Transcription Elongation Factor A-Like 7, regulated by miR-758-3p inhibits the malignant process of melanoma through decreasing the expression levels of c-Myc and AKT1

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Abstract

Background: The ectopic expression of transcription elongation factor A (SII)-like 7 (TCEAL7) has been observed in several kinds of cancers, but its role in melanoma is still unclear. This study was carried out to investigate TCEAL7 role in melanoma progression, and uncover the underlying mechanisms.

Methods: TCEAL7 expression level in melanoma tissues and cells were determined by using RT-PCR and western blotting. CCK-8, transwell chambers, flow cytometry, starch assay and tumorigenesis assay were applied to detect cell growth, invasion, apoptosis, migration and tumorigenesis, respectively.

Results: A low expression level of TCEAL7 was observed in melanoma tissues and cells, which associated with malignant process and poor prognosis. TCEAL7 negatively modulated AKT1, AKT2 and c-Myc expression and inhibited cancer progression via decreasing AKT1 and c-Myc expression. In addition, TCEAL7 was negatively modulated by miR-758-3p which promoted melanoma progression. Moreover, TCEAL7 overexpression abolished miR-758-3p-mediated melanoma progression.

Conclusion: This study demonstrated that TCEAL7, regulated by miR-758-3p inhibited the malignant process of melanoma through decreasing the expression levels of c-Myc and AKT1.

Introduction

Melanoma is a type of skin cancers, which is a malignant tumor with a high metastatic potential (1, 2). According to the most recent data in Cancer Genomics Consortium (http://www.cancergenomics.org/), the 5-year overall survival rate for melanoma is 92%. However, the 5-year-survival rate of metastatic melanoma is 25%. Thus, further explorations of the molecular mechanisms underlying the progression of malignant melanoma are desperately needed.

Transcription elongation factor A (SII)-like 7 (TCEAL7) encodes a cell death regulatory protein which is inactivated by methylation (3) and has a sequence similarity to brain-expressed (Bex) proteins, Tceal1 and Tceal6 proteins (4, 5). TCEAL7 has been identified to be frequently deregulated in several kinds of tumors, and the decreased expression of TCEAL7 often correlates with malignant process and poor prognosis of patients, such as ovarian cancer (4), gastric adenocarcinoma (6) and non-small cell lung cancer (7). Re-expression of TCEAL7 in ovarian cancer cell lines (OV 167, OV 177, OV 202, OV 207, OV 266, OVCAR-5 and SKOV-3) induces significant increases in cell death and inhibits cell colony formation efficiency (4). Knockdown of TCEAL7 increases the activity of oncogenic gene c-Myc in epithelial ovarian cancer cell lines (8). Downregulation of TCEAL7 enhances the expression levels of proliferative, angiogenic, inflammatory and anti-apoptotic genes in ovarian cancer cells through the nuclear factor kappa B (NF-κB) pathway (3). All of these findings suggest that TCEAL7 functions as an oncogene. However, the function of TCEAL7 in malignant melanoma still needs to be completely uncovered.

Increasing evidence demonstrates that microRNAs (miRNAs) is strongly implicated in tumorigenesis via degradation and/or translational suppression of mRNAs by binding to the 3'-untranslated regions (UTRs)

of their target genes (9, 10). miR-758 was found to be lowly expressed in retinoblastoma tissues and cell lines, and restoration of its expression caused significant inhibitions in cell proliferation, invasion and migration capacities, and increased cell apoptosis via targeting paired box protein 6 (PAX6) (11). It is predicted that TCEAL7 is a target of miR-758-3p using the online software miRanda and miRDB. However, whether miR-758-3p is involved in the progression of melanoma via targeting TCEAL7 remains unknown.

In this paper, we aimed to explore the role of TCEAL7 in the progression of melanoma and uncover whether miR-758-3p targets TCEAL7 and participate in melanoma progression.

Materials And Methods

Boinformatics analysis

TCEAL7 expression levels in melanoma tissues and normal tissues were analyzed by using the Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/). The correlation between the levels of TCEAL7, AKT1, AKT2 and c-Myc were analyzed by using starBase (http://starbase.sysu.edu.cn/). miRanda (http://microrna.org/microrna/home.do) and miRDB (http://www.mirdb.org/index.html) were applied to predict the miRNAs which target TCEAL7.

Patients and sample preparation

Ninety-eight fresh melanoma tissues were obtained from patients with melanoma. Thirty nevus tissues (benign proliferation of melanocytes) obtained from age and gender-matched individuals served as normal control. None of interventional treatment was performed before surgery.

Cell culture

A375, one human malignant melanoma cell line was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China); WM-115, another human melanoma cell line and the normal melanocytes PIG1 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in DMEM (Hyclone, USA) with 10% fetal bovine serum (FBS) (Hyclone, USA) in a humidified incubator at 37°C with 5% CO_2.

RNA interference and lentivirus construction

The small interfering RNAs (siRNAs) targeting the human TCEAL7 gene were designed and synthesized by the GenePharma Co., LTD (Sghanghai, China). The sequences are listed as follows:

si- TCEAL7-1: 5'- CCGAAGTCCTTATATTCCCGGGCTT-3';

si- TCEAL7-2: 5'- GAGCTGACGTGAACCGAAGTCCTTA-3';

si- TCEAL7-3: 5'- CCAGTCATTCGATGTTGCTGAGATT-3';

si-Scramble: 5'-CATCAATTGAACCGAGCCTTACGTA-3'. The three siRNAs-TCEAL7 and its negative control si-Scramble were transfected to cells by using the Lipofectamine 2000 (Invitrogen, MA, USA).

To upregulate TCEAL7 (Lenti-TCEAL7), miR-758-3p (mimics), AKT1 (Lenti-AKT1) and c-Myc (Lenti-c-Myc) in melanoma cells, the lentivirus vectors were constructed by GenePharma Co., LTD and infected into cells with the help of polybrene (Thermo Fisher Scientific, MA, USA). The infected cells were then probed with 6 μg/ml puromycin and/or 100 μg/ml G418 for 14 days to establish the stably transfected cells, which were used in the *in vivo* experiments.

Real-time Quantitative PCR (RT-PCR) analysis of mRNA levels

Total RNA isolation from tissues and cells was carried out with the help of Trizol reagent (Invitrogen, Carlsbad, CA, USA), which was then reverse-transcribed into cDNA using RT^2 First Strand Kit (Qiagen, Germany) in the light of manufacturer's instructions. Then, the mRNA levels were assayed by RT-PCR with TransStart Green qPCR SuperMix (TransGen, Beijing, China) in a DA7500 Real-time Nucleic Acid Amplification Fluorescence Detection System (Bio-Rad). The human GADPH was used to normalize the mRNA levels. The $2^{-\triangle\triangle Ct}$ method was used to calculate the changes of relative mRNA (12). The experiments were carried out in three biological replicates. The primers were synthesized by Sangon (China) and are listed in Table 1.

RT-PCR analysis of miRNA levels

The level miR-758-3p was detected by RT-PCR according to previous study (13). Briefly, total miRNAs were isolated from tumor samples and cell lines based on the manufacturer's protocol of miRcute miRNA Isolation kit (Tiangen Biotech Co., Ltd.). After that, the cDNA was synthesized using the miRcute miRNA cDNA kit (Tiangen Biotech Co., Ltd.), and miR-758-3p was then detected by the miRcute miRNA luciferase quantitative kit (Tiangen Biotech Co., Ltd.). U6 was used as an internal reference. The primer sequences were listed in Table 1.

