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Targeting GOLM1 by microRNA-200a in melanoma suppresses cell proliferation, invasion and migration via regulating PI3K/Akt signaling pathway and epithelial-mesenchymal transition

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Abstract. – OBJECTIVE: Metastatic melanoma, which is refractory to therapies, is one of the most aggressive types in skin cancers. microRNAs (miRNAs) have recently emerged as novel molecules which have therapeutic effects on melanoma. This study focused on the roles and mechanisms of miR-200a in melanoma progression.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed to examine the level of miR-200a expression in 46 pairs of melanoma tissues and para-cancerous specimens, and the relationship between miR-200a level and clinical features of melanoma patient prognosis was analyzed. MiR-200a expression in melanoma cells was further verified by qRT-PCR. In addition, we identified the biological target of miR-200a using TargetScan. To delineate the molecular mechanism underlying the tumor-suppressive roles of miR-200a in melanoma, Western blots were performed to determine the functions of miR-200a in PI3K/Akt pathway and EMT.

RESULTS: QRT-PCR analysis demonstrated prominent decrease of miR-200a in melanoma tissues, which was associated with the poor prognosis and malignant clinicopathologic features of melanoma patients. Moreover, functional assays indicated that miR-200a overexpression markedly repressed melanoma cell proliferation, invasion, and migration capacities. A luciferase reporter analysis showed that Golgi membrane protein 1 (GOLM1) was a functional target of miR-200a in melanoma cells. Western blot analysis revealed that miR-200a inhibited melanoma progression by regulating PI3K/Akt signaling pathway and epithelial-mesenchymal transition (EMT). It was also found that miR-200a upregulation markedly suppressed melanoma tumorigenesis *in vivo*. All these data showed that miR-200a served as a promising therapeutic target in melanoma patients.

CONCLUSIONS: We provided evidence that miR-200a was down-regulated in melanoma and was implicated in melanoma progression *via* inhibiting GOLM1 expressions and regulating PI3K/Akt signaling pathway and EMT. Decreased levels of miR-200a were related to poor prognosis of melanoma patients. Findings of the present study provided a novel insight into understanding melanoma pathogenesis, suggesting that miR-200a may function as a promising and potential therapeutic biomarker for melanoma treatments.

Key Words:
Melanoma, MiR-200a, GOLM1, PI3K/Akt signaling pathway, Epithelial-mesenchymal transition.

Introduction

Human melanoma is an aggressive skin cancer with high incidences and mortalities[1]. It is characterized by resistance to radiotherapy or chemotherapy, early metastases, and aggressive invasion[2]. Currently, the therapeutic strategies for melanoma patients mainly include surgical treatment, chemotherapy, radiotherapy, and subsequent biotherapy[3]. Cases with early diagnosis of melanoma can be cured by surgical resections[4]. On the other hand, advanced melanoma with distant or local metastasis is poorly responsive to

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existing treatments, leading to high mortalities[5], and poor prognosis with 5-year survival rates of no more than 15%[6]. Therefore, exploring the mechanism involved in melanoma tumorigenesis and progression is urgently needed for the development of potentially effective methods for melanoma treatment.

MicroRNAs (miRNAs) have appeared to be hotspots in cancer research, and growing evidence has indicated that dysregulations of miRNA frequently occur in tumors[7]. MiRNAs take important parts in regulating gene expressions *via* interacting with the 3'-UTRs of the target mRNA, giving rise to repressing or cleaving the translation of the target genes[8]. Emerging evidence has demonstrated that miRNA plays a critical role in various cellular processes, such as cell metastases, differentiation, apoptosis, and growth[9-11]. In the context of the tumor, miRNA may function as an oncogene or a tumor suppressor in tumorigenesis and cancer development. For instance, Liu et al[12] demonstrated that miR-19b promoted ovarian carcinoma cell metastasis by suppressing the PTEN/AKT signaling pathway. Wang et al[13] showed that miR-183 up-regulation promoted glioma cell proliferation and invasion by regulating NEFL. Shang et al[14] revealed that miR-192 repressed osteosarcoma progression *via* regulation of matrix metalloproteinase-11. However, the exact effects of miR-200a in melanoma progression remain to be thoroughly elucidated. Moreover, further understandings of the miR-200a functions in melanoma progression may be helpful in the identification of new therapeutic biomarkers for melanoma treatments.

Epithelial-mesenchymal transition (EMT), a cellular conversion from epithelial to mesenchymal property, plays pivotal roles in tumorigenesis[15]. EMT is one of the most essential processes in tumor invasion and metastases[16]. A great number of studies showed that activation of EMT was identified in multiple tumors and promoted tumorigenesis of cancers, including gastric carcinoma[17], breast carcinoma[18], and pancreatic carcinoma[19]. Thus, suppressing EMT plays key roles in repressing tumorigenesis. PI3K/Akt signaling pathway acts as an important driver in carcinogenesis[20]. Findings of some studies have shown that the abnormal activation of the PI3K/Akt signaling pathway is implicated in the initiation and development of numerous cancers, playing a vital role in the modulating several biological processes, such as metabolism, proliferation, and apoptosis survival[21]. Therefore, it was speculated that the EMT and PI3K/Akt are implicated in the regulations of melanoma mediated by miR-200a.

Golgi membrane protein 1 (GOLM1), a type II transmembrane protein of the Golgi cisternae, is typically expressed in the epithelial cells of normal human tissues[22]. It has been shown that Golgi plays active roles in cell metastasis *via* significant changes in the Golgi apparatus and posttranslational modifications, as verified by disruptions of biochemical compositions, structures, and functional levels observed in metastases and carcinogenesis[23]. Higher expressions of GOLM1 have been confirmed to be involved in a variety of tumors. Zhang et al[24] revealed that GOLM1 was up-regulated in non-small cell lung cancer (NSCLC), and the up-regulation correlated with malignant clinicopathological characteristics of NSCLC patients and promoted NSCLC cell proliferation and invasion. Therefore, our study focused on the functions of GOLM1 in melanoma.

Patients and Methods

Clinical Samples

46 pairs of melanoma tissue specimens and matched normal tissue specimens were obtained from Heilongjiang University of Chinese Medicine between October 2016 and March 2018. No patients had been treated with anticancer treatments before tissue collection. All the tissues were immediately frozen in liquid nitrogen and stored at -80°C until use. Written informed consents were obtained from all of the patients enrolled in the current study. This study was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University.

