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# MiR-155 inhibits proliferation, invasion and migration of melanoma *via* targeting CBL

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**Abstract. – OBJECTIVE: Malignant melanoma (MM), the deadliest form of skin malignancy, is a highly aggressive and malignant tumor with an increasing incidence rate in recent years. Increasing evidence suggested that dysfunctions of microRNAs (miRNAs) may play an important role in human tumors. However, the effect of miR-155 on malignant melanoma cell migration and invasion remains largely elusive. This research was designed to evaluate the potential function of miR-155 and CBL (Casitas B-lineage lymphoma) in malignant melanoma.**

**PATIENTS AND METHODS: Quantitative Real-time polymerase chain reaction (qRT-PCR) was utilized to detect miR-155 and CBL expression in malignant melanoma tissues and cell lines. 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay was performed to examine the regulation of miR-155 in melanoma proliferation. Transwell assay was carried out to detect the effect of miR-155 on the MM cell migration and invasion. Luciferase assay and biological analysis were used to predict and determine the target gene of miR-155.**

**RESULTS: miR-155 was down-regulated in malignant melanoma tissues and cell lines. Ectopic expression of miR-155 could inhibit migration and invasion in malignant melanoma cells. What's more, we found that CBL was a new target of miR-155. Additionally, CBL was negatively associated with miR-155 in malignant melanoma and overexpression of CBL attenuated miR-155-mediated inhibition on MM cell migration and invasion.**

**CONCLUSIONS: miR-155 inhibited malignant melanoma proliferation, migration and invasion. And high CBL expression was observed in MM tissues. This newly identified miR-155/CBL axis may provide new insight into the pathogenesis of malignant melanoma.**

*Key Words:*
MiR-155, CBL, Proliferation, Migration, Invasion, Malignant melanoma.

## Introduction

Malignant melanoma (MM), as the deadliest form of skin malignancy, is the seventh most common cancer in females and the fifth most common malignancy in males worldwide[1]. Malignant melanoma developing through the malignant transformation of melanocytes has shown obviously clinicopathological characteristics, such as aggressive invasion, poor prognosis, and resistance to chemotherapy or radiotherapy[2,3]. Despite remarkable advances in chemotherapy and immunotherapy have been achieved for the treatment of tumors[4], the availably therapeutic options for malignant melanoma are limited, and the prognosis of metastatic melanoma remains still unsatisfied[5]. The underlying causes of the unsatisfactory outcome are that there are insufficient basic knowledge about the biology mechanisms, which correlated with malignant melanoma carcinogenesis and progression. Therefore, it is urgently needed to identify molecular mechanisms underlying tumorigenesis and progression of malignant melanoma to offer effective therapeutic strategies for malignant melanoma. Emerged as a class of highly conserved and small non-coding RNAs (ranging in approximately 18-25 nucleotides), microRNAs (miRNAs) can negatively modulate the transcription and translation of genes through interacting with specific sequences in the 3'-untranslated region (3'-UTR)[6]. MiRNAs seem to play crucial role in various regulatory pathways such as differentiation, proliferation, apoptosis, and fat storage[7], many of these biological processes are altered in tumors. Accumulated evidence has revealed that aberrant expression of miRNAs have been associated with the development or metastasis of various types of tumors[8], including human malignant melanoma[9]. The host gene of miR-155 is B cell

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integration cluster, which is located in chromosome 21q21. The miRNAs function as oncogene or tumor suppressors in human cancers through targeting different genes[10], studies have suggested that miR-155 could act as all of the suppressor and promoter, and it can be considered quite contradictory[11-14]. Previous studies have indicated that miR-155 was downregulated in various types of cancers, such as human melanoma[14], hepatocellular carcinoma[13], and familial adenomatous polyposis[15]. And on the contrary, miR-155 was up-regulated in other tumors, for example acute pancreatitis[16], human colonic cancer[17]. Furthermore, miR-155 has been reported to be associated with the proliferation, invasion, apoptosis and cell cycle of tumor cells *in vivo* and *in vitro*[11]. However, the effect of miR-155 in mediating malignant melanoma cells migration and invasion remains largely elusive. Multiple studies[18,19] have shown that Receptor tyrosine kinases (RTKs) are associated with the cell cycle, proliferation, and differentiation of tumors cell. Therefore, targeting RTKs may be a new strategy for inhibiting tumor development. Casitas Blineage lymphoma (CBL) is an E3 ubiquitin ligase, which mediates the ubiquitinated degradation of activated RTKs, resulting in a halt in RTKmediated signaling[20,21]. Moreover, CBL is associated with the proliferation, invasion, migration and prognosis and is linked to the progression of tumors[22-25]. However, its underlying molecular mechanisms and the biological functions for its oncogenic roles in malignant melanoma remain unknown. We indicated that miR-155 was frequently down-regulated in malignant melanoma tissues and cell lines. MiR-155 ectopic expression could inhibit malignant melanoma cell proliferation, migration and invasion. CBL is a novel target gene of miR155 in malignant melanoma cells. Additionally, miR155 protein level was increased in malignant melanoma tissues, and CBL was negatively associated with miR-155 in malignant melanoma tissues. Moreover, over-expression of CBL ablated the inhibitory effects of miR-155. Therefore, our results illustrated that miR-155 functioned as oncogene in malignant melanoma and miR-155/CBL may be the novel therapeutic strategies in malignant melanoma.

## Patients and Methods

### Clinical Specimens

Sixty pairs of malignant melanoma tissues and the adjacent normal tissues were collected at People's Hospital of Rizhao (Rizhao, China), from January 2015 to March 2017. Neither radiotherapy nor chemotherapy was used in any patients before surgery. The Ethic Committee of People's Hospital of Rizhao approved this study. All tissues obtained from biopsy or surgery were frozen at -80°C.