Western blotting analysis

Total proteins from cells and tissues were obtained with RIPA buffer (Thermo Fisher Scientific) containing protease inhibitor cocktail. Then, the protein concentrations were determined by using the BCA Protein Assay Kit (Thermo Fisher Scientific) based on the instructions. After that, equal amounts of protein were resolved using 10% SDS-PAGE gels and then transferred to the polyvinylidene fluoride (PVDF; Millipore, Dallas, Tx, USA) membranes. The 5% skim milk was used to block the PVDF membranes, which was then incubated with the primary antibodies overnight at 4 °C, including TCEAL7 (cat no. 11218-1-AP, Proteintech, Wuhan, China), AKT1 (cat no. ab235958, Abcam, Cambridge, MA, USA), AKT2 (cat no. ab175354, Abcam), c-Myc (cat no. ab32072, Abcam), STAT3 (cat no. ab68153, Abcam), β-catenin (cat no. ab16051, Abcam), TGF-β (cat no. ab179695, Abcam), YAP (cat no. ab52771, Abcam), FOXO1 (cat no. ab52857, Abcam) and GAPDH (cat no. 60004-1-Ig, Proteintech). The membranes were then incubated

with secondary antibodies for 1 hour at room temperature. Western blotting luminol reagent (Millipore) was used to visualize the immunoreactive bands.

Cell proliferation assay

Cell viability was detected by Cell Counting Kit-8 (CCK-8, Dojindo, Japan) according to the manufacturer's instructions. In brief, 2×10^3 A375 and WM-115 cells were seeded into a 96-well plate overnight prior to transfection/infection. Next, 10 ml of CCK-8 and 90 ml cell culture medium were added into each well after 1, 2, 3, 4 or 5 days of cell transfection/infection, respectively. Following incubation for 2 hours at 37°C with 5% CO_2, the absorbance of each well was measured at 450 nm.

Apoptosis assay

The melanoma A375 and WM-115 cells were placed into 6-well plates at a concentration of 5 × 10^5/mL and allowed to grow at 37 °C overnight, followed by cell transfections/infections. After 48 hours, the cells were collected for apoptosis detection using Annexin-V-(FITC) and propidium iodide (PI) kit (BD Biosciences, San Jose, CA, USA). The double-stained cells were subsequently analyzed by the BD flow cytometer. At least 1 × 10^5 cells were detected each time.

Scratch assay

Melanoma cells were placed in 6-well plates with adequate numbers to reach 100% confluence in the next day. Then, the wounds were made by using 20 μl pipette tips. The non-adherent cells were removed by washing with PBS slowly. After that, each well of the 6-well plates were added 2 mL culture medium containing 1% FBS. The wound width was measured at 0 and 24 hours post the scratch.

Transwell chamber assay

To assess cell invasion, melanoma cells were suspended in FBS-free DMEM and inoculated in the Matrigel pre-coated membrane of the top chamber (BD Bioscience), with 1×10^5 cells for each well. The lower chamber was added 600 μL of 10% FBS-DMEM. At 24 hours post incubation, the non-invaded cells were removed with cotton swabs, and the invaded cells in the lower surface were fixed with 10 % methanol and stained with 0.1 % crystal violet (Solarbio Co., Ltd, Beijing, China) for 8 min. Pictures were taken under a microscope with a magnification of 200×, and the invaded cells were counted. Five randomly selected fields were recorded for each well.

Dual luciferase gene reporter assay

The 3'UTR of TCEAL7 with the putative miR-758-3p-binding site were chemically synthesized and cloned into the Renilla luciferase gene (pLUC-REPORT vector; Promega, Madison, WI, USA). WM-115 and A375 cells were co-transfected with 200 ng luciferase reporter vector and 100 nM miR-758-3p mimics or the negative controls. Luciferase activity was examined 48 hours after the transfection using the Dual-Luciferase Assay kit (Promega) according to manufacturer's instruction.

In vivo tumorigenesis assay

Nude mice experiment was approved by Animal Ethics Committee of China Japan Union Hospital of Jilin University. SPF grade male BALB/c nude mice were purchased from the Institute of Zoology, Chinese Academy of Sciences. The malignant melanoma cell lines A375 were re-suspended in 0.1 mL of PBS and subcutaneously injected into 6-week-old male nude mice (2×10^6 cells/mouse). The mice were euthanized 28 days after injection, and the tumors were took out and weighed.

Integrated analysis of miRNA target gene prediction database

Integrated analysis of the upstream miRNAs of TCEAL7 predication databases, including miRanda and miRDB (14-16), was conducted to identify the upstream miRNAs of TCEAL7.

Statistical analysis

All statistical analyses were performed by SPSS 19.0 software package (SPSS Inc., Chicago, IL, USA). Data from each group were expressed as mean ± standard error. Overall survival curves were calculated with the Kaplan-Meier method and were analyzed with the log-rank test. The Student's *t* test and a one-way analysis of variance followed by Tukey's post hoc test were conducted to analyze differences between 2 and ≥ 2 groups, and $P < 0.05$ was thought as statistical significance.

Results

TCEAL7 is downregulated in human melanoma tissues and cell lines

To determine the effects of TCEAL7 in the progression of melanoma, we first analyze TCEAL7 expression pattern in melanoma with the help of GEPIA database. In comparison with the normal group, TCEAL7 expression level was decreased in melanoma group (Figure 1A). We also explored the expression levels of TCEAL7 in melanoma tissues and cell lines using RT-PCR and western blotting. As shown in Figures 1B-1C, both of the mRNA and protein levels of TCEAL7 in melanoma tissues were obviously decreased as compared with that in the nevus tissues. In addition, we detected the mRNA and protein levels of TCEAL7 in human normal skin cell line PIG1 and melanoma cell lines, including A375 and WM-115. From the results, we found that the expression level of TCEAL7 was decreased dramatically in malignant melanoma cell line A375 as compared with PIG1, with null in WM-115 cells (Figures 1D-1E). These data demonstrated that TCEAL7 was lowly expressed in melanoma tissues and cell lines, suggesting TCEAL7 might play an important role in the progression of melanoma.

Clinical significance of TCEAL7 in melanoma

Next, we explored the relationship between the expression levels of TCEAL7 and the clinicopathologic features and prognosis of melanoma patients. As shown in Table 2, the expression levels of TCEAL7 showed a negative correlation with the tumor size, histological grade and TNM grade in melanoma patients. The overall survival of patients with low expression of TCEAL7 was lower than that of patients

with TCEAL7 high expression (Figure 1F). These results indicated that low expression of TCEAL7 was closely associated with the advanced process and poor prognosis in melanoma patients.

TCEAL7 inhibits melanoma cell proliferation, migration and invasion

Then, we revealed the effects of TCEAL7 on the progression of melanoma *in vitro*. TCEAL7 was upregulated in WM-115 and A375 cells, and downregulated in A375 cells. The expressions of TCEAL7 at both mRNA and protein levels in Lenti-TCEAL7 group were increased obviously when compared with the Lenti- NC group, and decreased in the si-TCEAL7-1/-2/-3 transfected cells as compared with the si-NC group (Figures 2A-2F). As si-TCEAL-1 showed the highest knockdown efficiency between the 3 siRNAs of TCEAL7, si-TCEAL7-1 was applied in the following experiments. Next, we determined the effects of TCEAL7 on cell proliferation, invasion, apoptosis and migration using CCK-8, transwell chambers, flow cytometry and scratch assays, respectively. The results showed that cell growth (Figures 2G-2H), invasion (Figures 2I-2J) and migration (Figures 2M-2N) abilities were significantly weakened in Lenti-TCEAL7 group when compared with the Lenti-NC group, and enhanced in si-TCEAL7 group in comparison with the si-NC group. Then, we tested the role of TCEAL7 on cell apoptosis by flow cytometry. In addition, overexpression of TCEAL7 induced cell apoptosis in both WM-115 and A375 cell lines, and knockdown of TCEAL7 resulted in apoptosis reduction (Figures 2K-2L). These results suggested that TCEAL7 functioned as a tumor suppressor in melanoma.

