Hsa_circRNA_000166 facilitated cell growth and limited apoptosis through targeting miR-326 / LASP1 axis in colorectal cancer

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Research

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Abstract

Background: Circular RNAs(circRNAs) belong to non-coding RNAs and widely expressed in a variety of cell species, including cancers. However, the function and mechanism of circRNAs in colorectal cancer (CRC) has not been well investigated. Methods: Microarray data of CRC from Gene Expression Omnibus (GEO) database was used to obtain DEGs. QRT-PCR and western blot assay were performed to determine the mRNA and protein levels of multiple genes, respectively. Cell growth and apoptosis assay were conducted to measure CRC cell proliferation and apoptosis, respectively. Luciferase assay was utilized to confirm the direct interaction between hsa_circRNA_000166 and miR-326. Results: We downloaded and analyzed the circRNA expression profile of CRC from the GEO database and identified 181 differentially expressed circRNAs between 10 pairs of CRC and adjacent normal tissues. Interestingly, we observed that the expression of hsa_circRNA_000166 was the top increased among these circRNAs. Then, we confirmed an upregulation of hsa_circRNA_000166 in CRC tissues and cell lines and observed that higher expression of hsa_circRNA_000166 was associated with poor 5-year survival rate of patients with CRC. Cell growth and apoptosis assay revealed that hsa_circRNA_000166 regulated the cell growth and apoptosis in CRC cell lines. Furthermore, we identified that hsa_circRNA_000166 targeted miR-326/LASP1 pathway using bioinformatic analysis and luciferase reporter assay. Finally, overexpression of miR-326 could sufficiently rescued the aberrant cell growth and apoptosis in CRC cell lines. Conclusion: Taken together, our results indicated that downregulation of hsa_circRNA_000166 inhibited the cell growth and facilitated apoptosis during CRC development by sponging miR-326 / LASP1 pathway.

1. Background

Colorectal cancer (CRC) is one of the malignant cancers with highest incidence and the fourth leading cause of cancer related mortality around the world [1]. Recently, according to changes in dietary patterns and physical activity, CRC has dramatically increased in China [2]. Despite the advanced surgery technologies and medicine treatments have been applicated in CRC treatment, the survival rate of patients with CRC still remains unsatisfactory [3–7]. Hence, the mechanisms underlying CRC progression is urgent calling for new breakthrough.

Circular RNAs (circRNAs), a subgroup of noncoding RNAs, have a crucial role in regulating gene expression and function in distinct biological processes [8–12]. Different with linear RNAs, circRNAs have covalently closed continuous loops, which result in increased stability [13, 14]. Multiple evidences have demonstrated that the expression of circRNAs are aberrant in various cancers(15–19). In general, circRNAs are mainly served as upstream regulator to control the expression of microRNA (miRNA) during tumorgenesis [16, 20–22]. Previous studies have shown that circRNAs have an essential role in CRC progression by the regulation of multiple miRNAs [18, 23, 24]. However, the function of dysregulated circRNAs during the development of CRC remains to be further elucidated.

In our study, we downloaded the circRNA expression profile of CRC from the Gene Expression Omnibus (GEO) database. After analysis, we identified 181 differentially expressed circRNAs and observed a top

overexpression of hsa_circRNA_000166 among them. Subsequently, we revealed the ectopic expression of hsa_circRNA_000166 in CRC tissues and cell lines, which was associated with 5-year survival rate of patients with CRC. Next, we knocked down the expression of hsa_circRNA_000166 using small interfering RNA (siRNA) to explore the potential roles of hsa_circRNA_000166 during CRC progression. We observed that the hsa_circRNA_000166 inhibited the cell proliferation and promoted apoptosis in CRC cell lines. Moreover, we evidenced that hsa_circRNA_000166 directly regulated miR-326/LASP1 pathway and the aberrant cell growth and apoptosis could be rescued after forced expression of miR-326 in CRC cell lines. In summary, our findings revealed that hsa_circRNA_000166 promoted the cell growth and repressed apoptosis via inducing miR-326/LASP1 pathway during CRC tumorgenesis, which might be a promising candidate for diagnostic and therapeutic application in CRC treatment.

2. Materials And Methods

Tissue collection

The CRC tissues and adjacent normal colon tissues were obtained from Inner Mongolia Medical University Affiliated Hospital between 2015 and 2018. Totally, 40 pairs of tissues were analyzed in the study. Patients with CRC didn't experience systemic treatment of chemotherapy or radiotherapy before surgery. All of patients had got the written informed consent. The study followed the ethics committee of Inner Mongolia Medical University Affiliated Hospital guidance. All specimens were stored at − 80℃ until use.

Cell culture

We cultured CRC cell lines SW1116, DLD-1, HCT116, SW480, SW620 and human normal colonic epithelial cells HCoEpiC in minimum essential medium (MEM) (Gibco, 41500034) with 1% Glutamax (Invitrogen, 35050-061), 1% Non-essential Amino Acids, 100X (Invitrogen, 11140-050) and 10% fetal bovine serum (FBS). Humidified atmosphere containing 5% CO2 at 37 °C was performed to incubate the cell lines mentioned above. We purchased the cell lines from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Science (Shanghai, China).