### Cell Culture and Transfection

The malignant melanoma cell lines (A375, SK-MEL-28 and A2058) and Human epidermal melanocytes (HEMs) were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The Roswell Park Memorial Institute-1640 (RPMI-1640) (Gibco, Rockville, MD, USA), which contained 15% of fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 1% of penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) was used for culturing the cells under the condition of 37°C with 5% $CO_2$. MiR-155 mimics (5'-UUA AUG CUA AUC GUG AUA GGG GU-3') and inhibitor (5'-AAU UAC GAU UAG CAC UAU CCC CA-3') or their corresponding negative controls (NC) were purchased from RiBoBio (Guangzhou, China). After the cells adhered, the transfection of the miR-155 mimic/inhibitor/negative control (NC) were performed with Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA), which could achieve the ectopic expression of miRNA.

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

Total RNA was extracted from malignant melanoma culture cells or tissues by using the TRIzol reagents (Invitrogen, Carlsbad, CA, USA). The RNA was reverse-transcribed and the levels of miR-155 were detected by qRT-PCR (SYBR Green method) with a miR-155 detection kit (Biomics Biotech; catalog number, BK3100). The CBL level was normalized relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and miR-155 level was normalized relative to U6 endogenous control by using the $2^{-\Delta\Delta CT}$ method. The primers were used as followed: miR-155 forward, 5′-ACACTCCAGCTGTAAACATCCTACACTCT-3′, reverse, 5′-CTCAACTGGTGTCGTGGA-3′; U6 forward, 5′-CTCGCTTCGGCAGCACA-3′, and reverse, 5′-AACGCTTCACGAATTTGCGT-3′. CBL forward, 5'GGACCAGTGAGTTGGGAGTTATTACT3' and reverse, CBL, 5'GGCAAGACTTCACTGTGAAGTCA3'; GAPDH forward, 5'AAGGTCGGA GTCACCGGATT3', and reverse, 5'GCCATCACGCCACAGTTTC3'.

### Cell Proliferation Assay In Vitro

Cell proliferative activity was determined using the 3-(4, 5-dimethyl-2-thazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) method (Beyotime Shanghai, China). A375 and SK-MEL-28 cells, transiently transfected with the miR-155 mimic, miR-155 inhibitor, negative control for 24 h, were seeded in 96-well plates at a density of $5\times10^3$ cells per well in 100 μL of growth medium and cultured for another 0 h, 24 h, 48 h, 72 h, 96 h, respectively. Subsequently, 10 μL of MTT solution (5 mg/mL) was added to each well. After 4 h incubation at 37°C, the cells were disrupted in 200 μL of dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA), and the absorbance was measured at 490 nm on a microplate reader (BioTek, Winooski, VT, USA).

### Transwell Assays

A transwell chamber (8 μm, 6.5 mm, Millipore, Billerica, MA, USA) was used to examine cell migration and invasion coating without (migration) or with (invasion) Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). A375 and SK-MEL-28 were seeded into 6-well plates with different transfection treatments. Cell transwell assays was conducted using chambers (Costar, Coppell, TX, USA) with or without 2 mg/mL Matrigel (Clontech, Laboratories, Mountain, View, CA, USA). Next, cells placed into the upper chambers, and lower chambers were filled with medium which containing 10% fetal bovine serum (FBS) as a chemo attractant. Cells were stained by Giemsa (JRDUN), and then used 1×phosphate-buffered saline (PBS) washing three times. Finally, stained cells were counted using the microscope.

### Western Blot

Cultured cells were collected and lysed with radioimmunoprecipitation assay (RIPA) lysis Buffer (Beyotime, Shanghai, China). The concentration of protein was detected by using Pierce® bicinchoninic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Protein samples (50 μg) were then transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking with 5% skimmed milk for 1 h at 37°C, the membranes were incubated in primary antibodies against CBL (1:1,000 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; Catalog No., sc-170,), β-actin (1:2,000 dilution; Santa Cruz Biotechnology, Inc.; Dallas, TX, USA; Catalog No., 4970), E-cadherin (1:1000 dilution; Santa Cruz Biotechnology, Inc.; Dallas, TX, USA; Catalog No., sc-71007), N-cadherin (1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA Catalog No., sc-59987) at 4°C overnight. After washing with Tris-buffered saline containing Tween-20 (TBST), the membranes were incubated with horseradish peroxidase (HRP)-labeled secondary antibodies (1:5,000 dilution; Beyotime Institute of Biotechnology; Shanghai, China, Catalog No.: A0208) at room temperature for 60 min.

### Luciferase Reporter

The bioinformatics analysis software TargerScan and miRanda were chosen for predicting the target gene of miR-155. We amplified the binding sites of miR-155 on 3'-UTR sequence of CBL by PCR. The binding region of CBL was inserted into the pMIR-REPORT luciferase vector named pMIR-CBL-wild type. Mutated the binding site of CBL in 3'-UTR and cloned into the plasmid, named pMIR-CBL-mutant. MiR-155 mimics and luciferase plasmid pMIR-CBL (wild-type/mutant, WT/MT) were transfected into A375 cells by using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After cultivated 48 h, the cells were harvested and lysed. Next, we used Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) for measuring the reporter activities.

### Statistical Analysis

Independent experiments were performed at least three times. Statistical results were analyzed by Statistical Product and Service Solutions (SPSS) 16.0 statistics software package (SPSS Inc., Chicago, IL, USA). The results are presented as the mean ± SD (Standard Deviation). The differences were analyzed by the Student's *t*-test. Correlation between mRNA and miRNA was estimated using the Spearman's correlation method. It was considered to be statistically significant when $p$-value of $<0.05$.

