Decreased expression of miR-490-3p in colorectal cancer predicts poor prognosis and promotes cell proliferation and invasion by targeting RAB14

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Abstract. Growing evidence indicates a potential role for miR-490-3p in tumorigenesis. However, its function in colorectal carcinoma (CRC) remains undefined. In this study, miR-490-3p was markedly downregulated in fifty colorectal cancer tissue samples compared with the corresponding adjacent non-cancerous specimens, by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The expression levels of miR-490-3p were closely associated with tumor differentiation and distant metastasis. In addition, both Kaplan-Meier and multivariate analyses indicated CRC patients with elevated miR-490-3p amounts had prolonged overall survival. Overexpression of miR-490-3p markedly reduced proliferation, colony formation and invasion in CRC cells by enhancing apoptosis and promoting G2/M phase arrest. Furthermore, ectopic expression of miR-490-3p resulted in decreased expression of RAB14, which was directly targeted by miR-490-3p, as shown by the dual-luciferase reporter gene assay. Finally, in a nude mouse model, miR-490-3p overexpression significantly suppressed the growth of CRC cells. The above results indicated that miR-490-3p might constitute a prognostic indicator and a novel molecular target for miRNA-based CRC therapy.

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Introduction

Colorectal carcinoma (CRC) is among the top three malignancies with increasing rates of morbidity and mortality worldwide (1). Despite significant advances in cancer treatment, individuals with relapse or metastases still exhibit poor prognosis (2). Therefore, the mechanisms of CRC progression require further investigation.

RAB proteins regulate diverse pathways in intracellular membrane trafficking and dynamics (3). Distinct RAB proteins exert different effects. For example, RAB-1 and RAB-2 modulate the innate immunity, neuron maturation and vesicle trafficking (4). Meanwhile, RAB14 is overexpressed in multiple human malignancies, including ovarian (5), non-small cell lung (6), breast (7) and gastric (8,9) cancer. RAB14 appears to act as an oncogene in human tumorigenesis and metastasis. However, how RAB14 affects CRC tumorigenesis remains unclear. Therefore, identifying and characterizing RAB14 is essential for understanding its roles in the pathogenesis of CRC.

MicroRNAs (miRNAs) constitute important mediators, which regulate target genes via binding to the 3'-untranslated region (UTR) of mRNAs (10). Multiple miRNAs are aberrantly expressed in CRC, which promotes malignant behavior (11,12). Previous findings by the authors demonstrated that the overexpression of miR-194 or miR-217 significantly decreased the proliferation and invasion of CRC cells. miR-199b might target Sirtuin 1 to suppress the metastasis of CRC to the liver (13-15). Meanwhile, miR-490-3p promotes cell proliferation and metastasis in liver cancer by targeting ERGIC and Golgi 3 (16) while inhibiting other malignant tumors, including gastric cancer (17,18), ovarian cancer (19), breast cancer (20) and osteosarcoma (21). Although Xu *et al* (22) and Zheng *et al* (23) reported that miR-490-3p was downregulated in colorectal carcinoma, the mechanism associated with its role is not completely elucidated.

The present study aimed to assess whether RAB14 is directly targeted by miR-490-3p, and evaluate the role of the miR-490-3p/RAB14 pathway in the progression of CRC.

Materials and methods

Cells and human tissue specimens. Human colorectal cancer cells, SW480, SW620, RKO, HT29, HCT116 and LoVo, were purchased from the American Type Culture Collection (Manassas, VA, USA). NCM460 cells were from INCELL Corporation LLC (San Antonio, TX, USA). HT29, LoVo, HCT116 and RKO cells were cultured in RPMI-1640. SW480 and SW620 were cultured in Leibovitz's L-15 medium. All cells were cultured at 37°C in a humidified environment containing 5% CO^2, and the medium was supplemented with 10% fetal bovine serum (FBS, Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 100 U/ml penicillin (Sigma-Aldrich; Merck KGaA), and 100 μg/ml streptomycin (Sigma-Aldrich; Merck KGaA).

A total of 90 patients with CRC were involved in the present study. Accordingly, 90 human colorectal cancerous specimens and the corresponding adjacent non-cancerous tissues were collected during operations at Peking University People's Hospital during January 2013 to December 2016 in subjects that underwent coloproctectomy according to the National Comprehensive Cancer Network guidelines for colon/rectal carcinoma (https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf or https://www.nccn.org/professionals/physician_gls/pdf/rectal.pdf, version 1. 2013-2016). The samples were kept at -80°C until use. Written informed consent was provided by all patients prior to sample collection. The present study received approval from the Research Ethics Committee of Peking University (Beijing, China).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The purification of total RNA and RT-qPCR were performed with PrimeScript RT reagent kit and SYBR Green PCR Master Mix, respectively (Takara Bio, Inc., Otsu, Japan) as described by the manufacturer. The thermocycling conditions were 5 sec at 95°C and 30 sec at 60°C. The data were analyzed using the $2^{-\Delta\Delta Cq}$ method as previously described by Livak and Schmittgen (24) and Wang *et al* (15). GAPDH and U6 RNA were employed as references for mRNAs and miRNAs, respectively. The primers used were: miR-490-3p forward, 5'-CAACCTGGAGGACTCCATGCTG-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3'; RAB14 forward, 5'-CGCTCGAGATGGCAACTGCACCATACAAC-3' and reverse, 5'-CGGAATTCCTAGCAGCCACAGCCTTCTC-3'; GAPDH forward, 5'-CCCCGGTTTCTATAAATTGAGC-3' and reverse, 5'-CACCTTCCCCATGGTGTCT-3'. The universal primer from Takara Bio, Inc. was used as the reverse primer for miR-490-3p and U6.

