Transmembrane protein 106A is silenced by promoter region hypermethylation and suppresses gastric cancer growth by inducing apoptosis

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Abstract

Inactivation of tumour suppressor genes by promoter methylation plays an important role in the initiation and progression of gastric cancer (GC). Transmembrane 106A gene (TMEM106A) encodes a novel protein of previously unknown function. This study analysed the biological functions, epigenetic changes and the clinical significance of TMEM106A in GC. Data from experiments indicate that TMEM106A is a type II membrane protein, which is localized to mitochondria and the plasma membrane. TMEM106A was down-regulated or silenced by promoter region hypermethylation in GC cell lines, but expressed in normal gastric tissues. Overexpression of TMEM106A suppressed cell growth and induced apoptosis in GC cell lines, and retarded the growth of xenografts in nude mice. These effects were associated with the activation of caspase-2, caspase-9, and caspase-3, cleavage of BID and inactivation of poly (ADP-ribose) polymerase (PARP). In primary GC samples, loss or reduction of TMEM106A expression was associated with promoter region hypermethylation. TMEM106A was methylated in 88.6% (93/105) of primary GC and 18.1% (2/11) in cancer adjacent normal tissue samples. Further analysis suggested that TMEM106A methylation in primary GCs was significantly correlated with smoking and tumour metastasis. In conclusion, TMEM106A is frequently methylated in human GC. The expression of TMEM106A is regulated by promoter hypermethylation. TMEM106A is a novel functional tumour suppressor in gastric carcinogenesis.

Keywords: transmembrane protein 106A • gastric cancer • apoptosis • epigenetic alteration

Introduction

Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer-related death worldwide. Despite considerable advances in surgical techniques, clinical diagnostics and new chemotherapy regimens, the clinical outcome of GC remains unsatisfactory [1], mainly because of a poor understanding of the molecular mechanism of GC development. However, epigenetic inactivation of tumour suppressor genes by promoter methylation is recognized to have a crucial role in gastric carcinogenesis and progression [2–4]. The identification of novel promoter hypermethylated genes may provide insights into the mechanisms of inactivation of tumour suppressive pathways and identify tumour markers for GC.

The TMEM106A gene on chromosome 17q21.31 encodes TMEM106A, a member of the TMEM106 family, comprising TMEM106A, TMEM106B and TMEM106C. TMEM106B is a transmembrane protein of unknown function, and is a bona fide risk factor for Frontotemporal lobar degeneration, especially in patients with Progranulin mutations [5–11]. TMEM106B is also associated with cognitive impairment in amyotrophic lateral sclerosis [12], and in the pathological presentation of Alzheimer disease [13]. TMEM106B is a type II integral membrane protein, localized in the late endosome/lysosome compartments and is regulated by lysosomal activities [14, 15]. Human TMEM106C is a differentially expressed transcript in ankylosing spondylitis [16], and porcine TMEM106C was a positional and functional candidate for arthrogryposis multiplex congenita [17]. Yu et al. identified 54 cellular mRNAs, including TMEM106A, which that appear to be Cyclin T1-dependent for their induction in activated CD4+ T
Jurkat T cells [18]. However, no detailed functional study has been reported.

In our human genomics project, we have cloned hundreds of functionally unknown human open reading frames (ORFs) by searching the human RefSeq and expressed sequence tag databases in GenBank. Using a cell-based high-throughput assay, we identified several novel genes associated with cell viability [19], including TMEM106A. The present study reports that TMEM106A is localized to mitochondria and the plasma membrane. Loss/reduction of TMEM106A expression is associated with promoter region hypermethylation in GC. Restoration of TMEM106A expression induced GC cell apoptosis and suppressed GC cell growth, suggesting that TMEM106A is a tumour suppressor in GC.
Adenoviral vectors

All of the recombinant adenovirus vectors were based on type 5 (E1/E3 deficient) adenovirus. Ad5-null and Ad5-GFP were purchased from Sino-GenoMax (Beijing, China). The complete coding sequence of the TMEM106A was subcloned into the BamHI and EcoRI sites of the pShuttle-CMV vector. The expression cassette of TMEM106A was then transferred into the adenoviral backbone vector pAdxsi, and the recombinant clones were confirmed by DNA sequencing. The recombinant viral vector of TMEM166 (Ad5-TMEM166) was linearized by PstI digestion and packaged into HEK293 cells. Viral particles were purified by caesium chloride density gradient centrifugation and titrated by TCID50 method. Ad5-EGFP was used to monitor the efficiency of cell infection and those with higher than 70% infection efficiency were used for further experiments.