## Results

### MiR-155 Expression is Down-Regulated in Malignant Melanoma and Inversely Correlates with CBL Expression

To investigate whether the miR-155 expression was altered in malignant melanoma tissues and paracancerous tissues, qRT-PCR was performed in 60 pairs of malignant melanoma tissues. The results suggested that the miR-155 level was observably lower in malignant melanoma tissues *vs.*

paracancerous tissues (Figure 1A). Furthermore, the expression of CBL in the malignant melanoma tissues and the adjacent normal tissues was also assessed by qRT-PCR. The CBL relative level was increased in the malignant melanoma tissues compared to paracancerous tissues as shown in Figure 1B. And then we used statistical analysis to further investigate the relationship between CBL and miR-155 expression in malignant melanoma tissues. Interestingly, statistical analysis showed that miR-155 was negatively associated with CBL mRNA in malignant melanoma tissues (Figure 1C). We then measured miR-155 and CBL expression in malignant melanoma cell lines (A375, SK-MEL-28 and A2058) compared to the human epidermal melanocytes (HEMs). The results showed that the miR-155 expression in A375, SK-MEL-28 and A2058 was significantly reduced relative to that in the HEMs (Figure 1D). The expression of CBL was up-regulated in these three malignant melanoma cell lines (A375, SK-MEL-28 and A2058) compared to the human epidermal melanocytes (HEMs) (Figure 1E).

### Characteristics of Malignant Melanoma Patients

Additionally, the relationship between miR-155 expression and clinicopathological characteristics in those malignant melanoma patients was analyzed. To investigate the potential associations between miR-155 expression and patients' clinicopathological variables, we divided the patients with melanoma into two groups based on mean value (0.763) of miR-155 expression: high expression group (>0.763, n=22), and low expression group (<0.763, n=38). Those results indicated that miR-155 was related to the progression of melanoma. As the results shown in Table I, the lower miR-155 expression level correlated with tumor thickness, TNM stage and lymph node

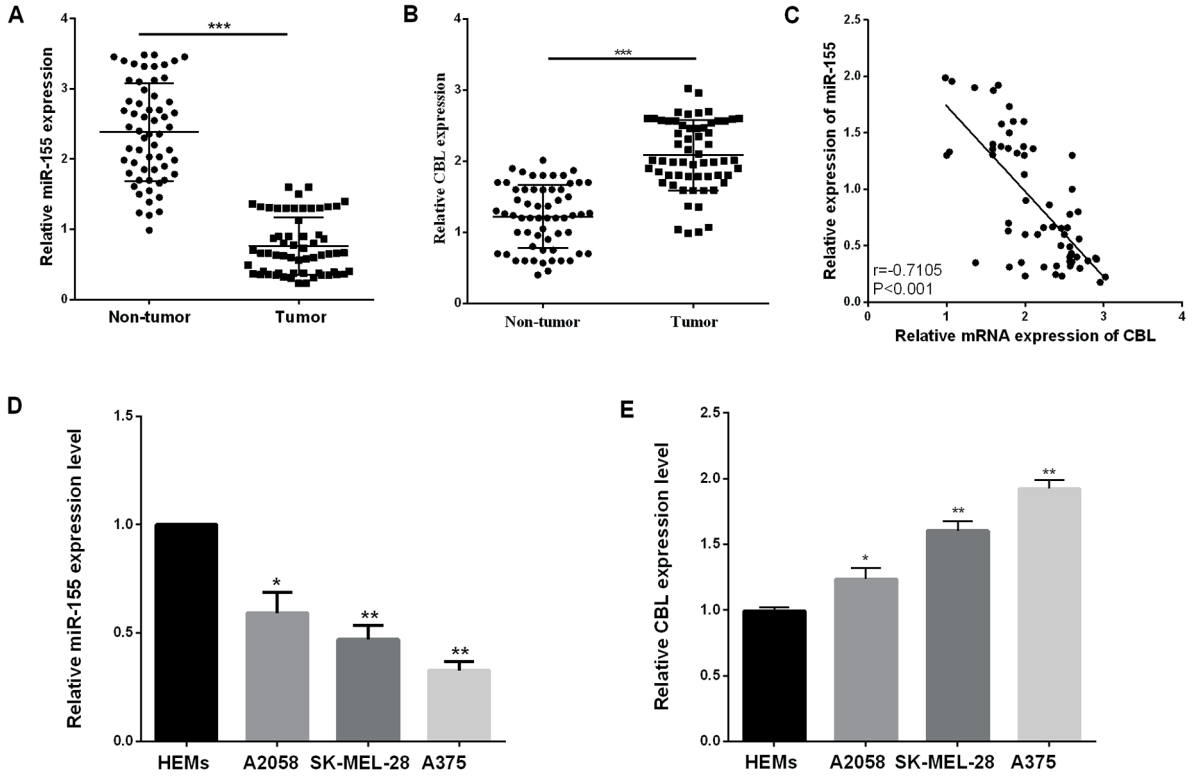


**Figure 1.** MiR-155 was down-regulated in malignant melanoma and inversely correlates with CBL expression**. A,** miR-155 expression was observed in 60 pairs of tissues samples. The miR-155 expression was detected by qRT-PCR using U6 as the internal control. **B,** Relative mRNA expression of CBL was observed in malignant melanoma tissues. **C,** Spearman correlation analysis of miR-155 and CBL level was obtained in malignant melanoma tissues ($\gamma = -0.7105$, $p<0.001$). **D,** miR-155 levels was identified in malignant melanoma cell lines (A375, SK-MEL-28 and A2058) compared to the human epidermal melanocytes (HEMs). **E,** CBL expression was examined in malignant melanoma cell lines (A375, SK-MEL-28 and A2058) compared to the human epidermal melanocytes (HEMs). $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$.