Cell transfection or infection. The miRNAs and siRNAs were synthesized by Guangzhou RiboBio Co., Ltd., (Guangzhou, China). RAB14 plasmids were manufactured by Invitrogen (Thermo Fisher Scientific, Inc.). Transfection was carried out with Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). RAB14 siRNA, miR-490-3p mimics and respective negative controls were used at 50 nM for transient transfection. Lentiviral vectors (3E+8 TU/ml) (Shanghai GeneChem Co., Ltd., Shanghai, China) expressing miR-490-3p (LV-miR-490-3p) or the negative control sequence were employed to infect CRC cells for *in vivo* experiments. The oligonucleotide sequences for transfection were: miR-490-3p mimics, 5'-CAACCUGGAGGACUCCAUGCUG-3'; miR-NC, 5'-UUUGUACUACACAAAAGUACUG-3' and RAB14 siRNA, 5'-TGCAAGGAATCTCACCAAT-3'.

Western blot assay. Cell lysis was performed with hypotonic lysis buffer (Thermo Fisher Scientific, Inc.). Equal quantities of protein (20 μg/lane) were resolved by 10% or 15% SDS-PAGE and electro-transferred onto nitrocellulose membranes (Pall Corporation, Pensacola, FL, USA). The detailed description of the assay was recently reported (14). Briefly, the membranes were blocked with 5% non-fat milk powder in Tris-buffered saline containing 0.1% Tween-20 and probed with primary antibodies overnight at 4°C. Following incubation with secondary antibodies in room temperature, the bands were visualized using enhanced chemiluminescence (Pierce; Thermo Fisher Scientific Inc.) and imaged using a ChemiDoc™ XRSC system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The primary antibodies used were: anti-GAPDH (1:1,000; cat. no. 2118; Cell Signaling Technology, Inc., Danvers, MA, USA) and anti-RAB14 (1:500; cat. no. ab28639; Abcam, Cambridge, UK). The secondary antibody used was anti-rabbit IgG (1:5,000; cat. no. ab286397074; Cell Signaling Technology, Inc.).

Cell proliferation and colony formation assay. Cell proliferation was assessed using the Cell Counting Kit-8 assay. After the cells (SW480 or SW620) were seeded into 96-well plates at 2,000 cells/well, incubation was carried out at 37°C for 48 h. Viable cells were quantified at various times at 450 nm on a microplate reader (Bio-Rad Laboratories, Inc.). Sextuplicate assays were performed for three times.

To assess the ability of the cells to form colonies, the cells were seeded into 6-well plates and cultured for two weeks at 37°C following transfection. Next, the cells were washed with PBS and fixed with 4% paraformaldehyde then subjected to staining with 0.1% crystal violet for 20 min at room temperature. Duplicate assays were carried out three times.

Cell cycle and apoptosis analysis. To assess cell cycle distribution, the cells (SW480 or SW620) were stained with the BD Cycletest™ plus DNA kit (BD Biosciences, Franklin Lakes, NJ, USA) as suggested by the manufacturer. To detect apoptosis, the transfected cells (72 h) were incubated with the Alexa Fluor® 488 Annexin V/Dead cell apoptosis kit (Invitrogen; Thermo Fisher Scientific, Inc.). Apoptosis analysis was performed according to the manufacturer's instructions. The temperature of incubation was different depending on the steps undertaken. Data analysis was carried out with FlowJo (version 7.0; Tree Star, Inc., Ashland, OR, USA) on a flow cytometer (BD Biosciences).

Cell invasion assay. Cell invasion assay was performed using the Transwell method. Cancer cells (SW480 or
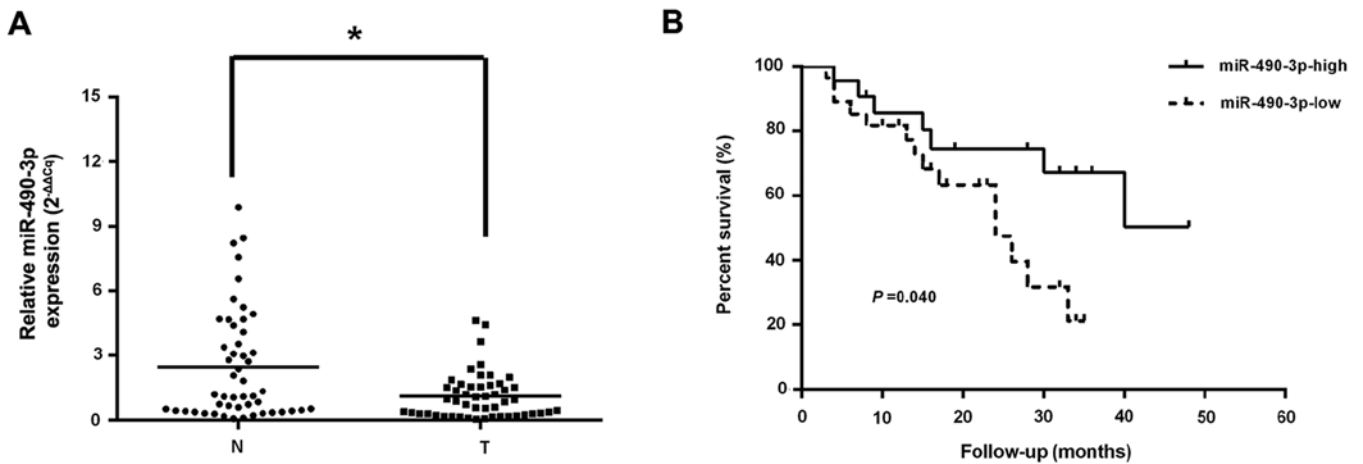
Figure 1. miR-490-3p expression in CRC tissue specimens and clinical significance. (A) Relative expression levels of miR-490-3p in human CRC (n=50) and CRN (n=50) tissue specimens, assessed by reverse transcription-quantitative polymerase chain reaction. CRC, colorectal carcinoma samples; CRN, matched adjacent non-cancerous colorectal tissue specimens. (B) Kaplan-Meier survival curves of CRC cases with elevated (n=22) and reduced (n=28) miR-490-3p. *P<0.05 assessed using log-rank test. CRC, colorectal carcinoma; miRNA, microRNA; N, adjacent normal colorectal samples; T, CRC tissue specimens.

