

MiR-154 inhibits cells proliferation and metastasis in melanoma by targeting AURKA and serves as a novel prognostic indicator

J. WANG, Y. FANG, Y.-F. LIU, X. WANG, X.-L. WANG, R.-Y. WANG, Z.-D. MENG

Department of Dermatology, Renmin Hospital, Hubei University of Medicine, Shiyan, Hubei, China

Jing Wang and Yong Fang contributed equally to this work

Abstract. – OBJECTIVE: Increasing evidence suggested that dysregulated miR-154 in several tumor tissues is involved in the clinical progress of cancers patients. The objective of this study was to explore the expression pattern of miR-154 and its potential effects in human melanoma.

PATIENTS AND METHODS: Microarray data from GEO datasets were analyzed to identify differentially expressed miRNAs. Real Time-Polymerase Chain Reaction (RT-PCR) was performed to determine the expressions of miR-154 in melanoma cell lines and tumor tissues. The associations between miR-154 levels and clinical progress were studied using a series of statistical methods. Cell viability, invasion, migration, and apoptosis were detected by Cell Counting Kit-8 (CCK-8) assays, transwell assay, wound healing assays, and flow cytometry, respectively. TargetScan system was used to identify the target genes of miR-154 and Luciferase activity analysis was carried out to demonstrate the possible target.

RESULTS: The expression levels of miR-154 were distinctly lower in tumor samples and melanoma cell lines than in normal controls ($p < 0.01$). The up-regulation of miR-154 in melanoma tissues was associated with advanced tumor stage ($p = 0.028$), ulceration ($p = 0.046$), and shorter overall survival ($p = 0.0035$). Moreover, the multivariate analysis suggested a decreased expression of miR-154 is an independent predictor of overall survival rates in melanoma patients. Functional observation showed that up-regulation of miR-154 suppressed the capability of proliferation, invasion, and migration, promoting apoptosis in melanoma cell lines. Bioinformatics analysis predicted AURKA (aurora kinase A) as a target of miR-154, which was confirmed using the luciferase activity assays. Besides, miR-154 overexpression rescued the suppressive effect of AURKA-mediated melanoma on cell proliferation, colony formation, and metastasis.

CONCLUSIONS: These results revealed that miR-154 has clinical implications for targeted

therapy of melanoma patients and indicated that miR-154 could represent a novel biomarker in predicting the clinical outcome for melanoma.

Key Words:

MiR-154, Melanoma, Prognosis, Migration, Invasion, AURKA.

Introduction

Melanoma, a common malignant tumor, is among the most aggressive and treatment-resistant human cancers and it is considered to be one of the best examples of an immunogenic tumor, whose incidence keeps increasing worldwide^{1,2}. In 2016, there was an estimation of 20,000 new cases and almost 11,000 deaths in China, accounting for approximately 70% percent of all skin cancer deaths³. Traditional therapeutic methods of malignant melanoma include excisional biopsies and chemotherapy. However, the prognosis of melanoma patients remains poor^{4,5}. In addition, despite recent advancements in targeted therapy and immunotherapy, melanoma rapidly develops resistance to the above treatments^{6,7}. Metastatic melanoma has a very poor outcome, with a median survival of 7-11 months and a five-year survival rate of 5-10%, and it is the most common cause for the high death rate of this disease^{8,9}. Thus, the research of the molecular pathogenesis of melanoma is urgently needed for the successful guidance of novel effective therapeutic methods. MicroRNAs (miRs), 18-25 nucleotides in length, are a class of noncoding RNAs, which have been previously considered to be “transcriptional noise”¹⁰. Emerging evidence in the past 20 years indicates that miRNAs act as post-transcriptional regulators that bind to the 3' untranslated regions

(UTRs) of specific targeting mRNAs, usually resulting in translational inhibition and silence of gene expression^{11,12}. Growing reports have shown that the aberrant expression of miRNAs have key roles in the tumorigenesis and progression of all types of tumors, where several functional miRNAs are identified as onco-miRs and some as tumor suppressors^{13,14}. Considering the dysregulation and critical roles of miRNAs during tumorigenesis, the use of miRNAs as potential novel biomarkers in the prognosis and diagnosis attracted increasing attention^{15,16}. In many other tumors, a large number of miRNAs have been identified and functionally characterized. However, there is precious little of the research of miRNAs in melanoma. Recently, a tumor-related miRNA, miR-154, was frequently observed to be down-regulated in some types of tumors such as lung cancer, glioblastoma, and laryngeal squamous cell carcinoma¹⁷⁻¹⁹. Besides, the tumor-suppressive roles of miR-154 in the above tumors cells and metastasis were also confirmed by gain-of-function assays *in vitro* experiments. However, whether miR-154 could serve as a possible regulator of progression of melanoma remains unknown. In this study, we firstly observed the up-regulation of miR-154 in melanoma. Moreover, we used various methods to explore the clinical significance and function involved in the tumor behaviors, as well as to investigate the mechanism of miR-154, which suppressed the progression of melanoma.