**Table I.** Relationship between miR-155 expression and their clinic-pathological characteristics of melanoma patients.

| Characteristics | Cases | miR-155 | | *p*-value |
|---|---|---|---|---|
| | | High (n=22) | Low (n=38) | |
| Age (years) | | | | 0.592 |
| ≥ 60 | 30 | 10 | 20 | |
| <60 | 30 | 12 | 18 | |
| Gender | | | | 0.319 |
| Male | 35 | 11 | 24 | |
| Female | 25 | 11 | 14 | |
| Tumor thickness | | | | 0.016* |
| < 1 cm | 26 | 14 | 12 | |
| ≥ 1 cm | 34 | 8 | 26 | |
| TNM stage | | | | 0.038* |
| I-II | 24 | 5 | 19 | |
| III-IV | 36 | 17 | 19 | |
| Lymph node metastasis | | | | 0.011* |
| No | 20 | 4 | 16 | |
| Yes | 40 | 18 | 22 | |

Statistical analyses were performed by the $\chi^2$ test. *$p$<0.05 was considered significant.

metastasis, whereas no statistical difference was found in the correlation of miR-155 expression with age and gender.

### MiR-155 Inhibits Malignant Melanoma Cell Proliferation, Migration and Invasion

We established cells transfected with miR-155 mimic/inhibitor to investigate the effect of miR-155 on the progression of malignant melanoma in both A375 and SK-MEL-28 cells. MTT and transwell chambers were conducted to test the malignant melanoma cells proliferation, migration and invasion ability with different transfection. The results demonstrated that over-expression of miR-155 inhibited malignant melanoma cell proliferation, and knockdown of miR-155 dramatically promoted proliferative abilities as detected by MTT assay (Figure 2A, 2B). We quantitated the expression levels of the EMT markers by Western blot analysis in A375 cells dysregulation miR-155. Compared with the mimic-NC group, the protein levels of N-cadherin decreased significantly in miR-155-overexpressing cells. Inversely, the expression level of E-cadherin increased. Additionally, compared with the inhibitor-NC group, the protein levels of N-cadherin increased significantly in miR-155 down-regulated cells. Inversely, the expression level of E-cadherin reduced (Figure 2C). Over-expression of miR-155 through transfected miR-155 mimics significantly suppressed A375 and SK-MEL-28 cell migration, whereas inhibiting miR-155 expression promoted A375 and SK-MEL-28 cell migration (Figure 2D). In the cell invasion assay, ectopic expression of miR-155 dramatically decreased A375 and SK-MEL-28 cell invasion, whereas inhibition of miR-155 expression significantly enhanced malignant melanoma cell invasion (Figure 2E). These results suggested miR-155 could inhibit migratory and invasive ability of malignant melanoma cells.

### CBL is a Novel Target Gene of miR155 in A375 Cells

Bioinformatics analysis software TargetScan and miRanda were chosen for predicting the miR-155 targets. We found one target gene for miR-155 was CBL, one putative binding site was found at 7711-7718 and this is highly conserved across species (Figure 3A). The luciferase assay was applied to confirm this predication. To further observe whether the 3'-UTR of CBL can be directly targeted by miR-155, WT pMIR-UTR or MT pMIR-UTR of CBL 3'-UTR vector was co-transfected into A375 cells with either miR-155 mimic/inhibitor or NC, then following by the measurement of luciferase reporter assays. Decreased expression of CBL was observed after transfecting with miR-155 mimic. However, miR-155 inhibitor can enhance CBL expression (Figure 3B). Furthermore, the CBL mRNA and protein expression were also increased by miR-155 inhibitor,

