European Review for Medical and Pharmacological Sciences 2019; 23: 5741-5751

Decreased expression of miR-551b predicts poor prognosis and promotes tumorigenesis by targeting PTP4A3 in human colorectal cancer

H.-Y. SUN[1], Z.-C. QU[2], D.-M. LIU[1], W. ZHANG[1], G. LIU[3], L. ZHU[1]

[1]Department Gastroenterology, Dongzhimen Hospital Beijing University of Chinese Medicine, Dongcheng, Beijing, China
[2]Emergency Department, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Dongcheng, Beijing, China
[3]Department of The Febrile Disease, University of Chinese Medicine, Dongcheng, Beijing, China

Abstract. – OBJECTIVE: MiR-551b has been reported to display tumor-suppressive and oncogenic potential in several cancers, but there has been no study on the roles of miR-551b in colorectal cancer (CRC). In this work, we aimed to explore the potential functions and mechanisms of miR-551b in the modulation of the CRC progression.

PATIENTS AND METHODS: The expressions of miR-551b were examined in 122 pairs of CRC cancer tissues and adjacent non-tumor samples by Real Time-Polymerase Chain Reaction (RT-PCR). The clinical significance of miR-551b in CRC patients was explored using a Chi-square test, Kaplan-Meier assays, and multivariate analysis. MiR-551b mimics and inhibitors were used to establish miR-551b upregulation and downregulation models in CRC cells to examine the functions of miR-551b on cells proliferation, migration, invasion, and apoptosis. Dual-luciferase reporter assays were conducted for the validation of the possible modulation of a putative target of miR-551b.

RESULTS: We showed that miR-551b was significantly down-regulated in CRC tissues and cell lines. It was observed that miR-551b expressions were correlated with lymph nodes metastasis, TNM stage, and poor prognosis. Multivariate analysis identifies low level of miR-551b expressions as an independent predictor for a shorter overall survival. The functional assessment suggested that forced miR-551b expression distinctly suppressed CRC cells growth, invasion, and migration, while the suppression of miR-551b displayed the opposite trend. Mechanistic studies confirmed that PTP4A3 was identified as a direct target of miR-551b in CRC. Interesting observations revealed that the cells capacities were higher in miR-551b +PTP4A3 group, when compared with those in miR-551b group, indicating that up-regulation of PTP4A3 rescued the repressive functions of miR-551b overexpression on CRC cells growth and migration.

CONCLUSIONS: The findings of our study first showed that miR-551b, a potential tumor suppressor, may be used as a promising prognostic predictor and therapeutic target for CRC.

Key Words:
MiR-551b, Colorectal cancer, Prognosis, PTP4A3, Metastasis.

Introduction

Colorectal cancer (CRC) is one of the most frequent cancers and the third leading cause of cancer-related deaths in both men and women, with an estimated 1.5 million new cases and approximately 78,500 deaths in 2016 worldwide[1,2]. In China, the incidence and mortality rates display an increased trend in recent years due to the changes of habits and customs[3]. Up to date, the use of treatment composition including chemotherapy, radiotherapy, and surgical operation has improved the clinical outcomes of many CRC patients with five-year survival ranging from 45% to 80%[4,5]. However, despite current development in clinical and experimental oncology, the five-year survival rate for CRC patients with metastasis remains relatively poor[6,7]. Thus, it is imperative to further illuminate the molecular mechanisms of CRC, which can help the identification of valuable diagnostic and prognostic biomarkers and sensitive therapeutic targets for CRC patients.

MiRNAs, a class of endogenous non-coding small RNAs with 18-25 nucleotides in length, negatively modulate genes expressions at the post-transcriptional level via inhibiting transla-

Corresponding Author: Li Zhu, MD; e-mail: xhmmlak7741@sina.com

tion via interaction with the 3'-UTR of target mRNAs[8,9]. The abnormal expressions of miRNAs in mammalian cells may be involved in the process of malignant organ transformation and tumor progression[10,11]. Increasing investigations[12,13] reveal that some special miRNAs paly a functional role in the regulation of numerous cells processes, such as differentiation, apoptosis, metastasis, and growth. Several studies[14,15] reveal that miRNAs act as regulators in human carcinogenesis as either anti-oncogenes or tumors promoters. Moreover, numerous clinical evidence[16,17] has shown associations between miRNAs levels and clinicopathological features or long-term survival. The current findings show the robust bases for the emphasis of miRNAs in the pathogenesis of CRC and highlighted the clinical indication of miRNAs in diagnosis, treatment, and outcome of CRC.

MiR-551b, a recently discovered miRNA located on chromosome 3q26.2, has been reported to play a critical role in regulating tumor growth and metastasis, as well as driving tumor progression[18,19]. Recently, Han et al[20] showed that miR-551b expressions were distinctly down-regulated in CRC specimens by performing bioinformatics analysis. However, the experiment evidence supporting the above conclusion was limited. In addition, the potential effects of miR-551b in CRC development remained unknown. In this report, we aimed to determine the possible roles and the underlying mechanism of miR-551b in the modulation of the CRC progression.

Patients and Methods

Patient Specimens

One hundred and twenty-two CRC specimens and adjacent normal samples were obtained from the Dongzhimen Hospital Beijing University of Chinese Medicine between May 2009 and September 2012. Before surgery, the patients received no anti-cancer treatment and signed written informed consents. The tissue specimens were stored at –80°C immediately after surgery resection. The research was approved by the Ethics Committee of Dongzhimen Hospital Beijing University of Chinese Medicine.

Cell Transfection

Human FHC cells (used as control cells) and four CRC cells (LOVO, HCT116, SW1116, SW480) were brought from YRgene Biological corporation (Changsha, Hunan, China). These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)-F12 media with 10% serum. The cell culture conditions were: 37°C and 5% CO_2. For miRNA mimics, inhibitors or plasmids transfection, Lipofectamine 2000 reagent kits (Ningbo, Zhejiang, China) were used following the protocols supplied in the kits. The miRNA mimics and inhibitors were brought from GenePharma Biological corporation (Suzhou, Jiangsu, China). The PTP4A3 cDNA was constructed into pcDNA3.1 empty vector to overexpressing PTP4A3. The construction was conducted by YuXi Biological corporation (Wuxi, Jiangsu, China).