Microarray data sets

Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/), a publicly available genomics database, is queried for all data sets. We downloaded the data set of CRC, which was the circRNA expression profile from GEO. The selected dataset in accordance with the following criteria: (1) They employed CRC tissue samples. (2) They took the adjacent normal tissues as control. (3) They utilized information on technology and platform.

Quantitative real time PCR assay

Total RNAs from tissues or cultured cells were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), following the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR), the reverse transcription

kit (Takara, Dalian, China) was used to reverse transcribed total RNA into cDNA according to the manufacturer's protocol; while a stem-loop RT-qPCR method was used to generate miRNAs. qRT-PCR was conducted in ABI StepOnePlusTM real-time PCR system (Applied Biosystems, Foster City, USA). U6 and GAPDH were applied as internal controls. The gene-specific primers are listed in Table 1.

Table 1
Gene-specific primers used for qRT-PCR

Gene	Primer	Sequence 5' to 3'
hsa_circRNA_000166	Forward	GCTTGGAACAGACTCACGGC
	Reverse	ATCTCCTGCCCAGTCTGACCT
miR-326	Forward	GGCGCCCAGAUAAUGCG
	Reverse	CGTGCAGGGTCCGAGGTC
LASP1	Forward	TGCGGCAAGATCGTGTATCC
	Reverse	GCAGTAGGGCTTCTTCTCGTAG
U6	Forward	TGCGGGTGCTCGCTTCGCAGC
	Reverse	CCAGTGCAGGGTCCGAGGT
GAPDH	Forward	ACACCCACTCCTCCACCTTT
	Reverse	TTACTCCTTGGAGGCCATGT

Plasmid and transfection

The siRNA- negative control (NC), siRNA-1, siRNA-2, miR-NC, miR-326 mimics, miR-326 I, hsa_circRNA_000166 wildtype (WT) and hsa_circRNA_000166 mutant (Mut) were constructed by GenePharma (Shanghai, China). According to the manufacturer's instructions, we transfected the plasmids into HCT116 and SW480 cells using Lipofectamine 2000 Transfection Reagent (Invitrogen).

Cell Counting Kit-8 assay

Cell Counting Kit-8 (CCK-8) assay was used to detect cell growth of HCT116 and SW480 cells. Each group was incubated with a density of 104 cells in 96-well plates. Cells in each well was incubated lasted for 2 hr at 1, 2 and 3 day with CCK-8 reagent (Doindo, Japan). We measured the optical density at 450 nm using an automatic microplate reader (Synergy4; BioTek).

Colony Formation Assay

We seeded the transfected cells into 6-well plates and cultured for 14 days and then fixed the cells with methanol and stained them with 0.5% crystal violet (Beyotime Biotechnology) for 30 min. Colonies with more than 10 cells were counted under a light microscope.

Flow cytometric assay

For apoptosis detection, the HCT116 and SW480 cells were transfected with different plasmids for 24 hours (h) before collection. Then, we used an Annexin V-FITC/PI apoptosis detection kit (Invitrogen) to label the HCT116 and SW480 cells with Annexin V and PI. Flow cytometry (FACScan; BD Biosciences) was used to detect and analyze the fluorescence (FL1) and red fluorescence (FL2).

Target predication

We obtained the sequence of hsa_circRNA_000166 from circbase (http://www.circbase.org). StarBase v2.0 (http://starbase.sysu.edu.cn) and circinteractome (https://circinteractome.nia.nih.gov) was utilized to predict the binding sites between hsa_circRNA_000166 and miRNAs.

Dual luciferase reporter assay

We constructed pGL3-promoter drived miR-326 luciferase reporter with containing the binding site for hsa_circRNA_000166. And then, we used Lipofectamine 2000 (Invitrogen) to transfect the luciferase reporter with hsa_circRNA_000166 WT and hsa_circRNA_000166 Mut into the HCT116 and SW480 cells. The firefly luciferase activity was detected at 48 hr after transfection using Dual Luciferase Reporter Assay system (Promega).

Western blot assay

For protein isolation after transfection, cells were lysed in the RIPA buffer (Beyotime, China). The SDS-PAGE gel assay was utilized to separate the proteins and then we transferred the separated proteins onto nitrocellulose membranes (GE Healthcare). Primary antibodies were used to incubate the membranes overnight at 4℃, following by washing the membranes for 5 times using phosphate buffered saline supplemented with Tween 20 (PBST). Subsequently, the corresponding horseradish peroxidase-conjugated secondary antibodies (Santa Cruz) were used to incubate the membranes for 2hr at room temperature. Finally, the Super Signal West Femto kit (Pierce, Rockford, IL) was utilized to bring the bands on the membranes into visualization in the final. The primary antibodies and secondary antibody were used as following: rabbit anti-LASP1 (1:2000, Abcam, ab117806), rabbit anti-GAPDH (1:5000, Abcam, ab181602) and goat anti-rabbit IgG H&L (HRP) (1:1500, Abcam, ab205718). We used GAPDH as the endogenous control in this assay.