SW620) were added to the upper chambers (24-well plate, pore size, 8 μm; Corning Incorporated, Corning, NY, USA) with Matrigel pre-coated membranes. Leibovitz's L-15 medium with 30% FBS was employed as a chemoattractant in the lower chambers. The Transwell plate was incubated for 2 days. The cells that passed through the membrane were washed with PBS. The cells were fixed with 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet for 20 min at room temperature. Subsequently, the cells were counted and imaged using an inverted microscope (magnification, x200; Leica DM IL LED; Leica Microsystems GmbH, Wetzlar, Germany). Duplicate assays were carried out three times.

Luciferase reporter assay. The miR-490-3p-binding site in RAB14 was predicted using TargetScan 7.1 (http://www.targetscan.org/), microRNA (http://www.microrna.org/) and miRDB (http://www.mirdb.org/). SW480 cells in 24-well plates were co-transfected with luciferase plasmids (wild-type or mutant, 500 ng/well) for RAB14 and miR-490-3p or NC mimics as directed by the manufacturer (RiboBio Co., Ltd.). The samples were incubated for 48 h at 37˚C, and luciferase activities were assessed using the Dual-Luciferase reporter assay system (Promega Corporation, Madison, WI, USA). The data were reported as a ratio of Firefly to *Renilla* luciferase activity.

Xenograft mice model. A total of 8 BALB/c-nude mice (female, 6-weeks-old; Vital River Laboratories, Beijing, China) were randomly allocated to two groups with 4 mice in each group to examine tumorigenicity. In this assay, 200 μl cell suspensions (1x10^7 SW480 cells) were subcutaneously administered to the right flank of each mouse. The volume of the tumors was assessed at 4-day intervals using the formula: V = 0.5 x L. The animals were scarified 40 days after cell inoculation and the tumors were extracted for volume and weight measurements. All animal experiments received approval from the Animal Research Committee of the Peking University People's Hospital (Beijing, China). The mice were handled following

guidelines provided by the Institutional and Animal Care and Use Committee.

Statistical analysis. The data are expressed as the mean ± standard deviation and were assessed with SPSS (version 20.0; SPSS, Inc., Chicago, IL, USA). Student's t-test was employed for group comparisons while LSD t-test was performed for multiple comparisons. The associations of miR-490-3p levels with clinicopathologic parameters in CRC were evaluated using the Pearson χ^2 test. The Kaplan-Meier method was employed to assess overall survival with group differences determined by the log-rank test. Cox regression analysis was used for multivariate analysis. The association between the expression of miR-490-3p and RAB14 was evaluated using the Spearman's correlation. P<0.05 indicated statistical significance.

Results

Low miR-490-3p levels indicate poor prognosis in CRC. RT-qPCR was employed to assess the levels of miR-490-3p, which were markedly reduced in CRC tissue specimens compared with adjacent normal colorectal samples (Fig. 1A). In addition, decreased miR-490-3p levels were highly correlated with late tumor-node metastasis (TNM) stage and lymph node metastasis, but not age, sex, tumor size, tumor differentiation and distant metastasis (Table I). Using the Kaplan-Meier method, it was identified that CRC cases expressing low levels of miR-490-3p had a reduced median survival compared with those exhibiting elevated miR-490-3p expression (Fig. 1B). Cox's multivariate analysis demonstrated that distant metastasis was closely associated with overall survival in CRC, and it was an independent predictor of survival (Table II).

miRNA-490-3p suppresses the proliferation of CRC cells in vitro and in vivo. As CRC cells express low levels of miR-490-3p (Fig. 2A), gain-of-function studies of miR-490-3p were performed by transfecting miR-490-3p mimics into

Table I. Association between miR-490-3p expression and clinicopathologic characteristics in patients with colorectal cancer.

| | miR-490-3p expression | | | |
Parameters	High (n=22)	Low (n=28)	Total (n=50)	P-value
Age, years				
≤60	7	11	18	0.585
>60	15	17	32	
Sex				
Female	11	14	25	1.000
Male	11	14	25	
Tumor size, cm				
≤2	9	11	20	0.907
>2	13	17	30	
Tumor differentiation				
Well/moderate	12	14	26	0.750
Poor	10	14	24	
TNM stage				
I+II	14	9	23	0.027[a]
III+IV	8	19	27	
Lymph node metastasis				
Positive	7	18	25	0.023[a]
Negative	15	10	25	
Distant metastasis				
Positive	5	12	17	0.136
Negative	17	16	33	
Vascular infiltration				
Positive	10	14	24	0.750
Negative	12	14	26	

[a]$P<0.05$. miR, miRNA; TNM, tumor-node metastasis.

