



# Upregulation LncRNA MEG3 expression suppresses proliferation and metastasis in melanoma via miR-208/SOX4

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## Abstract

Long non-coding RNAs (lncRNAs) regulate gene expression and play a significant role in cancer progression. Previously, downregulation of lncRNA MEG3 was shown to associate with poor clinical outcomes in melanoma patients. The basis for this association has not been described and the aims of this study were to identify a role for lncRNA MEG3 in melanoma and to describe its regulatory mechanism of action. RT-qPCR was used to detect lncRNA MEG3 expression in melanoma cells and tissues. Luciferase reporter assays were used to identify lncRNA MEG3 downstream targets. Melanoma cells were transfected with various expression vectors and these transfected cells were assessed for; migration, colony formation, proliferation, in vivo tumorigenesis, and metastatic potential. Melanoma cell lines were found to be sensitive to lncRNA MEG3 expression levels and overexpression was found to inhibit melanoma cell proliferation and invasion, both in vitro and in vivo. Luciferase reporter assays identified miR-208 and SOX4 as downstream targets of lncRNA MEG3. Overexpression of miR-208 and silencing of SOX4 rescued invasion and proliferation by cells that overexpressed lncRNA MEG3. Moreover, lncRNA MEG3 inhibited cancer stem cell differentiation and suppressed melanoma progression and metastasis through inhibition of miR-208 by SOX4.

**Keywords** LncRNA MEG3 · Melanoma · Proliferation · Metastasis · miR-208

## Introduction

Melanoma is a very aggressive skin cancer in that its metastatic progression is rapid. Early diagnosis is essential for melanoma treatment because advanced melanoma is

resistant to conventional therapy, resulting in poor survival outcomes [1, 2]. Long non-coding RNAs (lncRNAs) were originally considered non-functional, and were therefore termed “genomic dark matter” [3]. However, further study demonstrated lncRNAs to be important to cellular function. Many lncRNAs have been characterized but few have been functionally identified, although some lncRNAs have been shown to regulate tumorigenesis. For melanoma, lncRNAs have been shown to be differentially expressed and to regulate metastasis as well as cancer progression. LncRNA FOXD3-AS1 was demonstrated to enhance proliferation, migration, and invasion of dermal malignant melanoma by regulation of miRNA (miR)-325/mitogen-activated protein kinase kinase kinase 2 (MAP3K2) [4]. LncRNA LINC-PINT functions to suppress melanoma cell migration and proliferation through zeste 2 polycomb repressive complex 2 subunit (EZH2) enhancer recruitment [5]. LncRNA LINC00518 functions as a competitor of endogenous RNA, accelerating malignant melanoma metastasis through the miR-204-5p/adaptor relevant protein complex 1 subunit sigma 2 (AP1S2) axis [6].

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Maternally expressed 3 (MEG3) is a suppressor for multiple cancers including liver cancer and glioma [7, 8], but is also widely expressed within various normal tissues. LncRNA MEG3 suppresses laryngeal cancer cell proliferation by induction of apoptosis via the miR-23a/apoptotic protease activating factor 1 (APAF-1) axis [9]. LncRNA MEG3 impacts cell invasion, migration, and proliferation of ovarian cancer cells by regulation of the phosphatase and tensin homolog (PTEN) [10]. However, a role for lncRNA MEG3 in melanoma has not been described. Therefore, the aims of this study were to identify a role for lncRNA MEG3 in melanoma and to describe its regulatory mechanism of action.

## Materials and methods

### Tissue samples

We obtained 10 melanoma tumor tissues and paired normal skin tissues from The First Medical Center of PLA General Hospital. Tissues were maintained in liquid nitrogen. All patients supplied written informed prior to the study. The Ethics Committee of the First Medical Center of PLA General Hospital approved all research protocols (No. 2021-2-20).

### Cell culture

The melanoma cell lines, SK-MEL-2 and A357, and human normal epidermal melanocytes (PIG1) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) in a humidified incubator at 37 °C with 5% CO<sub>2</sub>.

### RNA interference and overexpression

We obtained; a miR-208 mimic (100 nM; mature miR-208: 5'-AUAAGACGAGCAAAAAGCUUGU-3'), an siRNA for SRY-box transcription factor 4 (si-SOX4, final concentration 20 nM; 5'-GCGACAAGAUCUUUCAUTT-3'), and a lncRNA MEG3 overexpression vector (lncRNA MEG3, final concentration 20 nM) from RiboBio (Guangzhou, China). Transfections were completed with Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

### Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

RNA was extracted with TRIzol reagent (Invitrogen) and cDNA synthesized with a pTRUEScript First Strand cDNA Synthesis Kit (Aidlab, Beijing, China). RT-qPCR utilized 2× SYBR Green qPCR Mix (Invitrogen) with an ABI 7900HT qPCR system (Thermo Fisher Scientific, Waltham, USA), with expression measured as fold-change using the  $2^{-\Delta\Delta CT}$  method. The RT-qPCR amplification primers were: lncRNA MEG3 forward: 5'-CTGCCCATCTACACCTCAG-3', reverse: 5'-CTCTCCGCCGTCTGCGCTAGGGGCT-3'; miR-208 RT primer: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTGCACCTGGATACGACACAAGCT-3', forward: 5'-TGCGGATAAGACGAGCAAAAAG-3'; SOX4 forward: 5'-GTGAGCGAGATGATCTCGGG-3', reverse: 5'-CAGGTTGGAGATGCTGGACTC-3'; U6 forward: 5'-CTCGCTTCGGCAGCACA-3', reverse: 5'-AACGCTTCATTTGCGT-3'; and GAPDH forward: 5'-AATCCATCACCATCTTCC-3', reverse: 5'-CATCACGCCACAGTTTCC-3'. We normalized expression levels of SOX4 and lncRNA MEG3 to GAPDH, and miR-208 expression to U6.