Patients and Methods

Specimen Collection

Tissue specimens were obtained from patients with melanoma (n=104) who underwent surgical resection at Renmin Hospital, the Hubei University of Medicine between April 2008 and August 2012. Before the resection, patients did not undergo anti-cancer therapy. The samples were immediately frozen after surgery and stored at -80°C . Written informed consents were received from the patients. The protocols were approved by the hospital Ethics Committee. Table I showed the patients' clinical characteristics.

Cell Culture and Transfection

Melanoma cells, including A375, SK-MEL-2, 1205Lu, UACC903, and CHL-1 were bought from BoYun Biological company (Hefei, Anhui, China). HEMA-LP cells (BoYun, Hefei, Anhui, China) were used as control cells. Cells were cultured using Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS). The cell transfection was carried out using Lipofectamine 3000 reagent. The miR-154 mimics, miR-154 inhibitors, and matched controls were bought from SBS Biological Company (Haidian, Beijing, China). The sequence of AURKA cDNA was constructed into a pcDNA3.1 empty vector to overexpress AURKA by BioTed Biological Company (Jinan, Shandong, China).

Table I. The correlation between tissues miR-154 expression and clinicopathological factors of melanoma.

Characteristics	No.	miR-154 expression		p-value
		High	Low	
Age				0.330
≤ 60	54	24	30	
> 60	50	27	23	
Sex				0.306
Male	60	32	28	
Female	44	19	25	
Tumor thickness (mm)				0.139
≤ 2.0	66	36	30	
> 2.0	38	15	23	
Ulceration				0.046
-	72	40	32	
+	32	11	21	
Lymph node metastasis				0.380
-	67	35	22	
+	37	16	21	
Tumor stage				0.028
I-II	58	34	24	
III-IV	46	18	29	

Real-Time PCR Analyses

TRIzol solution (GeneVector, Chengdu, Sichuan, China) was employed to extract the total RNAs in accordance with the manufacturer's instructions. After the RNA concentration was determined using a Thermo Scientific Nano-Drop One apparatus (JinYunBio, Changsha, Hunan, China), TaKaRa Reverse Transcription kits (GeneVector, Chengdu, Sichuan, China) were employed to transcribed total RNA (2 µg) into cDNA. Then, Real Time PCR analyses for mRNA detection were conducted using SYBR qPCR kits (HeFengBio, Suzhou, Jiangsu, China). The qPCR reaction conditions were as follows: pre-denaturation at 95°C for 30 s, 37 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 30 s. The miR-154 examination was conducted using miRNA quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) masterMix kits (KeGeno, Qingdao, Shandong, China) according to the protocols provided in the kits. The procedures of qPCR analyses for miRNAs included: 95°C for 3 min, followed by 39 cycles of 95°C for 12 s, 62°C for 60 s. The relative expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for mRNA detection or U6 for miRNA detection, and calculated according to the $2^{-\Delta\Delta Ct}$ method. Table II summarized the primers used in this work.

Western Blot Analysis

A375 cells after being treated with miR-154 mimics or inhibitors were harvested and lysed using the radioimmunoprecipitation assay (RIPA) buffer (BioFaire, Xi'an, Shanxi, China). Afterward, bicinchoninic acid (BCA) kits (SanFuBio, Hefei, Anhui, China) were used to examine the concentrations of total protein extracts. Next, 20 µg of protein extracts were separated using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After the proteins were transferred to polyvinylidene difluoride (PVDF) membranes, 5% bovine serum albumin (BSA) solution (SanFuBio, Hefei, Anhui, China) was used to block the membranes for 1-2 h. Then, anti-AURKA antibody (1:800; Huaan, Hangzhou, Zhejiang, China) or anti-GAPDH antibody (1:1500; BOSTER, Wuhan, Hubei, China) were employed to incubate the membranes. The membranes were placed at 4°C overnight. On the second day, after washing with Tris-Buffered Saline and Tween 20 (TBST) three times, the membranes were probed with matched secondary antibodies (1:5000; BOSTER, Wuhan, Hubei, China). After

Table II. Primers for qPCR assays.