Cell Culture

Human melanoma cells (A375, SK-HEP-1, WM35, and SK-MEL-28) were obtained from the American Type Culture Collection (ATCC: Manassas, VA, USA). Human epidermal melanocyte (HEM) obtained from ScienCell Research Laboratories, Inc. (San Diego, CA, USA) were cultured in melanocyte medium. Melanoma cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS: Thermo Fisher Scientific, Inc., Waltham, MA, USA). All cell lines were incubated in a humidified incubator containing 5% CO_2 at 37°C.

Cell Transfection

MiR-200a mimics, inhibitor and negative controls were purchased from GenePharma. (Shanghai, China). The transfections were performed by Lipofectamine 2000 (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's protocols.

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

TRIzol (Invitrogen, Carlsbad, CA, USA) was utilized to isolate the total ribonucleic acids (RNAs) from the tissues and cultured cells. Then, complementary deoxyribonucleic acid (cDNA) was prepared from total RNA using the PrimeScript RT reagent kit (Takara, Otsu, Shiga, Japan). QPCR was performed with SYBR Premix Ex Taq™ (Takara, Otsu, Shiga, Japan) on an ABI Prism 7500 Sequence Detection system (Applied Biosystems; Foster City, CA, USA). The primer sequences were listed in Table I. U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were internal controls for normalizations. The expressions were calculated using the $2^{-\Delta\Delta Ct}$ method.

MTT Assays

Cell proliferation ability of transfected melanoma cells was assessed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, thiazolyl blue tetrazolium bromide (MTT) assays (Sigma-Aldrich, St. Louis, MO, USA). Transfected melanoma cells were seeded into 96-well plates (Sigma-Aldrich, St. Louis, MO, USA). Then, the MTT solution was added to each well at 0, 24, 48, and 72 h post transfections and incubated at 37°C for an additional 4 h. After that, dimethyl sulfoxide (DMSO) was added into the wells to dissolve the remaining MTT formazan. The absorbance at 490 nm was detected using a microplate reader (BioTek, Winooski, VT, USA) to determine the cell proliferation activity.

Cell Migration and Invasion Assay

The 8-μm pore sized transwell chamber (BD Biosciences, San Jose, CA, USA) percolated with or without Matrigel was used to perform cell invasion and migration assays. The transfected cells were resuspended in serum-free medium and seeded in the upper chambers. The low chambers were filled with medium containing 10% FBS. After being incubated at 37°C for 48 h, cells remained on the top chambers were wiped off with cotton swabs, whereas the cells adhered to the undersurface of the membranes were fixed and stained. The cells were quantified with an inverted microscope (Olympus Corporation, Tokyo, Japan) under five randomly selected visual fields.

Western Blot Analyses

Total protein extractions were performed with lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA). bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Waltham, MA, USA) was utilized to measure the protein concentrations. An equal amount of protein samples were subjected to 10% dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE), followed by being transferred onto polyvinylidene difluoride (PVDF) membranes (Invitrogen, Carlsbad, CA, USA). After being blocked with 5% skimmed milk, the membrane was then incubated at 4°C overnight with primary antibodies against: GOLM1 (1:1000, Abcam, Cambridge, MA, USA), PI3K (1:1000, Abcam, Cambridge, MA, USA), p-PI3K (1:2000, Abcam, Cambridge, MA, USA), AKT (1:1000, Abcam, Cambridge, MA, USA), p-AKT (1:1000, Abcam, Cambridge, MA, USA), E-cadherin (1:2000, Abcam, Cambridge, MA, USA), N-cadherin (1:2000, Abcam, Cambridge, MA, USA), Vimentin (1:1000, Abcam, Cambridge, MA, USA) and GAPDH

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-200a forward	5'-AAGCGCCTTAACACTGTCTGG-3'
miR-200a reverse	5'-CAGTGCAGGGTCCGAGGT-3'
U6 forward	5'-CTCGCTTCGGCAGCACA-3'
U6 reverse	5'-AACGCTTCACGAATTTGCGT-3'
GOLM1 forward	5'-CCGGAGCCTCGAAAAGAGATT-3'
GOLM1 reverse	5'-ATGATCCGTGTCTGGAGGTC-3'
GAPDH forward	5'-ACCTGACCTGCCGTCTAGAA-3'
GAPDH reverse	5'-TCCACCACCCTGTTGCTGTA-3'

U6: small nuclear RNA, snRNA; GOLM1: Golgi membrane protein; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

(1:1000, Abcam, Cambridge, MA, USA). After that, the membranes were probed with appropriate horseradish peroxidase (HRP)-labeled secondary antibody (1:3,000, Abcam, Cambridge, MA, USA) at room temperature for 2 h. Finally, the protein bands were visualized with enhanced chemiluminescence (ECL) reagents (Millipore, Billerica, MA, USA). GAPDH was an internal control.

Luciferase Reporter Analysis

A wild-type (WT) or mutant (MUT) luciferase reporter plasmids of GOLM1 3'-UTRs containing the binding sites of miR-200a were chemically synthesized by GenePharma (Shanghai, China). Then, melanoma cells were cotransfected with GOLM1 3'-UTR-WT or GOLM1 3'-UTR-MUT and miR-200a mimics using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The Dual-Luciferase Reporter Assay Kit (Promega, Madison, WI, USA) was used to detect the luciferase activities 48 h post-transfection.

Immunohistochemistry (IHC)

IHC assays of human melanoma tissues were conducted to determine the expression levels of GOLM1. The tissue samples were fixed with 4% paraformaldehyde and dehydrated in gradient alcohol. Then, the tissues were subjected to being embedded in paraffin, deparaffinized in xylene and then rehydrated with gradient alcohol. Antigen retrieval was performed by microwaving the sections in citrate buffer. Endogenous peroxidase activity was eliminated with 3% H_2O_2. Following incubation with primary GOLM1 antibody (1:200, Abcam, Cambridge, MA, USA) overnight at 4°C, the slides were incubated with secondary goat anti-rabbit IgG (1:3000, Abcam, Cambridge, MA, USA) labeled by HRP. Then, the slides were stained with DAB, which was used as the chromogen, and counterstained with hematoxylin. A brightfield microscope (Olympus BX50; Olympus Corporation, Tokyo, Japan) was applied for imaging and analyzing the slides. Cells with yellow particles inside were viewed as positive cells, and the ratio of positive cells/all cells was used to calculate the expressions of FGFR1: the percentage > 25% was regarded as positive expressions (+), whereas the percentage < 25% was regarded as negative expressions (-)[25,26].

In Vivo Tumorigenesis Assay

Five-week-old nude mice were used for the tumor growth xenograft models. A375 cells transfected with (lenti-miR-200a or lenti-control) were injected subcutaneously into the lower left flank of mice. Tumor volumes were monitored every 3 days in accordance with the following formula: tumor volume (mm^3) = 1/2 × (length × $width^2$).