Real Time PCR Analyses

Total RNAs were extracted with TRIzol reagents kits (YiQi, Jinan, Shandong, China) following the protocols supplied in the kits. For PTP4A3 detection, reverse transcription reactions were conducted using cDNA synthesis kits (CytoRui, Xiamen, Fujian, China). Then, the qPCR analyses were conducted using TaKaRa SYBR Green qPCR detection kits (AnDu, Shenzhen, Guangdong, China) following the protocols provided in the kits. Relative PTP4A3 mRNA values were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression using the $2^{-\Delta\Delta Ct}$ method. For the miR-551b detection, Qiagen miREasy kits (DaiSong, Binhai, Tianjin, China) were used to extract the total miRNAs, and qPCR for miR-551b detection was conducted using One-step miRNA qPCR detection kits (KeenBio, Nanjing, Jiangsu, China) following the manufacture's protocols. The relative miR-551b levels were normalized to U6 expression. The primers used in this study were presented in Table I.

Clonogenic Formation Analyses

To assess the colony formation capacities of SW480 and HCT116 cells following miR-551b mimics or inhibitors treatment, clonogenic formation analyses were performed. The treated

Table I. The Primer sets used in the present study.

Names	Sequences (5′-3′)
miR-551b: F	GCGACCCATACTTGGTTTCAG
miR-551b: R	TCGTGAGATGAAGCACTGTAG
PTP4A3: F	GGACGGCATCACTGTTGTG
PTP4A3: R	GCGCTTCTGTCGGATGAAC
GAPDH: F	CTGGGCTACACTGAGCACC
GAPDH: R	AAGTGGTCGTTGAGGGCAATG

cells were trypsinized, collected, and re-placed into six-well plates (800 cells/well). The cells were placed in an incubator with proper culture conditions (37°C, 5% CO_2) and maintained for 14-16 days. Then, the cell colonies were observed, washed using phosphate-buffered saline (PBS), treated with paraformaldehyde and crystal violet (0.1%), and finally imaged by a microscope.

Cell Proliferation Detection

Cell Counting Kits-8 (CCK-8) detection kits (XianFeng, Nanjing, Jiangsu, China) were used to determine the cell proliferation. The cells were seeded (2.5×10^3 cells/well) in nighty-six well plates. After the cells were attached, they were incubated with CCK-8 reagents (25 μl/well) for 2.5 h at the designated timepoint (48 h, 72 h and 96 h). Subsequently, the plates were examined at the absorbance 450 nm by a microplate reader apparatus.

EdU Assay

The cellular proliferation was also detected by EdU assays using Invitrogen Click-iT EdU kits (QiWen, Wuhan, Hubei, China). In brief, the miR-551b mimics or inhibitors-treated SW480 or HCT116 cells were collected, seeded into forty-eight-well plates, and allowed to settle overnight. Then, we prepared EdU stock reagents and diluted them into media at a working concentration of 10 μM. Subsequently, the diluted media (250 μl) was placed into each well. The cells were then incubated at 37°C with 5% CO_2 for 2.5 h. The cells were stained using 4',6-diamidino-2-phenylindole (DAPI) for 15 min. After being fixed using paraformaldehyde, washed by PBS, the cells were imaged by an M5000 EVOS fluorescence microscope (Thermo, Pudong, Shanghai, China).

Flow Cytometry

The percentages of apoptotic cells were determined by flow cytometry using Annexin V-FITC apoptosis detection kits (YunJin, Nantong, Jiangsu, China). In short, the miR-551b mimics or inhibitors-treated SW480 or HCT116 cells were washed twice and collected in 350 μl binding buffer (provided in the kits). Then, the cells were double-stained using Annexin V-FITC (15 μg/ml) and PI (12 μg/ml). After incubation at 4°C in the dark for 25 min, the cells were collected by centrifuging (1000 g/min, 5 min, 4°C). After washing twice with PBS, the cells were subjected to flow cytometry analyses using a flow cytometer apparatus.

Cell Migration Analyses

Cellular migration was assessed by wound-healing assays. In brief, the miR-551b mimics or inhibitors-treated CRC cells were placed in twenty-four well plates. On the second day, the cells were formed monolayer (about 100% cell confluence). Then, the cell monolayer was scratched by a pipette tip (200 μl) and PBS was applied to remove the detached cells. Fresh media were added into the cells and the width of the wounds was recorded using a microscope at 0 h and 48 h.

Cell Invasion Analyses

The cell invasion was evaluated by transwell assays. First, the CRC cells were transfected with miR-551b mimics or inhibitors. 24 h post-transfection, serum-free media (170 μl) with 1.5×10^5 treated cells were placed into the 8 μm pore size Corning transwell inserts which was pre-coated with Matrigel. The lower sides of the transwell were filled with media containing 15% fetal bovine serum (FBS). 48 h later, cells invaded through the membranes were washed by PBS, treated with paraformaldehyde and crystal violet (0.1%), and finally imaged by a microscope.

Luciferase Activity Detection

The 3'UTR fragment (containing the wild-type or mutant miR-551b binding site) of PTP4A3 was separately constructed into a pGL3 luciferase reporter vector by YuXi Biological corporation (Wuxi, Jiangsu, China). The 293T cells were cultured in forty-eight-well plates using DMEM media with 10% FBS, and allowed to grown to 60-70% confluent. Then, miR-551b mimics were co-transfected with PTP4A3 wild-type or mutant type luciferase reporter plasmids into 293T cells using Lipofectamine 2000 reagent kits as described above. 48 h later, Promega luciferase activity detection kits (BioMy, Qidong, Jiangsu, China) were utilized for luciferase activity detection following the kit protocols.

Statistical Analyses

Statistical analyses were conducted using SPSS 20.0 (SPSS Inc, Chicago, IL, USA). The overall survival curves of CRC patients were assessed using the Kaplan-Meier method (with log-rank test). A two-tailed Student's *t*-test or one-way ANOVA was used for comparisons of two independent groups or more than two groups.

Multiple comparisons between groups were performed using the Student-Newman-Keuls post-hoc test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated by the use of the Cox proportional hazards model. A $p<0.05$ was considered as the statistical significance level.

