STING promotes invasion and migration of uveal melanoma through p38-MAPK signaling

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Abstract. Uveal melanoma (UM) is the most common intraocular malignant tumor in adults, with a lack of effective treatment for metastasis and a poor prognosis. Stimulator of interferon genes (STING, also known as TMEM173) plays an important role in tumor development by regulating cell proliferation, metastasis and other cellular processes. However, the function of STING in UM remains unclear and requires further investigation. The present study analyzed the expression status of STING to elucidate the mechanisms underlying UM. The correlation between STING and the prognosis of UM was evaluated based on UM RNA-seq data and clinical information extracted from The Cancer Genome Atlas database. Quantification of STING in UM cell lines and tissues was performed using the Wes Separation protein immunoassay. The effects of STING on the proliferation, migration and invasion of UM cells were investigated using Cell Counting Kit-8, Transwell and wound healing experiments. Survival analysis demonstrated that high levels of STING in UM tissues indicated a poor prognosis. The expression of STING in UM tissues was higher than that in the choroid membranes. Furthermore, it was found that downregulation of STING expression in UM cells suppressed migration and invasion, whereas overexpression of STING significantly promoted migration and invasion. Notably, STING had no significant effect on UM cell proliferation. It was also identified that STING positively upregulated the phosphorylation of p38 mitogen-activated protein kinase (p38-MAPK) in UM cells, enhancing cell migration and invasion, which the p38-MAPK inhibitor SB203580 reversed. Finally, the results of the present study demonstrated that high STING expression in UM indicates a poor prognosis. STING was revealed to promote the migration and invasion of UM cells through p38-MAPK signaling.

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Abbreviations: STING, stimulator of interferon genes; UM, uveal melanoma; p38-MAPK, p38 mitogen-activated protein kinase; TCGA, The Cancer Genome Atlas; CCK-8, Cell Counting Kit-8; BAP1, BRCA1-associated protein 1; TBK-1, TANK-binding kinase 1; RPMI-1640, Roswell Park Memorial Institute 1640; IFN, interferon; FBS, fetal bovine serum; PS, penicillin-streptomycin; shRNA, short hairpin RNA; Wes, Wes Separation; GEPIA, Gene Expression Profiling Interactive Analysis; ROC, receiver operating characteristic; OS, overall survival; DFS, disease-free survival; ERK, extracellular regulated protein kinases; JNK, c-Jun N-terminal kinase

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Key words: STING, p38-MAPK, uveal melanoma, invasion, migration

Introduction

Uveal melanoma (UM) is the most common intraocular malignancy among adults. Despite adequate and early primary tumor treatment, almost 50% of patients ultimately develop metastasis (1). Patients with metastasis have a disease-related death within 1 year (4 to 15 months), due to poor response to any treatments (2). Various features has been are known to related to UM metastasis, including larger tumor diameter and thickness, epithelioid cell type, loss of chromosome 3 heterozygosity, preferentially expressed antigen in melanoma expression, and loss of BRCA1-associated protein 1 (BAP1) mutations (3). UMs can be divided into Class 1 with low metastatic risk and Class 2 with high metastatic risk based on gene expression profiling including 12-genes expression (4). However, there is still a subset of patients categorized as Class 1 developing metastasis. It is necessary to identify additional and reliable biomarkers for metastatic prediction and potential therapeutic targets in UM.

Stimulator of interferon genes (STING, also known as TMEM173) is a transmembrane protein located in the endoplasmic reticulum and mitochondria, and exists in immune-related tissues, hematological malignancy and solitary tumor (5). An increasing number of studies demonstrated that in certain tumors, such as skin melanoma, breast cancer and gastrointestinal cancers, STING expression is lower than that in normal tissues and is positively associated with patients' prognosis by promoting intrinsic antitumour immunity (6-8).

However, the function of STING in tumors is complicated and controversial. In other tumors, STING levels increase and are negatively related to patient survival (9). STING can play a pro-tumorigenic role when activated by chemotherapy agents and facilitates cervical cancer progression during chronic inflammation (10,11). STING-dependent DNA sensing pathways suppress ferroptosis and promotes pancreatic tumorigenesis (12). In chromosomally unstable tumor cells, activated STING promotes cell invasion and metastasis (13). Increasing evidences demonstrated that STING is involved in tumorigenesis and metastasis.

In this context from literature review, it was observed that there had not been any studies on STING in UM. Therefore, a preliminary analysis on the relationship between STING and UM was performed by using publicly available data from The Cancer Genome Atlas (TCGA). In the present study, it was revealed that patients with higher STING expression had significantly shorter overall and disease-free survival times than those with lower STING expression. In addition, a significant correlation was identified between high risks of metastasis and higher expression of STING. This meaningful finding and the emerging controversial function of STING prompted the authors to investigate further its possible contribution in UM. Subsequently, this was investigated in patients with UM and cell lines and it was attempted to unveil the potential of STING. It was further confirmed that STING is abundant in UM tissues and cell lines and upregulated in UM tissues compared with para-UM tissues (choroid tissues). Increasing STING was found to promote the invasion and migration of UM cells *in vitro* by enhancing p38 mitogen-activated protein kinase (p38-MAPK) signaling. These findings suggested that STING plays a crucial role in the development and metastasis of UM, and inhibiting the STING-p38-MAPK pathway in intrinsic UM cells can be a potential therapeutic target.

Materials and methods

TCGA database analysis. The RNA-seq data and clinical information of 80 patients with UM were obtained from TCGA database (https://portal.gdc.cancer.gov/). Data analysis was performed using R x64 4.0.4 software (https://www.rstudios.co/). Overall survival (OS) and disease-free survival (DFS) analyses of STING expression were performed using the Gene Expression Profiling Interactive Analysis database (http://gepia.cancer-pku.cn) (14).

