European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2019; 23: 9267-9276

MicroRNA-130b-5p accelerates the migration and invasion of osteosarcoma *via* binding to TIMP2

Z.-H. CHENG, C. LUO, Z.-L. GUO

Department of Neurosurgery, Shanghai Ninth People's Hospital, Shang[illegible]iao To[illegible]University, School of Medicine, Shanghai, China

RETRACTED

Abstract. – **OBJECTIVE:** To elucidate the potential function of microRNA-130b-5p in the progression of osteosarcoma (OS) and the underlying mechanism.

MATERIALS AND METHODS: The relative level of microRNA-130b-5p in OS tissues and cell lines was determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The correlation between the microRNA-130b-5p level and the pathological characteristics of OS was analyzed by the Chi-square test. The Kaplan-Meier curves were introduced for assessing the survival of OS patients with high e[illegible]sion and low expression of microRNA-[illegible]. The regulatory effects of microRNA-130[illegible] on the migratory and invasive abilities of MG[illegible]d U2OS cells were evaluated by the transwe[illegible]say. The relative levels of matrix metalloprote[illegible]ase 2 (MMP2) and MMP9 in OS [illegible] with overexpression or knockdown of [illegible]130b-5p were determined. The bin[illegible]g relat[illegible]hip between microRNA-130b-5[illegible]d TIMP[illegible]as verified through the dual-l[illegible]se re[illegible] assay. Finally, a seri[illegible]s o[illegible] were performed to [illegible]over t[illegible] of microRNA-130b-5p/TIMP[illegible] the progre[illegible]f OS.

RESULTS: Mi[illegible]A-130b-5p wa[illegible]egulated in OS tiss[illegible]ell lines. The high expression of [illegible]oRNA[illegible]5p indicated a poor prognosis of OS patients[illegible]verexpression of microRN[illegible]0b-5p accelera[illegible]S cells to migrate a[illegible]nvade. Besides, the[illegible]lative levels of MMP[illegible]d MMP9 were upregulated in OS cells ove[illegible]ressin[illegible]icroRNA-130b-5p. TIMP2 was the [illegible]ta[illegible]t of mi[illegible]RNA-130b-5p, which was ne[illegible] regulat[illegible]by microRNA-130b-5p. [illegible] knoc[illegible]of T[illegible]2 reversed the regulato[illegible]ct of [illegible]130b-5p on the migratory an[illegible]asive ab[illegible]s of the OS cells.

C[illegible]CLUSIONS: MicroRNA-130b-5p is upregulat[illegible] OS. It accelerates the progression of O[illegible]g TIMP2 level.

[illegible]rds:
[illegible]NA-130b-5p, TIMP2, Osteosarcoma.

[illegible]troduction

Osteosarcoma (O[illegible]a primary malignant bon[illegible]r that mainly[illegible]ts children and ad[illegible] aged 15-25 years. OS often originates [illegible]m mesenchymal tissues and involves the [illegible]taphysis, es[illegible]ally in the distal femur and [illegible]imal tibia. [illegible]s highly destructive, and its d[illegible]ity rate, [illegible]rbidity, and metastatic rate rem[illegible]urrently, the etiology and the pathoge[illegible] of OS have not been comprehen[illegible]ly explored. Effective anti-osteosarcoma [illegible]lacking[3-5]. It is of great significance [illegible]ate the molecular mechanism of OS, thus developing novel strategies for OS treatment.

MicroRNA is a small RNA with 18-25 bp long that participates in various physiological processes in eukaryotic cells[6-8]. It is currently known that microRNAs are abnormally expressed in tumors and served as diagnostic hallmarks[9-11]. MicroRNA-130b locates on 22q11, which is upregulated in multiple types of cancers as an oncogene[12-17]. Conversely, microRNA-130b exerts a tumor-suppressor role in some other types of tumors[18-21]. The specific role of microRNA-130b in OS, however, has not been reported yet.

TIMPs are inhibitors of the MMPs family containing 184-194 amino acids with a molecular weight of 21 kD[22]. TIMPs have four subtypes, namely TIMP1, TIMP2, TIMP3, and TIMP4. The TIMPs family exerts a decisive role in the remodeling process of ECM. It is found[23] that TIMPs are abnormally expressed in different stages of tumors. Imbalanced MMPs/TIMPs leads to ECM deposition or degradation that affects the invasiveness and metastasis of tumors[24-27]. The potential relationship between microRNA-130b-5p and TIMP2 has not been reported yet.

Corresponding Author: Zhilin Guo, MD; e-mail: gzlysr@outlook.com

RETRACTED

This study aims to elucidate the role of microRNA-130b-5p in the progression of OS. Our conclusion may provide new therapeutic targets for OS.

Patients and Methods

OS Patients

48 paired tumor tissues and matched adjacent tissues were surgically resected from OS patients treated in Shanghai Ninth People's Hospital from December 2016 to October 2018. This study was approved by the Ethics Committee of Shanghai Ninth People's Hospital. They did not receive preoperative anti-tumor therapy and were pathologically diagnosed. All subjects volunteered to participate in the study and signed written informed consent.

Cell Culture and Transfection

Osteoblast cell line hFOB 1.19 and OS cell lines MG63, U2OS, Saos2, HOS, and 143B were provided by Cell Bank (Shanghai, China). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) 100 μg/mL penicillin and 0.1 mg/mL streptomycin, at 37°C, in a 5% CO_2 incubator.

