European Review for Medical and Pharmacological Sciences 2018; 22: 4837-4845

MiR-340-5p is a potential prognostic indicator of colorectal cancer and modulates ANXA3

L. YANG[1], W.-L. MEN[2], K.-M. YAN[3], J. TIE[4], Y.-Z. NIE[4], H.-J. XIAO[3,4]

[1]Department of General Surgery, Xianyang Central Hospital, Xianyang, Shaanxi, China
[2]Project Department, Consultancy Program Center, Chinese Academy of Engineering Beijing, China
[3]Department of Oncology, Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, China
[4]State Key Laboratory of Cancer Biology, National Clinical Research Center for Digestive Diseases and Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, Shaanxi, China

Abstract. – OBJECTIVE: MicroRNAs (miRNAs) are increasingly recognized as oncogenes or tumor suppressors in colorectal cancer (CRC). The aim of this study was to explore the expression and functions of miR-340-5p in CRC.

PATIENTS AND METHODS: The expression of miR-340-5p in CRC tissues and cell lines was detected by quantitative RT-PCR. Associations of miR-340-5p expression with clinicopathological factors and overall survival (OS) and progression-free survival (PFS) were statistically evaluated. Luciferase assay, RT-PCR, and Western blot were performed to verify the precise target of miR-340-5p. MTT assay, colony formation and transwell assay were performed to determine the proliferation, migration and invasion, respectively.

RESULTS: Our results showed that miR-340-5p was significantly down-regulated in CRC tissues and cell lines, and was associated with histological grade (p=0.020), lymph nodes metastasis (p=0.003) and TNM stage (p=0.007). Furthermore, Kaplan-Meier and log-rank tests revealed that patients with low expression of miR-340-5p had a shorter OS (p=0.0110) and PFS (p=0.0032) than those with high expression of miR-340-5p. We further validated Annexin A3 (ANXA3) was a direct target of miR-340-5p in CRC. The functional assay showed that up-regulation of miR-340-5p or down-regulation of ANXA3 can both inhibit CRC cell proliferation, migration, and invasion. Besides, the re-expression of ANXA3 reversed the miR-340-5p induced suppression of cell proliferation, migration and invasion.

CONCLUSIONS: Our data demonstrated that miR-340-5p exerted its tumor-suppressive function by directly targeting ANXA3 in CRC, suggesting that miR-340-5p might represent a novel prognostic biomarker and therapeutic target for CRC.

Key Words
miR-340-5p, Annexin A3, Proliferation, Migration, Invasion, Colorectal cancer, Prognosis.

Introduction

Colorectal cancer (CRC) is the 3rd most malignant cancer worldwide and the third most prevalent malignancy in women in China, with more than 200000 new cases reported annually[1,2]. Because of the variations in eating habits and lifestyles, the morbidity and mortality rates of CRC have significantly increased in China[3]. Due to the chemotherapy and radiation therapy, patients with CRC diagnosed at stage I-II have a better prognosis and five-year survival rate is 60-80%[4,5]. However, the 5-year survival rate decreases to estimate 5% in patients with distant metastases[6]. Like other malignancies, the tumorigenesis and progression of CRC are a multistep process which include various genetic and epigenetic changes[7,8]. However, up to date, the molecular mechanism underlying the disease remains largely unclear.

MicroRNAs (miRNAs) are a class of highly conserved, small noncoding RNAs that are approximately 22 nucleotides in length[9]. MiRNAs can degrade target mRNAs or suppress their translation by binding to the 3' UTRs[10]. It has been confirmed that miRNAs are implicated in a series of cellular biological pathways, including cell proliferation, cell cycle, and metastasis[11]. Increasing researches[12,13] have revealed that dysregulation of miRNA expression may be important in the progression and outcome of various diseases, including cancer. Studies have indicated that miRNAs such as miR-326[14], miR-135b[15], MiR-215-5p[16] and miR-874[17] function as oncogenes or tumor suppressors in CRC. miR-340 has been reported to be abnormally expressed in various tumors and participate in the regulation of proliferation and metastasis[18-20]. Although previous studies have

Corresponding Author: Hai-Juan Xiao, MD; e-mail: xhjdoc@163.com

suggested miR-340-5p as a tumor suppressor in CRC, more work need to be done to further investigate the role of miR-340-5p in CRC.

Annexin A3 (ANXA3), located on human chromosome 4q13-q22, belongs to annexin family[21]. The members of annexins are involved in many physiological activities, including anti-inflammatory, signal transduction cell proliferation and apoptosis[22]. Importantly, growing evidence shows that several annexins, such as Annexin A1 and Annexin A2, play an important role in development and progression of various tumors[23,24]. Nevertheless, the effect of ANXA3 has not been so much reported as compared to other annexins. ANXA3 was reported to be up-regulated in CRC, suggesting that ANXA3 functioned as a tumor promoter, which was confirmed by *in vitro* and *in vivo* assay[25,26]. Thus, elucidating the molecular mechanism of ANXA3 regulation in CRC is important for future CRC study.

In the present work, we detected the expression levels of miR-340-5p and its prognostic value in CRC patients. Then, we also identified the function of miR-340-5p on proliferation and metastasis in CRC cell lines and discovered the potential regulatory mechanism involved in ANXA3.