Statistical analysis

For significant difference analysis, GraphPad Prism 5.0 software was used to perform all the data. All results were analyzed using the two-tailed Student t-test and shown as the mean ± SD. *P < 0.05, **P < 0.01, *** P < 0.001.

3. Results

Microarray data information and DEGs analysis in colorectal cancer

We downloaded the circRNA expression microarray dataset GSE126094 associated with colorectal cancer from the Gene Expression Omnibus (GEO) database and normalized (Fig. 1A). Then, we screened the dataset to obtain differentially expressed genes (DEGs) using the limma package (|Log FC|> 1 and FDR < 0.05). Volcano plots showed the differential expression of multiple circRNAs from the microarray dataset (Fig. 1B). We obtained 181 DEGs from GSE126094 dataset, including 74 upregulated circRNAs and 107 downregulated circRNAs. R-heatmap software was used to draw a heatmap of the top 8 upregulated circRNAs (Fig. 1C). We found the expression of hsa_circRNA_000166 was the top 1 among upregulated circRNAs (Fig. 1C), which suggested that hsa_circRNA_000166 might play an vital role during CRC progression.

Upregulation of hsa_circRNA_000166 in CRC tissues and cell lines

To figure out the potential function of hsa_circRNA_000166 during CRC development, we firstly detected the expression level of hsa_circRNA_000166 in CRC tissues and adjacent normal colonic tissues. And we observed the overexpression of hsa_circRNA_000166 in CRC tissues compared with the matched normal tissues using qRT-PCR assay (Fig. 2A). Then, we divided the patients into two groups based on higher or lower hsa_circRNA_000166 expression in CRC tissues. Kaplan-Meier survival curve displayed that patients with higher hsa_circRNA_000166 expression had a poor 5-year survival rate rather than the patients with lower hsa_circRNA_000166 expression (Fig. 2B). Moreover, we also tested the expression of hsa_circRNA_000166 in CRC cells, such as SW1116, DLD-1, HCT116, SW480, SW620 and human normal colonic epithelial cells HCoEpiC. Consistent with that in CRC tissues, we found the transcriptional level of hsa_circRNA_000166 was significantly increased in CRC cell lines compared to normal colonic cells (Fig. 2C). Therefore, we verified the ectopic expression of hsa_circRNA_000166 in both CRC tissues and cell lines, which related to poor 5-year survival rate of CRC patients.

Downregulation of hsa_circRNA_000166 suppressed cell growth and promoted apoptosis in CRC cells

According to the overexpression of hsa_circRNA_000166 in CRC cells, we knocked down the transcriptional level of hsa_circRNA_000166 using small interfering RNA (siRNA) to study the role of hsa_circRNA_000166 during CRC tumorgenesis. After transfection with siRNAs, we observed that the expression of hsa_circRNA_000166 was obviously decreased in siRNA-1 treated CRC cells and no significant changes in siRNA-2 treated CRC cells compared with controls by qRT-PCR analysis (Fig. 3A), which certified that siRNA-1 could sufficiently knock down the transcriptional level of hsa_circRNA_000166. Subsequently, to demonstrate the function of hsa_circRNA_000166 in CRC cell growth, CCK-8 assay was performed in HCT116 and SW480 cell lines after siRNA-1 transfection. We observed the cell proliferation of CRC was limited in siRNA-1 treated groups compared with the controls in two CRC cell lines (Fig. 3B). Meanwhile, we also conducted the clone formation assay in HCT116 and SW480 cell lines and revealed a notably decrease of colony number in siRNA treated groups compared to that in the siRNA-NC groups in the two CRC cell lines (Fig. 3C). Furthermore, to inspect the role of hsa_circRNA_000166 in CRC apoptosis, flow cytometry was utilized to calculate the apoptotic cells in

both HCT116 and SW480 cell lines, and found that downregulation of hsa_circRNA_000166 resulted in the apoptosis rate was dramatically elevated in siRNA-1 treated groups rather than in controls (Fig. 3D). In brief, our findings manifested downregulation of hsa_circRNA_000166 could repressed cell growth and enhanced apoptosis in CRC cells.