The cells were pre-seeded in a 6-well plate and cultured until 80% of confluence. The transfection of 50 nmol/L microRNA-130b[illegible] anti-microRNA-130b-5p or [illegible] conducted using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The transfected cells for 48 h were harvested for *in vitro* experiments.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA in cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and subjected to reverse transcription. The extracted complementary deoxyribose nucleic acid (cDNA) was used for PCR using SYBR Green method (TaKaRa, Dalian, China). The primer sequences were as follows: MicroRNA-130b-5p: F: [illegible]CAGTGCAATGATGAA-3', R: 5'-[illegible]TGCA[illegible]GTCCGAGGT-3'; TIMP2: F: 5'-[illegible]AAGAGCCTGAACCACAGGT-3', R: 5'-[illegible]GGAGGAGATGTAGCAC-3'; MMP2: F: 5'-A[illegible]ACCTGGATGCCGTCGT-3', R: 5'-AGGCACCCTTGAAGAAGTAGC-3'; MMP9: F: 5'-GAACCAATCTCACCGACA[illegible] 5'-GCCACCCGAGTGTAACCATA[illegible]

Transwell

Diluted Matrigel was used to p[illegible] the transwell chamber overnight at [illegible]°C. Cell [illegible]ty was adjusted to 2×10^5/mL in [illegible]m-free me[illegible] μL of medium containing 10% FBS and 2[illegible] of cell suspension were added in the basolateral and apical chamber[illegible] 24-well plate, respectively. 24 h later, the cells [illegible] fixed in methanol for 30 min and stained with [illegible]% crystal violet for another [illegible] min. The in[illegible] cells were observed and photographed using an inverted microscope. The [illegible]ation assay was conducted in the same pro[illegible]s except for Matrigel pre[illegible]g (Promega, [illegible]on, WI, USA).

Western Blot

The total protein was extracted from cells [illegible]g radioimmunoprecipitation assay (RIPA; B[illegible]me, Shanghai, China) and quantified by bicin[illegible]d (BCA) method (Beyotime, Shanghai, China). The protein sample was loaded [illegible] electrophoresis and transferred on a polyvi[illegible]difluoride (PVDF; Millipore, Billerica, [illegible]A, [illegible]A) membrane. The membranes were blocked in 5% skim milk for 2 hours and subjected to incubation with primary and secondary antibodies. The bands were exposed by enhanced chemiluminescence (ECL; Pierce, Rockford, IL, USA) and analyzed by Image Software (NIH, Bethesda, MD, USA).

Dual-Luciferase Reporter Gene Assay

The cells seeded in the 24-well plate with 2×10^5 cells per well were co-transfected with WT TIMP2 3'UTR/MUT TIMP23'UTR and microRNA-130b-5p mimic/NC. 48 hours later, the cells were lysed for determining the relative luciferase activity (Promega, Madison, WI, USA).

Statistical Analysis

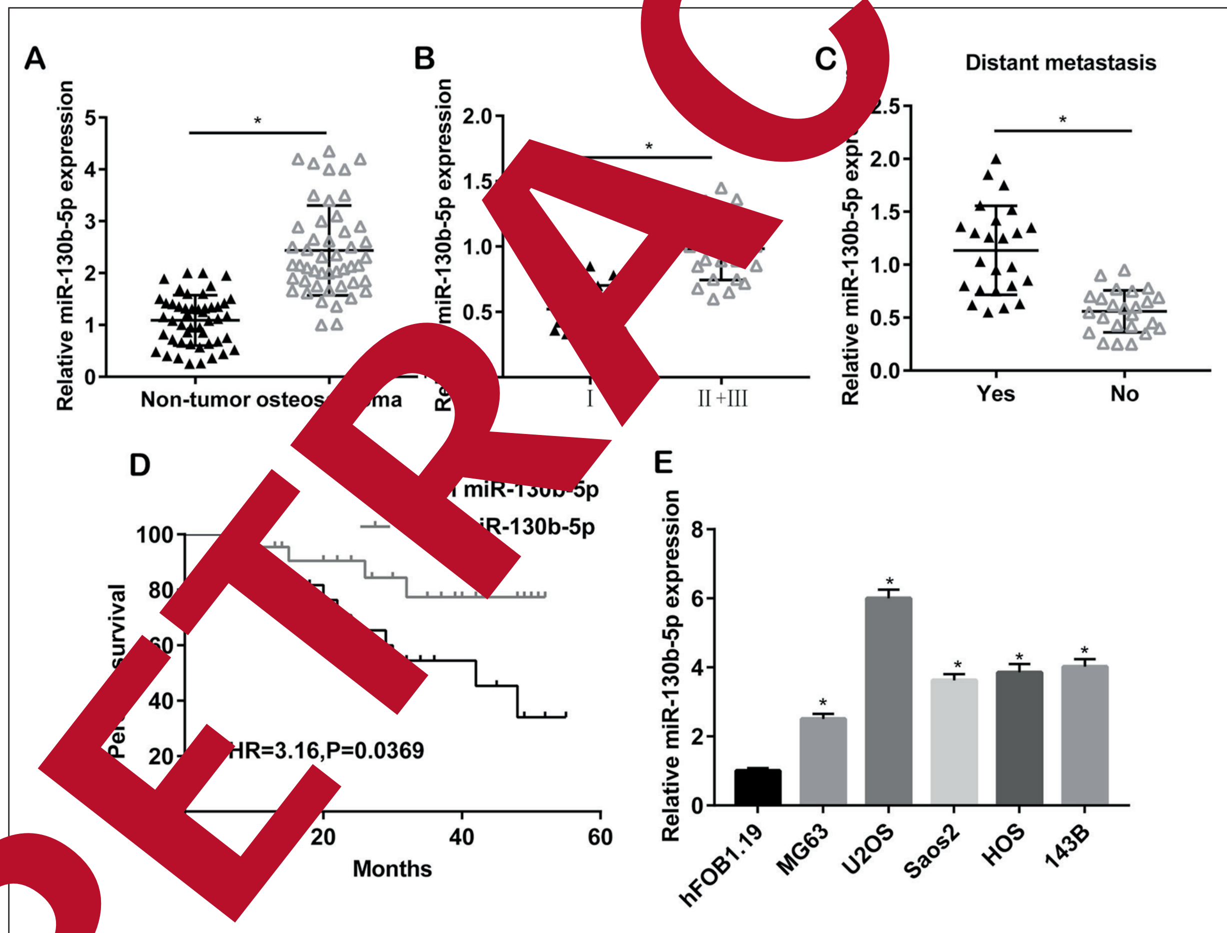
GraphPad Prism 7 (La Jolla, CA, USA) was used for data analyses. The data were expressed as mean ± standard deviation. The intergroup differences were analyzed by the *t*-test. The Chi-square test was performed for evaluating the correlation between the microRNA-130b-5p level and pathological indexes of OS patients. Survival analysis was carried out using the Kaplan-Meier method. $p<0.05$ was considered as statistically significant.