### Cell proliferation assay

Cells were seeded into 96-well plates (3000 cells/well), which were incubated in medium complemented with 10% FBS. After incubation for 1, 2, or 3 days, Cell Counting Kit-8 (CCK8) solution (Dojindo, Japan) was added to each well, incubated for 1–2 h, and absorbance measured at 450 nm. Experiments were completed in triplicate.

### Colony formation assay

To assess colony formation, melanoma cells, 1 days after transfection, were seeded into 24-well plates (400 cells/well). The culture medium was replaced every 3 days. After ~14 days, cells were washed with 1× phosphate-buffered saline (PBS), stained with common Crystal Violet dye, and counted using an inverted microscope (IX83; Olympus Corporation, Tokyo, Japan).

### In vitro migration assay

Melanoma cell invasion and migration were assessed by Transwell assay (24-well insert, 8-mm pore size with polycarbonate membrane; Corning Costar, Lowell, MA, USA). Transfected melanoma cells ( $1 \times 10^5$  cells/chamber) in 200 μL of serum-free DMEM, were seeded into the upper chamber (Becton Dickinson, Franklin Lakes, NJ, USA). DMEM

with 20% FBS was placed into the lower chamber as a chemoattractant. After 1 days, cells in the upper chamber were removed with a cotton swab and washed with PBS. The cells in the bottom chamber were fixed with 4% paraformaldehyde, stained with 0.1% Crystal Violet, and five randomly selected fields counted with a phase-contrast microscope (Olympus).

### Plate colony formation assay

Transfected SK-MEL-2 and A375 cells were placed into 6-well plates (200 cells/well) and cultured for 10 days. Cell shape was monitored and culture medium was changed every 3 days. Prior to the end of the experiment, images were captured with a fluorescence microscope before the cells were washed twice with PBS. Next, 500  $\mu$ L of Giemsa dye was added to the wells for 10–20 min. Cells were washed three times with double-distilled water and images captured with a digital camera.

### Tumor xenograft formation and metastasis assay

Four-week-old male BALB/c nude mice were used in this study. A375 cells were transfected with the lncRNA MEG3 overexpressing lentiviral vector (LV-lncRNA MEG3). Transfected and non-transfected A375 cells were injected ( $5 \times 10^6$  viable cells) into the right flank of nude mice. Tumor volume was calculated with vernier calipers every 5 days. Tumor size was calculated as volume =  $0.5 \times \text{length} \times \text{width}^2$ . One month after implantation, the mice were sacrificed and tissues stained with Ki67.

For metastatic analysis, A375 cells were transfected with a luciferase expression vector and injected into mice via the tail vein ( $2 \times 10^5$  cells per injection). After 1 month, A375 metastatic cells were detected after tail vein injection of luciferin (150 mg luciferin/kg body weight) by bioluminescence imaging.

The Animal Ethics Committee of the First Medical Center of PLA General Hospital approved all animal experiments (No. 2021-2-1). The Guide for the Care and Use of Laboratory Animals (8th Ed.) was followed strictly.

### Dual luciferase reporter assay

Reporter plasmids were synthesized by insertion of lncRNA MEG3 or SOX4 3'-UTR sequences into the pmir-GLO vector (Promega, Madison, USA). Reporter plasmids and miR-208 mimics were co-transfected into human embryonic kidney (HEK) 239T cells with Lipofectamine 2000. After 2 days of culture, Firefly and Renilla luciferase activities were assessed with a Dual Luciferase Reporter

Assay System (Promega, Sunnyvale, USA) following protocol.

### Statistics analysis

Statistical results are expressed as means  $\pm$  standard deviation (SD). GraphPad Prism (GraphPad, La Jolla, USA) was used to analyze differences between groups. *P* values  $\leq 0.05$  were considered statistically significant.