Hsa_circRNA_000166 regulated CRC progression by inducing miR-326/LASP1 axis

To elucidate the mechanism of hsa_circRNA_000166 in controlling CRC cell proliferation and apoptosis, we predicted that miR-326 was the candidate target of hsa_circRNA_000166 using the target prediction tool. Firstly, the venn analysis between the predicted target miRNAs of hsa_circRNA_000166 and differential expressed miRNAs in CRC cells indicated that 3 miRNAs, containing miR-326, were involved in CRC using starBase and circinteractome (Fig. 4A). To certify whether miR-326 was a putative downstream target of hsa_circRNA_000166, we constructed miR-326 with binding site of hsa_circRNA_000166 into pGL3 vector with luciferase reporter gene (Fig. 4B). Also, we detected the transcriptional level of miR-326 in CRC tissues, and found its downregulation in CRC tissues compared with that in matched normal tissues (Fig. 4C). Similarly, the expression of miR-326 was decreased in CRC cell lines compared to that in the normal cell line HCoEpiC (Fig. 4D). After transfection with siRNA-1 or siRNA-NC, we observed the transcriptional level of miR-326 was obviously upregulated in both HCT116 and SW480 cells (Fig. 4E). Then, the luciferase reporter plasmid was separately co-transfected with hsa_circRNA_000166 WT and hsa_circRNA_000166 Mut into HCT116 cells. Luciferase assay showed the relative luciferase activity in cells co-transfected with miR-326 luciferase reporter plasmid and hsa_circRNA_000166 WT was significantly decreased about 50% compared with the controls; while cells were co-transfected with miR-326 luciferase reporter plasmid and hsa_circRNA_000166 Mut, the relative luciferase activity was no obviously changes compared with the controls in HCT116 cells (Fig. 4F). Recent studies reported that miR-326 could directly controlled the expression of LIM and SH3 protein 1 (LASP1) to suppress cell proliferation and activat apoptosis in hepatocellular carcinoma (HCC)(25). Thus, we measured the transcriptional and translational levels of LASP1 after transfection with siRNA-1, and found a downregulation of LASP1 in both HCT116 and SW480 cells compared with the control groups using qRT-PCR and western blot assay (Fig. 4G, H). The outcomes intensively indicated hsa_circRNA_000166 might participate in CRC progression through targeting miR-326/LASP1 pathway.

Inhibition of miR-326 rescued the phenotype dominated by hsa_circRNA_000166

To further confirm that miR-326 mediated the function of hsa_circRNA_000166 during CRC development, we transfected cells with siRNA-1 and miR-326 I and inspected the role of miR-326 in the regulation of hsa_circRNA_000166 in CRC development. CCK-8 and clone formation assay demonstrated that miR-326 downregulation in siRNA-1 transfected cells could restore cell growth compared with the controls in HCT116 cells (Fig. 5A, B). Correspondingly, cells co-transfected with miR-326 I and siRNA-1 resulted in the number of apoptotic cells was significantly decreased compared with siRNA-1 transfected cells (Fig. 5C). In conclusion, our studies identified hsa_circRNA_000166 was overexpressed in CRC cells and evidenced the potential function of hsa_circRNA_000166 in regulating the cell growth and apoptosis in CRC cells

through directly interacting with miR326/LASP1 axis. Therefore, hsa_circRNA_000166 might be a promising target in diagnostic and therapeutic application of CRC patient treatment.

4. Discussion

Colorectal cancer (CRC) is one of the solid tumors with a higher mortality among cancer-related deaths worldwide. Though the advanced surgery technologies and medicine treatments have been applicated in treating patients with CRC, the survival rate of patients with CRC still poor. Therefore, there is an urgent demand for understanding the mechanisms underlying the development of CRC. In past decades, circRNAs are discovered to be a subgroup of noncoding RNAs and play an essential role in regulating gene expression and function associated with cancers [15–19]. In this study, we identified hsa_circRNA_000166 was one of the upregulated circRNAs with the highest expression level among all the upregulated circRNAs using bioinformatics analysis. Then, qRT-PCR assay was conducted to measure the expression of hsa_circRNA_000166 in CRC tissues and cell lines. We found the transcriptional level of hsa_circRNA_000166 was notably increased, which highly correlated with poor 5-year survival rate of CRC patients. Next, we inhibited the expression of hsa_circRNA_000166 to perform further investigation. After downregulation of hsa_circRNA_000166, we observed the cell growth and colony formation were limited and the cell apoptosis was activated using corresponding assay. These data strongly demonstrated that hsa_circRNA_000166 played an important role during CRC tumorgenesis.

Generally, circRNAs are sponging miRNAs to play its function in multiple biological processes, including tumorgenesis [20–22]. Previous studies have proved that multiple miRNAs mediated the function of circRNAs in CRC progression [23, 24]. Here, combined with bioinformatics analysis, we predicted that miR-326 might be a candidate target of hsa_circRNA_000166. Subsequently, luciferase assay was performed to confirm the directly interaction between hsa_circRNA_000166 and miR-326. Also, we measured the downregulation of miR-326 in CRC tissues and cell lines compared with the matched controls. Moreover, we revealed the LASP1, a downstream effector of miR-326 [25], was downregulated after siRNA-1 transfection comparted to the controls, which suggested miR-326/LASP1 pathway was involved in the regulation of hsa_circRNA_000166 during CRC progression. In the final, we verified that downregulation of miR-326 could compromised the phenotype in siRNA-1 treated groups. Taken together, our findings evidenced that hsa_circRNA_000166 activated the cell growth and repressed apoptosis by sponging miR-326/LASP1 axis during CRC tumorgenesis, which might benefit for diagnostic and therapeutic application in CRC treatment.

5. Conclusions

In this study, we used bioinformatics analysis to screen GSE126094 dataset in CRC, and identified hsa_circRNA_000166 was the top 1 among all upregulated circRNA. We further confirmed the overexpressions of hsa_circRNA_000166 in CRC tissues and cell lines, and found 5-year survival rate of CRC patients was highly related to the expression levels of hsa_circRNA_000166. Importantly, we confirmed the miR-326/LASP1 pathway mediated the regulation of hsa_circRNA_000166 during CRC

progression. Together, our findings manifested hsa_circRNA_000166 had a vital role in regulating CRC tumorgenesis, which implied hsa_circRNA_000166 had a promising value in early diagnosis and prevention of CRC.