Immunofluorescence and confocal microscopy

HeLa cells transfected with pcDB-TMEM106A plasmids were plated on glass coverslips. Twenty four hours after transfection, the cells were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. The cells were then incubated with blocking buffer (3% BSA in PBS) and stained with rabbit anti-TMEM106A antibody overnight at 4°C, followed incubation with FITC-goat anti-rabbit IgG. Nuclei were stained with Hoechst 33342 (H33342). The treated cells were observed and documented with an Olympus FV1000 confocal microscope (Olympus, Tokyo, Japan).

To observe membranes containing TMEM106A, HeLa cells were plated on coverslips the day prior to transfection with the indicated plasmids. Twenty four hours later, cells were incubated with mouse anti-MYC antibody or rabbit anti-TMEM106A antibody overnight at 4°C. Then the cells were stained with FITC-conjugated secondary antibodies for 2 hrs at 4°C, fixed with 4% paraformaldehyde, and observed as above.

5-aza-2’-deoxycytidine (5-Aza) treatment

Gastric cancer cell lines were split at low density (30% confluence) 12 hrs before treatment, and then treated with 2 μM of the DNA demethylating agent 5-aza-2’-deoxycytidine (5-Aza) for 96 hrs. The treated cells were harvested for DNA and RNA extraction.

Methylation-specific PCR (MSP)

Genomic DNA from GC cell lines and tissue specimens were prepared using the proteinase-K method [21], and modified by sodium bisulphite treatment, as described previously [22]. MSP primers for TMEM106A were designed (Table 1) and synthesized according to genomic sequences flanking the presumed transcription start sites. The MSP reaction was performed as previously described [21].

Bisulphite genomic sequencing (BGS)

Bisulphite-treated DNA was subjected to PCR using primers flanking the targeted MSP regions above. Sequencing primers were listed in Table 1. PCR cycle conditions were as follows: 95°C × 5 min.; 35 cycles (95°C × 30 sec., 55°C × 30 sec., 72°C × 40 sec.); 72°C × 5 min. PCR products were cloned into vector pCR2.1, according to the manufacturer’s protocol (Invitrogen). Six to ten colonies were randomly chosen and sequenced.

Immunohistochemistry

Immunohistochemistry was performed on paraffin sections of GC samples using rabbit anti-TMEM106A antibody. Assigning the percentage of positive tumour cells (0, none; 1, <20% of positive staining cells; 2, 20–50% of positive staining cells; 3, >50% of positive staining cells) scored the extent of TMEM106A staining.

Cell viability assay

Cells infected with Ad5-null or Ad5-TMEM106A were harvested and plated in 96-well plates at 2000 cells per well and incubated at 37°C. Cell viability was analysed using the Cell Counting Kit-8 [23].

 Colony formation assay

Cells were transfected with pcDB-TMEM106A or empty pcDB using lipofectamine 2000. After 48 hrs of transfection, cells were selected with G418 at 0.3 mg/ml for 2 weeks. G418-resistant colonies with ≥50 cells were counted after crystal violet staining.

Flow cytometry analysis

To detect the efficiency of adenovirus infection, cells were treated with Ad5-GFP at the indicated multiplicity of infection (MOI) for 24 hrs before harvesting and analysis on a FACScalibur flow cytometer. An FITC-Annexin V staining Detection kit (Biosea Biotechnology, Beijing, China) was used to analyse cell apoptosis, according to the manufacturer’s instructions [23].

Caspase activity assays

Caspase-2, caspase-3, caspase-9 and caspase-12 activities were measured using the Caspase Fluorometric Assay Kit (BioVision, Milpitas, CA, USA). All procedures were carried out according to the manufacturer’s instructions.

