TMIGD1 is Expressed at Low Levels in Abdominal Adhesion Tissue and Reduces Oxidative Stress in Peritoneal Mesothelial Cells

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Research

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Abstract

Background

Postoperative abdominal adhesion is one of the most commonly observed complications after abdominal surgery. However, there is no effective treatment for adhesion other than enterolysis. Mesothelial cell repair plays an extremely important role in the process of adhesion formation. Here, we hypothesize that transmembrane and immunoglobulin domain-containing 1 (TMIGD1) is expressed at low levels in abdominal adhesion tissue and can reduce oxidative stress and promote cell adhesion in peritoneal mesothelial cells.

Materials and Methods

First, we performed gene microarray analysis and used qPCR, western blotting, immunohistochemistry and immunofluorescence to detect the expression of TMIGD1 in rat adhesion tissue and normal peritoneal tissue. Then, we established a TMIGD1-overexpressing HMrSV5 cell line and detected ROS, apoptosis, and the mitochondrial membrane potential by the MTT assay, western blotting, flow cytometry with 2',7'-dichlorofluorescein diacetate (DCFH-DA) as a probe. Furthermore, we examined p38 phosphorylation in different TMIGD1-expressing cell lines and used a p38 inhibitor to determine whether the antioxidant effect of TMIGD1 is dependent on p38. Finally, we evaluated the adhesion ability of different TMIGD1 cell lines using scratch wound and adhesion assays.

Results

TMIGD1 was expressed at low levels in adhesion tissue and at lower levels in mesothelial cells. TMIGD1 overexpression alleviated H₂O₂-induced oxidative stress injury in human HMrSV5 cell lines. The phosphorylation level of p38 was higher in the TMIGD1-overexpressing cell line, and we found that the effect of TMIGD1 was inhibited by a p38 inhibitor. In addition, TMIGD1 overexpression inhibited mesothelial cell migration and promoted mesothelial cell adhesion.

Conclusion

TMIGD1 is expressed at low levels in abdominal adhesion tissue and can reduce H₂O₂-induced oxidative stress by promoting p38 phosphorylation. In addition, TMIGD1 can promote cell adhesion.

These authors contributed equally to this work.

1. Background

Postoperative abdominal adhesion (PAA) is one of the most commonly observed complications after abdominal surgery. Abdominal operations result in PAA formation in approximately 90–95% of patients, and PAA formation can further lead to intestinal obstruction, chronic abdominal pain, female infertility, etc. [1, 2]. However, there is no effective management strategy for PAA other than enterolysis, which is an

invasive method ^[3]. Although PAA can lead to serious health problems, few studies have focused on the prevention of PAA formation.

Abdominal adhesion formation is a complex process that involves the inflammatory response, collagen deposition and peritoneal mesothelial cell repair ^[4], and the underlying mechanism is poorly understood. After abdominal injury or trauma, the inflammatory response is activated, and fibrosis develops through the inflammatory cascade or storm. Additionally, the adhesion tissues that form are absorbed, and injured mesothelial cells are repaired within 5–7 days after surgery ^[5].

Mesothelial cell repair plays an extremely important role in the process of adhesion formation ^[6]. High levels of reactive oxygen species (ROS) are induced in the injured tissue under hypoxic conditions, which can induce higher expression of HIF-1 α and activate the TGF/Smad pathway ^[7]. An environment with high levels of ROS not only results in mesothelial cell apoptosis or necrosis but can also promote the transformation of mesothelial cells to stromal cells ^[8]. However, the mechanism underlying mesothelial cell repair is not well understood ^[9].

Transmembrane and immunoglobulin domain-containing 1 (TMIGD1) was recently reported to be a cell adhesion molecule. Studies have demonstrated that TMIGD1 can protect epithelial cells from oxidative injury ^[10]. TMIGD1 can also act as a tumor suppressor to regulate the expression of p21Cip1/p27Kip1 in renal cancer ^[11]. Here, we hypothesize that TMIGD1 is expressed at low levels in abdominal adhesion tissue and can reduce oxidative stress and promote cell adhesion in peritoneal mesothelial cells.

