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Original Article

LncRNA MIAT promotes the proliferation, migration, and invasion of melanoma cells through recruiting TCF12 and activating NFAT5

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Abstract: The present study aimed to explore the biological functions and mechanism of long non-coding RNA myocardial infarction-associated transcript (MIAT) in melanoma progression. MIAT expression in melanoma tissue samples and cells was detected by quantitative real-time PCR. After gain-of-function and loss-of-function models were constructed, cell counting kit-8, EdU, and Transwell assays were employed to detect the proliferation, migration, and invasion of melanoma cells. catRAPID database was employed and RNA pull-down assay and RNA immunoprecipitation assay were utilized to verify, the binding relationship between MIAT and transcription factor 12 (TCF12). The binding of TCF12 to the promoter region of the gene of nuclear factor of activated T cells 5 (NFAT5) was verified by chromatin immunoprecipitation-quantitative PCR assay and dual-luciferase reporter gene assay. The regulatory effects of MIAT and TCF12 on NFAT5 expression were detected via Western blot. The results showed that MIAT expression was increased in melanoma tissues and cells, and was significantly associated with the AJCC stage and the differentiation of melanoma tissues. MIAT overexpression markedly facilitated melanoma cells' multiplication, migration, and invasion, while MIAT knockdown inhibited the multiplication, migration, and invasion. MIAT showed direct interaction with TCF12. MIAT promoted the binding of TCF12 to NFAT5 promoter region, thereby promoting NFAT5 transcription. In conclusion, MIAT promotes melanoma progression through recruiting TCF12 and its interaction with NFAT5.

Keywords: MIAT, TCF12, NFAT5, melanoma, proliferation, migration

Introduction

Originating from melanocytes, melanoma causes about 55,500 deaths each year [1]. The preferred treatment option for primary melanoma is surgical removal of the tumor, and there are no other ideal treatments for metastatic melanoma [2]. In this context, patients with metastatic melanoma have an extremely poor prognosis [3]. Understanding the pathogenesis of melanoma is the key to develop novel therapeutic strategies [4].

Known as a kind of transcript characterized by over 200 nucleotides in length, long non-coding RNAs (lncRNAs) were once considered useless sequences due to their inability to encode proteins [5]. Recent research has shown that lncRNAs can take part in diverse physiological and pathological processes [6]. Abnormally expressed in multiple tumors, lncRNAs are reported to be involved in the tumorigenesis and cancer progression [7]. Among them, lncRNA myocardial infarction-associated transcript (MIAT) has been confirmed as a biomarker for the prognosis of melanoma, and it can also promote melanoma cell migration and invasion through regulation of the PI3K/AKT signaling pathway [8, 9]. Nevertheless, its specific molecular mechanism in melanoma progression is not completely clarified.

Identified as a member of the basic helix-loop-helix (bHLH) family, transcription factor 12 (TCF12) is implicated in the regulation of differentiation of various tissues/cells such as skeletal muscle, neuron, and lymphocytes [10]. Recent research has indicated that TCF12 plays

a cancer-promoting role in multiple tumors. For example, TCF12 facilitates colorectal cancer cells' migration and invasion by down-regulation of E-cadherin, and TCF12 overexpression promotes the expression of Wnt family member 5A (Wnt5A) to promote the aggressiveness of melanoma cells [11, 12]. Nuclear factor of activated T cells 5 (NFAT5), also known as tonicity enhancer binding protein (TonEBP), features prominently in embryonic development, development of autoimmune diseases, maintenance of blood pressure and cancer progression [13, 14]. In the present study, bioinformatics analysis suggested that MIAT could directly bind with TCF12, and had the potential to activate the transcription of NFAT5. Thus, the present study was designed to explore the biological functions of MIAT in melanoma and its regulatory functions on TCF12 and NFAT5, so as to provide potential targets for melanoma diagnosis and treatment.

Materials and methods

Case collection

63 melanoma patients who were newly diagnosed in the First Affiliated Hospital, College of Clinical Medicine of Henan University of Science and Technology were selected. After obtaining the informed consent signed by the patients, the cancerous tissues and the matched paracancerous tissues were removed during surgery. The surgically removed tissues were kept at -196°C in liquid nitrogen. None of the participants had received radiotherapy, chemotherapy, or any other therapy before the surgery. The current study was approved by the Ethics Committee of the First Affiliated Hospital of Henan University of Science and Technology (approval number: 201604).

Cell culture and transfection

The melanoma cell lines (HMCB cells, G-361 cells, and A375 cells) and HEK 293T cells used in this study were bought from the American Type Culture Collection (Manassas, VA, USA), and normal human melanocytes (HEMn-LP cells) were purchased from Cascade Biologics (Mansfield, UK). The cells were cultured in M-254 basal medium (Cascade Biologics, Mansfield, UK) containing neomycin (10 μg/ml), amphotericin B (0.25 μg/ml), penicillin (100 U/ml), human melanocyte growth supplement-2 (HMGS-2; Cascade Biologics, UK), and fetal bovine serum (FBS, 10%, Gibco, Carlsbad, CA, USA) in 5% CO_2 at 37°C [15]. The cells during logarithmic growth were passaged with 0.25% trypsin (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

In this study, pcDNA-MIAT overexpression vector (pcDNA-MIAT), pcDNA empty vector (NC), MIAT small interference RNAs (si-MIAT) and scrambled negative control of siRNA (si-NC), pcDNA-TCF12 overexpression vector (TCF12), and TCF12 small interference RNAs (si-TCF12) were designed and synthesized by RiboBio Co., Ltd. (Guangzhou, China). Cell transfection was conducted with Lipofectamine® 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. A quantitative real-time polymerase chain reaction (qRT-PCR) assay was carried out 48 h after the transfection to detect the transfection efficiency.