Results

MiR-551b Downregulated in Human CRC Specimens and Cell Lines

To understand the expression pattern of miRNAs in CRC tissues, R software was carried out for the assays of the microarray data from GSE41655 datasets. As shown in Figure 1A, we showed 97 upregulated and 83 downregulated miRNAs in CRC tissues using the heatmap. Then, using Venn diagram, we identified that several miRNAs such as miR-551b were lowly expressed in both low-grade CRC tissues and high-grade CRC tissues (Figures 1B and 1C). Then, our group further performed RT-PCR for the examination of miR-551b levels in 122 paired CRC specimens and matched normal tissues, finding that miR-551b expressions were distinctly downregulated in CRC tissues compared with those in matched normal tissues ($p<0.01$, Figure 1D). Furthermore, the expressions were further determined by qRT-PCR in 4 CRC cell lines and Human FHC cells. Our experiments indicated that miR-551b expressions were distinctly lower in four CRC cell lines than in FHC (Figure 1E). Based on the aforementioned data, our group suggested that miR-551b whose expressions were dysregulated in CRC may be involved in the progression of this disease.

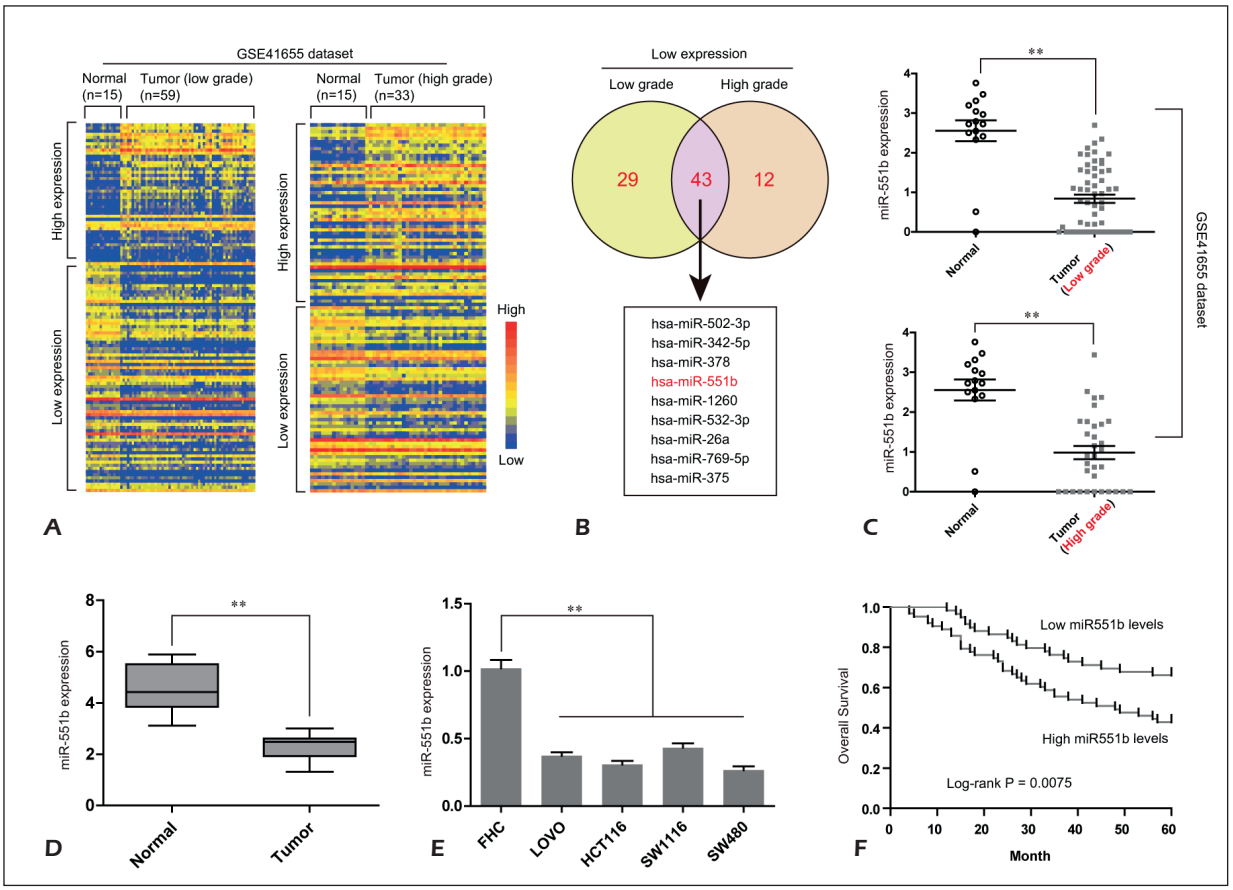

Figure 1. MiR-551b expressions in CRC cell lines and specimens, and its prognostic value for CRC. **A**, Heatmap of abnormally expressed miRNAs in CRC tissues compared to normal tissues according to an online cohort (GSE41655). **B**, Venn diagram of dysregulated miRNAs derived from two combinatorial comparisons and their overlapping regions. **C**, Expressions of miR-551b in normal colorectal tissues and CRC tissues with different stages based on the GSE41655 datasets. **D**, Mean expressions of miR-551b in 122 cases of CRC specimens and their matched non-tumor specimens by real-time PCR ($2^{-\Delta C_T}$, n=122). **E**, Levels of miR-551b were examined using RT-PCR in CRC cell lines SW480, SW116, HCT116 and LOVO. *$p<0.05$, **$p<0.01$.

Table II. Association between miR-551b expression and different clinicopathological features of human CRC.

Parameters	Group	Total	MiR-551b expression		*p*-value
			High	Low	
Gender	Male	64	34	30	0.360
	Female	58	26	32	
Age (years)	< 60	53	28	25	0.480
	≥ 60	69	32	37	
Tumor size (cm)	< 5	83	45	38	0.105
	≥ 5	39	15	24	
Lymph nodes metastasis	Negative	89	49	40	0.033
	Positive	33	11	22	
TNM stage	I-II	83	47	36	0.016
	III-IV	39	13	26	

Correlations Between miR-551b Expressions and Survival of CRC Patients

Subsequently, all patients suffering from CRC in our cohort were divided into two groups (High and Low) according to miR-551b expressions by the use of a cut-off value of 2.325. Next, miR-551b levels with the clinicopathological characteristics of patients with CRC were analyzed. As shown in Table II, miR-551b downregulations were correlated with lymph nodes metastasis (p=0.033) and TNM stage (p=0.016). However, no relation was found between miR-551b and other clinicopathologic features (all p>0.05). To further explore the influence of miR-551b in the overall survival of CRC, our group examined the associations between miR-551b levels and overall survival using Kaplan-Meier assays in 122 CRC cases, finding that the overall survival of CRC patients with lower-levels of miR-551b was distinctly lower than that with higher-levels (p=0.0075). Moreover, univariate regression analysis revealed that miR-551b expressions, lymph nodes metastasis, and TNM stage were potential survival predictors (all p>0.05). Multivariate regression assays enrolling the above distinct parameters revealed that BANCR expression (HR=2.582, 95% CI: 1.219-4.428, p=0.024), lymph nodes metastasis (HR=2.845, 95% CI: 1.159-4.238, p=0.028), and TNM stage (HR=2.892, 95% CI: 1.239-4.372, p=0.021) were independent prognostic markers for the overall survival of CRC patients (Table III).

