Japanese Journal of Gastroenterology and Hepatology

Research Article

ISSN: 2435-1210 | Volume 8

Microrna-145 Inhibits EMT and Fibrosarcoma Cell Proliferation, Migration and Invasion by Targeting HMGA2

Cheng M, Huang W, Cai W, Fang M, Chen Y, Wang C, Yan W* and Luo G

Department of Musculoskeletal Surgery of Fudan, University Shanghai Cancer Center, Shanghai 200030, P.R. China

***Corresponding author:**

Wangjun Yan,
Department of Musculoskeletal Surgery of Fudan, University Shanghai Cancer Center, 270 DongAn Road, Shanghai 200030, P.R. China,
E-mail: yanwj@fudan.edu.cn

Received: 11 Dec 2021
Accepted: 21 Dec 2021
Published: 27 Dec 2021
J Short Name: JJGH

Citation:

Yan W, Microrna-145 Inhibits EMT and Fibrosarcoma Cell Proliferation, Migration and Invasion by Targeting HMGA2. Japanese J Gstro Hepato. 2021; V8(1): 1-10

Keywords:

Fibrosarcoma; MicroRNA-145; High mobility group protein A2; Epithelial-to-mesenchymal transition; Migration

1. Abstract

1.1 Aim: Fibrosarcoma is a rare malignant tumor of subcutaneous tissue characterized by slow infiltrative growth and a high rate of local recurrence. The participation of microRNAs (miRNAs/miRs) in the pathogenesis of human diseases and the vital role of miR-145 in different types of cancer. However, the role of miR-145 in fibrosarcoma remains unclear. The present study aimed to investigate the effect of miR-145 in the progression of fibrosarcoma and its potential application as a new therapeutic target.

1.2. Methods: The present study screened out a differential gene named high mobility group protein A2 (HMGA2) via bioinformatics analysis. In the present study, the gene expression profiles of fibrosarcoma cells and normal cells were downloaded from GEO Datasets GSE10021 and GSE1774. The human fibrosarcoma cell line HT1080 were transfected with miR-145 mimics, mimic negative control (NC), pCMV6 vector, overexpression plasmid vector of HMGA2, (si)RNA-HMGA2 or siRNA-NC. Reverse transcription-quantitative PCR and western blot analysis were performed to examine the mRNA (miR-145) and protein (HMGA2, N-cadherin, Vimentin and E-cadherin) expression levels in HT1080 cells. Cell Counting Kit-8, wound healing and Transwell assays were used to examine the TH1080 cell viability, proliferation and migratory capacities, respectively. Bioinformatics prediction and luciferase reporter assay were performed to investigate the relationship between miR-145 and its potential target gene (HMGA2).

1.3. Results: The results demonstrated that overexpression of HMGA2 promoted cell proliferation, migration and invasion of HT1080 fibrosarcoma cells, whereas HMGA2 knockdown had no effect on the proliferation of HT1080 fibrosarcoma cells. However, overexpression of miR-145 reversed the facilitating effects of HMGA2 in tumor progression via epithelial-to-mesenchymal transition (EMT) modulation. Mechanistically, miR-145 exerted its inhibitory role in fibrosarcoma by directly binding to the 3'-untranslated region of HMGA2.

1.4. Conclusion: miR-145 plays an inhibitory role in fibrosarcoma progression by directly suppressing HGMA2 expression and EMT progression. Thus, miR-145 may act as a potential therapeutic target for malignant fibrosarcoma therapy.

2. Introduction

Soft tissue sarcomas (STSs) are a type of malignant tumor that mainly derive from mesenchymal tissues. Currently, >50 subtypes of STSs have been identified, among which pleomorphic sarcoma is the most common type, namely malignant fibrous histiocytoma. Most patients with STSs are 30-50 years old, with a higher proportion of men than women [1, 2]. STSs can occur anywhere in the body but limbs are the most common places of STSs [3-5]. Fibrosarcoma is a type of malignant tumor that consists of fibroblasts and the fibers they produce. It is relatively simple in shape and has a herringbone structure [6]. It can be divided into adult fibrosarcoma (>5 years) and congenital or infantile fibrosarcoma (<5 years), according to the age of patients [7]. Fibrosarcoma can take place after-burn scars and radiotherapy, with features of strong local invasion, invasive or destructive growth,

postoperative recurrence and distant metastasis [8]. Currently, treatment for fibrosarcoma includes surgery, radiotherapy, and chemotherapy [9]. However, ~40-50% of patients still die from recurrence and metastasis of fibrosarcoma, with an annual disease-free survival rate of 50% [8, 10, 11]. Fibrosarcoma has become a severe threat to human health and places a heavy burden on the medical security system.

MicroRNAs (miRNAs/miRs) are non-coding RNA molecules, ~18-25 nucleotides in length [12]. miRNAs have been extensively studied in association with different human diseases [13]. Increasing evidence suggest that miRNAs are involved in RNA silencing and post-transcriptional regulation of gene expression and can be a promising diagnostic tool for different types of cancer [14]. miR-145 has been reported to function as a main regulator in the progression of several diseases, including cardiopulmonary complications, osteosarcoma and colorectal cancer [15-17]. It has been demonstrated that overexpression of miR-145 reduces cancer migration by regulating fascin homolog 1 expression [18]. In addition, miR-145 acts as a tumor suppressor in breast [19], lung [20], colon [21] and gastric [22] cancers. However, the role of miR-145 in fibrosarcoma remains elusive and further investigations are required to determine its underlying molecular mechanisms.

High mobility group protein A2 (HMGA2) belongs to the HMGA family, which can selectively bind to DNA heterogeneous conformations, regulating replication, transcription and DNA repair [23]. HMGA2 is a transcription factor expressed during embryonic development, and is highly expressed in some malignancies, including breast cancer, non-small cell lung cancer (NSCLC), pancreatic cancer, retinoblastoma and squamous cell carcinoma, suggesting its importance in the malignant transformation of tumor cells [24-26]. In MG63 and U2SO cells, miR-106a-5p can target HMGA2 to regulate the biological process of osteosarcoma cells [27]. Previous studies have also demonstrated that let-7 takes part in tumor cell proliferation, migration and invasion by regulating HMGA2 expression [28,29]. However, the effects of HMGA2 in fibrosarcoma remain unclear.

The present study aimed to investigate the role of miR-145 and HMGA2 in fibrosarcoma and determine their molecular mechanisms. It was hypothesized that miR-145 acts as an inhibitor of fibrosarcoma cell proliferation, migration and invasion by targeting HMGA2 to intervene epithelial-to-mesenchymal transition (EMT).