qRT-PCR

The total RNA of the samples was extracted using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and a Nanodrop-spectrophotometer (NanoDrop, Waltham, MA, USA) was adopted to detect the purity and concentration of the total RNA. A PrimeScript™ RT reagent kit (Takara, Dalian, China) was utilized to reverse-transcribe total RNA into complementary DNA (cDNA). The SYBRGreen Master Mix kit (Takara, Dalian, China) was used for performing qRT-PCR in ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). $2^{-\Delta\Delta CT}$ method was applied to calculate the relative expression of target genes, with GAPDH as the internal reference. A Minute™ Cytoplasmic and Nuclear Extraction Kit (Invent Biotechnologies, Plymouth, MN, USA) were used to separate the nucleus and cytoplasm of melanoma cells. The expression of MIAT in the nucleus and cytoplasm were then measured respectively, with U6 and GAPDH as the nuclear and cytoplasmic internal control, respectively. The primer sequences are as follows (F stands for forward; R stands for reverse): MIAT primer sequence: F, 5'-TTTACTTTAACAGACCAGAA-3'; R, 5'-CTCCTTTGTTGAATCCAT-3'. U6 primer sequence: F, 5'-AAGTCCTCCAAGCTAGGGCCCUCU-3'; R, 5'-AGGUCGACCGUGUAAUGUGUU-3'. GAPDH primer sequence: F, 5'-GGGAGCCAAAAGGGTCAT-3'; R, 5'-GAGTCCTTCCACGATACCAA-3'.

Cell counting kit-8 (CCK-8) assay

The melanoma cell lines (G-361 and A375) during logarithmic growth were selected, trypsinized, and re-suspended. Subsequently, the cells were transferred to 96-well plates (2×10^3 cells/well). After culturing for 12, 24, 48, 72, and 96 h, 10 μL of CCK-8 reagent (Beyotime, Shanghai, China) was added to each well, and then the cells were incubated for 2 h. Subsequently, an automatic microplate reader was used to measure the absorbance at 450 nm of cells in each well.

EdU assay

The cells during logarithmic growth phase were transferred to 24-well plates and cultured routinely for 48 h. Then, each well was added with 200 μL of EdU medium (50 μmol/L; Invitrogen, Carlsbad, CA, USA). After 2 h of incubation, the cells were rinsed carefully with PBS and fixed with paraformaldehyde. After that, 200 μL of glycine (2 mg/ml) was added for incubation for 5 min, and the cells were then washed with PBS. Next, each well was added with 100 μL of PBS containing TritonX-100 (0.5%), and the cells were incubated for 10 min. After PBS washing, the cells were stained with Apollo for 30 min in the dark and stained with 1×Hoechst 33342 DNA staining solution for 30 min in the dark. Eventually, the cells were washed with PBS and counted under a fluorescence microscope.

Transwell assay

The cells during logarithmic growth phase were re-suspended in serum-free medium. An appropriate amount of cell suspension was added to the top compartment of the Transwell chamber (Corning, NY, USA), and the lower compartment of the Transwell chamber was added with 500 μL of complete medium containing 10% FBS. Then the cells were cultured for 24 h. Next, cotton swabs were employed to carefully wipe off the cells in the top compartment. The cells in the bottom of the membrane were fixed for 15 min with paraformaldehyde and washed with phosphate buffer saline (PBS) 3 times. Then the cells were stained with 0.1% crystal violet for 10 min and washed with PBS. Ultimately, the stained cells were counted in 5 randomly selected fields under the microscope.

RNA pull-down assay

A Pierce™ Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher, Waltham, MA, USA) was utilized to detect how MIAT interacted with TCF12. Briefly, the biotinylated MIAT was added to the nuclear extract of melanoma cells, and the incubation was continued for 1 h at 4°C in a rotary shaker. Then the mixture was incubated with magnetic beads in binding buffer, and then the RNA-protein complex was washed by washing buffer, then the eluent was used to elute the protein products on the magnetic beads, and next the protein expression was detected by Western blot.

RNA immunoprecipitation (RIP) assay

A Magna RIP™ RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA) was employed to conduct the experiment. Briefly, the cells during logarithmic growth phase were suspended in RIP lysis buffer. Then the lysate was incubated with anti-TCF12 antibody or negative control (IgG antibody) which was coupled with magnetic beads. After the immunoprecipitate was isolated, it was incubated with proteinase K to remove the protein. Then RNA was purified and reverse-transcribed to obtain cDNA. Subsequently, MIAT level in the immunoprecipitate was detected via qRT-PCR.

Chromatin immunoprecipitation (ChIP) assay

The experiment was conducted with a EZ-ChIP™ kit (Millipore, Billerica, MA, USA). The melanoma cells were fixed with formaldehyde. Next, the cells were scraped off and centrifuged to obtain the cell precipitates. Then PMSF-containing cell lysis buffer was added to suspend the cells. After lysate was centrifuged, the supernatant was removed to obtain the nuclear pellet. After ultrasonically shearing the DNA in an ice-water bath, 10% volume of the supernatant was used as the control, and the remaining 90% lysate was incubated with anti-TCF12 antibody coupled with magnetic beads overnight at 4°C. Then the complex containing the magnetic beads was collected, and the DNA in the complex was eluted with elution buffer. A DNA purification kit was employed to perform DNA purification, and then qRT-PCR was performed to detect the enrichment of the sequence of the promoter of NFAT5.

