TMEM100 negatively regulated by microRNA-106b facilitates cellular apoptosis by repressing survivin expression in NSCLC

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Abstract

Background

Non-small-cell lung cancer (NSCLC) is a common malignant tumor. Nevertheless, the 5-year survival rate of NSCLC patients remains poor. Thus, finding critical factors involved in regulating the progression of NSCLC is important for providing potential treatment targets.

Methods

CCK-8, colony formation, caspase-3 activity, luciferase reporter assay, and western blot assays were utilized in this study.

Results

We found that TMEM100 was significantly downregulated in NSCLC tissues compared with paired peritumoral tissues. Decreased TMEM100 expression was associated with poor clinical outcomes in NSCLC patients. Moreover, TMEM100 overexpression inhibited colony formation and facilitated apoptosis via repressing survivin expression in NSCLC cells, whereas TMEM100 knockdown had the opposite effect. In addition, miR-106b, a microRNA with controversial roles in different human cancers, was upregulated in NSCLC and directly downregulated TMEM100 expression. The roles of miR-106b in cell survival were mitigated by the restoration of TMEM100.

Conclusions

The above results indicate that TMEM100 induces cell apoptosis and inhibits cell survival by serving as a tumor suppressor and that miR-106b-degraded TMEM100 expression defines a potentially oncogenic pathway in NSCLC.

Background

Lung cancer is a common malignant tumor, and its cancer-related mortality ranks first among various types of tumors. There are two major pathological types of lung cancer: non-small-cell lung cancer (NSCLC) and small-cell lung cancer. NSCLC, as the main subtype, accounts for more than 75% of cases [1]. The standard treatment option of NSCLC is surgical resection combined with adjuvant radiotherapy/chemotherapy and molecule-targeted agents. However, many patients with NSCLC are diagnosed at a late stage and are not suitable for surgical treatment [2]. Currently, the molecular mechanisms of NSCLC progression are still incompletely understood and the treatment efficiency is far from what people expected. The 5-year survival rate is less than 15% in the persons with NSCLC. Accordingly, it is necessary to determine the oncogenic factors and/or tumor suppressors in the progression of NSCLC to provide potential treatment targets.

An important matter for tumor occurrence and development is the overgrowth of cancer cells without limitation, which greatly derived from the dysfunction of cell death. Thus, the regulators of cell apoptosis are regarded as tumor-related molecules, and inducing apoptosis in cancer cells is considered as an important method for mitigating tumors progression [3]. Accumulating evidence suggests that a few transmembrane proteins (TMEMs) are up- or downregulated in some types of tumors and participate in regulating tumor progression and tumorigenesis [4–6]. It has been reported that the upregulation of TMEM16A promotes cancer metestasis and is correlated with a poor prognosis in human gastric cancer [7]. TMEM88, which is upregulated in tumor, promotes the metastasis of cancer cells and facilitates tumorigenesis in triple-negative breast cancer and lung cancer [8, 9]. TMEM100, belonging to the family of transmembrane proteins, is well conserved in vertebrates. There are two putative transmembrane domains in TMEM100 [10]. The known cellular functions of TMEM100 mainly include arterial endothelium differentiation and vascular morphogenesis. In mouse embryos, TMEM100 knockdown leads to cardiovascular developmental disorders, heart defects, and a failure of vascular remodeling [11]. During atrioventricular canal cushion formation, the endothelial-mesenchymal transformation is impaired by TMEM100 deficiency [12]. Moreover, TMEM100 could mitigate the metastasis and growth of cancer cells in hepatocellular carcinoma [13]. However, the pathophysiologic roles of TMEM100 in the survival of NSCLC cells remain to be explored

In this research, we aimed to determine the regulatory roles of TMEM100 in the apoptosis of NSCLC cells and the corresponding mechanisms involved. We found that TMEM100, frequently downregulated in NSCLC tissues, induced the apoptosis by decreasing survivin expression in NSCLC. Furthermore, mechanistic studies implied that miR-106b was responsible for the decreased expression of TMEM100 and the roles of miR-106b in cell apoptosis were mitigated by TMEM100. These results reveal an important underlying mechanism of regulating NSCLC cells survival and provide potential treatment targets for this fatal disease.

