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Original Article

Long noncoding RNA MALAT1 promotes uveal melanoma cell growth and invasion by silencing of miR-140

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Abstract: Increasing evidences have demonstrated that long noncoding RNAs (LncRNAs) play a significant role in the development of tumor. However, the role of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in uveal melanoma remains unknown. In this study, we demonstrated that the expression of MALAT1 was upregulated in the uveal melanoma tissues compared to normal tissues. Among them, MALAT1 was upregulated in 72% (18/25) uveal melanoma tissues compared to their paired normal tissues. Knockdown of MALAT1 suppressed uveal melanoma cell proliferation, colony information, invasion and migration. Moreover, we showed that knockdown of MALAT1 promoted miR-140 expression and suppressed Slug and ADAM10 expression in the MUM-2C cell. In addition, we demonstrated that miR-140 was downregulated in the uveal melanoma tissues compared to normal tissues and cell lines. The expression level of MALAT1 was inversely correlated with the expression level of miR-140 in uveal melanoma tissues. These results suggested that MALAT1 served as an oncogenic LncRNA in the development of uveal melanoma.

Keywords: Long noncoding RNA, LncRNAs, MALAT1, uveal melanoma, mir-140

Introduction

Uveal melanoma is the most common primary intraocular malignancy in the adults, with an incidence about 0.7 per 100,000 [1-4]. Although development has been made in the therapy and diagnosis for the uveal melanoma, the 5-year survival rate still remains poor [5-7]. Most of the uveal melanoma patients have developed liver metastasis when diagnosed [8-10]. The molecular mechanisms of metastasis are still unknown [11, 12]. Therefore, it is important to identify new prognostic factors and therapy targets for uveal melanoma.

Long noncoding RNAs (lncRNAs) are a new class of non-protein-coding RNAs longer than 200 nucleotides [13-15]. Accumulating evidences have suggested that lncRNAs play a crucial role in many cellular processes including cell development, growth, differentiation, invasion and apoptosis [16-21]. LncRNAs are deregulated in a lot of cancers such as gastric cancer, osteosarcoma, colorectal cancer, cervical cancer and hepatocellular carcinoma [22-26]. Moreover, lncRNAs can act as tumor suppressor genes or oncogenes in the tumor [27-29]. However, the expression and functional roles of lncRNAs in uveal melanoma development are still unknown.

In this study, we demonstrated that the expression metastasis associated lung adenocarcinoma transcript 1 (MALAT1) was upregulated in uveal melanoma tissues and cell lines. Moreover, knockdown of MALAT1 expression suppressed uveal melanoma cell proliferation, colony information, invasion and migration partly through modulating miR-140 expression.

Materials and methods

Tissue samples and cell line cultured and transfection

Uveal melanoma tissues and their paired normal tissues were collected from uveal melanoma patients and immediately kept in the liquid