Patient tissues and cell cultures. A total of 20 clinical tissues were collected from patients who underwent primary enucleation at Eye & ENT Hospital of Fudan University (Shanghai, China), diagnosed with UM between January 2013 and December 2019. Among these, five were metastatic UM tissues obtained from patients who developed metastasis in the liver, lungs or other distant organs. By contrast, the other five were non-metastatic UM tissues obtained from patients who did not develop metastasis after more than five years of follow-up time. Retrospective data on survival and metastasis were obtained from medical records updating patients' follow-up information semi-annually. The remaining ten tissues comprised five primary UM tissues with surrounding para-UM tissues (choroid tissues), which were used to compare UM tissues and normal tissues. Written informed consent was obtained by all patients for using their tissues and information in researches. The present study was approved by The Ethics Committee of Eye & ENT Hospital of Fudan University (approval no. 2020044-1; Shanghai, China). Tissues were collected by an experienced pathologist immediately after enucleation and stored in liquid nitrogen. In the present study, none of the patients had received any treatment prior to enucleation. Clinicopathological characteristics (including age and sex distribution) are included in Table II.

The UM cell lines (Mel 202, 92.1, Mel270, Omm2.2, Omm2.3, Omm1 and Omm2.5) used in the present experiments were kindly provided by WuXi AppTec (https://www.wuxiapptec.com/zh-cn). THP-1 (Human Monocytic Cell Line), 293T (Human Embryonic Kidney Cell Line) and ARPE-19 (Adult Retinal Pigment Epithelial Cell Line) were purchased from the American Type Culture Collection. The UM cell lines, THP-1 and ARPE-19 were maintained in Roswell Park Memorial Institute 1640 (RPMI-1640) medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) and 293T in Dulbecco's Modified Eagle Medium (DMEM; all from Gibco; Thermo Fisher Scientific, Inc.) with 10% FBS and 1% PS. All the cell lines were cultured in a humidified incubator at 37˚C under 5% CO_2. These procedures were conducted in accordance with the highest ethical standards and best laboratory practices to ensure the validity and reliability of the findings.

Reagents. Primary antibodies targeting STING (cat no. 13647), p38-MAPK (cat. no. 8690), phosphorylated p38-MAPK (cat. no. 4511) and GAPDH (cat. no. 2118) were obtained from Cell Signaling Technology, Inc. The Anti-Rabbit Secondary antibody (cat. no. DM-001) Detection Module for Wes was purchased from ProteinSimple. The compound SB203580 (cat. no. S1076) was purchased from Selleck Chemicals. Wes Separation kits (12-230 kDa; cat. no. SM-W004; ProteinSimple) were used to perform our protein immunoassay procedures.

Transfection. Short hairpin RNA (shRNA) sequences (Genomeditech) were designed to downregulate STING expression, named shSTING, and the scramble shRNA control was named Scramble. The shRNA sequence targeting human STING and the scramble shRNA control sequences were as follows: 5'-TCTCAAGAGAAATCCGTGCGGA-3' (shSTING), and 5'-TTCTCCGAACGTGTCACGT-3' (Scramble). The double strands of shRNA were inserted into lentiviral vector through the pGMLV-SC5-PURO RNAi packaging plasmid. The concentrations of the two packaged lentiviral vectors were $5x10^8$ TU/ml. Human full-length STING cDNA was cloned into the expression plasmid pHBLV-CMV-MCS-3FLAG-EF1-ZsGreen-T2A-PURO by Hanbio Biotechnology Co. Ltd. to upregulate STING ($STING^+$), and the empty pHBLV-PURO lentiviral vector was used as negative control (Control). The concentrations of the two packaged lentiviral vectors were $4.5x10^8$ TU/ml and $3x10^8$ TU/ml, respectively.

UM cells mixed with targeted or negative control lentiviral vectors were seeded in 24-well-plates ($1.6x10^5$ cells vs. $3.2x10^6$ TU lentiviral vectors per well), incubated in a humidified incubator at 37˚C under 5% CO_2 for 4~6 h until

cell attachment, and then replaced with fresh mediums. Polybrene Reagent (cat. no. GM-040901; Genomeditech) was used for transfection with the concentration of 5 μg/ml. Stable cells with STING downregulated or upregulated were selected using puromycin with the concentration of 2 μg/ml. The transfected cells were used for further experiments after 48 h incubation.

Wes Separation protein immunoassay. Proteins were extracted from UM cells or tissues using RIPA buffer and a phosphatase inhibitor, followed using the Wes Separation (Wes) system according to the manufacturer's protocol. The Wes system is a next-generation hand-free capillary immunoassay platform combining protein separation with sensitive chemiluminescence and fluorescence immunodetection. This mature technology has been applied widely in vaccines, biopharmaceutical purification and other proteins-related researches (15-17).

For immunodetection, a Master Mix solution, sample buffer, ladder solution, and a chemiluminescence mixture were prepared. Next, the protein samples were mixed with the sample buffer and Master Mix and denatured at 95˚C for 5 min. The primary antibodies were diluted in 1:50~200. Ladder solution, protein samples, primary antibodies and secondary antibodies, chemiluminescence mixtures, and wash buffer were sequentially added to the reaction plate on-ice operation. The reaction plate and capillary tubes were placed into an automated analyzer that automatically loaded the proteins for separation and immunodetection in each capillary tube. Finally, immunoreactive protein bands were visualized according to chemiluminescence signals and quantified based on the signal intensities, using Compass Simple Western software (ProteinSimple).

Proliferation experiments. Cell proliferation was assessed using Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Inc.) according to the manufacturer's instructions. A total of $2.5x10^3$ UM cells were seeded into 96-well plates before transfection and treatment. 10 μl of CCK-8 reagent was added in each well and incubated at 37˚C for 2 h. Cell viability was measured at 450 nm at 0, 24, 48 and 72 h after transfection and drug treatment using microplate reader (Tecan Group, Ltd.).