Patients and Methods

Patients

Tumor tissues and adjacent non-tumor tissues were obtained from 107 CRC patients at at Xianyang Central Hospital and Affiliated Hospital of Shaanxi University of Chinese Medicine from January 2012 to February 2013. All resected tissue samples were immediately stored at -80°C until RNA or protein isolation. The diagnosis of all samples was histopathologically confirmed by two pathologists. None of the patients received any preoperative chemotherapy or radiotherapy. Complete clinical information of the patients was available. The clinicopathological characteristics of all patients were shown in Table I. This study was approved by the Ethics Committee of Xianyang Central Hospital and written informed consent was obtained from all patients.

Cell Culture and Transfection

The human CRC cell lines SW480, HCT116, LOVO and HT-29 and the human colonic epithelial cells HCoEpiC were obtained from Central Laboratory of Xianyang Central Hospital. Cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with 10% fetal bovine serum (FBS, HyClone, South Logan, UT, USA), penicillin (100 U/ml), and streptomycin (100 μg/ml), at 37°C in a humidified atmosphere containing 5% CO_2.

miR-340-5p mimics, pcDNA.3.1 ANXA3 and ANXA3 interference RNA lentiviral vector and their negative controls were designed and purchased from GenePharma Company (Hangzhou, Zhejiang, China). Transfection was using Lipofectamine 2000 Reagent (Invitrogen, Haidian, Beijing, China) in Opti-MEM (Addgene, Xiacheng, Nanjing, China). Then, the groups were designed as follows: 1) negative control group, 2) miR-340-5p mimics group, 3) ANXA3 shRNA group and 4) mixed group (pcDNA.3.1 ANXA3 and miR-340-5p mimics transfected). After 48 hours, cells were collected for Western blot, qRT-PCR analyses, and *in vitro* assay.

Real-Time Quantitative PCR

Total RNA was isolated from tissues and cell lines using the TRIzol Total RNA Reagent (Invitrogen, Carlsbad, CA, USA). First-strand complementary DNAs (cDNAs) were synthesized using 1 μg RNAs by PrimeScript 1st Strand cDNA Synthesis Kit (Applied Biosystems, Foster City, CA, USA). miR-340-5p levels were measured using Taqman MicroRNA Assays (Applied Biosystems, Foster City, CA, USA). qRT-PCR was performed with the standard SYBR-Green PCR kit on ABI 7500 system (Applied Biosystems, Foster City, CA, USA). GAPDH was used as the internal controls for normalization. The relative expression of miR-340-5p and ANXA3 mRNA was calculated by the $2^{-\Delta\Delta C}$ method. The primers were synthesized by TransGen Biotech. (Haidian, Beijing, China). The primers sequences used for the qPCR were as follows: miR-340-5p forward, 5'-GCGGTTATAAAGCAATGAGA-3' and reverse, 5'-GTGCGTGTCGTGGAGTCG-3'; GAPDH forward, 5'-GCTGGCGCTGAGTACGTCGTGGAGT-3' and reverse, CACAGTCTTCTGGGT GGCAGTGATGG-3'. All of the PCR reactions were repeated three times.

Dual Luciferase Reporter Assay

The target gene of miR-340-5p was predicted by TargetScan. The dual-luciferase reporter plasmids, p3'-UTR-ANXA3-mt and p3'-UTR-CYLD-mut (containing the mutant ANXA3 3'UTR; mut), were constructed. For the luciferase reporter assay, cells were seeded in 96-well plates

Table I. Correlation between miR-340-5p expression and different clinicopathological features in CRC patients.

Parameters	Group	Total	miR-340-5p expression		*p*-value
			High	Low	
Gender	Male	61	31	30	0.596
	Female	46	21	25	
Age (years)	< 60	53	28	25	0.386
	≥ 60	54	24	30	
Tumor size (cm)	< 5	54	30	24	0.146
	≥ 5	53	22	31	
Histological grade	Well and moderately	64	37	27	0.020
	Poorly	43	15	28	
Local invasion	T1-T2	65	36	29	0.081
	T3-T4	42	16	26	
Lymph nodes metastasis	Negative	65	39	26	0.003
	Positive	42	13	29	
TNM stage	I-II	62	37	25	0.007
	III-IV	45	15	30	

and transfected with 50 nM of miR-340-5p mimics or NC, and 100 ng of above luciferase reporter plasmid using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Two days later, the CRC cells were harvested and lysed in passive lysis buffer, and reporter activity was measured at 48 h after transfection using a dual-luciferase reporter assay system (Promega, Madison, WI, USA).

Western Blot Assay

Total proteins of CRC cells were collected using the Total Protein Extraction Kit (KeyGen, Nanjing, China). Equivalent amounts of protein were separated through 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane (PVDF; Millipore, Billerica, MA, USA). Next, 5% skim milk were added to block the membrane for 2 h. The membranes were incubated with primary antibodies, rabbit anti-ANXA3 and mouse anti-GADPH (both from Peking university, Beijing, China) at 4°C overnight. Following incubation with the horseradish peroxidase (HRP)-labeled secondary antibody (Invitrogen, Carlsbad, CA, USA). Finally, immune-reactive protein bands were detected with an odyssey scanning system (Li-Cor, Lincoln, NE, USA).

