MiR-128-1-5p inhibits cell proliferation and induces cell apoptosis via targeting PRKCQ in colorectal cancer

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Research Article

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Abstract

Background

MicroRNAs (miRNAs) are a type of highly conserved and endogenous noncoding RNA which can regulate various cellular processes, including cell proliferation, differentiation, apoptosis, migration and metastasis. Aberrant expression of miR-128-5p, has been shown to be a key regulator in tumorigenesis and cancer development. But the role and the underlying molecular mechanisms of miR-128 in CRC still remain largely unknown.

Methods

The expression and clinical significances of miR-128-1-5p in colorectal cancer tissues and cell lines was quantified by qRT-PCR. Stably transfected cell lines were established by infecting SW620 and LOVO cells with lentiviruses. The functions of miR-128-1-5p were measured by CCK-8 assay, Annexin V- DY-634 PI Apoptosis Staining / Detection, and Xenograft model. Western blotting, immunohistochemistry (IHC), luciferase reporter assays and Bioinformatic Analysis were also used in this study.

Results

MiR-128-1-5p was downregulated in CRC and was associated with optimal survival in CRC. Overexpression of miR-128-1-5p inhibited cell proliferation and induced cell apoptosis. Protein tyrosine kinase C theta isoform (PRKCQ) was a direct target of miR-128-1-5p and was involved in miR-128-1-5p-mediated proliferation and apoptosis.

Conclusions

These results provide novel insight into the role of miR-128-1-5p in CRC. Overexpression of miR-128-1-5p was associated with cell proliferation and apoptosis in CRC. Knockdown of miR-128-1-5p reduced CRC growth by modulating PRKCQ expression and is a possible new therapeutic target for patients with CRC.

Background

Colorectal cancer (CRC) is one of the most frequently diagnosed malignancies in the world[1]. The incidence of this disease is increasing, with about 1.8 million new cases and more than 880,000 deaths from CRC in 2018 [2]. Despite advances have made in current therapies, including surgical resection and adjuvant chemotherapy, the recurrence and metastasis of CRC are still two of the obstacles affecting its therapeutic effect [3, 4]. Therefore, it is urgent to explore the underlying molecular pathological mechanisms in CRC progression, and it may provide a new therapeutic strategy for improving the clinical outcome of patients with CRC.

MicroRNAs (miRNAs) are a type of highly conserved and endogenous noncoding RNA, with 17–25 nucleotides long in size, which can regulate various cellular processes, including cell proliferation, differentiation, apoptosis, migration and metastasis[5–7]. Growing evidences support that miRNAs have played crucial roles in progression of various cancers [6, 8]. Aberrant expression of miRNAs is frequently found in human cancers and associated with uncontrolled proliferation and metastasis [9–11]. It was reported that miR-128 exhibited both oncogenic and tumor-suppressive roles in different cancer types. For example, miR-128 expression was remarkably down-regulated in thyroid cancer and acted as a tumor suppressor to inhibit the growth of thyroid cancer by negatively regulating sphingosine kinase-1 (SPHK1) [12]. Low expression of miR-128 was correlated with shorter overall and disease-free survival in triple-negative breast cancer (TBNC) and miR-128 could inhibit glucose metabolism, mitochondrial respiration and proliferation of TNBC cells by targeting glucose metabolism, mitochondrial respiration and proliferation of TNBC cells [13]. However, high level of microRNA-128-3p (miR-128-3p) was found in non-small cell lung cancer[14]. Mechanistically investigations suggested that miR-128 directed cancer stem cells (CSCs) and epithelial mesenchymal transition (EMT) programming by activating β-catenin and TGF-β signaling [14]. Recently, miR-128-3p was reported to increase the sensitivity of CRC cells to oxaliplatin and could act as a clinical biomarker for oxaliplatin response [15]. Therefore, it is of great significance to explore what exact role of miR-128-1-5p plays and the relative mechanisms in CRC.

In this study, we explored the clinical significance and biological functions of miR-128-1-5p in CRC. Further experiments demonstrated that overexpression of miR-128-1-5p could inhibit CRC cell proliferation and facilitate CRC cell apoptosis by targeting PRKCQ. This finding might provide potential candidate for CRC therapies and highlight potential clinical applications of miR-128-1-5p in CRC.

Materials And Methods

Bioinformatic Analysis

CancerMIRNome (http://bioinfo.jialab-ucr.org/CancerMIRNome/) is a comprehensive database for facilitating the use of publicly available cancer miRNome data to assist in miRNA research in various cancers[16]. We focused the different expression between tumor and normal samples, ROC analysis, and KM survival analysis for miR-128-1-5p in COAD and READ by this database. Expression of miR-128-1-5p in serum in both healthy and different cancer types also explored in CancerMIRNome.

Human tissue samples

All human CRC and their adjacent normal tissues were obtained from patients who received surgery in the Department of Colorectal Surgery between January 2006 and October 2012. All patients did not receive anticancer treatments before operation and written informed consent was obtained from all patients enrolled in this study. The study protocol was reviewed and approved by the Institutional Human Experiment and Ethic Committee of Fudan University Cancer Hospital. After collection, the fresh tissues were immediately frozen in liquid nitrogen for RNA isolation.

Cell lines and cell culture

The human CRC cell lines (HT29, RKO, SW480, SW620 and LOVO) and the normal human intestinal epithelial cell line (HIEC) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured as described previously [17].

RNA extraction and Real-time PCR analysis

Total RNA was extracted using Trizol reagent (Invitrogen, Thermo Fisher Scientifc, Inc., Carlsbad, CA) according to the manufacturer's instructions. qRT-PCR was carried outas described previously[17]. The primers were obtained from Sangon (Shanghai, China), the primers sequences were as follows: miR-128-1-5p: forward, 5′-CCACAGAGAGGATTCCAGAA-3′, reverse, 5′-TCCAGGCTCTGTATAGAAGCT−3′; U6 RNA: forward, 5′-CTCGCTTCGGCAGCACA-3′, reverse, 5′-AACGCTTCACGAATTTGCGT-3′; PRKCQ:forward, 5′-ATGTCGCCATTTCTTCGGATT-3′, reverse, 5′-ACATACTCTTTGACGAGCACAG−3′; GAPDH: forward, 5′-AGCCTTCTCCATGGTGGTGAA-3′, reverse, 5′-ATCACCATCTTCCAGGAGCGA−3′.