3. Materials and Methods

3.1. Bioinformatics Analysis: TargetScan (Release 7.2; March 2018; https://www.targetscan.org) and PicTar (https://pictar.mdc-berlin.de/) were used to screen the candidate targets of miR-145. A two-fold cut-off with P<0.05 was used when identifying the genes. The datasets used in the present study were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The gene expression profiles of fibrosarcoma cells and normal cells from datasets GSE10021 (ID:300253204) and GSE1774 (ID:300030734). Differentially expressed genes (p.value<0.01, logFC<-2 or logFC>2) were analyzed using the online software, Gene Expression Omnibus 2 R (https://www.ncbi.nlm.nih.gov/geo/), and their function annotation was analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID; http://www.david.niaid.nih.gov website).

3.2. Cell lines and Cell Culture: The human fibrosarcoma cell line, HT1080, was purchased from the American Type Culture Collection and maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-glutamine (all purchased from Gibco; Thermo Fisher Scientific, Inc.), at 37˚C with 5% CO_2.

3.3. Cell Transfection: miR-145 mimics (miR-145) and overexpression plasmid vector of HMGA2 and their corresponding controls [mimic negative control (NC) and pCMV6 vector, respectively] were purchased from Shanghai GenePharma Co., Ltd. Small interfering (si)RNA-HMGA2 and siRNA-NC were purchased from Shanghai GeneChem Co., Ltd. HT1080 cells were seeded into six-well plates at a density of $1x10^5$ cells/well. Following incubation at 37˚C for 24 h, cells were transfected with overexpression plasmid vector of HMGA2 (1 μg), miR-145 mimics (100 nM) and siRNA-HMGA2 (100 nM), using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 24 h at 37˚C. Cells transfected with the corresponding controls were used as the control groups. After 24 h, the transfection efficiency was detected by reverse transcription-quantitative (RT-q) PCR. The following sequences were used: miR-145-5p mimic, 5'-GUCCAGUUUUCCCAGGAAUCCCU-3'; mimic NC, 5'-UUCUCCGAACGUGUCACGUTT-3'; siRNA-HMGA2, 5'-GGGCAAUCUUAUAUAUCUA-3'; and siRNA-NC, 5'-CCCAGUUACGAATCGCUUCCA-3'.

3.4. Cell Proliferation: The Cell Counting Kit-8 (CCK-8) assay was performed to detect cell proliferation. Following transfection for 24 h, cells were trypsinized, collected and re-suspended in DMEM. Cells were subsequently seeded into 96-well plates at a density of $3x10^3$ cells/well and cultured at 37˚C with 5% CO_2. After 24, 48, 72, 96 and 120 h at 37˚C, 10 μl CCK-8 solution (Beyotime Institute of Biotechnology) was added to each well and cells were incubated for an additional 4 h at 37˚C. Absorbance was measured at a wavelength of 450 nm (OD 450), using a Varioscan microwell reader (Thermo Fisher Scientific, Inc.).

3.5. Wound Healing Assay. HT1080 cells transfected with miR-145 mimics and siRNA-HMGA2 were seeded into 6-well plates and cultured until they reached ~100% confluence. Cell monolayers were scratched using sterile pipette tips to generate artificial wounds. Cells were cultured for 24 h at 37˚C in DMEM with 1% serum [30] and the wound distances before and after culture were observed under an IX71 microscope (magnification, x100; Olympus Corporation). Wound healing rate was calculated as follows: (wound width at 24 h/wound width at 0 h) x100. Cell monolayers were transfected with the corresponding NCs for the control groups.

3.6. Migration Assay*:* Following transfection, $1x10^5$ HT1080 cells were plated into the upper chambers of Transwell plates (Corning Inc.) with polycarbonate membranes (pore size, 8 μm; Corning Inc.), in 200 μl serum-free DMEM and the lower chamber was filled with 500 μl DMEM supplemented with 10% FBS. The chambers were removed following incubation at 37˚C for 24 h. Cells that failed to penetrate through the polycarbonate membranes were removed using a wet swab, while the migratory cells were fixed with 4% (w/v) paraformaldehyde at room temperature for 20 min and stained with 0.1% crystal violet at room temperature for 10 min. The chambers were washed, naturally air-dried, inverted onto a glass slide and photographed using an upright fluorescence microscope. Stained cells were counted in five randomly selected fields using an IX71 microscope (magnification, x100; Olympus Corporation).

3.7. Invasion Assay: Matrigel was diluted with serum-free DMEM at 1:7 (v/v). Each chamber was filled with 50 μl of diluted Matrigel and placed at 37˚C for 4 h for subsequent use. HT1080 cells were washed with PBS, trypsinized and re-suspended in serum-free medium (Gibco; Thermo Fisher Scientific, Inc.) following transfection. Cells were seeded into the Matrigel chamber at the density of $1x10^5$ cells/ml in 500 μl of DMEM supplemented with 10% FBS. The chambers were removed following incubation at 37˚C for 24 h. Cells that failed to penetrate through the polycarbonate membranes were removed using a wet swab, while the invasive cells were fixed, stained and counted under an IX71 microscope (Olympus Corporation).

3.7.8. Reverse Transcription-Quantitative (RT-q) PCR: Total RNA was extracted from HT1080 cells using TRIzol® reagent (Gibco; Thermo Fisher Scientific, Inc.), and the concentration and purity of RNA were quantified using a NanoDrop One/OneC (Thermo Fisher Scientific, Inc.). Total RNA (2 μg) was reverse transcribed into cDNA using PrimeScript™ RT Master Mix (Perfect Real Time; Takara Bio, Inc), according to the manufacturer's protocols. qPCR was subsequently performed using the One-Step TB Green® PrimeScript™ RT-PCR Kit II (cat. no. RR086A; Takara Bio, Inc.). The following primer sequences were used for qPCR: miR-145 Stem-loop Real-time (RT), 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGAT-3'; miR-145 forward, 5'-GGCTTAGTCCAGTTTTCCCAG-3' and reverse, 5'-GTGCAGGGTCCGAGGT-3'; HMGA2 forward, 5'-GGACAAGCAAGTTGATGAAT-3' and reverse, 5'-TGAGTGAGTAGACGAGTG-3', GAPDH forward, 5'-ACAACTTTGGTATCGTGGAAGG-3' and reverse, 5'-GCCATCACGCCACAGTTTC-3', U6 Stem loop RT, 5'-AACGCTTCACGAATTTGCGT-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3'. The following thermocycling conditions were used for qPCR: 94˚C for 30 sec; 40 cycles of 94˚C for 15 sec, 55˚C for 30 sec and 72˚C for 90 sec. Relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method [31] and normalized to the internal reference gene GAPDH and U6.