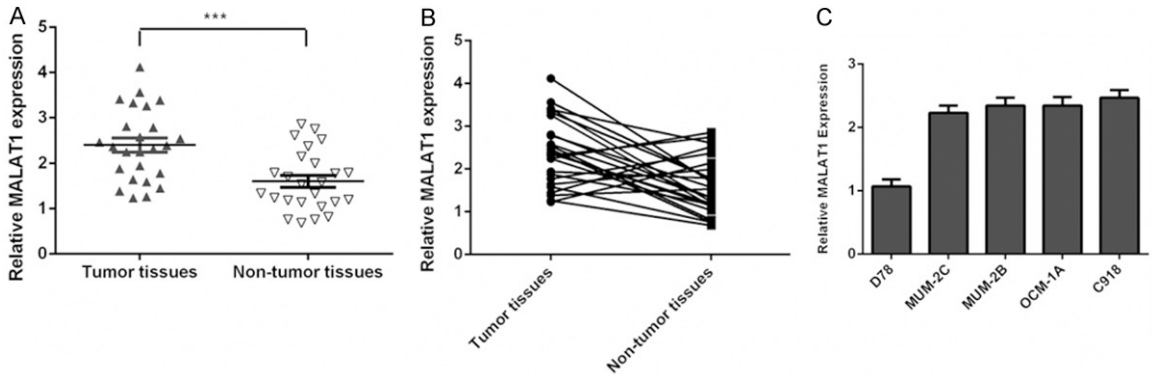

Figure 1. MALAT1 expression was increased in uveal melanoma tissues and cell. A. The expression of MALAT1 in the uveal melanoma tissues and normal tissues was measured using qRT-PCR. B. MALAT1 expression was upregulated in the 72% (18/25) uveal melanoma tissues compared to their paired normal tissues. C. The expression of MALAT1 in the uveal melanoma cell lines (MUM-2C, OCM-1A, MUM-2B and C918) and one melanocyte cell line (D78) was detected using qRT-PCR. ***p<0.001.

nitrogen. The uveal melanoma cell lines (MUM-2C, OCM-1A, MUM-2B and C918) and one melanocyte cell line (D78) were purchased from purchase Chinese Academy of Sciences (Beijing, China). The D78, OCM-1A and MUM-2C were cultured in the DMEM medium, and C918 and MUM-2B were maintained in RPMI 1640. Cell transfection was performed by using Lipofectamine 2000 (Invitrogen, USA) following to the manufacturer's information.

RNA extraction and qPCR analysis

Total RNA was isolated from the cells or tissues using TRIzol kit (Invitrogen, USA) following to the manufacturer's information. Quantitative Real-time PCR (qPCR) was performed to detect the expression of miR-140 and MALAT1 according to manufacturer's protocol on the iQ5 qPCR System (Bio-Rad, CA). U6 and GAPDH were used as control for miRNA and mRNA expression, respectively.

Western blot assay

The protein was extracted from cells or tissues and the protein concentration was determined by using BCA kit following to the manufacturer's information. Total protein was separated by 12% SDS-PAGE and transferred to PVDF (polyvinylidene fluoride) membrane. The membrane was blocked with non-fat milk and then inculcated with the primary antibody. The primary antibody was shown as following: PCNA, ki-67, Slug and ADAM10 (Abcam, USA).

Cell proliferation and colony information

Cell proliferation was detected using MTT assay. 20 ml MTT solution (Sigma, USA) was put to each well and the cells were continued to incubate for 4 h. The absorbance was detected at 490 nm in the Thermo (Thermo, USA). Cells were cultured in the 6-well plates and incubated for 2 weeks. Then, the cell colonies were fixed and stained with 10% crystal violet and counted.

Cell migration and invasion assays

Wound healing analysis was performed to measure the cell migration. Cells were cultured in the 6-well plate and seed to 100% confluence. The wound was created using a pipette tip and then the cells continue to culture for 48 hours. The closure rate was measured and described by a percentage. For cell invasion, the Transwell coated with Matrigel was used. Cells were placed on the upper chamber and 10% FBS was given to the lower chamber. Cells were culture for 48 hours and then cells invaded to the membrane were measure with crystal violet.

Statistical analysis

Data was shown as the mean ± SD (standard deviation). Difference between two groups was used by Student's t-test and one-way ANOVA was performed to measure the differences between more than two samples. P<0.05 was considered as significant difference.