Cell Proliferation

Cells were seeded into a 96-well plate at 3000 cells per well with 100 µl cultured medium and cultured at 37°C, 5% CO_2. At the start of harvesting, 20 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution were added into each well. Subsequently, 200 ml of dimethyl sulfoxide (DMSO) were added to each well to dissolve the crystals. The cell proliferation curves were plotted using the absorbance at 490 nm at each time point.

Colony Formation Assay

The cells transfected with the vectors were seeded into 6-well plates at 1,000/well and maintained in media containing 10% fetal bovine serum (FBS), replacing the medium every 4 days. Colonies were fixed with methanol 12 days later and stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). Next, the colony forming units were recorded and counted.

Migration and Invasion Assays

The migration and invasion of SW480 cells were evaluated with transwell assays. 3000 SW480 cells were seeded to the top chamber in 200 µL of serum-free medium and 400 µL of medium with 5% serum were added to the bottom. For the invasion experiments, the upper chamber was coated with 70 µL of Dulbecco's Modified Eagle Medium (DMEM) and Matrigel mixture. After 48 h incubation, non-invaded cells were carefully wiped out with a cotton bud. Cells on the lower surface of the membranes were then counted with an inverted microscope.

Statistical Analysis

All statistical analyses were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was determined using Student's *t*-test. The relationship between miR-340-5p expression level and various clinicopathological characteristics was analyzed by x^2-test. Overall survival (OS) and progression-free-survival (PFS) were calculated by Kaplan-Meier survival analysis. A probability value of less than 0.05 was chosen for statistical significance.

Results

The Expression of miR-340-5p was Downregulated in CRC Tissues and Cell Lines

We firstly detected the expression levels of miR-340-5p in CRC tissues, cell lines and normal controls by RT-qPCR. As shown in Figure 1A, the expression levels of miR-340-5p were found to be distinctly decreased in CRC tissues compared to the paired adjacent noncancerous tissues ($p<0.01$). Then, we further determined whether dysregulation of miR-340-5p in CRC cell lines. As shown in Figure 1B, the results showed that miR-340-5p was down-expressed in CRC cell lines, including HT-29, LOVO, SW480, and HCT116 cell lines, in comparison to that of in the human colonic epithelial cells (HCoEpiC). We concluded that dysregulation of miR-340-5p might be associated with the development of CRC.

Decreased Levels of miR-340-5p Are Predictive of Poor Prognosis in Patients with CRC

In order to analyze the correlation between miR-340-5p expression level and clinicopathological features, the 107 CRC patients were classified into two groups (High and Low) according to the median expression level of miR-340-5p.