3.9. Western Blotting: Cells transfected with miR-145 mimics for 36 h were lysed using RIPA buffer (Thermo Fisher Scientific, Inc.) for 30 min on ice to obtain total protein. Total protein concentration was quantified via the standard BCA protein quantification method and homogenized via dilution. A total of 10 μg protein sample was separated by 10% SDS-PAGE, transferred onto PVDF membranes (EMD Millipore) and immersed in TBST (0.05% Tween 20)-prepared 5% BSA (Thermo Fisher Scientific, Inc.) at room temperature for 2 h, prior to washing with TBST. The membranes were incubated with primary antibodies against HMGA2 (cat. no. ab207301), E-cadherin (cat. no. ab1416), N-cadherin (cat. no. ab76011) and Vimentin (cat. no. ab92547, all 1:1,000 and purchased from Abcam) overnight at 4˚C with shaking. Following the primary incubation, membranes were incubated with HRP-conjugated secondary antibody (cat. no. ab6721; Abcam), which was formulated with 5% BSA at a ratio of 1:10,000, for 2 h at room temperature. Protein bands were visualized using the chemiluminescence using the ECL kit (Pierce; Thermo Fisher Scientific, Inc.) and analyzed using ImageJ software (version 1.52p 22 June 2019; National Institutes of Health) to quantify protein expression.

3.10. Dual-Luciferase Reporter Assay: The dual-luciferase reporter assay was performed to verify whether HMGA2 is the direct target of miR-145. The putative miR-145 binding site within the 3'-untranslated region (UTR) of HMGA2 (NCBI transcript NM_003483) was predicted using TargetScan. pmirGLO dual-luciferase miRNA target expression vector with HGMA2 3'-UTR [HMGA2-wild-type (WT)] and its control vector mutant pmirGLO-HMGA2-3'-UTR [HMGA2-mutant (MUT)] were purchased from Shanghai GenePharma Co., Ltd. HT1080 cells were seeded into 12-well plates at the density of $5x10^4$ cells/ml and transfected with pmirGLO-HMGA2 (100 pmol), miR-145 mimics (50 pmol) or miR-145 mimic NC (50 pmol), using Lipofectamine® 3000. After 48 h, firefly and *Renilla* luciferase activities were detected in HT1080 cells using the Dual-Luciferase® Reporter Assay kit (Beyotime Institute of Biotechnology). The activity of firefly luciferase was normalized to that of *Renilla* luciferase.

3.11. Statistical Analysis: Data are presented as the mean ± SD. All experiments were repeated ≥ three times. The data of two groups were assessed using unpaired Student's t-test. Multiple comparisons were analyzed via one-way ANOVA followed by Tukey's post hoc test. All data were analyzed using SPSS 18.0 statistical software (SPSS, Inc.) $P<0.05$ was considered to indicate a significantly significant difference.

4. Results

Overexpression of HMGA2 promotes fibrosarcoma cell proliferation, migration and invasion via EMT. The present study analyzed the GSE10021 chip from fibrosarcoma cells and normal cells and obtained 515 differentially expressed genes (Fig. 1A). In addition, the GSE1774 chip from fibrosarcoma cells and MT1-MMP inhibitor groups was analyzed and 607 differentially expressed genes were

acquired (Fig. 1B-C). Differentially expressed genes were analyzed using the online software, Gene Expression Omnibus 2 R, and their function annotation was analyzed using the Database for Annotation, Visualization and Integrated Discovery. A total of 19 genes were revealed to be highly expressed in fibrosarcoma cells (Fig. 1 and Table 1), and the present study predicted the function of these candidate genes (Table 2). The results confirmed that the HMGA2 gene is directly associated with miR-145 [32].

To further determine the role of HMGA2 in fibrosarcoma, HMGA2 was overexpressed and knocked down in HT1080 cells, respectively. The efficiency of HMGA2 overexpression and knockdown was assessed via RT-qPCR analysis (Fig. S1). The overexpression of HMGA2 significantly upregulated the expression of HMGA2 compared with that in the NC plasmid group, suggesting that HMGA2 was overexpressed successfully in HT1080 cells. The expression of HMGA2 was significantly decreased in the si-HMGA2 group compared with that in the si-NC group, suggesting that HMGA2 was silenced successfully in TH1080 cells. The effect of overexpressing HMGA2 on cell proliferation, migration and invasion was assessed via the CCK-8, wound healing and Transwell assays, respectively. As presented in (Fig. 2A-C), overexpression of HMGA2 significantly promoted fibrosarcoma cell proliferation, migration and invasion, whereas HMGA2 knockdown had no effect on the proliferation of fibrosarcoma cells (Fig. S2). Mechanistically, western blot analysis demonstrated that overexpression of HMGA2 increased the protein expression levels of N-cadherin and Vimentin, while decreasing E-cadherin expression, representing a signal of EMT (Fig. 2D). Taken together, these results suggest that HMGA2 promotes cell migration and invasion via EMT.

Over expression of miR-145 inhibits cell proliferation, migration and invasion of fibrosarcoma. To determine the direct function of miR-145 in fibrosarcoma cells, miR-145 was overexpressed in HT1080 cells via transfection with its mimics. The efficiency of miR-145 mimics was assessed via RT-qPCR analysis (Fig. S3). The expression levels of miR-145 were significantly increased in the miR-145 mimic group compared with those in the mimic NC group, indicating that the miR-145 overexpression was successfully constructed. As presented in (Fig. 3A), overexpression of miR-145 significantly inhibited the proliferation of HT1080 cells within 5 days. Subsequently, the present study investigated the suppressive effects of miR-145 on the migratory ability of fibrosarcoma cells via the wound healing and Transwell migration assays. The results of the wound healing assay demonstrated that miR-145 effectively impaired the migratory ability of HT1080 cells (Fig. 3B and C). The results of the Transwell migration assay further confirmed the inhibitory effects of miR-145 on the migratory ability of fibrosarcoma cells (Fig. 3C). Collectively, these results suggest that overexpression of miR-145 remarkably inhibits proliferation, migration and invasion of fibrosarcoma cells.