Declarations

Acknowledgements

Not applicable.

Author Contributions: Conceptualization, Q.H. and ZT.Zhang.; Methodology and data analysis, Q.H. and ZT.Zhang.; Data interpretation and manuscript drafting, Q.H.; Manuscript reviewand editing, Q.H.; Funding acquisition, ZT.Zhang. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The data that support the fndings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was performed with the approval by the Ethics Committee of Inner Mongolia People's Hospital.

Consent for publication

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Abbreviations

CRC: Colorectal cancer; GEO: Gene Expression Omnibus; CircRNAs: Circular RNAs; qRT-PCR: Quantitative real-time PCR; miRNA: MicroRNA; GEO: Gene Expression Omnibus; siRNA: Small interfering RNA; MEM: Minimum essential medium; FBS: Fetal bovine serum; NC: Negative control; WT: Wildtype; CCK-8: Cell Counting Kit-8.

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Figures

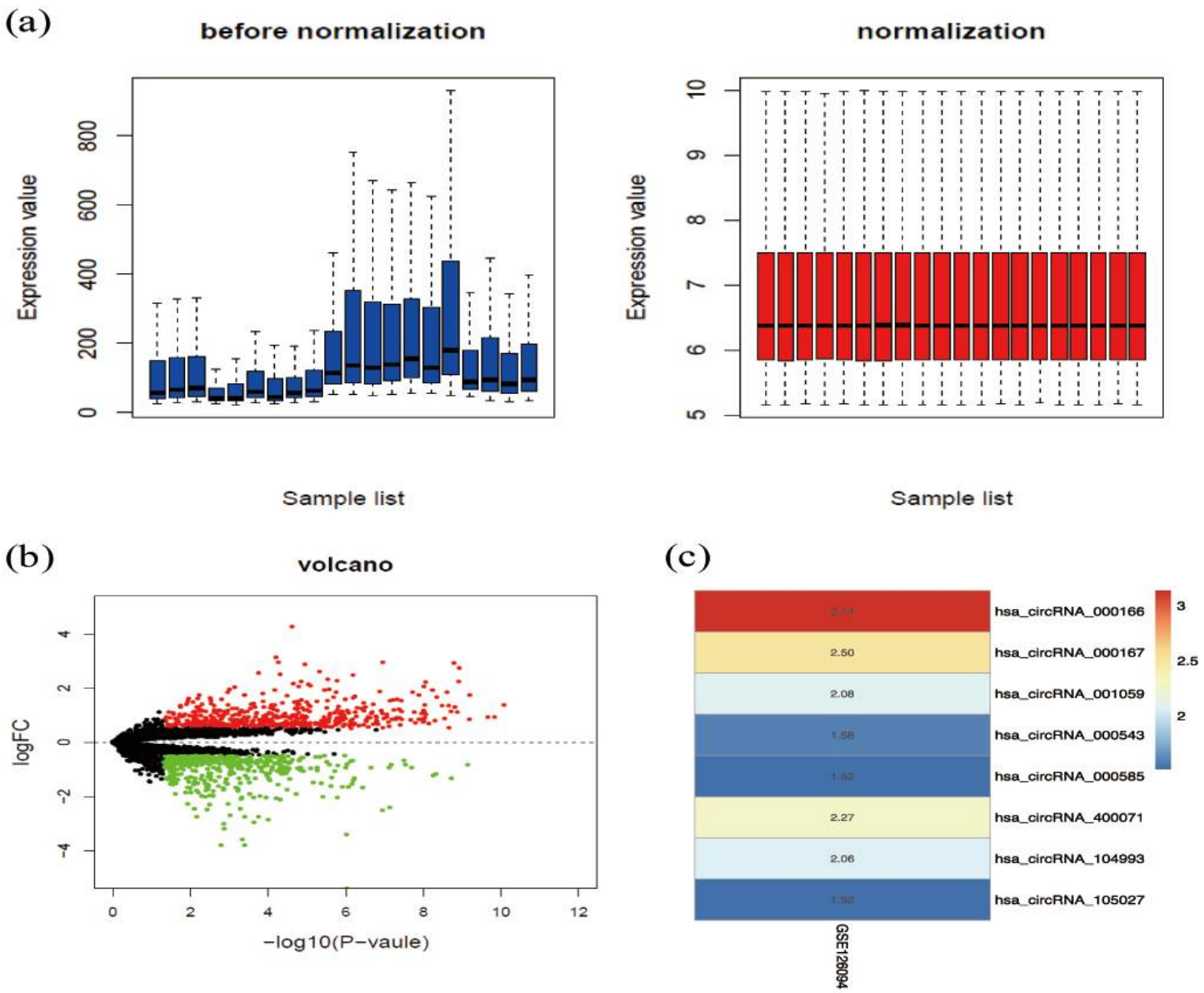

Figure 1

Microarray data information and DEGs analysis in colorectal cancer. (a) The standardization of GSE126094 data. The data before normalization were displayed as the blue bar, while the normalized data were shown as the red bar. (b) The volcano plots of GSE126094 data. The red and green points respectively represented upregulated and downregulated genes screened on the basis of |fold change(FC)|>2.0 and a corrected P-value < 0.05. Genes with no significant difference were shown as the black points. (c) The abscissa was defined as GEO ID, and the ordinate was defined as the gene name. The values in the box represent the logFC values.