Names	Sequence (5'-3')
miR-154: F	TCCACACTATTTCCCTTAGC
miR-154: R	GCCTGAGATGAAGCACTGT
AURKA: F	GAGGTCCAAAACGTGTTCTC
AURKA: R	ACAGGATGAGGTACACTGGTTG
GAPDH: F	GGGAGCCAAAAGGGTCATCA
GAPDH: R	TGATGGCATGGACTGTGGTC

incubation for 2 h, the membranes were washed using TBST buffer three times. Then, membranes were treated using enhanced chemiluminescence (ECL) assay kits (HongJieBio, Xiamen, Fujian, China) according to the protocols in the kits. Finally, the protein bands were visualized by a Gel Imager Machine (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Proliferation Analyses

A375 and CHL-1 cells were treated with indicated miRNA mimics or inhibitors. After transfection for about 10-12 h, cells were digested and collected. After seeding cells (2×10^3 /well) into 96-well plates, 10 µl (per well) CCK-8 reagent (HanYun Bio, Changsha, Hunan, China) was added into the plates. 2 h later, the absorbance of 450 nm was examined with the use of a microplate reader system.

Clonogenic Assay

A375 or CHL-1 cells after being treated with indicated miRNA mimics or inhibitors were digested and collected. After the cell number was counted, about 500 cells were placed into 6-well plates. The plates were then kept in an incubator (37°C, 5% CO₂) for the appropriate 15 days. The colonies were fixed and treated using crystal violet solution (0.1%), followed by capturing pictures using a microscope.

Apoptosis Analyses

Apoptosis analyses kits (DaXi Bio, Kunming, Yunnan, China) were used to measure the apoptosis. After the cells were treated with indicated miRNA mimics or inhibitors, they were digested and collected in 500 µl phosphate-buffered saline (PBS). Then, annexin V-FITC/PI was added into the cell suspensions, followed by incubation of the plates in the dark for 30 min. Finally, a flow cytometer was employed to assess the percentages of apoptotic cells.

Caspase-3/9 Activity Detection

Caspase-3/9 activity analyses kits (Beyotime, Nantong, Jiangsu, China) were employed to measure the activities of caspase-3/9. In short, the lysis buffer was used to lyse the cells. Subsequently, supernatants of the extracts were collected by centrifugation. After the Ac-DEVD-pNA reagent was added into the supernatants, the absorbance of 405 nm was evaluated using a microplate reader machine.

Wound Healing Assay

Cells after treatment grew to about 100% confluence. Then, a pipette tip (200 μ l) was used to scratch the cell monolayers. After washing using PBS, the cells were allowed to grow for 48 h. Then, a microscope was used to capture the wound closure pictures at the beginning of scratch (0 h) and 48 h after scratching.

Transwell Assay

2×10^5 treated cells (suspended in 250 μ l serum-free media) were planted into the upper side of the transwell chamber (pre-coated using Matrigel). Thereafter, the medium with 15% serum was placed into the lower chamber. The transwell plates were then placed in an incubator (37°C, 5% CO₂) for 24 h. After that, the cells on the lower sides of the transwell membranes were treated using crystal violet solution (0.1%), followed by capturing pictures using a microscope.

Luciferase Reporter Analyses

The predicted miR-154 binding site in the 3'-UTR of AURKA (wild-type 3'-UTR of AURKA: WT) and mutant-type AURKA 3'UTR missing the binding site of miR-154 (MUT) was separately constructed into pMIR empty luciferase reporter vectors by Haomin GeneTech Company (Wuhan, Hubei, China). Then, miR-154 mimics or control mimics were co-transfected with indicated luciferase reporter vectors (WT or MUT) into A375 or CHL-1 cells. Subsequently, luciferase reporter analyses kits (Promega, Madison, WI, USA) were employed to determine the luciferase activity.

Statistical Analysis

SPSS software (SPSS Inc., Chicago, IL, USA) was employed to conduct statistical analyses. Comparison among groups used a two-tailed Student's *t*-test or one-way ANOVA test. Tukey's Post-Hoc test was used to validate the ANOVA for pairwise comparisons. The Kaplan-Meier method (with log-rank test) was employed to assess the

overall survival. Clinical data were further analyzed using univariate and multivariate Cox regression analyses. The statistical significance was set to $p < 0.05$.

Results

The Expression of miR-154 is Down-Regulated in Melanoma Tissues and Cell Lines

To determine whether miR-154 was abnormally expressed in melanoma, we firstly analyzed ncRNAs alterations between melanoma and non-tumor tissues in GSE35579 obtained from GEO datasets. We found that 156 upregulated and 132 downregulated miRNAs in melanoma tissues using heatmap and volcano plot (Figures 1A and 1B). Of note, we observed a down-regulation of miR-154 expression in melanoma tissues compared with non-tumor tissue ($p < 0.05$, Figure 1C). To further confirm the analysis results, our group performed qRT-PCR assays to detect the levels of miR-154 in 104 paired melanoma tissues and the results showed that the levels of miR-154 were lower in melanoma samples than that in the normal tissues ($p < 0.01$, Figure 1D). Furthermore, the levels of miR-154 were further determined in the melanoma cell line or the human normal HEMa-LP cells. As shown in Figure 1E, our group observed that miR-154 was significantly down-regulated in melanoma cell lines compared with HEMa-LP cells. Overall, these data indicate that miR-154 was frequently down-regulated in melanoma and related to melanoma progression.