Analysis

All experiments were independently repeated at least 3 times. All data were analyzed by SPSS 23 (SPSS Inc., Chicago, IL, USA). Differences between 2 groups were determined by Student's *t*-test. Comparison between groups was done using One-way ANOVA test followed by Post Hoc Test (Least Significant Difference). Overall survival rates were generated by Kaplan-Meier analysis and log-rank tests. $p<0.05$ was considered as statistically significant.

Results

Down-Regulation of MiR-200a in Melanoma Tissue Samples Was Confirmed and Conferred a Poor Prognosis to Melanoma Patients

To identify the functions of miR-200a involved in melanoma progression, we first measured miR-200a expression levels in melanoma tissues by qRT-PCR. Results indicated that miR-200a was remarkably down-regulated in melanoma tissue samples in contrast with the matched normal tissues (Figure 1A). We then classified the melanoma patients into high miR-200a expression group and low miR-200a expression group according to the mean miR-200a expression level. As shown in Table II, low miR-200a expressions were associated with adverse phenotypes of melanoma patients. Moreover, we also investigated the functional significance of miR-200a in the prognosis of melanoma patients by Kaplan-Meier analysis, and it was found that the overall survival rate of patients in lower miR-200a expression group was significantly shorter than that of patients in high miR-200a expression group (Figure 1B).

MiR-200a Restoration Inhibited Melanoma Cell Proliferation

We then detected miR-200a expressions in melanoma cells and the qRT-PCR analysis showed that miR-200a was also remarkably downregulated in melanoma cells compared to the normal cells (Figure 2A). To further analyze the functional effects of miR-200a in melanoma, we

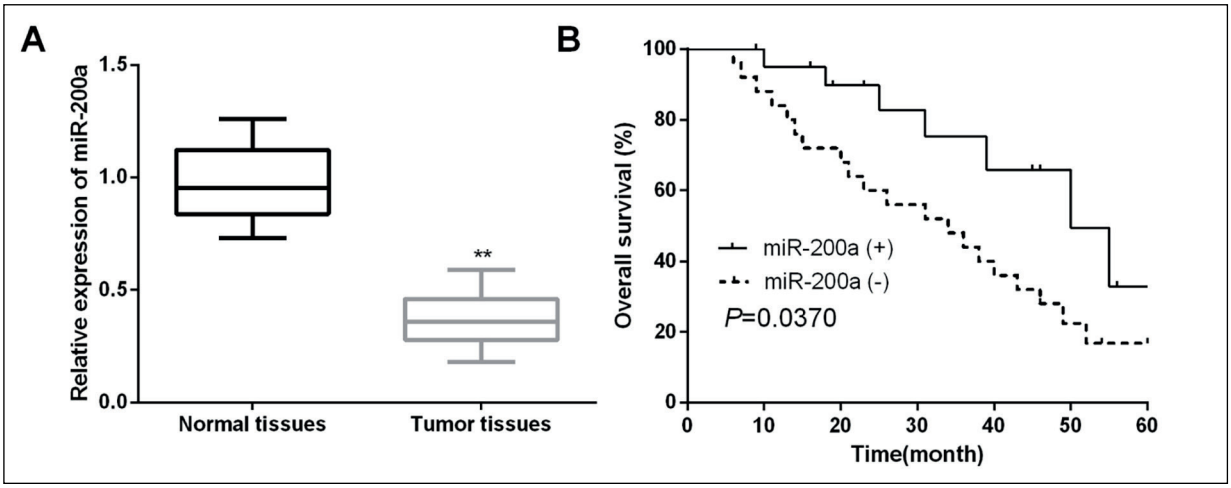

Figure 1. MiR-200a was notably down-regulated in melanoma tissue samples. ***A,*** The expressions of miR-200a were measured by qRT-PCR. ***B,*** Kaplan-Meier analysis presented shorter OS of melanoma patients with low miR-200a expressions.

overexpress or inhibit miR-200a expressions by transfecting miR-200a mimics or inhibitor into A375 or SK-HEP-1 cells based on their relatively low and high endogenous miR-200a expressions. The transfection efficiencies were confirmed by qRT-PCR (Figure 2B, 2C). Then, the MTT assays were carried out to investigate the influence of miR-200a on melanoma cell proliferation and it was shown that miR-200a upregulation in A375 cells significantly suppressed the proliferation ability (Figure 2D). In contrast, the proliferation capacity of miR-200a suppressed SK-HEP-1 cells was prominently enhanced compared to the control group (Figure 2E).

MiR-200a Upregulation Inhibited Melanoma Cell Invasion and Migration

As we observed that miR-200a suppressed melanoma cell proliferation, the transwell assays were further carried out to investigate the impacts of miR-200a on melanoma cell invasion and migration. Results revealed that miR-200a overexpression in A375 cells markedly inhibited the invasion and migration abilities (Figure 3A, 3B). We also found that miR-200a inhibition significantly promoted SK-HEP-1 cell invasion and migration (Figure 3C, 3D). All the findings revealed that miR-200a functioned as an anti-tumor miRNA in melanoma progression.

Table II. Relationship between miR-200a expression and the clinic-pathological characteristics of melanoma patients.

Characteristics	Cases (n = 46)	MiR-200a a expression		p-value
		High (n = 19)	Low (n = 27)	
Age (years)				0.722
≥ 60	18	8	10	
< 60	28	11	17	
Gender				0.431
Male	23	9	14	
Female	23	10	13	
Tumor thickness				0.012*
< 1 cm	21	14	7	
≥ 1 cm	25	5	20	
TNM stage				0.033*
I-II	20	14	6	
III-IV	26	5	21	
Lymph node metastasis				0.075
No	21	12	9	
Yes	25	7	18	

TNM: tumor-node-metastasis. a The mean expression level of miR-200a was used as the cutoff. *Statistically significant.