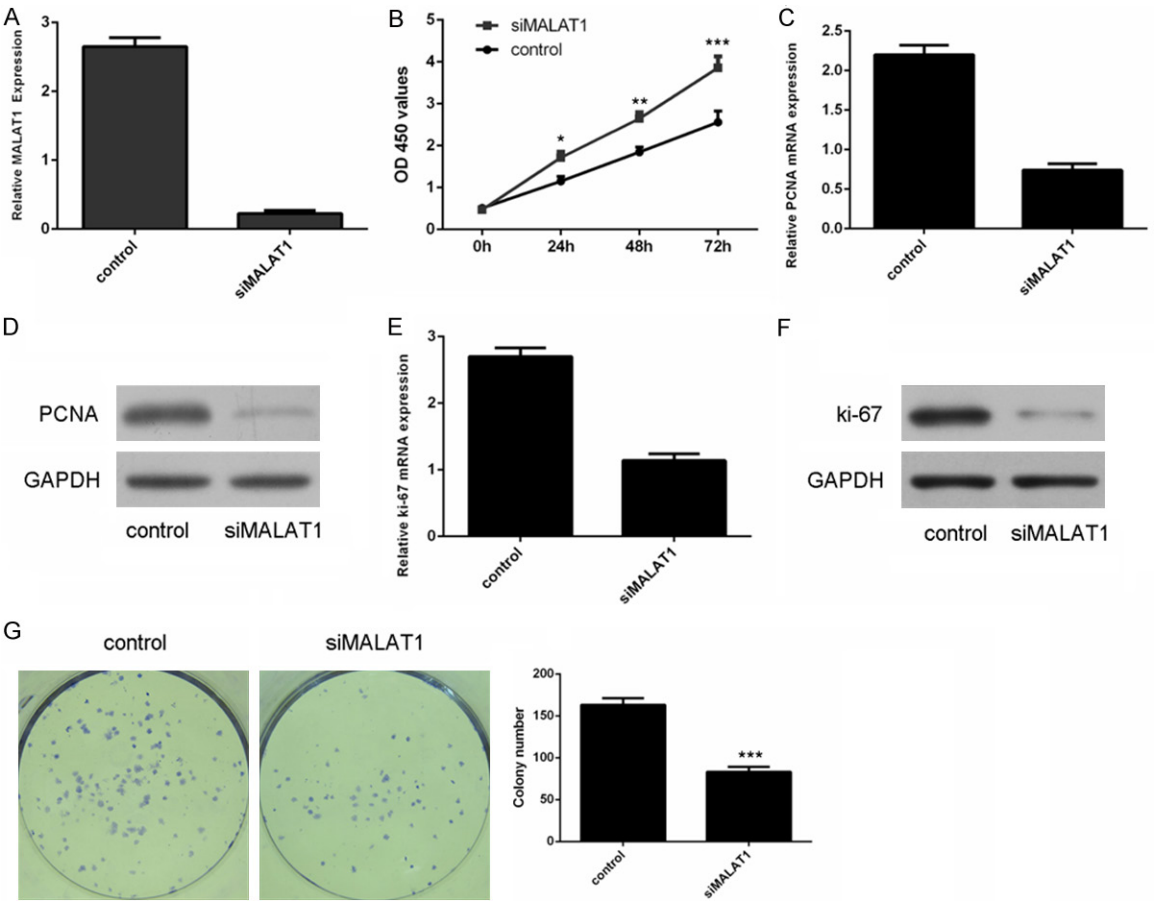

Figure 2. Inhibition of MALAT1 decreased the uveal melanoma cell proliferation and colony formation. A. Relative expression of MALAT1 in the MUM-2C cells after transfected with MALAT1 siRNA. B. Knockdown of MALAT1 suppressed the MUM-2C cells proliferation. C. Knockdown of MALAT1 inhibited the mRNA expression of PCNA. D. The protein expression of PCNA was detected using western blot. E. Knockdown of MALAT1 inhibited the mRNA expression of ki-67. F. The protein expression of ki-67 was detected using western blot. G. Knockdown of MALAT1 suppressed the MUM-2C cells colony formation. *p<0.05, **p<0.01, ***p<0.001.

Results

MALAT1 expression was increased in uveal melanoma tissues and cell

We found that MALAT1 was upregulated in uveal melanoma tissues compared to normal tissues (**Figure 1A**). Among them, MALAT1 expression was upregulated in 72% (18/25) uveal melanoma tissues compared to their paired normal tissues (**Figure 1B**). In addition, we demonstrated that MALAT1 was upregulated in uveal melanoma cell lines (MUM-2C, OCM-1A, MUM-2B and C918) compared to melanocyte cell line (D78).

Inhibition of MALAT1 decreased uveal melanoma cell proliferation and colony formation

The expression level of MALAT1 was decreased in MUM-2C cells after transfected with siMALAT1 (**Figure 2A**). Inhibition of MALAT1 suppressed uveal melanoma cell proliferation (**Figure 2B**). In addition, siMALAT1 inhibited the mRNA expression of PCNA in MUM-2C cells (**Figure 2C**). PCNA was downregulated in MUM-2C cells after transfected with siMALAT1 (**Figure 2D**). Moreover, the mRNA expression of ki-67 was downregulated in the MUM-2C cells after transfected with siMALAT1 (**Figure 2E**). SiMALAT1 inhibited the protein expression of ki-67 in MUM-2C cells (**Figure 2F**). We also demonstrated that inhibition of MALAT1 suppressed MUM-2C cell colony formation (**Figure 2G**).