TCEAL7 negatively regulates AKT1, AKT2 and c-Myc expression

To reveal the molecular mechanisms of TCEAL7 by which inhibits the progression of melanoma, we explored the effects of TCEAL7 on the expression of key proteins of signaling pathways. Compared with the lenti-NC group, the expression levels of AKT1, AKT2 and c-Myc were significantly increased following TCEAL7 overexpression in WM-115 and A375 cells at both mRNA and protein levels (Figures 3A-3D), with no obvious change in the expression levels of STAT3, β-catenin, TGF-β, YAP and FOXO1. To further clarify the relationship between TCEAL7 and AKT1, AKT2 and c-Myc, the starBase database was applied to analyze the correlation between the levels of TCEAL7 and AKT1, AKT2 and c-Myc in skin cutaneous melanoma (SKCM). The results showed that TCEAL7 level was negatively correlated with AKT1, AKT2 and c-Myc levels in SKCM samples, with significances for AKT1, AKT2 and c-Myc (Figures 3E-3G). However, the Pearson correlation analysis showed that TCEAL7 levels was only significantly negatively correlated with AKT1 and c-Myc levels in melanoma samples (Figures 3H-3J).

TCEAL7 inhibits melanoma cell proliferation, migration and invasion through downregulating AKT1 and c-Myc

Then, we explored whether AKT1 and c-Myc play a role in TCEAL7-mediated inhibition in melanoma progression. Compared with the lenti-TCEAL7 group, cell proliferation (Figures 4A-4D), invasion (Figures 4E-4F) and migration (Figures 4I-4L) abilities were significantly enhanced after overexpression of either AKT1 or c-Myc in WM-115 and A375 cell lines, while cell apoptosis was reduced (Figures 4G-4H). These

results demonstrated that TCEAL7 suppressed melanoma cell proliferation, migration and invasion through downregulating AKT1 and c-Myc.

MiR-758-3p downregulates TCEAL7 expression in melanoma cells

To further reveal the underlying mechanisms of TCEAL7 in melanoma progression, the databases of miRanda and miRDB were used to search the upstream miRNAs which modulates TCEAL7. The results showed that the co-regulator of TCEAL7 from both miRanda and miRDB databases was miR-758-3p (Figure 5A). We then explored miR-758-3p expression levels in melanoma tissues and cells. The RT-PCR results showed that miR-758-3p was upregulated in melanoma tissues and cell lines A375 and WM-115 (Figures 5B-5C). The high expression level of miR-758-3p predicted a shorter overall survival in patients with melanoma (Figure 5D). Next, we explored the relationship between miR-758-3p and TCEAL7. The expression level of miR-758-3p was significantly elevated when A375 and WM-115 cells were transfected with mimics-miR-758-3p as compared with the control group (Figure 5E). However, the expression of TCEAL7 was reduced obviously when miR-758-3p was upregulated in A375 and WM-115 cells (Figure 5F). Moreover, the luciferase gene reporter assay showed that the transcriptional activity of TCEAL7 was repressed following of miR-758-3p upregulation in A375 and WM-115 cells (Figure 5G). All of these results indicated that miR-758-3p was overexpressed in melanoma and negatively regulated TCEAL7 expression.

miR-758-3p facilitates melanoma progression via inhibiting TCEAL7 expression

We next investigated whether miR-758-3p was involved in the progression of melanoma. CCK-8, transwell and starch assays results showed that cell proliferation (Figures 6A-6B), invasion (Figure 6C) and migration (Figures 6E-6F) capacities were enhanced when WM-115 and A375 cells were infected with mimics-miR-758-3p, but these roles were impaired when TCEAL7 was overexpressed on the base of mimics-miR-758-3p treatment. In addition, cell apoptosis was inhibited when miR-758-3p was upregulated in WM-115 and A375 cells via infection with mimics-miR-758-3p, but this role was impaired when WM-115 and A375 cells were transfected with Lentiv-TCEAL7 on the base of mimics-miR-758-3p (Figures 6D). These results indicated that miR-758-3p served as an oncogene in melanoma progression through inhibiting TCEAL7 expression. The *in vivo* xenotransplantation assay showed that upregulation of TCEAL7 repressed the tumorigenesis of WM-115 cells, while miR-758-3p overexpression promoted tumor formation. In addition, TCEAL7 overexpression significantly abolished the pro-tumor formation effect of miR-758-3p (Figure 7A). Furthermore, the expressions of miR-758-3p, AKT1, AKT2 and c-Myc were elevated and TCEAL7 expression was decreased in tumor tissues in the mimics group, whereas this effect was impaired following TCEAL7 overexpression (Figures 7A-7D). Taken together, these above findings demonstrated that miR-758-3p facilitated melanoma progression by targeting TCEAL7.

Discussion

The malignant melanoma is characterized by aberrant proliferation, apoptosis reduction, and high motility and invasive potentials, which are of importance to maintain the aggressive clinical course of

melanoma (17, 18). Here, we explored the effects of TCEAL7 on cell proliferation, apoptosis, invasion, migration and tumorigenesis in melanoma, as well as the underlying mechanisms. The results showed that TCEAL7, negatively regulated by miR-758-3p, served as a tumor suppressor in melanoma through downregulating AKT1 and c-Myc.

TCEAL7 is a newly-identified pro-apoptotic protein that shares amino-acid sequence homology with the TCEAL1 (p21/SIIR/pp21) and the pp21 homolog (WBP5/TCEAL6) (4). TCEAL7 was identified to be frequently downregulated in tumors as compared with that in the corresponding normal tissues, such as ovarian cancer, endometrial carcinoma and gastric adenocarcinoma (4, 19, 20). The present study demonstrated, for the first time, that TCEAL7 was also lowly expressed in melanoma tissues and cell lines. In addition, we observed that the low expression of TCEAL7 was closely associated with shorter overall survival and the more malignant features in melanoma patients, including larger tumor size, higher histological and TNM grades. Consistently, Huang et al. (20) reported that low expression level of TCEAL7 was closely correlated with larger tumor size, higher histological grade, lower survival and worse nodal status in gastric adenocarcinoma.

Increasing evidence has identified that TCEAL7 exerts a tumor-suppressive role. For instance, Chien et al (4) reported that TCEAL7 induced cell death and inhibited cell colony formation efficiency in ovarian cancer cell lines. Guo et al (19) found that TCEAL7 overexpression reduced cell proliferation and colony formation ability and downregulated the expression levels of the pro-proliferative genes, such as c-Myc and cyclin D1, and inhibited NF-κB activation. In the present study, we upregulated TCEAL7 in TCEAL7-null WM-115 cells, and both upregulate and downregulate TCEAL7 in A375 cells to study TCEAL7 role in melanoma progression. We found that TCEAL7 overexpression caused significant inhibitions in cell proliferation, invasion and tumorigenesis and induced cell apoptosis in melanoma WM-115 and A375 cells, indicating that TCEAL7 serves as a tumor suppressor in melanoma.

In mechanism, we assessed the effects of TCEAL7 on the expression levels of proteins-related to key signaling pathways. The results showed that TCEAL7 overexpression led to significant decreases in the expression levels of AKT1, AKT2 and c-Myc in WM-115 and A375 cells at both mRNA and protein levels. Further studies have shown that TCEAL7 level in melanoma samples showed significantly negative correlation with the expression levels AKT1 and c-Myc. Upregulation of either AKT1 or c-Myc abolished TCEAL7 roles in inhibiting cell proliferation, invasion and migration and inducing cell apoptosis in both A375 and WM-115 cells, indicating that TCEAL7 inhibited melanoma progression via decreasing AKT1 and c-Myc expression. Consistently, previous studies have revealed that TCEAL7 negatively regulated c-Myc expression in cancers (8, 21).