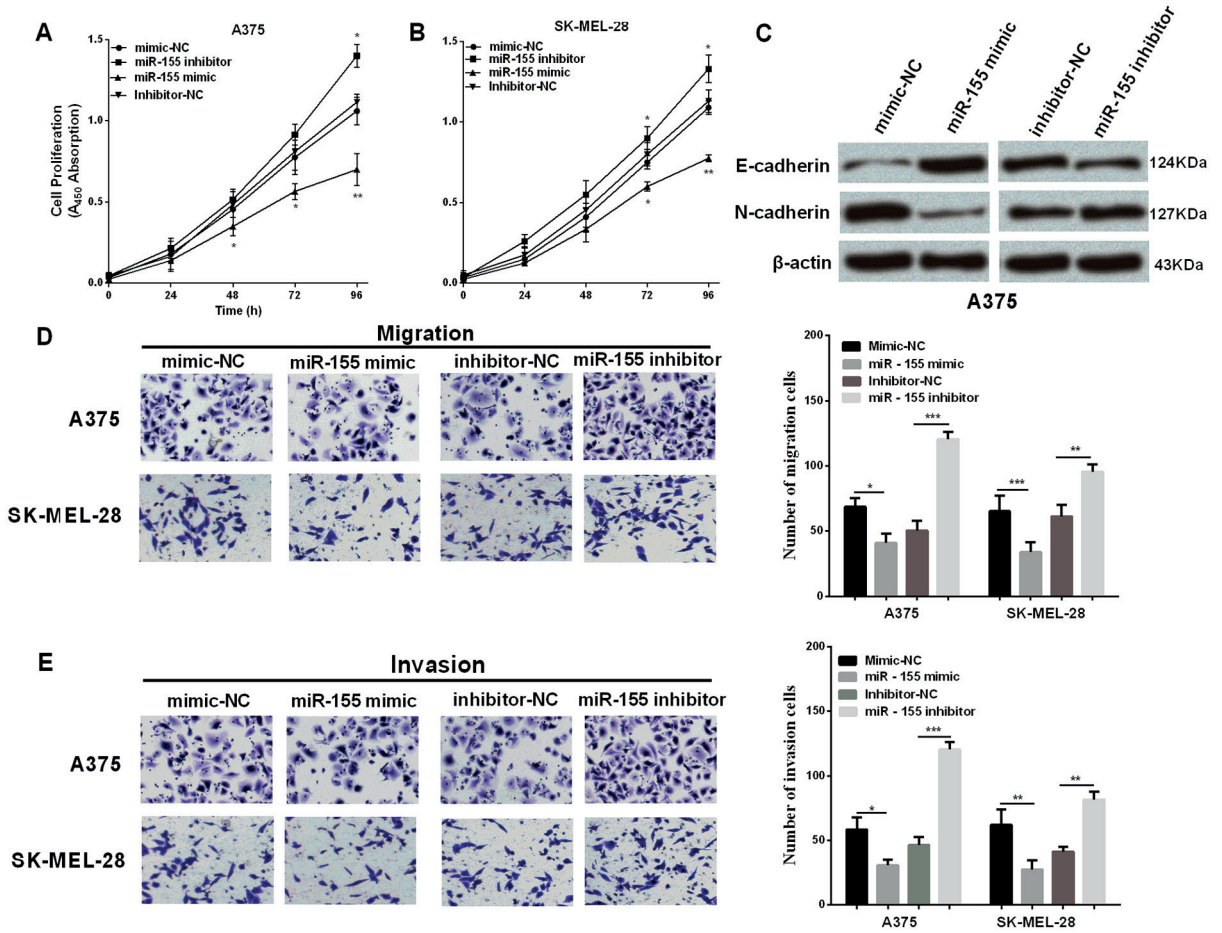


**Figure 2.** MiR-155 inhibited malignant melanoma proliferation, invasion and migration. **A-B,** MTT assay results showed that miR-155 suppressed viability of two cells lines after transfection of miR-155 mimic/inhibitor/NC. **C,** miR-155 leads to EMT associated markers dysregulation in A375 cells. E-cadherin and N-cadherin expression was analyzed by Western blotting in A375 cells transfected with miR-155 mimic/inhibitor/NC. **D,** Ectopic miR-155 expression significantly suppressed A375 and SK-MEL-28 cells migration, whereas low miR-155 expression promoted melanoma cells migration (magnification: 40×). **E,** miR-155 over-expression dramatically inhibited A375 and SK-MEL-28 cell invasion, whereas inhibition of miR-155 expression significantly promoted cell invasion (magnification: 40×). ***$p$<0.001, **$p$<0.01.

and miR-155 mimic played an opposing role on CBL expression (Figure 3C-3D).

### *Over-Expression of CBL Ablated the Inhibitory Effects of miR-155*

Evidence have indicated that CBL was the direct target of miR-155 in the A375 cell, CBL might take part in miR-155-mediated inhibition of malignant melanoma cells migration and invasion. To detect whether over-expression of CBL would simulate miR-155-mediated effect, CBL vector was transfected into A375 cells to enhance its expression. The results of qRT-PCR and Western blot assays suggested that both the CBL mRNA levels and protein level were reduced by miR-155 mimic, and the levels were restored after con-transfected CBL vector and miR-155 mimic (Figure 4A-B). And then we calculated the functions of CBL over-expressing on the inhibition of miR-155 in A375 cell migration and invasion. Over-expressing of CBL reversed the inhibitory effects of miR-155 on malignant melanoma cell migration (Figure 4C) as well as invasion (Figure 4D).

## Discussion

Changes of miRNAs expression frequently occur in cancer cells and are usually associated with tumorigenesis and the development of cancer[26,27]. Due to the important roles of miRNAs in the progression and development of tumors, more

and more researchers pay attention to miRNAs[6]. Accumulating evidence suggested that miR-155 could function as tumor suppressor or oncogene in multiple cancers. For example, Li et al[28] reported that miR-155 regulates lymphoma cell proliferation and apoptosis through targeting SOCS3/JAK-STAT3 signaling pathway. Wei et al[29] indicated that miR-155 affects renal carcinoma cell proliferation, invasion and apoptosis through regulating GSK-3beta/beta-catenin signaling pathway. On the contrary, miR-155 was down-regulated in human cardiomyocyte progenitor cells and it affected renal carcinoma cell proliferation, invasion and apoptosis through regulating GSK-3beta/beta-catenin signaling pathway[30]. However, the biological function of miR-155 in malignant melanoma proliferation, migration and invasion remains unclear. We firstly measured miR-155 expression in 60 pairs of malignant melanoma tissues and in malignant melanoma cell lines (A375, SK-MEL-28 and A2058). We found that miR-155 expression was down-regulated in malignant melanoma tissues and cell lines compared with the paracancerous tissues and the human epidermal melanocytes (HEMs). Recently, large numbers of researches indicated miRNAs play critical roles in various cancers and participated in many biological processes. Authors[29,31] reported miR-155 was took part in development and metastasis in tumors. This research firstly demonstrated the synthesized analysis of miR-155 effects on malignant melanoma. MiR-155 was dramatically down-regulated in malignant melanoma cell lines and tissues. Further studies identified that miR-155 inhibited malignant melanoma cells proliferation, migration and invasion. These results together demonstrated that miR-155 could be a powerful anti-malignant melanoma candidate. To investigate the peculiar regulatory mechanism of miR-155 on the development of melanoma, we retrieved two important databases, TargetScan 7.0 and miRanda, and found CBL may be an important downstream targeting gene, and we confirmed that the CBL