Low MiR-154 Expression is Associated with Poor Prognosis of Patients with Melanoma

For the exploration of the clinical significance of miR-154 expression in melanoma, we divided the 104-melanoma patients into high expression group ($n = 51$) and low expression group ($n = 53$) using as the cutoff value of the miR-154 expression in all melanoma samples. The associations between tissues miR-154 expression and clinicopathological parameters were summarized in Table II. We observed that low expressions of miR-154 were associated with ulceration ($p = 0.046$) and tumor stage ($p = 0.028$). However, no significant differences were observed between miR-154 levels and patient gender, age or tumor thickness (all $p > 0.05$). In addition, the associations between miR-154 levels and overall survival

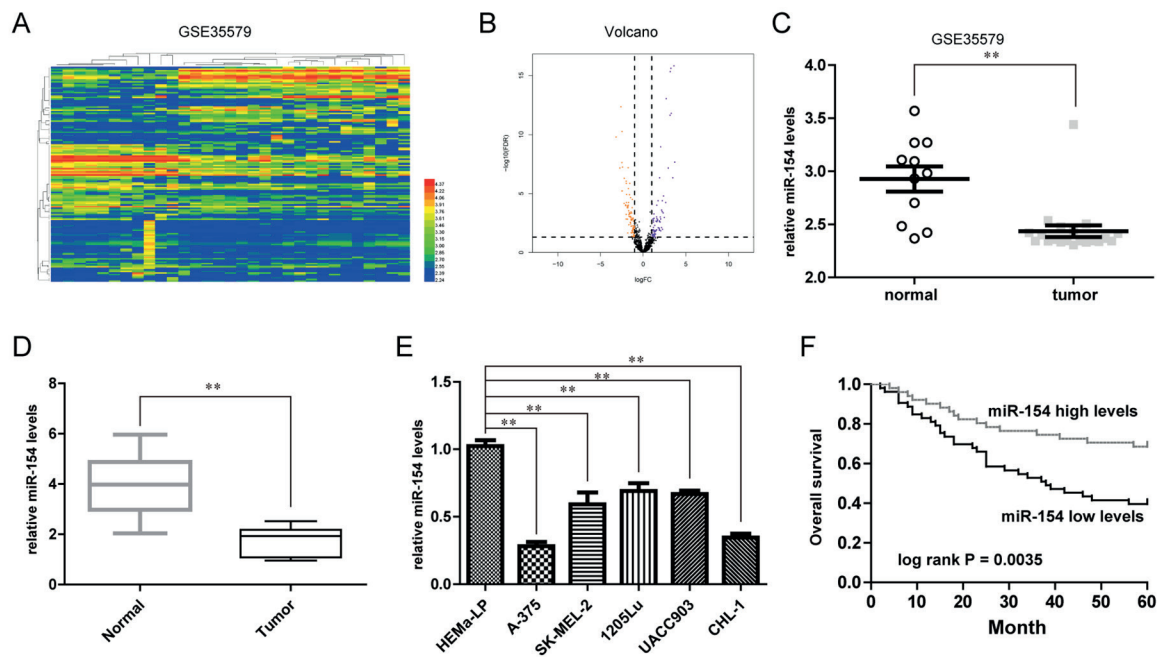


Figure 1. The expression of miR-154 and its prognostic significance in melanoma. **A**, Significantly differentially expressed miRNA in melanoma tissues reflected by heat map by analyzing GSE35579. **B**, Volcano plots show differentially expressed miRNAs in melanoma tissues. **C**, Expression of miR-154 was up-regulated in melanoma by analyzing GSE35579. **D**, MiR-154 is significantly decreased in primary human melanoma tissues in comparison to matched skin tissues. **E**, Expression level of miR-154 was compared between five melanoma cell lines and the HEMa-LP cells. **F**, Kaplan-Meier survival curves of patients with melanoma based on miR-154 expression status. * $p < 0.05$, ** $p < 0.01$.

were explored using the Kaplan-Meier analysis, as well as a log-rank test. As present in Figure 1F, the results demonstrated that patients with low miR-154 expression had a significantly shorter overall survival ($p = 0.008$). Moreover, the data from the univariate analysis revealed that tumor stage and miR-154 expression were distinctly correlated with the overall survival of melanoma patients ($p < 0.05$, Table III). Multivariate assays enrolling the parameters mentioned above further confirmed that miR-154 expression (HR=3.145,

95% CI: 1.129-4.687, $p = 0.011$) and tumor stage (HR=3.018, 95% CI: 1.185-3.859, $p = 0.021$) were independent prognostic markers for melanoma patients (Table III).