Through degrading mRNA of target genes, miRNAs serve important roles in tumor development and progression (22, 23). It was previously reported that endogenous miR-182 can degrade TCEAL7 mRNA and inhibit its translation (19). In the present study, with the help of bioinformatics analysis, TCEAL7 was predicted to be a target of miR-758-3p. MiR-758-3p has been identified to be abnormally expressed and implicated in various kinds of cancers. For example, miRNA-758-3p expression was repressed by

chemotherapy drugs in esophageal cancer (24). MiR-758 was downregulated in papillary thyroid cancer (25), bladder cancer (26), glioblastoma (27), cervical cancer (28)and non-small cell lung cancer (29) and served as a tumor-suppressor. In the present study, we found that miR-758-3p was overexpressed in melanoma tissues and cells, and promoted the growth, migration, invasion and tumorigenesis, and repressed the apoptosis of melanoma cells through downregulating TCEAL7 expression, indicating that miR-758-3p served as an oncogene in melanoma. We conjectured the different cancer type caused the difference in miR-758-3p role in cancers. Evidence has demonstrated that the same miRNA may exert opposite roles in different types of cancers. For instance, miR-93-5p has been shown to promote cell proliferation in osteosarcoma (30), gliomas (31), gastric cancer (32, 33) and prostate [10] and enhanced cell invasion in nasopharyngeal carcinoma (34). In contrast, miR-93-5p suppresses cell invasion, migration and proliferation in breast cancer (35) and ovarian carcinoma (36).

In conclusion, our results showed that TCEAL7, regulated by miR-758-3p inhibited cell proliferation, migration, invasion and tumorigenesis and induced apoptosis in melanoma through upregulating AKT1, AKT2 and c-Myc expression levels (Figure 8). Our study provides a potential therapeutic target (miR-758-3p/TCEAL7) for the treatment of melanoma.

Abbreviations

TCEAL, Transcription Elongation Factor A-Like 7; 3'UTR, 3'-untranslated regions; miRNAs, microRNAs; CCK-8, Cell Counting Kit-8.

Declarations

Ethics approval and consent to participate All patients knew this research and signature on the informed consent. The study was approved by the Human Research Committee of China Japan Union Hospital of Jilin University and was performed in accordance with the Helsinki Declaration. Nude mice experiment was approved by Animal Ethics Committee of China Japan Union Hospital of Jilin University.

Consent for publication Not applicable.

Availability of data and materials All data generated or analysed during this study are included in this published article.

Competing interests The authors declare there have no conflict of interest.

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Authors' contributions Hong Li provided the conception of the study and revised the manuscript; Xilin Liu and Xianji Song did the experiments and data analysis, and completed the draft.

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Figures

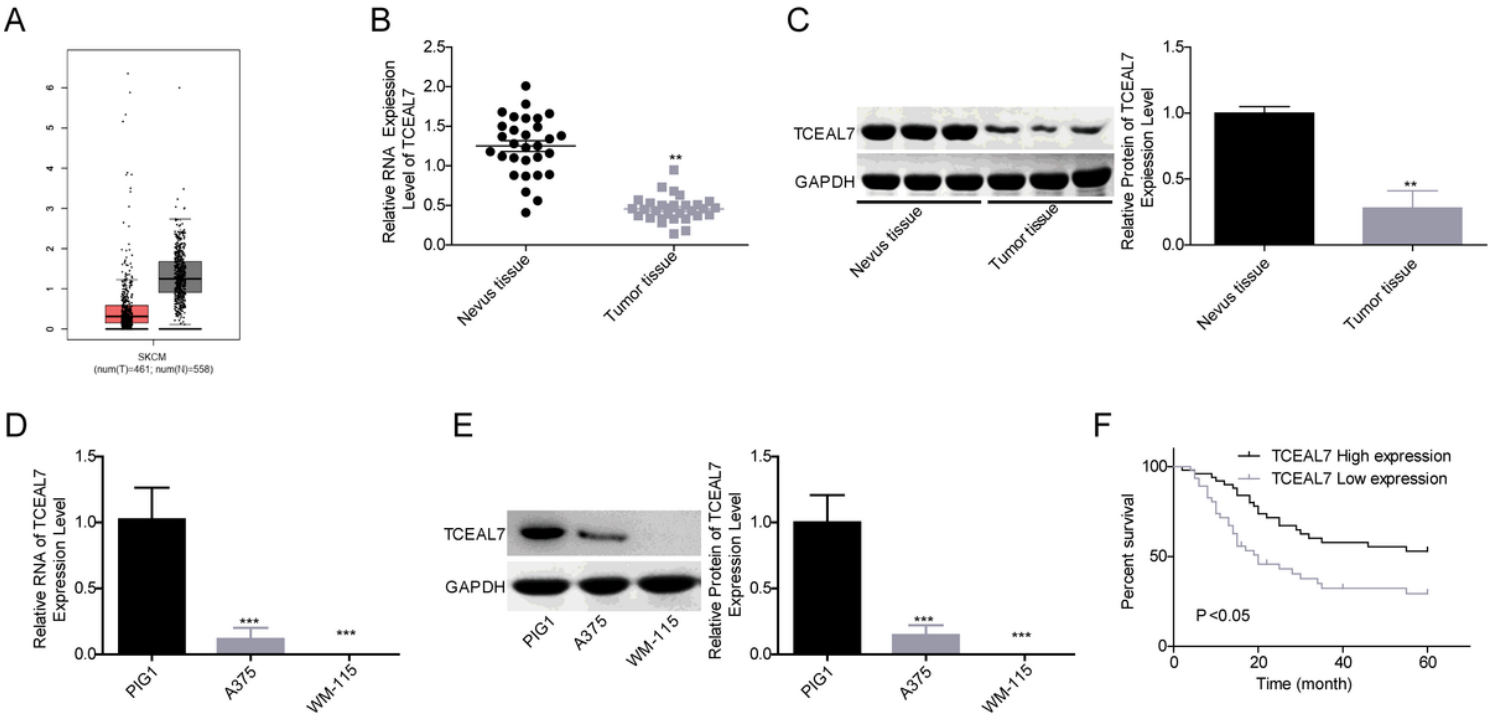

Figure 1

Analysis of the expression patterns of TCEAL7 in melanoma and its clinical significance in melanoma. A. GEPIA database was applied to analyze the expression level of TCEAL7 in melanoma tissues and normal tissues. B-C. The expression levels of TCEAL7 in melanoma tissues and nevus tissues at mRNA and protein levels were determined by using RT-PCR and western blotting assays, respectively. D-E. RT-PCR and western blotting were performed to detect the mRNA and protein levels of TCEAL7 in normal melanocyte cell line PIG1 and melanoma cell lines WM-115 and A375. F. Kaplan-Meier was used to analyze the prognostic value of TCEAL7 in melanoma patients. (**P<0.01, ***P<0.001)