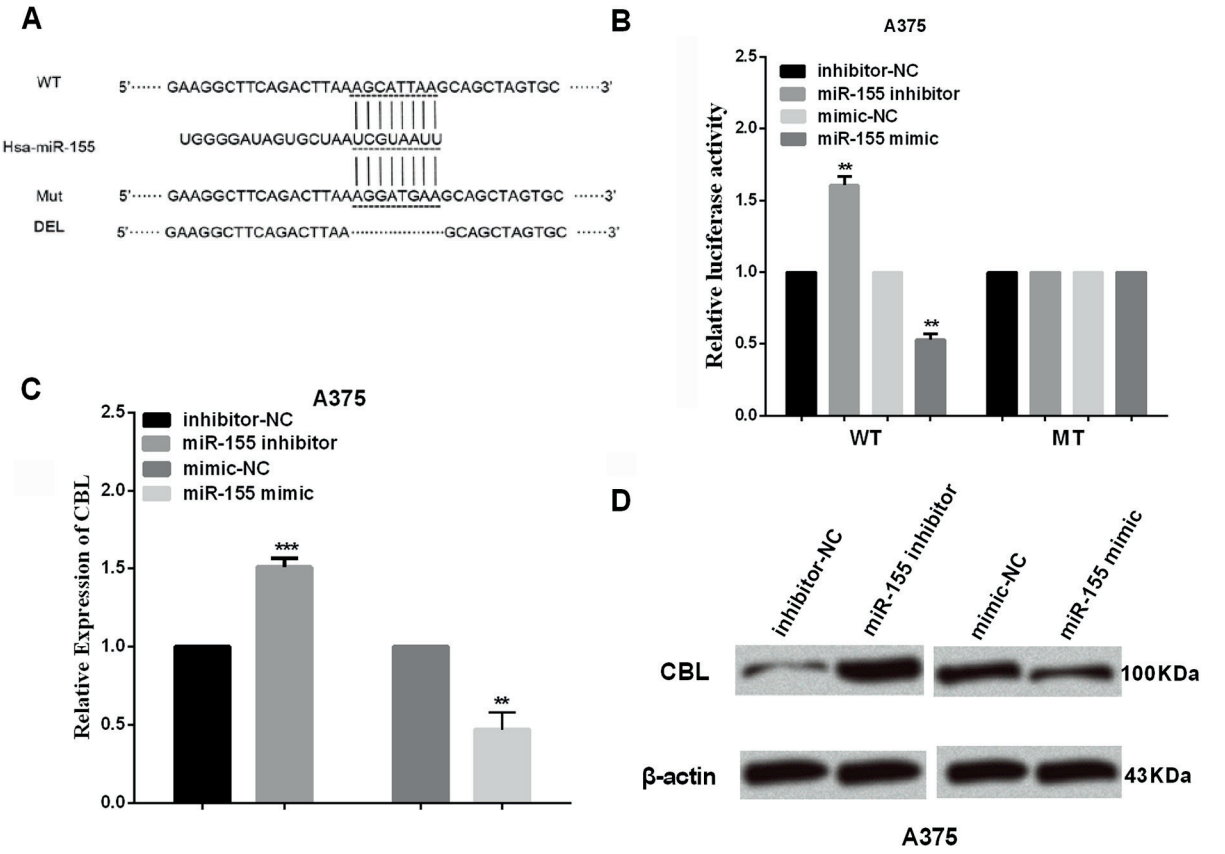


***Figure 3.*** CBL is the direct target of miR-155. **A,** MiR-155 has the binding sites of CBL. **B,** Luciferase reporter assay with the pMIR-CBL-3′-UTR-WT or pMIR-CBL-3′-UTR-MT were co-transfected with miR-155 mimic/inhibitor/NC into A375 cells. **C-D,** CBL expression level in A375 cells transfected with miR-155 mimic/inhibitor/NC was examined by qRT-PCR and Western blotting. ***$p<0.001$, **$p<0.01$.

