European Review for Medical and Pharmacological Sciences 2019; 23: 5332-5342

MicroRNA-22 targets FMNL2 to inhibit melanoma progression via the regulation of the Wnt/β-catenin signaling pathway and epithelial-mesenchymal transition

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Abstract. – OBJECTIVE: Melanoma is regarded as one common malignancy in skin cancers, and there is growing evidence that microRNAs (miRNAs) play a vital role in the oncogenesis of tumors. This study aimed to investigate the roles and mechanism of miR-22 in melanoma.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was utilized to detect the expressions of miR-22 and mRNA. The functions of miR-22 in melanoma cell proliferation, migration and invasion were investigated with functional assays, including MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and transwell assay. Western blots were utilized to examine the protein expressions. Luciferase reporter analysis was conducted to confirm the interactions between formin-like 2 (FMNL2) and miR-22 in melanoma cells. FMNL2 expression levels in melanoma tissues were investigated by immunohistochemistry (IHC) assays.

RESULTS: The qRT-PCR analysis demonstrated significant decreased miR-22 expressions in melanoma tissues. Decreased miR-22 in melanoma tissues were correlated with adverse clinicopathologic features and poor prognosis. Functional assays indicated that upregulation inhibited melanoma cell proliferation, invasion and migration capacities. Luciferase reporter assays showed that FMNL2 was targeted by miR-22 in melanoma cells. Western blots indicated that miR-22 exerted anti-tumor functions by regulating the Wnt/β-catenin and epithelial-mesenchymal transition (EMT).

CONCLUSIONS: Our findings showed that miR-22 served as a tumor suppressor in melanoma progression, implying that miR-22 may function as a novel therapeutic target and prognostic biomarker for melanoma treatments.

Key Words
Melanoma, MiR-22, FMNL2, Wnt/β-catenin signaling pathway, Epithelial-mesenchymal transition.

Introduction

Human melanoma is a frequently malignant kind in skin tumors with high mortalities[1]. The typical characteristics of melanoma are early metastases and uncontrollable growth[2]. Despite the advanced improvements in the therapeutic approaches of melanoma, the prognosis of melanoma patients remains to be significantly improved[3]. Therefore, multidisciplinary and comprehensive treatments, including immunotherapies and molecularly targeted therapies, should be applied to the treatments of advanced melanoma[4,5]. However, due to the lack of effective targets, these regimens have received little benefit[6]. Hence, finding new biomarkers and exploring the underlying mechanisms involved in melanoma development are pivotal to the treatments of malignant melanoma and have attracted increasing attention.

MicroRNAs (miRNAs) can suppress mRNA translations and induce mRNA degradations *via*

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binding to the 3'-UTRs of the target mRNAs, having emerged as critical regulators of gene expressions[7]. Alterations in miRNA expressions have been found in varieties of malignancies, playing a critical role in tumorigenesis by regulation of wide ranges of biological processes including cell cycle, cell apoptosis, proliferation, and metastases[8-10]. In human cancers, abnormal expression of miRNAs can serve as an oncogene or a tumor suppressor based on the nature of the targets. For instance, Yan et al[11] found that miR-383 inhibited colorectal cancer cell invasion and proliferation *via* regulating paired box 6. In contrast, studies by Yao et al[12] showed that miR-544 promoted colorectal cancer progression by targeting forkhead box O1. Accordingly, considerable efforts are essential to elucidate the expressions, functions and underlying mechanisms of tumor-associated miR-22 in melanoma, and the outcomes may be particularly helpful to provide promising therapeutic biomarkers for in melanoma patients.

Epithelial-mesenchymal transition (EMT), in which epithelial cells can be converted into the mesenchymal cells in a specific condition, has been proved to be closely associated with the tumor progression[13]. Park et al[14] have found that EMT phenomenon takes crucial parts in distant metastases and invasion of various tumors. Aberrant activation of the Wnt/βcatenin may result in uncontrolled proliferation, thereby leading to tumorigenesis[15]. Moreover, the abnormal activations of this signaling pathway have already been confirmed in diverse human tumors, including hepatocellular carcinoma[16], astrocytomas[17], and melanoma[18]. Depending on these previous studies, we speculated that EMT and Wnt/βcatenin signaling are involved in the functions of miR-22 in melanoma progression.

Formin-like 2 (FMNL2) is a member of DRFs (diaphanous-related formins), which remodel exerted actin to dominate morphogenesis, adhesion, cytokinesis and cell shape *via* the formin homology domains[19]. Dysregulated FMNL2 has been verified in several solid tumors, participating in invasive behaviors and development of cancers[20,21]. Increasing studies[22] have demonstrated that FMNL2 takes critical parts in carcinogenesis. High FMNL2 expressions in colorectal cancer cells were associated with increased invasiveness, implying the metastasis-related roles of FMNL2 in tumors[23]. Therefore, it has attracted our attention in the expressions and functions of FMNL2 in melanoma.

Patients and Methods

Tissue Specimens

The current study was approved by the Ethics Committee of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. 48 pairs of melanoma and matched non-cancer tissues were collected from The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University from September 2016 to April 2018. All patients involved in the current study provided written informed consents. None of the patients received preoperative anticancer treatment. All fresh tissue samples were frozen in liquid nitrogen immediately and stored at -80°C for further use.