RETRACTED

Results

MicroRNA-130b-5p Was Upregulated in OS Tissues and Cell Lines

Compared with matched non-tumor tissues, microRNA-130b-5p was upregulated in the OS tissues as the qRT-PCR data revealed (Figure 1A). The enrolled OS patients were divided into high expression group and low expression group based on the median level of microRNA-130b-5p. The correlation analyses demonstrated that high expression of microRNA-130b-5p was closely related to tumor stage and distant metastasis of OS (Table I). Identically, the microRNA-130b-5p level remained higher in OS patients with stage II+III relative to those with stage I (Figure 1B). OS patients accompanied by distant metastasis presented a higher abundance of microRNA-130b-5p than those without distant [illegible] (Figure 1C). The Kaplan-Meier cur[illegible] revealed worse prognosis in OS patients w[illegible] high expression of microRNA-130b-5p c[illegible]red to those with low expression (Figure 1D)[illegible]dition, the microRNA-130b-5p level [illegible] OS c[illegible]s was also detected. Compared [illegible]n normal os[illegible] microRNA-130b-5p w[illegible]ighly expressed [illegible] OS cell lines (Figure[illegible]. Amon[illegible]he five OS cell lines determined in[illegible]2OS and MG63 cells expressed t[illegible] high[illegible]owest l[illegible] of microRNA-130b[illegible], respectiv[illegible]nc[illegible]ey were selected fo[illegible]nstructing the[illegible]down and overexpre[illegible]els *in vitro*. [illegible]e above data suggeste[illegible]he po[illegible]l involvement of microRNA-130b-5p in the p[illegible]sion of OS.



Fi[illegible]0b-5p was upregulated in OS tissues and cell lines. **A,** The relative level of miR-130b-5p in OS tissues and [illegible]ched non-tumor tissues. **B,** The relative level of miR-130b-5p in OS patients with stage II+III and stage I. **C,** The relative [illegible]f miR-130b-5p in OS patients either with distant metastasis or not. **D,** The Kaplan-Meier curves introduced for the su[illegible] OS patients with high expression and low expression of miR-130b-5p. **E,** The relative level of miR-130b-5p in osteo[illegible] cell line hFOB1.19 and OS cell lines MG63, U2OS, Saos2, HOS, and 143B.

Table I. Correlation between miR-130b-5p level and pathological characteristics of OS patients (n=48).

Clinicopathologic features	Number of cases	miR-130b-5p expression		*p*-value
		Low (n = 24)	High (n = 2[illegible]	
Age (years)				0.386
≤ 20	23	10	1[illegible]	
> 20	25	14	[illegible]	
Gender				[illegible]
Male	25	13	12	
Female	23	11	12	
Tumor size				[illegible]562
≤ 5 CM	22	10	[illegible]	
> 5 CM	26	14	[illegible]	
TNM stage				0.009*
I	27	18	9	
II+III	21	6	15	
Distant metastasis				0.042*
Yes	21	14	7	
No	27	10	[illegible]	

RETRACTED

MicroRNA-130b-5p Promoted OS Cells to Migrate and Invade

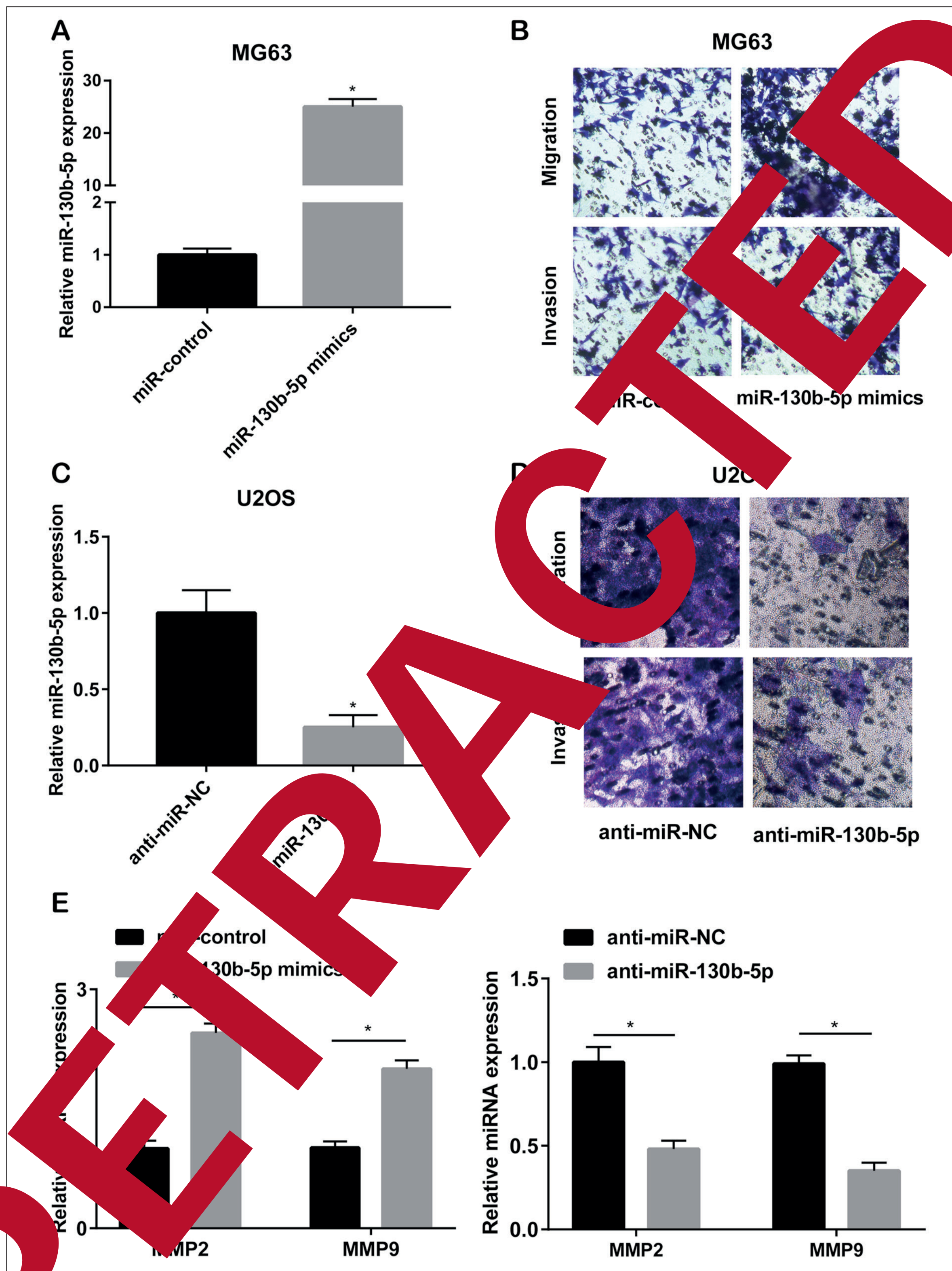
MicroRNA-130b-5p mimics and anti-microRNA-130b-5p were constructed to explo[illegible] biological function of microRNA-130b-5[illegible] progression OS. The transfection of [illegible]oRNA-130b-5p mimics in MG63 cells ma[illegible]ly upregulated microRNA-130b-5p level (Fi[illegible] 2A). The migratory and invasive abilities w[illegible] elevated in MG63 cells overe[illegible] microRNA-130b-5p (Figure 2B). [illegible] tra[illegible]tion of anti-microRNA-130b-5p [illegible]ficiently [illegible]ownregulated microRNA-130b[illegible]el in [illegible] (Figure 2C). The tra[illegible]swe[illegible] the knockdown of [illegible]croRN[illegible]5p attenuated U2OS cells [illegible]migrate and i[illegible] (Figure 2D). Previous [illegible]ve reported [illegible] crucial role of MMP[illegible]n re[illegible]g the migratory and invasive abilities of OS [illegible] Here, the relative levels of [illegible]P2 and MMP9 [illegible]upregulated in MG63 [illegible]s overexpressing mi[illegible]oRNA-130b-5p. Con[illegible]ely, th[illegible] transfection of anti-microRNA[illegible]5p m[illegible]kedly reduced their levels (Figure 2[illegible]e data [illegible]strated that microRNA-130b[illegible]luen[illegible] the OS cells to migrate [illegible]vade v[illegible]ng MMPs.