Lentivirus production and transduction

Lentivirus-medicated miR-128-1-5p overexpression vector was purchased from GenePharma (Shanghai, China). Lentivirus was produced by transfecting 293T **cell line**with Lenti-Pac HIV Expression Packaging Mix and the lentiviral vector using Lipofectamine 2000 (Life Technologies Corporation, Carlsbad, CA, USA). Stably transfected cell lines were established by infecting SW620 and LOVO cells with lentiviruses containing 2 μg/mL puromycin. The full-length sequence of PRKCQ was cloned into the pcDNA3.1 vector and empty vector was used as negative control simultaneously.

Cell proliferation and colony formation assay

The CCK-8 assay was performed as our previous study [17]. In brief, 2×10^3 cells were cultured in a 96-well plate and cultured for 24-48-72-96 h. Then 10 μL CCK-8 solution was added to each well and incubated for 2 h at 37˚C. Finally, the absorbance was measured at 450 nm. For colony formation assay, 8×10^2 cells were seeded in 6-well plates and cultured for 2 weeks at 37°C. Cell colonies were fixed with methanol and stained with 0.5% crystal violet. Colonies containing more than 50 cells were counted and photographed.

Flow cytometric analysis

Cells were dissociated with trypsin without EDTA, subsequently incubated with Annexin V- DY-634 PI Apoptosis Staining / Detection Kit (Abcam, Cambridge, United Kingdom). The ratio of apoptotic cells was detected with a flow cytometry (FACScan, BD Biosciences).

Vector constructs and luciferase reporter assay

The full-length of wild type 3’UTR of PRKCQ was cloned into the psi-CHECK2 vector (Promega, Madison, WI, USA) to form the reporter vector PRKCQ-wild-type (PRKCQ-WT). The corresponding mutant was

created by mutating the miR-128-1-5p seed region binding site, which was named PRKCQ-MUT. All clones were verified by DNA sequencing. MiR-128-1-5p or vector was co-transfected with luciferase reporter vectors into 293T cells using Lipofectamine^{TM} 2000.Cells were harvested 36 h post-transfection and luciferase activities were analyzed by the dual-luciferase reporter assay system (Promega) in a luminescence reporter gene assay system (PerkinElmer, Norwalk, CT, USA) according to the manufacturer's instructions.

Protein isolation and western blotting

Total protein was extracted using RIPA lysis buffer from Cell Signaling Technology (CST, Beverly, MA, USA) according to the manufacturer's protocol. Western blotting was performed using standard protocols as described previously[17]. The primary antibodies used in this study included: PRKCQ (Proteintech Group, Inc, IL, USA), Caspase-3 (CST), Bax (CST), Bcl-2 (CST), and GAPDH (Proteintech Group, Inc).

Xenograft model

4–6-week-old female immune-deficient nude mice (BALB/c null) were purchased from Slaccas (Slaccas Laboratory Animal, Shanghai, China). All animal procedures were performed in accordance with guidelines of the care and use of laboratory animals of Fudan University and approved by the Animal Ethics Committee of Zhongshan hospital. Stable miR-128-1-5p-overexpressing LOVO and control cells (1×10^7 cells) in 100 ul serum free medium were subcutaneously injected into the flank region of the mice (8 for one group). Tumor size was measured with a slide caliper every week. Mice were sacrificed 4 weeks later and the tumors were removed for further investigation.

Immunohistochemistry Staining

Immunohistochemistry (IHC) staining was carried out as our previously described (23). Briefly, formalin-fixed tissues were embedded in paraffin and cut into 4-μm-thick sections. Subsequently, the specimens were dewaxed, hydrated, antigen retrieved by heat, blocked, and then incubated overnight at 4°C with primary antibody Ki67 (CST). Following, sections were incubated with HRP conjugated secondary antibodies. Finally, slides were treated with 3, 3'-diaminobenzidine (DAB) substrate and visualized under a Nikon Eclipse Ti inverted microscope (Nikon, Amstelveen, The Netherlands).

Statistical analysis

The results were presented as the mean ± standard error of the mean (SEM). Student's t-test was used to assess differences between two groups. One-way analysis of variance (ANOVA) was used to compare differences among three groups or more. Association between miR-128-1-5p and PRKCQ expression was assessed using Spearman rank correlation test. The $\chi 2$ test was performed to analyze the relationship between miR-128-1-5p expression and the clinicopathological characteristics. Statistical analysis used SPSS 18.0 (SPSS, Chicago, USA) and a $P < 0.05$ was considered statistically significant.

Results

MiR-128-1-5p levels are downregulated in CRC with poor survival

Using CancerMIRNome database for the identification of prognostic miRNA biomarkers, we found that expression of miR-128-1-5p was investigated to be strongly associated with optimal survival in C`1qolon adenocarcinoma (COAD) based on Hazard Ratio (HR=0.39, p□0.001; Figure 1A). Then, the expression of miR-128-1-5p in serum was found to be significantly downregulated in most human cancers, including CRC, compared with the healthy subjects (Figure 1B, a, b). The comparison of miR-128-1-5p expression between cancer and adjacent normal tissue in CancerMIRNome database revealed significantly lower miR-128-1-5p expression in colon adenocarcinoma (COAD) (p□0.001; Figure 1C, a) and rectum adenocarcinoma (READ) (p□0.001; Figure 1C, d). To evaluate the association between miR-128-1-5p and prognosis, the Kaplan–Meier survival analysis was performed by CancerMIRNome. Patients with high miR-128-1-5p expression levels had significantly higher overall survival in COAD (HR=0.39, p □0.001; Figure 1C,b). Similar trend was also found in READ, although there was no significant difference (HR=0.39, p=0.17; Figure 1C,e). Further, The ROC curve showed that miR-128-1-5p has better diagnostic ability in COAD (AUC=0.91; Figure 1C, c) and READ (AUC= 0.97, Figure 1C, f).