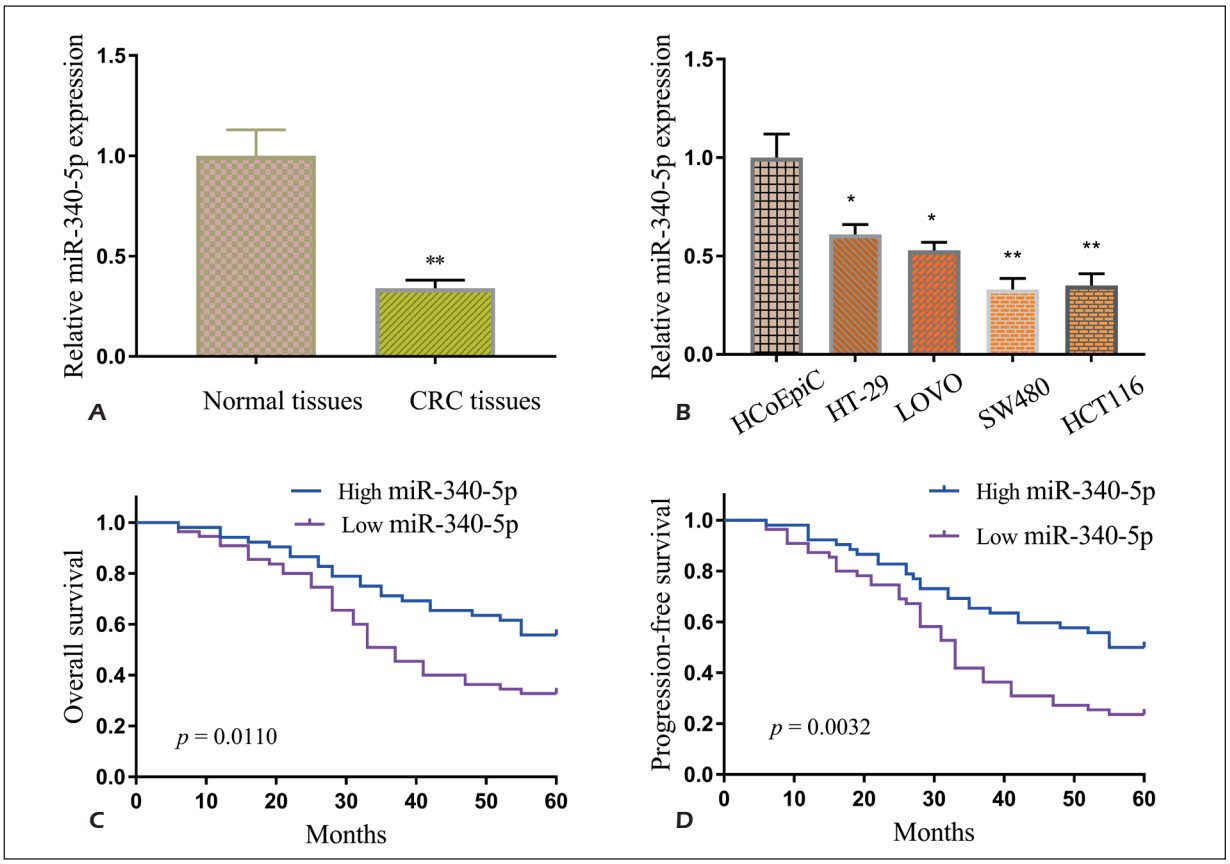

Figure 1. Expression of miR-340-5p in human CRC tissues and its correlation with prognosis of CRC patients. **A**, The expression of miR-340-5p was examined in 107 paired human CRC and matched normal tissues via qRT-PCR. **B**, qRT-PCR analysis of miR-340-5p expression in four CRC cell lines (HT-29, LOVO, SW480, and HCT116) and the human colonic epithelial cells (HCoEpiC). **C-D**, Kaplan-Meier curves for overall survival and progression-free survival in patients with CRC based on miR-340-5p expression levels. Statistically significant differences were observed (*$p<0.05$, **$p<0.01$).

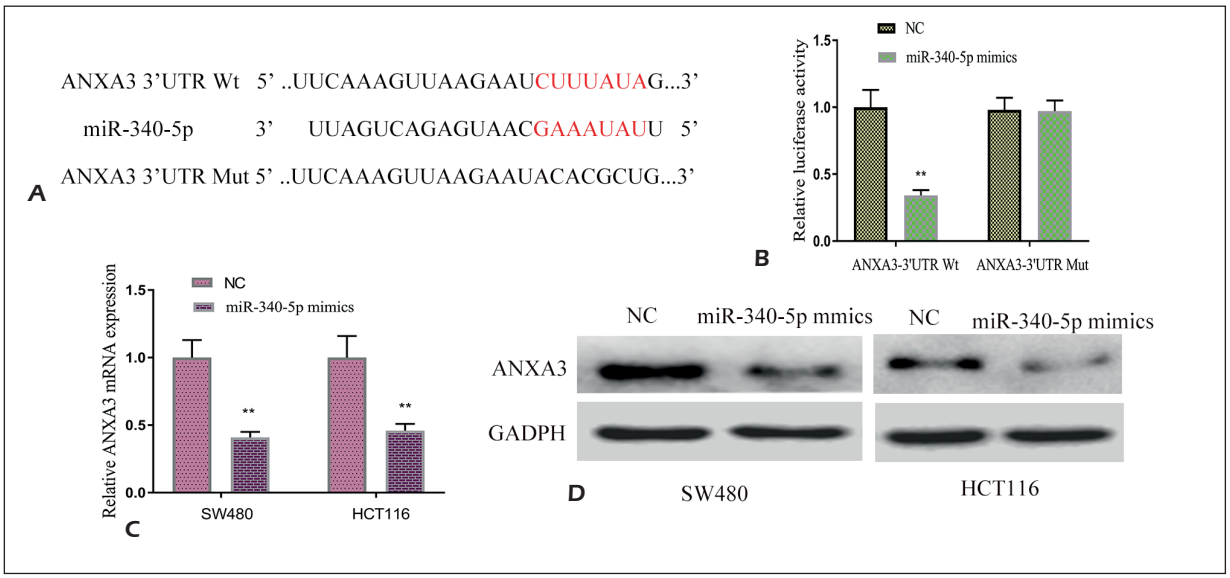

Figure 2. MiR-340-5p suppresses ANXA3 expression by directly targeting the ANXA3 3' UTR. **A**, The predicted binding sites of miR-340-5p in the wild-type ANXA3 3'-UTR and the mutant sequence. **B**, Luciferase activity assay was performed to measure the interaction between ANXA3 3'-UTR and miR-340-5p. **C**, ANXA3 mRNA expression levels were detected by qRT-PCR in SW480 and HCT116 cells transfected with the miR-340-5p mimics or NC. **D**, ANXA3 protein expression was measured by Western blot in SW480 and HCT116 cells transfected with the miR-340-5p mimics or NC. Statistically significant differences were observed (*p<0.05, **p<0.01).

The results were shown in Table I. It was observed that decreased miR-340-5p expression was significantly correlated with histological grade (p=0.020), lymph nodes metastasis (p=0.003) and TNM stage (p=0.007). However, no significant correlation between miR-340-5p expression and gender, age, tumor size or local invasion was observed (all p>0.05). To further explore whether miR-340-5p expression predicted prognosis of CRC patients, we performed Kaplan-Meier analysis. As shown in Figure 1C and 1D, patients with low expression of miR-340-5p had shorter OS (p=0.0110) and PFS (p<0.0032) as compared with the miR-340-5p -high group. Our results indicated that miR-340-5p may serve as a prognostic marker in CRC.