Transwell and wound healing experiments. In brief, hydrated Transwell chambers (pore size, 8-μm, Corning, Inc.) were coated with Matrigel by RPMI-1640 medium in the cell incubator at 37˚C for 2 h. UM cells ($1x10^5$) were seeded into each Transwell chambers in RPMI-1640 medium without FBS. The Transwell chambers were immersed in 24-well plates containing RPMI-1640 medium with 10% FBS and the plates were incubated at 37˚C for 24 h. The cells were fixed in chambers using 4% paraformaldehyde at room temperature for 20 min and stained with 1% crystal violet at room temperature for 15 min. The cells attached to the upper surface of the chambers were wiped out, and cells that invaded through the pores were counted in six randomly selected fields (magnification, x100) using the light microscope (Leica Microsystems GmbH). The UM cells ($5x10^5$) were placed into six-well plates and cultured for 24 h. A 200-μl pipette was then used to scrape the cell layer in a straight line, creating a scratch. The cells were washed twice with RPMI-1640 medium without FBS, then cultured in RPMI-1640 medium supplemented with 1% FBS. Images were captured after 48 h, and the cell migration rate was compared with that of the initial scratch.

In the Transwell assays and wound healing experiments, although four UM cell lines were treated in different time and repeated experiments were performed, the backgrounds of the images compared at the same time/cell lines were consistent whether in Transwell assays or wound healing experiments.

Statistical analysis. To compare the differences between two groups of data, the unpaired Student's t-test was employed. MEDCAL software was used to identify the cutoff value of STING expression through receiver operating characteristic (ROC) curve analysis. The Kaplan-Meier test (followed by log-rank test) was used to analyze OS and DFS. The correlation between the clinical information of patients with UM and STING expression was assessed using the chi-square test. All statistical analyses were performed using IBM SPSS Statistics software (version 25.0; IBM Corp.). $^*P<0.05$ was considered to indicate a statistically significant significance.

Results

Upregulation of STING in UM indicates a poor prognosis. A total of 80 mRNA-seq datasets and the corresponding clinical information of patients with UM were retrieved from the TCGA database. An area under the curve was generated based on STING expression to predict the 5-year survival of patients. The area under the ROC curve was 0.787, indicating that expression of STING was a favorable predictor of survival in patients with UM. The cutoff value for distinguishing between high and low STING expression was determined to be 10.693 Fragments per kilobase of transcript per million mapped reads (Fig. 1A and Table I). Survival analysis of patients with UM demonstrated that those with higher STING expression had significantly shorter overall and disease-free survival times than those with lower STING expression (Fig. 1B and C; $P<0.01$). Furthermore, the chi-square test was conducted to evaluate the relationship between STING expression and clinical characteristics. The results revealed a significant association between high risks of metastasis and higher expression of STING, including the histological types of epithelioid cells, higher UM clinical stage, and survival with tumors (Table II; $P<0.05$). These findings suggested that upregulation of STING expression indicates a poor prognosis in patients with UM.

STING is widely expressed in UM and significantly higher than in normal tissues. To determine the expression levels of STING in UM, a Wes Separation protein immunoassay was used to detect STING in UM cell lines, UM tissues and para-UM tissues (choroid tissues). In UM cell lines, STING exhibited variable expression levels relative to those in THP-1, 293T and ARPE-19 cells (Fig. 2A). The present findings also revealed that STING in UM tissues was significantly higher in UM tissues than in para-UM tissues, suggesting that STING may be involved in the development of UM (Fig. 2B; $P<0.05$). However, STING protein expression levels did not significantly differ between metastatic and non-metastatic UM tissues (Fig. 2C; $P>0.05$).

Table I. Parameters of receiver operating characteristic curve based on the expression of stimulator of interferon genes to predict survival of patients.

Area under the curve	Standard error	95% confidence interval	P (Area=0.5)	Sensitivity	Specificity	Cut-off value (FPKM)
0.787	0.135	0.556-0.933	0.033	77.78%	91.67%	≤10.693

FPKM, Fragments per kilobase of transcript per million mapped reads.