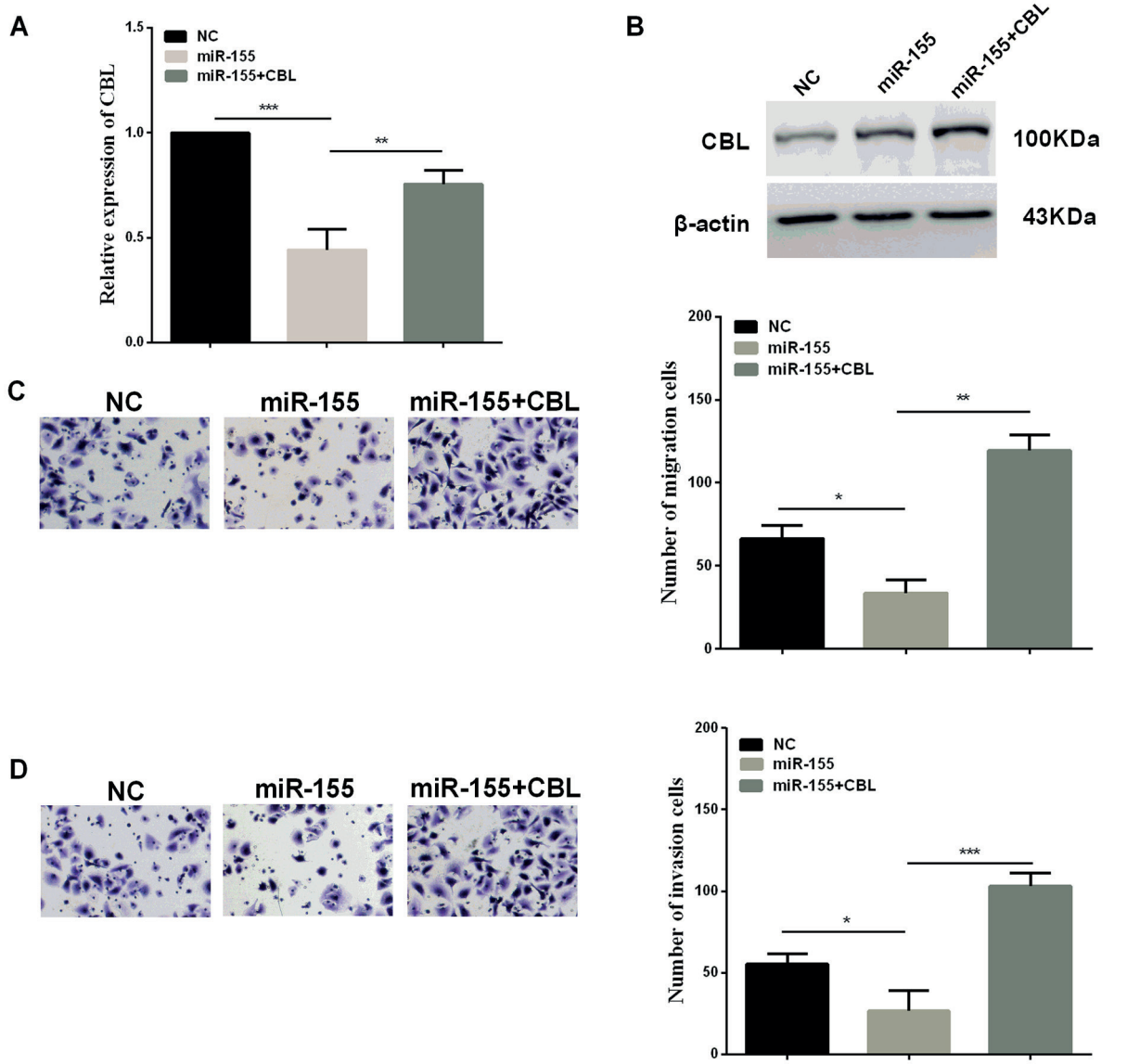


**Figure 4.** Over-expression of CBL ablated the miR-155 inhibitory effects. **A-B,** CBL expression was identified in A375 cells. **C-D,** Transwell assay was performed in A375 cells after transfecting with miR-155 mimic or CBL vector (magnification: 40×). ***$p$<0.001, **$p$<0.01, *$p$<0.05.

protein level was significantly inversely correlated with the miR-155 level in melanoma tissues. In this study, CBL was identified as a direct target of miR-155 using qRT-PCR, luciferase reporter assay and Western blot analysis as previous study demonstrate[11]. CBL is an E3 ubiquitin ligase, which plays important roles in the tumor migration and adhesion[32]. Cells deficient of CBL was reported to have severe defects on cell migration, suggesting that CBL has an important part in cell migration. Increasing CBL expression resulted in decreased cell growth, increased cell apoptosis and inhibition of tumor development in a mouse model[33]. In addition, CBL was found to function as a tumor promoter role in malignant melanoma cells[34], which was opposite to the effects of miR-155. We determined CBL as the target of miR-155 in malignant melanoma, a significantly high expression of CBL was observed after transfection with miR-155 inhibitor. However, the CBL expression was inhibited by miR-155 mimic in malignant melanoma cells. Furthermore, for the first time, over-expression of CBL ablated the inhibitory effects of miR-155, suggesting that the interaction between miR-155 and CBL may have a fundamental biological role.

## Conclusions

miR-155 was dramatically down-regulated in malignant melanoma cells and tissues. MiR-155 over-expression can inhibit the proliferation, migration and invasion in malignant melanoma *in vitro*. In addition, CBL were detected as a direct and functional target of miR-155, and it can mediate the effect of miR-155 on malignant melanoma cell proliferation, migration and invasion. Collectively, we believed that miR-155 played an essential role in the progression of human melanoma and might represent as a therapeutic target for human melanoma.

### Conflict of Interests

The Authors declare that they have no conflict of interests.