2. Results

2.1 TMIGD1 is Expressed at Low Levels in Abdominal Tissues

By analyzing the microarray data from the GSE4715 dataset in GEO (gene 2R software; normal tissue vs adhesion tissue; screening criteria: $|\text{LogFC}| > 1$ and $P < 0.05$; <https://www.ncbi.nlm.nih.gov/geo/>, as shown in Supplemental Fig. 1), we found that TMIGD1 was expressed at low levels in adhesion tissue compared to normal tissues on day 3. To further verify these findings, we detected TMIGD1 expression in rat abdominal adhesion tissues compared to normal tissue by qPCR, IHC and western blotting. The results demonstrated that the expression of TMIGD1 was lower in adhesion tissue than in normal tissue on days 3 and 10 after the operation (Fig. 1A, Fig. 1B, Fig. 1C, Fig. 1D and Fig. 1E). Then, we used double immunofluorescence staining to verify whether TMIGD1 was expressed in mesothelial cells and at low levels in adhesion tissue. Immunofluorescence staining showed that in normal peritoneal tissue, the green fluorescence signal of TMIGD1 was high in CK19 (green fluorescence)-expressing mesothelial cells; however, the green fluorescence intensity was weakened in adhesion tissue on days 3 and 5 (Fig. 1F and Fig. 1G).

2.2 TMIGD1 Overexpression Alleviates H₂O₂-Induced Oxidative Stress Injury in Human HMrSV5 Cell Lines

TMIGD1 has been reported to act as an antioxidative molecule in renal cells. Thus, we wondered whether TMIGD1 can induce oxidative stress injury in mesothelial cells. First, we constructed a HMrSV5 cell model stably expressing TMIGD1 (Fig. 2A and Fig. 2B). Cell immunofluorescence showed that TMIGD1 is mainly expressed in the cell membrane and cytoplasm (Fig. 2C). Then, we treated both the control and TMIGD1-overexpressing cell lines with different doses of H₂O₂, and the MTT assay revealed that the viability of cells expressing TMIGD1 at high levels was higher than that of control cells. Then, we evaluated the apoptosis level in different TMIGD1-expressing cell lines treated with 500 μM H₂O₂. Western blotting showed that the expression of the apoptosis-related proteins Bax and PARP-γ was higher and that the Bcl-2 level was lower in the TMIGD1-expressing group than in the control group, as shown in Fig. 3B. Flow cytometry analysis confirmed this result, and the ratio of apoptotic cells in the H₂O₂-treated TMIGD1-overexpressing group was decreased (Fig. 3C). To further demonstrate the effect of TMIGD1 on cell apoptosis, we detected the mitochondrial membrane potential level of cells by JC-1 staining and found that the mitochondrial membrane potential was increased in the H₂O₂-treated TMIGD1-overexpressing cell line (Fig. 3D). Finally, we detected the ROS level in the different TMIGD1-expressing and H₂O₂-treated groups and found that the ROS level was low in the H₂O₂-treated TMIGD1-overexpressing cell line, as shown in Fig. 3E.

2.3 TMIGD1 Reduces Oxidative Stress Injury by Promoting p38 Phosphorylation

In a previous study, TMIGD1 was demonstrated to increase the phosphorylation of p38^[10]. Here, we found that the phosphorylation level of p38 was higher in the TMIGD1-overexpressing cell line. To determine whether TMIGD1 alleviates ROS and apoptosis by promoting p38 phosphorylation, we treated the TMIGD1-overexpressing cell line with 10 μM SB203580, a p38 inhibitor, and found that TMIGD1 protected cells from H₂O₂-induced apoptosis and inhibited mitochondrial damage and ROS (Fig. 4C, Fig. 4D, and Fig. 4E).

2.4 TMIGD1 Overexpression Inhibits Migration and Promotes Cell Adhesion of Mesothelial Cells

TMIGD1 is a member of the immunoglobulin (Ig) domain-containing cell adhesion molecule family. Here, we wanted to determine whether TMIGD1 can promote mesothelial cell adhesion. The cell scratch assay revealed that the cell migration rate of the TMIGD1-expressing cell line was decreased compared to that of the control cell line (Fig. 5A and Fig. 5B). Then, we evaluated cell adhesion and found that cell adhesion was promoted in the TMIGD1-overexpressing cell line, as shown in Fig. 5C.

3. Discussion

Peritoneal mesothelial cell repair plays an important role in the formation of PAA. Under inflammatory conditions, mesothelial cells may undergo apoptosis, die or be transformed into mesenchymal cells. Thus, elucidating the underlying mechanism of mesothelial cell repair is very important for understanding how PAA forms [4, 12, 15]. In this study, we demonstrated that TMIGD1 was expressed at low levels in abdominal adhesion tissue and reduced H₂O₂-induced oxidative stress by promoting p38 phosphorylation. In addition, TMIGD1 promoted cell adhesion.