Ectopic Expression of miR-154 Suppressed Melanoma Cells Proliferation and Promoted Apoptosis

The biological functions of miR-154 in the development of melanoma were investigated. To achieve that, gain-of-function (GOF) research

Table III. Univariate and multivariate analyses of prognostic factors in melanoma patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.548	0.673-2.134	0.422	-	-	-
Sex	1.692	0.832-2.389	0.127	-	-	-
Tumor thickness	2.015	0.586-2.669	0.285	-	-	-
Ulceration	2.224	1.216-3.018	0.069	-	-	-
Lymph node metastasis	1.782	0.895-2.557	0.115	-	-	-
Tumor stage	3.562	1.374-4.281	0.013	3.018	1.185-3.859	0.021
miR-154 expression	3.472	1.482-5.216	0.004	3.145	1.129-4.687	0.011

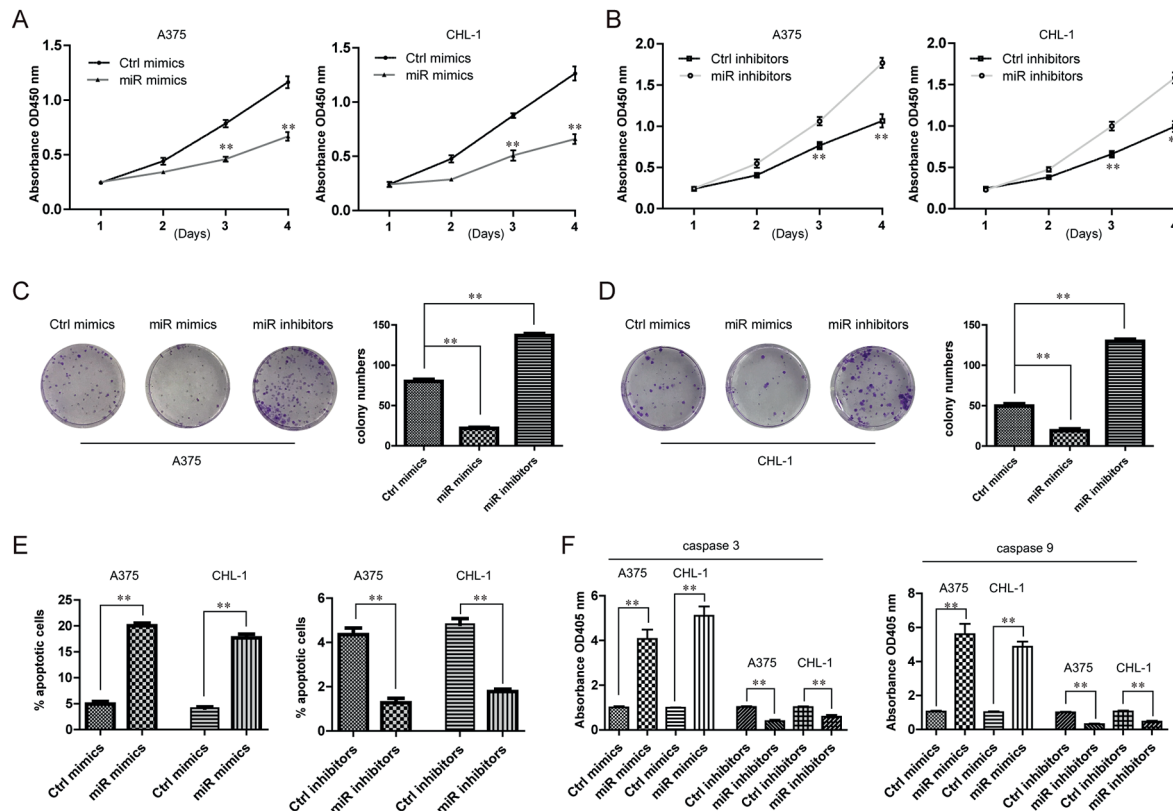


Figure 2. MiR-154 reduced cell proliferation and induced apoptosis in melanoma cells. *A-B*, Cell proliferation as measured by CCK-8 assays was inhibited by up-regulation of miR-154 and elevated by down-regulation of miR-154. *C-D*, Clonogenic assays evaluated the impact of miR-154 expressing changes on colony formation capabilities of melanoma cells. *E*, Apoptosis experiment. Overexpression of miR-154 promoted the apoptosis of melanoma cells and silence of miR-154 suppressed apoptosis. *F*, Caspase 3/9 activity analyses experiment. * $p < 0.05$, ** $p < 0.01$.