## References


1) Trotter SC, Sroa N, Winkelmann RR, Olencki T, Bechtel M. A global review of melanoma follow-up guidelines. J Clin Aesthet Dermatol 2013; 6: 18-26.
2) Achkar T, Tarhini AA. The use of immunotherapy in the treatment of melanoma. J Hematol Oncol 2017; 10: 88.
3) Kee D, McArthur G. Immunotherapy of melanoma. Eur J Surg Oncol 2017; 43: 594-603.
4) Davar D, Tarhini AA, Kirkwood JM. Adjuvant immunotherapy of melanoma and development of new approaches using the neoadjuvant approach. Clin Dermatol 2013; 31: 237-250.
5) Olszanski AJ. Current and future roles of targeted therapy and immunotherapy in advanced melanoma. J Manag Care Spec Pharm 2014; 20: 346-356.
6) Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.
7) Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.
8) Saumet A, Lecellier CH. microRNAs and personalized medicine: evaluating their potential as cancer biomarkers. Adv Exp Med Biol 2015; 888: 5-15.
9) Latchana N, Ganju A, Howard JH, Carson WR. MicroRNA dysregulation in melanoma. Surg Oncol 2016; 25: 184-189.
10) Ventura A, Jacks T. MicroRNAs and cancer: short RNAs go a long way. Cell 2009; 136: 586-591.
11) Yu H, Xu W, Gong F, Chi B, Chen J, Zhou L. MicroRNA-155 regulates the proliferation, cell cycle, apoptosis and migration of colon cancer cells and targets CBL. Exp Ther Med 2017; 14: 4053-4060.
12) Qu Y, Zhang H, Sun W, Han Y, Li S, Qu Y, Ying G, Ba Y. MicroRNA-155 promotes gastric cancer growth and invasion by negatively regulating transforming growth factor-beta receptor 2. Cancer Sci 2018; 109: 618-628.
13) Chen G, Wang D, Zhao X, Cao J, Zhao Y, Wang F, Bai J, Luo D, Li L. miR-155-5p modulates malignant behaviors of hepatocellular carcinoma by directly targeting CTHRC1 and indirectly regulating GSK-3beta-involved Wnt/beta-catenin signaling. Cancer Cell Int 2017; 17: 118.
14) Levati L, Pagani E, Romani S, Castiglia D, Piccinni E, Covaciu C, Caporaso P, Bondanza S, Antonetti FR, Bonmassar E, Martelli F, Alvino E, D'Atri S. MicroRNA-155 targets the SKI gene in human melanoma cell lines. Pigment Cell Melanoma Res 2011; 24: 538-550.
15) Prossomariti A, Piazzi G, D'Angelo L, Miccoli S, Turchetti D, Alquati C, Montagna C, Bazzoli F, Ricciardiello L. miR-155 is downregulated in familial adenomatous polyposis and modulates WNT signaling by targeting AXIN1 and TCF4. Mol Cancer Res 2018; 16: 1965-1976.
16) Wang D, Tang M, Zong P, Liu H, Zhang T, Liu Y, Zhao Y. MiRNA-155 regulates the Th17/Treg ratio by targeting SOCS1 in severe acute pancreatitis. Front Physiol 2018; 9: 686.
17) Cao H, Huang S, Liu A, Chen Z. Up-regulated expression of miR-155 in human colonic cancer. J Cancer Res Ther 2018; 14: 604-607.
18) Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science 2002; 298: 1912-1934.
19) Robinson DR, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. Oncogene 2000; 19: 5548-5557.
20) Bacher U, Haferlach C, Schnittger S, Kohlmann A, Kern W, Haferlach T. Mutations of the TET2 and CBL genes: novel molecular markers in myeloid malignancies. Ann Hematol 2010; 89: 643-652.
21) Tan YC, Mirzapoiazova T, Won BM, Zhu L, Srivastava MK, Vokes EE, Husain AN, Batra SK, Sharma S, Salgia R. Differential responsiveness of MET inhibition in non-small-cell lung cancer with altered CBL. Sci Rep 2017; 7: 9192.
22) Sumara I, Maerki S, Peter M. E3 ubiquitin ligases and mitosis: embracing the complexity. Trends Cell Biol 2008; 18: 84-94.
23) Zhang HG, Wang J, Yang X, Hsu HC, Mountz JD. Regulation of apoptosis proteins in cancer cells by ubiquitin. Oncogene 2004; 23: 2009-2015.
24) Bernassola F, Karin M, Ciechanover A, Melino G. The HECT family of E3 ubiquitin ligases: multiple players in cancer development. Cancer Cell 2008; 14: 10-21.
25) Jing Z, Li L, Wang X, Wang M, Cai Y, Jin ZI, Zhang YE. High c-Cbl expression in gliomas is associated with tumor progression and poor prognosis. Oncol Lett 2016; 11: 2787-2791.
26) Pandima DK, Rajavel T, Daglia M, Nabavi SF, Bishayee A, Nabavi SM. Targeting miRNAs by polyphenols: Novel therapeutic strategy for cancer. Semin Cancer Biol 2017; 46: 146-157.

27) Muluhngwi P, Klinge CM. Identification of miRNAs as biomarkers for acquired endocrine resistance in breast cancer. Mol Cell Endocrinol 2017; 456: 76-86.

28) Li XD, Li XM, Gu JW, Sun XC. MiR-155 regulates lymphoma cell proliferation and apoptosis through targeting SOCS3/JAK-STAT3 signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 5153-5159.

29) Wei RJ, Zhang CH, Yang WZ. MiR-155 affects renal carcinoma cell proliferation, invasion and apoptosis through regulating GSK-3beta/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 5034-5041.

30) Liu J, van Mil A, Aguor EN, Siddiqi S, Vrijsen K, Jaksani S, Metz C, Zhao J, Strijkers GJ, Doevendans PA, Sluijter JP. MiR-155 inhibits cell migration of human cardiomyocyte progenitor cells (hCMPCs) via targeting of MMP-16. J Cell Mol Med 2012; 16: 2379-2386.

31) Zhao XD, Zhang W, Liang HJ, Ji WY. Overexpression of miR -155 promotes proliferation and invasion of human laryngeal squamous cell carcinoma via targeting SOCS1 and STAT3. PLoS One 2013; 8: e56395.

32) Huang C. Roles of E3 ubiquitin ligases in cell adhesion and migration. Cell Adh Migr 2010; 4: 10-18.

33) Severe N, Dieudonne FX, Marty C, Modrowski D, Patiño Garcia A, Lecanda F, Fromigue O and Marie PJ. Targeting the E3 ubiquitin casitas B lineage lymphoma decreases osteo¬sarcoma cell growth and survival and reduces tumorigenesis. J Bone Miner Res 2012; 27: 2108 2117.

34) Nihal M, Wood GS. c-CBL regulates melanoma proliferation, migration, invasion and the FAK-SRC-GRB2 nexus. Oncotarget 2016; 7: 53869-53880.