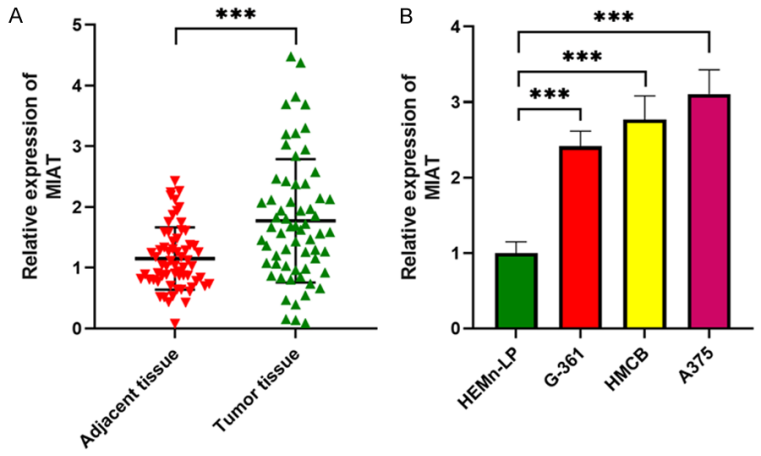


Figure 1. MIAT expression in melanoma. MIAT expression in 63 cases of melanoma and para-tumorous tissues was detected by qRT-PCR (A). MIAT expression in HEMn-LP cells and melanoma cell lines (HMCB, G-361, and A375) was detected by qRT-PCR (B). All of the experiments were performed in triplicate. *** indicates $P<0.001$.

Dual-luciferase reporter gene assay

To construct pGL3-NFAT5-wild type (NFAT5-WT) and pGL3-NFAT5-mutant (NFAT5-MUT) reporter vectors, the target fragments of wild-type NFAT5 and mutant NFAT5 were amplified and integrated into the pGL3 vector (Promega, Madison, WI, USA). The melanoma cells were co-transfected with NFAT5-WT (or NFAT5-MUT) and TCF12 overexpression plasmid, si-TCF12, MIAT overexpression plasmid, siMIAT, and the negative controls, respectively. The luciferase activity was measured with a dual-luciferase reporter assay system (Promega, Madison, WI, USA) following the manufacturer's instruction 48 h after the transfection.

Western blot assay

The cells during logarithmic growth phase were collected and incubated with RIPA lysis buffer (Beyotime, Shanghai, China) for 20 min on ice. Subsequently, the lysate was centrifuged at 4°C at 13000 r/min for 20 min, and the supernatant was collected as the protein sample. A BCA protein quantification kit (Beyotime, Shanghai, China) was employed to quantify the protein. After the protein samples were mixed with loading buffer and denatured, the protein was dissolved by SDS-PAGE and then transferred to the PVDF membrane. The membranes were blocked with 3% bovine serum albumin (BSA) for 1.5 h at room temperature. After the membranes were incubated primary antibodies at 4°C overnight, the membranes were washed with TBST 3 times. Subsequently, the membranes were incubated with the secondary antibodies for 40 min at room temperature, and then, the membranes were washed 2 times with TBST. After that, Enhanced Chemiluminescence Western Blotting Substrate (Dongguan Biotech, Shandong, China) was used for developing the protein bands. ImageJ was used for analyzing the gray value of each protein band, and the ratio of the gray value of the target protein to that of GAPDH indicated the relative expression of the target protein. The antibodies used in the present work: anti-TCF12 antibody (1:1000, ab70746), anti-NFAT5 antibody (1:1000, ab3446), anti-GAPDH antibody (1:1000, ab9485), and Goat Anti-Rabbit IgG H&L (1:3000, ab205718). All the antibodies were bought from Abcam (Shanghai, China).

Statistical analysis

SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software was used as the tool to analyze the experimental data. Kolmogorov-Smirnov test was used to test the normality of the data. For the data which were normally distributed, an independent sample *t*-test was used. For the data with skewed distribution, a paired sample Wilcoxon signed-rank test was used. To make the comparison among three or more groups, a one-way ANOVA test was used, with Turkey's post-hoc test to make the comparison between two groups. Enumeration data were represented in a contingency table and analyzed using χ^2 test. A difference was of statistical significance when $P<0.05$.

Results

MIAT expression is up-regulated in melanoma and is associated with poor clinicopathological indicators

First of all, qRT-PCR was conducted to detect MIAT expression in melanoma tissues, and it was revealed that MIAT expression was markedly elevated in melanoma tissue samples (**Figure 1A**). In comparison to normal human

Table 1. Correlation between MIAT expression and clinicopathological features in melanoma

Pathological parameters	Numbes (n=63)	MIAT expression		χ^2	*P*-Value
		High (n=31)	Low (n=32)		
Gender					
Male	28	15	13	0.3842	0.5353
Female	35	16	19		
Age (years)					
<50	41	18	23	1.3215	0.2503
≥50	22	13	9		
Tumor thickness (mm)					
<10	39	15	24	4.7289	0.0297*
>10	24	16	8		
AJCC stage					
I, II	35	13	22	4.5853	0.0322*
III, IV	28	18	10		
Lymph node metastasis					
No	42	16	26	6.2238	0.0126*
Yes	21	15	6		
Degree of differentiation					
Low, medium	26	19	7	10.0927	0.0015*
High	37	12	25		

*$P<0.05$.

melanocytes (HEMn-LP cells), MIAT expression in melanoma cell lines (HMCB, G-361, and A375 cells) was significantly increased (**Figure 1B**). Chi-square test revealed that highly expressed MIAT was closely associated with the tumor thickness, higher AJCC stage, lymph node metastasis, and decreased degree of differentiation in melanoma (**Table 1**).

Effects of MIAT on the proliferation and migration of melanoma cells

To delve deeper into the biological functions of MIAT in melanoma cells, we transfected pcDNA-MIAT into G361 cells and si-MIAT into A375 cells, which was confirmed via qRT-PCR to be successful (**Figure 2A**). Subsequently, a CCK-8 assay was conducted to detect the cell multiplication, and it was revealed that in comparison to the NC group, cell proliferation in the pcDNA-MIAT group was remarkably higher; in comparison to the si-NC group, the proliferation in the si-MIAT group was markedly reduced (**Figure 2B**). Next, an EdU assay was conducted, and consistently, it was revealed that MIAT overexpression could remarkably facilitate G361 cell proliferation, whereas knocking down MIAT dramatically suppressed A375 cell proliferation (**Figure 2C**). Transwell assay showed that MIAT overexpression noticeably facilitated G361 cell migration and invasion while knocking down MIAT notably suppressed A375 cell migration and invasion (**Figure 2D**, **2E**).