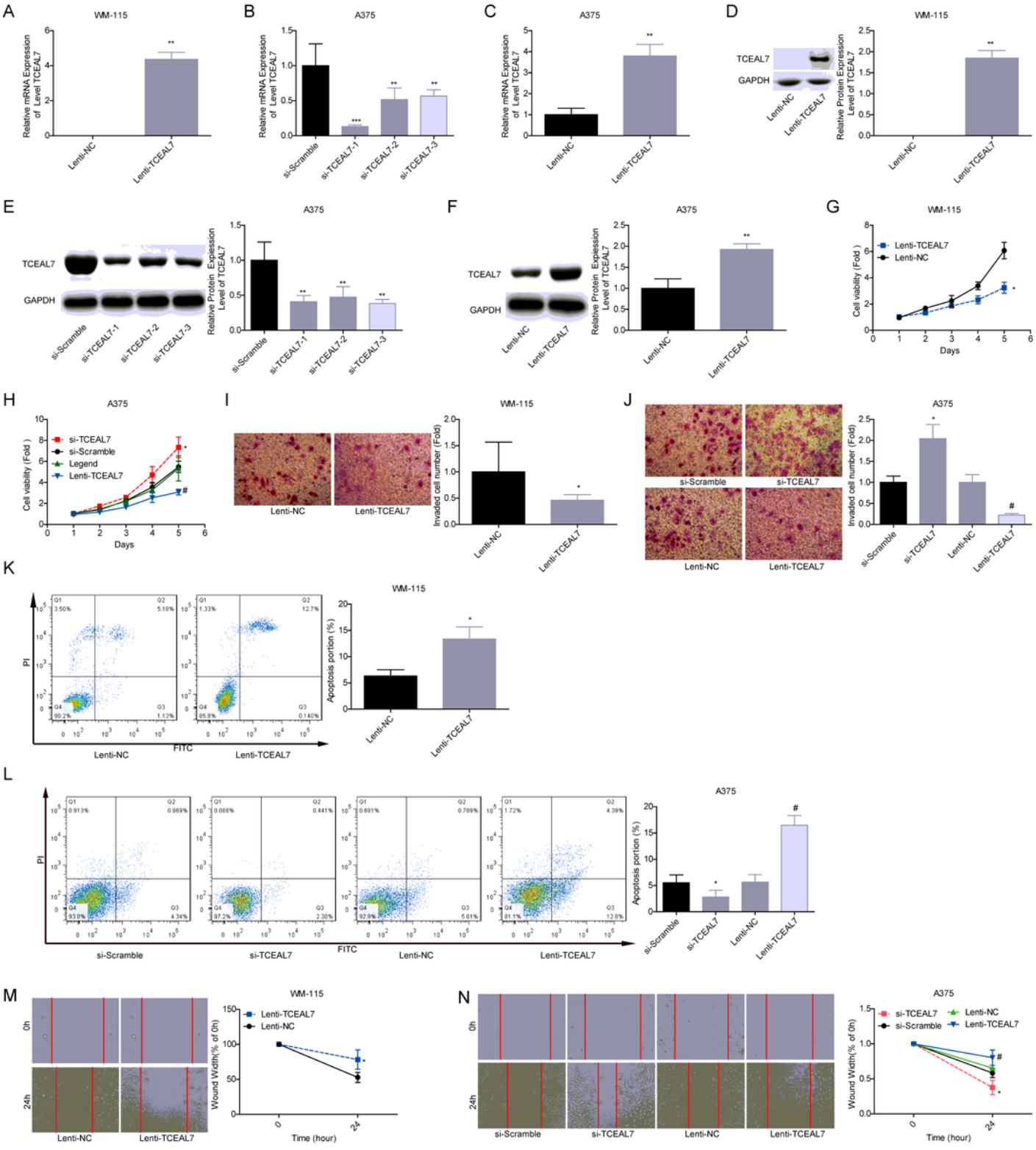

Figure 2

TCEAL7 inhibited melanoma cell proliferation, invasion and migration and induced cell apoptosis. A-F. RT-PCR and western blotting assays were used to detect the knockdown and overexpression efficiencies of TCEAL7 in A375 and WM-115 cell lines. G-H. CCK-8 assay was used to test cell proliferation following TCEAL7 was overexpressed/silenced in WM-115 and A375 cells. I-J. Transwell chamber assay was used to test cell invasion ability following TCEAL7 was overexpressed/silenced in WM-115 and A375 cells. K-L.

The effects of TCEAL7 on the apoptosis of WM-115 and A375 cells were detected by flow cytometry. M-N. Starch assay was used to test cell migration ability following TCEAL7 was overexpressed/silenced in WM-115 and A375 cells (*P<0.05, **P<0.01 and ***P<0.001, compared with Lenti-NC group; ###P<0.001, compared with si-Scramble group)

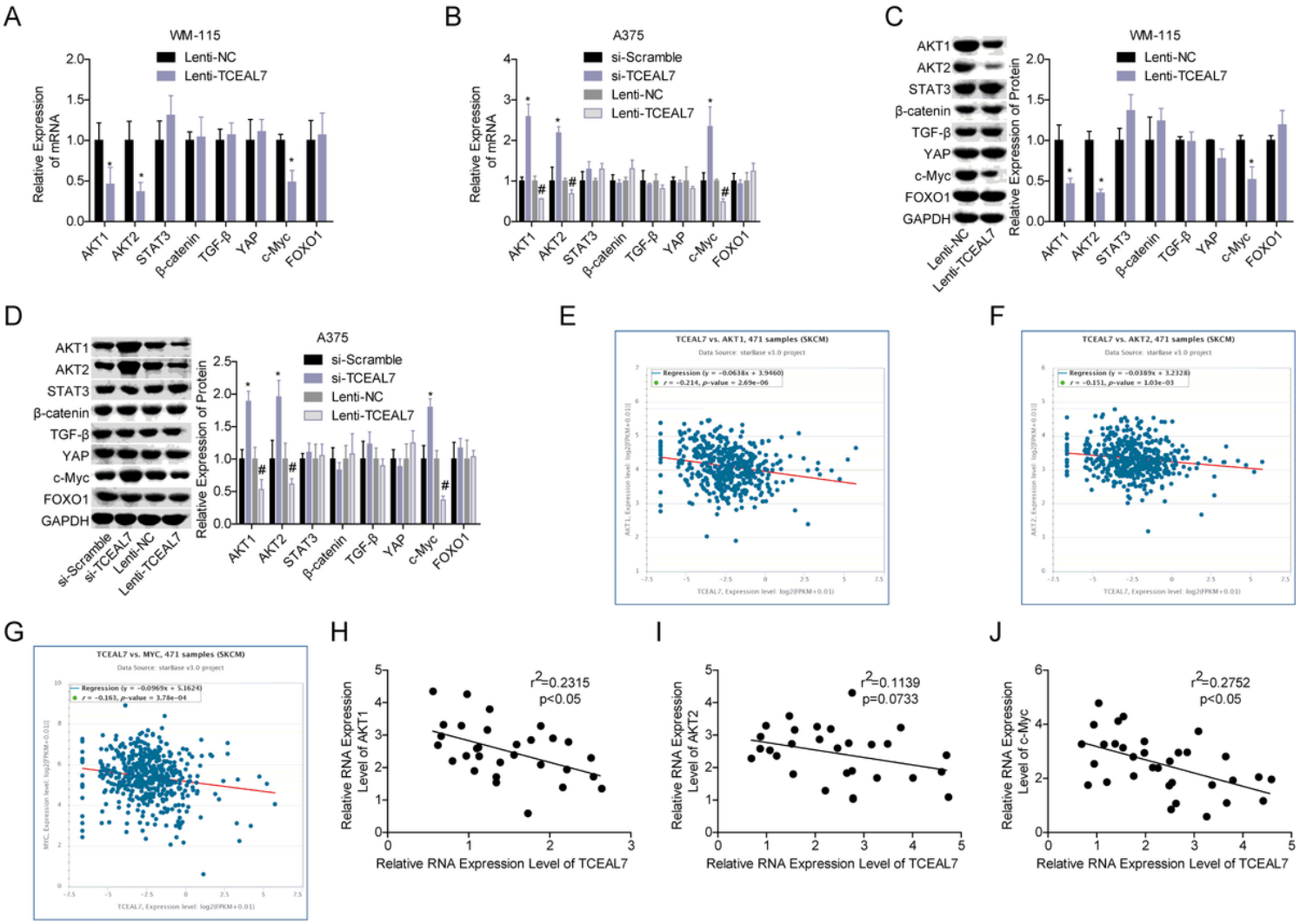

Figure 3

TCEAL7 increased the expression levels of AKT1, AKT2 and c-Myc in melanoma cells. A-D. The mRNA and protein levels of AKT1, AKT2, c-Myc, STAT3, β-catenin, TGF-β, YAP and FOXO1 in Lenti-TCEAL7/si-TCEAL7 treated WM-115 and A375 cells were determined by RT-PCR and western blotting. E-G. The correlations between the expression levels of TCEAL7 and AKT1, AKT2 and c-Myc in melanoma samples were predicted by using the starBase database. H-J. The correlations between the expression levels of TCEAL7 and AKT1, AKT2 and c-Myc in 30 melanoma tissues samples were determined. (*P<0.05, **P<0.01 and ***P<0.001, compared with Lenti-NC group; ###P<0.001, compared with si-Scramble group)