Table II. Multivariate analysis of factors that are associated with overall survival in patients with colorectal cancer.

| | Multivariate analysis | |
Variables	HR (95% CI)	P-value
Age	1.021 (0.989-1.054)	0.197
Sex	0.560 (0.165-1.900)	0.352
Tumor size	0.956 (0.321-2.847)	0.936
Tumor differentiation	1.292 (0.467-3.572)	0.622
TNM stage	9.829 (0.724-133.442)	0.086
Lymph node metastasis	0.257 (0.036-1.838)	0.274
Distant metastasis	6.538 (1.615-26.473)	0.009[a]
miR-490-3p	0.707 (0.205-2.439)	0.583
RAB14	2.019 (0.658-6.202)	0.220

[a]$P<0.05$. CI, confidence interval; HR, hazard ratio;miR, miRNA; TNM, tumor-node metastasis.

cells (Fig. 2B). After upregulating miR-490-3p, proliferative ability was markedly repressed in SW480 and SW620 cells (Fig. 2C). Additionally, SW480 and SW620 cells transfected with miR-490-3p mimics formed markedly fewer colonies compared with the NC controls (Fig. 2D). To further confirm these findings, an *in vivo* study was carried out where SW480 cells were infected with LV-miR-490-3p and LV-miR-NC. Tumors from the LV-miR-490-3p-infected cells grew much more slowly compared with the LV-miR-NC group (Fig. 2E and F). In addition, miR-490-3p levels in the LV-miR-490-3p-infected tumors were higher compared with the control values (Fig. 2G).

miR-490-3p induces cancer cell cycle arrest. miR-490-3p overexpression resulted in the accumulation of SW480 (1.235±0.078 vs. 5.175±0.106%, P<0.01) and SW620 (2.655±0.332 vs. 8.110±0.665%, P<0.01) cells in the G2-M phase (Fig. 3A). However, miR-490-3p overexpression seemed to have no significant effects on apoptosis in CRC cells (Fig. 3B).

miR-490-3p suppresses the invasive ability of CRC cells. To assess the function of miR-490-3p in CRC metastasis, miR-490-3p mimics were transfected into SW480 and SW620 cells. Transwell assays indicated that miR-490-3p overexpression decreased the invasive abilities of SW480 and SW620 cells (Fig. 3C).
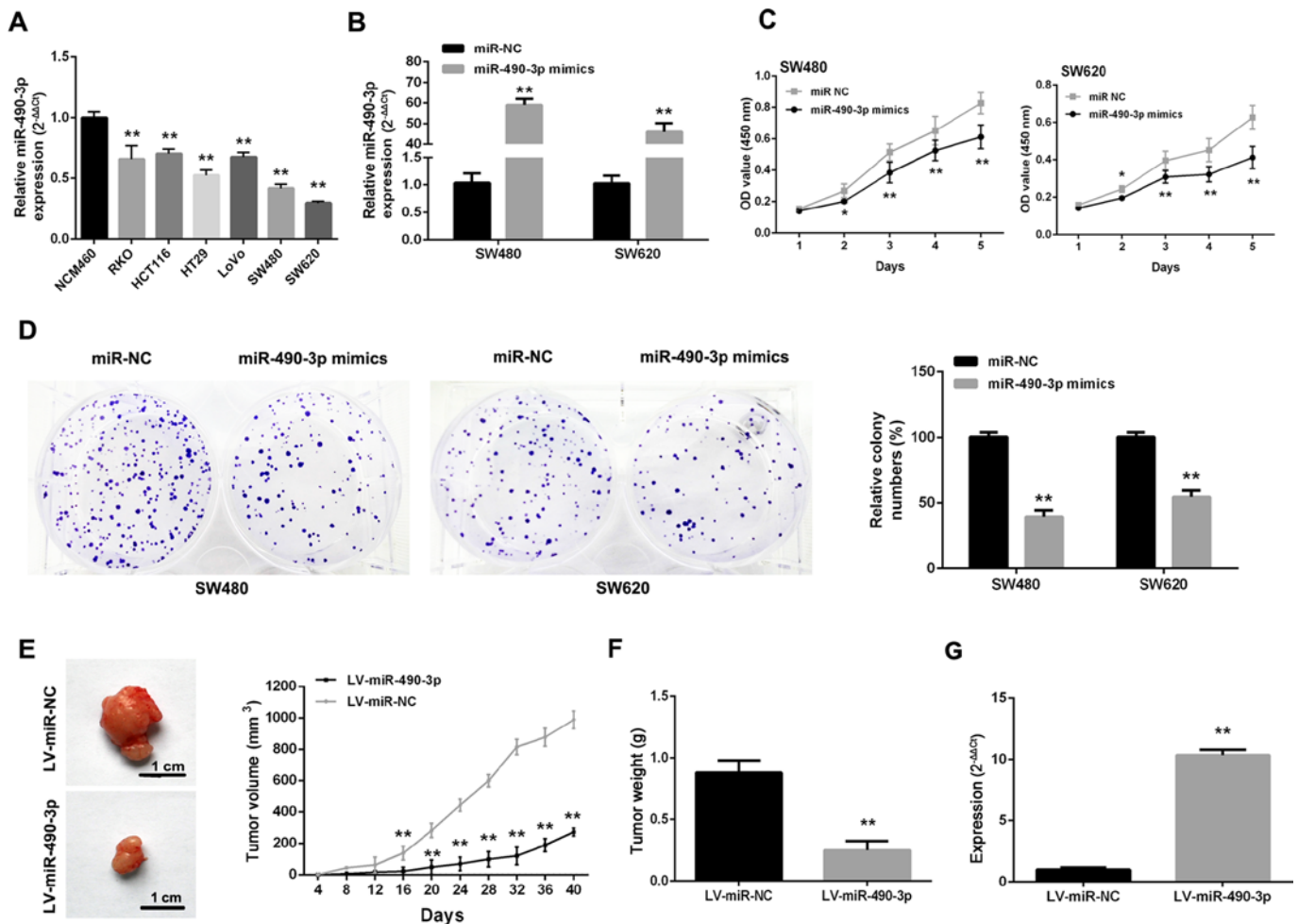
Figure 2. miR-490-3p represses the growth of CRC cells *in vitro* and in mice. (A) Relative miR-490-3p expression levels in 6 CRC cell lines and the normal colorectal cell line, NCM460. Mean gene expression in NCM460 was considered to be 1. (B) Relative expression levels of miR-490-3p upon transfection with miR-490-3p mimics or miR-NC as evaluated by reverse transcription-quantitative polymerase chain reaction. Mean miRNA levels in miR-NC were considered to be 1. (C) Growth curves of SW480 and SW620 cells following the overexpression of miR-490-3p as assessed by Cell Counting Kit-8 assay. (D) Colony formation ability following the overexpression of miR-490-3p. (E) Effects of miR-490-3p on the growth of xenograft CRC tumor in mouse models. Infected SW480 cells were injected into nude mouse models. Tumor growth curves were generated by assessing tumor volumes at 4-day intervals. (F) Following the sacrifice of the mice, the weight of the tumors was quantified. Data are expressed as the mean ± standard deviation. (G) Relative miR-490-3p expression levels in extracted tumors, normalized to the reference control U6 RNA. *P<0.05, **P<0.01. CRC, colorectal carcinoma; miRNA, microRNA; NC, negative control.