MIAT interacts with TCF12/NFAT5

LncMAP database (http://bio-bigdata.hrbmu.edu.cn/LncMAP/) was adopted to predict the underlying mechanism of MIAT, and it was discovered that MIAT could probably promote NFAT5 transcription via recruiting the transcription factor TCF12 to the promoter region of NFAT5 gene (**Figure 3A**). Next, qRT-PCR was performed to determine the subcellular location of MIAT in G361 and A375 cells, and it was revealed that MIAT was mainly distributed in the nucleus of melanoma cells (**Figure 3B**), suggesting that it could probably regulate transcription. Next, catRAPID database (http://service.tartaglialab.com/page/catrapid_group) was used to predict the binding relationship between TCF12 and MIAT, and the interaction map illustrated that MIAT and TCF12 could bind to each other (**Figure 3C**). Subsequently, RNA pull-down assay showed that TCF12 was

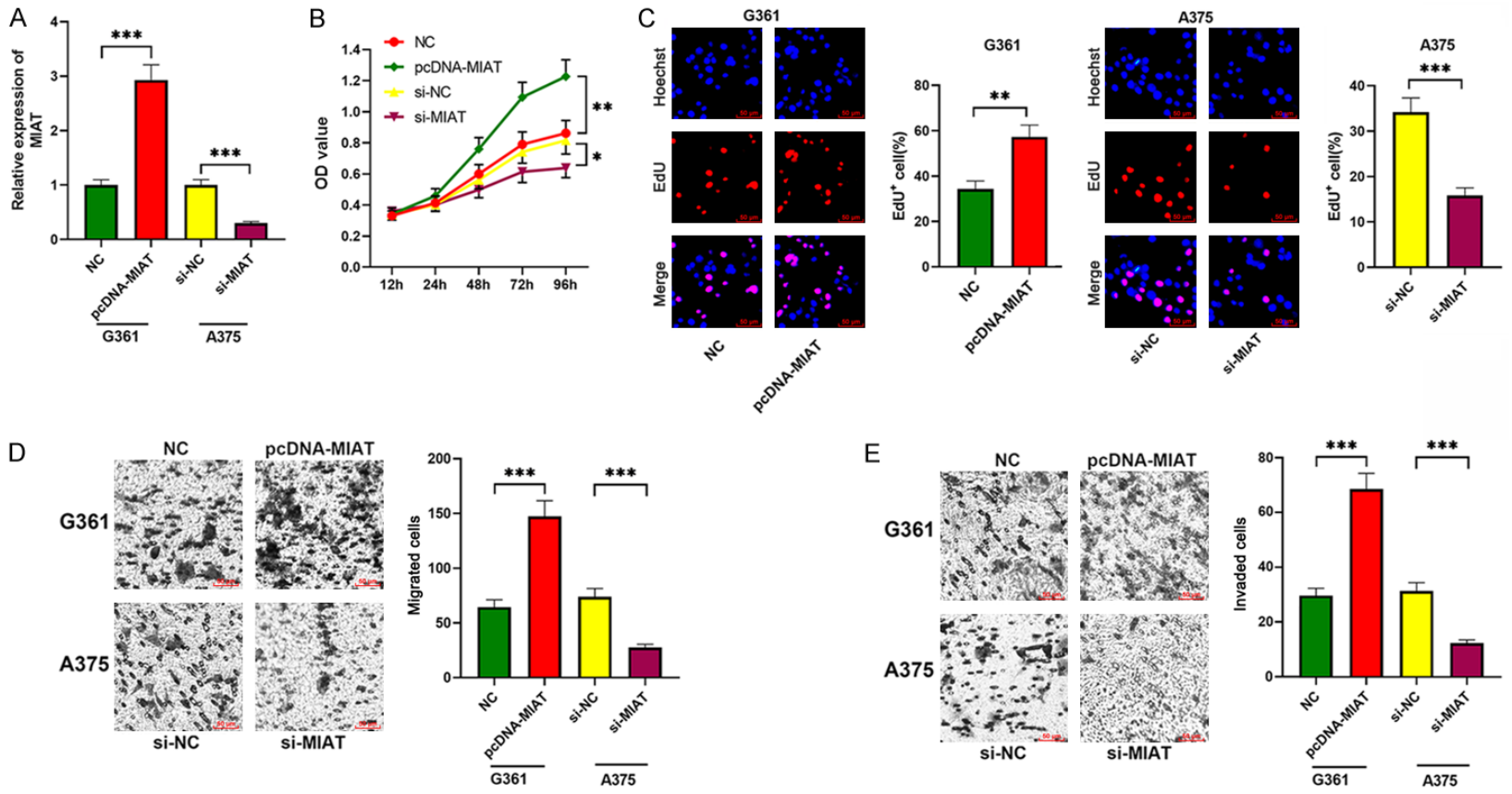


Figure 2. Effects that MIAT has on the multiplication, migration, and invasion of melanoma cells. G361 cells were transfected with empty vector or MIAT overexpression plasmids, and si-NC or si-MIAT was transfected into A375 cells; the transfection efficiency was detected through qRT-PCR (A). CCK-8 assay and EdU assay were utilized to detect the changes in G361 and A375 cell proliferation (B, C, 200×). Transwell assay was conducted to examine the changes in G361 and A375 cell migration and invasion (D, E, 200×). All of the experiments were performed in triplicate. * indicates $P<0.05$, ** indicates $P<0.01$, and *** indicates $P<0.001$.

