Migfilin sensitizes cisplatin-induced apoptosis in human glioma cells in vitro

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Aim: Filamin binding LIM protein 1, also known as migfilin, is a skeleton organization protein that binds to mitogen-inducible gene 2 at cell-extracellular matrix adhesions. The aim of this study was to investigate the role of migfilin in cisplatin-induced apoptosis in human glioma cells, to determine the functional domains of migfilin, and to elucidate the molecular mechanisms underlying the regulation of cisplatin-related chemosensitivity.

Methods: The human glioma cell lines Hs683, H4, and U-87 MG were transfected with pEGFP-C2-migfilin to elevate the expression level of migfilin. RNA interference was used to reduce the expression of migfilin. To determine the functional domains of migfilin, U-87 MG cells were transfected with plasmids of migfilin deletion mutants. After treatment with cisplatin (40 µmol/L) for 24 h, the cell viability was assessed using the MTS assay, and the cell apoptotic was examined using the DAPI staining assay and TUNEL analysis. Expression levels of apoptosis-related proteins were detected by Western blot analysis.

Results: Overexpression of migfilin significantly enhanced cisplatin-induced apoptosis in Hs683, H4, and U-87 MG cells, whereas down-regulation of migfilin expression inhibited the chemosensitivity of these cell lines. The N-terminal region of migfilin alone was able to enhance the cisplatin-induced apoptosis. However, despite the existence of the N-terminal region, mutants of migfilin with any one of three LIM domains deleted led to a function loss. Furthermore, apoptotic proteins (PARP and caspase-3) and the anti-apoptotic protein Bcl-xL were modulated by the expression level of migfilin in combination with cisplatin.

Conclusion: The LIM1-3 domains of migfilin play a key role in sensitizing glioma cells to cisplatin-induced apoptosis through regulation of apoptosis-related proteins.

Keywords: filamin binding LIM protein 1 (migfilin); glioma; cisplatin; apoptosis; PARP; caspase-3; Bcl-xL

Introduction

Glioma, the most common type of brain tumor, originates from glial cells of the central nervous system[1]. Glioblastoma multiforme (GBM) is the grade IV glioma that represents the most invasive form of malignant brain tumors. The growth of glioblastoma cells exhibits infiltration into contiguous tissue throughout the brain, which challenges the complete resection of gliomas and might be partially responsible for the high rates of disease recurrence. Thus, in spite of extensively applied multimodal therapies, glioblastoma patients commonly receive a poor prognosis with median survival duration of less than 14 months[1–3]. However, in recent years, targeted therapy has provided novel therapeutic insights into improving the sensitivity of malignant cells to systemic radiotherapy or chemotherapy[2].

The connection between the extracellular matrix (ECM) and adjacent cells plays a significant role in cell fate determination, including differentiation, proliferation, migration and apoptosis[4]. Filamin binding LIM protein 1 (FBLIM1), also known as migfilin, is characterized as a skeleton organization protein that binds to mitogen-inducible gene 2 (Mig-2) at cell-ECM adhesions[5-7]. It has been elucidated that migfilin colocalizes with Mig-2 and binds to Mig-2 through its C-terminal domain, which is functionally required for the morphological maintenance of cells[5]. Moreover, the N-terminus of migfilin mediates integrin activation through competitively binding to the site of filamin immunoglobulin-like domain IgLFN21, disaggregating the filamin/integrin complex and promoting talin/integrin interaction[8-9]. Notably, migfilin consists of...
three structural parts, which possess disparate functions and act as indispensable binding domains, including an N-terminal region, a central proline-rich region and a C-terminal region. The C-terminal region has three sequential LIM domains[8]. In addition, two types of migfilin isoforms have been differentiated as migfilin(s) and FBLP-1[4, 8]. Migfilin(s) is a splicing variant with a proline-rich region deleted, resulting in the loss of the VASP binding site[4, 5]. FBLP-1 is missing the third LIM domain, which most likely interferes with the interaction of Mig-2[4, 6]. Thus, it appears that the functional domains of migfilin, partially presented in migfilin variants, are responsible for distinct downstream signaling through binding to corresponding proteins.