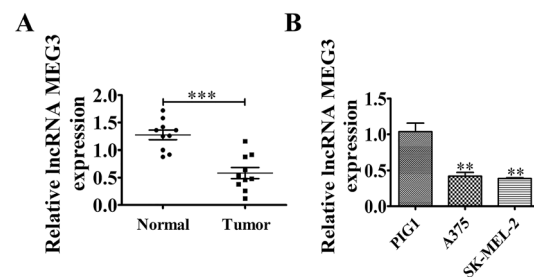
## Results

### lncRNA MEG3 is downregulated in melanoma cells and tissues

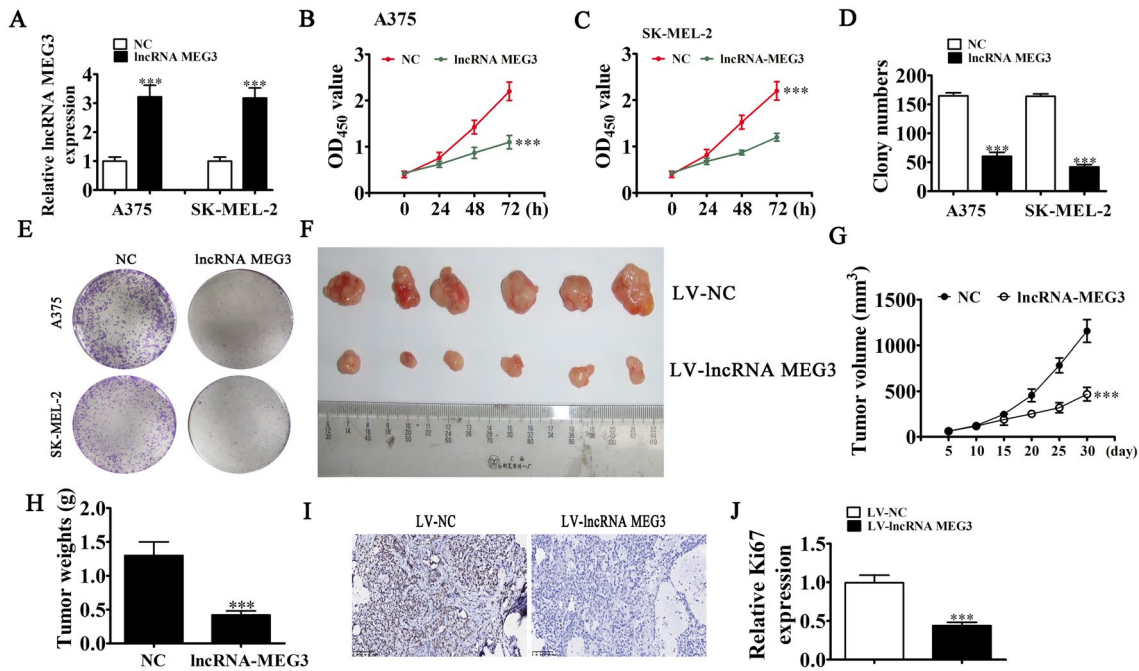
lncRNA MEG3 expression was assessed in 10 melanoma samples by RT-qPCR. Compared to normal skin tissue, circ\_0079593 expression was downregulated in melanoma tumor tissue (Fig. 1A). lncRNA MEG3 expression was also significantly lower in SK-MEL-2 and A375 cells compared to PIG1 cells (Fig. 1B).

### lncRNA MEG3 overexpression suppresses melanoma cell proliferation and tumor formation

A lncRNA MEG3 overexpression vector (LV-lncRNA MEG3) was transfected into SK-MEL-2 and A375 cells. RT-qPCR demonstrated lncRNA MEG3 expression levels to be increased significantly in SK-MEL-2 and A375 cells after transfection with the overexpression vector, compared to control (NC) (Fig. 2A). CCK8 (Fig. 2B, C) and colony formation assays (Fig. 2D, E) demonstrated overexpression of lncRNA MEG3 to suppress proliferation of



**Fig. 1** Expression of lncRNA MEG3 in melanoma. **A** RT-qPCR was used to measure lncRNA MEG3 expression in melanoma and normal skin tissues. Results are presented as means  $\pm$  SD. \*\*\**P* < 0.001 versus Normal. **B** lncRNA MEG3 expression in melanoma cell lines (SK-MEL-2 and A375) and PIG1 cells measured by RT-qPCR. Results are presented as means  $\pm$  SD. \*\**P* < 0.01 versus PIG1 cells



**Fig. 2** LncRNA MEG3 overexpression suppresses melanoma cell proliferation and tumor formation. **A** LncRNA MEG3 transfection efficiencies for SK-MEL-2 and A375 cells were validated by RT-qPCR. Results are presented as means  $\pm$  SD. \*\*\* $P$  < 0.001 versus NC. **B** and **C** CCK8 assay of A375 and SK-MEL-2 cells. Results are expressed as means  $\pm$  SD. \*\*\* $P$  < 0.001 vs NC. **D** and **E** Colony formation assay of A375 and SK-MEL-2 cells. Results are presented as means  $\pm$  SD. \*\*\* $P$  < 0.001 versus NC. **F** Representative images of

A375 xenograft tumors in nude mice. **G** Tumor size was measured every 5 days. Results are shown as means  $\pm$  SD. \*\*\* $P$  < 0.001 versus LV-NC. **H** Tumor weight 1 month after grafting. Results are shown as means  $\pm$  SD. \*\*\* $P$  < 0.001 versus LV-NC. **I** and **J** Immunohistochemical staining for Ki67 in tumor tissue of the LV-NC and LV-lncRNA MEG3 groups. Results are presented as means  $\pm$  SD. \*\*\* $P$  < 0.001 vs. LV-NC

SK-MEL-2 and A375 cell lines. In nude mice, LV-lncRNA MEG3-transfected A375 cells produced smaller xenografts (Fig. 2F–H) and reduced tumor weights (Fig. 2H) compared to control. Immunohistochemistry demonstrated lncRNA MEG3 overexpression to inhibit Ki67 expression (Fig. 2I, J). Taken together, these results suggest lncRNA MEG3 suppresses melanoma cell proliferation and tumor growth.