Inhibition of MALAT1 suppressed uveal melanoma cell migration and invasion

To measure the role of MALAT1 on the invasive and migratory capability, transwell chamber

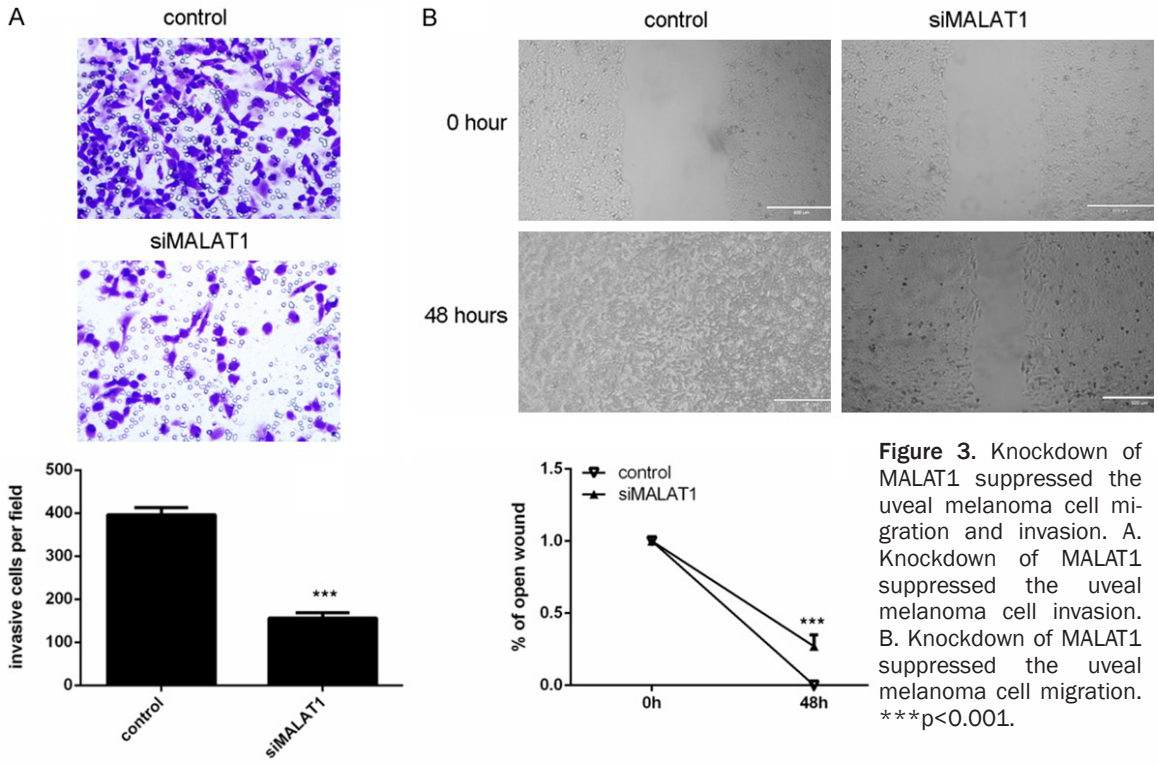

Figure 3. Knockdown of MALAT1 suppressed the uveal melanoma cell migration and invasion. A. Knockdown of MALAT1 suppressed the uveal melanoma cell invasion. B. Knockdown of MALAT1 suppressed the uveal melanoma cell migration. ***p<0.001.

and wound healing assay were done in the MUM-2C cell. We demonstrated that knockdown of MALAT1 inhibited MUM-2C cell invasion (**Figure 3A**). Moreover, knockdown of MALAT1 suppressed cell migration of the MUM-2C cell (**Figure 3B**).