A

Cancer Type	LncRNA ID	LncRNA Symbol	TF ID	TF Symbol	Gene ID	Gene Symbol	P-Value Discovery	P-Value Validation
SKCM	ENSG00000225783	MIAT	ENSG00000140262	TCF12	ENSG00000102908	NFAT5	1.02e-01	8.21e-01

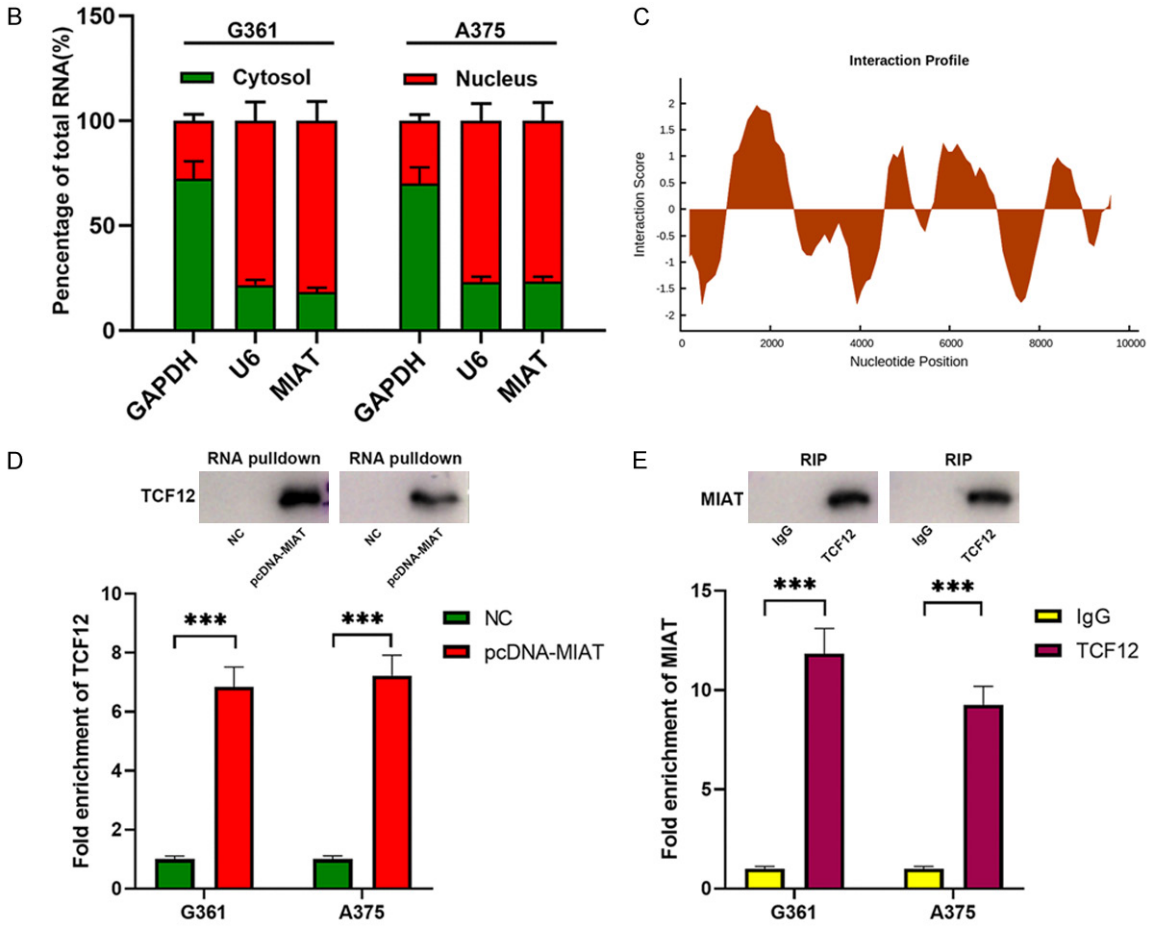
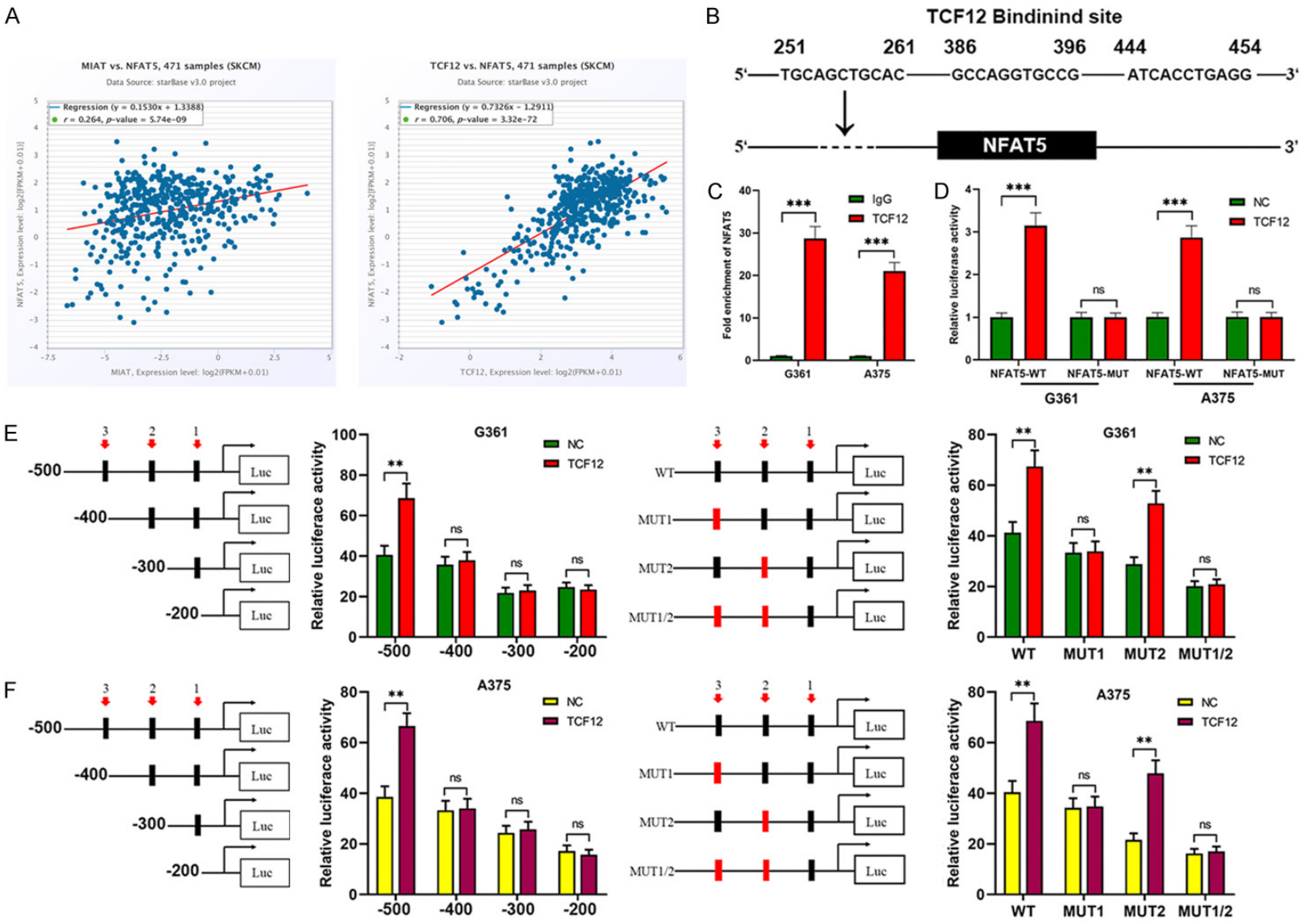