miR-145 acts as an HMGA2 sponge to mitigate EMT. Western blot analysis demonstrated that miR-145 negatively regulated the expression levels of HMGA2, N-cadherin and Vimentin, but promoted E-cadherin expression in HT1080 cells (Fig. 3D). Bioinformatics analysis revealed that HMGA2 was a predicted target of miR-145. To confirm this, the dual-luciferase reporter assay was performed to determine whether miR-145 directly targets HMGA2 in fibrosarcoma cells. HT1080 cells were co-transfected with pmirGLO Dual-Luciferase miRNA Target Expression Vector with HGMA2-3'-UTR (HMGA2-WT) or its control vector mutant pmirGLO-HMGA2-3'-UTR (HMGA2-MUT) and miR-145 mimics or NC. The results demonstrated that luciferase activity was significantly lower in cells transfected with HMGA2-WT and miR-145 compared with cells transfected with HMGA2-MUT and miR-145, but exhibited negligible difference with that in the cells transfected with HMGA2 MUT and miR-145 (Fig. 4A). These results suggest that miR-145 targets the 3'-UTR of HMGA2 directly and negatively regulates HMGA2 expression in HT1080 cells, which was consistent with the downregulated HMGA2 expression in HT1080 cells transfected with miR-145 (Fig. 4B).

To further confirm that the effects of miR-145 on HT1080 cell function are mediated by HMGA2, rescue experiments were performed using HT1080 cells co-transfected with miR-145 mimics, with or without the HMGA2 overexpression plasmid. As presented in (Fig. 4C), HMGA2 protein expression increased in HT1080 cells co-transfected with miR-145 mimics and HMGA2 overexpression plasmid compared with those that were co-transfected with miR-145 mimics alone. Furthermore, the CCK-8 assay demonstrated that HMGA2 restoration in HT1080 cells significantly reversed the inhibition of cell proliferation following overexpression of miR-145 (Fig. 4D). In addition, overexpression of HMGA2 rescued the inhibitory effects of miR-145 on HT1080 cell migration and invasion, as determined by the wound healing (Fig. 5A and C) and Transwell migration and invasion (Fig. 5B, D and E) assays, respectively.

Table 1: Predicted genes and their full names.

Gene	Full name	Gene	Full name
ACTL8	Actin-like protein 8	IL13RA2	Interleukin 13 receptor subunit alpha 2
BAGE	B melanoma antigen	MCAM	Melanoma cell adhesion molecule
BCL2A1	BCL2 related protein A1	MMP14	Matrix metallopeptidase 14
CORO1C	Coronin 1C	NLRP3	NLR family pyrin domain containing 3
EIF1AY	Eukaryotic translation initiation factor 1A Y-linked	SCLY	Selenocysteine lyase
EPHB2	EPH receptor B2	SSX1	SSX family member 1
ESM1	Endothelial cell specific molecule 1	SSX2B	SSX family member 2B
FABP4	Fatty acid binding protein 4	ZFY	Zinc finger protein Y-linked
GAGE1	G antigen 1	ZWILCH	Zwilch kinetochore protein
HMGA2	High mobility group protein A2		

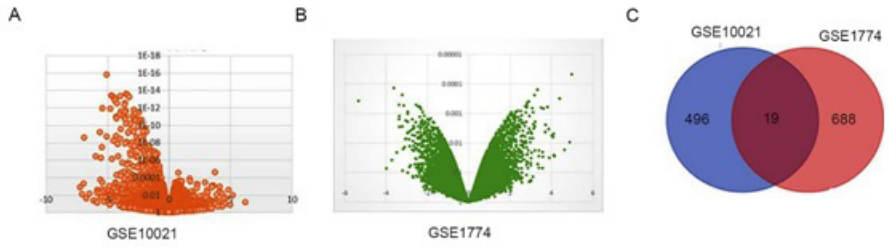
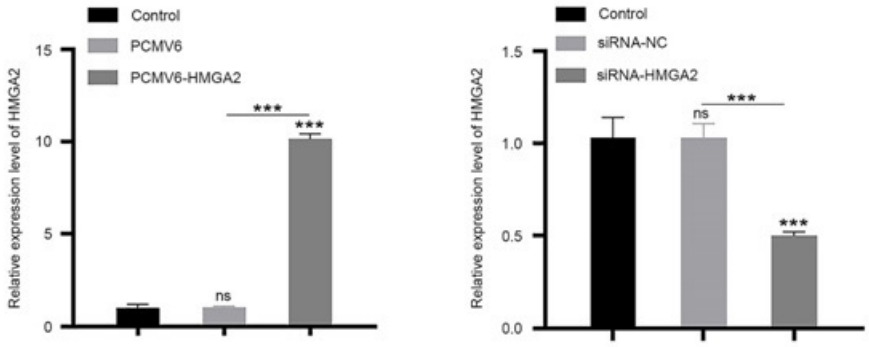


Figure 1: Bioinformatics analysis. (A) The GSE10021 chip was analyzed to identify genes expressed in fibrosarcoma HT1080 cells. (B) The GSE1774 chip was analyzed to identify genes expressed in fibrosarcoma HT1080 cells. (C) The numbers of genes expressed in fibrosarcoma HT1080 cells.

Table 2: Predicted functions of candidate genes.

Predicted functions of candidate genes
Transcriptional misregulation in cancer_Homo sapiens_hsa05202
Selenocompound metabolism_Homo sapiens_hsa00450
Regulation of lipolysis in adipocytes_Homo sapiens_hsa04923
NOD-like receptor signaling pathway_Homo sapiens_hsa04621
GnRH signaling pathway_Homo sapiens_hsa04912
PPAR signaling pathway_Homo sapiens_hsa003320
Pertussis_Homo sapiens_hsa05133
TNF signaling pathway_Homo sapiens_hsa04668
NF kappa B signaling pathway_Homo sapiens_hsa04064
Apoptosis_Homo sapiens_hsa04210



Supplementary Figure S1: HMGA2 is successfully overexpressed and downregulated in HT1080 cells. (A) RT-qPCR analysis was performed to detect HMGA2 expression in HT1080 cells transfected with HMGA2 overexpression plasmid. (B) RT-qPCR analysis was performed to detect HMGA2 expression in HT1080 cells transfected with siRNA-HMGA2. ***P<0.001 vs Control, pCMV6 or siRNA-NC. RT-qPCR, reverse transcription-quantitative PCR; HMGA2, high mobility group protein A; si, small interfering; NC, negative control; ns, no significance.