MALAT1 negatively regulated miR-140 expression

We also confirmed that knockdown of MALAT1 can promote the miR-140 expression in the MUM-2C cell (**Figure 4A**). Moreover, inhibition of MALAT1 could suppress the expression of Slug in MUM-2C cell (**Figure 4B** and **4C**). qRT-PCR analysis demonstrated that inhibition of MALAT1 could suppress the expression of ADAM10 in the MUM-2C cell (**Figure 4D** and **4E**).

MiR-140 was downregulated in uveal melanoma tissues and cell

We found that miR-140 was downregulated in the uveal melanoma tissues compared to normal tissues (**Figure 5A**). Among them, miR-140 expression was upregulated in the 68% (17/25) uveal melanoma tissues compared to their paired normal tissues (**Figure 5B**). The expression level of miR-140 was inversely correlated with the MALAT1 expression level in uveal melanoma tissues (**Figure 5C**). In addition, we demonstrated that the expression of miR-140 was downregulated in uveal melanoma cell lines (MUM-2C, OCM-1A, MUM-2B and C918) compared to one melanocyte cell line (D78) (**Figure 5D**).

Discussion

In this study, we demonstrated that MALAT1 was upregulated in the uveal melanoma tissues compared to normal tissues. Among them, MALAT1 expression was upregulated in the 72% (18/25) uveal melanoma tissues compared to their paired normal tissues. Knockdown of MALAT1 suppressed uveal melanoma cell proliferation, colony information, invasion and migration. Moreover, we showed that knockdown of MALAT1 promoted miR-140 expression and suppressed the Slug and ADAM10 expression in the MUM-2C cell. In addition, we demonstrated that miR-140 was downregulated in uveal melanoma tissues compared to normal tissues and cell lines. The expression level of MALAT1 was inversely correlated with the expression level of miR-140 expression in uveal melanoma tissues. These results suggested