Oxidative stress is a normal process that occurs during the healing of peritoneal tissue [16]. However, we know little about the molecular mechanism involved in oxidative stress. In this study, we demonstrated that TMIGD1 promotes p38 phosphorylation to reduce ROS levels, which may be useful to prevent adhesion formation of mesothelial cells. In a previous study, TMIGD1 was demonstrated to be an ROS scavenger gene in renal tissue; here, we showed it has the same effect on peritoneal tissue [10]. The peritoneal tissue environment is proinflammatory after surgery. Necrotic cells, clots and recruited inflammatory cells release a large amount of ROS [17, 18]. Although a certain concentration of ROS is beneficial for tissue repair, a large amount of ROS can damage peritoneal mesothelial cells [17, 19]. The p38 signaling pathway participates in many physiological processes involved in tissue healing [20]. It has been reported that p38 can promote SOD2 transcription. A series of studies demonstrated that many antioxidant relays on p38 alleviate ROS [21, 22]. Consistent with a previous study, we found that the antioxidant activity of TMIGD1 was related to p38 and that a p38 inhibitor reduced these effects of TMIGD1.

The other function of TMIGD1 is associated with cell adhesion. During peritoneal tissue repair, peritoneal mesothelial cells that repair the peritoneum may originate from normal peritoneal mesothelial cells at the edge of the incision, stem cells that differentiated into mesothelial cells, and free peritoneal mesothelial cells in the abdominal cavity [23]. Under inflammatory conditions, mesothelial cells may be shed and die. Thus, promoting cell adhesion can promote poor conditions and cause cells floating in the abdominal cavity to adhere to the peritoneal tissue [24]. Here, we demonstrated that TMIGD1 promotes cell adhesion, which may be another mechanism by which TMIGD1 participates in abdominal adhesion formation.

Though, in this study we found that TMIGD1 is low expressed in the adhesion tissues. However, we did not detect the expression of TMIGD1 in humans because TMIGD1 is expressed at very low levels in human tissues other than renal tissues. This may limit the ability of TMIGD1 to prevent abdominal adhesion in humans.

4. Materials And Methods

4.1 Induction of Adhesion via Peritoneal Injury

A rat abdominal adhesion model was established as previously reported [12, 13]. After the rats were anesthetized and sterilized, a 2- to 3-centimeter incision was made in the middle of the abdomen. A PAA model was established by scraping the lower right abdominal wall and the adjacent cecum until pinprick hemorrhagic spots appeared. Then, the cecum was placed in the abdominal cavity adjacent to the damaged peritoneum, and the abdominal cavity was closed. The rats were sacrificed 3 or 7 days after the operation, and adhesion tissue specimens with surrounding normal cecum and abdominal wall were collected for the subsequent experiment.

4.2 Cell or Tissue Immunofluorescence and Tissue Immunohistochemical Staining

Tissues collected from the rat models were first soaked in 10% formalin for 24 hours. Then, 4- μ M-thick paraffin sections were obtained. For cell immunofluorescence, 1×10^5 cells were seeded on cell slides and fixed with 4% paraformaldehyde. Immunostaining and immunofluorescence were performed according to the manufacturer's instructions, and the samples were incubated overnight at 4 °C with primary antibodies against TMIGD1 (1:100, Bioss, Beijing, China, 1:1000 dilution) and CK19 (1:50, Wuhan Google biotechnology co., LTD, Wuhan, China). A Nikon Eclipse C1 confocal laser scanning microscope (Nikon Corporation, Tokyo, Japan) was used to take a series of images.

4.3 Cell Culture

The human mesothelial cell line HMrSV5 was obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. These cells were cultivated in MEM (Gibco, Thermofisher, Beijing, China) supplemented with 10% FBS (Gibco BRL, Carlsbad, CA, USA) at 5% CO₂ at 37 °C. An overexpression lentivirus was purchased from Gemma Biotechnology Co., Ltd. and used according to the instructions.

4.4 Western Blotting

Western blotting was performed as described in the literature [14]. Protein was extracted with the RIPA Protein Extraction Kit (HeTe, Xi'an, Shaanxi, China). A 12% sodium dodecyl sulfate polyacrylamide gel was used for electrophoresis. The primary antibodies used in this study included anti-TMIGD1 (Bioss, Beijing, China, 1:1000 dilution), anti-Bax (Proteintech, Chicago, USA, 1:5000 dilution), anti-Bcl2 (Proteintech, Chicago, USA, 1:5000 dilution), anti-PARP- γ (Proteintech, Chicago, USA, 1:5000 dilution), anti-p38 (Immunoway, Chicago, USA, 1:5000 dilution), anti-p-p38 (Immunoway, Chicago, USA, 1:5000 dilution), anti-GAPDH (Proteintech, Chicago, USA, 1:5000 dilution) antibodies. Protein expression was detected by using a chemiluminescence detection system (Millipore, Billerica, MA, USA).