ANXA3 is a Direct Target of miR-340-5p

Previous study[27] has revealed that forced miR-340-5p expression could suppress tumor growth of CRC. However, the potential mechanism remains unclear. Then, it was found that ANXA3 mRNA was a potential target of miR-340-5p after computational analysis using TargetScan (Figure 2A). Next, whether miR-340-5p directly targeted ANXA3 in CRC was investigated using a Luciferase reporter system, which was employed by co-transfection of miR-340-5p and luciferase reporter plasmids containing 3'UTR of ANXA3, or mutated ANXA3. Figure 2B showed that up-regulation of miR-340-5p markedly inhibited the luciferase activity of the ANXA3 3-Wt 3'UTR, without having an effect on ANXA3 3-Mut 3'UTR in the SW480 cells. Furthermore, qRT-PCR and Western blot analyses confirmed that forced miR-340-5p expression significantly downregulated the expression of SATB1 at the mRNA and protein levels in both SW480 and HCT116 cell lines (Figure 2C and 2D). Collectively, these results revealed that ANXA3 may be a direct target of miR-340-5p in CRC.

MiR-340-5p Promoted Cell Proliferation of SW480 Cells Through Targeting ANXA3

In order to explore the inhibitor effect of miR-340-5p on the proliferation of SW480 cells, we divided SW480 into four groups. qRT-PCR was performed to detect the expression levels of miR-340-5p and ANXA3 mRNA in SW480 after transfection. As shown in Figure 3A, up-regulation of miR-340-5p was observed in the miR-340-5p mimics and Mixed groups, while down-regulation of ANXA3 mRNA was observed in miR-340-5p mimics and ANXA3 shRNA groups (Figure 3B). The results of the MTT assay showed that cell proliferation was significantly impaired in SW480 cells transfected with

miR-340-5p mimics and ANXA3 shRNA. However, there is no significant difference between the Mixed group and the control group (Figure 3C). To further determine the effect of miR-340-5p on long-term SW480 cell proliferation, colony formation assays were performed. The results also showed that cell proliferation was significantly impaired in SW480 cells transfected with miR-340-5p mimics and ANXA3 shRNA. In addition, there is no significant difference between the Mixed group and the control group (Figure 3D). Taken together, our results showed that miR-340-5p exerted its inhibitory effect on CRC cell proliferation through targeting ANXA3.

MiR-340-5p suppressed the Migration and Invasion of SW480 Cells by Targeting ANXA3

Then, we further explored whether miR-340-5p suppressed migration and invasion of SW480 cells by targeting ANXA3. The result of transwell experiment showed that SW480 cells transfected with miR-340-5p mimics and ANXA3 shRNA had weaker migration compared with the control group (Figure 4A), while there is no significant difference between the Mixed group and the control group. Moreover, we also observed that SW 480 cells transfected with miR-340-5p mimics and ANXA3 shRNA had weaker migration compared with the control group (Figure 4B). Taken together, our results suggested that miR-340-5p exerted its inhibitory effect on migration and invasion of CRC through targeting ANXA3.

Discussion

MiR-340-5p has been found aberrantly deregulated in tumorigenesis. For instance, Huang et al[28] reported that miR-340 was significantly lowly expressed in prostate cancer and its overexpression suppressed prostate cancer cell proliferation and migration by modulating the MDM2-p53 pathway. Hou et al[29] showed that miR-340 functioned as a negative regulator in progression of breast cancer by targeting ZEB. Li et al[30] found that overexpression