To better understand the role of miR-128-1-5p in CRC, we collected 107 pairs of CRC tissues and their respective adjacent normal tissues. Our results showed that the expression of miR-128-1-5p in CRC tissues was significantly down-regulated compared with their corresponding normal tissues (p□0.001; Figure 1D,a). The expression levels of miR-128-1-5p were associated with recurrence (p□0.001; Figure 1D,b) and AJCC stage (Figure 1D,c). Similarly, the expression of miR-128-1-5p in CRC cell lines (HT29, RKO, SW480, SW620 and LOVO) was significantly lower compared with the immortalized colon epithelial cell line HIEC (Figure 1D,d). To evaluate whether miR-128-1-5p expression levels are correlated with CRC progression, the relationship between miR-128-1-5p levels and clinicopathological parameters of CRC patients was assessed. As shown in table 1, miR-128-1-5p levels were correlated with AJCC (p = 0.0333) and distant metastasis (p = 0.0271). Survival analysis using the Kaplan-Meier method revealed that higher miR-128-1-5p levels were associated with longer overall survival in CRC patients (HR=0.49, p=0.02, Figure 1D,e). These results suggested that miR-128-1-5p might play a critical role in the development and progression of colorectal cancer.

MiR-128-1-5p inhibits CRC cell proliferation and induces cell apoptosis

To confirm the biological effects of miR-128-1-5p on CRC development, we overexpressed miR-128-1-5p in SW620 and LOVO CRC cell lines which had lower endogenous miR-128-1-5p among aforementioned cell lines. As shown in Figure 2A, overexpression of miR-128-1-5p led to a significant increase of miR-128-1-5p level in SW620 and LOVO cells, as determined by qRT-PCR. Overexpression of miR-128-1-5p decreased the proliferation rate of SW620 (Figure 2B, a) and LOVO cells (Figure 2B, b). Colony formation assay also indicated that upregulation of miR-128-1-5p resulted in a significant decrease of colony number (Figure 2C, a, b). To explore the possible mechanism underlying the inhibitory effect on cell growth by miR-128-1-5p, cell apoptosis was performed. Flow cytometry showed that the apoptosis rate of

CRC cells in the miR-128-1-5p overexpression cells was significantly higher than that in the vector (Figure 2D, a, b). Western blot also showed that overexpression of miR-128-1-5p increased apoptosis-related proteins caspase-3 and Bcl2-associated x protein (Bax), and reduced apoptosis inhibitory protein B-cell lymphoma 2(Bcl-2) (Figure 2E, a, b). These data implicated that miR-128-1-5p acted as a suppressor gene by regulating cell growth.

PRKCQ is a direct target of miR-128-1-5p

To investigate the mechanism of miR-128-1-5p in CRC, we used online algorithm to search potential targets of miR-128-1-5p (Figure 3A) and found the 3'-UTR of PRKCQ had binding site of miR-128-1-5p (Figure 3B). To confirm whether PRKCQ was a direct target of miR-128-1-5p in CRC cells, luciferase reporter assay was performed to assess luciferase activities and the results showed that the activity of PRKCQ 3'-UTR-WT was obviously reduced after transfection with miR-128-1-5p (Figure 3C). However, there was no significant difference in activity of PRKCQ 3'-UTR-MUT(Figure 3C). Furthermore, miR-128-1-5p overexpression markedly suppressed the mRNA and protein expression of PRKCQ in CRC cells (Figure 3D). Moreover, the qRT-PCR results showed that the level of PRKCQ was significantly upregulated in CRC tissues when compared with their respective adjacent normal tissues (Figure 3E). Pearson correlation assay suggested that miR-128-1-5p expression was negatively correlated with the mRNA level of PRKCQ (Figure 3F). Taken together, these data demonstrated that PRKCQ was a direct target of miR-128-1-5p.

MiR-128-1-5p modulates CRC cells proliferation and apoptosis by targeting PRKCQ

To investigate whether miR-128-1-5p may inhibit cell growth by silencing the PRKCQ expression in CRC cells, we assessed the effect of PRKCQ on cell proliferation by transfecting PRKCQ in miR-128-1-5p overexpression SW620 and LOVO cells. Western blotting showed that the expression of PRKCQ was notably increased by transfecting PRKCQ in miR-128-1-5p overexpression SW620 and LOVO cells (Figure 4A, a, b). Interestingly, overexpression of PRKCQ significantly reversed the inhibition of cell growth induced by miR-128-1-5p (Figure 4B, a, b). The colony formation assays also demonstrated that upregulating PRKCQ in miR-128-1-5p overexpression cells increased colony numbers comparing control groups (Figure 4C). The results of the cell apoptosis assays suggested that overexpression of PRKCQ abolished the increased cell apoptosis resulting from miR-128-1-5p (Figure 4D). Furthermore, the levels of caspase-3 and Bax were upregulated together with Bcl-2 downregulated by PRKCQ overexpression (Figure 4A). Collectively these findings clearly described that PRKCQ is a mediator of miR-128-1-5p in progression of CRC.

In vivo effect of miR-128-1-5p on CRC

To further investigate whether miR-128-1-5p affect CRC growth *in vivo*, LOVO cells stably overexpressing miR-128-1-5p were subcutaneously injected into nude mice to examine the effects of miR-128-1-5p on tumor growth *in vivo*. As shown in Figure 5A, overexpression of miR-128-1-5p could suppress the tumor growth, demonstrated by decreased mean tumor volume and weight (Figure 5A). Furthermore, the expression of miR-128-1-5p was significantly increased in the xenograft tumor tissues (Figure 5B, b),

whereas the mRNA expression of PRKCQ was significantly decreased in the xenograft tumor tissues (Figure 5B, a). In addition, the immunohistochemical staining showed that the protein expression of Ki-67、Bcl-2 and PRKCQ was significantly downregulated together with caspase-3 upregulated in the tumor tissues of miR-128-1-5p overexpression group (Figure 5C). Taken together, our results supported the notion that miR-128-1-5p inhibited CRC cell growth via regulating PRKCQ.