using miR-154 mimics or loss-of-function study with miR-154 inhibitors were conducted. As determined by CCK-8 assays, the proliferative rates were significantly lower in A375 and CHL-1 cells treated with miR-154 mimics, while the knock-down of miR-154 (using miR-154 inhibitors) notably elevated the cellular growth (Figures 2A and B). Consistent with the results of CCK-8 assays, clonogenic assays certified that ectopic expression of miR-154 led to a remarkable decline of cellular colony number, while miR-154 inhibitors markedly restored the colony formation capacities (Figures 2C and D). Furthermore, as measured by flow cytometry, the percentages of apoptotic cells were markedly increased after the elevation of miR-154, while the silence of miR-154 significantly reduced the apoptotic rates of A375 and CHL-1 cells (Figure 2E). Correspondingly, the activity of apoptosis relevance molecules was examined using caspase 3/9 activity detection analyses. The data demonstrated that caspase 3/9 activities were

notably elevated in A375 and CHL-1 cells treated with miR-154 mimics, while transfection of miR-154 inhibitors significantly repressed the activities of caspase 3/9 (Figure 2F). Thus, our data proved that miR-154 inhibited cell proliferation and promoted apoptosis of melanoma.

MiR-154 Inhibited the Mobility of Melanoma Cells

To determine whether the alternation of miR-154 levels was relevant to the metastatic potentials of melanoma cells, we carried out wound-healing and transwell assays. As detected by wound-healing assays, transfection of miR-154 mimics remarkably suppressed the wound closures of A375 and CHL-1 cells (Figure 3A). Conversely, repressing the expression of miR-154 notably promoted the migration abilities of melanoma cells (Figure 3B). Additionally, transwell invasion assays demonstrated that the number of melanoma cells passing via the transwell

membrane was remarkably reduced after ectopic expression of miR-154 (Figure 3C). In contrast, the inhibition of miR-154 significantly promoted the invasive capacities of A375 and CHL-1 cells (Figure 3D). Therefore, these data indicated that miR-154 played important roles in modulating the metastatic potentials of melanoma cells.

AURKA was Identified as a Functional Target of MiR-154

MiRNAs had been found to exert their functions via suppressing downstream target genes. Therefore, we next employed “starBase” to predict the possible targets of miR-154, and found that miR-154 was capable to potentially bind the 3'UTR of AURKA, a well-known oncogene (Figure 4A). Real Time PCR analyses results indicated that miR-154 overexpression depressed the AURKA levels, while miR-154 silencing significantly promoted the expressing levels of AURKA in A375 and CHL-1 cells (Figure 4B). Data of Western blot analyses also confirmed similar results (Figure 4C). Moreover, bioinformatics analyses using the Cancer Genome Atlas (TCGA) datasets indicated that melanoma tissues expressed markedly higher AURKA (aurora kinase A), than adjacent normal specimens (Figure 4D). Luciferase activity analyses revealed that miR-154 mimics markedly reduced the luciferase activity of cells transfected with wild-type 3'UTR of AURKA

(WT) reporter plasmids (Figure 4E). Besides, CCK-8 assays proved that enhancing AURKA expression remarkably reversed the proliferation of melanoma cells, which was impeded by miR-154 mimics (Figure 4F). Moreover, AURKA could also abrogate the depressing impact of miR-154 on melanoma cell migration (Figure 4G). In summary, the data demonstrated that AURKA was a target of miR-154 and miR-154 was capable to modulate the malignant phenotypes of melanoma.

Discussion

Up to date, the increasing incidence of melanoma has been observed in the USA with approximately 71,000 patients diagnosed and around 10,000 died from this malignancy²⁰. The poor prognosis of melanoma patients with metastasis encouraged scientists to explore the nosogenesis of this tumor and further develop novel therapeutic methods⁸. In recent years, an increasing number of studies reported^{21,22} the critical effects of miRNAs in the modulation of the cellular process by targeting several important biological proteins. In addition, the possible roles of miRNAs as a tumor suppressor or oncogenes also attracted increasing attention^{23,24}. With the development of measuring method for ncRNAs and bioinformatics, the use of miRNAs for the diagnosis and