TIM[illegible] Was the Direct Target of Mi[illegible]0b-5p

[illegible]rough [illegible]argetScan prediction, the binding [illegible]ences between microRNA-130b-5p and T[illegible] were depicted in Figure 3A. Furthermore, mic[illegible]NA-130b-5p overexpression reduced the [illegible]ferase activi[illegible]n wild-type TIMP2 3'UTR; w[illegible]s microR[illegible]-130b-5p knockdown elevated [illegible]). The transfection of microRNA-130[illegible] mimics in MG63 cells downregu[illegible]ed the mRNA and protein levels of TIMP2 [illegible]igure 3C, upper in Figure 3D). The [illegible]pos[illegible] trends were observed after transfection of anti-microRNA-130b-5p in U2OS cells (right in Figure 3C, bottom in Figure 3D). Therefore, TIMP2 was verified to be the direct target of microRNA-130b-5p.

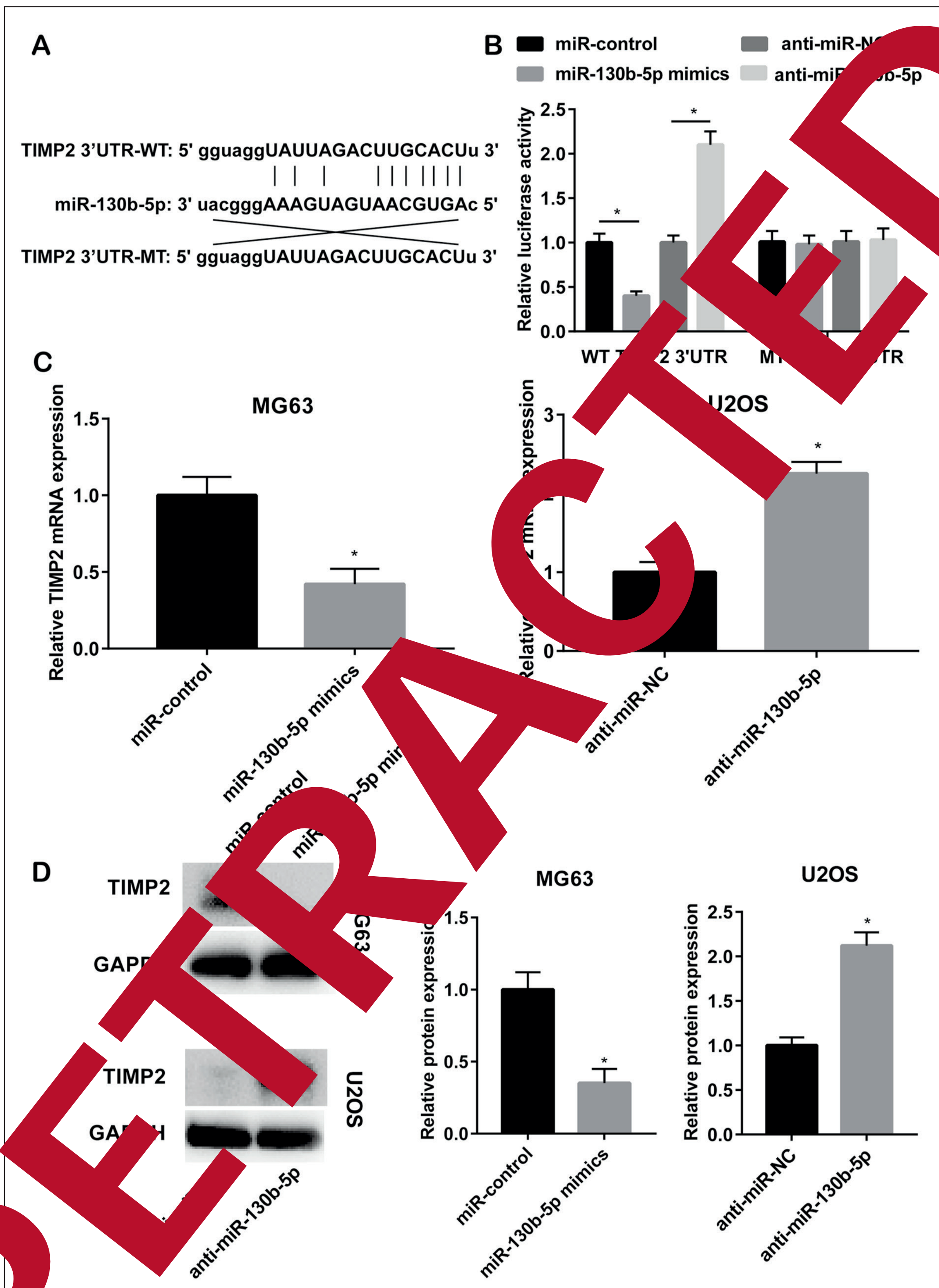
TIMP2 Reversed the Role of MicroRNA-130b-5p in the Progression of OS