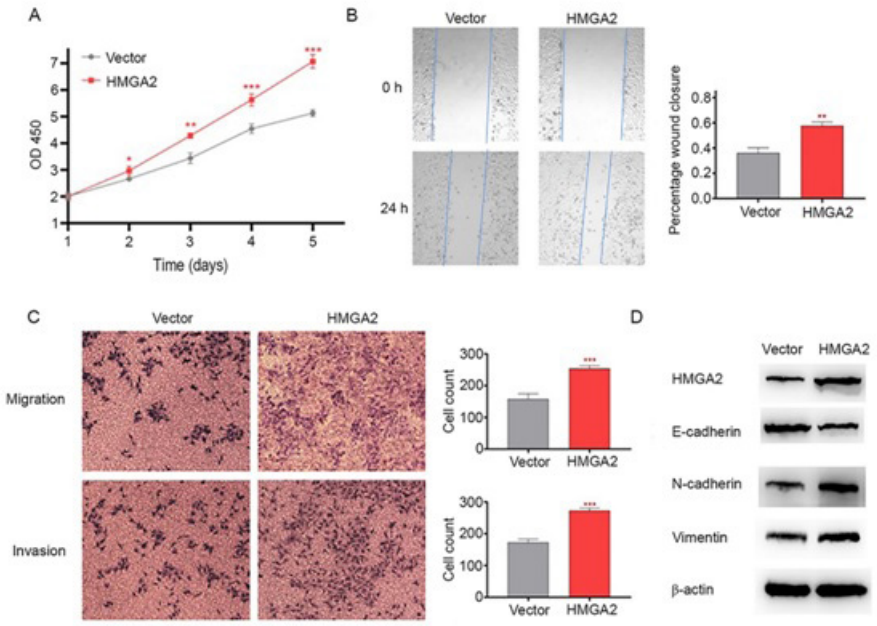
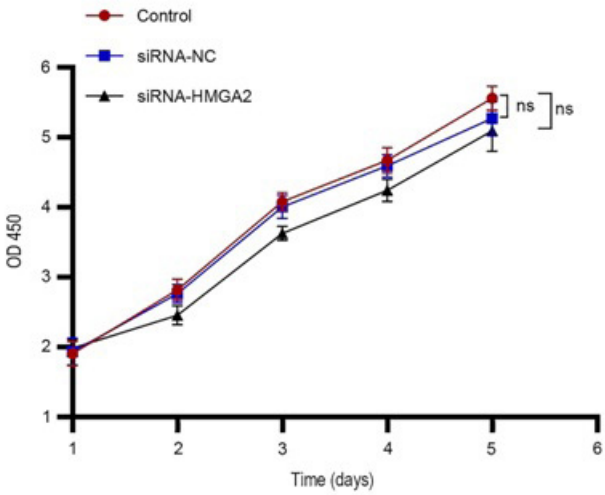
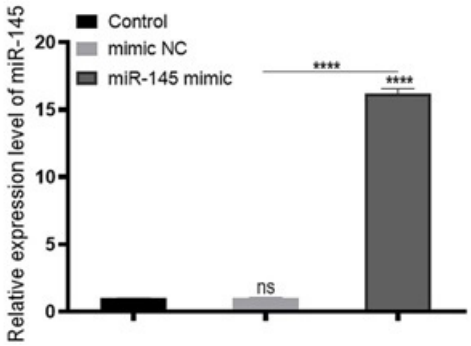


Figure 2: Overexpression of HMGA2 promotes HT1080 cell proliferation, migration and invasion. (A) The Cell Counting Kit-8 assay was performed to detect the proliferation of HT1080 cells transfected with HMGA2 for 24, 48, 72, 96 and 120 h. (B) The wound healing assay was performed to detect the migratory ability of HT1080 cells transfected with HMGA2 for 24 h (magnification, x200). (C) The Transwell assay was performed to detect the migratory and invasive abilities of HT1080 cells transfected with HMGA2 for 24 h (magnification, x200). (D) Western blot analysis was performed to detect the protein expression levels of HMGA2, E-cadherin, N-cadherin and Vimentin. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ vs. Vector. HMGA2, high mobility group protein A2; OD, optical density.



Supplementary Figure S2: Cell Counting Kit-8 assay is used to detect the proliferation of HT1080 cells following HMGA2 knockdown for 24, 48, 72, 96 and 120 h. HMGA2, high mobility group protein A; si, small interfering; NC, negative control; OD, optical density; ns, no significance.



Supplementary Figure S3: miR-145 is successfully overexpressed in HT1080 cells. (A) RT-qPCR analysis was performed to detect miR-145 expression in HT1080 cells transfected with miR-145 mimic the. $^{****}P<0.0001$ vs control group and mimic NC. RT-qPCR, reverse transcription-quantitative PCR; miR, microRNA; NC, negative control; ns, no significance.

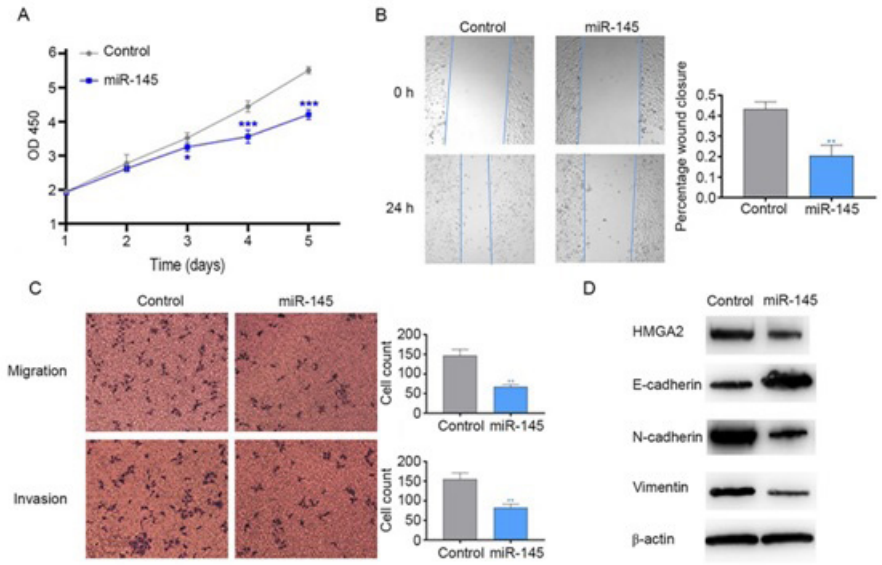


Figure 3: Overexpression of miR-145 inhibits HT1080 cell proliferation, migration and invasion. (A) The Cell Counting Kit-8 assay was performed to detect the proliferation of HT1080 cells transfected with miR-145 for 24, 48, 72, 96 and 120 h. (B) The wound healing assay was performed to detect the migratory ability of HT1080 cells transfected with miR-145 for 24 h. (C) The Transwell assay was performed to detect the migratory and invasive abilities of HT1080 cells transfected with miR-145 for 24 h. (D) Western blot analysis was performed to detect the protein expression levels of HMGA2, E-cadherin, N-cadherin and Vimentin. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ vs. Vector. miR, microRNA; OD, optical density.