Currently, the DNA-alkylating agent temozolomide is widely used to treat glioblastoma patients, showing a quite modest survival benefit[10, 11]. Unfortunately, no effective second-line chemotherapy drug can be utilized for glioblastoma patients when chemoresistance to temozolomide occurs[12]. Meanwhile, the mechanism of chemoresistance to regular chemotherapeutic agents is poorly understood[10, 12]. Cisplatin is an anti-cancer drug that specifically causes DNA damage, including DNA interstrand and intrastrand cross-links, and is widely applied for the treatment of malignant solid tumors[13, 14]. Overcoming the ineffectiveness of cisplatin(s) and FBLP-1[4, 8] and is widely applied for the treatment of malignant solid tumors[13, 14]. Overcoming the ineffectiveness of cisplatin in gliomas and possibly providing an alternative therapeutic strategy for glioma patients would be beneficial. In this study, we report that migfilin plays an important role in sensitizing glioblastoma cells to cisplatin-induced apoptosis, which might be a novel therapeutic target for gliomas.

Materials and methods

Cell lines and culture conditions

As previously described, the human glioma cell lines Hs683, H4, and U-87 MG (purchased from Cell Resource Centre, IBMS, CAMS/PUMC), were cultured in DMEM (GIBCO) culture medium containing 10% fetal bovine serum (GIBCO)[15]. Cells were incubated at 37°C in a humidified atmosphere with 5% CO2.

Reagents

Mouse monoclonal anti-migfilin antibody was produced as previously described[5]. Antibodies specific to Bcl-xL, Bcl-2, Caspase-3, and PARP were purchased from Cell Signaling Technology Inc. Anti-p53 antibody was purchased from Santa Cruz Biotechnology Inc. Anti-beta-actin antibody was obtained from Sigma. Goat anti-mouse IgG and goat anti-rabbit IgG were purchased from Promega Inc. The anti-cancer drug cisplatin was diluted to the concentration of 1 mg/mL (obtained from Cancer Hospital, Chinese Academy of Medical Sciences).

Plasmids, siRNAs and transient transfections

The plasmids containing different types of cDNAs of migfilin deletion mutants, which were inserted into pEGFP-C2 vectors encoding green fluorescent protein (GFP), were generated by Clontech[16]. The siRNAs utilized were as follows: migfilin siRNA (5’-AGGGGCAUCCACAGACAUU-3’) and control siRNA (5’-UUUCAGUGCUGACGU-3’). U-87 MG cells were seeded and cultured without antibiotics at 37°C overnight. Cells were then transfected with plasmids or siRNAs using Lipofectamine 2000 (Invtrogen) in serum-free DMEM. After a 4-h incubation, the serum-free culture medium was changed to DMEM with 10% FBS. Cells were then incubated for 24–48 h in preparation for subsequent assays.

MTS assay

Cells were seeded into 96-well culture plates at 5×10^3 cells per well, incubated overnight, and transfected with plasmids or siRNAs. At 24 h post-transfection, cisplatin was supplemented in the DMEM medium at a final concentration of 40 μmol/L, and cells were maintained for 24 h. Twenty microliters of MTS (CellTiter 96 aqueous one solution reagent, Promega) diluted in 100 μL of culture medium was added to each well of the 96-well culture plates. Cells were incubated for 2 h, and then the absorbance of each well at 490 nm was recorded using a 96-well plate reader (Bio-Rad Inc). All of the experiments were performed in triplicate and repeated three times.

DAPI staining

Cells were cultured on glass coverslips in 6-well plates for 24 h, transfected with plasmids or siRNAs and then treated with or without cisplatin (following the drug treatment described above). Cells subsequently were fixed with ice-cold 100% methanol overnight at 4°C. After triple washes in phosphate-buffered saline (PBS) for 5 min each, cells were incubated with 100 nmol/L DAPI (Sigma) for 5 min at room temperature in the dark. Following triple rinses in PBS for 5 min each, coverslips were placed on slides and the edges sealed. Slides were viewed using upright fluorescence microscopy (BX51, Olympus) and pictures were captured by the Image-Pro Discovery software. Values were calculated in at least 9 different views and presented as percentage of positive apoptotic cells stained.

TUNEL apoptosis assay

TdT-mediated dUTP-biotin nick-end labeling (TUNEL) is a method used for apoptosis detection by determining the exposure end of the fragmentation of nuclear chromatin, which is one of the criteria of late stage apoptosis. The in situ cell death detection kit POD (Roche) was used to determine early stage apoptotic cells.