### Overexpression of lncRNA MEG3 suppresses melanoma cell metastasis and migration

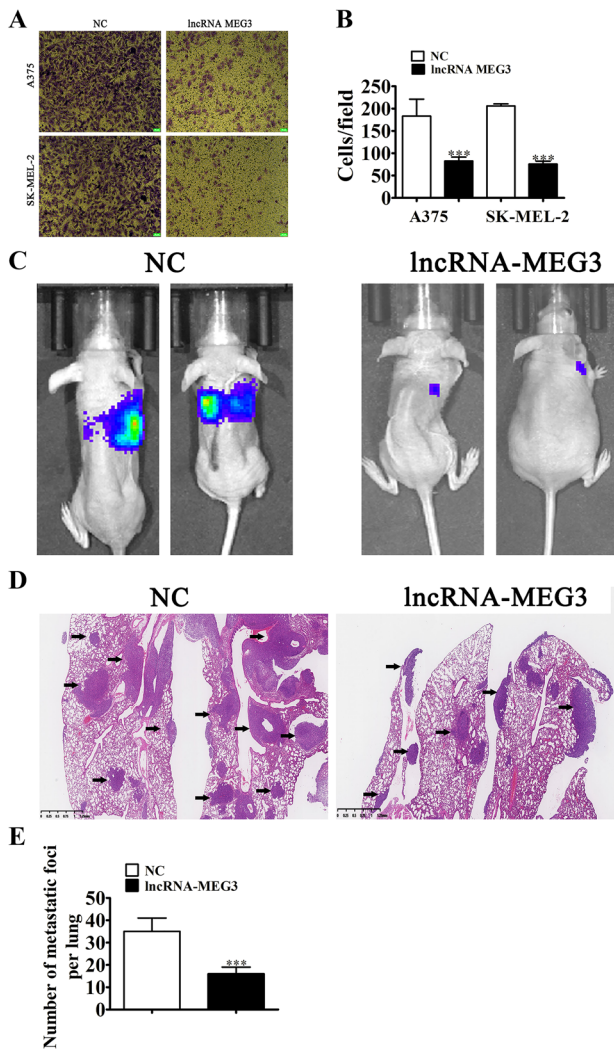
Transwell assays demonstrated overexpression of lncRNA MEG3 to suppress migration of SK-MEL-2 and A375 cells (Fig. 3A, B). Following injection of A375 cells, live imaging demonstrated metastasis to the lungs. Overexpression of lncRNA MEG3 decreased pulmonary metastasis, as judged by decreased numbers of metastatic foci in lung tissue (Fig. 3C–E). These findings suggest that lncRNA MEG3 suppresses melanoma cell invasion and metastasis.

### MiR-208 and SOX4 are downstream targets of lncRNA MEG3

Bioinformatics analysis predicted interactions among lncRNA MEG3 and miRNAs including; miR-143-3p, miR-556-3p, miR-145-5p, miR-219a-5p, miR-4782-3p, and miR-208. Luciferase reporter assays demonstrated miR-208 suppression of luciferase activity in WT cells, but not in MUT cell lines (Fig. 4A, B), demonstrating miR-208 to be a lncRNA MEG3 target.

Bioinformatics analysis predicted SOX4 to be a miR-208 downstream target. To verify this prediction, MUT or WT 3'-UTR-SOX4 sequences containing the miR-208 binding site were inserted into the luciferase reporter vector (Fig. 4C). The luciferase reporter vector was then transfected into HEK293 cells treated with the miR-208 mimic or not. Results demonstrated miR-208 mimic suppression of luciferase activity in WT cells but not in MUT cell lines (Fig. 4D), confirming SOX4 as a miR-208p target.





**Fig. 3** LncRNA MEG3 overexpression suppresses melanoma cell migration and metastasis. **A** and **B** Transwell assays were conducted to analyze A375 and SK-MEL-2 cell migration. Data are presented as means  $\pm$  SD. \*\*\* $P < 0.001$  vs NC. **C** Live imaging of A375 cells 5 weeks after intravenous tail injection. **D** and **E** The number of metastatic foci in lung tissues was assessed by hematoxylin and eosin staining. Results are presented as means  $\pm$  SD. \*\*\* $P < 0.001$  vs. NC

### MiR-208 overexpression and SOX4 suppression restore proliferation and invasion of melanoma cells overexpressing lncRNA MEG3