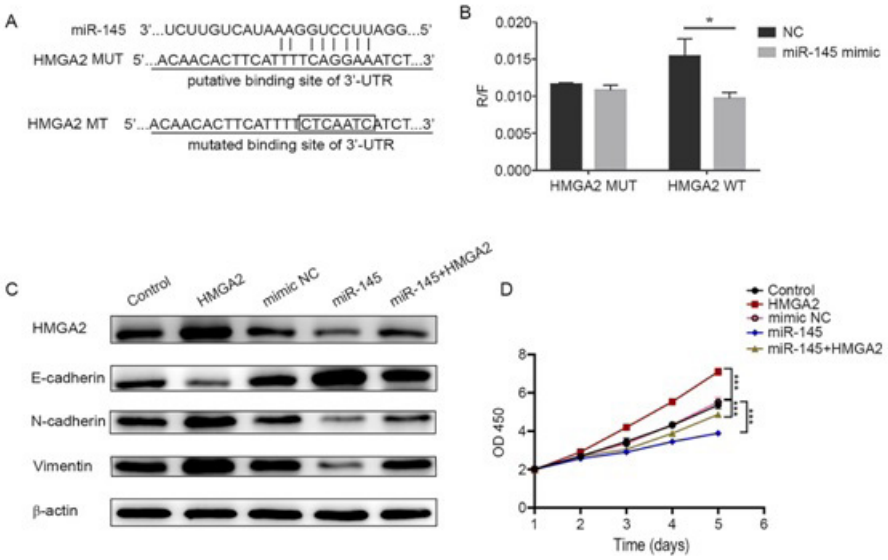


Figure 4: miR-145 acts as an HMGA2 sponge to mitigate epithelial-to-mesenchymal transition. (A) The binding site of miR-145 within the 3'-UTR of HMGA2. (B) The dual-luciferase reporter assay confirmed that miR-145 targets HMGA2. (C) Western blot analysis was performed to detect the protein expression levels of HMGA2, E-cadherin, N-cadherin and Vimentin in HT1080 cells transfected with miR-145 mimics, in the presence or absence of HMGA2 overexpression. (D) The Cell Counting Kit-8 assay was performed to detect the proliferation of HT1080 cells transfected with miR-145 mimics, in the presence or absence of HMGA2 overexpression. miR, microRNA; HMGA2, high mobility group protein A2; UTR, untranslated region; WT, wild-type; MUT, mutant; NC, negative control; OD, optical density; R/F, Relative luciferase activity.

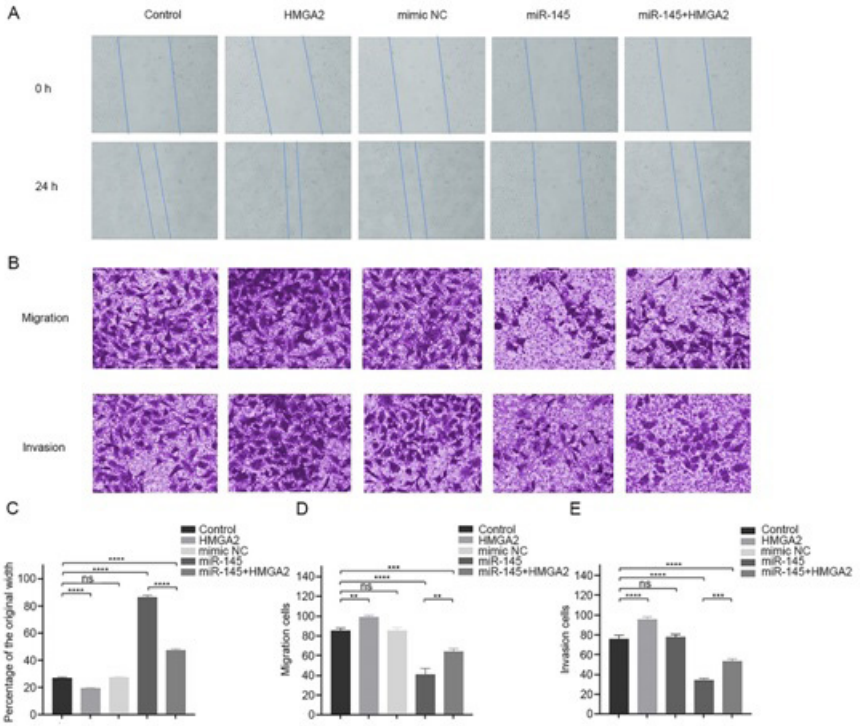


Figure 5: Overexpression of HMGA2 reverses the inhibitory effects of miR-145 on HT1080 cell migration and invasion. (A) The wound healing assay was performed to detect the migratory ability of HT1080 cells transfected with miR-145 mimics, in the presence or absence of HMGA2 overexpression for 24 h. (B) The Transwell assay was performed to detect the migratory and invasive abilities of HT1080 cells transfected with miR-145 mimics, in the presence or absence of HMGA2 overexpression for 24 h. (C) Quantified results from the wound healing assay. (D) Quantified results from the Transwell migration assay. (E) Quantified results from the Transwell invasion assay. $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$ vs. Vector. miR, microRNA; HMGA2, high mobility group protein A; NC, negative control; ns, no significance.

5. Discussion

Recently, increasing evidence suggest that miRNAs play important roles in the pathogenesis of several diseases, including cancer [33,34]. The recently discovered miRNA, miR-145, plays important roles in different diseases, according to recent studies. For example, it has been confirmed that miR-145 is associated with spontaneous hypertension by sponging SLC7A1 [35], and is also associated with breast cancer [36] and laryngeal cancer [37]. In addition, in NSCLC, miR-145 inhibits the expression of epidermal growth factor receptor, which increases sensitivity to receptor inhibitors, and significantly suppresses the proliferation of NSCLC cells [38]. However, the association between miR-145 and fibrosarcoma remains unclear. The results of the present study demonstrated that miR-145 suppressed fibrosarcoma cell proliferation, migration and invasion by blocking EMT through directly targeting an EMT-related key regulator, HMGA2.