Materials And Methods

Cell culture

H358, H1650, H1299, A549, H1975, and H460 cells were got from ATCC (Manassas, USA). Cells were grown on cell culture flasks in DMEM supplemented with 10% fetal bovine serum (FBS). Cells were cultured under conditions of 5% CO2 atmosphere at 37 °C.
Western Blot Assay

Cell lysates at equal amounts (50 μg protein) were loaded on sodium dodecyl sulfate–polyacrylamide gels, and then the gels were transferred to polyvinylidene difluoridemembranes (Roche, Basel, Switzerland). After 2 hours of transferring, the membranes were incubated with 5% skimmed milk for 1 hour for blocking, and then the primary antibodies were added on the membranes overnight at 4 °C. Primary antibodies included: TMEM100 (1:500; Abcam, Hong Kong, China), Bim and cytochrome C (1:500; Cell Signaling Technology), survivin (1:500; abcam, Hong Kong, China), or beta-actin (1:3000; Cell

Signaling Technology). After extensive washing with TBS-T in about 40 minutes, the corresponding horseradish-peroxidase-conjugated secondary antibodies (1:5000; Cell Signaling Technology) were added onto the membranes for 1 h at room temperature. After extensive washing with TBS-T in about 40 minutes, the signal was next visualized with chemiluminescence substrate (Pierce Biotechnology) by chemiluminescence detection.

Real-time PCR

We performed Real-time PCR as previously described [36]. TRIzol reagent (Invitrogen) and miRNA isolation kit (Ambion) were used to extract total RNA according to the protocol provided by the supplier, respectively. For miRNA analysis, qRT-PCR Taqman probes for miR-106b was got from Applied Biosystems and Taqman premix (Takara, Shiga, Japan) was used for qRT-PCR. For mRNA analysis, A SYBR Green PCR system (Applied Bio-systems) was applied after reverse transcription. Actin and snRNA U6 were utilized as normalization control to quantify mRNA and miRNA expression levels, respectively.

Cell Viability Assay

Cell Counting Kit-8 (CCK-8, Dojindo, Japan) was used to determine cell viability. Cells were cultured into a 96-well plate at 3×10^3 cells per well. After adding 10 μL of CCK-8 solution to each well for 2 h at 37 °C, the absorbance was recorded with a microplate reader at 450 nm.

Colony Formation Assay

Cells were cultured at 0.8×10^3 cells (H358 cells) per well or 3×10^3 cells (H460 cells) per well in 6-cm petri dish. 15 days later, 500 μL 4% paraformaldehyde were added into each well for 10 minutes. After that, 1% crystal violet (Sigma-Aldrich) was utilized to stain the cells. Then colonies could be observed directly with the unaided eye and only clearly visible colonies (megascopic cell colonies) were counted and analyzed.

Caspase-3 Activity Assay

Caspase-3 assay kit (Invitrogen, USA) was utilized to detect the activity of caspase-3. Briefly, 50 μL cell lysis buffer was added into the harvested cells on ice for 30 minutes. Then we centrifuged the lysed cells at 5000 rpm for 5 minutes at 4 °C. After adding 50 μL of 2X substrate working solution, the mixture was incubated at room temperature for 30 min. The fluorescence was then measured by using a microplate reader (excitation: 342 nm and emission: 441 nm).

Luciferase Experiments

As previously described, we conducted a dual luciferase reporter gene assay [36]. Briefly, the full-length human TMEM100 3'UTRs containing the WT (wild-type) sites or MT (mutant-type) sites were cloned into the pGL3-Promoter vector (Promega). By using Lipofectamine 2000 (Invitrogen), control vectors, pGL3-TMEM100 3'UTR and miRNA mimics (Life Technologies) were transfected into HEK293 cells. According

to the protocol provided by the supplier (Promega), the dual luciferase reporter assay was performed and the firefly luciferase luminescence was recorded by a VICTOR multilabel counter.

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM) from at least three independent experiments. We used Student’s t-test or one-way ANOVA followed by Dunnett's test to evaluate the statistical significance. $P < 0.05$ was defined as statistical significance.

Results

Downregulation of TMEM100 is associated with poor clinical outcomes in NSCLC patients

The expression of TMEM100 on Oncomine (a public data set, www.oncomine.org) was analyzed, and we found that TMEM100 expression was downregulated in some types of cancer tissues, including breast cancer, colorectal cancer, and lung cancer (Fig. 1A). To determine the role of TMEM100 in the progression of NSCLC, we first examined TMEM100 expression in NSCLC tissues and paired peritumoral tissues via two public data sets (GSE19804 and GSE27262). As shown in Fig. 1B and 1C, the mRNA levels of TMEM100 were obviously decreased in 60 cases of tumor tissues compared with paired peritumoral tissues. We acquired the consistent results in the other data set. The expression of TMEM100 in tumor tissues was downregulated compared with paired peritumoral tissues in 25 patients. (Fig. 1D and 1E).