Western blotting

Eighty micrograms of protein extracts was loaded and separated by 10% SDS-PAGE gel (100 V, 1 h) and then transferred to PVDF membrane (12 V, 2 h). After blocking with 2% bovine serum albumin (BSA) at room temperature for 30 min, the membrane was incubated overnight with specific primary apoptosis-related antibodies, such as anti-migfilin (1:1000), anti-Bcl-xL (1:500), anti-Bcl-2 (1:500), anti-PARP (1:500), anti-Caspase-3 (1:500), and anti-p53 (1:1000). Mouse anti-beta-actin (1:5000) monoclonal antibody was used as control with a 1 h
incubation. Next, goat-anti-mouse IgG (1:2000) and goat-anti-rabbit IgG (1:3000) conjugated with horseradish peroxidase (HRP) were used to probe the membrane at room temperature for 1 h. The membrane was rinsed in triplicate in PBS/Tween 0.1% for 5 min each. The chemiluminescent substrate was then added to the membrane. Photographs were taken by Image Reader LAS-4000 (LAS-4000, Fujifilm Inc.) and analyzed by Multi Gauge V3.2 software.

Statistic analysis
Data were presented as the mean±SD. For the comparison of two groups, we used the Student's t-test to determine statistically significant differences. P values less than 0.05 were considered to be statistically significant.

Results
Migfilin sensitizes cisplatin-induced apoptosis in glioma cells
We first investigated whether migfilin played an important role in the cisplatin-induced apoptosis in gliomas. The human glioma cell lines Hs683, H4, and U-87 MG were transiently transfected with the pEGFP-C2-migfilin plasmid, causing migfilin overexpression, and also with migfilin siRNA, leading to the knockdown of migfilin (Figures 1A, 1B, and 1C). Using dose-response experiments, we determined that the final treatment concentrations of cisplatin at 35, 50, and 40 µmol/L caused 50% cell death in Hs683, H4, and U-87 MG cells, respectively.

With the treatment of cisplatin, cell viability rates of the cell lines Hs683, H4, and U-87 MG were significantly reduced when migfilin expression levels were upregulated (P<0.05, P<0.05, and P<0.01, respectively) (Figures 1D, 1E, and 1F). The viability rates of Hs683, H4, and U-87 MG cells were markedly increased with reduced expression levels of migfilin after cisplatin treatment (P<0.05, P<0.05, and P<0.05, respectively) (Figure 1D, 1E, and 1F).

Morphological evaluation using DAPI staining was performed to analyze the rates of apoptotic cells showing nuclear condensation and fragmentation. Significant increases in cisplatin-induced apoptotic cells in the cell lines Hs683, H4, and U-87 MG were detected when expression levels of migfilin were elevated (P<0.01, P<0.05, and P<0.05, respectively) (Figures 1G, 1H, and 1I). However, apoptotic cell levels decreased significantly in migfilin-knockdown groups in Hs683, H4, and U-87 MG cells, in contrast to respective cell line control groups (P<0.05, P<0.05, and P<0.05, respectively) (Figures 1G, 1H, and 1I).

In addition, we observed similar results using the TUNEL staining assay. After exposure to cisplatin, the level of early-apoptotic Hs683, H4, and U-87 MG cells was positively correlated with migfilin expression levels (Figures 2A, 2B, and 2C). However, the modulation of migfilin did not directly influence the apoptotic rates of the glioma cells without cisplatin supplement. Therefore, migfilin can sensitize cisplatin-induced apoptosis, whereas downregulation of migfilin can inhibit cisplatin-induced apoptosis in glioma cells.

The LIM domain of migfilin plays a key role in cisplatin-induced apoptosis
To assess the functional domains of migfilin that affect the chemosensitivity of glioma cells, we transfected cells with various deletion-mutants of migfilin. Specifically, plasmids encoding different types of GFP-tagged migfilin mutants were transfected to express mutant forms of migfilin in the U-87 MG cell line. As shown in Figure 3, migfilin(s), a splicing variant of migfilin, lacks a proline-rich region. PR-LIM1-3 lacks the N-terminal region, whereas the vector N-ter only expresses the N-terminal residues of migfilin. LIM1-3 lacks of the N-terminus and the proline-rich regions, whereas ALIM1N possesses a mutant LIM1 domain in addition to the mutations of the LIM1-3 vector. The C243 and C306 vectors possess the LIM2 domain mutant and the LIM3 domain mutant, respectively.