RT-qPCR confirmed an increase in lncRNA MEG3 expression following transfection with the lncRNA MEG3 overexpression vector. Overexpression of miR-208 or silencing of SOX4 did not influence lncRNA MEG3 expression in A375 and SK-MEL-2 cells (Fig. 5A, B), confirming that miR-208 and E2F3 were downstream of lncRNA MEG3. RT-qPCR demonstrated lncRNA MEG3 overexpression to decrease miR-208 expression. Silencing of SOX4 did not affect lncRNA MEG3-induced expression

of miR-16-5p (Fig. 5C, D), confirming that miR-208 was a lncRNA MEG3 downstream target. Furthermore, overexpression of lncRNA MEG3 increased SOX4 expression. However, when miR-208 was overexpressed, the lncRNA MEG3-induced upregulation of SOX4 expression was blocked. With silencing of SOX4, expression of SOX4 was decreased significantly (Fig. 5E, F), suggesting that lncRNA MEG3 enhanced SOX4 expression by inhibiting miR-208.

By CCK8 assay, SOX4 inhibition and miR-208 overexpression restored proliferation of SK-MEL-2 and A375 cells that overexpressed lncRNA MEG3 (Fig. 5G, H). Transwell migration assays demonstrated miR-208 overexpression and SOX4 silencing restored invasion of SK-MEL-2 and A375 cells that overexpressed lncRNA MEG3 (Fig. 5I–K).

### LncRNA MEG3 influences cancer stem cell differentiation by regulation of miR-208/SOX4

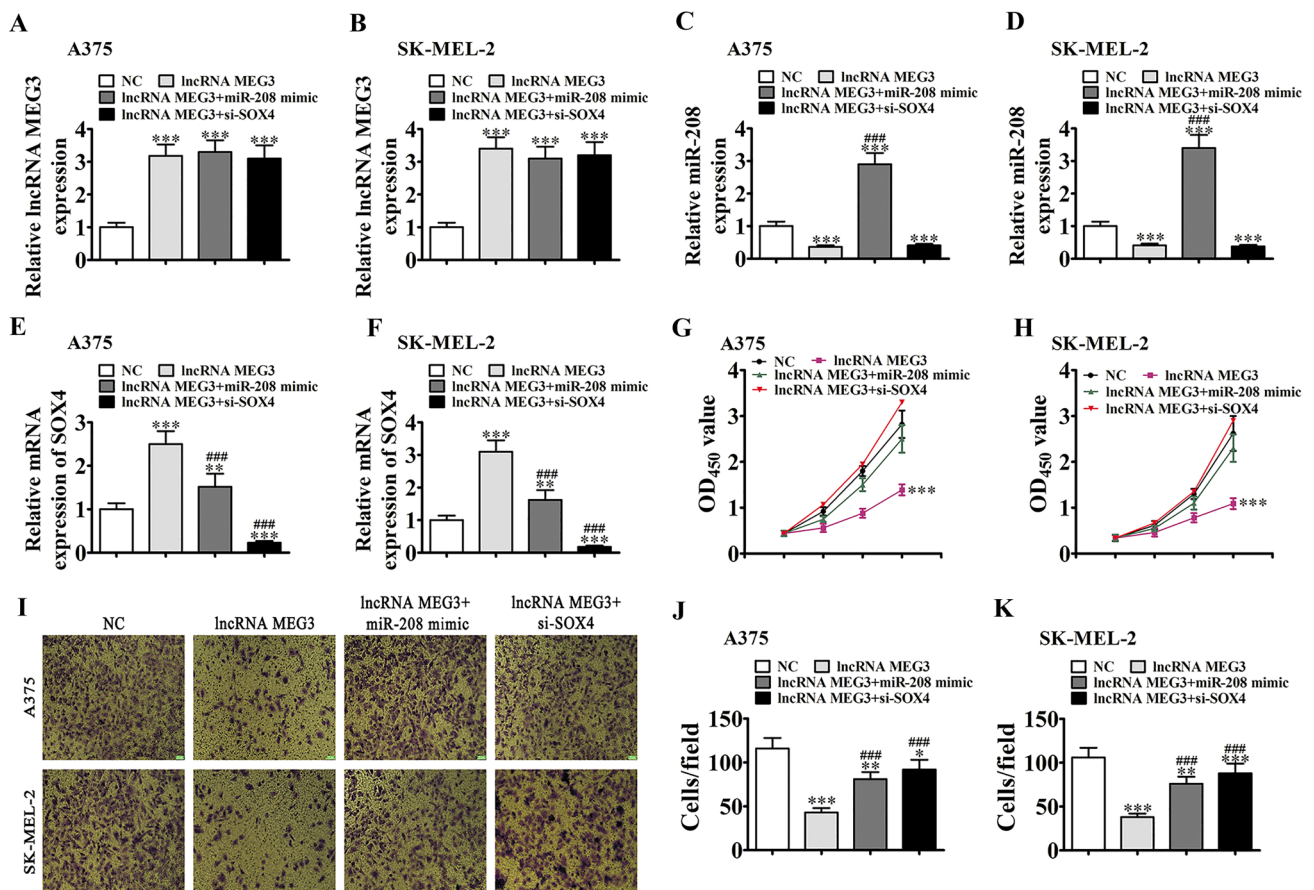
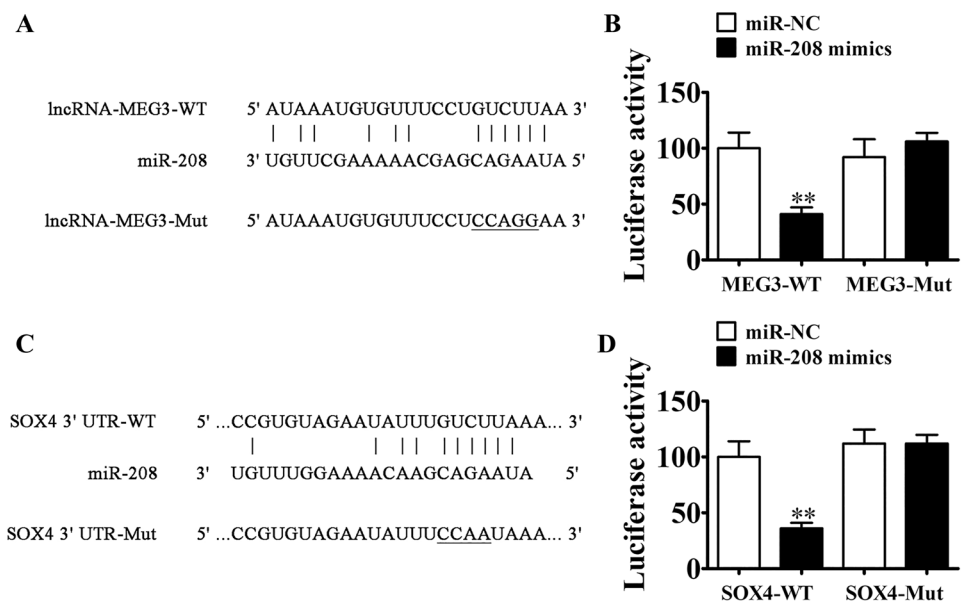
By tumor sphere formation assay, overexpression of lncRNA MEG3 inhibited SK-MEL-2 and A375 cell division. Overexpression of miR-208 and inhibition of SOX4 restored tumor sphere formation for cells that overexpressed lncRNA MEG3 (Fig. 6A–C).