Western blot analysis

Total proteins were extracted and measured by the BCA protein assay reagent (Pierce, Rockford, IL, USA). Equal amounts of protein were separated by 12.5% SDS-PAGE and transferred onto polyvinylidene fluoride membranes. The membranes were blocked and incubated with the indicated antibodies. Blots were visualized using an IRDye 800CW-conjugated secondary antibody, and the fluorescence images were obtained using an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA).
In vivo tumourigenicity

A nude mouse xenograft model was established using 6-week-old female BALB/c nude mice (Experimental Animal Center, Peking University Health Sciences Center, Beijing, China). Mice were housed and maintained in a pathogen-free facility, and the Institutional Authority for Laboratory Animal Care of Peking University approved all experimental procedures and protocols. For in vivo treatments, HGC-27 cells infected with Ad5-TMEM106A, Ad5-null or mock were injected subcutaneously into the left axilla of BALB/c nude mice in a total volume of 100 µl (4 × 10^6 cells), respectively. The tumours were measured using callipers every 2 days for 3 weeks. The tumour volume for each mouse was determined (in cubic millimetres) by measuring in two dimensions and calculated as tumour volume = length × (width)^2/2.

Statistical analysis

All analyses were performed with the SAS statistical package. Data were presented as the mean ± SD. Statistical analyses was carried out with the Student’s t-test, the non-parametric tests or the chi-squared test. A P < 0.05 was considered statistically significant.

Results

Bioinformatics analysis and expression profile of human TMEM106A

The human TMEM106A gene is located on chromosome 17q21.31 and encompasses nine exons and eight introns (Fig. 1A). The full length of human TMEM106A cDNA is 2795 bp comprising an ORF encoding a predicted 28.9 kD protein of 262 amino acids (Fig. S1A) with an isolectric point of 7.04. The alignment of TMEM106A sequences from various eukaryotic organisms showed that TMEM106A is highly evolutionarily conserved (Fig. S1B and s1C). Transmembrane (TM) analysis (http://www.cbs.dtu.dk/services/TMHMM-2.0/) [24] suggested that TMEM106A is a type II TM protein with a conserved TM domain (94-116 aa) (Fig. S1A, dashed lines). As far as we know, no functional studies have been performed on TMEM106A.

The presence of the TMEM106A mRNA was confirmed by quantitative real-time PCR and semi-quantitative reverse transcription-PCR in a variety of normal human tissues and cell lines. The TMEM106A mRNA is expressed in various tissues and organs (Fig. 1B and Fig. S2A). TMEM106A mRNA was detected at a low level or was absent in various cell lines (Fig. 1C and Fig. S2B). The rabbit anti-TMEM106A specific antibody, prepared by using chemically
TMEM106A is a type II membrane protein and localizes to plasma membrane and mitochondria

Bioinformatic prediction program suggests TMEM106A is a type II membrane protein that lacks an N-terminal signal peptide and may contain one TM domain. To confirm this prediction, HeLa cells were transfected with empty vector or TMEM106A-MYC (MYC is in the C-terminus) plasmids for 24 hrs, stained with anti-MYC monoclonal antibody, and observed by confocal microscopy. Overexpressed TMEM106A-MYC is localized in the living HeLa cell membrane, with its C-terminus facing extracellular space (Fig. 2A). The membrane-expressed TMEM106A protein was also recognized by the rabbit anti-TMEM106A antibodies (Fig. 2A, right). Consistent with the confocal observation, we also did flow cytometry analysis and found that TMEM106A-MYC appeared in the cell membrane surface in living cells (Fig. S3).

To observe the subcellular localization of TMEM106A, HeLa cells were transfected with pCDB-TMEM106A and marker plasmids (DsRed-ER and DsRed-Golgi) or dye (MitoTracker and LysoTracker) for subcellular compartments stained by immunofluorescence assay, and observed by confocal microscopy. Overexpressed TMEM106A was colocalized predominantly with MitoTracker (Fig. 2B), but not with DsRed-ER, DsRed-Golgi and LysoTracker (data not shown). Taken together, these results indicate that TMEM106A is localized in the plasma membrane and mitochondria.