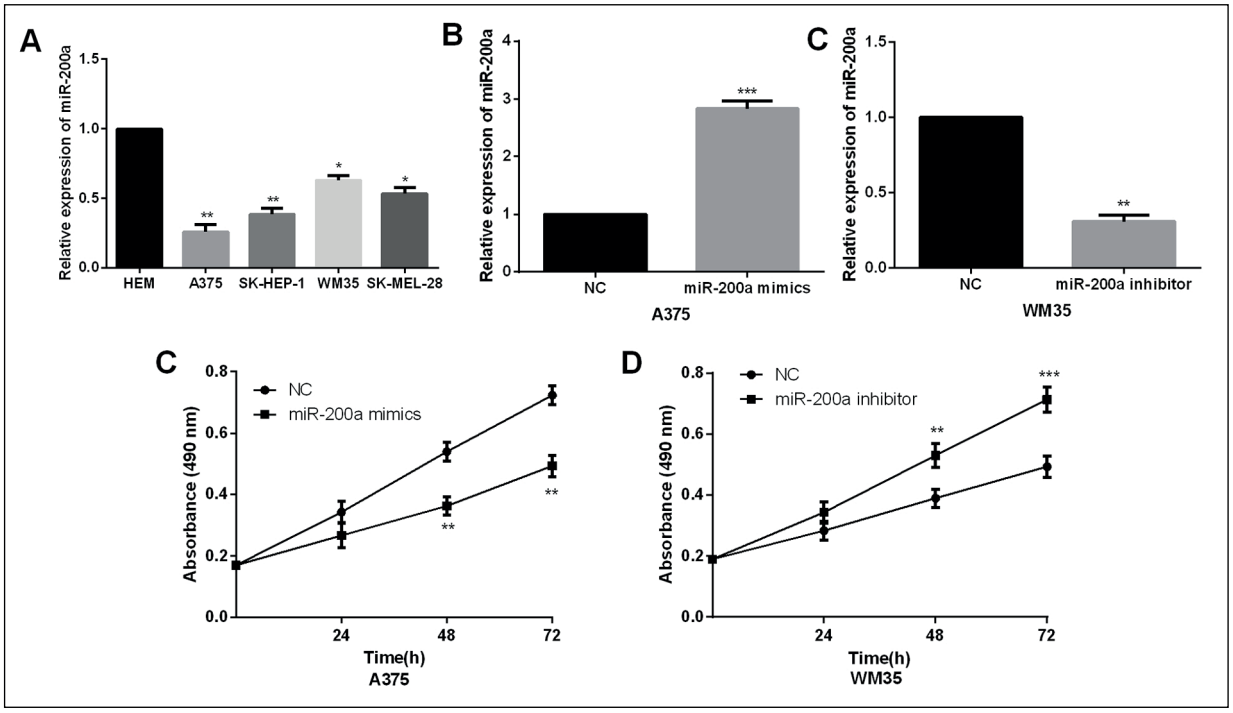

Figure 2. MiR-200a overexpression inhibited melanoma cell proliferation. ***A,*** MiR-200a expressions in melanoma cells were determined by qRT-PCR. ***B,*** MiR-200a expressions in miR-200a-overexpressed A375 cells. ***C,*** MiR-200a expressions in miR-200a-suppressed SK-HEP-1 cells. ***D, E,*** The proliferation abilities of A375 or SK-HEP-1 cells treated with miR-200a mimics or inhibitor were determined by MTT assays.

GOLM1 Was a Direct Target of MiR-200a in Melanoma Cells

Accumulating evidence implied that miRNAs played roles in tumorigenesis *via* the regulation of target genes. Therefore, we identified the biological target of miR-200a using the TargetScan. Results showed that GOLM1 was a potential target of miR-200a (Figure 4A). Then, the dual-luciferase reporter assay was conducted to validate the association between miR-200a and GOLM1. As shown in Figure 4B, the luciferase activities of GOLM1-3'UTR-WT were remarkably suppressed by miR-200a overexpression while the luciferase activities of GOLM1-3'UTR-MUT were not significantly affected by miR-200a overexpression. Furthermore, the regulatory functions of miR-200a in GOLM1 expressions were investigated. It was found that miR-200a restoration evidently represses GOLM1 expressions in A375 cells whereas miR-200a inhibition in SK-HEP-1 cells markedly facilitated the GOLM1 expressions (Figure 4C, 4D). Collectively, GOLM1 was identified to be an important functional target of miR-200a in melanoma cells.

MiR-200a Modulated PI3K/Akt Signaling Pathway and EMT in Melanoma Cells

As we confirmed that GOLM1 was a functional target of miR-200a in melanoma cells, we next investigated the clinical value of GOLM1 in melanoma patients. Firstly, IHC assays showed that GOLM1 was mainly localized at the cytoplasm (Figure 5A). Additionally, GOLM1 was remarkably upregulated in melanoma tissues than normal tissues (Figure 5B). Moreover, the Kaplan-Meier analysis indicated that melanoma patients with high GOLM1 expressions presented shorter overall survival rate (Figure 5C). To delineate the molecular mechanism underlying the tumor-suppressive roles of miR-200a in melanoma, Western blots were performed to determine the functions of miR-200a in PI3K/Akt pathway and EMT. It was found that the expression of E-cadherin was significantly increased whereas expression levels of N-cadherin and Vimentin were prominently decreased by miR-200a upregulation in A375 cells. On the contrary, miR-200a inhibition had the opposite functions in SK-HEP-1 cells (Figure 5D). In the meantime, Western blot analysis also indicated that the expressions of p-Akt and p-PI3K were