Cell Culture

Human epidermal melanocytes (HEM) obtained from ScienCell Research Laboratories, Inc. (San Diego, CA, USA) were cultured in melanocyte medium (ScienCell Research Laboratories, San Diego, CA, USA) according to the manufacturer's instructions. Four melanoma cell lines (A375, SK-MEL-1, WM35 and SK-MEL-28) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA). All cells were grown in a humidified incubator at 37°C with 5% CO_2.

Cell Transfection

MiR-22 mimics, inhibitor and negative controls were obtained from GenePharma (Shanghai, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was applied to transfect the miRs into melanoma cells following the manufacturer's instructions.

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

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to isolate the total RNA from tissues and cells following the manufacturers' protocols. Reverse transcription was carried out by PrimeScript RT reagent (TaKaRa, Otsu, Shiga, Japan) to synthesize cDNA. Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was carried out using SYBR Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan) on an ABI Prism 7500 Sequence Detection system (Applied Bio-

systems, Foster City, CA, USA). U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as internal reference for miR-22 and mRNA (Primers were shown in Table I). The expressions were analyzed with the $2^{-\Delta\Delta Ct}$ method.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide) Assays

Cell proliferation ability of melanoma cells was assessed by MTT assays (Sigma-Aldrich, St. Louis, MO, USA). In brief, after transfections, melanoma cells were passed into a 96-well plate, being incubated for 0, 24, 48, and 72 h at 37°C. After that, the cells were treated with MTT (5 mg/mL) solution for 4 h at 37°C. Then, dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was added into the wells to dissolve the remaining MTT formazan. The optical density $(OD)_{490}$ values were determined using a microplate reader (BioTek, Winooski, VT, USA).

Transwell Assay

Transwell assay was carried out to evaluate the invasion and migration abilities using transwell chamber (8-μm pores, Costar, Cambridge, MA, USA). For invasion assays, the transwell chambers were coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Indicated melanoma cells were suspended in serum-free medium and seeded into the upper chambers. In the meantime, the lower chambers were filled with medium containing 10% FBS, serving as a chemoattractant. After being incubated for 48 h at 37°C in a 5% CO_2 atmosphere, cells which passed through the membranes were fixed and stained with methanol and crystal violet, and quantified using a microscope (Olympus, Tokyo, Japan). For migration assays, the transwell chambers were not precoated with Matrigel.

Western Blot Analyses

The cell lysate was prepared in cold radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) lysis containing protease-inhibitor (Roche, Basel, Switzerland). A bicinchoninic acid (BCA) protein assay kit (Invitrogen, Carlsbad, CA, USA) was used to examine the protein concentrations. Then, proteins were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), which were blocked with 5% skim milk in Tris-Buffered Saline and Tween (TBST; Sigma-Aldrich, St. Louis, MO, USA) for 2 h at room temperature. After that, the membranes were incubated overnight at 4°C with primary antibodies: cyclin D1 (1:1000, Abcam, Cambridge, MA, USA), c-Myc (1:2000, Abcam, Cambridge, MA, USA), β-catenin (1:1000, Abcam, Cambridge, MA, USA), p-GSK3β (1:1000, Abcam, Cambridge, MA, USA), total GSK3β (1:2000, 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 (1:1000, Abcam, Cambridge, MA, USA). Following three washes with TBST, the membranes were incubated with the corresponding horse reddish peroxidase (HRP)-labeled secondary antibody (1:3,000, Abcam, Cambridge, MA, USA) at room temperature for 2 h. Then, target proteins were quantified by electrochemiluminescence (ECL; Beyotime, Shanghai, China) assays. GAPDH was used as an internal control.

Luciferase Reporter Analysis

Luciferase reporter plasmids containing wild-type (WT) or mutant (MUT) sequences of miR-22 in FMNL2 3′-UTR were chemically synthesized by GenePharma. For Luciferase report assays, mela-

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-22 forward	5'- GCCTGAAGCTGCCAGTTGA-3'
miR-22 reverse	5'- GTGCAGGGTCGAGGT-3'
U6 forward	5'-CTCGCTTCGGCAGCACA-3'
U6 reverse	5'- AACGCTTCACGAATTTGCGT-3'
FMNL2 forward	5'- TAATCAGCATTAGCATTTCTGAGG-3'
FMNL2 reverse	5'- AGGAGAGTAAGGCCAGGTTCC -3'
GAPDH forward	5'-ACCTGACCTGCCGTCTAGAA-3'
GAPDH reverse	5'-TCCACCACCCTGTTGCTGTA-3'

U6: small nuclear RNA, snRNA. FMNL2: Formin-like 2. GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

noma cells were cotransfected with miR-22 mimics and FMNL2 3'-UTR-WT or FMNL2 3'-UTR-MUT by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. 48 h after the transfection, a Dual-Luciferase Reporter assay system (Promega, Madison, WI, USA) was applied for the detection of the Luciferase activity.