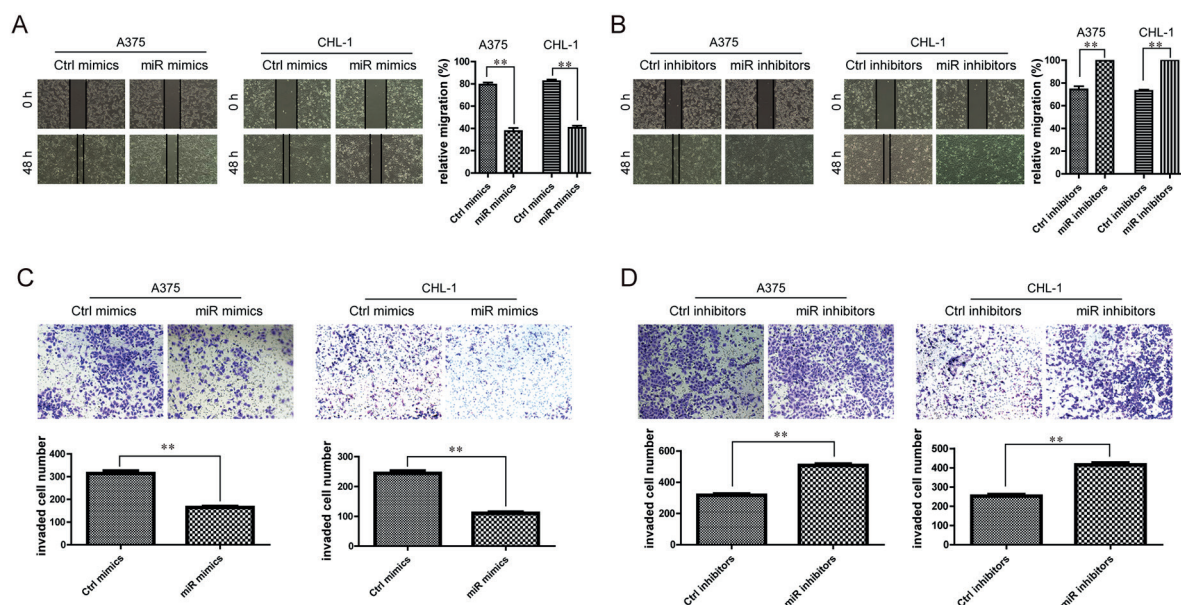


Figure 3. MiR-154 suppressed cellular mobility in melanoma cells. **A-B**, Wound-healing assays detected the migration of melanoma cells. **C-D**, Transwell assays assessed the invasion ability of A375 and CHL-1 cells. * $p < 0.05$, ** $p < 0.01$.