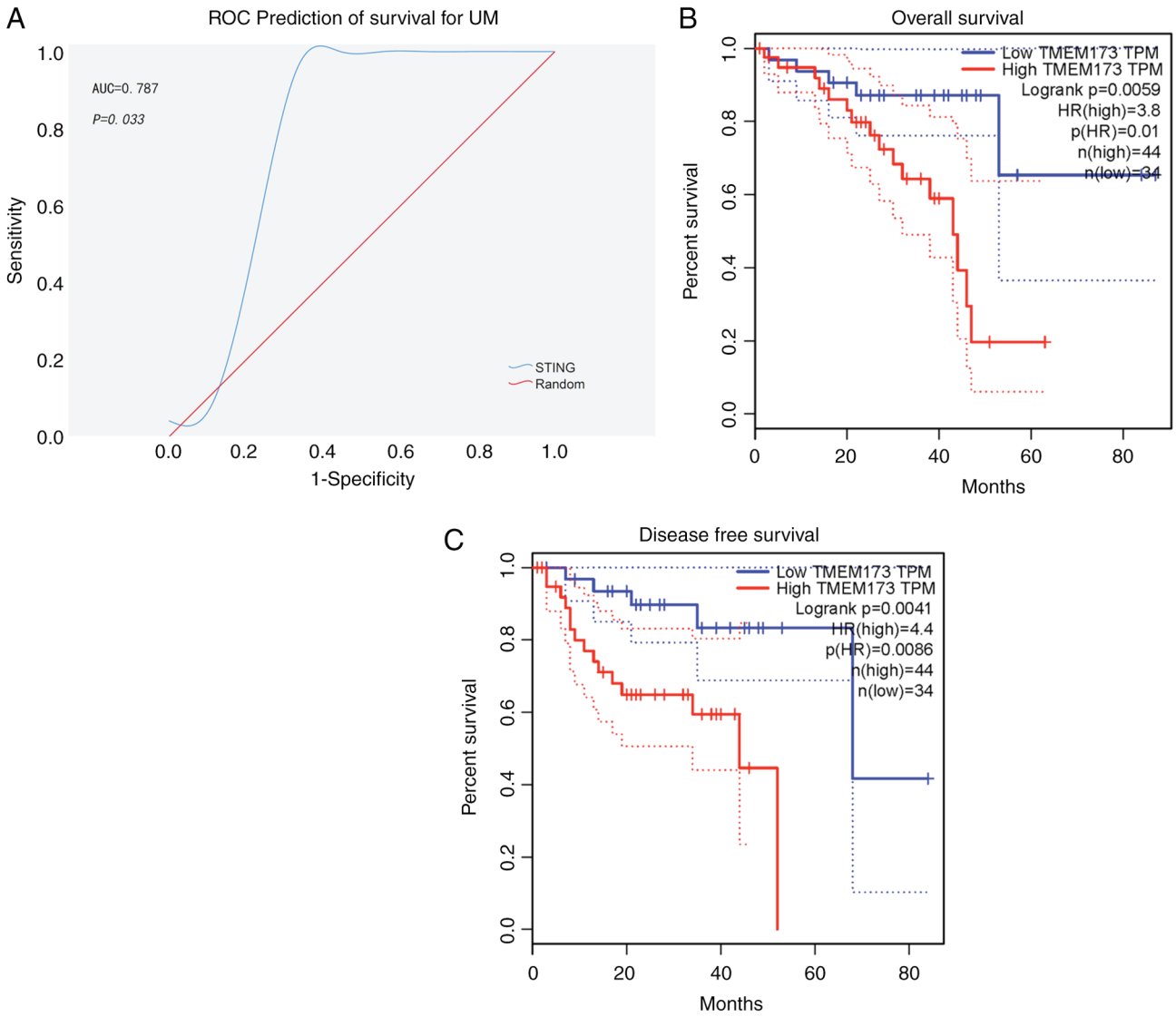

Figure 1. Upregulation of STING is significantly related to poor prognosis of patients with UM according to The Cancer Genome Atlas database. (A) ROC curve based on the expression of STING to predict 5-year survival of patients (AUC=0.787, P=0.033). (B) Overall survival of patients with high (n=44) and low expression (n=34) of STING (P=0.0059, P-values were calculated by log-rank test). (C) Disease-free survival of patients with high (n=44) and low expression (n=34) of STING (P=0.0041). P-values were calculated using the log-rank test. STING, stimulator of interferon genes; ROC, receiver operating characteristic.

STING promotes invasion and migration of UM cells. Transwell experiments with Matrigel and wound healing experiments were performed to evaluate the invasion and migration ability. Loss-of-function experiments were carried out on Mel270 and Mel202 cells, which exhibit high STING expression, while gain-of-function experiments were carried out on Omm2.3 and Omm2.5 cells, with low STING expression levels. First, the shRNA targeting STING (shSTING) was transfected into Mel270 and Mel202 cells to downregulate STING with 55~66 and 42~59% reduction respectively, compared with the Scramble groups (Fig. S1A; P<0.001). Transwell experiments and wound healing experiments demonstrated that the downregulation of STING in Mel270 and Mel202 significantly inhibited their invasive

Table II. Correlations between clinical characteristics of patients with uveal melanoma and the expression of STING.

Clinical characteristics	Number	Level of STING mRNA		χ^2	P-value
		High	Low		
Sex				0.08119	0.7757
Male	45	27	18		
Female	35	19	16		
Age, years				0.05115	0.8211
≤60	40	24	16		
>60	40	22	18		
Episcleral invasion				[a]1.780	0.1821
Yes	7	6	1		
No	68	35	33		
Histopathological features				[a]6.537	0.0106
Epithelioid cell	13	11	2		
Spindle cell	30	11	19		
Clinical stage				8.345	0.0039
II	39	17	22		
III + IV	40	28	12		
Tumor status				6.041	0.014
Tumor-free	61	31	30		
Tumor	18	15	3		
Ciliary body location				2.494	0.1143
Yes	24	17	7		
No	56	29	27		

[a]Chi-square test with Yates' correction. STING, stimulator of interferon genes.

and migratory abilities (Fig. 3A-D; P<0.05). In parallel, the STING cDNA (STING$^+$) was transfected into Omm2.3 and Omm2.5 to overexpress STING with 29~32-fold and 15~18-fold overexpression rate respectively (Fig. S1B; P<0.001), compared with the Control groups. The experiment assay revealed that overexpression of STING in Omm2.3 and Omm2.5 significantly promoted their invasive and migratory abilities (Fig. 3E-H; P<0.05). However, changes in STING expression did not significantly affect UM cell proliferation (Fig. S2A-D; P>0.05).