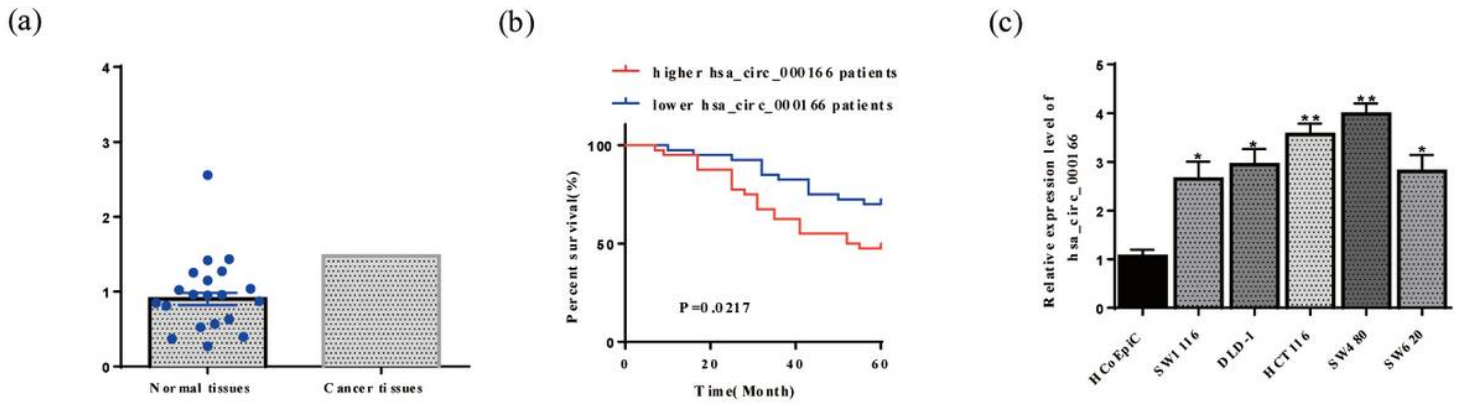

Figure 2

Hsa_circRNA_000166 was upregulated in CRC tissues and cell lines. (a) qRT-PCR assay showed the expression of hsa_circRNA_000166 was increased in CRC tissues (n=40) compared with adjacent normal lung tissues (n=40). (b) Kaplan–Meier curve displayed 5-year survival rate of CRC patients with different hsa_circRNA_000166 expression levels (n=40, p=0.0217). (c) qRT-PCR assay showed an upregulation of hsa_circRNA_000166 in CRC cell lines compared with normal colonic cells.