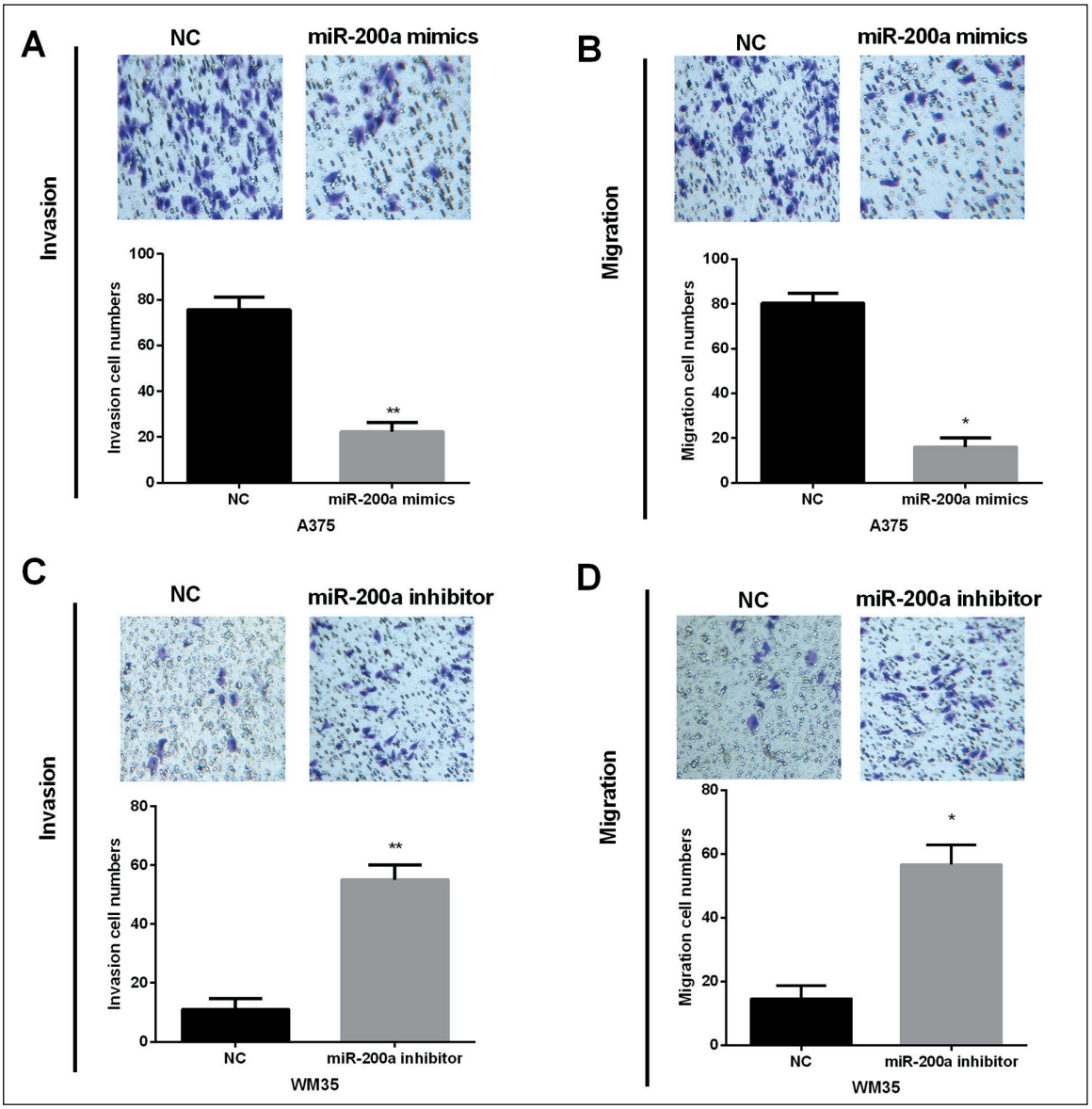

Figure 3. MiR-200a upregulation suppressed melanoma cell invasion and migration. ***A, B,*** Transwell assays were carried out to detect the invasion and migration capacities of miR-200a-overexpressed A375 cells. ***C, D,*** The invasion and migration capacities of miR-200a-suppressed SK-HEP-1cells were observed by transwell assays.

prominently decreased by miR-200a overexpression in A375 cells while there was no statistically significant effect on PI3K and Akt expressions; in contrast, miR-200a inhibition in SK-HEP-1 cells led to significant increase of p-PI3K and p-Akt expressions (Figure 5D).

MiR-200a Inhibited Melanoma Tumorigenesis In Vivo

The functions of miR-200a in melanoma were further investigated in nude mice models. As shown in Figure 6A, 6B, miR-200a restoration markedly repressed the volume of xenograft tumors and the tumor growth rate when compared to the negative controls. Therefore, these data revealed that miR-200a played suppressive roles in melanoma progression.

Discussion

Malignant melanoma arises from a melanocyte and remains one leading factor of skin tumor-associated deaths[27]. High mortalities of melanoma

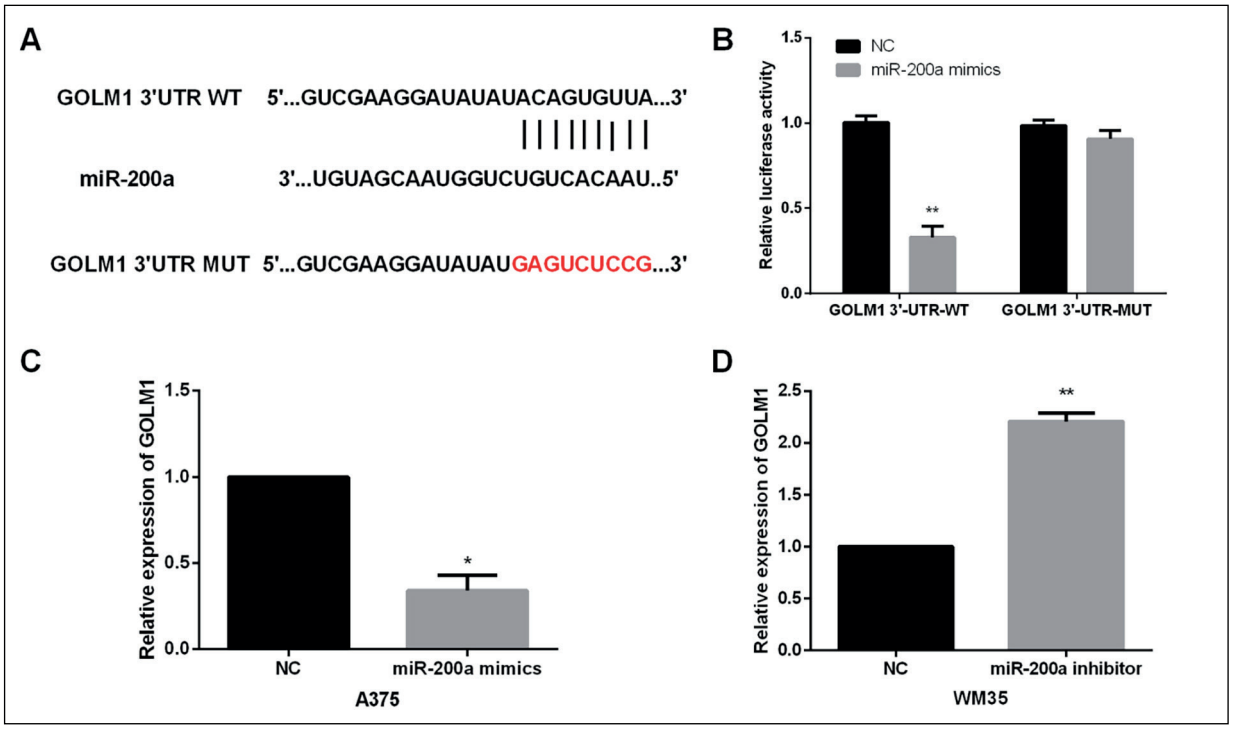

Figure 4. GOLM1 was identified as a direct target of miR-200a in melanoma cells. ***A,*** The binding sites of miR-200a in the GOLM1 3′-UTR. ***B,*** Relative luciferase activities in melanoma cells were determined after the transfection with WT or MUT GOLM1 3′-UTRs and miR-200a mimics. ***C, D,*** GOLM1 expressions in miR-200a-overexpressed A375 cells or miR-200a-suppressed SK-HEP-1cells.