MiR-551b Was Able to Attenuate Cell Growth and Accelerate Apoptosis of CRC Cells

To discover the possible functions of miR-551b in CRC progression, we first conducted miRNA mimics-mediated gain-of-function research and miRNA inhibitors-mediated loss-of-function studies in CRC cells. The data from the real-time PCR analyses demonstrated that the miR-551b levels of CRC cells in the presence of miR-551b mimics were notably increased, while the miR-551b expression was significantly suppressed in cells when they were treated with miR-551b inhibitors (Figure 2A). Then, clonogenic formation analyses were performed and the data suggested that increasing miR-551b expression remarkably reduced the colony formation abilities of SW480 and HCT116 cells, whereas decreasing miR-551b levels significantly promoted the colony formation (Figure 2B). Subsequently, we carried out

Table III. Univariate and multivariate analyses for overall survival in CRC patients.

	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Gender	1.146	0.745-1.943	0.327	-	-	-
Age	1.442	0.527-2.523	0.156	-	-	-
Tumor size	1.664	0.832-2.414	0.122	-	-	-
Lymph nodes metastasis	3.024	1.354-4.723	0.011	2.845	1.159-4.238	0.028
TNM stage	3.365	1.482-5.271	0.001	2.892	1.239-4.372	0.021
MiR-551b expression	2.985	1.395-4.882	0.015	2.582	1.219-4.428	0.024

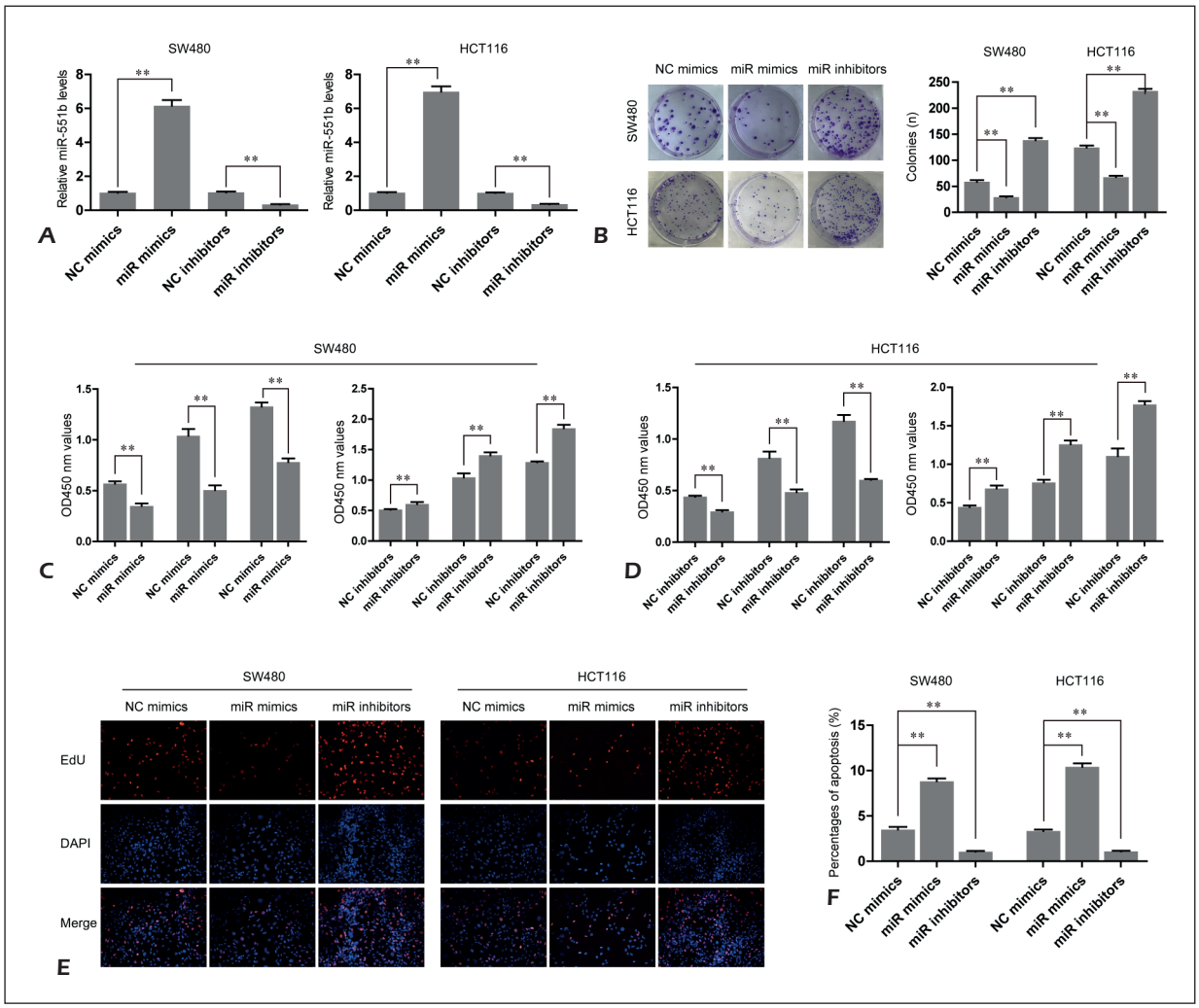

Figure 2. MiR-551b was associated with CRC cell growth and apoptosis. **A**, Effectively enhancing expression of miR-551b using miR-551b mimics and effective silencing of miR-551b levels by miR-551b inhibitors. **B**, Upregulation of miR-551b decreased the number of colonies and repressing miR-551b expression increased the colony number (Magnification: 10×). **C-D**, CCK-8 assays evaluated the CRC cell growth after treatment with miR-551b mimics or inhibitors. E, EdU assays assessed the proliferation of CRC cells. Proliferative cells were labeled red and nuclei were labeled blue (DAPI staining) (Magnification: 100×). **F**, Flow cytometry detected the cell apoptosis. *p<0.05, **p<0.01.