miR-490-3p directly targets RAB14 by interacting with its 3'UTR. To explore the mechanisms by which miR-490-3p suppresses the malignant behavior of CRC cells, TargetScan 7.1, microRNA and miRDB were employed to identify potential miR-490-3p targets. The databases revealed that the miRNA responsive element within the 3'UTR of RAB14 is a putative miR-490-3p target (Fig. 4A). Therefore, dual-luciferase reporters comprising the 3'-UTR of RAB14 with wild-type and mutated seed sequence were cloned (Fig. 4A). Following the co-transfection of miR-490-3p or NC mimics with RAB14-UTR-WT or RAB14-UTR-MUT plasmid into SW480 cells, the relative luciferase activity in the wild-type group was markedly reduced, while the mutant group exhibited no significant changes (Fig. 4B). In addition, the effects of miR-490-3p on endogenous RAB14 expression were assessed by RT-qPCR and immunoblotting. Interestingly, the overexpression of miR-490-3p markedly reduced the levels of RAB14 mRNA (Fig. 4C) and protein (Fig. 4D).

Association of miR-490-3p with RAB14 expression in colorectal tissue specimens. Next, the association of miR-490-3p with RAB14 expression was examined in CRC and normal colorectal tissue specimens. RAB14 levels were markedly elevated in CRC tissues compared with non-cancerous specimens (P<0.01; Fig. 5A). Notably, an inverse correlation was obtained between miR-490-3p and RAB14 levels in CRC (r=-0.3700, P<0.05) and normal (r=-0.3202, P<0.01) tissues by Pearson's correlation analysis (Fig. 5B and C).