It is speculated that TIMP2 may participate in the microRNA-130b-5p-mediated progression of OS. The relative level of TIMP2 was markedly upregulated in U2OS cells transfected with anti-microRNA-130b-5p, which was further reduced after transfection of si-TIMP2 (Figure 4A). The downregulated MMP2 and MMP9 in U2OS cells transfected with anti-microRNA-130b-5p were reversed by TIMP2 knockdown (Figure 4B). Moreover, the transwell assay showed that the knockdown of microRNA-130b-5p attenuated the migratory and invasive abilities of U2OS cells, which were further reversed by transfection of si-TIMP2 (Figure 4C). Collectively, microRNA-130b-5p accelerated the migratory and invasive abilities of the OS cells *via* targeting TIMP2.



RETRACTED

Fi... ...0b-5p promoted OS cells to migrate and invade. **A,** The transfection efficacy of miR-130b-5p mimics in ...3 cells. **B,** The transwell assay showed the migration and invasion in MG63 cells transfected with miR-control or miR-...p mimics (magnification: 40×). **C,** The transfection efficacy of anti-miR-130b-5p in U2OS cells. **D,** The transwell assay sh... ...e migration and invasion in U2OS cells transfected with anti-miR-NC or anti-miR-130b-5p (magnification: 40×). **E,** The r... ...ve levels of MMP2 and MMP9 in OS cells transfected with miR-130b-5p mimics or anti-miR-130b-5p.



RETRACTED

Fi[illegible]2 was the direct target of miR-130b-5p. **A,** The predicted binding sequences between miR-130b-5p and [illegible]P2. **B,** The dual-luciferase reporter gene assay showed the relative luciferase activity in cells co-transfected with miR-[illegible]5p mimics/anti-miR-130b-5p and WT TIMP2 3'UTR/MUT TIMP2 3'UTR. **C,** The relative level of TIMP2 in OS cells tra[illegible]d with miR-130b-5p mimics or anti-miR-130b-5p. **D,** The protein level of TIMP2 in OS cells transfected with miR-130b-[illegible]imics or anti-miR-130b-5p.