Discussion

CRC is the one of the most aggressive malignancies with the Third leading cause of cancer-related deaths in United States [18]. Current management strategies for CRC treatment mainly include curative surgery and post-operative adjuvant chemotherapy[19–21]. Further studies of the underlying mechanisms of CRC initiation and progression are required to reduce the mortality caused by this cancer. In our present study, we attempted to explore the expression and role of miR-128-1-5p in CRC.

Previous studies have shown that numerous miRNAs were deregulated in CRC, and correlate with CRC pathological stage and prognosis[22–24]. MiR-128-1-5p has been identified as a tumor suppressor and found to be downregulated in certain malignancies. MiR-128 has been reported to be down-regulated in thyroid carcinoma and overexpression of miR-128 suppressed thyroid carcinoma cancer cell growth, migration and invasion by negatively regulating SPHK1 expression[12]. MiR-128 was also found to be downregulated in glioma tissues and cells and overexpression of miR-128 could inhibit LN229 and U251 cells proliferation and invasion[25]. A recent study has shown that miR-128 might act as a tumor suppressor in CRC[26].

Among them, our data showed that miR-128-1-5p was significantly downregulated in CRC tissues and cells and low miR-128 expression was associated with advance AJCC stage and distant metastasis and indicated a poorer prognosis. Furthermore, we used CCK-8 and colony formation assay to detect its effect on cell proliferation. Here, we have confirmed that overexpression of miR-128-1-5p significantly inhibited the proliferation of CRC cells.

To reveal the potential mechanisms of how miR-128-1-5p affected cell proliferation, cell apoptosis was detected and cytometry analysis showed that miR-128-1-5p induced cell apoptosis with significantly alteration of Bcl-2, Bax and caspase3.

MiRNAs regulate gene expression by binding to partially complementary sequences of 3’ untranslated region (3’UTR) of target genes to suppress mRNA translation[7]. To identify potential miR-128-1-5p targets in CRC cells, we screened predicted targets by online target tools and forecasted as upregulated in CRC tissues according to literature. The potential candidate PRKCQ, a member of the PKC family[27, 28], was regarded as a direct target of miR-128-1-5p. Luciferase reporter activity confirmed that PRKCQ was directly targeted by miR-128-1-5p. A negative correlation between miR-128-1-5p and PRKCQ mRNA levels was observed in CRC tissues. Interestingly, restoration of PRKCQ expression could significantly reverse the effects of miR-128-1-5p overexpression on cell viability and apoptosis, suggesting that miR-128-1-5p functions through lowering the expression of PRKCQ.

Moreover, we identified that miR-128-1-5p was frequently down-regulated in human CRC tissues and cells and functioned as a suppressor gene to inhibit cell proliferation *in vitro* and *in vivo*. Mechanistically, miR-128-1-5p suppressed the cell proliferation by directly inhibiting PRKCQ expression. Our study might provide novel insights into understanding the molecular pathogenesis of CRC and offer a potential target for the diagnosis and therapy of patients with CRC.

Conclusions

Our study proposed that miR-128-1-5p suppressed cell proliferation and induced cell apoptosis during CRC via regulating the expression of BAX and BCL-2 through targeting to the 3'-UTR of PRKCQ. In summary, we have determined that PRKCQ might be a direct target of miR-128-1-5p. Our findings enrich the functional value of miR-128-1-5p in CRC, and indicate a novel potential therapeutic target with CRC.

Abbreviations

CRC: colorectal cancer; PRKCQ: Protein tyrosine kinase C theta isoform; TBNC: triple-negative breast cancer; CSCs: cancer stem cells; EMT: endothelial mesenchymal transition; Bcl-2: B-cell lymphoma 2; Bax: Bcl2-associated x protein; qRT-PCR: quantitative reverse transcription polymerase chain reaction; GAPDH: Glyceraldehyde3-phosphate dehydrogenase; IHC: immunohistochemistry; ANOVA: analysis of variance; OS: overall survival; COAD: Colon Cancer; READ: Rectal Cancer.

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Contributions

QAJ, XL and LL contributed to the study design and analyses. QAJ and LL performed the majority of the bioinformatics analyses. XL and YFL collated the data, designed and developed the database. BHX drafted and prepared the manuscript. All authors approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

All authors have agreed to publish this manuscript.

Competing interests

The authors declare that they have no competing interests.