TMEM106A mRNA is down-regulated or silenced by promoter hypermethylation in GC cell lines

Our results suggested that TMEM106A expression was reduced or absent in most tumour cell lines (Fig. 1C and Fig. S2B). Promoter region methylation is thought to play an important role in gene expression regulation. Bioinformatic analysis indicated that TMEM106A contains a typical CpG island in the promoter region. Therefore, we explored the possibility of promoter region methylation and TMEM106A mRNA expression. Using real-time quantitative PCR, we detected the levels of TMEM106A mRNA in eight GC cell lines and non-tumour stomach tissue. By comparison with non-tumour stomach, the levels of TMEM106A mRNA in detected GC cell lines were significantly decreased. Nevertheless, the TMEM106A mRNA expressed in these eight cell lines was different. The expression of TMEM106A mRNA was almost absent in HGC-27, NUGC3, MKN45 and MGC803 cell lines, while other cell lines such as BGC823, SGC7901, AGS and N87 displayed higher levels of TMEM106A mRNA compared to the former (Fig. 3A). Figure 3B was the results of MSP analysis. It was noted that the TMEM106A promoter was partially methylation in MGC803, BGC823, SGC7901, AGS, PHM82 and N87 cells, but the methylation level of N87 is the lowest. The almost complete methylation was detected in MKN45, HGC-27 and NUGC3 cells. Comparison of Figure 3A and B, we found that there was a good correlation between expression down-regulation and methylation of TMEM106A in these cell lines. That is to say, fully methylated cell lines (MKN45, HGC-27 and NUGC3) almost did not express TMEM106A mRNA, whereas partially methylated cell lines (SGC7901, AGS and N87) expressed higher levels of TMEM106A mRNA, except for MGC803. To validate the MSP results and further analyse the methylation status of the TMEM106A promoter, BGS was performed which confirmed the MSP analysis (Fig. 3C).

We further investigated if TMEM106A expression could be rescued by pharmacological demethylation. MKN45, HGC-27 and NUGC3 cell lines were treated with the DNA methyltransferase inhibitor 5-Aza. Results from quantitative PCR assay demonstrated that the re-expression of TMEM106A was observed in these cells treated by 5-Aza (Fig. 3D). Taken together, our data suggested that TMEM106A expression is regulated by promoter methylation in GC cells.
To explore the methylation status of TMEM106A in primary GCs, 105 cases of GCs were examined by MSP. Frequent methylation of TMEM106A was detected in GCs (93/105, 88.6%; Fig. 4A), while only two cases were methylated among 11 cancer adjacent non-tumour samples (2/11, 18.1%; Fig. 4B). Further analysis suggested that TMEM106A methylation in primary GCs was significantly correlated with smoking ($P = 0.0422$), and tumour metastasis ($P = 0.0462$). No association was found with age, gender, alcohol abuse, differentiation and TNM stage (Table 2).

Immunohistochemistry was performed to evaluate TMEM106A protein expression in 49 paired GCs and their cancer adjacent non-tumour tissues. A blinded pathologist scored the expression level of TMEM106A in individual samples. As shown in Figure 4C and D and Table 3, the expression of TMEM106A protein was positive (45/49, 91.8%) in most cancer adjacent non-tumour tissues (Fig. 4C, Table 3), but was near negative (48/49, 98.0%) in primary GCs (Fig. 4D, Table 3).

**TMEM106A suppressed GC cell growth**

The frequent silencing of TMEM106A in GC cell lines and primary cancers, but not in normal gastric mucosa, suggested that TMEM106A is a tumour suppressor. Therefore, we examined the effect of ectopic expression of TMEM106A on the growth and viability in HGC-27 and NUGC3 cells with complete methylation and silencing of TMEM106A. Western blotting showed that the TMEM106A protein expression significantly increased in HGC-27 and NUGC3 cells infected with Ad5-TMEM106A (Fig. 5A). We selected MOI 200 for HGC-27 and MOI 50 for NUGC3 for subsequent experiments. The cell viability of the aforementioned cell lines, infected by Ad5-Null or Ad5-TMEM106A for different times, was assessed using the CCK-8 assay. The growth of HGC-27 and NUGC3 cells infected by Ad5-TMEM106A was inhibited significantly more than with Ad5-Null treatment, and the inhibition was time dependent (Fig. 5B). This effect on GC was further proved by a colony formation assay. TMEM106A re-expression significantly suppressed the colony-forming ability in HGC-27 and NUGC3 cells, compared with the control group (Fig. 5C1 and C2). If TMEM106A was silenced in BGC823 cells which were transfected by effective shRNAs against TMEM106A, the colony-forming ability was increased compared with shRNA control cells (Fig. S4). Thus, TMEM106A inhibits the growth of GC cells, functioning as a potential tumour suppressor.