CCK-8 analyses to detect the impact of miR-551b on CRC cell growth. The ectopic expression of miR-551b markedly depressed cellular growth compared with the control, while miR-551b depletion significantly promoted the cell proliferation rates (Figures 2C and D). In addition, EdU assays were also conducted to evaluate the influence of miR-551b on CRC cell proliferation. The data confirmed that over-expressed miR-551b resulted in a notable reduction in the cell growth of CRC cells, while silencing miR-551b facilitated the cell growth (Figure 2E). Next, flow cytometry was applied to examine the cell apoptosis of CRC cells after their miR-551b was over-expressed or knocked down. As the data presented in Figure 2F, forced expression of miR-551b led to markedly increased apoptotic rates, while miR-551b depletion notably suppressed the percentages of apoptotic cells. These data indicated that miR-551b not only inhibited the growth of CRC cells, but also induced cell apoptosis.

MiR-551b Impaired the Mobility of CRC Cells

To ascertain whether there was influence of miR-551b on the metastatic potentials of CRC cells, miR-551b mimics or inhibitors were separately transfected into SW480 and HCT116 cells,

and wound-healing assays as well as transwell assays were performed to assess the migration and invasion, respectively. Wound-healing assays revealed that, in contrast with the miR-551b-silenced cells, the wounded closures of CRC cells treated with miR-551b mimics were significantly slower, which implied that miR-551b could repress the migration of CRC cells (Figures 3A and B). Furthermore, as demonstrated by transwell assays, the cells invaded through the Matrigel-coated membranes were notably fewer when they were treated with miR-551b mimics, while repressing the levels of miR-551b significantly elevated the invaded cell number (Figure 3C). Therefore, these results suggested that miR-551b was capable to suppress the metastatic potentials of CRC cells.

MiR-551b Exerted its Functions Via Directly Targeting PTP4A3 in CRC Cells

We next thought to elucidate the molecular mechanisms by which miR-551b exerted its tumor suppresser functions in CRC cells. Since miR-551b was down-regulated in CRC specimens, its target genes were most probably up-regulated in CRC tissues. Hence, we first conducted bioinformatics analyses using The Cancer Genome Atlas (TCGA) dataset, and found that there were 3891 genes highly expressed in CRC tissues (Figure 4A). Then, we used "starbase" algorithm to predict the potential target genes of miR-551b. We found that 40 predicted genes were also highly expressed in CRC tissues (Figure 4B). Among these 40 genes, we selected PTP4A3, which was reported to act as tumor suppresser in diverse types of cancers, for further

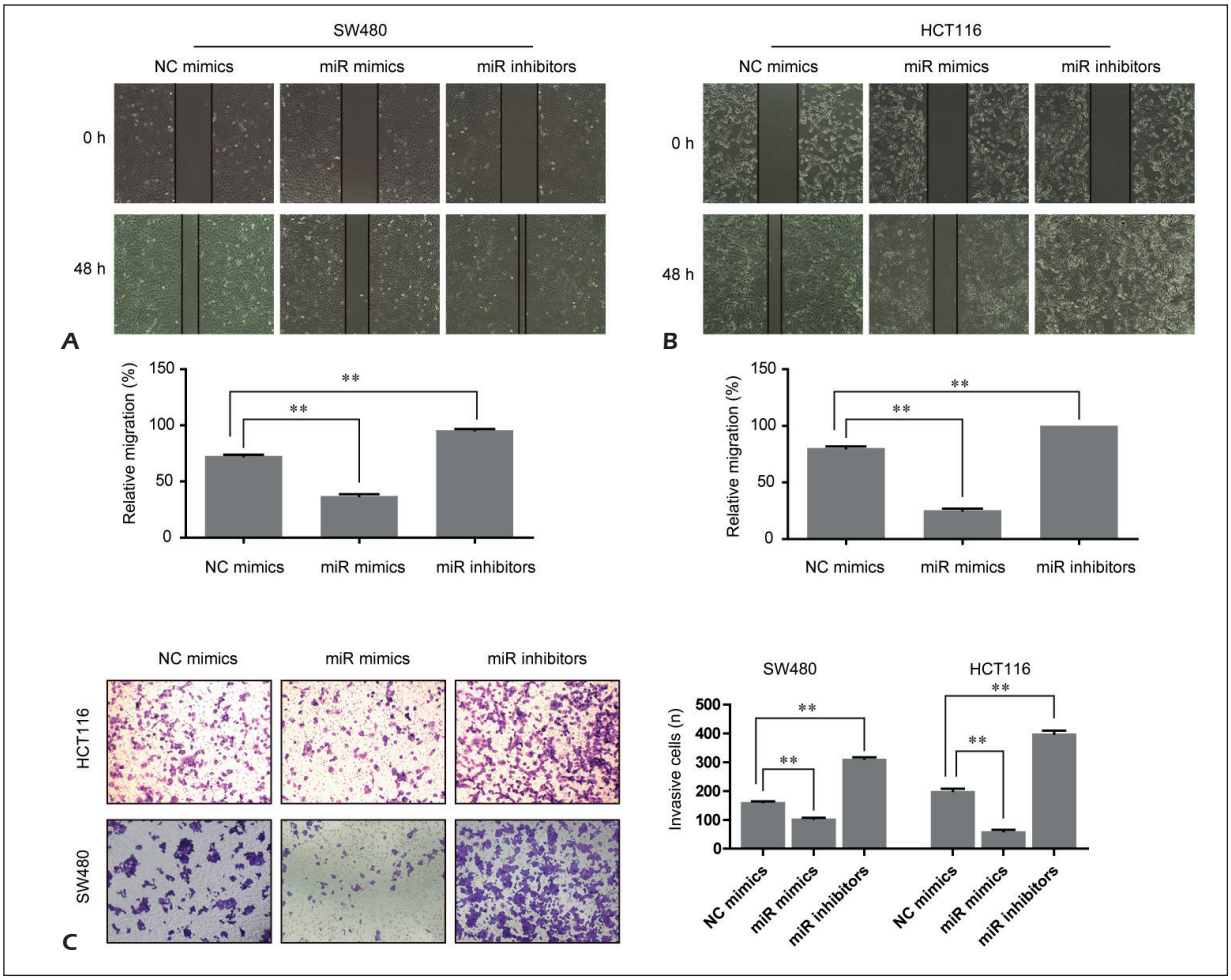

Figure 3. Effects of miR-551b on CRC cell motility. **A-B**, Cell migration was detected by the wound-healing assays at 0 h and 48 h. The quantification of wound closure rates in SW480 and HCT116 cells after treatment was also conducted (Magnification: 10×). **C**, Invasion capacities of SW480 and HCT116 cells after treatment were analyzed by transwell assays (Magnification: 40×). *p<0.05, ** p<0.01.