Figure 3. The binding relationship between MIAT and TCF12. LncMAP database was employed to predict the binding relationship among MIAT, TCF12, and NFAT5 (A). Cytoplasmic and nuclear expression of MIAT in G361 and A375 cells were measured via qRT-PCR. GAPDH and U6 acted as the cytoplasmic and nuclear controls, respectively (B). CatRAPID database was employed to predict the binding domain between MIAT and TCF12 (C). RNA pull-down assay was performed to analyze the binding relationship between MIAT and TCF12 in G361 and A375 cells (D). RIP assay was conducted to analyze the binding relationship between MIAT and TCF12 in G361 and A375 cells (E). All of the experiments were performed in triplicate. *** indicates $P<0.001$.

enriched in the biotinylated MIAT group, while TCF12 was almost not expressed in the control group (**Figure 3D**). Additionally, RIP assay suggested that as opposed to the IgG group, MIAT was significantly enriched by anti-TCF12 antibody in both G361 and A375 cells, but not enriched by the control IgG (**Figure 3E**).

MIAT promotes NFAT5 transcription via recruiting TCF12 to the promoter region of NFAT5

StarBase database (http://starbase.sysu.edu.cn/) showed that MIAT expression and TCF12 expression were positively correlated with NFAT5 expression in skin cutaneous melanoma (SKCM) samples (**Figure 4A**), suggesting that MIAT and TCF12 could regulate the expression of NFAT5. PROMO database (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo) showed that TCF12 could bind to the promoter sequence of NFAT5 gene (**Figure 4B**). Therefore, it was supposed that TCF12 might promote the transcription of NFAT5. Next, TCF12-specific antibodies were used to perform ChIP-qPCR assay in G361 cells and A375 cells, and it was revealed that as against the IgG group, TCF12 binding com-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MIAT vs. NFAT5, 471 samples (SKCM)
Data Source: starBase v3.0 project
Regression (y = 0.1530x + 1.3388)
r = 0.264, p-value = 5.74e-09
NFAT5, Expression level: log2[FPKM+0.01]
MIAT, Expression level: log2(FPKM+0.01)
TCF12 vs. NFAT5, 471 samples (SKCM)
Data Source: starBase v3.0 project
Regression (y = 0.7326x - 1.2911)
r = 0.706, p-value = 3.32e-72
NFAT5, Expression level: log2[FPKM+0.01]
TCF12, Expression level: log2(FPKM+0.01)
B
TCF12 Bindinind site
251
261
386
396
444
454
5'—TGCAGCTGCAC——GCCAGGTGCCG——ATCACCTGAGG—3'
5'
NFAT5
3'
C
Fold enrichment of NFAT5
IgG
TCF12
***
***
G361
A375
D
Relative luciferase activity
NC
TCF12
***
***
ns
ns
NFAT5-WT
NFAT5-MUT
NFAT5-WT
NFAT5-MUT
G361
A375
E
-500
-400
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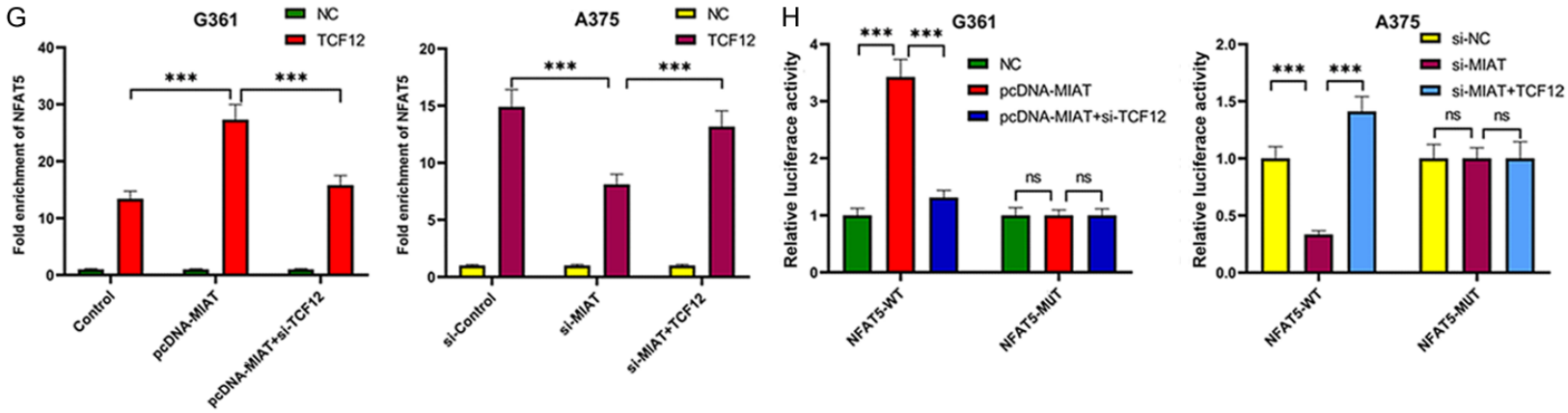