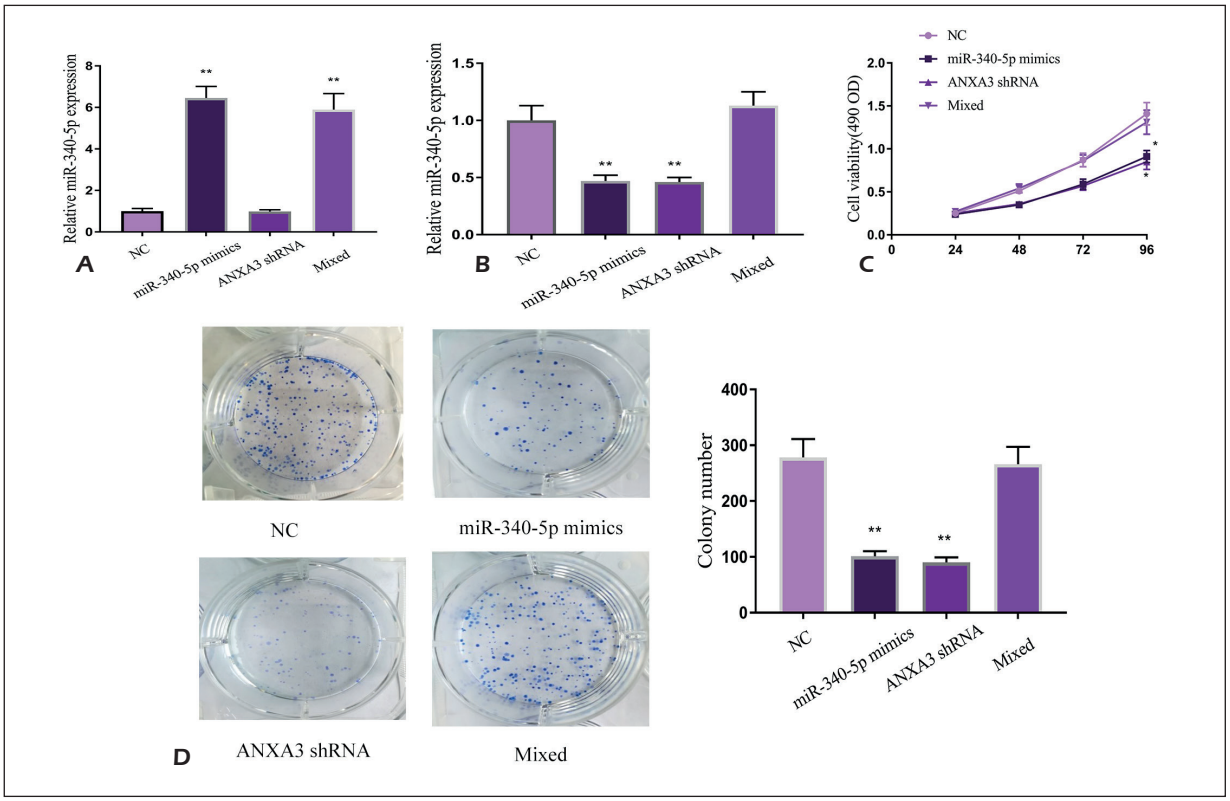

Figure 3. MiR-340-5p suppresses SW480 cell growth by targeting ANXA3. **A**-**B**, The expression levels of miR-340-5p and ANXA3 mRNA in SW480 cells were determined after transfection. **C**, MTT assay was performed to measure the cellular viability. **D**, Colony formation assay was used to detect the colony formation activity. Statistically significant differences were observed (*p<0.05, **p<0.01).

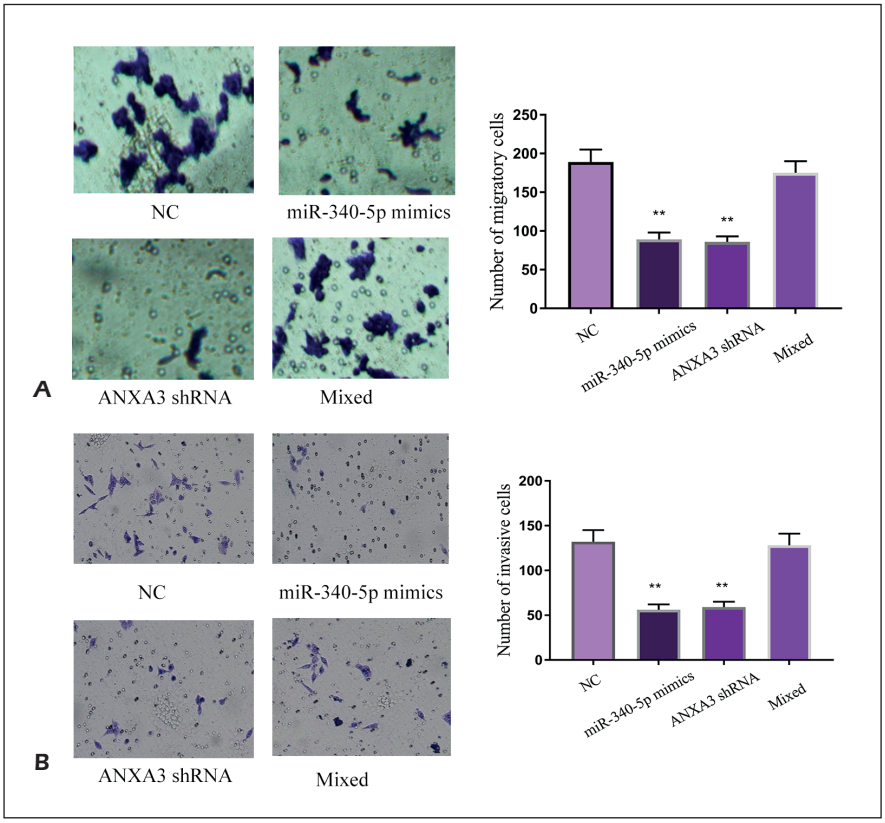

Figure 4. MiR-340-5p inhibits the invasion and migration of SW480 cells. (**A**-**B**) The migration and invasion abilities of SW480 cells were analyzed using transwell migration and invasion assay. Statistically significant differences were observed (*p<0.05, **p<0.01).

of miR-340 induced cell apoptosis and inhibited metastasis in ovarian cancer cells by downregulation of NF-κB1. Those results suggested miR-340 as a tumor suppressor in tumorigenesis and progression of tumors. For CRC, it had been found that decreased expression of miR-340 was associated with poor prognosis of CRC patients, and forced miR-340 expression resulted in CRC growth inhibition by targeting RLIP76[31,32]. However, the potential mechanism by which miR-340 suppressed CRC behaviors remains largely unclear.

In the present study, on line with previous study, our results from RT-PCR also showed miR-340-5p down-regulation in both CRC tissues and cell lines. By clinical assay, we found that decreased miR-340-5p expression was significantly correlated with histological grade, lymph nodes metastasis and TNM stage, suggesting that down-regulation of miR-340-5p contributed to the clinical progression of CRC patients. Moreover, Kaplan-Meier analysis revealed that patients with decreased miR-340-5p expression had poorer OS and PFS than those with elevated expression of miR-340-5p. These samples from our hospital supported miR-340-5p as a tumor suppressor in CRC.