Immunohistochemistry (IHC)

We performed immunohistochemistry (IHC) assays to determine the expressions of FMNL2 in melanoma tissues. The tissue specimens were fixed with 4% paraformaldehyde, dehydrated with graded ethanol, embedded in paraffin, and cut into 4-μm-thick paraffin sections. Then, the slides were subjected to routine dewaxing and gradient hydration. Antigen retrieval was conducted with citrate buffer in a microwave oven and endogenous peroxidase was blocked by 3% hydrogen peroxide. After that, the sections were incubated with primary FMNL2 antibody (1:100, Abcam, Cambridge, MA, USA) at 4°C overnight, followed by incubation with HRP-conjugated secondary antibodies (1:2000, Abcam, Cambridge, MA, USA). Finally, the sections were stained with diaminobenzidine (DAB) as the chromogen and counterstained with hematoxylin. The expression status of FMNL2 in the cytoplasm was determined by the average percentage of positive cells in 5 random fields under a light microscope (Olympus BX50; Olympus Corporation, Tokyo, Japan). The scores were calculated according to positive cell ratio: stained cells/all cells<25% was considered as negative (-) while >25% was positive[24, 25].

Nude Mice Xenografts

Animal assays were approved by the Animal Care and Use Committee of the Wenzhou Medical University. For *in vivo* tumorigenesis assays, A375 cells stably expressing miR-22 or negative control (lenti-miR-22 or lenti-control) were subcutaneously implanted into the flanks of nude mice (4-6-week old). Tumor volumes were calculated every three days following the formula: tumor volume (mm^3) =1/2× (length×$width^2$).

Statistical Analysis

Data were from at least three independent duplicates and analyzed using Statistical Product and Service Solutions (SPSS) 23 (SPSS Inc., Chicago, IL, USA). The Student's *t*-test was applied for the comparison between the two groups, and One-way analysis of variance (ANOVA) followed by Tukey's comparison test was used for comparison between at least three groups. The Kaplan-Meier analysis and the log-rank test were used to determine the overall survival rate. $p<0.05$ were indicated statistical significance.

Results

MiR-22 Expressions Were Significantly Decreased in Melanoma Tissues and Indicated a Poor Prognosis

To determine the impacts of miR-22 in melanoma, we first detected the expressions of miR-22 in melanoma tissue samples using qRT-PCR. The results demonstrated a significant decrease of miR-22 expressions in melanoma tissue samples in comparison to the matched non-tumor tissue samples (Figure 1A). Furthermore, the Kaplan-Meier analysis indicated that patients with low miR-22 expressions exhibited a shorter overall survival rate than those with higher miR-22 expressions (Figure 1B). Additionally, we also explored the relationship between miR-22 expressions and the clinicopathologic characteristics of melanoma patients. Melanoma patients involved in the current study were assigned into high and low miR-22 expression groups based on the mean level of miR-22 expressions. Findings showed that melanoma patients with lower miR-22 expressions presented adverse phenotypes (Table II).

MiR-22 Overexpression Repressed Melanoma Cell Proliferation

As we confirmed the decreased miR-22 expressions in melanoma tissues, we further examined miR-22 expressions in melanoma cells by qRT-PCR. As expected, miR-22 in melanoma cells were significantly downregulated in comparison with the normal cells (Figure 2A). A375 and SK-MEL-28 cells were selected to further perform the miR-22 gain-function and loss-function assays to determine the functions of miR-22 in melanoma progression. A375 cells were transfected with miR-22 mimics to overexpress miR-22 due to its relatively low endogenous miR-22 expressions (Figure 2B). In the meantime, miR-22 expressions in SK-MEL-28 were repressed *via* the transfection of miR-22 inhibitor (Figure 2C). Then, MTT assay was conducted to detect the impacts of miR-22 on melanoma cell proliferation. It was found that miR-22 overexpression in A375 cells prominently repressed the proliferation abilities whereas miR-22 inhibition markedly promoted SK-MEL-28 cell proliferation (Figure 2D and 2E).

