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miR-137 suppresses tumor growth of malignant melanoma by targeting aurora kinase A

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ABSTRACT

As an oncogene, aurora kinase A (AURKA) is overexpressed in various types of human cancers. However, the expression and roles of AURKA in malignant melanoma are largely unknown. In this study, a miR-137-AURKA axis was revealed to regulate melanoma growth. We found a significant increase in levels of AURKA in melanoma. Both genetic knockdown and pharmacologic inhibition of AURKA decreased tumor cell growth *in vitro* and *in vivo*. Further found that miR-137 reduced AURKA expression through interaction with its 3′ untranslated region (3′UTR) and that miR-137 was negatively correlated with AURKA expression in melanoma specimens. Overexpression of miR-137 decreased cell proliferation and colony formation *in vitro*. Notably, re-expression of AURKA significantly rescued miR-137-mediated suppression of cell growth and clonality. In summary, these results reveal that miR-137 functions as a tumor suppressor by targeting AURKA, providing new insights into investigation of therapeutic strategies against malignant melanoma.

1. Introduction

Melanoma is one of the highly aggressive cutaneous malignant tumors with increasing incidence and lethality in the world [1].

Most patients are diagnosed at advanced stage and the median overall survival is short, so effective therapies against malignant melanoma are urgently needed. Although several signaling molecules such as BRAF, MEK involved in malignant melanoma have been identified and provided potential targets for treatment [2,3], the molecular mechanisms involved in the development of melanoma are still not entirely clear.

Aurora kinase A (AURKA) is one of the mitotic serine/threonine kinase family consisting of Aurora A, Aurora B, and Aurora C kinases, which are essential components of the chromosomal segregation and cell division [4]. AURKA is known to induce formation of the mitotic spindle, cell proliferation and migration. Moreover, overexpression or dysfunction of AURKA is associated with invasion, senescence, poor prognosis and is observed in several tumor types, including colon, liver, prostate, gastric cancers, breast cancers and lung cancers [5–7]. However, little is known about the expression and regulation of AURKA in malignant melanoma and especially its post-transcriptional regulation by microRNAs.

MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression through a post-transcriptional manner. General mechanism of miRNA function is the repression of translation or direct mRNA cleavage after binding complementary sequences in the 3′-UTR of mRNAs [8]. It is well known that several microRNAs function as oncogenes and others act as tumor suppressors [9,10]. Therefore, we hypothesize that if some specific miRNAs can affect the expression of AURKA in malignant melanoma.

Here, we revealed a miR-137-AURKA axis that regulated cell growth in malignant melanoma. Re-expression of AURKA significantly restored miR-137- mediated inhibition of cell growth and clonality. Meanwhile, we explore the feasibility of using miR-137 functions as a tumor-suppressor by targeting AURKA, providing new insights into investigation of treatment strategies against malignant melanoma.

2. Materials and methods

2.1. Tissue specimens, cell lines and transfection

A total of 40 snap-frozen normal and malignant melanoma

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specimens were collected. Human A2058, A375, A875, SKMEL5 melanoma cells, normal skin epithelial TE353-SK cells and immortal keratinocyte Hacat cells were obtained from ATCC. All cells were cultured in DMEM/RPMI 1640 (Hyclone) medium supplemented with 10% FBS (Hyclone), 100 U/ml penicillin and 100 μg/ml streptomycin in humidified air at 37 °C with 5% CO_2. The small-molecule AURKA inhibitor MLN8237, were provided by Selleck Chemicals (Houston, TX, USA). Control miRNA (pre-miR-control) and pre-miR-137 (Applied Biosystems, Foster City, CA, USA), siRNA specific for AURKA and control siRNA (si-Control), control pcDNA3.1 empty vector and pcDNA3.1-AURKA were transiently transfected into cells using Lipofectamine 3000 Transfection Reagent (Invitrogen, CA, USA).

2.2. Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cells and tissues using Trizol reagent (Invitrogen, CA, USA). qRT-PCR was performed according to the manufacturer's protocol (TaKaRa) and the corresponding data analysis was described earlier [11]. The expression of miRNAs and mRNAs was normalized with reference to U6 small nuclear RNA and GAPDH respectively.

2.3. Western blot and immunohistochemistry (IHC)

10^6 cells were seeded into 6-well plates and subjected to indicated treatment. The whole-cell protein lysates were isolated using RIPA and protein concentrations were examined using the Bradford protein assay (Bio-Rad, Hercules, CA, USA), and western blot analysis was performed as described previously [12]. IHC staining of total and phosphorylated AURKA protein in tissues and xenograft tumor were done as previously described [11]. Photomicrographs were taken with an Olympus microscope BX51. AURKA (D3E4Q) Rabbit mAb and phospho-AURKA (Thr288, p-AURKA) rabbit mAb were purchased from Cell Signaling Technology (Denvers, MA, USA).