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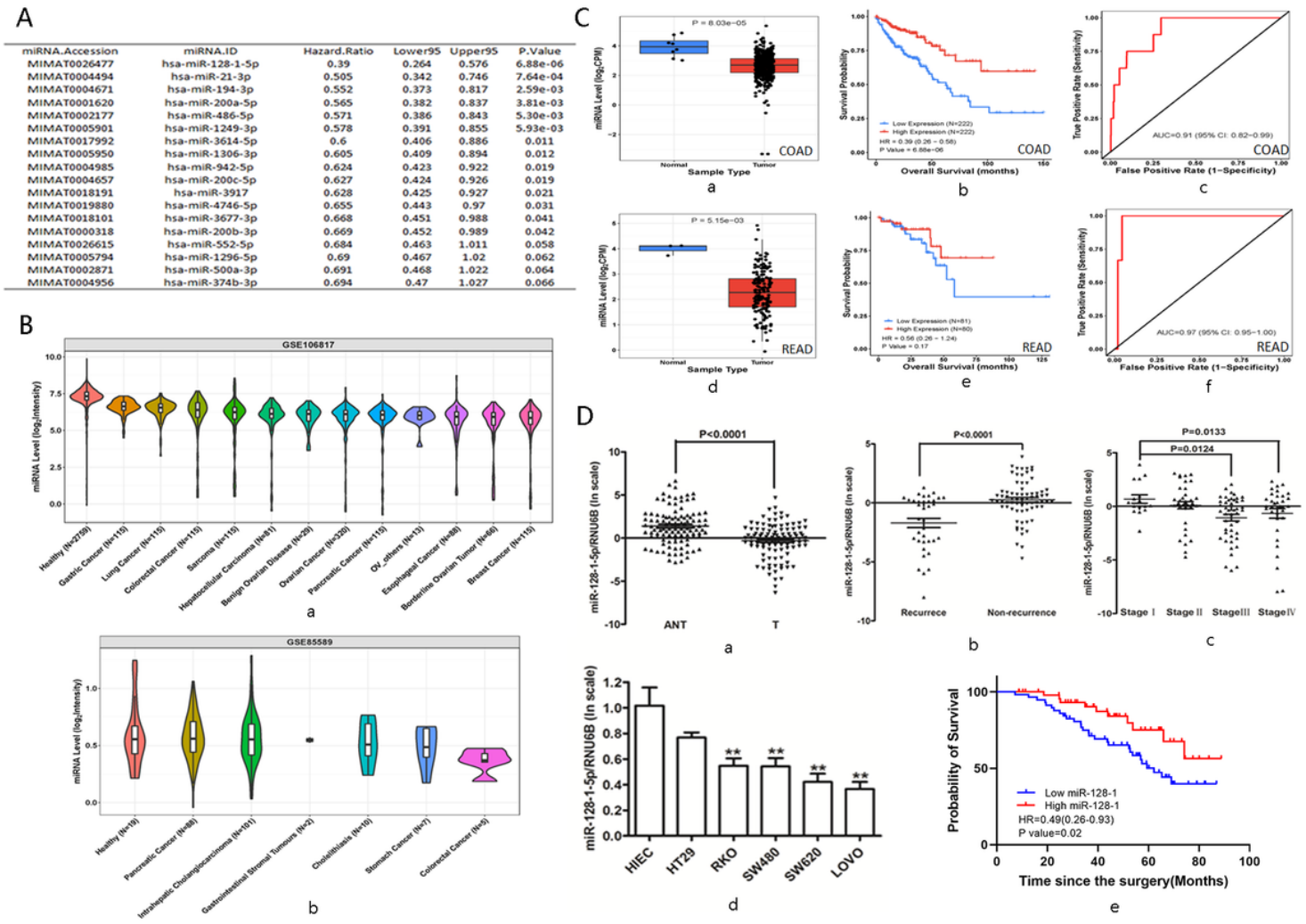
Tables

Table 1. Relationship between clinicopathological factors and miR-128-1-5p expression in CRC tissues

Variable	MiR-128-1-5p expression		P-value
	High (n = 57)	Low (n = 50)	
Age (years)			0.8461
≤ 50	30	28	
> 50	27	22	
Gender			0.6999
Male	32	30	
Female	25	20	
Tumor size			0.41
≤ 5 cm	36	36	
> 5 cm	21	14	
Location			0.6984
Colon	29	28	
Rectum	28	22	
Vascular invasion			0.8126
Absent	42	38	
Present	15	12	
Lymph metastasis			0.0672
Absent	40	43	
Present	17	7	
Distant metastasis			0.0271
Absent	41	45	
Present	16	5	

Note: CRC patients were divided into miR-128-1-5p high group and low group according to the results of qRT-PCR. Differences among variables were evaluated by $\chi 2$ or Fisher's exact $\chi 2$-test.

Figures



miRNA.Accession	miRNA.ID	Hazard.Ratio	Lower95	Upper95	P.Value
MIMAT0026477	hsa-miR-128-1-5p	0.39	0.264	0.576	6.88e-06
MIMAT0004494	hsa-miR-21-3p	0.505	0.342	0.746	7.64e-04
MIMAT0004671	hsa-miR-194-3p	0.552	0.373	0.817	2.59e-03
MIMAT0001620	hsa-miR-200a-5p	0.565	0.382	0.837	3.81e-03
MIMAT0002177	hsa-miR-486-5p	0.571	0.386	0.843	5.30e-03
MIMAT0005901	hsa-miR-1249-3p	0.578	0.391	0.855	5.93e-03
MIMAT0017992	hsa-miR-3614-5p	0.6	0.406	0.886	0.011
MIMAT0005950	hsa-miR-1306-3p	0.605	0.409	0.894	0.012
MIMAT0004985	hsa-miR-942-5p	0.624	0.423	0.922	0.019
MIMAT0004657	hsa-miR-200c-5p	0.627	0.424	0.926	0.019
MIMAT0018191	hsa-miR-3917	0.628	0.425	0.927	0.021
MIMAT0019880	hsa-miR-4746-5p	0.655	0.443	0.97	0.031
MIMAT0018101	hsa-miR-3677-3p	0.668	0.451	0.988	0.041
MIMAT0000318	hsa-miR-200b-3p	0.669	0.452	0.989	0.042
MIMAT0026615	hsa-miR-552-5p	0.684	0.463	1.011	0.058
MIMAT0005794	hsa-miR-1296-5p	0.69	0.467	1.02	0.062
MIMAT0002871	hsa-miR-500a-3p	0.691	0.468	1.022	0.064
MIMAT0004956	hsa-miR-374b-3p	0.694	0.47	1.027	0.066

Figure 1

MiR-128-1-5p expression is down-regulated in colorectal cancer and correlated with prognosis. (A) MiR-128-1-5p is an optimal prognostic biomarker in COAD. (B) MiR-128-1-5p was significantly downregulated in serum in most human cancers compared with the healthy subjects based on GEO datasets GSE106817 (a) and GSE85589(b). (C) Different expression analysis, ROC analysis, and K-M survival analysis for miR-128-1-5p in COAD and READ (a-f). (D) qRT-PCR analysis of miR-128-1-5p expression in 107 pairs of colorectal cancer and corresponding normal tissues (a). The relative expression of miR-128-1-5p in colorectal cancer tissues with different clinical stages(b). Relative miR-128-1-5p expression levels in CRC with recurrence (n = 36) were significantly lower than in CRC tissues without recurrence (n = 71) (c). Relative miR-128-1-5p expression levels in CRC cell lines and the immortalized colon epithelial cell line HIEC (d). Kaplan-Meier survival analysis of CRC patients' overall survival based on miR-128-1-5p expression (e). Data are represented as mean ± SEM. **P < 0.01.