Upregulation of RAB14 counteracts the inhibitory effects of miR-490-3p on malignant behavior in SW480 cells. To confirm the role of RAB14 in miR-490-3p associated anticancer process, rescue experiments were carried out by upregulating RAB14 after transfecting miR-490-3p mimics. The overexpression of miR-490-3p and RAB14 had no significant effects on proliferation (Fig. 6A) and invasion (Fig. 6B) in SW480 cells.
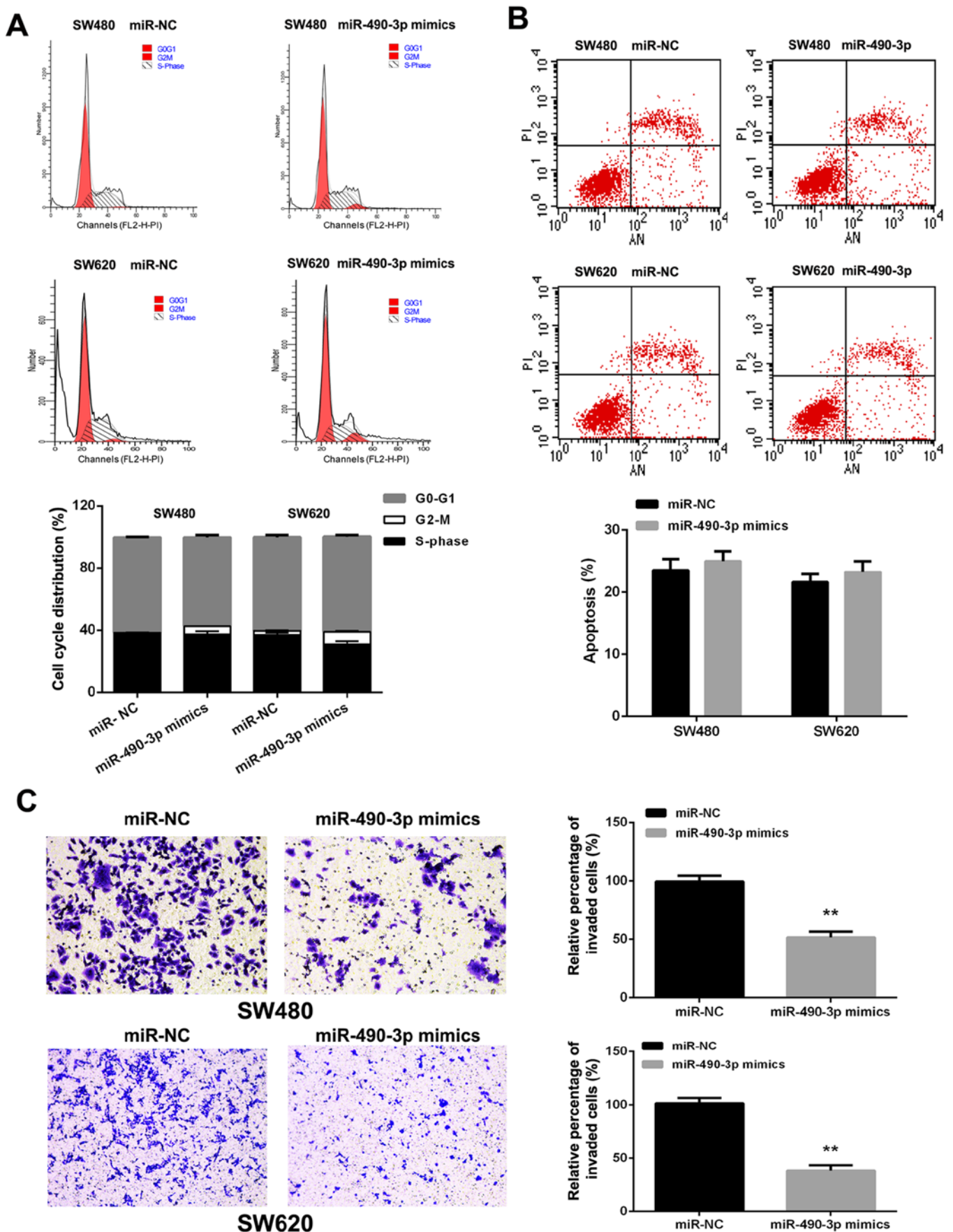
Figure 3. Ectopic miR-490-3p expression promotes G2/M phase arrest and inhibits invasion in CRC cells. (A) Cell cycle distribution was assessed by flow cytometry following treatment with mimics. miR-490-3p promoted cell cycle arrest at the G2-M phase. (B) Transfection with miR-490-3p mimics had no effects on apoptosis in CRC cells as indicated by flow cytometric analysis. (C) Transwell assay was performed to evaluate the invasive abilities of SW480 and SW620 cells following miR-490-3p upregulation. The stained cells in the lower chambers were counted under a microscope (magnification, x200). **P<0.01. CRC, colorectal carcinoma; miRNA, microRNA; NC, negative control.
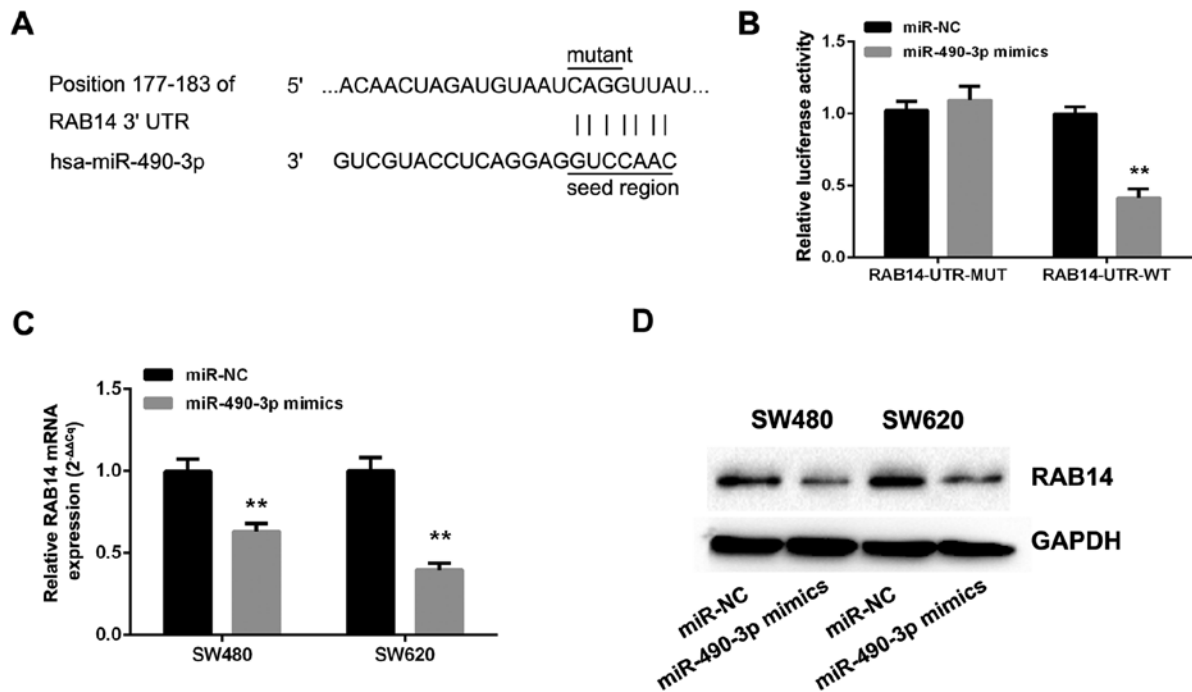
Figure 4. RAB14 is directly targeted by miR-490-3p. (A) Prediction of the binding site of human miR-490-3p to the 3'-UTR of RAB14 by TargetScan 7.1. The mutated binding site of miR-490-3p in the 3'UTR of RAB14 is illustrated. (B) A reporter plasmid comprising wild-type or mutant 3'UTR of RAB14 was co-transfected with miR-490-3p mimics or miR-NC. Relative luciferase activity was determined as firefly luciferase activity normalized to that of *Renilla* luciferase. Effects of miR-490-3p upregulation on RAB14 expression at (C) mRNA and (D) protein levels. Gene and protein expression levels were evaluated 48 and 72 h after transfection by reverse transcription-quantitative polymerase chain reaction and immunoblotting, respectively. **P<0.01. miR, miRNA; MUT, mutated; NC, negative control; UTR, untranslated region; WT, wild-type.
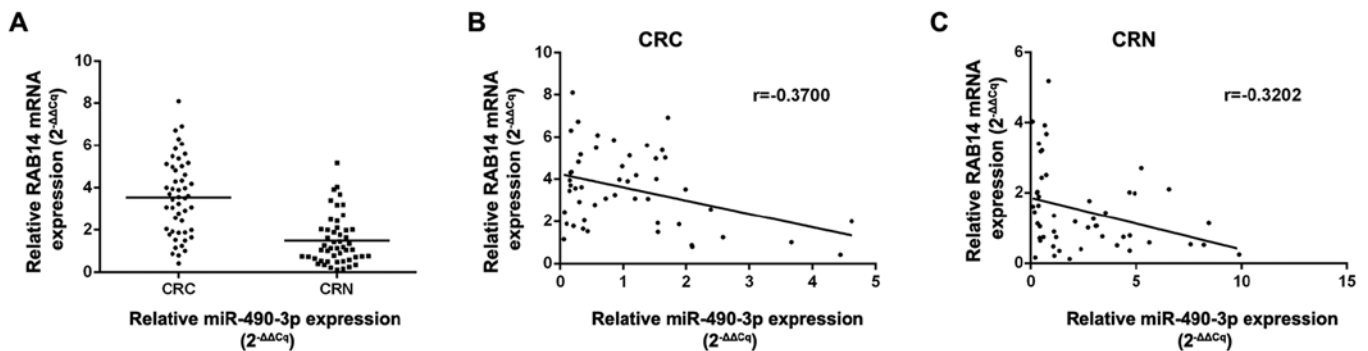