STING promotes the invasion and migration of UM through p38-MAPK signaling. The roles of MAPKs signaling and BAP1 in UM cell proliferation and metastasis have been extensively investigated (18,19). Studies have shown that STING promotes the development of tumors through canonical and non-canonical nuclear factor-kappa B (NF-κB) pathways as well as p21 (13,20,21). To gain insights into the mechanism of STING-induced UM invasion and migration, Wes separations were performed to detect the expression of MAPKs (p38-MAPK, extracellular regulated protein kinases/ERK, c-Jun N-terminal kinase/JNK), BAP1, NF-κB (p65, p100/52), and p21 in UM cells. The present results demonstrated that STING downregulation and overexpression consistently modulated the levels of phosphorylated p38-MAPK (Fig. 4A), whereas the expression of ERK, JNK, BAP1, NF-κB (p65, p100/52) and p21 remained unchanged. The phosphorylation of p38-MAPK decreased when STING was downregulated, but increased upon STING overexpression. Furthermore, Transwell and wound healing experiments revealed that SB203580, a potent p38-MAPK inhibitor (at 10 μM), effectively reversed STING-enhanced cell invasion and migration (Fig. 4B-E; P<0.05). Notably, p38-MAPK inhibition had no significant effect on UM cell proliferation, irrespective of STING expression levels (Fig. S3A-D; P>0.05).

Discussion

An increasing number of studies have demonstrated that STING directly influences the tumorigenesis and metastasis of tumor cells. However, the functions of STING in tumors is complicated and controversial. Previous studies have consistently indicated that STING can restrain tumorigenesis and that STING agonists inhibit tumor development in animal studies and clinical trials (22,23). However, other studies have also reported that STING promotes tumorigenesis of certain tumors and that elevated STING expression is significantly associated with a poorer prognosis (24,25). On these bases, in the present study, it was found that upregulation of STING in UM indicates a poor prognosis according to TCGA data analysis. STING is abundant in both UM tissue and cell lines and

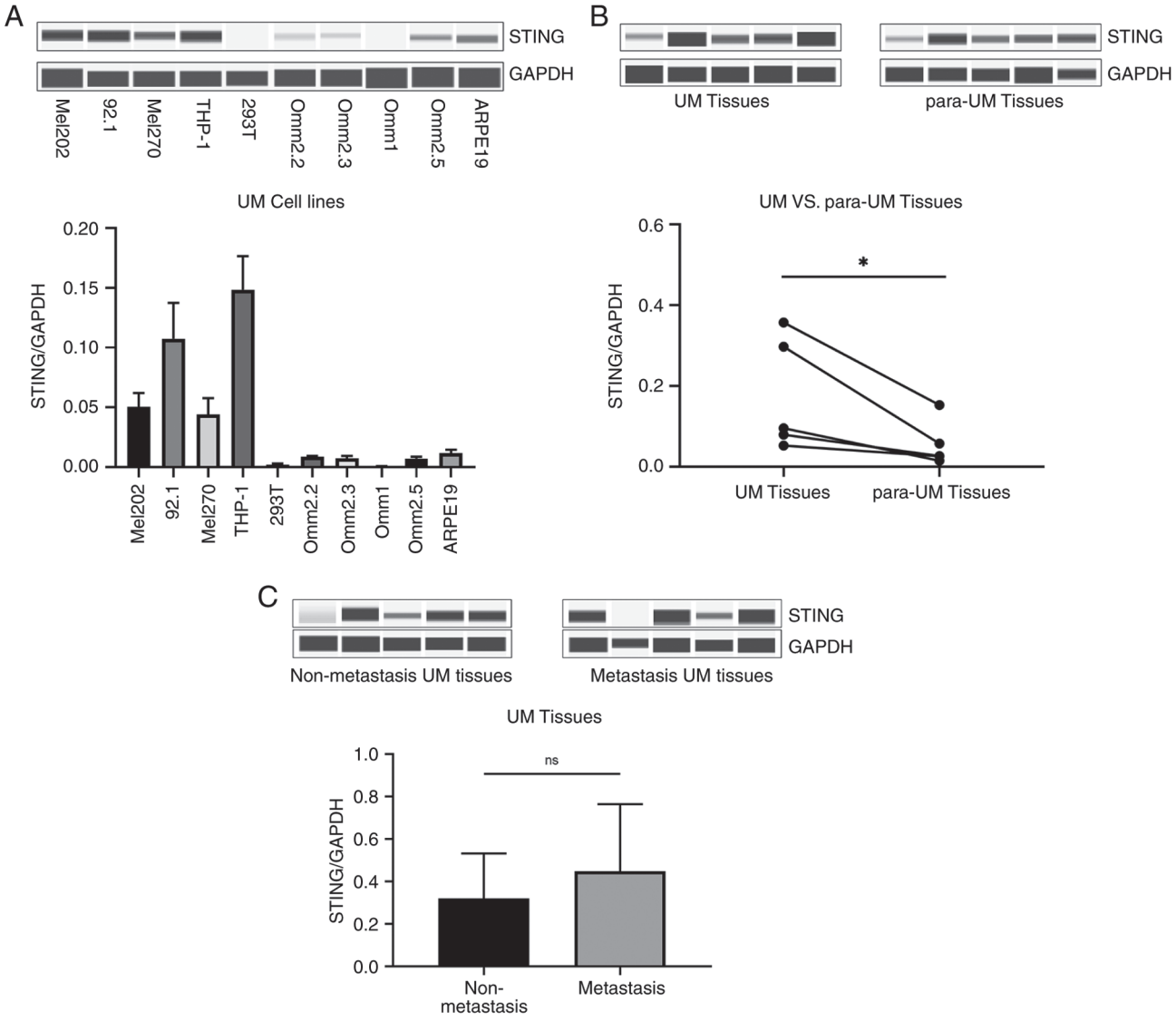

Figure 2. STING is widely expressed in UM and higher than in para-UM tissues (Choroid tissues). (A) Wes Separation Protein Immunoassay of STING in UM cell lines, THP-1, 293T and ARPE-19. (B) Wes Separation Protein Immunoassay of STING in UM tissues and para-UM tissues (Choroid tissues). (C) Wes Separation Protein Immunoassay of STING in Non-metastasis and metastasis UM tissues. Mel202 and 92.1 cell line protein bands are non-adjacent bands but were run on the same reaction plate of Wes Separation Protein Immunoassay, owing to the deleted bands of another cell line. *P<0.05. STING, stimulator of interferon genes; UM, uveal melanoma; ns, not significant (P>.0.05).

the level of STING was higher in UM tissues than para-UM tissues (choroid membranes). Moreover, STING regulated the levels of phosphorylated p38-MAKP and promoted invasion and migration of UM cells, which could be reversed by a p38-MAPK inhibitor. All the findings demonstrated that STING promotes invasion and migration of UM through p38-MAPK signaling.