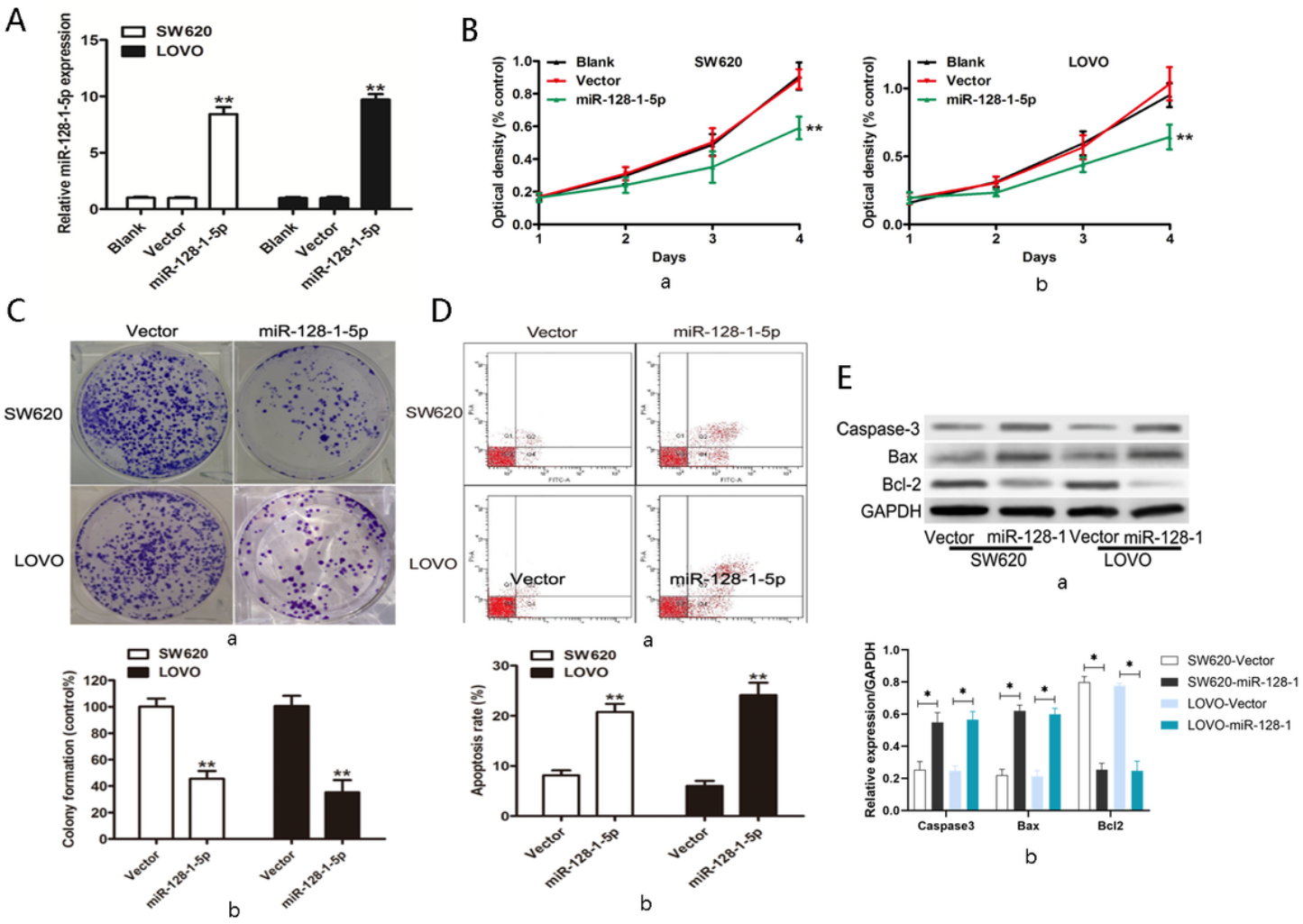


Figure 2

Overexpression of miR-128-1-5p affects colorectal cancer cell proliferation and apoptosis. (A) miR-128-1-5p expression was detected by qRT-PCR in SW620 and LOVO cells transfected with miR-128-1-5p. (B) Cell proliferation assays were performed in SW620 (a) and LOVO (b) cells transfected with vector or miR-128-1-5p. (C) Represent images of colony formation in SW620 and LOVO cells transfected with vector or miR-128-1-5p (a, b). (D) Flow cytometry was used to analyze apoptosis ratio of SW620 and LOVO cells transfected with vector or miR-128-1-5p (a, b). (E) Western blot detected the expression of certain apoptosis-related proteins in SW620 and LOVO cells transfected with vector or miR-128-1-5p (a, b). Data are represented as mean ± SEM. **P < 0.01.