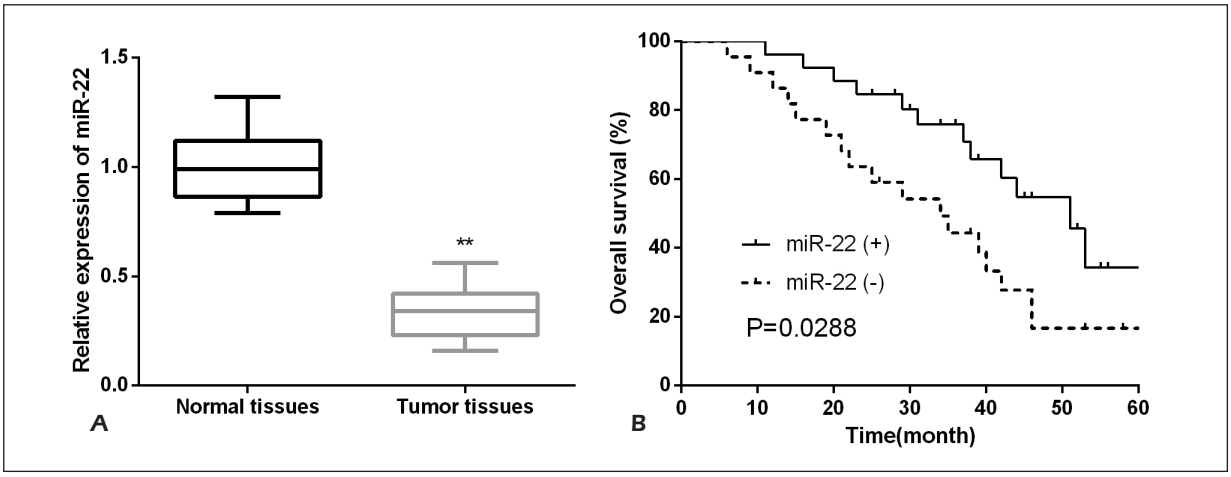

Figure 1. Low miR-22 expressions in melanoma tissues indicated poor prognosis of melanoma patients. **A**, The expressions of miR-22 were measured by qRT-PCR. **B**, Kaplan-Meier analysis presented shorter OS of melanoma patients with low miR-22 expressions.

MiR-22 Upregulation Suppressed Melanoma Cell Invasion and Migration

We then performed transwell assays to examine the functions of miR-22 in melanoma cell invasion and migration capacities. As shown in Figure 3A and 3B, we found that the invasion and migration capacities of A375 were dramatically repressed by miR-22 overexpression. In contrast, miR-22 inhibition in SK-MEL-28 cells prominently enhanced the invasion and migration capacities (Figure 3C and 3D). All the above findings indicated that miR-22 served anti-tumor functions in melanoma.

FMNL2 Was a Functional Target of MiR-22

The potential targets of miR-22 were searched by bioinformatic analysis. According to TargetScan, FMNL2 was predicted as a candidate target of miR-22 (Figure 4A). We next performed Luciferase reporter assay to verify this interaction between FMNL2 and miR-22 in FMNL2 cells. The results showed that the miR-22 overexpression markedly reduced the Luciferase activity of FMNL2-3'UTR-WT, while having no evident influence on the Luciferase activities of FMNL2-3'UTR-MUT (Figure 4B). Subsequently,

Table II. Relationship between miR-22 expression and the clinic-pathological characteristics of melanoma patients.

Characteristics	Cases (No=48)	miR-487a expression		*p*-value
		High (n=20)	Low (n=28)	
Age (years)				0.612
≥ 60	18	8	10	
< 60	30	12	18	
Gender				0.331
Male	24	10	14	
Female	24	10	14	
Tumor thickness				0.014*
< 1 cm	22	14	8	
≥ 1 cm	26	6	20	
TNM stage				0.024*
I-II	22	15	7	
III-IV	26	5	21	
Lymph node metastasis				0.063
No	22	12	10	
Yes	26	8	18	

TNM: tumor-node-metastasis. [a]The mean expression level of miR-22 was used as the cutoff. *Statistically significant.