study (Figure 4C)[21,22]. To clarify whether PTP4A3 was an exact target gene of miR-551b, we next carried out Real Time PCR analyses to measure the levels of PTP4A3 in SW480 and HCT116 cells after treatment with miR-551b mimics or inhibitors. The results indicated that PTP4A3 expression was reversely correlated in miR-551b mimics-treated CRC cells and miR-551b inhibitors-treated CRC cells, which indicated that miR-551b was capable to suppress the expression of PTP4A3 (Figure 4D). Therefore, we next employed luciferase activity analyses to certify whether miR-551b could directly bind to the 3'UTR of PTP4A3. The data validated that the luciferase activity was remarkably decreased in 293T cells co-transfected with miR-551b mimics and PTP4A3 wild-type luciferase reporter vectors, while there was no influence on luciferase activity in cells when they were co-transfected with miR-551b mimics and PTP4A3 mutant luciferase reporter vectors (Figure 4E). We, then, investigated whether miR-551b exerted the suppressing effects on the malignant phenotypes of CRC via targeting PTP4A3. Therefore, CCK-8 assays were performed and the data suggested that PTP4A3 was able to restore the cell growth which was inhibited by miR-551b overexpression (Figure 4F). In addition,

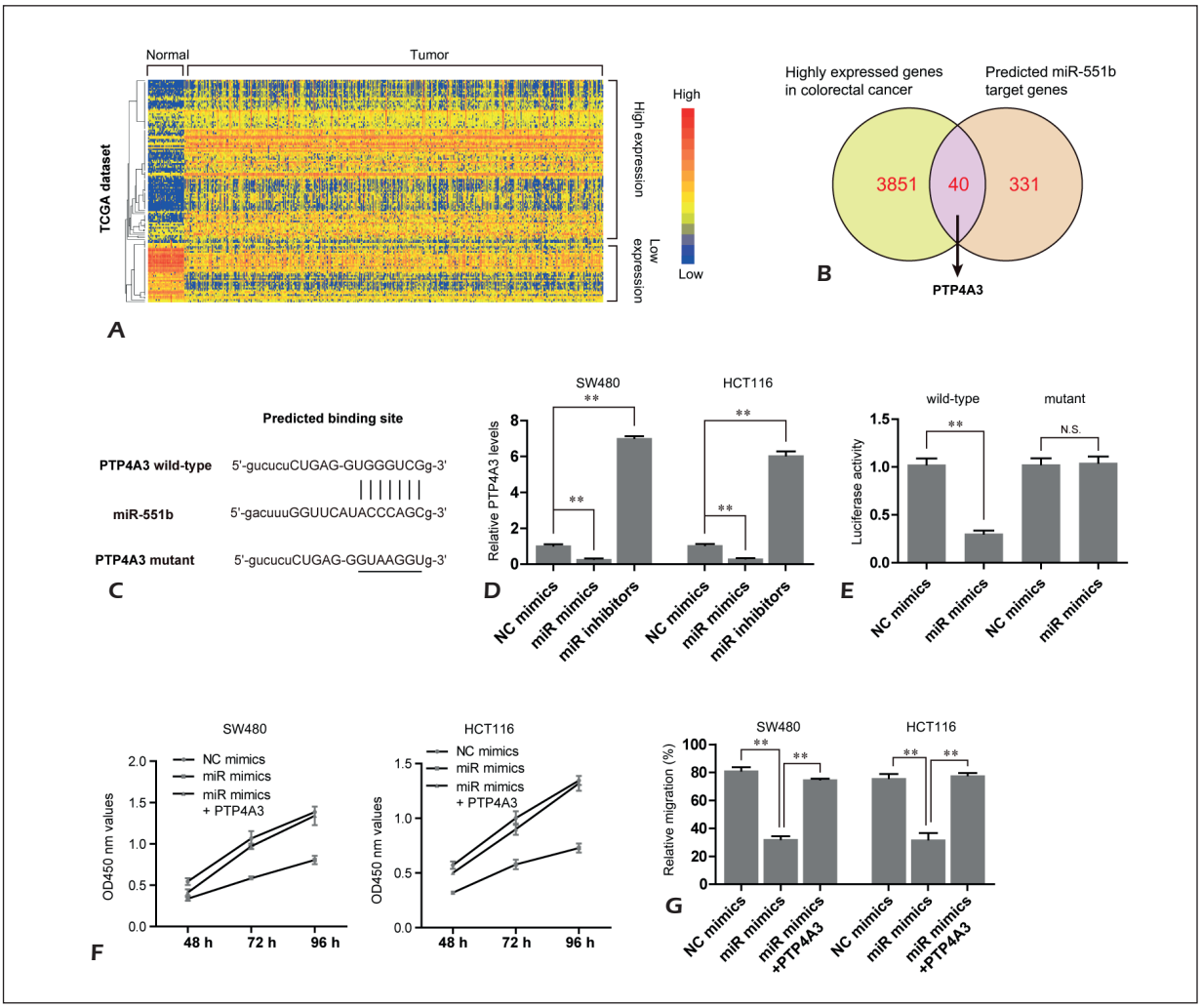

Figure 4. Regulation of miR-551b on CRC cell growth and migration was mediated via directly targeting PTP4A3. **A**, Differentially expressed genes in CRC tissues were analyzed by bioinformatics analyses using TCGA dataset. **B**, Common genes (40 genes) between the highly expressed genes group in CRC specimens and predicted miR-551b target genes group were obtained by Venny bioinformatics tool (http://bioinfogp.cnb.csic.es/tools/venny/index.html). **C**, Bioinformatics tool "starbase" predicted the binding site between miR-551b and the 3'UTR of PTP4A3. **D**, Real Time PCR analyses detected the PTP4A3 levels in CRC cells after miR-551b mimics or inhibitors transfection. **E**, Dual-luciferase reporter assays examine the luciferase activity in 293T cells. **F**, CCK-8 assays evaluated the CRC cell proliferation. **G**, Wound-healing assays detected the cell migration after treatment. *p<0.05, **p<0.01.

the re-introduction of PTP4A3 could also abrogate the inhibition of miR-551b on cell mobility, as determined by wound-healing assays (Figure 4G). Therefore, these studies certified that PTP4A3 was a direct target of miR-551b in CRC cells.