Previous studies have revealed that STING expression in gastrointestinal cancer tissues is markedly lower than that in normal tissues (7,26). A STING deficiency in tumor cells accelerates tumor progression, and high STING expression is associated with an improved prognosis in gastrointestinal cancer, hepatocellular carcinoma and cervical cancer (27-30). Activation of cGAS-STING pathway can inhibit the growth of tumor in numerous animal models by promoting anti-tumor immunity (8). However, a previous study demonstrated that STING is higher in squamous carcinoma of the tongue than in normal tissues and can promote tumor development by increasing infiltration of regulatory T cells (Tregs) (9). Additionally, high STING expression is associated with an increased risk of relapse in breast and ovarian cancers treated with adjuvant chemotherapy (31). Low-grade serous ovarian carcinomas and serous borderline tumors exhibit uniformly high STING expression (32). This suggests that high STING expression reflects pathway activation and that the mechanisms may vary across different tumors. The present results are consistent with those of previous studies, wherein the upregulation of STING in UM indicates a poor prognosis. A possible explanation for the complicated role of STING is that the activation patterns and downstream signaling of STING may depend on the tumor types and cell conditions. STING activation patterns include phosphorylation, ubiquitination, SUMOylation and palmitoylation. The downstream signaling includes TANK-binding kinase 1 (TBK1), canonical/non-canonical NF-κB, DHHC, TRIM56 and so on (33).

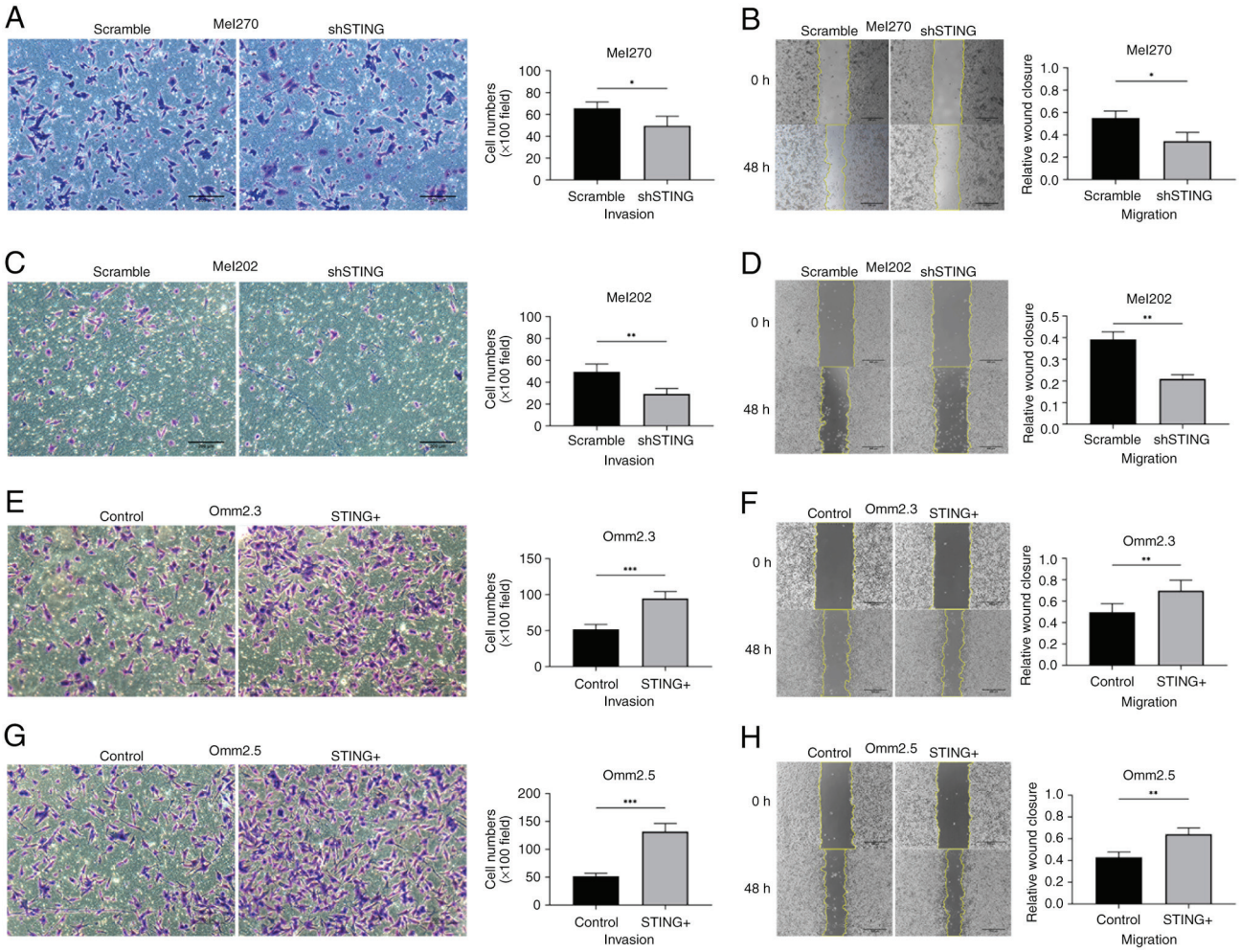

Figure 3. STING promotes invasion and migration of UM cells *in vitro*. (A-D) Downregulation of STING (shSTING) in Mel270 and Mel202 cells inhibited invasion and migration as assessed by (A and C) Transwell and (B and D) wound healing experiments, compared with scramble shRNA lentiviral vectors (Scramble). (E-H) Overexpression of STING (STING$^+$) in Omm2.3 and Omm2.5 cells enhanced invasion and migration as assessed by (E and G) Transwell and (F and H) wound healing experiments, compared with empty control lentiviral vectors (Control). *P<0.05, **P<0.01 and ***P<0.001. STING, stimulator of interferon genes; UM, uveal melanoma; sh-, short hairpin.