Western blotting was conducted to detect GFP protein expression levels in migfilin-mutant transfectants to assess the efficacy of the transient transfections (Figure 4A). As with wild-type migfilin, U-87 MG cells transfected with migfilin(s), N-ter, PR-LIM1-3, and LIM1-3 demonstrated decreased survival rates when treated with cisplatin (P<0.05) (Figure 4B). Moreover, migfilin(s), N-ter, PR-LIM1-3, and LIM1-3 vector transfectants showed increased apoptotic cell rates when treated with cisplatin, compared to treated control vector transfectants (approximately 1.71-fold, 2.41-fold, 1.59-fold, and 2.24-fold changes, respectively; P<0.05). In contrast, ALIM1N, C243 and C306 vector transfectants did not significantly differ from the control group (P>0.05) (Figure 4C). TUNEL-stained apoptotic areas were significantly increased in cisplatin-treated cells transfected with migfilin(s), N-ter, PR-LIM1-3, and LIM1-3 vectors (P<0.05), and apoptotic areas were relatively static in cells transfected with the ALIM1N, C243, and C306 vectors (P>0.05) (Figure 4D). Therefore, we observed that the N-terminus alone affects cisplatin-induced apoptosis in U-87 MG cells. In addition, cells transfected with PR-LIM1-3 and LIM1-3 vectors containing a deleted N-terminal region were also sensitive to cisplatin-induced apoptosis. Furthermore, we found that cell transfection with the C243 and C306 vectors, which possess the entire length of the N-terminus but contain mutated LIM domains, led to a loss of protein function, thus demonstrating that the C-terminal LIM domains may play a key role in wild-type migfilin and migfilin(s) protein activities. Furthermore, mutants of migfilin with any one of LIM domain deletions also resulted in function loss, indicating the importance of the integrity of all three LIM domains.

Migfilin enhances cisplatin-induced apoptosis through the regulation of Bcl-XL and Caspase-3 expression
To investigate the underlying molecular mechanisms involved in the cisplatin-related sensitivity of U-87 MG cells, we detected expression levels of apoptosis-associated proteins in the cellular extracts of migfilin transfectants. With strict efficacy control of transient transfection with vectors and siRNA (Figure 5A and 5C), we found that without cisplatin treat-
ment, there were no alterations in the expression of apoptotic proteins such as PARP and Caspase-3 in migfilin transfectants (Figure 5B). However, with cisplatin treatment, regulation of migfilin was positively related to the expression of cleaved

Figure 1. Regulation of migfilin sensitizes cisplatin-induced apoptosis. (A) Hs683 cells, (B) H4 cells, and (C) U-87 MG cells were examined for expression levels of migfilin by Western blotting after transfection of control vector, pEGFP-C2-migfilin vector, control siRNA, and migfilin siRNA, respectively. (D) Hs683 cells, (E) H4 cells, and (F) U-87 MG cells were examined for the effects of migfilin expression on cell viability and were analyzed by the MTS viability assay. Cells were treated with cisplatin for 24 h. (G) Hs683 cells, (H) H4 cells, and (I) U-87 MG cells were analyzed by DAPI staining for cisplatin-sensitivity. Apoptotic cells were stained with light blue (400×objective). The scale bars stand for 10 μm. Mean±SD. n=3. \( ^{b}P<0.05, ^{c}P<0.01 \) vs control.
Figure 2. Regulation of migfilin sensitizes cisplatin-induced apoptosis. (A) Hs683 cells, (B) H4 cells, and (C) U-87 MG cells were analyzed by the TdT-mediated dUTP-biotin nick-end labeling (TUNEL) assay for the detection of early-stage-apoptotic cells. Apoptotic cells were determined by both DAB-stained positive areas (brown areas) and fluorescein-stained areas (green areas) (400×objective). The scale bars stand for 20 um. Apoptotic cell rates were calculated under 9 different views. Mean±SD. n=3. *P<0.05, †P<0.01 vs control.
PARP and cleaved Caspase-3 (Figure 5B).