EMT refers to the loss of epithelial cell phenotype and the gradual acquisition of stromal cell phenotype of tumor cells. In this process, tumor cells acquire enhanced migratory and invasive abilities [39,40]. Therefore, EMT is considered an important molecular mechanism in tumor cell migration and invasion [41]. HMGA2 belongs to the family of high mobility group proteins and is a transcription factor that is highly expressed in some malignancies, including breast cancer, NSCLC, pancreatic cancer, retinoblastoma and squamous cell carcinoma, suggesting its role in the malignant transformation of tumor cells [24-26]. It has been reported that HMGA2 can bind to the promoter of Snail following direct or indirect binding with Smad [42]. This binding results in the suppression of E-cadherin transcription and promotion of Vimentin and N-cadherin expression, which promote the EMT process [42]. Previous studies have demonstrated that suppression of HMGA2 by miRNAs can inhibit EMT and thus suppressing tumor cell migration and invasion in breast cancer, NSCLC and pancreatic cancer [43-45]. Zhao et al [18] reported that miR-145 suppresses breast cancer cell migration by targeting FSCN-1 and inhibiting EMT, suggesting the molecular association between miR-145-5p, FSCN-1 and EMT in the regulation of breast cancer migration. The results of the present study confirmed that HMGA2 is a candidate target of miR-145 in fibrosarcoma cells via bio informatics analysis. Although Kim et al [32] has reported that miR-145 serves as a prognostic biomarker and a tumor suppressor that regulates HMGA2 expression in ovarian cancer, its underlying molecular mechanisms remain unclear. Thus, further studies are required to determine whether the molecular mechanism of miR-145 in fibrosarcoma inhibits EMT by targeting HMGA2. To investigate this, the present study overexpressed miR-145 in fibrosarcoma cells. The results demonstrated that overexpression of miR-145 reversed EMT and significantly decreased HT1080 cell proliferation, migration and

invasion, the effects of which were reversed following overexpression of HMGA2. Furthermore, western blot analysis demonstrated that overexpression of miR-145 notably decreased HMGA2 expression. The results of the dual-luciferase reporter assay verified that miR-145 can directly bind to HGMA2, which are consistent with the results of Kim et al [32], confirming that HMGA2 is a direct target of miR-145 [32]. Taken together, the results of the present study suggest that miR-145 plays an important role in the pathogenesis of fibrosarcoma by targeting HMGA2, an oncogenic protein that promotes the migration and invasion of fibrosarcoma cells via EMT[46]. Therefore, the miR-145/HMGA2 pathway may be a potential strategy for fibrosarcoma therapy.

The present study is not without limitations. Only HT1080 cells were assessed to demonstrate that miR-145 can inhibit EMT and fibrosarcoma cell proliferation, migration and invasion by targeting HMGA2. Thus, prospective studies will assess the effects in multiple cell lines, as well HMGA2 expression in different cell lines.

In conclusion, the results of the present study suggest that miR-145 plays an inhibitory role in fibrosarcoma progression by directly suppressing HGMA2 expression and EMT progression. Thus, miR-145 may be a potential target of gene therapy for malignant fibrosarcoma.

6. Funding

The present study was supported by the project of National Natural Science Foundation of China (grant no. 81872179).

References

1. Voltan K, Baptista AM, Etchebehere M. Extremities Soft Tissue Sarcomas, more Common and as Dangerous as Bone Sarcomas. Rev Bras Ortop (Sao Paulo). 2021; 56(4): 419-424.
2. Gaiser T, Sauer C, Marx A, Jakob J, Kasper B. Molecular and Pathological Profiling of Corresponding Treatment-Naive and Neoadjuvant Pazopanib-Treated High-Risk Soft Tissue Sarcoma Samples of the GISG-04/NOPASS Study. Biology (Basel). 2021; 10(7): 639.
3. Teicher BA. Searching for molecular targets in sarcoma. Biochem Pharmacol. 2012; 84(1): 1-10.
4. Bright CJ, Hawkins MM, Winter DL, Alessi D, Allodji RS. Risk of Soft-Tissue Sarcoma Among 69 460 Five-Year Survivors of Childhood Cancer in Europe. J Natl Cancer Inst. 2018; 110(6): 649-660.
5. Chudgar NP, Brennan MF, Tan KS, Munhoz RR, D'Angelo SP. Is Repeat Pulmonary Metastasectomy Indicated for Soft Tissue Sarcoma?. Ann Thorac Surg. 2017; 104(6): 1837-1845.
6. Davis DD, Shah SJ and Kane SM. Fibrosarcoma. In: StatPearls, Treasure Island (FL). 2021.
7. Akcali M, Yapicioglu H, Akay E, Özlü F, Kozanoğlu B. A congenital soft tissue Ewing sarcoma in a newborn patient. Turk J Pediatr. 2017; 59(1): 76-79.
8. Grieser T, Nobauer-Huhmann IM. Postoperative and posttherapeutic changes after primary bone tumors: What's important for radiologists?. Radiologe 2017; 57(11): 938-957.
9. Zhu B, Wu Y, Niu L,Yao W, Xue M. Silencing SAPCD2 Represses Proliferation and Lung Metastasis of Fibrosarcoma by Activating Hippo Signaling Pathway. Front Oncol. 2020; 10: 574383.
10. Kasper B, Wardelmann E. Outcome prediction in patients with localized soft tissue sarcoma: which tool is the best?. Ann Oncol. 2018; 29(2): 297-298.
11. Villalobos VM, Byfield SD, Ghate SR, Adejoro O. A retrospective cohort study of treatment patterns among patients with metastatic soft tissue sarcoma in the US. Clin Sarcoma Res. 2017; 7: 18.
12. Mirzaei S, Saebfar H, Gholami MH. MicroRNAs regulating SOX2 in cancer progression and therapy response. Expert Rev Mol Med. 2021; 23: e13.
13. Wong ACH, Rasko JEJ. Splice and Dice: Intronic microRNAs, Splicing and Cancer. Biomedicines. 2021; 9(9): 1268.
14. Smolle MA, Leithner A, Posch F, Szkandera J, Liegl-Atzwanger B. MicroRNAs in Different Histologies of Soft Tissue Sarcoma: A Comprehensive Review. Int J Mol Sci. 2017; 18(9): 1960.
15. Thomas S, Manivannan S, Sawant D. miR-145 transgenic mice develop cardiopulmonary complications leading to postnatal death. Physiol Rep. 2021; 9(17): e15013.
16. Cao C, Shu X. Suppression of circ_0008932 inhibits tumor growth and metastasis in osteosarcoma by targeting miR-145-5p. Exp Ther Med. 2021; 2294): 1106.
17. Kadkhoda S, Taslimi R, Noorbakhsh F. Importance of Circ0009910 in colorectal cancer pathogenesis as a possible regulator of miR-145 and PEAK1. World J Surg Oncol. 2021; 19(1): 265.
18. Zhao H KX, Xia X, Wo L, Gu X, Hu Y. miR-145 suppresses breast cancer cell migration by targeting FSCN-1 and inhibiting epithelial-mesenchymal transition. Am J Transl Res. 2016; 8(7): 3106-3114.
19. Johannessen C, Moi L, Kiselev Y. Expression and function of the miR-143/145 cluster in vitro and in vivo in human breast cancer. PLoS One 2017; 12(10): e0186658.
20. Mataki H, Seki N, Mizuno K. Dual-strand tumor-suppressor microRNA-145 (miR-145-5p and miR-145-3p) coordinately targeted MTDH in lung squamous cell carcinoma. Oncotarget. 2016; 7(44): 72084-72098.
21. Yu Y, Nangia-Makker P, Farhana L, Rajendra SG, Levi E. miR-21 and miR-145 cooperation in regulation of colon cancer stem cells. Mol Cancer. 2015; 14: 98.
22. Zeng JF, Ma XQ, Wang LP, Wang W. MicroRNA-145 exerts tumor-suppressive and chemo-resistance lowering effects by targeting CD44 in gastric cancer. World J Gastroenterol. 2017; 23(13): 2337-2345.
23. Zhao XP, Zhang H, Jiao JY, Tang DX, Wu YL. Overexpression of HMGA2 promotes tongue cancer metastasis through EMT pathway. J Transl Med. 2016; 14(26): 1-11.
24. Sun X, Xu M, Liu H, Ming K. MicroRNA-219 is downregulated in non-small cell lung cancer and inhibits cell growth and metastasis by targeting HMGA2. Mol Med Rep. 2017; 16(3): 3557-3564.
25. Zhuo HC, Song YF, Ye J, Lai GX, Liu DL. MicroRNA-154 functions as a tumor suppressor and directly targets HMGA2 in human non-small cell lung cancer. Genet Mol Res. 2016; 15(3).