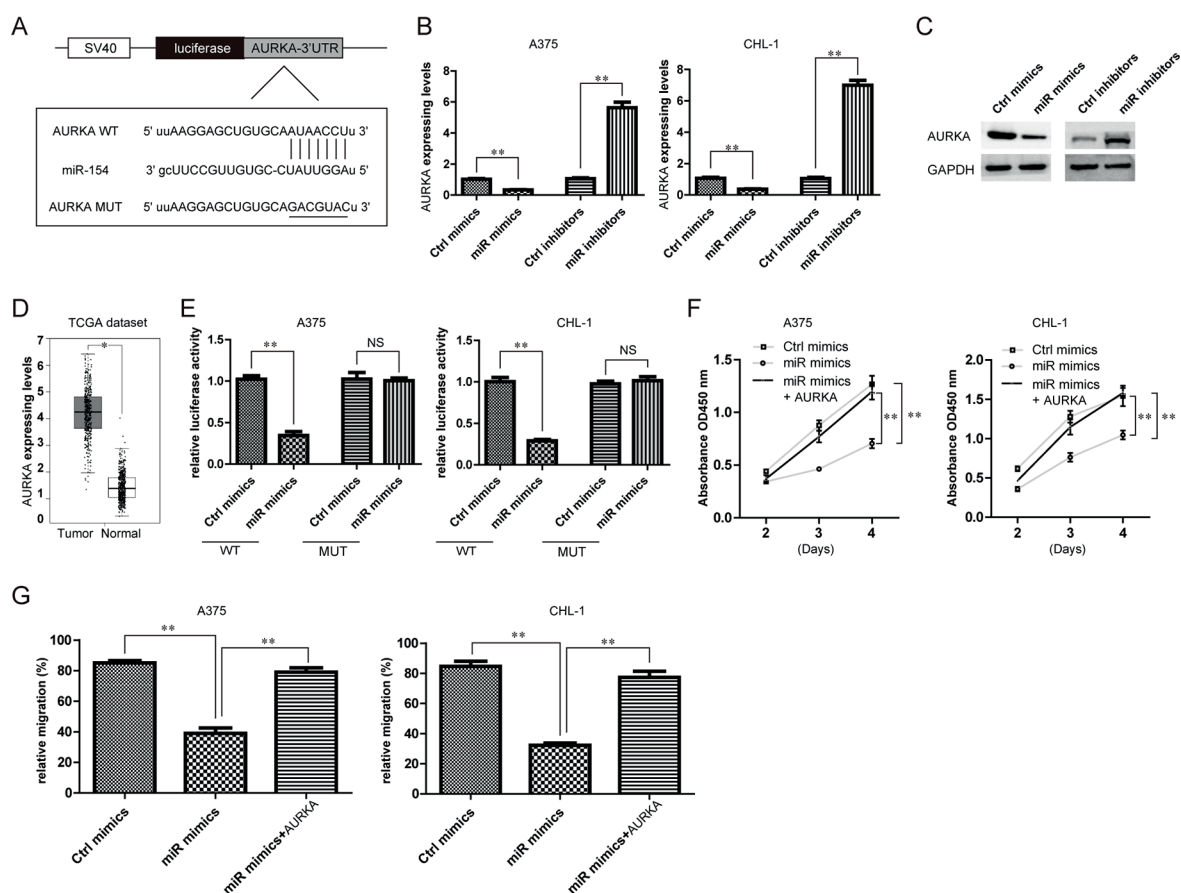


Figure 4. MiR-154 directly targeted AURKA. **A**, Schematic representation of the predicted target site for miR-154 in the 3'UTR of AURKA. **B**, Real Time PCR analyses detected the AURKA levels in A375 and CHL-1 cells. **C**, Western blot analyses examined the protein expression of AURKA in A375 cells. **D**, "GEPIA" analyzed the TCGA dataset and showed the relative expressing levels of AURKA in melanoma tissues. **E**, Luciferase reporter analyses. **F**, CCK-8 assays measured the proliferation in A375 and CHL-1 cells. **G**, Relative migration of melanoma cells was evaluated by wound-healing assays. * $p < 0.05$, ** $p < 0.01$.

prognosis of melanoma patients has raised great expectations.

In this work, we identified a melanoma-related miRNA, miRNA-154 whose down-regulation has been reported in several cancers. Here, our group also observed that miR-154 expression was distinctly reduced in melanoma by performing microarray analysis from GEO datasets and RT-PCR. Then, the observation from the clinical investigation showed that higher levels of miR-154 in melanoma was associated with ulceration and advanced tumor stage, suggesting that miR-154 may be an indicator for the clinical outcome of melanoma patients, which was observed by the results of the Kaplan-Meier assays. Moreover, the multivariate analysis showed that miR-154 played a significant role of independent prognostic markers in overall survival rates. Interestingly,

the prognostic value of miR-154 was also found in several other tumors, such as. Thus, our finding, together with previous findings, revealed that miR-154 may be a typical prognostic indicator for common tumors.

Previous studies in recent years have detected miR-154 expression was abnormal in a series of tumors. In laryngeal squamous cell carcinoma, miR-154 was found to be down-regulated and its overexpression suppressed tumor growth via targeting GALNT7¹⁹. In glioblastoma, miRNA-154 was observed to be lowly expressed and its up-regulation suppressed tumor cells proliferation and metastasis by modulating PIWIL1¹⁸. Previous findings indicated that miR-154 acted as an oncogene in the above tumors. However, Lin et al²⁵ firstly suggested that miR-154 expression was reduced in renal cell carcinoma and asso-

ciated with poor prognosis of patients. Besides, the oncogenic roles of miR-154 were also proved. These findings suggested that the effects of miR-154 possessed differentiation according to the types of tumors. In this research, using miR-154 mimics, we created miR-154-overexpressed A375 and CHL-1 cell lines, which was found using RT-PCR. Subsequently, a series of functional investigations were performed *in vitro*. As expected, the overexpression of miR-154 suppressed melanoma cells proliferation and promoted apoptosis. In addition, the forced miR-154 expression also negatively regulated melanoma cells' migration/invasion. Thus, our results revealed miR-154 as a novel therapeutic target against metastasis of melanoma. AURKA located in 20q13.2, is a cell cycle-regulated kinase²⁶. In recent years, the overexpression of AURKA was frequently reported in various tumors, such as bladder cancer, ovarian cancer, and esophageal cancers²⁷⁻²⁹. Functional research suggested that AURKA acted as a positive regulator in the progression of tumors via promoting cell proliferation, metastasis, and cancer stem cell behaviors³⁰. To explore the potential mechanism by which miR-154 suppressed melanoma behaviors, predicted target genes of miR-154 were analyzed using online databases and AURKA may be one of the targets. Then, a luciferase report assays showed that miR-154 directly downregulates AURKA by binding its 3'-UTR. The negative modulation of AURKA by miR-154 was also supported using RT-PCR. In addition, further functional assays revealed that the overexpression of AURKA rescued the inhibitory effects of miR-154 in the ability of growth and metastasis of melanoma cells. Overall, our findings suggested that miR-154 repressed melanoma growth and metastasis through targeting AURKA. However, the downstream signaling pathways of AURKA in melanoma require further investigation.

Conclusions

We demonstrated that miR-154 is decreased in melanoma and its down-regulation is correlated with poor outcome of melanoma patients. Functionally, miR-154 regulates cells growth and metastasis by targeting AURKA. Our findings showed a novel possibility for the improvement of the clinical outcome of melanoma patients with the therapeutic effects of miR-154 suppression by suppressing AURKA levels.

Conflict of interest

The authors declare no conflicts of interest.

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