Figure 4. Effects of MIAT and TCF12 on NFAT5 expression in melanoma cells. The StarBase database was adopted to analyze the correlation of NFAT5 expression with MIAT expression (left) and TCF12 expression (right) in skin cutaneous melanoma (SKCM) samples (A). PROMO database was used to predict binding site between TCF12 and NFAT5 promoter sequence (B). ChIP-qPCR assay was utilized to detect the binding of TCF12 to NFAT5 promoter region in G361 and A375 cells (C). MIAT overexpression plasmid and si-TCF12 were transfected into G361 cells, and si-MIAT and TCF12 overexpression plasmid were transfected into A375 cells, and the effects of MIAT and TCF12 on NFAT5 mRNA expression were detected through qRT-PCR (D). Dual-luciferase reporter gene assay was conducted to detect the specific sites of TCF12 binding to NFAT5 promoter region in G361 cells (3, 2, and 1 indicate site 3, 2, and 1, respectively) (E). Dual-luciferase reporter gene assay was performed to detect the specific sites of TCF12 binding to NFAT5 promoter region in A375 cells (3, 2, and 1 indicate site 3, 2, and 1, respectively) (F). MIAT overexpression plasmid and si-TCF12 was co-transfected into G361 cells, and si-MIAT and TCF12 overexpression plasmid was co-transfected into A375 cells. The chIP-qPCR assay was utilized to detect the impacts of MIAT and TCF12 on the binding of TCF12 to NFAT5 promoter region (G). MIAT overexpression plasmid and si-TCF12 were co-transfected into G361 cells, and si-MIAT and TCF12 overexpression plasmid were co-transfected into A375 cells, and dual-luciferase reporter gene assay was applied to detect the impacts of MIAT and TCF12 on the binding relationship between MIAT and TCF12 (H). All of the experiments were performed in triplicate. ** $P<0.01$ and *** $P<0.001$; ns: no significance.

plexes were significantly enriched in the NFAT5 promoter (**Figure 4C**). Dual-luciferase reporter gene assay showed that TCF12 overexpression markedly enhanced the luciferase activity of NFAT5-WT, but failed to significantly influence that of NFAT5-MUT (**Figure 4D**). To determine the specific binding sites between TCF12 and NFAT5 promoter, the aforementioned binding sequence was truncated or mutated, and a dual-luciferase reporter gene assay was carried out; it was found that site 3 was the specific site where TCF12 protein bound to the NFAT5 promoter region (**Figure 4E**, **4F**). Additionally, it was revealed that MIAT overexpression could enhance the binding between TCF12 and NFAT5 promoter, while knocking down MIAT could weaken the binding (**Figure 4G**). The dual-luciferase report gene assay results suggested that MIAT overexpression promoted NFAT5-WT's luciferase activity, while TCF12 knockdown showed the opposite effects; knocking down MIAT inhibited the luciferase activity of NFAT5-WT, while up-regulating TCF12 expression could also reverse this effect (**Figure 4H**).

MIAT exerts its functions in melanoma through TCF12

Subsequently, to further verify the effects of MIAT and TCF12 on melanoma cells, si-TCF12, and MIAT overexpression plasmid were co-transfected into G361 cells, and si-MIAT and TCF12 overexpression plasmid were co-transfected into A375 cells; then NFAT5 expression was detected through Western blot (**Figure 5A**). Next, CCK-8, EdU, and Transwell assays were performed, and it was revealed that in comparison to the control group, MIAT overexpression could promote G361 cell multiplication, migration, and invasion, whereas TCF12 knockdown could counteract the effects of MIAT; on the other hand, MIAT knockdown suppressed A375 cell multiplication, migration, and invasion, while up-regulating TCF12 expression could counteract the inhibitory impact of MIAT knockdown on the malignant phenotypes of melanoma cells (**Figure 5B-E**).

Discussion

In melanoma, a lot of lncRNAs have been reported to be aberrantly expressed and participate in melanoma progression [16, 17]. Among them, MIAT has drawn wide attention. Specifically, MIAT can increase the expression of c-Myc and cyclin D1 by promoting the phosphorylation of PI3K and AKT, thereby facilitating the migration and invasion of melanoma cells [8]. Another study reported that MIAT can promote melanoma progression via repressing miR-150 [17]. Consistently, the present study revealed that MIAT was highly expressed in melanoma and correlated with the unfavorable pathological characteristics of melanoma. With MIAT knockdown and overexpression models, it was revealed that MIAT overexpression promoted the malignancy of melanoma cells, while MIAT knockdown had the opposite effects. These data further support that MIAT is a promising prognostic biomarker and a therapy target for melanoma.