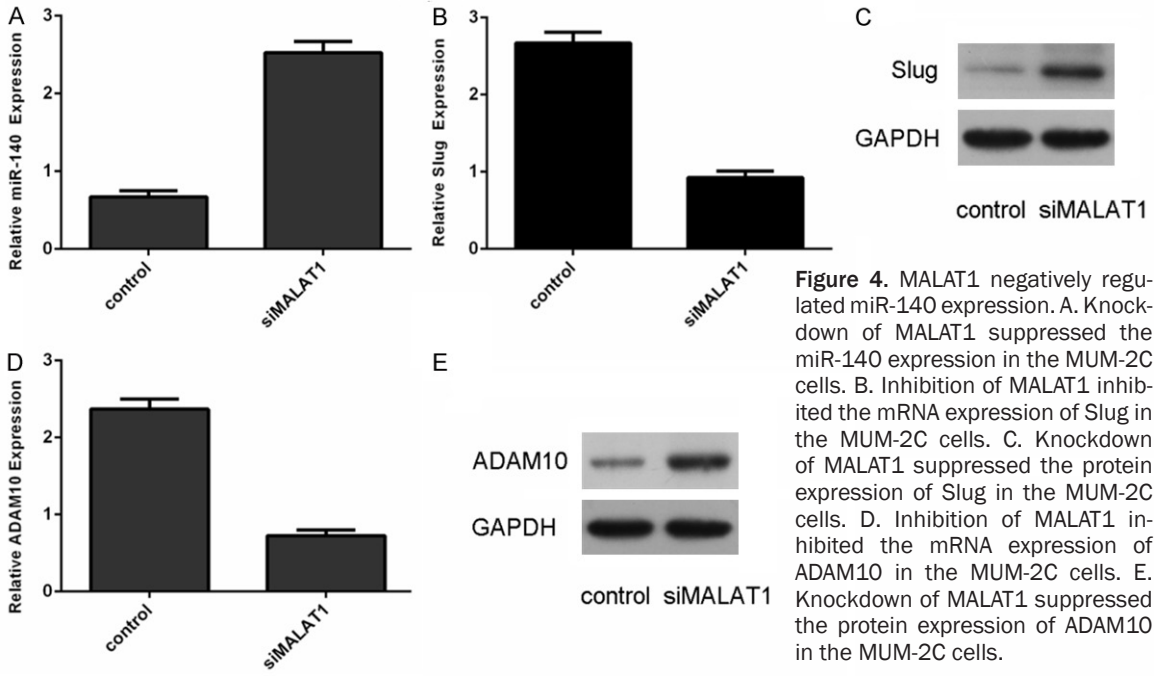

Figure 4. MALAT1 negatively regulated miR-140 expression. A. Knockdown of MALAT1 suppressed the miR-140 expression in the MUM-2C cells. B. Inhibition of MALAT1 inhibited the mRNA expression of Slug in the MUM-2C cells. C. Knockdown of MALAT1 suppressed the protein expression of Slug in the MUM-2C cells. D. Inhibition of MALAT1 inhibited the mRNA expression of ADAM10 in the MUM-2C cells. E. Knockdown of MALAT1 suppressed the protein expression of ADAM10 in the MUM-2C cells.

that MALAT1 played an oncogenic role in the development of uveal melanoma.

MALAT1, also named as NEAT2 (non-coding nuclearenriched abundant transcript 2), is a highly conserved lncRNA with about 8000-nt in length [14, 23, 28, 30]. Previous studies demonstrated that MALAT1 expression was upregulated in many tumors such as bladder cancer, gastric cancer, osteosarcoma and pancreatic cancer [26, 31-33]. For example, Pang et al [34]. demonstrated that MALAT1 expression was higher in the pancreatic cancer than in non-cancerous tissues and correlated with tumor size, clinical stage, distant metastasis and lymph node metastasis. Jin et al [35]. showed that MALAT1 expression was increased in the triple-negative breast cancer tissues. Knockdown of MALAT1 suppressed cell motility, proliferation, and increased cell apoptosis. Downregulation of MALAT1 promoted expression of miR-1 in breast cancer cell. Zhang et al [36]. demonstrated that MALAT1 expression was upregulated in clear cell renal cell carcinoma (ccRCC) tissues ad cells. Knockdown of MALAT1 inhibited renal cancer cell migration, proliferation and invasion. However, the role of MALAT1 still remains unknown. In this study, we demonstrated that MALAT1 was upregulated in the uveal melanoma tissues compared to normal tissues. Among them, MALAT1 expression was upregulated in the 72% (18/25) uveal melanoma tissues compared to their paired normal tissues. Knockdown of MALAT1 suppressed the uveal melanoma cell proliferation, colony information, invasion and migration. These data suggested that MALAT1 plays an oncogene LncRNA in the uveal melanoma.

Previous study revealed that knockdown of MALAT1 promoted miR-140 expression in the glioma cells [37]. In line with this data, we also showed that knockdown of MALAT1 could promote miR-140 expression in the MUM-2C cell. Increasing studies have suggested that miR-140 acts an important role in many tumors including non-small cell lung cancer, colorectal cancer, ovarian cancer and esophageal cancer [38-41]. Moreover, Li et al [41]. demonstrated that miR-140 was downregulated in esophageal cancer tissues and knockdown of miR-140 increased esophageal cancer cell invasion through targeting Slug expression. Kai et al [42]. showed that miR-140-5p could inhibit tongue squamous cell carcinoma invasion and migration by directly targeting ADAM10 expression. In our study, we revealed that miR-140 was downregulated in the uveal melanoma tissues and cell lines. The expression level of MALAT1 was inversely correlated with miR-140 expression level in uveal melanoma tissues. In addition, knockdown of MALAT1 promoted the expression of Slug and ADAM10, which were the direct target genes of miR-140. These data