## Discussion

LncRNA MEG3 is an important tumor suppressor that regulates cancer cell migration, proliferation, apoptosis, caspase-8, Bmi1/RNF2, and angiogenesis in various cancers by P53 targeting [11–14]. This investigation found lncRNA MEG3 expression to be lower in melanoma tumor tissues and melanoma cell lines than in normal tissues or cells. Overexpression of lncRNA MEG3 suppressed melanoma proliferation and metastasis in vivo and in vitro, suggesting that lncRNA MEG3 functions in the regulation of melanoma cell progression.

Recent investigation have reported lncRNAs to exert their function by targeting miRNAs [15, 16]. By luciferase reporter analysis, we found lncRNA MEG3 to interact with miR-208. Furthermore, we found that upregulation of lncRNA MEG3 decreased miR-208 expression, and that miR-208 overexpression reduced proliferation and invasion of cells that overexpressed lncRNA MEG3. Previously, miR-208 was shown to induce pancreatic cancer cell epithelial to mesenchymal transition, and to enhance tumor cell invasion and metastasis [17]. MiR-208 has also been shown to enhance cell proliferation of human esophageal squamous cell carcinoma through SOX6 inhibition [18]. MiR-208-3p has been reported to enhance hepatocellular carcinoma invasion and proliferation by regulation of ARID2 expression [19]. These results demonstrated miR-208 overexpression to

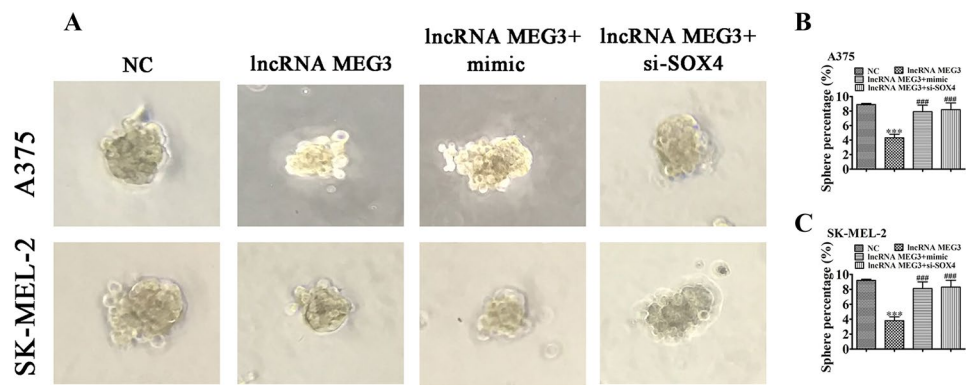
**Fig. 4** MiR-208 and SOX4 are lncRNA MEG3 downstream targets. **A** Predicted miR-208 binding sites for lncRNA MEG3. Mutated versions of lncRNA MEG3. **B** Relative luciferase activity was measured 48 h after transfection of HEK293T cells with miR-208 mimic/NC or with lncRNA MEG3 wild/Mut. Results are presented as means  $\pm$  SD.  $**P < 0.01$ . **C** Predicted miR-208 binding sites within the 3'-UTR of lncRNA MEG3. The mutated version of 3'-UTR-SOX4. **D** HEK293T cell relative luciferase activity was determined 2 days after transfection with miR-208 mimic/NC or with 3'-UTR-SOX4 wild/Mut. Results are presented as means  $\pm$  SD.  $**P < 0.01$