We then investigated the clinical significance of TMEM100 in NSCLC tissues (GSE3141). The patients were divided into two groups based on the mRNA levels of TMEM100 (the high TMEM100 group and the low TMEM100 group). As shown in Fig. 1F, our results showed that patients with low TMEM100 expression were associated with poorer overall survival (median overall survival time, 28.4 months; $P < 0.01$) than patients with high TMEM100 expression (median overall survival time, 55.4 months). Thus, these results imply that TMEM100 could be considered a potential molecule for predicting the prognosis in the persons with NSCLC.

Overexpression of TMEM100 represses cell growth and elicits apoptosis in NSCLC cells

To investigate the cellular function of TMEM100 in the progression of NSCLC, the endogenous expression of TMEM100 was then detected in six NSCLC cell lines (Fig. 2A). Based on the endogenous expression of TMEM100 in these cells, TMEM100 was stably overexpressed in H358 cells (low expression levels of endogenous TMEM100) via lentiviral infection. Real-time PCR and western blot analyses were utilized to confirm the overexpression efficiency (Fig. 2B and 2C). As shown in Fig. 2D and 2E, the overexpression of TMEM100 decreased cell viability and inhibited colony formation in H358 cells. Moreover, the expression of key proapoptotic proteins (Bim and cytochrome c) in mitochondrial apoptosis and the activity of caspase-3 were examined to validate apoptosis in NSCLC cells. The results showed that the overexpression of TMEM100 induced the expression of Bim and enhanced cytochrome c release into the cytoplasm (Fig. 2F and 2G). Consistent with these results, the activity of caspase-3 was significantly

increased by TMEM100 overexpression in H358 cells (Fig. 2H). These results imply that TMEM100 induces the apoptosis in NSCLC cells.

TMEM100 Knockdown Facilitates Cell Survival In NSCLC

To represent the physiological functions of TMEM100 on cell survival in NSCLC, TMEM100 expression was knocked down with a shRNA. As shown in Fig. 3A and 3B, the efficiency of knockdown was confirmed by real-time PCR and western blot analyses in H460 cells (high expression levels of endogenous TMEM100). We then utilized serum deprivation (SD) as an apoptotic model to examine the effects of TMEM100 on cell apoptosis. We found that the knockdown of TMEM100 obviously increased cell viability and enhanced colony formation after treatment with SD in H460 cells (Fig. 3C and 3D). Moreover, the protein levels of Bim and cytochrome c in the cytoplasm were both attenuated by TMEM100 knockdown in starved H460 cells (Fig. 3E and 3F). The knockdown of TMEM100 suppressed the activation of caspase-3 induced by SD in H460 cells (Fig. 3G). These results show that TMEM100 knockdown promotes cell survival and inhibits cell apoptosis in NSCLC.

microRNA-106b acts as an upstream regulator of TMEM100 in NSCLC

MicroRNAs, which can block the translation or initiate the transcript degradation of target mRNAs, play a critical role in reducing protein expression. To search for miRNAs that potentially target TMEM100 and to identify the upstream regulator of TMEM100, TargetScan and miRanda were utilized to determine miRNAs that potentially target the 3' untranslated region (3'UTR) of TMEM100 mRNA. Among these miRNAs, microRNA-106b (miR-106b) was significantly increased in the NSCLC tissues compared with paired peritumoral tissues in both public NSCLC data sets (GSE63805 and GSE36681) (Fig. 4A and 4B).

We then studied whether TMEM100 was regulated by miR-106b. As shown in Fig. 4C, there was a specific sequence conserved in the 3'UTR of TMEM100 mRNA, which was predicted to integrate with miR-106b. A luciferase reporter assay was utilized to demonstrate the predicted combination of miR-106b and the 3'UTR of TMEM100 mRNA. Our results showed that the luciferase activity of the WT plasmid was significantly repressed by miR-106b, while the luciferase activity of the MT plasmid was not affected by miR-106b (Fig. 4D). Moreover, we also found that the mRNA and protein expression of TMEM100 was obviously downregulated by miR-106b (Fig. 4E and 4F), whereas a miR-106b-specific inhibitor (anti-miR-106b) led to the increased expression of TMEM100 (Fig. 4G and 4H). These results indicate that miR-106b serves as an upstream regulator of TMEM100 in NSCLC.