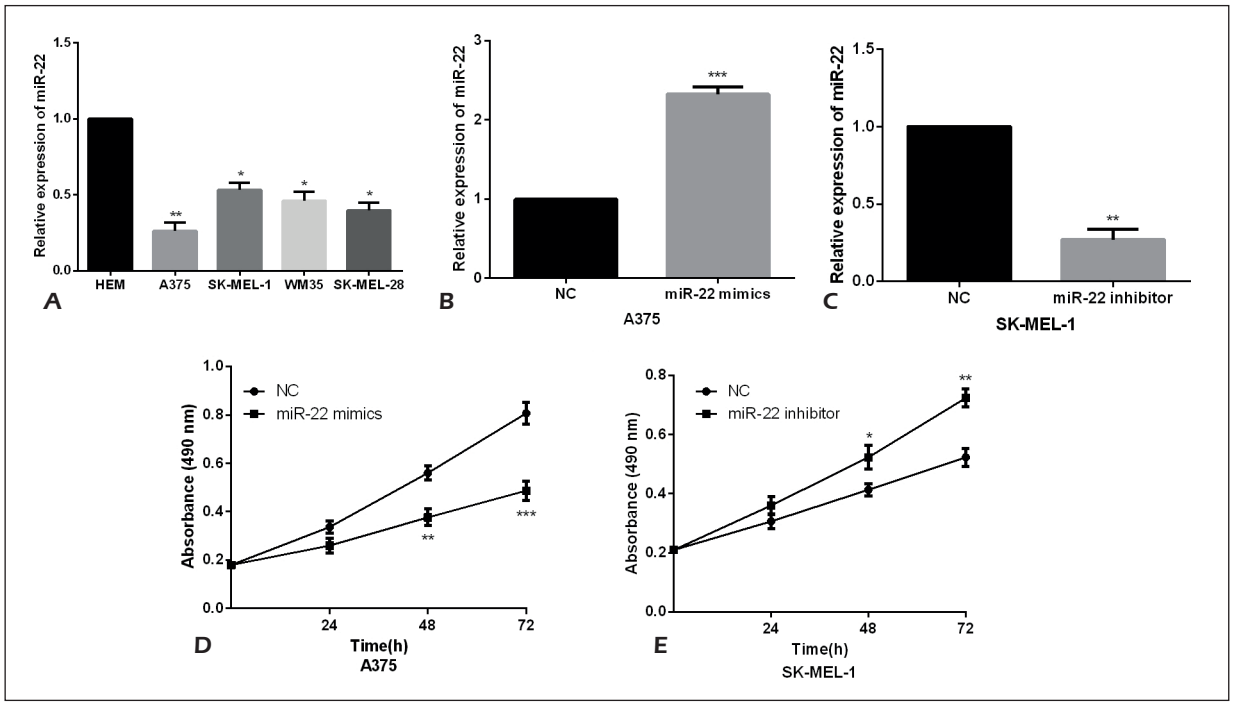

Figure 2. MiR-22 overexpression inhibited melanoma cell proliferation. **A**, miR-22 expressions in melanoma cells were detected by qRT-PCR. **B**, miR-22 overexpression in A375 cells was found using qRT-PCR. **C**, qRT-PCR was carried out to show the inhibition of miR-22 in SK-MEL-28 cells. **D**-**E**, MTT assay was conducted to detect the proliferation abilities of melanoma cells treated with miR-22 mimics or inhibitor.

to further confirm whether miR22 has regulation effects on endogenous FMNL2 in melanoma, we measured FMNL2 expressions in A375 and SK-MEL-28 cells after transfection with miR-22 mimics or inhibitor. We found that miR22 upregulation dramatically inhibited FMNL2 expressions in A375 cells whereas miR-22 inhibition in SK-MEL-28 cells significantly promoted FMNL2 expressions (Figure 4C and 4D).

MiR-22 Regulated EMT and Wnt/β-Catenin Signaling Pathway in Melanoma Cells

As we confirmed that FMNL2 was an important target of miR-22 in melanoma cells, we further investigated the clinical significance of FMNL2 in melanoma patients. IHC assays showed that FMNL2 was mainly localized at the cytoplasm (Figure 5A). Additionally, we also found that FMNL2 was remarkably upregulated in melanoma tissues than the matched normal tissues (Figure 5B). Furthermore, the Kaplan-Meier analysis was utilized to investigate the prognostic significance of FMNL2 in melanoma patients. Data showed that patients with higher FMNL2 expressions presented shorter overall survival rates (Figure 5C). Subsequently, to determine the underlying mechanism responsible for the anti-tumor functions in melanoma mediated by miR-22, we investigated the functions of miR-22 in melanoma cell EMT and Wnt/β-catenin by Western blots. As shown in Figure 5D, miR-22 overexpression in A375 cells prominently facilitated the expressions of E-cadherin while significantly reduced N-cadherin and Vimentin expression levels. On the other hand, in miR-22 suppressed SK-MEL-28 cells, the E-cadherin expression was markedly downregulated whereas N-cadherin and Vimentin expressions were upregulated. Moreover, the impacts of miR-22 on the Wnt/β-catenin signaling pathway in melanoma cells was determined. The results indicated that the expression levels of cyclin D1, c-Myc, activated β-catenin, and p-GSK3β were significantly reduced by miR-22 overexpression (Figure 5D).

MiR-22 Overexpression Inhibited Melanoma Tumorigenesis In Vivo

To validate the *in vivo* functions of miR-22 in melanoma tumor growth, we established nude mice xenografts. The results revealed that miR-22 upregulation could dramatically repress volume of xenograft tumors. The suppressive function of miR-22 in tumor growth rate was also showed *in vivo* (Figure 6A and 6B).