RETRACTED

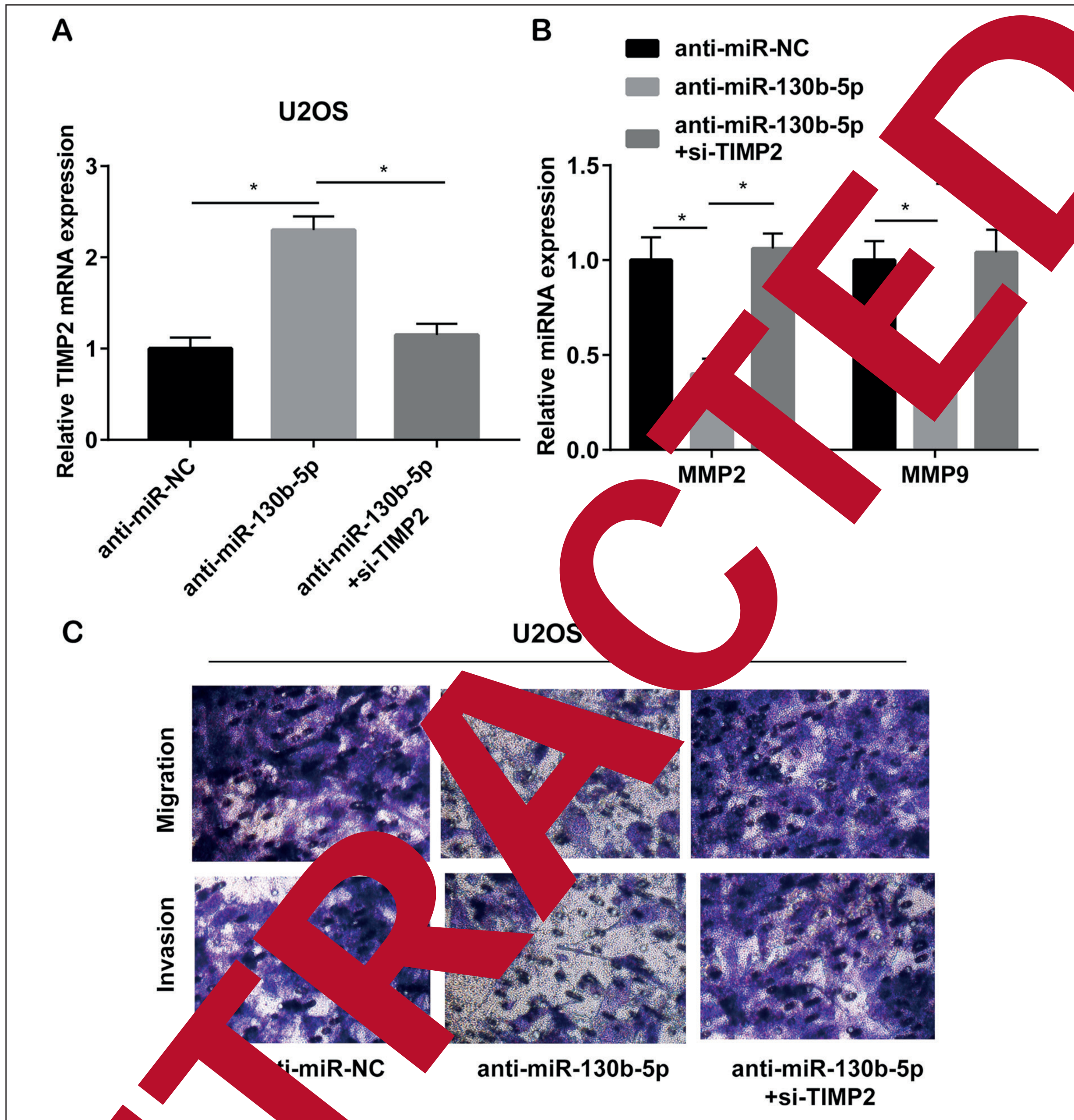


Figure [illegible] TIMP2 reversed the role [illegible] miR-130b-5p in the progression of OS. **A,** The relative level of TIMP2 in U2OS cells t[illegible]ected with anti-miR-NC, anti-miR-130b-5p or anti-miR-130b-5p+si-TIMP2. **B,** The relative levels of MMP2 and MM[illegible] U2OS [illegible] transfected with anti-miR-NC, anti-miR-130b-5p or anti-miR-130b-5p+si-TIMP2. **C,** The transwell assay [illegible] th[illegible]igration a[illegible] invasion in U2OS cells transfected with anti-miR-NC, anti-miR-130b-5p or anti-miR-130b-5p+si-TI[illegible]gnificatio[illegible]×).

Discussion

[illegible]S is a p[illegible]ary malignant tumor with high ma[illegible]ncy and poor prognosis. It is estimated that the lo[illegible]m survival of patients with metastatic or recurr[illegible]OS is less than 20%. With the development of new examination technologies and therapeutic strategies, the treatment efficacy of OS has made great progress in recent years. Nevertheless, the overall survival of OS is unsatisfactory[28,29]. Therefore, further explorations on the molecular mechanism of the OS development have a vital significance.

The potential interaction between abnormally expressed microRNAs and tumors has been well concerned. MicroRNAs could degrade or inhibit the translation of target mRNAs by binding to mRNA 3'UTR, thus silencing the target genes[30,31]. A great number of studies[32-37] have shown that abnormally expressed microRNAs are closely related to tumor diagnosis and prognosis. In this paper, microRNA-130b-5p was upregulated in OS and correlated to poor prognosis of OS patients. Furthermore, *in vitro* experiments demonstrated that the overexpression of microRNA-130b-5p could accelerate the migratory and invasive abilities of the OS cells. Therefore, microRNA-130b-5p may be a potential target contributing to OS treatment.

TIMPs are endogenous inhibitors of the matrix metalloproteinase family, which can effectively inhibit MMPs activity, reduce ECM destruction, and maintain cell-cell integrity. TIMPs also prevent tumor metastasis from improving the prognosis of the affected patients[38,39]. Currently, clinical evidence has identified the involvement of TIMP2 in tumor progression. For example, the serum level of TIMP2 is relatively l[illegible] in patients with invasive gastric cancer, re[illegible] cell carcinoma, esophageal cancer, or no[illegible]all cell lung carcinoma[40,41]. Low level of TIM[illegible] in tumor tissues may be related to the increa[illegible] invasiveness of tumors. On the contrary, the ov[illegible] expression of TIMP2 sufficie[illegible]es tum[illegible] growth and enhances chem[illegible]rapy-[illegible]itivity[42]. Several microRNAs have [illegible]n identif[illegible] to influence tumor cell behavio[illegible]rgeti[illegible] For instance, miR-20[illegible]b in [illegible] cells, [illegible] 106A in gastric can[illegible]cells, an[illegible]221 in renal cancer cells are c[illegible]ble of regula[illegible]or cell behaviors *via* t[illegible]TIMP2[44-46]. H[illegible]we verified the bin[illegible] rel[illegible]hip between microRNA-130b-5[illegible] and TIM[illegible]icroRNA-130b-5p negativel[illegible]gulated the TIM[illegible]el in OS cells. Notabl[illegible]e knockdown of TI[illegible]P2 reversed the regul[illegible]y effe[illegible] of microRNA-130b-5p on the OS[illegible] To[illegible]n up, microRNA-130b-5p was believ[illegible]t the ca[illegible]ogenic role in OS *via* inhibiting [illegible]2 lev[illegible]

Conclusions

[illegible] that microRNA-130b-5p is upreg[illegible]d in OS and predicts poor prognosis of OS p[illegible] It accelerates the progression of OS *via* inhi[illegible]g TIMP2 level.

Conflict of Interest

The Authors declare that they have no confli[illegible]