ANXA3 has been found to play critical roles in the regulation of molecular and cellular processes. Also, ANXA3 have been reported to be closely correlated with various cancers and serve as an oncogene[33]. It was reported that ANXA3 was highly expressed in breast cancer and associated with poor prognosis of breast cancer patients. *In vitro* assay indicated that ANXA3 promoted cell proliferation of breast cancer by affecting the Bcl-2/Bax balance[34]. In addition, up-regulation of ANXA3 was observed in gastric cancer and its knockdown inhibited cell proliferation and tumor growth. Similarly, high ANXA3 expression was associated with poor prognosis of gastric cancer patients[35]. Furthermore, dysregulation of ANXA3 was found in CRC patients[36]. Bai et al[37] reported that inhibition of ANXA3 could suppressed the invasion of CRC cells through the up-regulation of p53. They also confirmed that ANXA3 was a target gene of miR-495. Those results indicated that ANXA3 acted as a tumor promoter in various tumors, including CRC, and encouraged us to further explore the potential mechanism of ANXA3 in progression of CRC. Then, we found that there was a putative binding site in the 3' UTR of ANXA3 mRNA to which miR-340-5p seed sequence can

bind by searching TargetScan. Then, we performed dual luciferase reporter gene assay and the results confirmed that miR-340-5p directly bound with the 3'-UTR of ANXA3 mRNA in SW480 cells. In addition, both RT-PCR and Western blot indicated that up-regulation of miR-340-5p could suppress the levels of ANXA3 at both mRNA and protein levels. Moreover, a series of *in vitro* experiments also confirmed that miR-340-5p suppressed CRC proliferation and metastasis by targeting ANXA3. Overall, these data suggested that miR-340-5p was a direct regulator of ANXA3 expression in CRC.

Conclusions

We found that the low expression of miR-340-5p levels was associated with poor PFS and OS in CRC patients. In addition, ectopic miR-340-5p expression inhibited CRC cell proliferation, migration, and invasion through direct targeting of ANXA3. Therefore, miR-340-5p is a valuable marker for CRC progression and prognosis.

Acknowledgements

This study was supported by The National Natural Science Foundation of China, Grant numbers: 81421003; National Clinical Research Center for Digestive Diseases,Grant numbers: 2015BAI13B07;China's Post-doctoral Science Fund,Grant number: 2017M613351

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg 2009; 22: 191-197.
2) Zheng ZX, Zheng RS, Zhang SW, Chen WQ. Colorectal cancer incidence and mortality in China, 2010. Asian Pac J Cancer Prev 2014; 15: 8455-8460.
3) Chen YS, Xu SX, Ding YB, Huang XE, Deng B, Gao XF, Wu DC. Colorectal cancer screening in high-risk populations: a survey of cognition among medical professionals in Jiangsu, China. Asian Pac J Cancer Prev 2014; 14: 6487-6491.
4) Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med 2005; 352: 476-487.
5) Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer 2009; 9: 489-499.
6) Din FV, Theodoratou E, Farrington SM, Tenesa A, Barnetson RA, Cetnarskyj R, Stark L, Porteous ME, Campbell H, Dunlop MG. Effect of aspirin and NSAIDs on risk and survival from colorectal cancer. Gut 2010; 59: 1670-1679.
7) Song M, Garrett WS, Chan AT. Nutrients, foods, and colorectal cancer prevention. Gastroenterology 2015; 148: 1244-1260.
8) Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJ, Watanabe T. Colorectal cancer. Nat Rev Dis Primers 2015; 1: 15065.
9) Inui M, Martello G, Piccolo S. MicroRNA control of signal transduction. Nat Rev Mol Cell Biol 2010; 11: 252-263.
10) He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522-531.
11) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
12) Bernardo BC, Ooi JY, Lin RC, McMullen JR. miRNA therapeutics: a new class of drugs with potential therapeutic applications in the heart. Future Med Chem 2015; 7: 1771-1792.
13) Zhang Y, Yang P, Wang XF. Microenvironmental regulation of cancer metastasis by miRNAs. Trends Cell Biol 2014; 24: 153-160.
14) Wu L, Hui H, Wang LJ, Wang H, Liu QF, Han SX. MicroRNA-326 functions as a tumor suppressor in colorectal cancer by targeting the nin one binding protein. Oncol Rep 2015; 33: 2309-2318.
15) Akbari A, Ghahremani MH2, Mobini GR3, Abastabar M4, Akhtari J5, Bolhassani M6, Heidari M7. Down-regulation of miR-135b in colon adenocarcinoma induced by a TGF-β receptor I kinase inhibitor (SD-208). Iran J Basic Med Sci 2015; 18: 856-861.
16) Vychytilova-Faltejskova P, Merhautova J, Machackova T, Gutierrez-Garcia I, Garcia-Solano J, Radova L, Brchnelova D, Slaba K, Svoboda M, Halamkova J, Demlova R, Kiss I, Vyzula R, Conesa-Zamora P, Slaby O. MiR-215-5p is a tumor suppressor in colorectal cancer targeting EGFR ligand epiregulin and its transcriptional inducer HOXB9. Oncogenesis 2017; 6: 399.
17) Zhao B, Dong AS. MiR-874 inhibits cell growth and induces apoptosis by targeting STAT3 in human colorectal cancer cells. Eur Rev Med Pharmacol Sci 2016; 20: 269-277.
18) Yu W, Zhang G, Lu B, Li J, Wu Z, Ma H, Wang H, Lian R. MiR-340 impedes the progression of laryngeal squamous cell carcinoma by targeting EZH2. Gene 2016; 577: 193-201.
19) Fiore D, Donnarumma E, Roscigno G, Iaboni M, Russo V, Affinito A, Adamo A, De Martino F, Quintavalle C, Romano G, Greco A, Soini Y, Brunetti A6,7, Croce CM, Condorelli G. miR-340 predicts glioblastoma survival and modulates key cancer hallmarks through down-regulation of NRAS. Oncotarget 2016; 7: 19531-19547.
20) Wei P, Qiao B, Li Q, Han X, Zhang H, Huo Q, Sun J. microRNA-340 suppresses tumorigenic potential