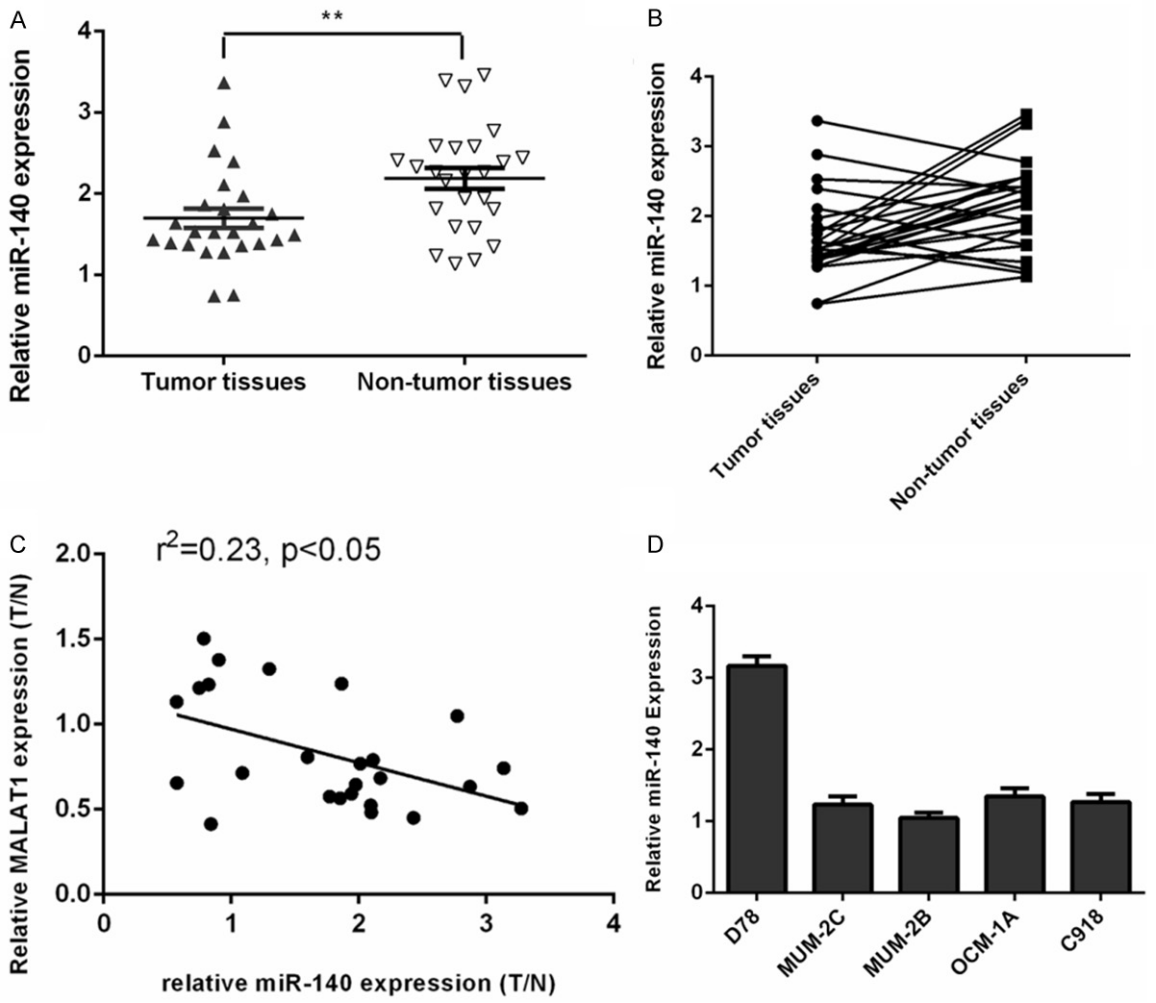

Figure 5. miR-140 was downregulated in uveal melanoma tissues and cell. A. The expression of miR-140 in the uveal melanoma tissues and normal tissues was measured using qRT-PCR. B. miR-140 expression was upregulated in the 68% (17/25) uveal melanoma tissues compared to their paired normal tissues. C. The expression level of miR-140 was inversely correlated with the MALAT1 expression level in uveal melanoma tissues. D. The expression of miR-140 in the uveal melanoma cell lines (MUM-2C, OCM-1A, MUM-2B and C918) and one melanocyte cell line (D78) was detected using qRT-PCR. **p<0.01.

suggested that MALAT1 suppressed the uveal melanoma cell proliferation and invasion through modulating miR-140 expression.

In conclusion, we demonstrated that MALAT1 was upregulated in the uveal melanoma tissues and cell lines. Moreover, knockdown of MALAT1 suppressed the uveal melanoma cell proliferation, colony information, invasion and migration partly through modulating miR-140 expression.

Acknowledgements

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