Figure 5. RAB14 is upregulated in CRC and inversely correlated with miR-490-3p expression in tissue samples. (A) Increased expression of RAB14 was detected in CRC specimens compared with adjacent normal counterparts by reverse transcription-quantitative polymerase chain reaction. Pearson's correlation analysis of the association of RAB14 expression with miR-490-3p in (B) CRC and (C) non-cancerous samples. **P<0.01. CRC, colorectal carcinoma; CRN, adjacent normal colorectal samples; miR, miRNA.
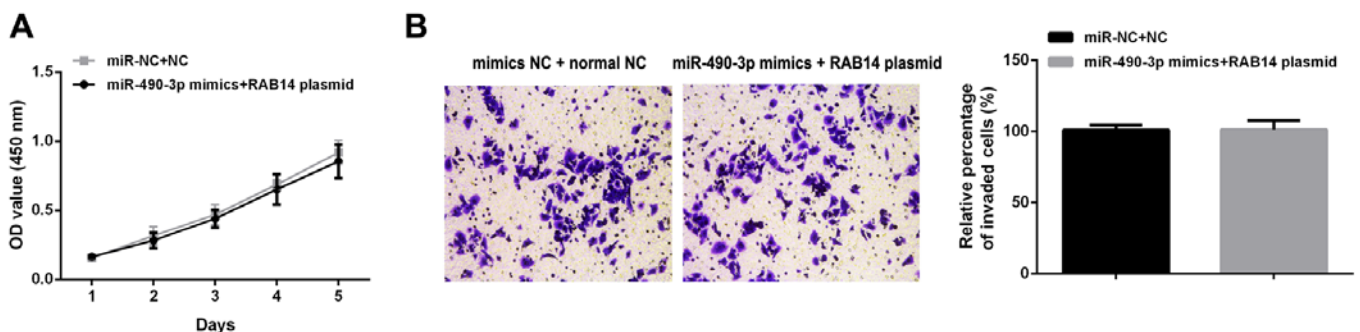

Figure 6. Effects of rescuing miR-490-3p ectopic expression by simultaneous overexpression of RAB14. (A) Cell proliferation was assessed in SW480 cells at 1, 2, 3, 4 and 5 days post-transfection. (B) SW480 cell invasion across an 8-μm pore size membrane containing Matrigel (magnification, x200). miR, miRNA; NC, negative control.

Discussion

The biological roles of many miRNAs have been described in malignancy, revealing that they might function as oncogenes or tumor suppressors (25,26). The present study assessed miR-490-3p, which was reported to be aberrantly expressed in various malignancies (17-20). However, the roles of miR-490-3p in tumorigenesis and cell growth are unclear.

In the present study, the clinicopathological importance of miR-490-3p in CRC was first assessed. The mean miR-490-3p expression in CRC samples were markedly reduced compared with paired adjacent non-cancerous specimens. In addition, miR-490-3p levels in patients with CRC were correlated with the pathological stage and lymph node metastasis. Furthermore, low miR-490-3p expression was associated with poor survival in CRC. As the sample size in the present study was small, additional studies with larger sample sizes are required to confirm the clinical and prognostic values of miR-490-3p in CRC. Additionally, Cox's multivariate analysis revealed distant metastasis as an independent predictive factor of overall survival in CRC.

The overexpression of miR-490-3p markedly suppressed the proliferation and colony formation of CRC cells, inducing cell cycle arrest at the G2/M phase. Reportedly, miR-490-3p regulated cyclin dependent kinase 1 that resulted in G2/M phase arrest in ovarian cancer (19). Therefore in colorectal cancer, miR-490-3p might exert in a similar mechanism. However, in the present study, the upregulation of miR-490-3p had no effects on cell apoptosis. This difference in results might be because the effects of miRNA and its diverse downstream targets are dependent on the type of cancer.

Zhang *et al* (16) revealed that miR-490-3p promoted the growth and metastasis of hepatocellular carcinoma cells by repressing cell apoptosis. By contrast, Shen *et al* (17) reported that miR-490-3p targeted regulator of chromatin subfamily D member 1 (SMARCD1) to stimulate apoptosis in gastric cancer. Moreover, the *in vivo* experiments in the present study firstly confirmed miR-490-3p upregulation markedly inhibited the growth of CRC xenograft tumors in nude mice. These findings strongly indicated that miR-490-3p inhibited tumorigenesis in CRC.