Numerous studies have demonstrated that STING signaling contributes to cancer development by participating in immune response, autophagy, cell proliferation and metastasis. For example, cancer cells migrating to brain activate the STING pathway and produce inflammatory cytokines in astrocytes to enhance the growth and chemoresistance of metastatic brain cancers (34). Activated STING in the human papillomavirus-related carcinogenesis of tongue squamous cells increases infiltration of Tregs via the c-jun/CCL22 signaling, potentially leading to tumor immune escape (9). IFI16 promotes cervical cancer progression *in vitro* and *in vivo* through activating the STING-TBK1-NF-kB pathway (11). The immune microenvironment is indispensable for STING immune regulation in tumor. However, UM is an immune-privileged tumor with an immunosuppressive microenvironment, due to the blood-ocular barrier and the lack of lymphatic vessels (35). Therefore, the intrinsic effects of STING on UM cells were investigated in the present study. Cheradame *et al* (31) demonstrated that STING downregulation decreased cell survival and increased sensitivity to genotoxic treatments in breast cancer cell lines in a cell-autonomous manner. Bakhoum *et al* (13) reported that STING, activated by unstable chromosomes in breast cancer, enhanced cell migration and invasion, promoting the downstream non-canonical NFκB2 (p52/Rel B) pathway. Bakhoum *et al* (36) also revealed that chromosomal instability is widespread in high-risk UM and drives UM cell migration in a STING-dependent manner. The *in vitro* results of the present study are consistent with studies that STING promotes invasion and migration of UM cells, which may be activated by chromosomal instability in tumor cells.

However, the molecular mechanisms downstream of STING underlying UM metastasis need to be further elucidated. MAPK signaling promotes the development of UM cells with Gαq pathway mutations, and the inhibition of MAPK can suppress progression of UM effectively (37). The lack of BAP1 expression in UM tissues significantly predicts metastasis and indicates a lower metastasis-free survival (19). By contrast, studies have identified that STING promotes the development of tumors through the canonical and non-canonical NF-κB pathways and p21 (13,20,21). Based on the aforementioned studies, it was planned to sift out potential signaling involved in the downstream pathway of STING to promote UM, including MAPKs (p38-MAPK, ERK, and JNK), BAP1, NF-κb (p65 and p100/52) and p21.