4.5 Real-Time PCR

Total RNA was isolated from tissue by using TRIzol (Invitrogen, Thermo Fisher Scientific, California, USA), and complementary DNA (cDNA) was synthesized with the PrimeScript RT Reagent Kit (TaKaRa, Osaka, Japan). Real-time PCR was conducted with an IQ5 instrument (Bio-Rad, CA, USA) using SYBR Green fluorescence signal detection assays (TaKaRa, Osaka, Japan) and primers. mRNA expression was

analyzed by the $-2^{\Delta\Delta Ct}$ method. The primers used in this study were as follows: sense and antisense primers targeting rat TMIGD1 (5'-TCCTGTCATGCCGATAGAGC-3' and 5'-TCTTTCACGCAGAGCTTCAT-3') and sense and antisense primers targeting human TMIGD1 (5'-CTCCCATGCCATCCCTTGTTA-3' and 5'-CGATCCTTTGCGAATGGAGAAAT-3').

4.6 Detection of ROS

To detect ROS levels in cells, 5×10^6 cells were seeded on coverslips. After 24 hours, the cells were treated with 500 $\mu\text{mol/L}$ H_2O_2 (Sigma Chemical Co., St. Louis, MO, USA) for 12 hours. Then, the cells were washed twice and incubated with 10 μM 2',7'-dichlorofluorescein diacetate (DCFH-DA) at 37 °C for 30 min in the dark. Finally, the cells were washed again and detected with a Leica microscope. The fluorescence intensity was analyzed by ImageJ software.

4.7 MTT Assay

Five thousand cells were seeded in 96-well plates, and after 12 hours, the cells were treated with different concentrations of H_2O_2 for 24 hours and then with 20 μL of 5 mg/mL MTT solution for 4 hours. The MTT crystals were dissolved in DMSO, and cell viability was evaluated with a microplate reader (Thermo Fisher, Waltham, USA).

4.8 Detection of Apoptosis and the Mitochondrial Membrane Potential

Cell apoptosis and the mitochondrial membrane potential were detected with relevant kits (apoptosis: Affinity BioReagents; mitochondrial membrane potential detection with JC-1: Solebo Biotechnology Co., Ltd.) according to the manufacturer's instructions.

4.9 Scratch Wound and Adhesion Assays

The migratory potential of cells expressing TMIGD1 at different levels was detected by a scratch wound assay. Cells were seeded in 6-well plates and grown to confluence. Then, the cells were scratched with a 200- μL pipette tip and cultured for 48 hours. Images were taken with a photomicroscope (Leica DFC950 camera; Leica Microsystems, Wetzlar, Germany), and cell migration was quantitated using Scion Image software (beta 4.0.2, Scion, Frederick, MD). Cell adhesion was evaluated using a commercial kit (Bestbio, Beijing, China) following the manufacturer's instructions.

4.10 Statistical Analysis

The data collected in this study were analyzed by SPSS 18.0 (Chicago, IL, USA), and all results are expressed as the mean $\pm$ standard deviation. Normally distributed data were analyzed by *t* test or one-way ANOVA (comparisons between two groups were analyzed using the least significant difference (LSD) test). Nonnormally distributed data were analyzed by the Kruskal-Wallis test. The χ -square test or Fisher's exact test was used to analyze quantitative data. *P*-values less than 0.05 were considered statistically significant.

5. Conclusion

In our study, we found that TMIGD1 was expressed at low levels in rat adhesion tissue including the peritoneal mesothelial cell. Overexpression of TMIGD1 can reduce H₂O₂-induced oxidative stress by promoting p38 phosphorylation in human mesothelial cell line HMrSV5. Besides, as a member of the extracellular adhesion family of molecules TMIGD1 can promote cell adhesion.

Abbreviations

Abbreviations	Full name
PAA	Postoperative abdominal adhesion
TMIGD1	Transmembrane and immunoglobulin domain-containing 1
ROS	Reactive oxygen species
CK19	Cytokeratin 19
MTT	3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide

Declarations

Ethics approval and consent to participate

All the animal used in this study was well treated and animal experiments were approved by the ethics committee of medical science department of Xi 'an Jiaotong university.

Consent for publication

Not applicable.