Moreover, we observed that after exposure to cisplatin, the migfilin-mutant transfectants, including migfilin(s), N-ter, PR-LIM1-3, and LIM1-3, showed increased expression of cleaved PARP and cleaved Caspase-3, compared to the control group, whereas ∆LIM1N, C243, and C306 transfectants had no significant impact on the expression of apoptotic proteins (Figure 5D).

We further investigated upstream apoptosis-related proteins. Expression levels of Bcl-2 and p53 showed no differences in migfilin-overexpression and migfilin-knockdown transfectants with or without cisplatin treatment (Figure 6A). With cisplatin treatment, expression of the anti-apoptotic protein Bcl-xL was negatively associated with the expression level of migfilin (Figure 6A). Transfectants of functional migfilin-mutant vectors, including migfilin(s), N-ter, PR-LIM1-3, and LIM1-3, consistently resulted in the downregulation of Bcl-xL (Figure 6B). Thus, it has been suggested that migfilin plays an important role in the chemosensitivity of glioma cells through modulating Bcl-xL and Caspase-3 expression.

**Discussion**

Programmed cell death (PCD) has been regarded as a key point in development, and PCD disorders play a role in a wide range of diseases, such as neuro-degeneration, autoimmune diseases, cardiovascular diseases, and cancer[17]. Apoptosis-resistance, growth signal autonomy and insensitivity to anti-growth signals are major factors in the malignant transformation of cells, ultimately leading to unlimited cell proliferation[33]. In this study, we have demonstrated that migfilin plays a key role in regulating cisplatin-induced cellular apoptosis in glioblastoma cells. In addition, we found that U-87 MG cells transfected with N-terminus, PR-LIM1-3 and LIM1-3 vectors containing mutant migfilin were sensitized to cisplatin-induced apoptosis. However, C243 and C306 vector transfectants that contained a full length N-terminus and mutated LIM domains led to a function loss. Therefore, the LIM domain likely plays a key role in wild-type migfilin and migfilin(s) protein activities. It is also probable that the N-terminus fragment is involved in a different mechanism of regulating chemosensitization than the LIM region. We speculate that the LIM1-3 domain might function to influence the conformational space or protein-binding sites of the N-terminus to ultimately perform the regulation of cisplatin-induced apoptosis in wild-type migfilin. Moreover, in addition to interacting with Mig-2[5], the C-terminal LIM domains have been implicated in the localization of migfilin to cell-cell adhesions through regulation of the cadherin–β-catenin complex[6, 16]. Previous studies have reported that the regulation of cell-cell junctions by E-cadherin could influence cisplatin-based chemoresistance[15–21]. In human glioma cells, expression levels of E-cadherin, β-catenin and PARP are regulated in ZEB2-mediated cellular apoptosis[22]. Therefore, it seems likely that the LIM domain of migfilin participates in cisplatin-induced apoptosis via remodeling of cell-cell or cell-matrix adhesions.

In addition to acting as a focal adhesion protein involved in cell-cell and cell-ECM adhesion, migfilin is a candidate oncogene that has distinct potential in becoming a biological marker of human leiomyosarcomas (LMS)[23]. In human mammary epithelial cells, downregulation of migfilin directly leads to anoikis by interacting with Src[24]. Therefore, migfilin appears to be an anti-apoptotic candidate oncogene in addition to being a gene with the apoptotic functions described in this paper. While paradoxical, this is not the first incidence of an oncogene promoting apoptosis. Activation of the oncogene Ras sensitizes chemotherapeutic agent-induced apoptosis both in HCT116 cells[25] and in embryonic stem cells[26]. The oncogene c-myc has also been reported to enhance cisplatin-induced apoptosis by inhibiting NF-κB signaling, which represses the expression of Bcl-2 families in mouse pancreatic cancer cells[27]. In addition, E1A has been proposed to be an immortalization oncoprotein that exhibits the anti-tumor properties of cell-cycle arrest and apoptosis induction[25, 26, 29]. The paradox caused by the dual function of oncogenes might derive from the functions of targeted genes downstream of the oncogenes, which could counteract the intrinsic anti-apoptotic function of oncogenes[25, 30–32]. It has also been proposed that cell growth and apoptosis are closely linked and that apoptosis might trigger the early progression of tumorigenesis[33, 34].