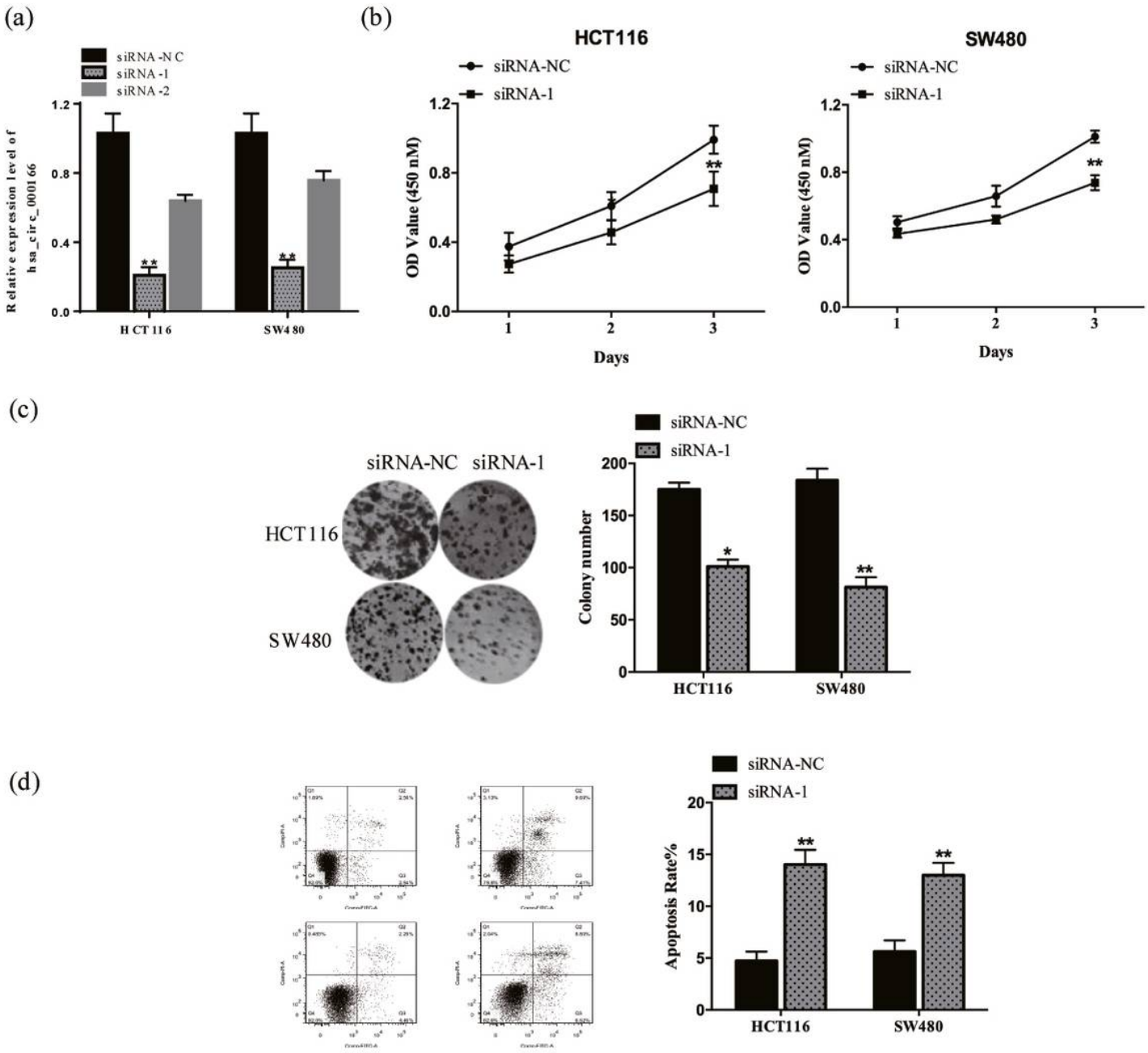

Figure 3

Decreased hsa_circRNA_000166 expression affected cell growth and apoptosis in CRC cells. (a) The transcriptional level of hsa_circRNA_000166 was measured by qRT-PCR in both HCT116 and SW480 cells after transfected with siRNA-1 or siRNA-NC. (b) Cell proliferation assay showed decreased hsa_circRNA_000166 expression limited the growth of HCT116 and SW480 cells after siRNA-1 transfection compared with the controls. (c) Clone formation assay demonstrated the number of colonies was significantly decreased after siRNA-1 transfection compared with the controls in HCT116 and SW480 cells. (d) Flow cytometric analysis displayed the number of apoptotic cells was noticeably increased in siRNA-1 transfected groups compared with the controls.