Metastasis results from a multistep-process with cancer cells that respond to multiple intrinsic and extrinsic stimuli, detach from the primary tumor, invade the contiguous stroma, migrate to distant sites and colonize different organs (27,28). Among distortions in the epigenome, aberrant expression or function of miRNAs considerably contributes to metastasis (29,30). An increasing number of studies involving preclinical models of different human tumor types indicated that specific miRNAs have functions in various steps of CRC metastasis (31-33). Therefore, how miRNAs affect CRC metastasis might provide a basis for developing new therapeutics for CRC. As aforementioned, miR-490-3p was closely related to TNM stage and lymph node metastasis. In addition, the overexpression of miR-490-3p resulted in significant repression of invasive ability in CRC cells. These findings suggested that restoring miR-490-3p in CRC might represent a novel therapeutic approach for CRC, particularly in metastatic cases.

The context-dependent roles of miRNAs in various types of cancer are likely to depend on the signaling pathways targeted. miR-490-3p was predicted to target numerous cancer-associated genes, including SMARCD1 in gastric cancer (17), TNKS2 in breast cancer (20), HMGA2 in osteosarcoma (21) and ABCC2 in ovarian cancer (34).

Notably, RAB14 was also predicted to be a target of miR-490-3p. RAB14 belongs to the RAB family of proteins. RAB14 is involved in intracellular vesicle trafficking as well as regulating signal transduction and recycling of diverse membrane receptors (35). RAB14 has a role in the progression of human cancer; however, its biological significance in CRC is largely unknown. The present study assessed the importance of RAB14 in individuals with CRC. Survival analysis indicated no significant difference between the high and low RAB14 expression groups. However, overall survival was prolonged in CRC cases with reduced RAB14 levels. Consistent with these findings, the downregulation of RAB14 in cancer cells suppressed cell proliferation and invasion as observed following the overexpression of miR-490-3p. These results confirmed that RAB14 promotes the progression of CRC.

Finally, whether RAB14 was directly targeted by miR-490-3p was examined. The ectopic expression of miR-490-3p induced marked RAB14 downregulation at the mRNA and protein levels. In addition, in CRC tissues, a low expression level of miR-490-3p was observed with a high expression level of RAB14. Conversely, a high expression level of miR-490-3p was detected with decreased RAB14 in colorectal normal tissues. These findings supported that RAB14 was a direct target of miR-490-3p and that the downregulation of RAB14 constitutes a mechanism by which miR-490-3p suppresses tumor growth.

To further test this hypothesis, a rescue experiment was carried out. The overexpression of RAB14 markedly reversed miR-490-3p-induced inhibition of proliferation and invasion in SW480 cells. However, due to distinct differences in body structures and functions of cells between animals and humans, mouse xenograft models in the present study work do not closely reflect clinical CRC, notably concerning metastasis. Therefore, further *in vivo* investigations are warranted to clarify the effects of miR-490-3p on the metastasis of CRC.

Recently, Xu *et al* (22) and Zheng *et al* (23) reported that miR-490-3p inhibits the metastatic and aggressive phenotype in colorectal cancer by targeting transforming growth factor (TGF)-β and Wnt/β-catenin signaling pathways. TGF-β, a cytokine with multiple biological effects, was first reported as an inducer of epithelia-mesenchymal transition (EMT) in normal mammary epithelial cells. Several subsequent studies reported important roles of TGF-β-induced EMT in tumor metastasis, including colorectal cancer (36). The canonical Wnt signaling pathway is aberrantly activated in the majority of patients with CRC due to mutations in adenomatous polyposis coli or β-catenin, and it was demonstrated that the hyperactivation of Wnt signaling has an essential role in the pathogenesis of CRC (37,38). Recent studies have indicated that TGF-β and TGFβ-induced factor homeobox 1 were able to activate the Wnt/β-catenin signaling pathway in colorectal cancer (39), breast cancer (40) and systemic sclerosis (41). Hou *et al* (5) revealed that the Wnt inhibitor abolished the effect of RAB14 on cell proliferation and Wnt target genes

in ovarian cancer, demonstrating that RAB14 regulated cell aggressiveness via the Wnt/β-catenin pathway. Furthermore, RAB family protein, RAB11, with similar structure to RAB14, was reported to directly regulate the recycling of TGF-β receptors irrespective of the activation state of the receptors (42). Therefore, based on these recent studies, RAB14 might be an upstream key factor in regulating, or at least, affecting the functions of TGF-β and the Wnt/β-catenin signaling pathway. In the present study, miR-490-3p targeted RAB14 to affect the biological functions of colorectal cancer. RAB14 might be the main target of miR-490-3p in regulating the malignant phenotype of colorectal cancer, which remains to be verified in further investigations.

In summary, the present study indicated that human CRC tissue samples and cells have decreased miR-490-3p expression compared with colorectal normal tissues and cells respectively, which was also reflected by a poor prognosis of patients with CRC. Meanwhile, miR-490-3p suppressed tumorigenesis and aggressiveness in CRC by directly targeting RAB14. These findings provide novel insights into targeting miR-490-3p/ RAB14 interactions as a therapeutic option for CRC.

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Availability of data and materials

The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

BW, YY and ZS were responsible for the study design, drafting and editing of the original article, data acquisition and data analysis. BW, CC and HJ performed the experiments. MY, KJ and SW were responsible for data acquisition and analysis. MY, CC and KJ were responsible for data interpretation and methodology. YY, ZS and SW were responsible for supervision. BW, MY, YY and ZS revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The animal studies were conducted according to the recommendations outlined in the Guide for the Care and Use of Laboratory Animals in the Weatherall report. The animal experiments were approved by the Animal Research Committee of the Peking University People's Hospital (Beijing, China).

Patient consent for publication

Written consent for research and publication was obtained from each patient involved in this study.

Competing interests

The authors declare that they have no competing interests.

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