On the basis of the above observations, we then tested whether TMEM106A could suppress the growth of GC cells in nude mice in vivo. The tumour growth curves of HGC-27 cells infected with mock, Ad5-null or Ad5-TMEM106A alone in nude mice are shown in Figure 5D1. The Ad5-null and mock groups developed grossly
visible tumours at the site of injection within 22 days. By comparison, Ad5-TMEM106A displayed smaller tumours that became invisible at the end of the period. At the end of the experiment, xenograft tumours were isolated and photographed (Fig. 5D2). Compared with Ad5-null and mock group, there was no visible tumour in the Ad5-TMEM106A group, indicating TMEM106A inhibits tumourigenicity. In addition, no toxicity was observed in any of the control or treatment groups throughout these studies, as monitored by weight loss (Fig. 5D3).

**TMEM106A induces apoptosis of GC cells via the caspase activation**

A key biochemical hallmark of apoptosis is the translocation of phosphatidylserine (PS) from the cytoplasmic surface of the cell membrane to the external cell surface. Exposure of PS on the surface of apoptotic cells can be easily identified by flow cytometry using fluorescence-labelled Annexin V, which specifically binds PS. To characterize TMEM106A-induced growth arrest, we detected PS surface exposure in Ad5-TMEM106A-treated HGC-27 and NUGC3 cells. A representative result of the flow cytometry (Fig. 6A) revealed that HGC-27 and NUGC3 cells treated by Ad5-TMEM106A displayed more apoptotic cells than those cells infected with Ad5-Null.

Caspase-mediated apoptosis is the best-defined cell apoptosis for countering tumour growth. To confirm the correlation between the activation of caspase cascades and TMEM106A levels, HGC-27 cells infected with either Ad5-TMEM106A or Ad5-Null were assayed for caspase-2, caspase-3, caspase-9 and caspase-12 activities. The Ad5-TMEM106A-infected cells exhibited elevated caspase-2, caspase-3 and caspase-9 activities compared with Ad5-Null, but there was no obvious difference for caspase-12 (Fig. 6B). To further determine if Ad5-TMEM106A-induced cell apoptosis was caspase dependent, HGC-27 cells were pre-treated with 50 μM z-VAD-fmk for 2 hrs before the addition of Ad5-TMEM106A for another 48 hrs. Annexin V-binding assays showed that pre-treatment with z-VAD-FMK partly decreased cell apoptosis (Fig. 6C), indicating that caspase activation at least partly mediated Ad5-TMEM106A-induced cell apoptosis.

Caspase-2 initiates apoptosis by cleaving the BCL-2 family member BID to form cleaved BID (tBID) [25, 26], which results in
activation of the mitochondrial pathway and cleavage of caspase-3. Western blotting indicated that compared with the null cells, the overexpression of TMEM106A (Fig. 6D1) in HGC-27 cells markedly decreased the levels of the full-length BID and increased the levels of tBID (Fig. 6D2), following augmented cleaved caspase-3 (Fig. 6D3). Cleaved poly(ADP-ribose) polymerase (PARP), which is associated with caspase-3 activation, was present in Ad5-TMEM106A-infected cells, but rarely detected in control cells (Fig. 6D4). Taken together, these results demonstrated the importance of caspase signalling cascades in Ad5-TMEM106A-mediated cell apoptosis.