TCF12 is an important member of the bHLH family, and has been reported to facilitate the development of some cancers (e.g. prostate cancer, gallbladder cancer, breast cancer and ovarian cancer) via multiple mechanisms, such as promoting epithelial-mesenchymal transition, inducing the activation of cancer-related fibroblasts [18-23]. Moreover, TCF12 can up-regulate the expression of Yes1-associated transcriptional regulator by promoting Wnt5A to accelerate melanoma progression [12]. In the present work, we report that, TCF12 is the mediator by which MIAT exerts its biological function in melanoma. However, the specific mechanism by which TCF12 exerts its tumor-promoting effect in melanoma has not been fully clarified. In the present work, we demonstrated that MIAT could interact with TCF12 to facilitate the transcription of NFAT5 in melanoma cells. NFAT5 belongs to the REL family and has a Rel-homology domain, and it can directly bind with DNA to exert its biological functions [24, 25]. NFAT5 is considered as a key transcription factor to maintain cell homeostasis in a hypertonic environment, and recent evidence has shown that it also features prominently in regulating the function of immune cells [25-27]. Recently, some studies reported that NFAT5 can promote or inhibit the tumorigenesis and development of various tumors. For example, NFAT5 can facilitate the growth and migration of cancer cells through up-regulating S100A4 expression in clear cell renal cell carcinoma [28]. In breast cancer, NFAT5 promotes cancer progression via inducing the expression of vascular endothelial growth factor-A [29]. A study has confirmed that targeting NFAT5 can signifi-

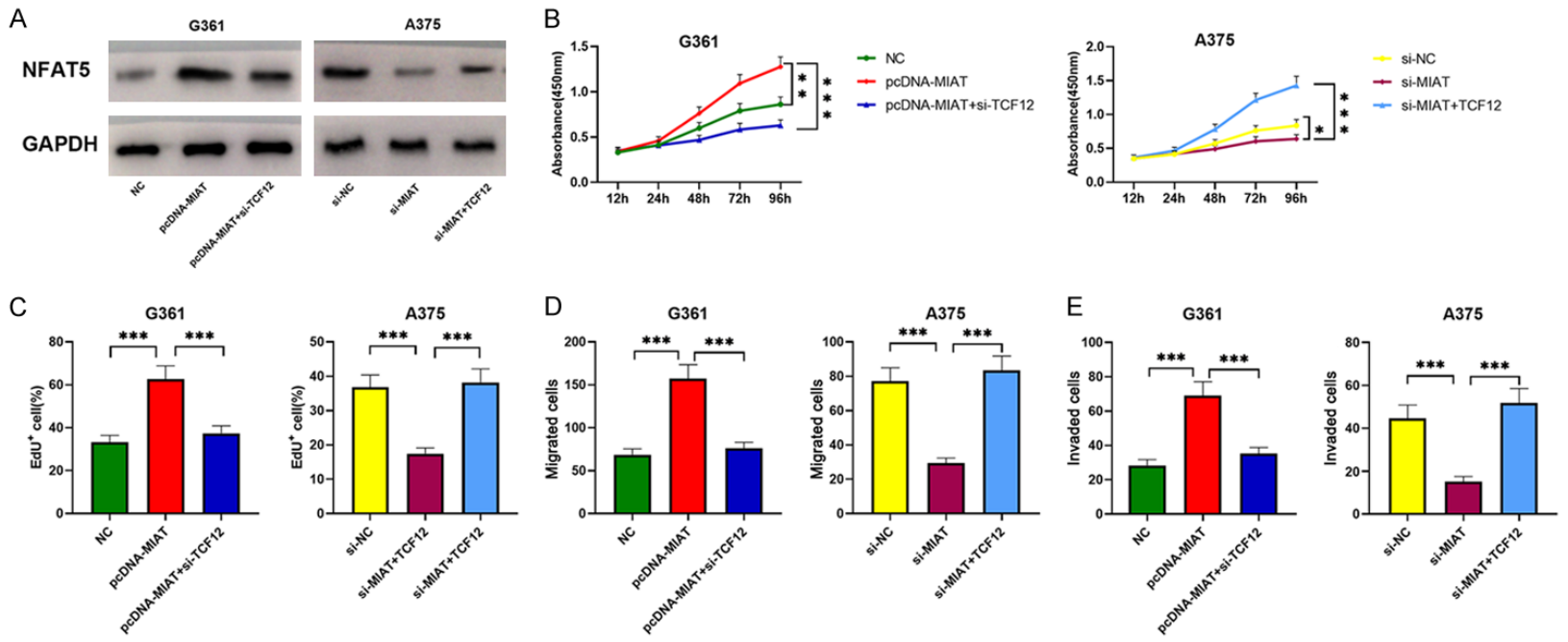

Figure 5. Effects of MIAT and TCF12 on melanoma cell proliferation, migration, and invasion. Empty vector, MIAT overexpression plasmid, or MIAT overexpression plasmid + si-TCF12 were transfected into G361 cells, respectively, and si-NC, si-MIAT, or si-MIAT + TCF12 overexpression plasmid were transfected into A375 cells, respectively. The transfection efficiency was detected by Western blotting (A). CCK-8 assay and EdU assay were carried out to detect the changes in the proliferation of G361 and A375 cells (B and C). Transwell assay was employed to detect the changes of the migration and invasion of G361 and A375 cells (D and E). All of the experiments were performed in triplicate. * indicates $P<0.05$, ** indicates $P<0.01$, and *** indicates $P<0.001$.

cantly inhibit the progression of melanoma [30]. In this study, bioinformatics suggested that TCF12 could directly bind to NFAT5 promoter region, and it has been shown that MIAT overexpression could enhance the binding of TCF12 to NFAT5 promoter, thus promoting NFAT5 transcription. Collectively, these results suggest that MIAT/TCF12/NFAT5 axis was a novel mechanism of melanoma progression.

There are several shortcomings in the present work. First of all, only *in vitro* models were used in the experiments, and *in vivo* studies are required to further validate the biological function of MIAT in melanoma in the following work. Additionally, the relationship between the expression level of MIAT and the prognosis of the patients needs to be explored in the future. Lastly, the downstream mechanism of NFAT5 by which it regulates melanoma progression is still obscure, which is needed to be further investigated.

To sum up, MIAT expression is enhanced in melanoma, and its high expression implies adverse prognosis of the patients. In terms of mechanism, MIAT promotes NFAT5 transcription by recruiting TCF12 to the NFAT5 promoter region, subsequently inducing the transcription of NFAT5, thereby promoting melanoma cell proliferation, migration and invasion. The above evidence suggests that MIAT is expected to become a molecular therapeutic target for melanoma.

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Disclosure of conflict of interest

None.

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