Discussion

Colorectal cancer (CRC) remains a serious clinical problem, resulting in more than 650,000 deaths each year. Estimated 25-45% of patients suffering from CRC developed far-off metastasis and local tumor recurrences after system treatments targeting primary lesions[23,24]. For the improvement of patient's prognosis, sensitive cancer markers which could be used for the prediction of the risk of metastasis in CRC are imperative[25,26]. In recent years, several studies highlighted the tremendous potential of miRNAs as novel cancer biomarkers due to its frequent dysregulation and extensive regulations targeting various tumor-related genes. In this study, we focused on a new miRNA, miR-551b whose down-regulation has been demonstrated in several tumors[27,28]. By analyzing microarray data, we confirmed it as a down-regulated in CRC tissues, which was further confirmed by experiment of RT-PCR. Then, higher levels of miR-551b were observed to be strongly associated with lymph nodes metastasis and advanced TNM stage, suggesting that miR-551b may be involved in progress of distant metastasis. Additionally, survival assays revealed miR-551b as a positive regulator in CRC patients because its down-regulation predicted a shorter overall survival. Moreover, Cox regression using multivariate assays demonstrated miR-551b as an independent biomarker predicting CRC prognosis, highlighting the important clinical values of miR-551b. As far as we know, this is the first clinical assay indicating distinct correlations between miR-551b levels and the clinical outcome of CRC.

Numerous studies have reported that miR-551b, serving as a tumor suppressive or promotive miRNA based on the types of tumors, is involved in the biological regulation in several cancers and some other diseases. For instance, Zhang et al[29] reported that the serum levels of miR-551b were significantly up-regulated in severe acute pancreatitis patients and its knockdown acted as a negative role in the modulation of inflammatory responses. Wei et al[28] showed that miR-551b levels were up-regulated in ovarian tumor and induced tumor promotive activities. Song et al[19] showed that miR-551b levels were reduced in gastric cancer cells, besides aggravated the tumor cells metastasis via targeting ERBB4 expression. These findings suggested miR-551b as a tumor-related miRNA possessing differentiation effects. Thus, we wondered whether miR-551b functioned in CRC cells behaviors. Above findings have confirmed miR-551b was lowly expressed in CRC. Therefore, we supposed that miR-551b may be a tumor suppressor in CRC. Using gain-of-function and lost-of-function assays, as well as *in vitro* assays, our group provided evidence that the overexpression of miR-551b inhibited the proliferation, migration, and invasion, while its silence displayed opposite results. Those findings demonstrated our suggestion that miR-551b served as tumor suppressor in CRC, which was line with its effects in gastric cancer.

Protein-tyrosine phosphatase 4A3 (PTP4A3) is a member of the PTPs that is categorized into a subgroup which is named as dual-specificity phosphatases[30]. This gene is extensively expressed in various human cells so the cellular bases of its etiological contribution to oncogenesis may be associated with both cancer and stromal cells[30,31]. In recent years, several researches[32-34] have shown that PTP4A3 was overexpressed in several tumors and plays a significant role in local lymph node metastasis from these tumors, such as CRC, uveal melanoma, and glioblastoma. Indeed, the regulation mechanisms of miRNAs are very complicated, as miRNAs can target mRNAs to further influence cells progress[35]. In this study, we used the TargetScan for the screen of target genes for miR-551b and found that PTP4A3 may be a target of miR-551b. Then, our group proved that up-regulations of miR-551b suppressed the protein levels of PTP4A3 in both SW480 and HCT116 cells. The results of Luciferase assays also revealed that PTP4A3 is one of the direct downstream target genes of miR-551b in CRC cells. Furthermore, we proved that miR-551b up-regulation distinctly reversed PTP4A3-mediated cell proliferation and migration. Based on the above findings of soft predictions and experimental assays, our group verified that PTP4A3 was a direct target of miR-551b in human CRC.