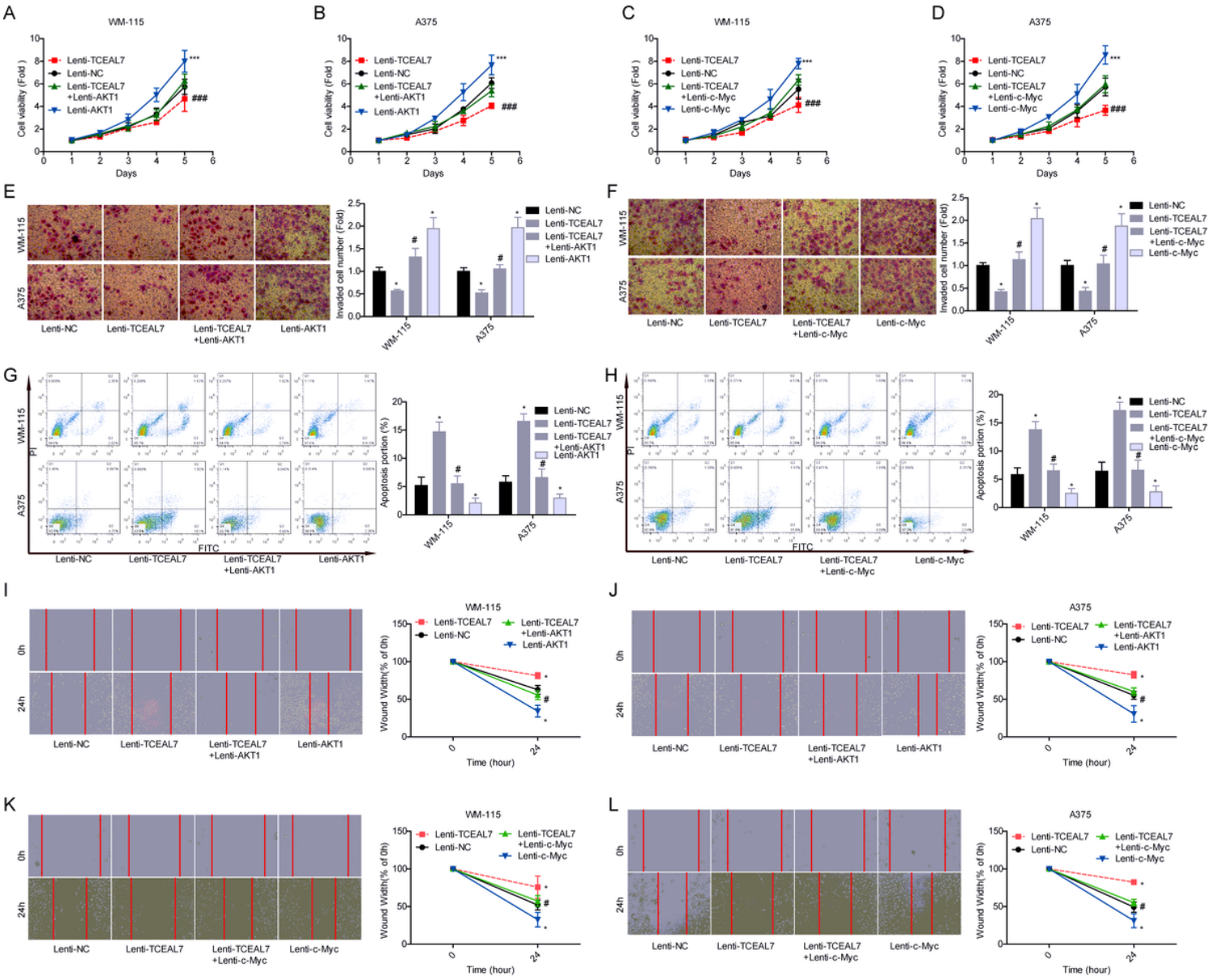

Figure 4

TCEAL7 inhibited the progression of melanoma through decreasing the expression of AKT1 and c-Myc. WM-115 and A375 cells were divided into Lenti-TCEAL7, Lenti-NC, Lenti-TCEAL7+Lenti-AKT1/c-Myc and Lenti-AKT1/c-Myc groups and submitted to the following assays. A-D. Cell proliferation was detected by using the CCK-8 assay. E-F. Transwell chamber assay was used to detect cell invasion. G-H. Flow cytometry assay was applied to detect cell apoptosis. I-L. Scratch assay was used to assess cell migration ability. (*P<0.05 and ***P<0.001, compared with Lenti-NC group; #P<0.05 and ###P<0.001, compared with Lenti-TCEAL7 group)