References

1) Rosenberg AE. Primary [illegible]alignan[illegible]umors. Curr Opin Rheumatol 1[illegible], 2: 102-11[illegible]
2) Inbar-Feigenberg M, C[illegible]ani S, Butcher DT, [illegible] M, Weksberg R. B[illegible] concep[illegible] of epigene[illegible]s. Fertil Steril 2013[illegible]07-615[illegible]
3) Ziyan W, Shuhua Y, [illegible] Xiaoyun [illegible]croR-NA-21 is in[illegible]ed in o[illegible]rcoma c[illegible]nvasion and migra[illegible] Med Onco[illegible]28[illegible]9-1474.
4) Lewis B[illegible] CB, Bartel D[illegible]served seed pairin[illegible]nked by aden[illegible]es, indicates that[illegible]usan[illegible]uman genes are microRNA targets. Cell 200[illegible]15-20.
5) C[illegible]CM. Causes a[illegible]sequences of microR-[illegible]sregulation in can[illegible]. Nat Rev Genet 2009; 10: 704-714.
6) Liu CG, Calin [illegible], Volinia S, Croce CM. MicroRNA expression p[illegible]ing using microarrays. Nat Protoc 2008; 3: 5[illegible]578.
7) [illegible]an MQ, [illegible]rdon JA, Beloti MM, Croce CM, [illegible], Stein JL, Stein GS, Lian JB. A network [illegible]ecting Runx2, SATB2, and the miR-23a~27a~24-2 cluster regulates the osteoblast [illegible]erentiation program. Proc Natl Acad Sci U S A [illegible]07: 19879-19884.
8) Nagadia R, Pandit P, Coman WB, Cooper-White J, Punyadeera C. MiRNAs in head and neck cancer revisited. Cell Oncol (Dordr) 2013; 36: 1-7.
9) Zhao C, Lu F, Chen H, Zhao F, Zhu Z, Zhao X, Chen H. Clinical significance of circulating miRNA detection in lung cancer. Med Oncol 2016; 33: 41.
10) Riquelme I, Letelier P, Riffo-Campos AL, Brebi P, Roa JC. Emerging role of miRNAs in the drug resistance of gastric cancer. Int J Mol Sci 2016; 17: 424.
11) Pal MK, Jaiswar SP, Dwivedi VN, Tripathi AK, Dwivedi A, Sankhwar P. MicroRNA: a new and promising potential biomarker for diagnosis and prognosis of ovarian cancer. Cancer Biol Med 2015; 12: 328-341.
12) Wang W, Peng B, Wang D, Ma X, Jiang D, Zhao J, Yu L. Human tumor microRNA signatures derived from large-scale oligonucleotide microarray datasets. Int J Cancer 2011; 129: 1624-1634.
13) Chen H, Yang Y, Wang J, Shen D, Zhao J, Yu Q. MiR-130b-5p promotes proliferation, migration and invasion of gastric cancer cells via targeting RASAL1. Oncol Lett 2018; 15: 6361-6367.
14) Sand M, Skrygan M, Sand D, Georgas D, Gambichler T, Hahn SA, Altmeyer P, Bechara FG. Comparative microarray analysis of microRNA expression profiles in primary cutaneous malignant melanoma, cutaneous malignant melanoma metastases, and benign melanocytic nevi. Cell Tissue Res 2013; 351: 85-98.

RETRACTED

RETRACTED

15) Scheffer AR, Holdenrieder S, Kristiansen G, von Ruecker A, Müller SC, Ellinger J. Circulating microRNAs in serum: novel biomarkers for patients with bladder cancer? World J Urol 2014; 32: 353-358.
16) Colangelo T, Fucci A, Votino C, Sabatino L, Pancione M, Laudanna C, Binaschi M, Bigioni M, Maggi CA, Parente D, Forte N, Colantuoni V. MicroRNA-130b promotes tumor development and is associated with poor prognosis in colorectal cancer. Neoplasia 2013; 15: 1086-1099.
17) Wu X, Weng L, Li X, Guo C, Pal SK, Jin JM, Li Y, Nelson RA, Mu B, Onami SH, Wu JJ, Ruel NH, Wilczynski SP, Gao H, Covarrubias M, Figlin RA, Weiss LM, Wu H. Identification of a 4-microRNA signature for clear cell renal cell carcinoma metastasis and prognosis. PLoS One 2012; 7: e35661.
18) Yip L, Kelly L, Shuai Y, Armstrong MJ, Nikiforov YE, Carty SE, Nikiforova MN. MicroRNA signature distinguishes the degree of aggressiveness of papillary thyroid carcinoma. Ann Surg Oncol 2011; 18: 2035-2041.
19) Dong P, Karaayvaz M, Jia N, Kaneuchi M, Hamada J, Watari H, Sudo S, Ju J, Sakuragi N. Mutant p53 gain-of-function induces epithelial-mesenchymal transition through modulation of the miR-130b-ZEB1 axis. Oncogene 2013; 32: 3286-3295.
20) Leone V, Langella C, D'Angelo D, Mussnich P, Wierinckx A, Terracciano L, Raverot G, Lachuer J, [illegible] di S, Jaffrain-Rea ML, Trouillas J, Fusco A[illegible] and miR-130b expression is downregul[illegible] in p[illegible]tuitary adenomas. Mol Cell Endocrinol 20[illegible]90: 1-7.
21) Zhao G, Zhang JG, Shi Y, Qin Q, Liu Y, Wa[illegible] Tian K, Deng SC, Li X, Zhu S, Gong Q, Niu Y, W[illegible] CY. MiR-130b is a prognostic [illegible] and inhib[illegible] cell proliferation and invasi[illegible] p[illegible]atic cancer through targeting ST[illegible]. PLoS [illegible] 2013; 8: e73803.
22) Nagase H, Visse R, Mur[illegible] Struc[illegible] tion of matrix meta[illegible]protei[illegible] TIMPs. Ca[illegible]diovasc Res 200[illegible]9: 562-57[illegible]
23) Jackson HW, [illegible] V, Waterhou[illegible]okha R. TIMPs: vers[illegible]acellular regula[illegible] in cancer. Nat R[illegible] Canc[illegible]7; 17: 38-53.
24) Gialeli C, Theocharis [illegible]ramanos NK. Roles of ma[illegible] metalloproteina[illegible] cancer progression[illegible] their pharmacologic[illegible]argeting. FEBS J 20[illegible] 278: 16-27.
25) [illegible]CB, Hsi[illegible]J, Hsieh YH, Chien MH, Chiou HL, [illegible] A[illegible]metastatic effects of norcantharidin o[illegible]ellular ca[illegible]oma by transcriptional inhibitio[illegible]MP-9 [illegible]ough modulation of NF-kB [illegible]ctivity. [illegible]012; 7: e31055.
26) [illegible]Y, Wan D, [illegible] W. Dryofragin inhibits the mi[illegible]tion and invasion of human osteosarcoma [illegible] cells by suppressing MMP-2/9 and elevat[illegible] through PI3K/AKT and p38 MAPK signaling pathways. Anti-Cancer Drugs 2016; 27: 660-668.
27) [illegible]P, Sähr H, Lehner B, Fischer C, Seebach E, Fel[illegible]erg J. Elevated ratio of MMP2/MMP9 activity is associated with poor response to chemotherapy in osteosarcoma. BMC Ca[illegible] 16: 223.
28) Li X, Chen L, Wang W, Meng FB, [illegible]o RT, Chen Y. MicroRNA-150 inhibits cell in[illegible]on and migration and is downregulated [illegible]an osteosarcoma. Cytogenet Genome Re[illegible] 146: 124-135.
29) Lu T, Zhang C, Chai M[illegible]an YB, Jia JL. [illegible] promotes the prolif[illegible]on of osteosarco[illegible] proliferation by ta[illegible]ing Axi[illegible] Int J Clin [illegible]xp Pathol 2015; 8: [illegible]0783[illegible]
30) Oliveto S, Ma[illegible]cino [illegible] N, Biffo [illegible]ole of microRNAs [illegible]ranslati[illegible]lation [illegible] cancer. World J B[illegible]chem 2017; [illegible]
31) Miao Y, [illegible]an Q, Li S, Feng [illegible]bition of proliferat[illegible] [illegible]n, and invasion by knockdown of p[illegible]vate ki[illegible]2 (PKM2) in ovarian cancer SKOV3 and OVC[illegible] cells. Oncol Res 2016; 24: [illegible]475.
32) [illegible] Siverly AN, Chen D, Wang M, Yuan Y, Wang Y, Lee H, Zhang J, Muller WJ, Liang H, Gan B, Yang X, Sun Y, You [illegible], Ma L. Ablation of miR-10b suppresses onco[illegible]e-induced mammary tumorigenesis and met[illegible]asis and reactivates tumor-sup[illegible]ssive path[illegible]ys. Cancer Res 2016; 76: 6424-[illegible]
33) Han [illegible], [illegible]ang YD, Zhang CH, Yang YM, Pang D, Song YN, Zhang GQ. A novel panel of serum [illegible]21/miR-155/miR-365 as a potential diagnos[illegible]marker for breast cancer. Ann Surg Treat Res 2017; 92: 55-66.
34) Sato H, Shien K, Tomida S, Okayasu K, Suzawa K, Hashida S, Torigoe H, Watanabe M, Yamamoto H, Soh J, Asano H, Tsukuda K, Miyoshi S, Toyooka S. Targeting the miR-200c/LIN28B axis in acquired EGFR-TKI resistance non-small cell lung cancer cells harboring EMT features. Sci Rep 2017; 7: 40847.
35) Ali S, Ahmad A, Aboukameel A, Ahmed A, Bao B, Banerjee S, Philip PA, Sarkar FH. Deregulation of miR-146a expression in a mouse model of pancreatic cancer affecting EGFR signaling. Cancer Lett 2014; 351: 134-142.
36) Khanizadeh S, Ravanshad M, Hosseini SY, Davoodian P, Almasian M, Khanlari Z. The effect of the hepatitis C virus (HCV) NS3 protein on the expression of miR-150, miR-199a, miR-335, miR-194 and miR-27a. Microb Pathog 2017; 110: 688-693.
37) Hirahata M, Osaki M, Kanda Y, Sugimoto Y, Yoshioka Y, Kosaka N, Takeshita F, Fujiwara T, Kawai A, Ito H, Ochiya T, Okada F. PAI-1, a target gene of miR-143, regulates invasion and metastasis by upregulating MMP-13 expression of human osteosarcoma. Cancer Med 2016; 5: 892-902.
38) Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochim Biophys Acta 2010; 1803: 55-71.
39) Zhu L, Yu H, Liu SY, Xiao XS, Dong WH, Chen YN, Xu W, Zhu T. Prognostic value of tissue inhibi-