| Clinicopathological parameters | n | TMEM106A methylation status | P-value |
|-------------------------------|---|----------------------------|---------|
|                              |   | Methylated n = 93 (88.6%) |         |
|                              |   | Unmethylated n = 12 (11.4%)|         |
| Age (years)                  |   |                           |         |
| <50                          | 20| 17 (85%)                  | 3 (15%) | 0.5787 |
| ≥50                          | 85| 76 (89.4%)                | 9 (10.6%)|       |
| Gender                       |   |                           |         |
| Male                         | 77| 69 (89.6%)                | 8 (10.4%)| 0.5808 |
| Female                       | 28| 24 (85.7%)                | 4 (14.3%)|       |
| Alcohol abuse                |   |                           |         |
| Negative                     | 75| 68 (90.7%)                | 7 (9.3%) | 0.2883 |
| Positive                     | 30| 25 (83.3%)                | 5 (16.7%)|       |
| Smoking                      |   |                           |         |
| Negative                     | 71| 66 (93.0%)                | 5 (7.0%) | 0.0422*|
| Positive                     | 34| 27 (79.4%)                | 7 (20.6%)|       |
| Differentiation              |   |                           |         |
| Poor/Moderate                | 95| 84 (88.4%)                | 11 (11.67%)| 0.5337 |
| Well                         | 3 | 3 (100.0%)                | 0 (0.0%) |       |
| T                            |   |                           |         |
| T2/T3                        | 7 | 7 (100.0%)                | 0 (0.0%) | 0.3276 |
| T4                           | 98| 86 (87.8%)                | 12 (12.2%)|       |
| N                            |   |                           |         |
| N0                           | 17| 13 (76.5%)                | 4 (23.5%)| 0.0882 |
| N1/N2/N3                    | 88| 80 (90.9%)                | 8 (9.1%) |       |
| M                            |   |                           |         |
| M0                           | 81| 69 (85.2%)                | 12 (14.8%)| 0.0462*|
| M1                           | 24| 24 (100.0%)               | 0 (0.0%) |       |
| TNM Stage                    |   |                           |         |
| II/III                       | 84| 72 (85.7%)                | 12 (14.3%)| 0.0670 |
| IV                           | 21| 21 (100.0%)               | 0 (0.0%) |       |

*P < 0.05.
Discussion

We cloned the entire ORF of the human gene transmembrane protein 106A (TMEM106A). Sequencing analysis revealed that TMEM106A is conserved in human, chimpanzee, dog, bovine, mouse, rat and rhesus monkey, indicating that it may have important functions in vertebrates. Bioinformatics analysis suggested that TMEM106A is a type II TM protein. TMEM106A is located in the plasma membrane and mitochondria, and is expressed at a low level in various tissues and cell

| Groups                              | n  | TMEM106A expression | P-value   |
|-------------------------------------|----|---------------------|-----------|
| Gastric cancer                      | 49 | 48 1 0 0            | <0.0001   |
| Cancer adjacent non-tumour tissues  | 49 | 4 11 16 18          |           |

Table 3 Comparison of TMEM106A protein expression in gastric cancer and cancer adjacent tissues

Fig. 5 TMEM106A inhibits tumour cell growth. (A) HGC-27 and NUGC3 cells were infected with Ad5-TMEM106A at 10, 20, 50, 100, 200 multiplicity of infection (MOI) or Ad5-null at 200 MOI for 24 hrs. The dose-dependent expression of TMEM106A protein was analysed by Western blotting. ACTB was detected as the protein loading control. (B) HGC-27 and NUGC3 cells were infected with Ad5-TMEM106A or Ad5-null for indicated time, cell viability was detected by the CCK8 assay. Data are presented as mean ± SD. ***P < 0.001. (C1) Representative images of the colony formation in HGC-27 and NUGC3 cells transfected with Ad5-TMEM106A or Ad5-Null were shown. (C2) Quantitative analysis of colony numbers is shown for three independent experiments. Data are mean ± SD; ***P < 0.001. (D) Ad5-TMEM106A inhibited the growth of tumours in vivo. (D1) HGC-27 cells infected with Ad5-TMEM106A or Ad5-Null were injected subcutaneously in BALB/c nude mice. Development of tumours (mean volume ± SD) was monitored using callipers every 2 days. (D2) Excised xenograft tumours were photographed on day 22. (D3) Bodyweight (mean ± SD) was determined over time in different treatment groups.

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Protein expression of TMEM106A was significantly decreased in primary GC tissues compared with adjacent tissues, suggesting that TMEM106A is important in gastric carcinogenesis.

We showed that the silencing or down-regulation of TMEM106A was closely related with promoter hypermethylation, as demonstrated by methylation-specific PCR, BGS analysis and by the restored expression of TMEM106A in silenced cancer cells obtained using 5-Aza treatment. Thus, promoter hypermethylation of TMEM106A results in transcriptional silencing, which may contribute to the development of GC.