emphasize the need for relevant research. Accumulating evidence has shown that alteration of miRNA expressions is identified in a variety of cancers, playing essential roles in tumor initiation and progression[28]. Therefore, extensive investigations of miRNAs have been carried out for the identification of promising biomarkers in melanoma diagnosis and prognosis in order to develop effective therapeutic strategies[29]. For instance, miR-33a was confirmed to serve tumor suppressive functions in melanoma by regulating HIF-1alpha[30]; miR-137 repressed melanoma cell proliferation *via* downregulating GLO1[31]; miR-769 was verified to enhance melanoma cell proliferation *via* suppressing GSK3B[32]. However, there are few studies on the correlations between miR-200a and melanoma.

The roles of miR-200a in cancer progression have recently received more attention. Numerous studies have demonstrated that miR-200a is implicated in kinds of cancers. For instance, Shi et al[33] found that miR-200a promoted endometrial cancer cell EMT by negative regulation FOXA2; Suo et al[34] reported that miR-200a promoted ovarian carcinoma cell invasion and migration by regulating PTEN; Tsouko et al[35] proposed that miR-200a suppressed triple-negative breast cancer cell metastasis through direct inhibition of EPHA2. These conflicting studies suggest that the biological roles of miR-200a in cancer carcinogenesis and progression are tissue-specific and that miR-200a could serve as a therapeutic biomarker for the treatment of these specific tumors. In the current study, miR-200a was found to be downregulated in melanoma, indicating a poor prognosis and malignant phenotypes of melanoma patients. Furthermore, functional assays also demonstrated that miR-200a could repress melanoma cell proliferation, invasion and migration abilities *via* modulating PI3K/Akt signaling pathway and EMT. All these findings indicated that miR-200a exerted anti-melanoma functions in melanoma development.

Wu et al[36] demonstrated that miR-200a can inhibit tumor development by targeting multiple mRNAs, such as PTEN, ZEB2, and CTNNB1[37]. Identifying new targets of miR-200a is useful for elucidating its regulatory mechanism in melanoma. In the current study, GOLM1 was confirmed to be an important functional tar-

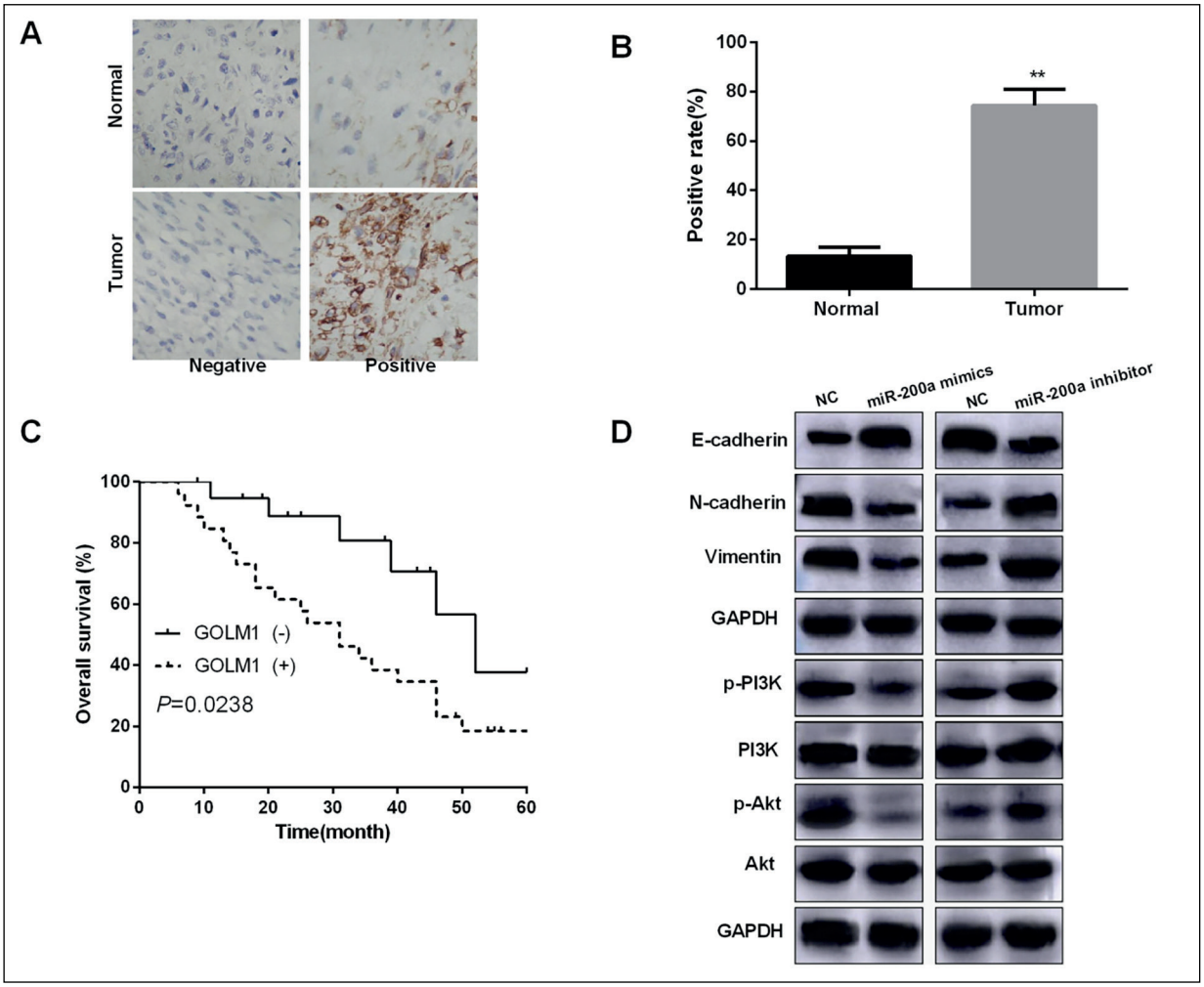

Figure 5. GOLM1 was upregulated in melanoma tissue samples and miR-200a regulated the PI3K/Akt signaling pathway and EMT. ***A, B,*** GOLM1 expressions in melanoma tissues were measured by IHC assays. ***C,*** Kaplan-Meier analysis of melanoma patients with different GOLM1 expressions. ***D,*** The effects of miR-200a on PI3K/Akt signaling pathway and EMT melanoma cells.