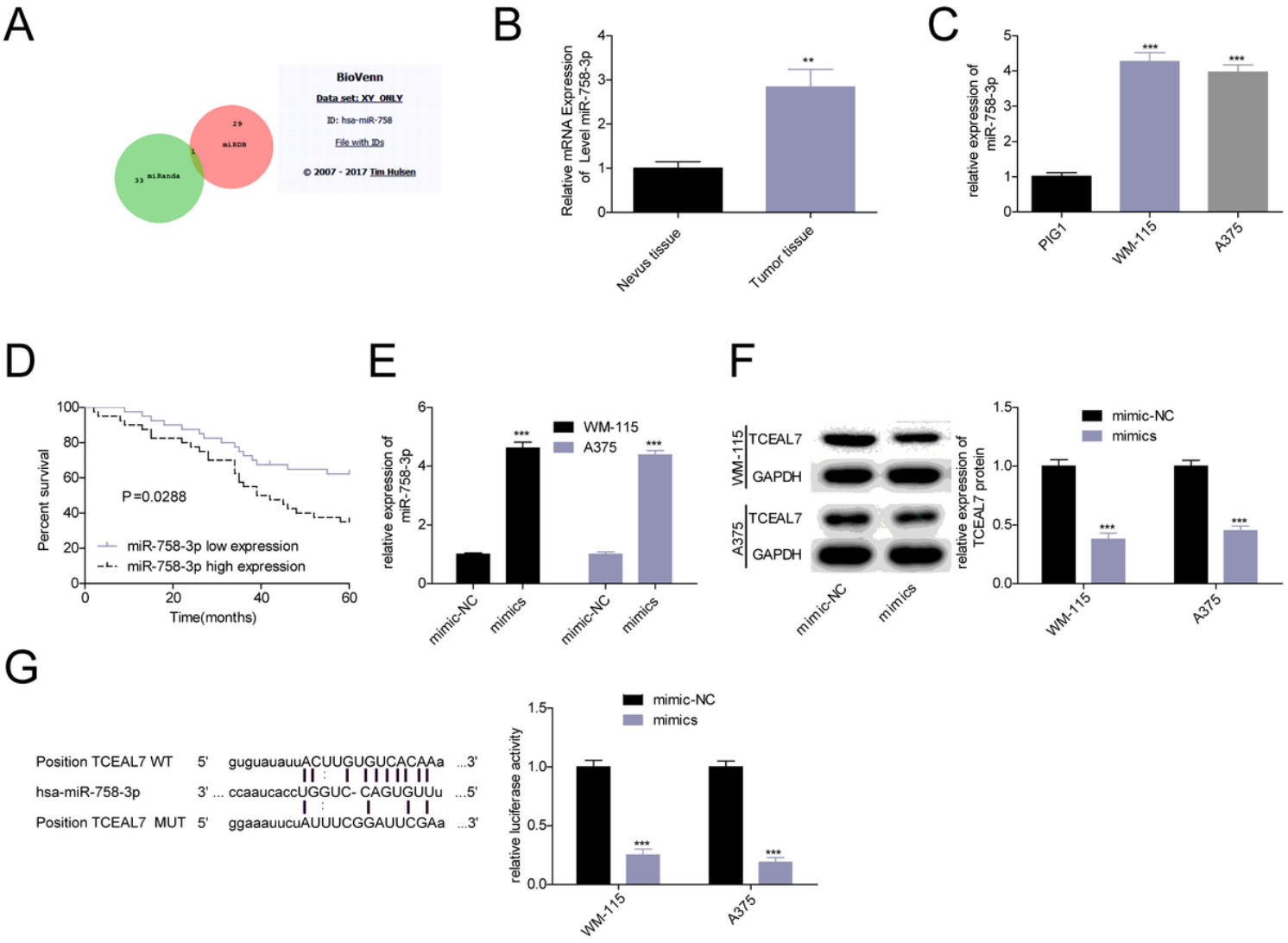

Figure 5

miR-758-3p negatively modulated TCEAL7 expression. A. BioVenn (https://www.biovenn.nl/index.php) was built to find the co-regulator of TCEAL7 from miRanda and miRDB diabetes. B. RT-PCR analysis of the expression levels of miR-758-3p in melanoma tissues and nevus tissues tissues. C. RT-PCR was applied to determine miR-758-3p levels in normal melanocyte PIG1 and and melanoma cell lines WM-115 and A375. D. Kaplan-Meier was used to analyze the prognostic value of miR-758-3p in melanoma patients. E. RT-PCR analysis of the expression levels of miR-758-3p following cell infection with mimics-miR-758-3p or mimic-NC in WM-115 and A375 cells. F. Western blotting analysis of the effect of miR-758-3p on the expression of TCEAL7 protein. G. Dual-luciferase gene reporter system was used to assess the effect of miR-758-3p on the transcriptional activity of TCEAL7. (**P<0.01, ***P<0.001)

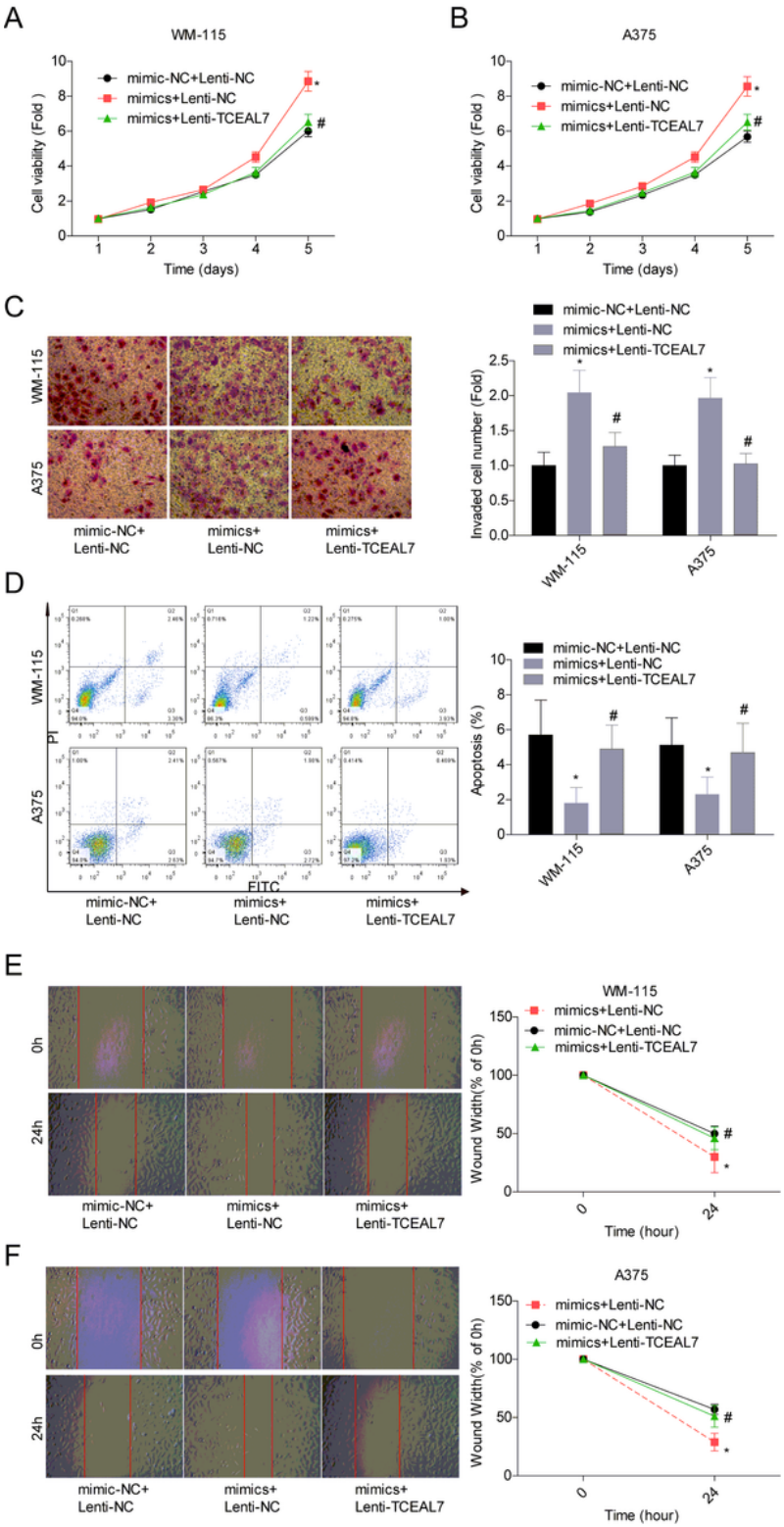

Figure 6

miR-758-3p promoted cell growth, migration and invasion and reduced cell apoptosis in melanoma cells via targeting TCEAL7. WM-115 and A375 cells were divided into mimic-NC+Lenti-NC, mimics+Lenti-NC and mimics+Lenti-TCEAL7 groups, and submitted to the following assays. A-B. CCK-8 assay was used to test cell proliferation. C. Transwell chamber assay was used to detect cell invasion. D. Flow cytometry

assay was applied to detect cell apoptosis. E-F. Scratch assay was used to assess cell migration ability. (*P<0.05, compared with mimic-NC+Lenti-NC group; #P<0.05, compared with mimics+Lenti-NC group)