miR-106b facilitates cell survival by serving as an oncogenic factor

We then examined the regulatory effects of miR-106b on cell survival in NSCLC. Our results showed that the inhibition of miR-106b mitigated cell growth and repressed colony formation (Fig. 5A and 5B). The protein levels of Bim and Caspase-3 activity were significantly increased by treatment with anti-miR-106b in H358 cells (Fig. 5C and 5D). In contrast, miR-106b increased cell viability and promoted colony formation after treatment with SD in H460 cells, which was attenuated by the reintroduction of TMEM100

(Fig. 5E and 5F). Additionally, miR-106b-inhibited Bim expression and caspase-3 activation were eliminated by the restoration of TMEM100 in starved H460 cells (Fig. 5G and 5H). These results imply that miR-106b inhibits apoptosis by reducing TMEM100 expression in NSCLC cells.

Effects of TMEM100 on cell apoptosis are attenuated by the reintroduction of survivin

It is reported that survivin, a critical regulator of cell apoptosis, plays important roles in the progression of NSCLC [14]. As shown in Fig. 6A, the expression of survivin was significantly upregulated in 60 cases of NSCLC tissues (GSE19804) compared with paired peritumoral tissues (Fig. 6A). There were highly negative correlations between TMEM100 expression and survivin expression in the same NSCLC tissues ($r=-0.46$, $P < 0.001$) (Fig. 6B). We then examined whether survivin is involved in the inhibitory effects of TMEM100 on cell survival. Our results showed that the overexpression of TMEM100 mitigated the protein levels of survivin, while the expression of survivin was increased by TMEM100 knockdown (Fig. 6C and 6D). Furthermore, to determine the roles of survivin in TMEM100-regulated cell apoptosis, survivin expression was increased by the transfection of a recombinant plasmid (Fig. 6E). We found that the inhibitory effects of TMEM100 on cell viability were mitigated by the increased expression of survivin (Fig. 6F). In addition, TMEM100-enhanced Bim expression and caspase-3 activation were antagonized by the restoration of survivin (Fig. 6G and 6H). These results indicate that the roles of TMEM100 in cell survival are, at least in part, mediated by negatively regulating survivin in NSCLC.

Discussion

Mounting evidence has indicated that transmembrane proteins play important roles in the progression and development of some malignancies. Cytosolic TMEM88, which is highly expressed in NSCLC specimens and breast cancer tissues, stimulates the invasion and metastasis of cancer cells. Higher expression of TMEM88 is closely correlated with poorer differentiation, higher TNM stage, and worse overall survival [9]. The increased expression of TMEM16A is found in some tumors, including gastric cancer [7], prostate carcinoma [15], and head and neck squamous cell carcinoma [16]. Additionally, the increased expression of TMEM16A is positively related to tumor stage and negatively correlated with the overall survival of patients [7]. TMEM100, a member of the transmembrane protein family, also participates in regulating the progression of some cancers. A previous study showed that TMEM100 inhibits metastasis and proliferation, and the levels of TMEM100 correlated with tumor size, TNM stage, overall survival and disease-free survival in hepatocellular carcinoma [13]. Han et al. found that TMEM100 overexpression suppresses migration and invasion in NSCLC cell lines [17]. However, the roles of TMEM100 in the survival of cancer cells are not fully understood thus far. In the present study, we provide new evidence to indicate that TMEM100, negatively regulated by miR-106b, induces cell apoptosis and inhibits the progression of NSCLC by repressing survivin expression.

It is known that apoptosis acts as a negative regulator in cell growth. Accumulating evidence has indicated that the inhibition of cell apoptosis is commonly observed in some cancer tissues, and genes involved in modulating cell apoptosis are regarded as the new class of tumor-related genes [18, 19].