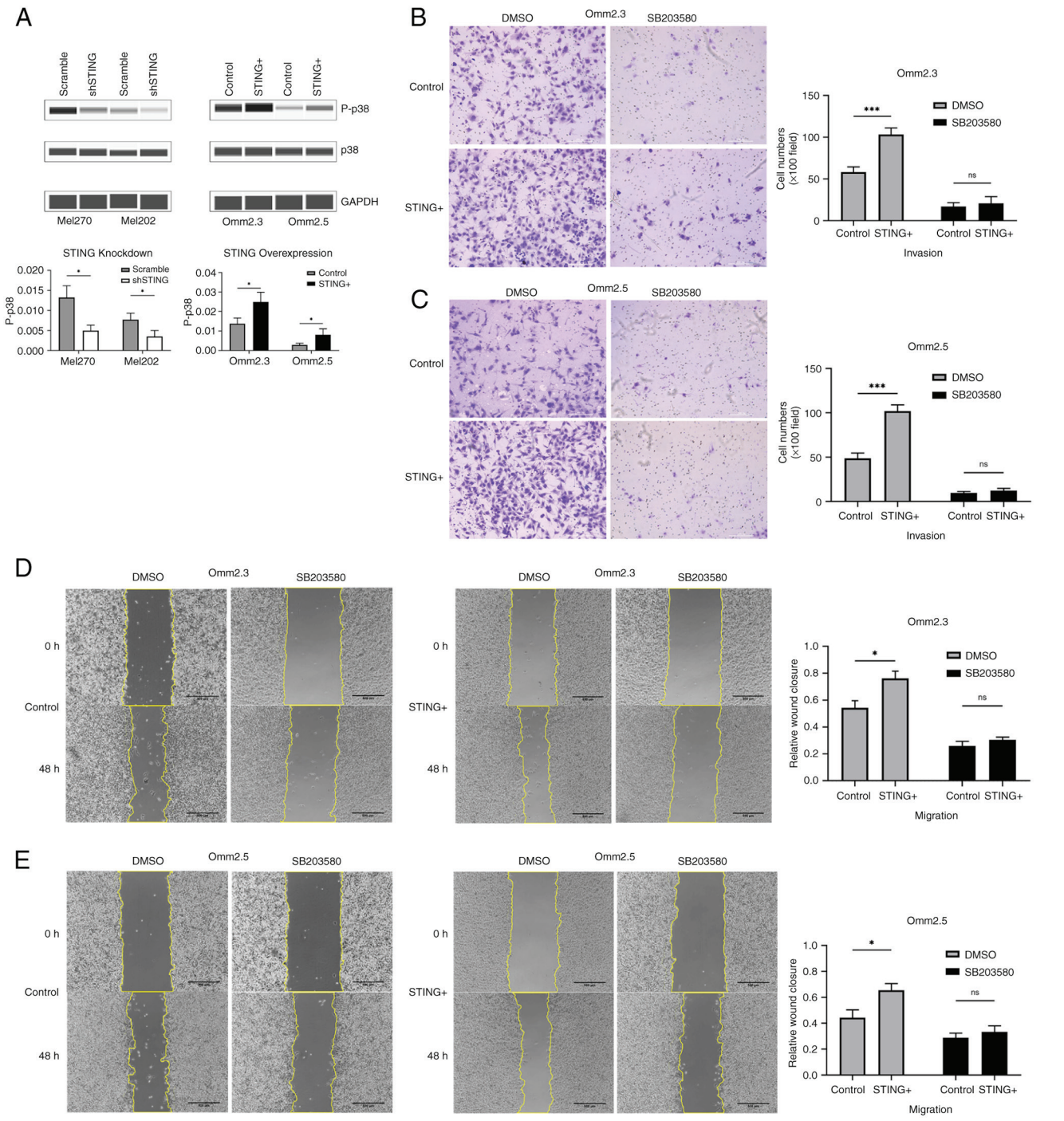

Figure 4. STING regulates the levels of p-p38-MAKP and STING-enhanced invasion and migration of UM cells can be reversed by a p38-MAPK inhibitor. (A) Wes Separation Protein Immunoassay of total and p-p38-MAPK with downregulation (shSTING) vs. overexpression (STING^{+}) of STING in UM cells, compared with scramble shRNA (Scramble) and empty control lentiviral vectors (Control). (B and C) Transwell experiments in STING-overexpressing and control Omm2.3 and Omm2.5 treated with p38-MAPK inhibitor (SB203580, 10 μM). (D and E) Wound healing experiments in STING-overexpressing and control Omm2.3 and Omm2.5 cells treated with p38-MAPK inhibitor (SB203580, 10 μM). *P<0.05 and ***P<0.001. STING, stimulator of interferon genes; p-, phosphorylated; UM, uveal melanoma; sh-, short hairpin; ns, not significant (P>.0.05).

The current findings revealed that phosphorylated p38-MAPK levels decreased in STING-knockdown cells but increased in STING-overexpressing cells. The p38-MAPK, a member of the canonical MAPKs family, plays crucial roles in cell proliferation, apoptosis and motility through phosphorylation and dephosphorylation processes (38). Previous studies have demonstrated that p38-MAPK promotes metastasis in various types of cancers, including hepatocellular carcinoma, head and neck squamous cell carcinoma, gliomas, renal carcinoma and gastric adenocarcinoma (39-43). The p38-MAPK signaling upregulates the expression and secretion of IL-6 in human uveal melanocytes, and IL-6/STAT3 signaling contributes to UM metastasis via epithelial-mesenchymal transition (44,45). Based on previous studies, it was hypothesized that p38-MAPK signaling is involved in the progression of UM. Functional experimental results confirmed that STING

promotes metastasis of UM through p38-MAPK signaling. It is worth considering why the downstream pathways of STING promoting metastasis in UM differ from breast cancer reported by Bakhoum *et al* (13). One possible explanation for this is tumor heterogeneity. Activation patterns and downstream signaling of STING may depend on the tumor type and cellular conditions. While the mechanism of STING in UM was being explored by the authors, other research teams also were paying attention to the role of STING in UM. Recently, Bakhoum *et al* (36) reported that the level of STING was negatively correlated with overall survival and tumor-related metastasis in UM patients, and treatment with the inhibitor of STING reduced the migratory phenotype in UM cell line, which is consistent with the conclusion of the present study. More recently, on the other hand, Tao *et al* (46) identified that the photodynamic polymer couple with cationic platinum agent activated cGAS-STING pathway improving the survival rate of animal model of UM *in vivo* by promoting immune response, which conflicted with the conclusion of Bakhoum *et al* (36) and the present conclusions. These observations suggest that STING plays a pro-tumor role in intrinsic UM cells but an antitumor role in tumor microenvironment. However, the UM animal model used in the study by Tao *et al* (46) was subcutaneous tumor model. It is widely known that UM is located in the eye, a relative immune-privileged environment obviously different from other tissues and organs. This means that the effects of STING on pro-immunity may not play a decisive role in patients with UM. Moreover, the bearing tumor in animal experiments in the aforementioned study originated from OCM-1, a cell line with controversial origin.

The limitations of the present study include the absence of *in vivo* functional experiments for STING and the small size of the patient cohort used to analyze the correlation between STING and UM prognosis. Further studies are required to understand the downstream pathway of p38-MAPK underlying UM metastasis and the activation pattern of STING in UM. Despite its preliminary characteristics and limitations, the present study, to the best of the authors' knowledge, is the first to report the downstream pathways of STING in UM cells, and to establish that STING directly promotes the invasion and migration of UM cells via p38-MAPK signaling in a tumor cell-autonomous manner.

In summary, the results of the present study indicated that STING promotes the invasion and migration of UM cells through upregulating the p38-MAPK signaling pathway. Therefore, STING expression may can serve as a biomarker for predicting the prognosis of patients with UM, and STING and p38-MAPK may serve as potential treatment targets for metastatic UM.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JQ, XZ, JW and BX designed the experiments. XZ, YC and FM performed the experiments and collected the data. XZ, RM, YC and BX analyzed and interpreted the data. XZ and FM drafted the manuscript. BX, RM and FM revised the language of the manuscript. JQ and JW supervised the present study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. XZ, FM and BX confirm the authenticity of all the raw data. All the authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. 2020044-1) by the Institutional Review Board of the Eye & ENT Hospital of Fudan University (Shanghai, China). Written informed consent was signed by all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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