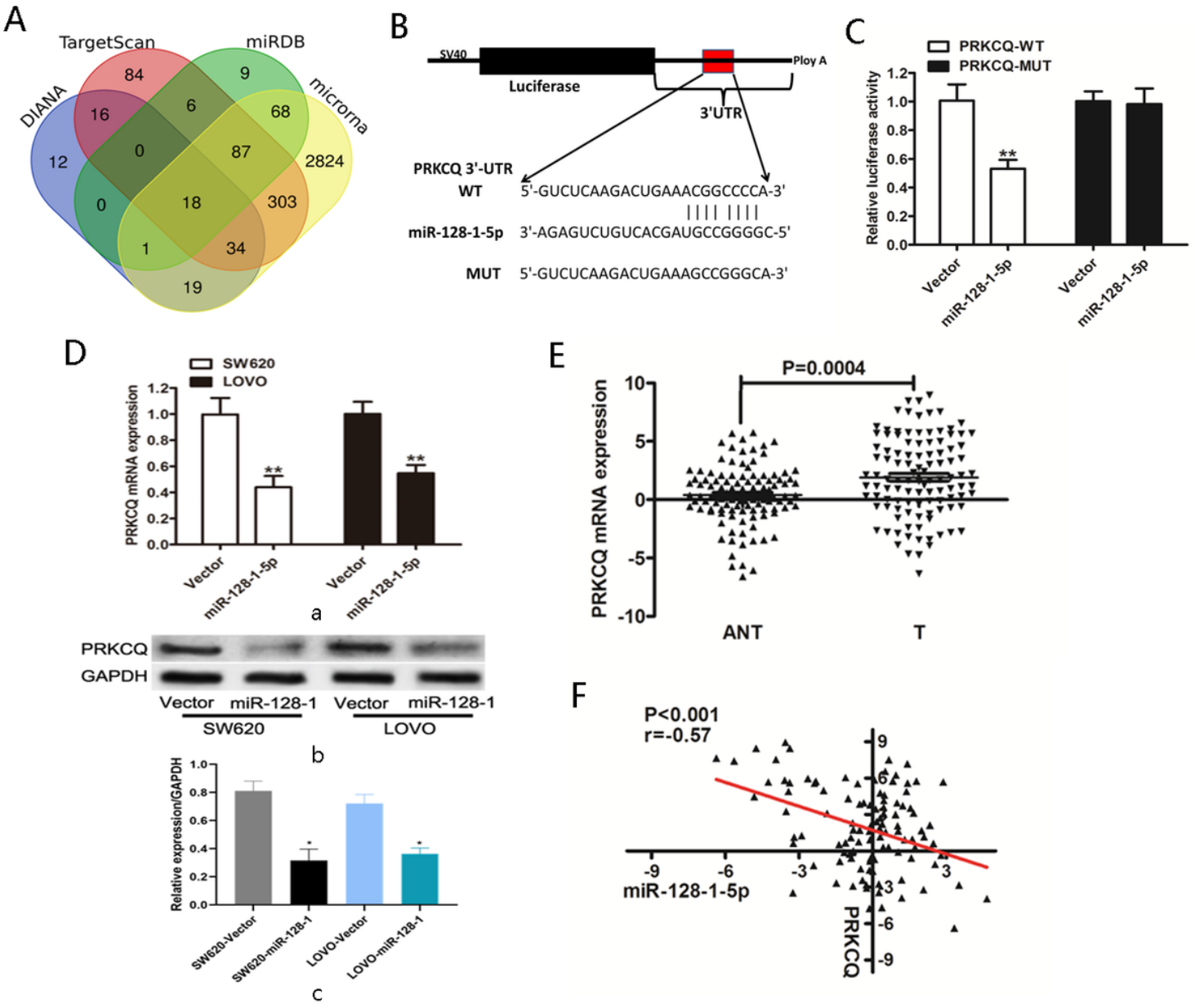


Figure 3

PRKCQ *directly bind to miR-128*-1-5p in human CRC cell lines. (A) Venn diagram showing the overlapping of targets from four target genes prediction programs. (B) Predicted binding site of miR-128-1-5p to the 3'-UTR of the human PRKCQ gene, and the diagram of luciferase reporter vectors containing the wild-type (WT) or mutated (MT) 3'-UTR of PRKCQ. (C) Determination of the luciferase activity of PRKCQ in 293T cells transfected with PRKCQ-MUT or PRKCQ-WT and miR-128-1-5p or vector. (D) The mRNA and protein expression level of PRKCQ were examined in SW620 and LOVO cells transfected with vector or miR-128-1-5p. (E) Relative mRNA expression levels of PRKCQ were significantly upregulated in CRC tissues. (F) The correlation between PRKCQ mRNA level and miR-128-1-5p expression was assessed by Pearson correlation analysis in cancer samples. Data are represented as mean ± SEM. **P < 0.01.