Availability of data and materials

The data to support the findings of this study are available from the corresponding author upon request

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

Yunhua Wu and Xuqi Li contributed to the experimentation and manuscript composition and contributed equally to this work. Yunhua Wu, Cong Shen, Zijun Wang contributed to the animal experiments. Yunhua Wu, Guangbing Wei, Qiuying Gao and Xuqi Li contributed to the in vivo experiments. Yunhua Wu, Guangbing Wei and Dong Liu had contributed to the data collection and analysis. All authors read and approved the submission and publication of the final version of manuscript. The authors vouch for the accuracy and completeness of the experiment data.

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Figures

Figure 1

TMIGD1 is expressed at low levels in abdominal adhesion tissue (A). qPCR demonstrated that TMIGD1 was expressed at low levels in adhesion tissue of a rat abdominal adhesion model (compared to normal tissue) on day 3 and day 10. N=6, * compared to normal tissue, $P < 0.05$; t test. (B/C).

Immunohistochemical staining demonstrated that TMIGD1 was expressed at low levels in adhesion tissue of a rat abdominal adhesion model (compared to normal tissue) on day 3 and day 10. N=6, the

black arrow shown the normal peritoneum or adhesion tissue, with 200× magnification in the top right corner. * compared to normal tissue, $P<0.05$; t test. (D/E). Western blotting demonstrated that TMIGD1 was expressed at low levels in adhesion tissue of a rat abdominal adhesion model (compared to normal tissue) on day 3 and day 10. $N=6$, * compared to normal tissue, $P<0.05$; t test. (F/G). Immunofluorescence staining for CK19 (red fluorescence) and TMIGD1 (green fluorescence) in adhesion tissue and the corresponding fluorescence intensities. $N=6$, the yellow arrow shown the normal peritoneum or adhesion tissue, with 400× magnification. * compared to normal tissue, $P<0.05$; t test.

Figure 2

Construction and identification of a cell line stably transfected with TMIGD1 (A). qPCR was used to verify TMIGD1 expression in the control and high TMIGD1-expressing cell lines. ** compared to the control, P<0.05. (B). Western blotting was used to verify TMIGD1 expression in the control and high TMIGD1-expressing cell lines. (C). Cell immunofluorescence was used to evaluate TMIGD1 expression in the control and high TMIGD1-expressing cell lines. with 200× magnification.

Figure 3

TMIGD1 overexpression alleviates H2O2-induced oxidative stress injury (A). The MTT assay showed that oxidative stress injury induced by different concentrations of H2O2 was reduced in the high TMIGD1-expressing cell line. * compared to the control, $P<0.05$; t test. (B). Western blotting showed that TMIGD1 overexpression reduced apoptosis-related protein expression after H2O2 treatment. (C). The apoptosis level of cells transfected with a normal control (NC) or TMIGD1 overexpression vector after H2O2 treatment for 24 hours was examined by flow cytometry (mean $\pm$ SD, $n=3$). * compared to the control, $P<0.05$; t test. (D). The mitochondrial membrane potential level of cells was examined by JC-1 staining. The flow cytometry scatter plot shows the distribution of cell populations with JC-1 aggregates (red) and JC-1 monomers (green). Histogram of the relative ratio of red to green fluorescence (mean $\pm$ SD, $n=3$). * compared to the control, $P<0.05$; t test. (E). ROS levels in cells transfected with an NC or TMIGD1 overexpression vector and treated with H2O2 (mean $\pm$ SD, $n=3$). With 100 $\times$ magnification. * compared to the control, $P<0.05$; t test.

Figure 4

TMIGD1 reduces oxidative stress injury via phosphorylation of p38 (A). Western blotting was used to evaluate the expression of total and phosphorylated p38 in different TMIGD1-expressing cells. (B). The

p38 inhibitor SB203580 inhibited the phosphorylation of p38 in the TMIGD1-overexpressing cell line. (C). The p38 inhibitor SB203580 inhibited the protective effect of TMIGD1 against cell apoptosis. * compared to the control, $P<0.05$; one-way ANOVA. (D). The effect of TMIGD1 on mitochondrial damage was reduced by the 38 inhibitor SB203580. * compared to the control, $P<0.05$; one-way ANOVA. (E). ROS levels in control and TMIGD1-overexpressing cells treated with the p38 inhibitor SB203580. * compared to the control $P<0.05$; one-way ANOVA.

Figure 5

TMIGD1 overexpression inhibits migration and promotes cell adhesion of mesothelial cells (A). The cell scratch assay was used to evaluate cell migration in different TMIGD1-expressing cell lines at different time points. (B). The wound scratch healing rate of different TMIGD1-expressing cell lines at different time points. * compared to the control $P<0.05$; t test. (C). Cell adhesion conditions in different TMIGD1-expressing cell lines. * compared to the control, $P<0.05$; t test.

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