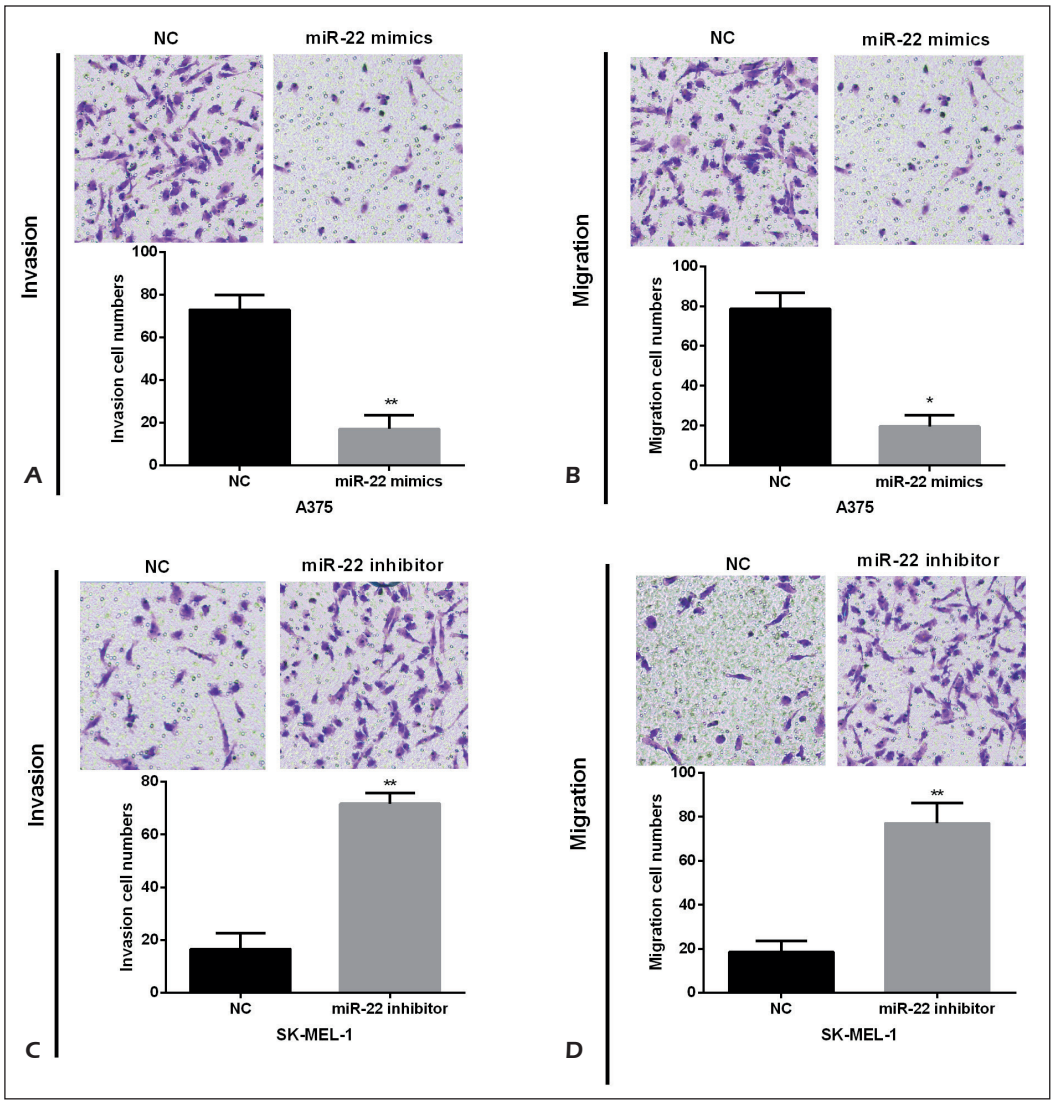

Figure 3. MiR-22 upregulation repressed melanoma cell invasion and migration. ***A*-*B***, The invasion and migration abilities of A375 cells treated with miR-22 mimics were assessed by transwell assays. ***C*-*D***, The transwell assay was conducted to examine the invasion and migration capacities of SK-MEL-28 cells treated with miR-22 inhibitor.

Discussion

As an aggressively neoplastic skin disease, melanoma has a high mortality rate and a poor prognosis when diagnosed at advanced stages[26]. So far, the therapeutic efficiencies of melanoma remain limited, which is partially due to the subsequent malignant process following histological biopsies, the potential risk of metastases or the late detection by current diagnostic approaches[27,28]. Accumulating evidence showed that miRNA played key roles in melanoma development, acting as a potential therapeutic biomarker and target. Pu et al[29] found that miR-146a facilitated melanoma cell migration and invasion by regulating SMAD4; Yang et al[30] revealed that miR-124 suppressed melanoma progression by regulating versican; Zhou et al[31] demonstrated that miR-33a exerted anti-tumor functions in melanoma *via* regulation of HIF-1alpha. Thus, it is important to further reveal more functional miRNAs in melanoma.

MiR-22 has been identified as a regulator of multiple cancers. For example, miR-22 could repress esophageal squamous cell carcinoma progression[32]. Moreover, miR-22 was found to inhibit gastric cancer growth and invasion[33]. According to Fan et al[34], miR-22 functioned as a cancer suppressor in clear cell renal cell carcinoma. How-