Cisplatin is a strong, widely used anti-cancer therapeutic drug that causes DNA damage and programmed cell death and is activated in a p53-independent pathway[35, 36]. Here, we have demonstrated that with the treatment of cisplatin, expression levels of cleaved Caspase-3 and PARP were elevated and accompanied by increased migfilin expression. Bcl-xL expression levels were negatively modulated by the expression level of migfilin and did not affect the expression level of p53 in glioblastoma cells. The anti-apoptotic factors Bcl-2 and Bcl-xL appear to be the most significant regulators involved in irradiation- and chemotherapy-induced apoptosis, particularly in a p53-dispensable pathway[27, 34, 35, 36]. The suppression of Bcl-xL induced by intrinsic or extrinsic upstream signals can result in the activation of apoptosis[37]. In the nervous system, expres-
Figure 4. Functional domains of migfilin were determined in U-87 MG cells. (A) Cellular extracts of transfectants of migfilin mutant vectors with the GFP flag were detected for expression levels of GFP by Western blotting. (B) The MTS viability assay was performed to demonstrate the cell viability of transfectants of migfilin, migfilin(s), N-ter, PR-LIM1-3, LIM1-3, ∆LIM1N, C243, and C306 with cisplatin treatment (n=3. Mean±SD). (C) Apoptotic cell rates of migfilin-mutant transfectants with or without cisplatin treatment were examined by DAPI staining. Apoptotic cells were stained with light blue (400×objective; n=3. Mean±SD). (D) Using the TUNEL staining assay, apoptotic cells of migfilin-mutant transfectants with or without cisplatin treatment were determined by both DAB-stained positive areas (brown areas) and fluorescein-stained areas (green areas) (400×objective). Apoptotic cell rates were calculated under at least 9 different views. Mean±SD. n=3. "P<0.05, "P<0.01 vs control.
The expression of endogenous Bcl-xL plays a vital role in the protection of neurons from DNA damage-induced apoptosis, while Bcl-xL deficiency causes activation of caspase-3 and neuronal cell death with or without p53-deficiency[39]. Essentially, the level of Bcl-xL has been associated with the chemosensitivity and regulation of apoptosis[40]. Suppression of Bcl-xL deamidation causes chemoresistance to cisplatin-induced apoptosis, which is maintained through suppressing the proapoptotic ability of BH3 domain-only proteins in fibroblasts[41]. In this study, upregulation of migfilin was found to sensitize glioblastoma cells to cisplatin-induced apoptosis through a mitochondria-dependent pathway. Migfilin regulates functional Bcl-xL expression to respond to the DNA damage caused by cisplatin. Given that a variety of tumors lack p53 activity, chemotherapeutic agent-induced apoptosis relies on p53-independent signaling[41, 42].

Figure 5. Expression of proapoptotic proteins in U-87 MG transfectants. (A) Migfilin expression levels were examined by Western blotting to determine the efficacy of transient transfections of migfilin vectors and siRNAs. (B) Expression levels of the proapoptotic proteins PARP and Caspase-3 were determined by Western blotting in migfilin-overexpression and migfilin-knockdown transfectants with or without cisplatin treatment. (C) Cellular extracts of transfectants of U-87 MG-migfilin mutants were examined for the expression levels of GFP protein by Western blotting. (D) Expression levels of PARP and Caspase-3 were detected by Western blotting in migfilin-mutant transfectants with or without cisplatin treatment.

Figure 6. Expression of apoptosis-related proteins in U-87 MG transfectants. (A) Expression levels of the apoptosis-related proteins Bcl-xL, Bcl-2, and p53 were determined by Western blotting in migfilin-overexpression and migfilin-knockdown transfectants with or without cisplatin treatment. (B) Expression levels of Bcl-xL were detected by Western blotting in migfilin-mutant transfectants with or without cisplatin treatment.
Our results establish a link between the functional domain of migfilin and cisplatin-mediated chemosensitivity in human gliomas. Moreover, migfilin might be considered a promising novel therapeutic target, through its interference with chemotherapy-induced cytotoxicity in glioblastomas, and could serve as an important molecular target for drug discovery.

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Author contribution
Yong-mei SONG and Chun-jiang YU designed this research; Yong-mei SONG and Qi-min ZHAN supervised the research; Yun-wei OU and Jing FAN performed the research; Yun-wei OU analyzed the data; Jing FAN wrote the paper; Chuan-yue WU contributed plasmids and anti-migfilin antibody.

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