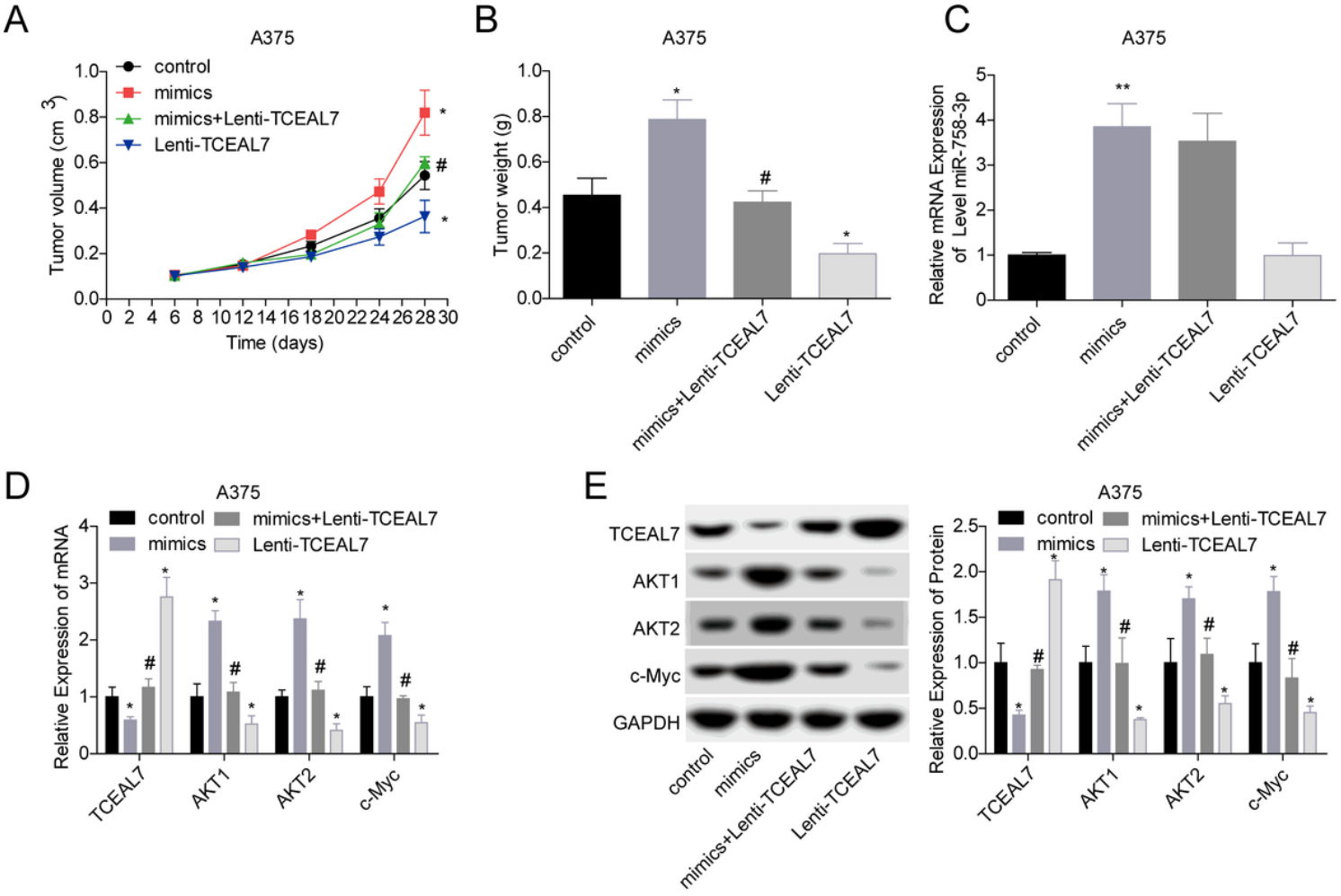

Figure 7

miR-758-3p promoted the tumorigenesis of melanoma cells via targeting TCEAL7. A. In vivo tumor formation assay was carried out to detect the tumorigenesis in A375 cells from mimic-NC+Lenti-NC, mimics+Lenti-NC and mimics+Lenti-TCEAL7 groups. B. RT-PCR analysis of the expression levels of miR-758-3p in tumor tissues. C-D. RT-PCR and western blotting assays were applied to detect the mRNA and protein levels of TCEAL7, AKT1, AKT2 and c-Myc in tumor tissues. (*P<0.05, compared with mimic-NC+Lenti-NC group; #P<0.05, compared with mimics+Lenti-NC group)

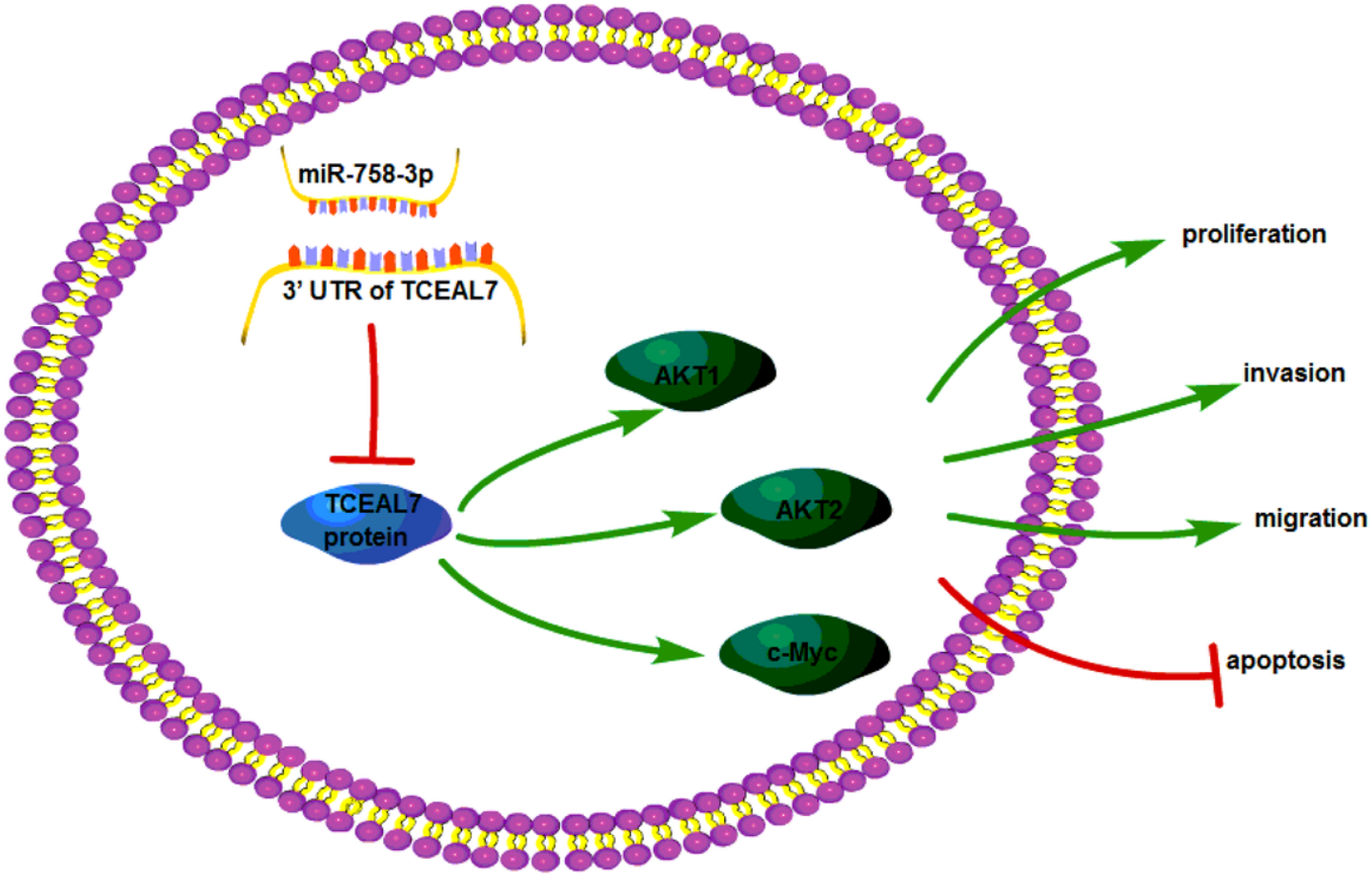

Figure 8

Schematic diagram of miR-758-3p/TCEAL7 in melanoma progression. TCEAL7, regulated by miR-758-3p inhibited cell proliferation, migration, invasion and tumorigenesis and induced apoptosis in melanoma through upregulating AKT1, AKT2 and c-Myc expression levels.