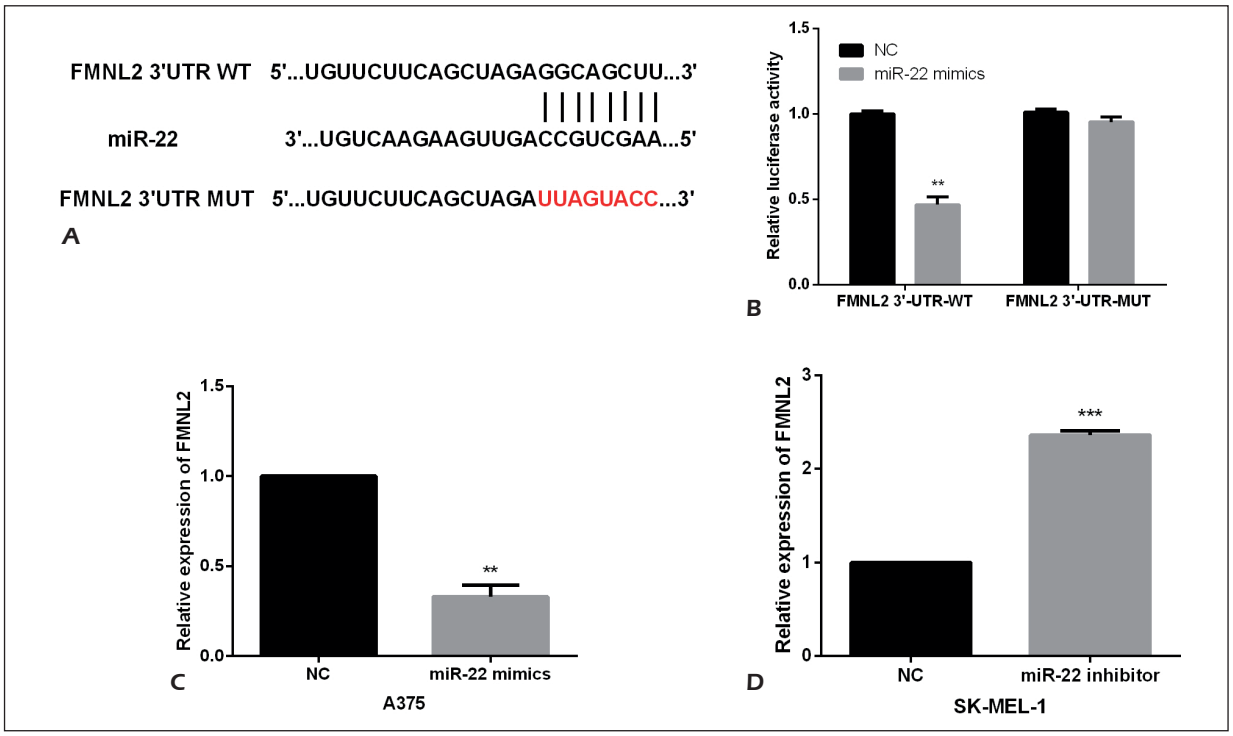

Figure 4. FMNL2 was a direct target of miR-22 in melanoma cells. **A**, Predicted miR22 target sequences in FMNL23'UTR. **B**, Luciferase activities in melanoma cells were determined after cotransfection with WT or MUT FMNL2 3'-UTRs and miR-22 mimics. **C-D**, Effects of miR-22 on FMNL2 expressions in melanoma cells.

ever, the functions and mechanisms of miR-22 in melanoma development need to be fully elucidated. In the current work, miR-22 was prominently down-regulated in melanoma, and the decreased miR-22 expressions were observed to be associated with the poor prognosis and adverse clinicopathologic features of patients with melanoma patients. Additionally, we also investigated the biological effects of miR-22a on melanoma cells. The functional assays indicated that miR-22 restoration prominently represses melanoma cell proliferation, invasion and migration. It was also found that miR-22 upregulation inhibited melanoma tumorigenesis *in vivo*. The underlying mechanisms of miR-22 in melanoma were further examined, and the results showed that miR-22 exerted its anti-melanoma functions by modulating EMT and Wnt/β-catenin. All of these results manifested that miR-22 inhibited melanoma progression.

Several targets of miR-22 have been identified, including HMGB1 in osteosarcoma[35] and YWHZA in hepatocellular carcinoma[36]. We identified FMNL2 as a direct functional target of miR-22 in melanoma cells. In some studies, FMNL2 has been demonstrated to be implicated in various cancers. For example, up-regulation of FMNL2 enhanced oral squamous cell carcinoma progression[37]. Additionally, FMNL2 overexpression was closely associated with colorectal cancer metastases[38]. In this work, we provided further evidence that FMNL2 was a prognostic biomarker for melanoma patients and implicated in the biofunctions of miR-22 in melanoma.

Conclusions

We demonstrated the tumor-suppressive roles of miR-22 in melanoma. The results in the current study revealed that miR-22, which was dramatically down-regulated in melanoma, associated with poor prognosis and malignant phenotypes of melanoma patients. Furthermore, we provided significant evidence that miR-22 upregulation inhibited melanoma cell proliferation, invasion and migration by modulating EMT and Wnt/β-catenin. Moreover, it was found that FMNL2 was involved in the anti-melanoma functions mediated by miR-22. MiR-22 may serve as a therapeutic target and diagnostic marker for melanoma patients.