**Fig. 5** MiR-208 overexpression and SOX4 inhibition restored proliferation and invasion by melanoma cells that overexpressed lncRNA MEG3. **A–F** Melanoma cell expression of lncRNA MEG3, miR-208, and SOX4 as judged by RT-qPCR. Data are presented as means  $\pm$  SD;  $**P < 0.01$ ,  $***P < 0.001$  vs NC;  $###P < 0.001$  vs. lncRNA MEG3. **G**

and **H** CCK8 measurement of SK-MEL-2 and A375 cell proliferation. Data are presented as means  $\pm$  SD.  $***P < 0.001$  versus NC. **I–K** Transwell assay demonstrating SK-MEL-2 and A375 cell migration. Results are presented as means  $\pm$  SD.  $**P < 0.01$ ,  $***P < 0.001$  versus NC,  $###P < 0.001$  versus lncRNA MEG3

**Fig. 6** LncRNA MEG3 affects cancer stem cells by regulation of miR-208/SOX4. **A–C** Tumor sphere formation images of SK-MEL-2 and A375 cells. Results are presented as means  $\pm$  SD. \*\*\* $P < 0.001$  versus NC ### $P < 0.001$  vs. lncRNA MEG3



reinstate metastasis and proliferation by cells overexpressing lncRNA MEG3.

Previous investigations suggested that miR-208 interaction with SOX4 3'-UTR suppressed SOX4 mRNA levels. Herein, luciferase reporter analysis demonstrated SOX4 to be a miR-208 target, and that upregulation of lncRNA MEG3 promoted SOX4 expression. Silencing of SOX4 restored cellular proliferation and invasion in cells that overexpressed lncRNA MEG3. SOX4 is a transcriptional activator that regulates cell type maturation and differentiations [20]. SOX4 also regulates cancer stem cell differentiation [21]. Cancer stem cells are known to function in cellular differentiation, proliferation, migration, and angiogenesis [22–24]. Therefore, inhibition of cancer stem cells would suppress cancer proliferation and invasion. Herein, lncRNA MEG3 overexpression was shown to decrease cancer stem cell differentiation by regulation of miR-208/SOX4.

In summary, lncRNA MEG3 overexpression was shown to suppress melanoma cell invasion and proliferation by regulation of miR-208/SOX4 signaling. Furthermore, lncRNA MEG3 was identified as a candidate melanoma diagnostic biomarker and/or drug target. These results suggest this lncRNA to be a potential diagnostic tool and therapeutic treatment option for melanoma.

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**Author contributions** YY, LJ, YC, and ZL contributed to study concept and design. All authors collected the data and performed data analysis. JH, RW and YW interpreted data and completed the Figures and Tables. JB and YY prepared the initial draft of the article and approved the submitted version.

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**Data availability** The datasets used and/or analyzed for this study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** All the authors declare that they have no conflict of interest.

**Ethical approval and patient consent statement** Ethical approval was given by the Ethics Committee of The First Medical Center of PLA General Hospital.

**Informed consent** All patients provided written informed consent.

## References

- Sanchez JA, Robinson WA (1993) Malignant melanoma. *Annu Rev Med* 44:335–342. <https://doi.org/10.1146/annurev.me.44.020193.002003>
- Brandner JM, Haass NK (2013) Melanoma's connections to the tumour microenvironment. *Pathology* 45(5):443–452. <https://doi.org/10.1097/PAT.0b013e328363b3bd>
- Safa A, Gholipour M, Dinger ME, Taheri M, Ghafouri-Fard S (2020) The critical roles of lncRNAs in the pathogenesis of melanoma. *Exp Mol Pathol* 117:104558. <https://doi.org/10.1016/j.yexmp.2020.104558>
- Chen X, Gao J, Yu Y, Zhao Z, Pan Y (2019) lncRNA FOXD3-AS1 promotes proliferation, invasion and migration of cutaneous malignant melanoma via regulating miR-325/MAP3K2. *Biomed Pharmacother* 120:109438. <https://doi.org/10.1016/j.biopha.2019.109438>
- Xu Y, Wang H, Li F, Heindl LM, He X, Yu J et al (2019) Long non-coding RNA LINC-PINT suppresses cell proliferation and migration of melanoma via recruiting EZH2. *Front Cell Dev Biol* 7:350. <https://doi.org/10.3389/fcell.2019.00350>
- Luan W, Ding Y, Ma S, Ruan H, Wang J, Lu F (2019) Long non-coding RNA LINC00518 acts as a competing endogenous RNA to promote the metastasis of malignant melanoma via miR-204-5p/AP1S2 axis. *Cell Death Dis* 10(11):855. <https://doi.org/10.1038/s41419-019-2090-3>
- Zheng Q, Lin Z, Xu J, Lu Y, Meng Q, Wang C et al (2018) Long noncoding RNA MEG3 suppresses liver cancer cells growth through inhibiting  $\beta$ -catenin by activating PKM2 and inactivating PTEN. *Cell Death Dis* 9(3):253. <https://doi.org/10.1038/s41419-018-0305-7>