tor of metalloproteinase-2 expression in patients with non-small cell lung cancer: a systematic review and meta-analysis. PLoS One 2015; 10: e124230.

40) Lu H, Yang Z, Zhang H, Gan M, Zhou T, Wang S. The expression and clinical significance of matrix metalloproteinase 7 and tissue inhibitor of matrix metalloproteinases 2 in clear cell renal cell carcinoma. Exp Ther Med 2013; 5: 890-896.

41) Groblewska M, Mroczko B, Kozlowski M, Niklinski J, Laudanski J, Szmitkowski M. Serum matrix metalloproteinase 2 and tissue inhibitor of matrix metalloproteinases 2 in esophageal cancer patients. Folia Histochem Cytobiol 2012; 50: 590-598.

42) Shao J, Xu Z, Peng X, Chen M, Zhu Y, Xu L, Zhu H, Yang B, Luo P, He Q. Gefitinib synergizes with irinotecan to suppress hepatocellular carcinoma via antagonizing Rad51-mediated DNA-repair. PLoS One 2016; 11: e0146968.

43) Liang B, Yin JJ, Zhan XR. MiR-301a promotes cell proliferation by directly targeting TIM[illegible] ple myeloma. Int J Clin Exp Pathol [illegible]; 8: [illegible] 9174.

44) Dai Y, Xia W, Song T, Su X, Li J[illegible], Chen Y, Wang W, Ding H, Liu X, Li H, Z[illegible] Shao N. MicroRNA-200b is overexpresse[illegible]dometrial adenocarcinomas and e[illegible]hances [illegible] activity by downregulating TIM[illegible] in human e[illegible]tri[illegible] cancer cell line HEC[illegible] cells. Nucleic A[illegible] 2013; 23: 29-34.

45) Zhu M, Zhang N[illegible] Lui Y[illegible] G, Zhao L. MicroRNA-106a targe[illegible] regulate [illegible]vasion and metasta[illegible] of gas[illegible]er. FEBS [illegible]ett 2014; 588: 600-6[illegible]

46) Lu GJ, [illegible] YQ, Zhang QM, [illegible] Jiao LY, Gao QZ, Z[illegible] MiRNA-221 pro[illegible]otes proliferation, [illegible]gratio[illegible] invasion by targeting TIMP2 in renal cell carcin[illegible] Int J Clin Exp Pathol 2015; 8: 5224-5229.

RETRACTED