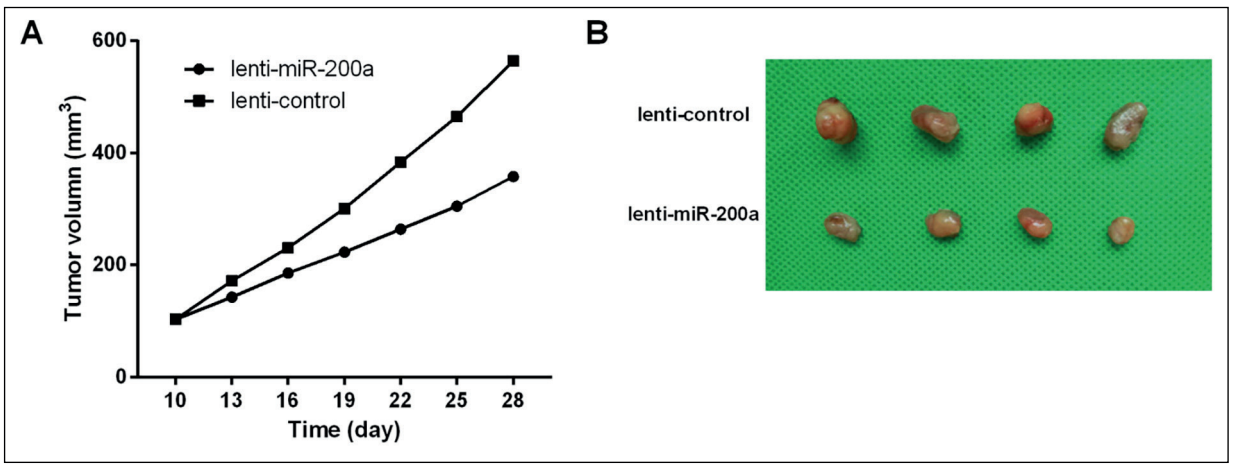

Figure 6. MiR-200a overexpression repressed melanoma tumor growth *in vivo*. ***A,*** Tumor growth curves of the groups with different treatments. ***B,*** Schematic representation of A375 xenograft tumors in the lenti-miR-200a and lenti-control groups.

get of miR-200a in melanoma cells. Increased GOLM1 predicted unfavorable overall survival in lung adenocarcinoma[38]. In addition, GOLM1 promoted prostate carcinoma progression *via* activation of PI3K/AKT/mTOR[39]. Our findings also indicated that increased GOLM1 expressions in melanoma indicated poor prognosis and participated in the functions mediated by miR-200a in melanoma.

Conclusions

We provided evidence that miR-200a was down-regulated in melanoma and was implicated in melanoma progression *via* inhibiting GOLM1 expressions and regulating PI3K/Akt signaling pathway and EMT. Decreased levels of miR-200a were related to poor prognosis of melanoma patients. The findings of the present study provided a novel insight into understanding melanoma pathogenesis, suggesting that miR-200a may function as a promising and potential therapeutic biomarker for melanoma treatments.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Madhunapantula SV, Robertson GP. Chemoprevention of melanoma. Adv Pharmacol 2012; 65: 361-398.
2) Yu L, Li J, Xiao M. Picrocrocin exhibits growth inhibitory effects against SKMEL-2 human malignant melanoma cells by targeting JAK/STAT5 signaling pathway, cell cycle arrest and mitochondrial mediated apoptosis. J BUON 2018; 23: 1163-1168.
3) Eggermont AM, Suciu S, Rutkowski P, Kruit WH, Punt CJ, Dummer R, Sales F, Keilholz U, de Schaetzen G, Testori A. Long term follow up of the EORTC 18952 trial of adjuvant therapy in resected stage IIB-III cutaneous melanoma patients comparing intermediate doses of interferon-alpha-2b (IFN) with observation: ulceration of primary is key determinant for IFN-sensitivity. Eur J Cancer 2016; 55: 111-121.
4) Kang K, Zhang J, Zhang X, Chen Z. MicroRNA-326 inhibits melanoma progression by targeting KRAS and suppressing the AKT and ERK signalling pathways. Oncol Rep 2018; 39: 401-410.
5) Samarkos M, Papaxoinis G, Athanasoula K, Benopoulou O, Bouros S, Anastasopoulou A, Diamantopoulos P, Gogas H, Mantzourani M. Significance of survivin mRNA blood levels in patients with melanoma. J BUON 2018; 23: 96-103.
6) Yang J, Xiao P, Sun J, Guo L. Anticancer effects of kaempferol in A375 human malignant melanoma cells are mediated via induction of apoptosis, cell cycle arrest, inhibition of cell migration and downregulation of m-TOR/PI3K/AKT pathway. J BUON 2018; 23: 218-223.
7) Zhang Y, Jiang F, He H, Ye J, Mao X, Guo Q, Wu SL, Zhong W, Wu CL, Lin N. Identification of a novel microRNA-mRNA regulatory biomodule in human prostate cancer. Cell Death Dis 2018; 9: 301.
8) Zhao L, Zhang Z, Lou H, Liang J, Yan X, Li W, Xu Y, Ou R. Exploration of the molecular mechanisms of cervical cancer based on mRNA expression profiles and predicted microRNA interactions. Oncol Lett 2018; 15: 8965-8972.
9) Luo H, Li Y, Liu B, Yang Y, Xu ZD. MicroRNA-15b-5p targets ERK1 to regulate proliferation and apoptosis in rat PC12 cells. Biomed Pharmacother 2017; 92: 1023-1029.
10) Pan BL, Tong ZW, Wu L, Pan L, Li JE, Huang YG, Li SD, Du SX, Li XD. Effects of microRNA-206 on osteosarcoma cell proliferation, apoptosis, migration and invasion by targeting ANXA2 through the AKT signaling pathway. Cell Physiol Biochem 2018; 45: 1410-1422.
11) Cong F, Wu N, Tian X, Fan J, Liu J, Song T, Fu H. MicroRNA-34c promotes osteoclast differentiation through targeting LGR4. Gene 2017; 610: 1-8.
12) Liu DT, Yao HR, Li YY, Song YY, Su MY. MicroRNA-19b promotes the migration and invasion of ovarian cancer cells by inhibiting the PTEN/AKT signaling pathway. Oncol Lett 2018; 16: 559-565.
13) Wang ZY, Xiong J, Zhang SS, Wang JJ, Gong ZJ, Dai MH. Up-regulation of microRNA-183 promotes cell proliferation and invasion in glioma by directly targeting NEFL. Cell Mol Neurobiol 2016; 36: 1303-1310.
14) Shang G, Mi Y, Mei Y, Wang G, Wang Y, Li X, Wang Y, Li Y, Zhao G. MicroRNA-192 inhibits the proliferation, migration and invasion of osteosarcoma cells and promotes apoptosis by targeting matrix metalloproteinase-11. Oncol Lett 2018; 15: 7265-7272.
15) Lyons JG, Lobo E, Martorana AM, Myerscough MR. Clonal diversity in carcinomas: its implications for tumour progression and the contribution made to it by epithelial-mesenchymal transitions. Clin Exp Metastasis 2008; 25: 665-677.
16) Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002; 2: 442-454.
17) Xu C, Li M, Zhang L, Bi Y, Wang P, Li J, Jiang X. MicroRNA-205 suppresses the invasion and epithelial-mesenchymal transition of human gastric cancer cells. Mol Med Rep 2016; 13: 4767-4773.
18) Xu H, Qian M, Zhao B, Wu C, Maskey N, Song H, Li D, Song J, Hua K, Fang L. Inhibition of RAB1A sup-