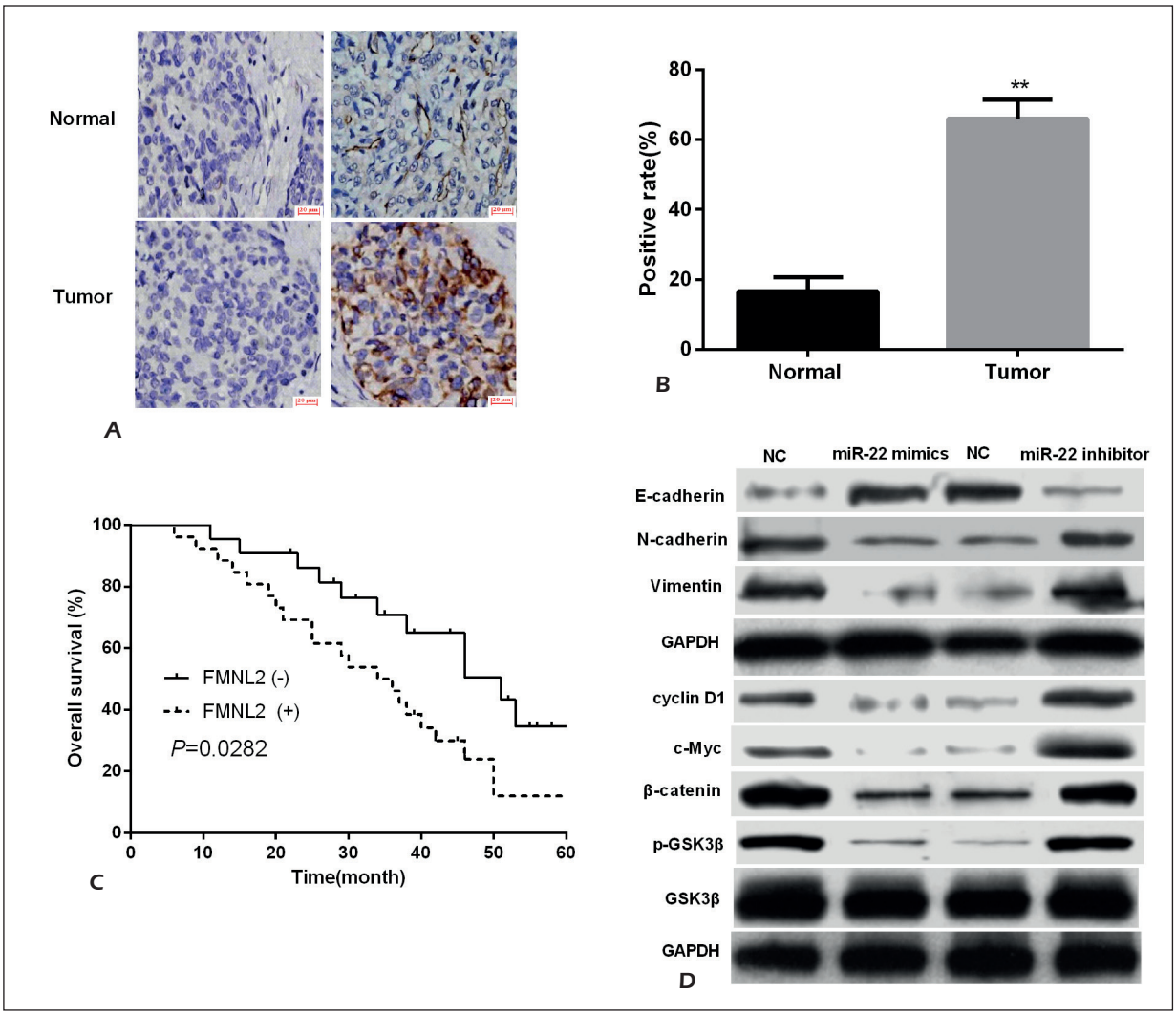

Figure 5. MiR-22 regulated EMT and Wnt/β-catenin signaling pathway in melanoma cells. **A-B**, IHC assay was conducted to determine the FMNL2 expressions in melanoma tissues. **C**, Kaplan-Meier analysis of melanoma patients with high and low FMNL2 expressions. **D**, Western blots were performed to assess the influence of miR-22 on EMT and Wnt/β-catenin in melanoma cells.

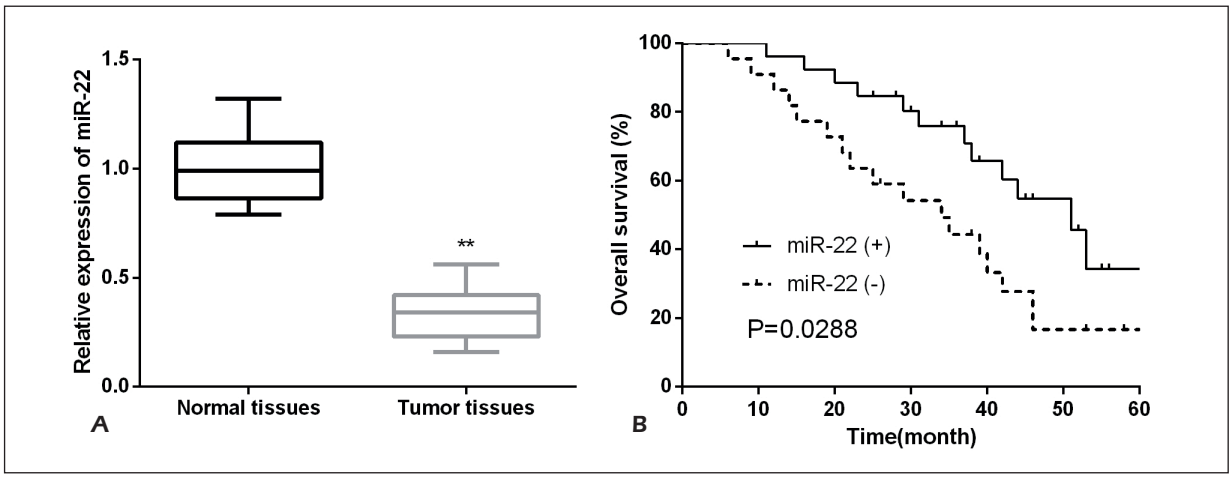

Figure 6. MiR-22 suppressed melanoma tumorigenesis in vivo. **A**, Growth curves of tumor volumes were drafted. **B**, Representative images of tumors in the different treatment groups.

Conflict of Interests

The authors declare that they have no conflict of interest.

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