK, Deng WY, Zhou Q, Jiao BH. Expression of long non-coding RNA CRNDE in glioma and its correlation with tumor progression and patient survival. Eur Rev Med Pharmacol Sci. 2016;20(19):3992–6.
13. Shao K, Shi T, Yang Y, Wang X, Xu D, Zhou P. Highly expressed lncRNA CRNDE promotes cell proliferation through Wnt/beta-catenin signaling in renal cell carcinoma. Tumour Biol. 2016. [E-pub ahead of print]
14. Zhang K, Li Q, Kang X, Wang Y, Wang S. Identification and functional characterization of lncRNAs acting as ceRNA involved in the malignant progression of glioblastoma multiforme. Oncol Rep. 2016;36(5):2911–25.
15. Sui J, Li YH, Zhang YQ, Li CY, Shen X, Yao WZ, Peng H, Hong WW, Yin LH, Pu YP, Liang GY. Integrated analysis of long non-coding RNA-associated ceRNA network reveals potential lncRNA biomarkers in human lung adenocarcinoma. Int J Oncol. 2016;49(5):2023–36.
16. Liu X, Jiao T, Wang Y, Su W, Tang Z, Han C. Long noncoding RNA GAS5 acts as a molecular sponge to regulate miR-23a in gastric cancer. Minerva Med. 2016. [E-pub ahead of print]
17. Conte F, Fiscon G, Chiara M, Colombo T, Farina L, Paci P. Role of the long non-coding RNA PVT1 in the dysregulation of the ceRNA-ceRNA network in human breast cancer. PLoS One 2017;12(2):e0171661.
18. Li H, Wang W, Zhang L, Lan Q, Wang J, Cao Y, Zhao J. Identification of a long noncoding RNA-associated competing endogenous RNA network in intracranial aneurysm. World Neurosurg. 2017;97:684–92.e4.
19. Zhang Y, Xu Y, Feng L, Li F, Sun Z, Wu T, Shi X, Li J, Li X. Comprehensive characterization of lncRNA-mRNA related ceRNA network across 12 major cancers. Oncotarget 2016; 7(39):64148–67.
20. Gao H, Song X, Kang T, Yan B, Feng L, Gao L, Ai L, Liu X, Yu J, Li H. Long noncoding RNA CRNDE functions as a competing endogenous RNA to promote metastasis and oxaliplatin resistance by sponging miR-136 in colorectal cancer. Onco Targets Ther. 2017;10:205–16.
21. Han P, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, Liu YL, Cui BB. The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/beta-catenin signaling. Mol Cancer 2017;16(1):9.
22. Dong R, Liu XQ, Zhang BB, Liu BH, Zheng S, Dong KR. Long non-coding RNA-CRNDE: A novel regulator of tumor growth and angiogenesis in hepatoblastoma. Oncotarget 2017. [Epub ahead of print]
23. Du J, Liu S, He J, Liu X, Qu Y, Yan W, Fan J, Li R, Xi H, Fu W, Zhang C, Yang J, Hou J. MicroRNA-451 regulates stemness of side population cells via PI3K/Akt/mTOR signaling pathway in multiple myeloma. Oncotarget 2015;6(17):14993–5007.