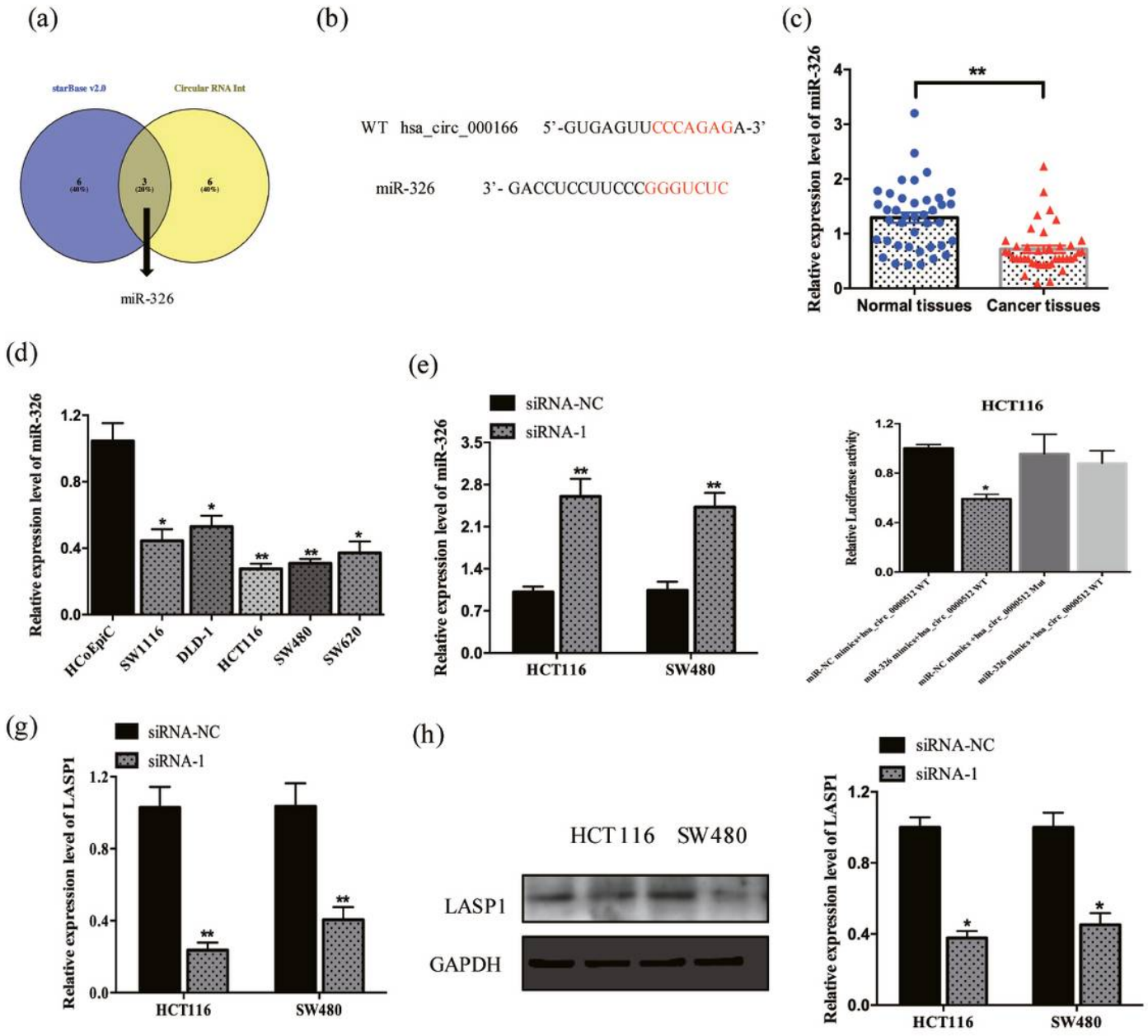

Figure 4

Hsa_circRNA_000166 regulated CRC progression by targeting miR-326/LASP1 axis. (a) The venn analysis implied that 3 miRNAs, including miR-326, were involved in CRC. The blue represented 9 predicted miRNAs analyzed by starBase v2.0, and the yellow represented 9 predicted miRNAs analyzed by circinteractome. (b) Binding site of hsa_circRNA_000166 in miR-326 was predicted by starBase. (c) qRT-PCR analysis showed the transcriptional level of miR-326 was dramatically decreased in CRC tissues (n=40) compared to the matched normal tissues (n=40). (d) qRT-PCR analysis showed miR-326 was notably downregulated in CRC cells compared with the normal colonic cells. (e) qRT-PCR assay displayed the miR-326 was significantly upregulated in siRNA-1 transfected groups compared with the controls in both HCT116 and SW480 cells. (f) Luciferase reporter assay indicated WT hsa_circRNA_000166 dramatically repressed the miR-326 luciferase activity but not Mut hsa_circRNA_000166 in HCT116 cells. (g) and (h), qRT-PCR and western blot assay showed the transcriptional and translational levels of LASP1

were obviously downregulated in siRNA-1 transfected groups compared with the controls in both HCT116 and SW480 cells.

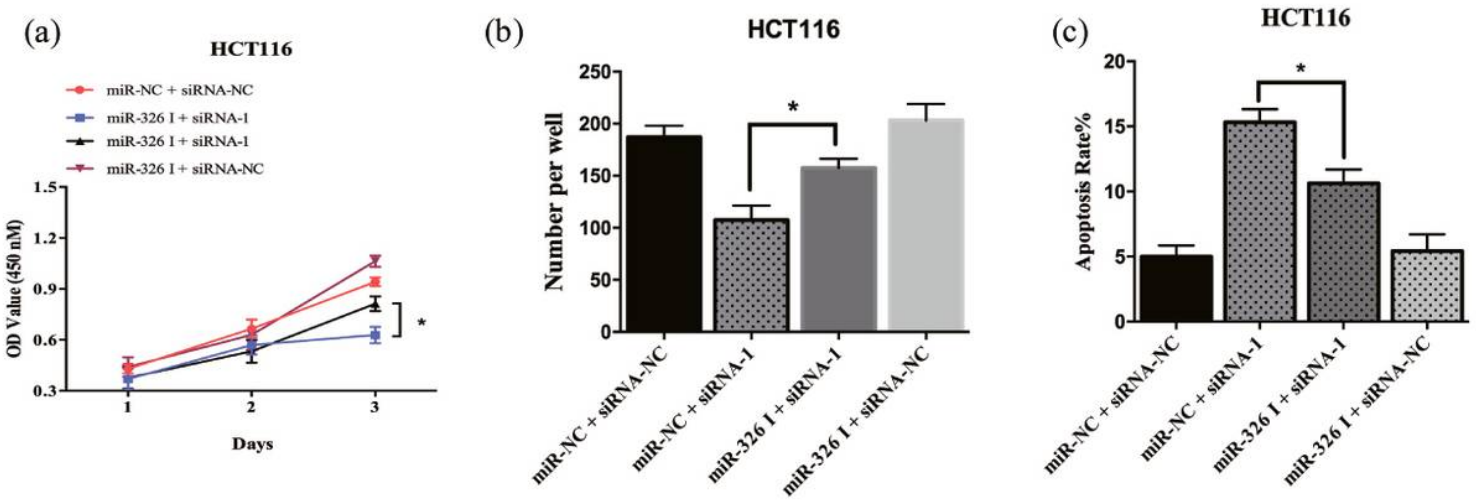

Figure 5

miR-326 downregulated rescued the phenotype dominated by hsa_circRNA_000166. (a) and (b), CCK-8 and clone formation assay demonstrated cells co-transfected with siRNA-1 and miR-326 I promoted cell growth compared with siRNA-1 and miR-NC co-transfected groups in HCT116 cells. (c) The number of apoptotic cells in siRNA-1 and miR-326 I co-transfected groups was less than the number in siRNA-1 and miR-NC co-transfected groups transfected groups in HCT116 cells.