of prostate cancer cells by targeting high-mobility group nucleosome-binding domain 5. DNA Cell Biol 2016; 35: 33-43.
21) Harashima M, Niimi S, Koyanagi H, Hyuga M, Noma S, Seki T, Ariga T, Kawanishi T, Hayakawa T. Change in annexin A3 expression by regulatory factors of hepatocyte growth in primary cultured rat hepatocytes. Biol Pharm Bull 2006; 29: 1339-1343.
22) Laohavisit A, Davies JM. Annexins. New Phytol 2011; 189: 40-53.
23) Biaoxue R, Xiguang C, Shuanying Y. Annexin A1 in malignant tumors: current opinions and controversies. Int J Biol Markers 2014; 29: 8-20.
24) Wang CY, Lin CF. Annexin A2: its molecular regulation and cellular expression in cancer development. Dis Markers 2014; 2014: 308976.
25) Yip KT, Das PK, Suria D, Lim CR, Ng GH, Liew CC. A case-controlled validation study of a blood-based seven-gene biomarker panel for colorectal cancer in Malaysia. J Exp Clin Cancer Res 2010; 29: 128.
26) Xie YQ, Fu D, He ZH, Tan QD. Prognostic value of Annexin A3 in human colorectal cancer and its correlation with hypoxia-inducible factor-1α. Oncol Lett 2013; 6: 1631-1635.
27) Sun Y, Zhao X, Zhou Y, Hu Y. miR-124, miR-137 and miR-340 regulate colorectal cancer growth via inhibition of the Warburg effect. Oncol Rep 2012; 28: 1346-1352.
28) Huang K, Tang Y, He L, Dai Y. MicroRNA-340 inhibits prostate cancer cell proliferation and metastasis by targeting the MDM2-p53 pathway. Oncol Rep 2016; 35: 887-895.
29) Hou LK, Yu Y, Xie YG, Wang J, Mao JF, Zhang B, Wang X, Cao XC. miR-340 and ZEB1 negative feedback loop regulates TGF-β- mediated breast cancer progression. Oncotarget 2016; 7: 26016-26026.
30) Li P, Sun Y, Liu Q. MicroRNA-340 Induces Apoptosis and inhibits metastasis of ovarian cancer cells by inactivation of NF-x03BA;B1. Cell Physiol Biochem 2016; 38: 1915-1927.
31) Takeyama H, Yamamoto H, Yamashita S, Wu X, Takahashi H, Nishimura J, Haraguchi N, Miyake Y, Suzuki R, Murata K, Ohue M, Kato T, Takemasa I, Mizushima T, Ishii H, Mimori K, Doki Y, Mori M. Decreased miR-340 expression in bone marrow is associated with liver metastasis of colorectal cancer. Mol Cancer Ther 2014; 13: 976-985.
32) Zhang LL, Xie FJ, Tang CH, Xu WR, Ding XS, Liang J. miR-340 suppresses tumor growth and enhances chemosensitivity of colorectal cancer by targeting RLIP76. Eur Rev Med Pharmacol Sci 2017; 21: 2875-2886.
33) Wu N, Liu S, Guo C, Hou Z, Sun MZ. The role of annexin A3 playing in cancers. Clin Transl Oncol 2013; 15: 106-110.
34) Zeng C, Ke Z, Song Y, Yao Y, Hu X, Zhang M, Li H, Yin J. Annexin A3 is associated with a poor prognosis in breast cancer and participates in the modulation of apoptosis in vitro by affecting the Bcl-2/Bax balance. Exp Mol Pathol 2013; 95: 23-31.
35) Wang K, Li J. Overexpression of ANXA3 is an independent prognostic indicator in gastric cancer and its depletion suppresses cell proliferation and tumor growth. Oncotarget 2016; 7: 86972-86984.
36) Yip KT, Das PK, Suria D, Lim CR, Ng GH, Liew CC. A case-controlled validation study of a blood-based seven-gene biomarker panel for colorectal cancer in Malaysia. J Exp Clin Cancer Res 2010; 29: 128.
37) Bai Z, Wang J, Wang T, Li Y, Zhao X, Wu G, Yang Y, Deng W, Zhang Z. The MiR-495/Annexin A3/P53 Axis inhibits the invasion and EMT of colorectal cancer cells. Cell Physiol Biochem 2017; 44: 1882-1895.