2.4. Cell proliferation assay

3000 cells were seeded in triplicate into 96-well plates. After incubated overnight, cells were treated with MLN8237 as indicated. In some experiments, the cells were transfected with gene-specific siRNA or pCDNA3.1 vector, and were subjected to serum-free medium. After treatment for an additional 4 d, cells were collected and subjected to trypan blue staining. The viable cells (cells that excluded trypan blue) were counted using a haemocytometer.

2.5. Soft agar colony formation assay

After transfection or treated with MLN8237, cells were harvested. Cloning ability in soft agar was measured under a stereoscopic microscope in triplicate of a 6-well plate as described previously [13].

2.6. In vivo studies

The BALB/c female mice (6-week-old) were purchased from the Weitonglihua Biotechnology (Beijing, China). 10^6 cells were resuspended in 100 μl PBS and subcutaneously injected into the flanks of nude mice. When the tumors reached a mean size of 50–100 mm^3, mice were assigned into 3 groups (6 mice each) receiving vehicle orally (100 μl of 10% 2-hydroxypropyl-β-cyclodextrin [Sigma-Aldrich]/1% sodium bicarbonate) or MLN8237 (15 mg/kg, and 30 mg/kg in a final formulation in 10% 2-hydroxypropyl-β-cyclodextrin/1% sodium bicarbonate) for 27 consecutive days. Tumor volumes were monitored and calculated every 3 days by the formula π (length $\times$ width^2)/6.

2.7. Luciferase activity assay

A375 and SKMEL5 cells were cultured in 6-well plates, receiving transfection with either pGL3-AURKA-3′UTR vector or pGL3-AURKA-3′UTR-Mut vector, together with pre-miR-124 or control. At 48 h post-transfection, relative luciferase activity was measured using a Dual-Luciferase system (Promega, Madison, Wisconsin) according to the manufacturer's instructions.

2.8. Statistical analysis

All data are presented as mean ± s.e.m. Two-tailed *t*-test or one-way ANOVA were used for analysis of differences between groups. Statistical analyses were performed using GraphPad Prism program version 5 (GraphPad Software, USA), with *P* values less than 0.05 considered statistically significant.

3. Results

3.1. AURKA is overexpressed in malignant melanoma

To determine the relevance of AURKA in human melanoma carcinoma, we firstly evaluated AURKA expression by qRT-PCR in 10 normal skin tissues and 30 melanoma tissues. It was demonstrated that AURKA expression was significantly upregulated in melanoma, compared to normal skin tissues, consistent with the results from reanalysis of a previously published dataset (GSE46517) (Fig. 1A), suggesting that AURKA may be involved in melanoma development and progression. The overexpression of AURKA in melanoma tissues was further confirmed by IHC staining (Fig. 1B). Moreover, the levels of AURKA in several malignant melanoma cell lines were also determined using qRT-PCR, demonstrating a overexpression of AURKA in melanoma cell lines (Fig. 1C).

To evaluate the tumor-promoting action of AURKA in melanoma development, we further examined the effects of AURKA on cell growth and colony formation. The overexpression of AURKA was verified, and increased phosphorylation of AURKA (p-AURKA) was shown in A2058 and A375 cells after pcDNA3.1-AURKA transfection (Fig. 1D). As shown in Fig. 1E, AURKA upregulation by transfecting the plasmids carrying AURKA (pcDNA3.1-AURKA) markedly enhanced the proliferation and clone formation abilities of melanoma cells.

Together, these results demonstrated that AURKA was overexpressed in malignant melanoma, and may functions tumor-promoting action in melanoma development, making AURKA as a potential target in melanoma therapy.

3.2. Downregulation of AURKA inhibits malignant melanoma cell growth

To further investigated potential roles of AURKA in melanoma treatment, we inhibited AURKA activity by either genetic knockdown or pharmacologic inhibitor. As shown in Fig. 2A, transfection with AURKA-targeting siRNA, but not control siRNA, decreased the levels of AURKA expression and phosphorylation in A375 and SKMEL5 cells. As expected, silencing AURKA significantly attenuated the proliferation and clone formation abilities of these cells compared to control cells (Fig. 2B). Furthermore, We applied MLN8237, an available small-molecule AURKA inhibitor in preclinical studies to inhibit AURKA phosphorylation. It was demonstrated that MLN8237 effectively inhibited AURKA activity and effectively suppressed cell growth and clone formation in a dose-