The biological function of TMEM106A in human GC was further investigated in vitro and in vivo. Ectopic expression of TMEM106A in the silenced HGC-27 and NUGC3 cells significantly inhibited cell viability and colony formation ability. In GC cell lines, ectopic expression of TMEM106A induced significant cell apoptosis. We then studied the tumour suppressive effect of TMEM106A against gastric tumour
formation in vivo: tumour growth was significantly inhibited in nude mice inoculated with HGC-27/Ad5-TMEM106A compared with those inoculated with HGC-27/Ad5-Null. Thus, the in vitro and in vivo results indicated that TMEM106A functions as a tumour suppressor in gastric carcinogenesis. This anti-tumour effect of TMEM106A in GC suggests that restoration of the function of TMEM106A could halt or reverse GC, thus having a potential therapeutic effect.

The molecular basis of TMEM106A’s tumour suppressor property in GC involved activation of the caspase cascade, partly mediated the enhanced apoptosis ability by TMEM106A and subsequent cleavage of substrates. Caspase-2, an initiator caspase, was enhanced by TMEM106A restoration. Caspase-2 is activated by dimerization and then initiates apoptosis via the mitochondrial apoptotic pathway [26]. Initiator caspases generally function at the apex of their respective signalling cascades and promote activation of executioner caspases, directly or indirectly. Caspase-2-mediated cleavage of the pro-apoptotic BCL-2 family member BID is generally considered the mode through by which caspase-2 initiates apoptosis [27]. Restoration of TMEM106A activates caspase-2, which activates BID by transforming it into truncated BID (tBID). Once BID is cleaved, the tBID translocates onto mitochondria and promotes mitochondrial membrane permeability and cytochrome c release, thereby triggering the formation of the apoptosome and caspase-9 activation. Activated caspase-9 processes effector caspase-3 to initiate a caspase cascade. Caspase-3 further initiates the proteolytic cleavage of the nuclear enzyme PARP, which causes loss of DNA repair, cellular disassembly and finally, apoptosis. Z-VAD-fmk, a caspase inhibitor, partly rescued cell viability from TMEM106A-induced apoptosis, confirming that activation of the caspase cascade is involved in TMEM106A-induced apoptosis.

To investigate the clinical application of TMEM106A in gastric tumorigenesis in vivo, we examined promoter methylation of TMEM106A primary GCs and normal controls. TMEM106A gene promoter was methylated in 88.6% of GCs compared with 18.1% of normal controls. If TMEM106A is indeed a tumour suppressor, inactivation of the gene by promoter methylation would favour tumour progression and a worse outcome. Therefore, we investigated the clinical significance of TMEM106A promoter methylation and its associations with patient clinicopathological factors. TMEM106A promoter methylation was significantly associated only with smoking and tumour metastasis. Increasing evidence suggests that promoter methylation could be a predictive biomarker in many human cancers [28–36]. Therefore, TMEM106A tumour-specific promoter methylation may become an epigenetic biomarker for GC. Further studies with additional patient cohorts are required to better understand the prognostic significance of TMEM106A in GC.

Gastric cancer is a major health burden worldwide, and conventional treatments for GC (chemotherapy and radiotherapy) have limited effectiveness. Thus, new treatment strategies are needed for GC. Increasingly, therapeutic strategies to inhibit anti-apoptotic signals selectively in tumour cells are being sought that could provide powerful tools to treat GC. Gene therapy is one of the approaches for inducing cancer cell apoptosis, and TMEM106A may show its pro-apoptotic effect in specific and combinatorial treatment approaches in the future.

In conclusion, the novel functional tumour suppressor gene TMEM106A is inactivated by promoter methylation in GC, and has important roles in suppressing cell proliferation and inducing apoptosis. TMEM106A induces cancer cell apoptosis through caspase-2/BID activation and the mitochondrial pathway. TMEM106A is a candidate tumour suppressor, and TMEM106A methylation may serve as an epigenetic biomarker in GC.

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Conflicts of interest

The authors confirm that there are no conflicts of interest.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 TMEM106A is an evolutionarily conserved protein.

Figure S2 Levels of TMEM106A mRNA analysed by reverse transcription-PCR.

Figure S3 Distribution of TMEM106A.

Figure S4 Knockdown of TMEM106A increases the colony-forming ability in BGC823 cells.

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