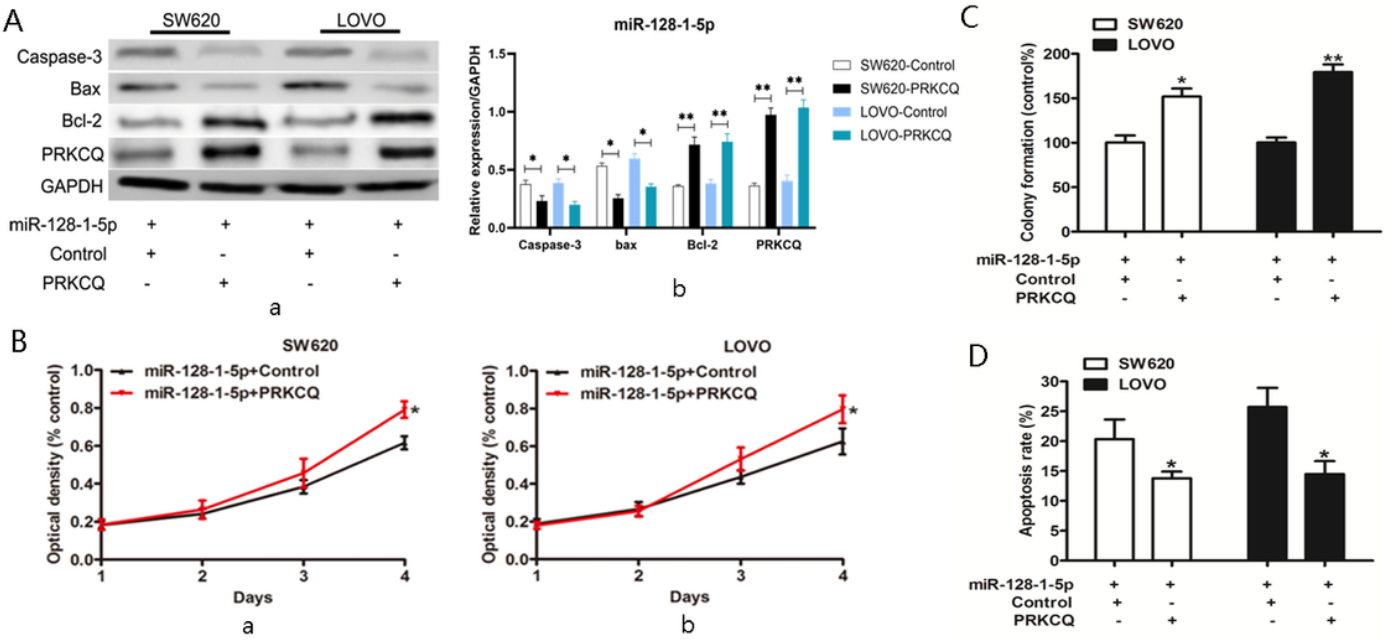


Figure 4

PRKCQ is responsible for miR-128-1-5p-mediated cell proliferation and apoptosis. (A) Western-blot analysis of Bax, Bcl-2 and caspase3 protein expression in miR-128-1-5p overexpressed SW620 and LOVO cells transfected with control or PRKCQ. CCK8 assay (B) and colony formation assays (C) were performed to determine the cell viability in miR-128-1-5p overexpressed SW620 and LOVO cells transfected with control or PRKCQ. (D) The percentage of apoptotic cells was determined by flow cytometric analysis as above. Data are represented as mean ± SEM. *P < 0.05; **P < 0.01.

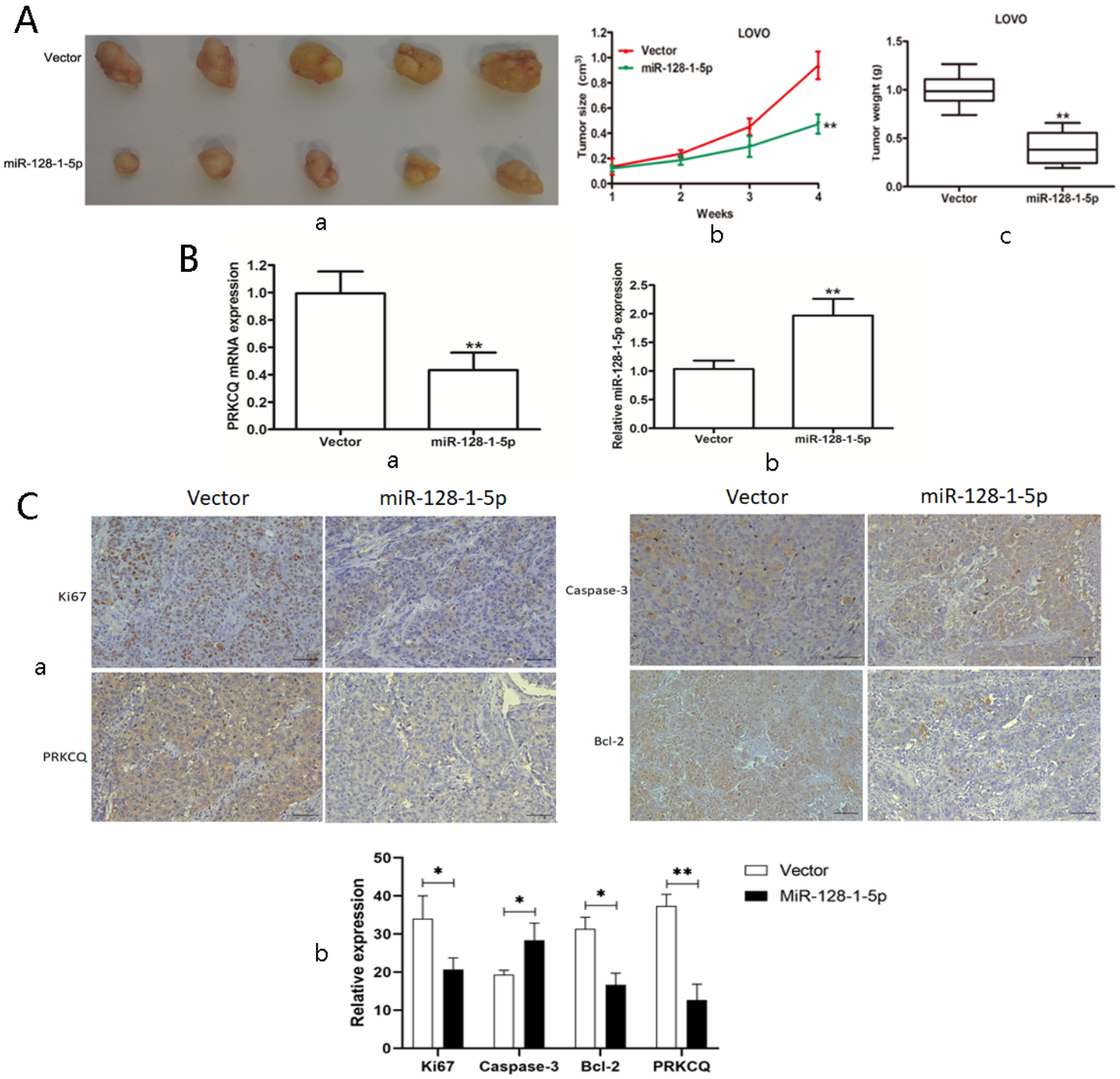


Figure 5

miR-128-1-5p suppresses CRC growth *in vivo*. (A) Representative images of mice bearing tumors from vector and miR-128-1-5p groups (a). The tumor volume (b) growth curves after injections and the tumor weight (c) was measured after mice sacrificed. (B) Detection of the expression of miR-128-1-5p (a) and PRKCQ (b) by qRT-PCR. (C) Immunohistochemistry analysis of Ki-67, PRKCQ, caspase-3 and Bcl-2 protein levels in xenograft tumor tissues from above mice. Data are represented as mean ± SEM. **p < 0.01.

Supplementary Files

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- supplementaryfiles.rar