presses epithelial-mesenchymal transition and proliferation of triple-negative breast cancer cells. Oncol Rep 2017; 37: 1619-1626.

19) Yang Y, Tao X, Li CB, Wang CM. MicroRNA-494 acts as a tumor suppressor in pancreatic cancer, inhibiting epithelial-mesenchymal transition, migration and invasion by binding to SDC1. Int J Oncol 2018; 53: 1204-1214.

20) Robbins HL, Hague A. The PI3K/Akt pathway in tumors of endocrine tissues. Front Endocrinol (Lausanne) 2016; 6: 188. doi: 10.3389/fendo.2015.00188. eCollection 2015.

21) Matheny RJ, Adamo ML. Effects of PI3K catalytic subunit and Akt isoform deficiency on mTOR and p70S6K activation in myoblasts. Biochem Biophys Res Commun 2009; 390: 252-257.

22) Zhou Y, Li L, Hu L, Peng T. Golgi phosphoprotein 2 (GOLPH2/GP73/GOLM1) interacts with secretory clusterin. Mol Biol Rep 2011; 38: 1457-1462.

23) Hu L, Li L, Xie H, Gu Y, Peng T. The Golgi localization of GOLPH2 (GP73/GOLM1) is determined by the transmembrane and cytoplamic sequences. PLoS One 2011; 6: e28207.

24) Zhang Y, Hu W, Wang L, Han B, Lin R, Wei N. Association of GOLPH2 expression with survival in non-small-cell lung cancer: clinical implications and biological validation. Biomark Med 2017; 11: 967-977.

25) Hu XT, Chen W, Zhang FB, Shi QL, Hu JB, Geng SM, He C. Depletion of the proteasome subunit PSMA7 inhibits colorectal cancer cell tumorigenicity and migration. Oncol Rep 2009; 22: 1247-1252.

26) Hu XT, Chen W, Wang D, Shi QL, Zhang FB, Liao YQ, Jin M, He C. The proteasome subunit PSMA7 located on the 20q13 amplicon is overexpressed and associated with liver metastasis in colorectal cancer. Oncol Rep 2008; 19: 441-446.

27) Xiong TF, Pan FQ, Li D. Expression and clinical significance of S100 family genes in patients with melanoma. Melanoma Res 2019; 29: 23-29.

28) Zheng R, Pan L, Gao J, Ye X, Chen L, Zhang X, Tang W, Zheng W. Prognostic value of miR-106b expression in breast cancer patients. J Surg Res 2015; 195: 158-165.

29) He J, Tian N, Yang Y, Jin L, Feng X, Hua J, Lin S, Wang B, Li H, Wang J. miR-185 enhances the inhibition of proliferation and migration induced by ionizing radiation in melanoma. Oncol Lett 2017; 13: 2442-2448.

30) Zhou J, Xu D, Xie H, Tang J, Liu R, Li J, Wang S, Chen X, Su J, Zhou X, Xia K, He Q, Chen J, Xiong W, Cao P, Cao K. MiR-33a functions as a tumor suppressor in melanoma by targeting HIF-1alpha. Cancer Biol Ther 2015; 16: 846-855.

31) Lv N, Hao S, Luo C, Abukiwan A, Hao Y, Gai F, Huang W, Huang L, Xiao X, Eichmuller SB, He D. MiR-137 inhibits melanoma cell proliferation through downregulation of GLO1. Sci China Life Sci 2018; 61: 541-549.

32) Qiu HJ, Lu XH, Yang SS, Weng CY, Zhang EK, Chen FC. MiR-769 promoted cell proliferation in human melanoma by suppressing GSK3B expression. Biomed Pharmacother 2016; 82: 117-123.

33) Shi W, Wang X, Ruan L, Fu J, Liu F, Qu J. MiR-200a promotes epithelial-mesenchymal transition of endometrial cancer cells by negatively regulating FOXA2 expression. Pharmazie 2017; 72: 694-699.

34) Suo HB, Zhang KC, Zhao J. MiR-200a promotes cell invasion and migration of ovarian carcinoma by targeting PTEN. Eur Rev Med Pharmacol Sci 2018; 22: 4080-4089.

35) Tsouko E, Wang J, Frigo DE, Aydogdu E, Williams C. MiR-200a inhibits migration of triple-negative breast cancer cells through direct repression of the EPHA2 oncogene. Carcinogenesis 2015; 36: 1051-1060.

36) Wu Q, Lu RL, Li JX, Rong LJ. MiR-200a and miR-200b target PTEN to regulate the endometrial cancer cell growth in vitro. Asian Pac J Trop Med 2017; 10: 498-502.

37) Xia H, Ng SS, Jiang S, Cheung WKC, Sze J, Bian X, Kung H, Lin MC. MiR-200a-mediated downregulation of ZEB2 and CTNNB1 differentially inhibits nasopharyngeal carcinoma cell growth, migration and invasion. Biochem Bioph Res Co 2010; 391: 535-541.

38) Liu X, Chen L, Zhang T. Increased GOLM1 expression independently predicts unfavorable overall survival and recurrence-free survival in lung adenocarcinoma. Cancer Control 2018; 25: 1146213265.

39) Yan G, Ru Y, Wu K, Yan F, Wang Q, Wang J, Pan T, Zhang M, Han H, Li X, Zou L. GOLM1 promotes prostate cancer progression through activating PI3K-AKT-mTOR signaling. Prostate 2018; 78: 166-177.