Conclusions

We suggested that miR-551b is a molecular hub involved in the inhibition of the progression of CRC, as such, possess potential values to be used as a prognostic/diagnostic biomarker and a drug-target for CRC patients.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.
2) Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, Jemal A. Colorectal cancer statistics, 2017. CA Cancer J Clin 2017; 67: 177-193.
3) Gu MJ, Huang QC, Bao CZ, Li YJ, Li XQ, Ye D, Ye ZH, Chen K, Wang JB. Attributable causes of colorectal cancer in China. BMC Cancer 2018; 18: 38.
4) Tampellini M, Bironzo P, Di Maio M, Scagliotti GV. Thymidine phosphorylase: the unforeseen driver in colorectal cancer treatment? Future Oncol 2018; 14: 1223-1231.
5) Kawczyk-Krupka A, Bugaj AM, Latos W, Zaremba K, Wawrzyniec K, Kucharzewski M, Sieron A. Photodynamic therapy in colorectal cancer treatment--the state of the art in preclinical research. Photodiagnosis Photodyn Ther 2016; 13: 158-174.
6) Yu IS, Cheung WY. Metastatic colorectal cancer in the era of personalized medicine: a more tailored approach to systemic therapy. Can J Gastroenterol Hepatol 2018; 2018: 9450754.
7) Kotelevets L, Chastre E, Desmaele D, Couvreur P. Nanotechnologies for the treatment of colon cancer: from old drugs to new hope. Int J Pharm 2016; 514: 24-40.
8) Singh NK. MicroRNAs databases: developmental methodologies, structural and functional annotations. Interdiscip Sci 2017; 9: 357-377.
9) Kappel A, Keller A. MiRNA assays in the clinical laboratory: workflow, detection technologies and automation aspects. Clin Chem Lab Med 2017; 55: 636-647.
10) Osaki M, Okada F, Ochiya T. MiRNA therapy targeting cancer stem cells: a new paradigm for cancer treatment and prevention of tumor recurrence. Ther Deliv 2015; 6: 323-337.
11) Mei Q, Li X, Guo M, Fu X, Han W. The miRNA network: micro-regulator of cell signaling in cancer. Expert Rev Anticancer Ther 2014; 14: 1515-1527.
12) Fiammengo R. Can nanotechnology improve cancer diagnosis through miRNA detection? Biomark Med 2017; 11: 69-86.
13) Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. Nat Struct Mol Biol 2012; 19: 586-593.
14) Asadzadeh Z, Mansoori B, Mohammadi A, Aghajani M, Haji-Asgarzadeh K, Safarzadeh E, Mokhtarzadeh A, Duijf PHG, Baradaran B. MicroRNAs in cancer stem cells: biology, pathways, and therapeutic opportunities. J Cell Physiol 2019; 234: 10002-10017.
15) Hao JP, Ma A. The ratio of miR-21/miR-24 as a promising diagnostic and poor prognosis biomarker in colorectal cancer. Eur Rev Med Pharmacol Sci 2018; 22: 8649-8656.
16) Mousavi S, Moallem R, Hassanian SM, Sadeghzade M, Mardani R, Ferns GA, Khazaei M, Avan A. Tumor-derived exosomes: potential biomarkers and therapeutic target in the treatment of colorectal cancer. J Cell Physiol 2019; 234: 12422-12432.
17) Moreno EC, Pascual A, Prieto-Cuadra D, Laza VF, Molina-Cerrillo J, Ramos-Munoz ME, Rodriguez-Serrano EM, Soto JL, Carrato A, Garcia-Bermejo ML, Guillen-Ponce C. Novel molecular characterization of colorectal primary tumors based on miRNAs. Cancers (Basel) 2019; 11. pii: E346.
18) Jiang X, Jiang M, Xu M, Xu J, Li Y. Identification of diagnostic utility and molecular mechanisms of circulating miR-551b-5p in gastric cancer. Pathol Res Pract 2019; 215: 900-904.
19) Song G, Zhang H, Chen C, Gong L, Chen B, Zhao S, Shi J, Xu J, Ye Z. MiR-551b regulates epithelial-mesenchymal transition and metastasis of gastric cancer by inhibiting ERBB4 expression. Oncotarget 2017; 8: 45725-45735.
20) Han B, Feng D, Yu X, Zhang Y, Liu Y, Zhou L. Identification and interaction analysis of molecular markers in colorectal cancer by integrated bioinformatics analysis. Med Sci Monit 2018; 24: 6059-6069.
21) Gronroos T, Teppo S, Mehtonen J, Laukkanen S, Liuksiala T, Nykter M, Heinaniemi M, Lohi O. Overexpression of PTP4A3 in ETV6-RUNX1 acute lymphoblastic leukemia. Leuk Res 2017; 54: 1-6.
22) den Hollander P, Rawls K, Tsimelzon A, Shepherd J, Mazumdar A, Hill J, Fuqua SA, Chang JC, Osborne CK, Hilsenbeck SG, Mills GB, Brown PH. Phosphatase PTP4A3 promotes triple-negative breast cancer growth and predicts poor patient survival. Cancer Res 2016; 76: 1942-1953.
23) Passardi A, Canale M, Valgiusti M, Ulivi P. Immune checkpoints as a target for colorectal cancer treatment. Int J Mol Sci 2017; 18. pii: E1324.
24) Weinberg BA, Marshall JL, Salem ME. The growing challenge of young adults with colorectal cancer. Oncology (Williston Park) 2017; 31: 381-389.
25) Tabernero J, Cervantes A, Rivera F, Martinelli E, Rojo F, von Heydebreck A, Macarulla T, Rodriguez-Braun E, Eugenia Vega-Villegas M, Senger S, Ramos FJ, Rosello S, Celik I, Stroh C, Baselga J, Ciardiello F. Pharmacogenomic and pharmacoproteomic studies of cetuximab in metastatic colorectal cancer: biomarker analysis of a phase I dose-escalation study. J Clin Oncol 2010; 28: 1181-1189.
26) Xu Y, Xu Q, Yang L, Liu F, Ye X, Wu F, Ni S, Tan C, Cai G, Meng X, Cai S, Du X. The effect of colonoscopy on whole blood gene expression profile: an experimental investigation for colorectal cancer biomarker discovery. J Cancer Res Clin Oncol 2015; 141: 591-599.
27) Xu X, Wells A, Padilla MT, Kato K, Kim KC, Lin Y. A signaling pathway consisting of miR-551b, catalase and MUC1 contributes to acquired apoptosis resistance and chemoresistance. Carcinogenesis 2014; 35: 2457-2466.
28) Wei Z, Liu Y, Wang Y, Zhang Y, Luo Q, Man X, Wei F, Yu X. Downregulation of Foxo3 and TRIM31 by miR-551b in side population promotes cell proliferation, invasion, and drug resistance of ovarian cancer. Med Oncol 2016; 33: 126.

29) Zhang Y, Yan L, Han W. Elevated level of miR-551b-5p is associated with inflammation and disease progression in patients with severe acute pancreatitis. Ther Apher Dial 2018; 22: 649-655.
30) Sharlow ER, Wipf P, McQueeney KE, Bakan A, Lazo JS. Investigational inhibitors of PTP4A3 phosphatase as antineoplastic agents. Expert Opin Investig Drugs 2014; 23: 661-673.
31) Salamoun JM, McQueeney KE, Patil K, Geib SJ, Sharlow ER, Lazo JS, Wipf P. Photooxygenation of an amino-thienopyridone yields a more potent PTP4A3 inhibitor. Org Biomol Chem 2016; 14: 6398-6402.
32) Wang L, Liu J, Zhong Z, Gong X, Liu W, Shi L, Li X. PTP4A3 is a target for inhibition of cell proliferatin, migration and invasion through Akt/mTOR signaling pathway in glioblastoma under the regulation of miR-137. Brain Res 2016; 1646: 441-450.
33) McQueeney KE, Salamoun JM, Ahn JG, Pekic P, Blanco IK, Struckman HL, Sharlow ER, Wipf P, Lazo JS. A chemical genetics approach identifies PTP4A3 as a regulator of colon cancer cell adhesion. FASEB J 2018; 32: 5661-5673.
34) Maacha S, Anezo O, Foy M, Liot G, Mery L, Laurent C, Sastre-Garau X, Piperno-Neumann S, Cassoux N, Planque N, Saule S. Protein tyrosine phosphatase 4A3 (PTP4A3) promotes human uveal melanoma aggressiveness through membrane accumulation of matrix metalloproteinase 14 (MMP14). Invest Ophthalmol Vis Sci 2016; 57: 1982-1990.
35) Pradhan AK, Emdad L, Das SK, Sarkar D, Fisher PB. The enigma of miRNA regulation in cancer. Adv Cancer Res 2017; 135: 25-52.