26. Wu J, Zhang S, Shan J, Liu X, Chen L. Elevated HMGA2 expression is associated with cancer aggressiveness and predicts poor outcome in breast cancer. Cancer Lett. 2016; 376(2): 284-292.
27. He QY, Wang GC, Zhang H, Tong Da-Ke, Dinge C. miR-106a-5p eSuppresses the Proliferation, Migration, and Invasion of Osteosarcoma Cells by Targeting HMGA2. DNA Cell Biol. 2016; 35(9): 506-520.
28. Tang P, Xiong Q, Ge W. Zhang Z. The role of microRNAs in osteoclasts and osteoporosis. RNA Biol. 2014; 11(11): 1355-1363.
29. Damanakis AI, Eckhardt S, Wunderlich A. MicroRNAs let7 expression in thyroid cancer: correlation with their deputed targets HMGA2 and SLC5A5. J Cancer Res Clin Oncol. 2016; 142(6): 1213-1220.
30. Guo T, Wang J, Cheng G, Huang H. miR-590-5p may regulate colorectal cancer cell viability and migration by targeting PDCD4. Exp Ther Med. 2020; 20(5): 55.
31. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4): 402-408.
32. Kim TH, Song JY, Park H. miR-145, targeting high-mobility group A2, is a powerful predictor of patient outcome in ovarian carcinoma. Cancer Lett. 2015; 356(2 Pt B): 937-945.
33. Moazzendizaji S, Sevbitov A, Ezzatifar F. microRNAs: small molecules with a large impact on colorectal cancer. Biotechnol Appl Biochem. 2021.
34. Konoshenko MY, Bryzgunova OE, Laktionov PP. miRNAs and androgen deprivation therapy for prostate cancer. Biochim Biophys Acta Rev Cancer. 2021; 1876(2):188625.
35. Wang Y, Jin L. miRNA-145 is associated with spontaneous hypertension by targeting SLC7A1. Exp Ther Med 2018; 15(1): 548-552.
36. Quan Y, Huang X, Quan X. Expression of miRNA-206 and miRNA-145 in breast cancer and correlation with prognosis. Oncol Lett. 2018; 16(5): 6638-6642.
37. Guo L, Cai X, Hu W, Hua W, Yan W. Expression and clinical significance of miRNA-145 and miRNA-218 in laryngeal cancer. Oncol Lett. 2019; 18(1): 764-770.
38. Amri J, Molaee N, Baazm M, Karami H. Targeting Epidermal Growth Factor Receptor by MiRNA-145 Inhibits Cell Growth and Sensitizes NSCLC Cells to Erlotinib. Asian Pac J Cancer Prev. 2019; 20(9): 2781-2787.
39. Ray T, Ryusaki T, Ray PS. Therapeutically Targeting Cancers That Overexpress FOXC1: A Transcriptional Driver of Cell Plasticity, Partial EMT, and Cancer Metastasis. Front Oncol. 11: 721959.
40. Ashrafizadeh M, Mirzaei S, Hashemi F. New insight towards development of paclitaxel and docetaxel resistance in cancer cells: EMT as a novel molecular mechanism and therapeutic possibilities. Biomed Pharmacother 2021; 141: 111824.
41. Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer 18(2): 128-134.
42. Thuault S, Tan EJ, Peinado H, Cano A, Heldin CH. HMGA2 and Smads co-regulate SNAIL1 expression during induction of epithelial-to-mesenchymal transition. J Biol Chem. 2008; 283(48): 33437-33446.
43. Mansoori B, Duijf PHG, Mohammadi A. MiR-142-3p targets HMGA2 and suppresses breast cancer malignancy. Life Sci. 2021; 276: 119431.
44. Han X, Huang T, Han J. Long noncoding RNA VPS9D1-AS1 augments the malignant phenotype of non-small cell lung cancer by sponging microRNA-532-3p and thereby enhancing HMGA2 expression. Aging (Albany NY). 2020; 12(1): 370-386.
45. Mao XB, Sheng T, Zhuang LP, Wu CK, Zhao GG. [Expression of miRNA let-7a and HMGA2 and Diagnostic Value of Serum miRNA let-7a Level in Pancreatic Cancer]. Sichuan Da Xue Xue Bao Yi Xue Ban. 2020; 51(4): 540-545.
46. Wang J, Chen Y, Zeng Z. HMGA2 contributes to vascular development and sprouting angiogenesis by promoting IGFBP2 production. Exp Cell Res. 2021; 408(1): 112831.