8. Zhang S, Guo W (2019) Long non-coding RNA MEG3 suppresses the growth of glioma cells by regulating the miR-96-5p/MTSS1 signaling pathway. *Mol Med Rep* 20(5):4215–4225. <https://doi.org/10.3892/mmr.2019.10659>
9. Zhang X, Wu N, Wang J, Li Z (2019) LncRNA MEG3 inhibits cell proliferation and induces apoptosis in laryngeal cancer via miR-23a/APAF-1 axis. *J Cell Mol Med* 23(10):6708–6719. <https://doi.org/10.1111/jcmm.14549>
10. Wang J, Xu W, He Y, Xia Q, Liu S (2018) LncRNA MEG3 impacts proliferation, invasion, and migration of ovarian cancer cells through regulating PTEN. *Inflamm Res* 67(11–12):927–936. <https://doi.org/10.1007/s00011-018-1186-z>
11. Wei GH, Wang X (2017) lncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway. *Eur Rev Med Pharmacol Sci* 21(17):3850–3856
12. Jia HY, Zhang K, Lu WJ, Xu GW, Zhang JF, Tang ZL (2019) LncRNA MEG3 influences the proliferation and apoptosis of psoriasis epidermal cells by targeting miR-21/caspase-8. *BMC Mol Cell Biol* 20(1):46. <https://doi.org/10.1186/s12860-019-0229-9>
13. Kumar MM, Goyal R (2017) LncRNA as a therapeutic target for angiogenesis. *Curr Top Med Chem* 17(15):1750–1757. <https://doi.org/10.2174/1568026617666161116144744>
14. Li J, Jiang X, Li C, Liu Y, Kang P, Zhong X et al (2019) LncRNA-MEG3 inhibits cell proliferation and invasion by modulating Bmi1/RNF2 in cholangiocarcinoma. *J Cell Physiol* 234(12):22947–22959. <https://doi.org/10.1002/jcp.28856>
15. Fan CN, Ma L, Liu N (2018) Systematic analysis of lncRNA-miRNA-mRNA competing endogenous RNA network identifies four-lncRNA signature as a prognostic biomarker for breast cancer. *J Transl Med* 16(1):264. <https://doi.org/10.1186/s12967-018-1640-2>
16. Militello G, Weirick T, John D, Döring C, Dimmeler S, Uchida S (2017) Screening and validation of lncRNAs and circRNAs as miRNA sponges. *Brief Bioinform* 18(5):780–788. <https://doi.org/10.1093/bib/bbw053>
17. Liu A, Shao C, Jin G, Liu R, Hao J, Song B et al (2014) miR-208-induced epithelial to mesenchymal transition of pancreatic cancer cells promotes cell metastasis and invasion. *Cell Biochem Biophys* 69(2):341–346. <https://doi.org/10.1007/s12013-013-9805-3>
18. Li H, Zheng D, Zhang B, Liu L, Ou J, Chen W et al (2014) Mir-208 promotes cell proliferation by repressing SOX6 expression in human esophageal squamous cell carcinoma. *J Transl Med* 12:196. <https://doi.org/10.1186/1479-5876-12-196>
19. Yu P, Wu D, You Y, Sun J, Lu L, Tan J et al (2015) miR-208-3p promotes hepatocellular carcinoma cell proliferation and invasion through regulating ARID2 expression. *Exp Cell Res* 336(2):232–241. <https://doi.org/10.1016/j.yexcr.2015.07.008>
20. Foronda M, Martínez P, Schoeftner S, Gómez-López G, Schneider R, Flores JM et al (2014) Sox4 links tumor suppression to accelerated aging in mice by modulating stem cell activation. *Cell Rep* 8(2):487–500. <https://doi.org/10.1016/j.celrep.2014.06.031>
21. Peng X, Liu G, Peng H, Chen A, Zha L, Wang Z (2018) SOX4 contributes to TGF- $\beta$ -induced epithelial-mesenchymal transition and stem cell characteristics of gastric cancer cells. *Genes Dis* 5(1):49–61. <https://doi.org/10.1016/j.gendis.2017.12.005>
22. Battle E, Clevers H (2017) Cancer stem cells revisited. *Nat Med* 23(10):1124–1134. <https://doi.org/10.1038/nm.4409>
23. Yoshida GJ (2018) Emerging roles of Myc in stem cell biology and novel tumor therapies. *J Exp Clin Cancer Res* 37(1):173. <https://doi.org/10.1186/s13046-018-0835-y>
24. Vlashi E, Pajonk F (2015) Cancer stem cells, cancer cell plasticity and radiation therapy. *Semin Cancer Biol* 31:28–35. <https://doi.org/10.1016/j.semcancer.2014.07.001>

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