Inducing cellular apoptosis is one of the most effective strategies for relieving the progression of tumors. A previous study showed that mitochondrial dysfunction is an early-stage event that triggers the intrinsic apoptotic pathway [20]. Bim is a critical mediator of the mitochondrial apoptotic pathway and participates in regulating the function of mitochondria. Bim can cause a decrease in the mitochondrial membrane potential and induce the opening of the mitochondrial permeability transition pore either by directly activating proapoptotic Bax/Bak or by antagonizing antiapoptotic Bcl-2 [21]. A disruption in the mitochondrial membrane potential leads to the release of cytochrome c from the mitochondria into the cytoplasm, which in turn triggers the activation of caspase cascades and elicits cell apoptosis [22]. To study the role of TMEM100 in the survival of NSCLC cells, we examined the expression of Bim, cytochrome c release and caspase-3 activation under conditions of TMEM100 overexpression or knockdown. Our results showed that the overexpression of TMEM100 induced the expression of Bim, promoted the release of cytochrome c into the cytoplasm, and increased the activity of caspase-3. In contrast, TMEM100 knockdown had the opposite effects. These results imply that TMEM100 elicits cell apoptosis and inhibits the progression of NSCLC by acting as a tumor suppressor.

An important finding of this study is that TMEM100 facilitates cell apoptosis by repressing survivin expression in NSCLC. Survivin belongs to the inhibitor of apoptosis (IAP) gene family and plays important roles in the progression of some human malignancies, including NSCLC [14, 23]. Its roles in cell survival and apoptosis have been widely studied in NSCLC. It has been reported that the inhibitory effects of nicotine on chemotherapeutic drug-induced cell apoptosis are mediated by upregulating XIAP and survivin [24]. Ezponda et al. reported that SF2/ASF promotes the stability of survivin mRNA and that the downregulation of SF2/ASF induces apoptosis by reducing the expression of survivin in NSCLC cells [25]. In LKB1-deficient lung adenocarcinomas, survivin is responsible for promoting malignant progression by acting as the downstream mediator of YAP [26]. In this study, our results showed that the expression of survivin was negatively correlated with TMEM100 expression in NSCLC tissues. Moreover, the roles of TMEM100 in cell apoptosis were attenuated by the reintroduction of survivin. These results imply that TMEM100 facilitates cell apoptosis, at least in part, by inhibiting survivin expression in NSCLC.

Another notable finding of this study is that TMEM100 is negatively regulated by miR-106b in NSCLC cells. MicroRNAs, a large family of short, noncoding endogenous RNAs, can bind to a specific sequence conserved in the 3'UTR of their target genes and posttranscriptionally regulate their expression [27]. Currently, a number of microRNAs have been indicated to regulate various types of pathological processes, including the initiation and development of cancers [28–30]. However, the effects of miR-106b on different human cancers are not consistent. Previous studies have proved that miR-106b enhances the metastasis and proliferation of cancer cells and boosts tumorigenesis in colorectal cancer [31], hepatocellular carcinoma [32], and glioma [33]. In contrast, it has been reported that miR-106b, as a tumor suppressor, is significantly downregulated in clinical samples of giant bone cell tumors and inhibits metastasis and tumorigenesis in thyroid cancer [34] and breast cancer [35]. Until now, the precise roles of miR-106b in NSCLC have been inconclusive. In this research, our results showed that miR-106b, which was obviously upregulated in NSCLC tissues, interacted with the 3'UTR of TMEM100 mRNA and depressed its expression. Moreover, the inhibitory roles of miR-106b in cell apoptosis were eliminated by

the restoration of TMEM100 in NSCLC. These results imply that miR-106b-mitigated TMEM100 expression promotes the progression of NSCLC.

Conclusions

Our results reveal that TMEM100 induces cell apoptosis in NSCLC by acting as a tumor suppressor. Moreover, miR-106b is responsible for the decreased expression of TMEM100 in NSCLC. These results imply an important underlying regulatory mechanism and provide potential treatment targets for NSCLC.

Abbreviations

NSCLC: non-small-cell lung cancer; TMEMs: transmembrane proteins; ATCC: American Tissue Culture Collection; DMEM: Dulbecco's modified Eagle's medium; FBS: fetal bovine serum; TBS-T: Tris-HCl Buffered Saline-Tween; qRT-PCR: quantitative Real-Time Polymerase Chain Reaction; miRNA: micro RNA; CCK-8: Cell Counting Kit-8; WT: wild-type; SEM: standard error of the mean; SD: serum deprivation; miR-106b: micro RNA 106b; TNM: tumor,nodes,metastasis; IAP: inhibitor of apoptosis;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests" in this section.

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Authors' Contributions:

Jihong Wu , Jun Ma and Lei Zhang designed the experiments; Jun Ma and Tingting Yan performed the experiments; Yongrui Bai, Ming Ye and Xiumei Ma analyzed the results; Xiumei Ma, Jihong Wu, and Lei Zhang prepared the submission.

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Figures

Figure 1

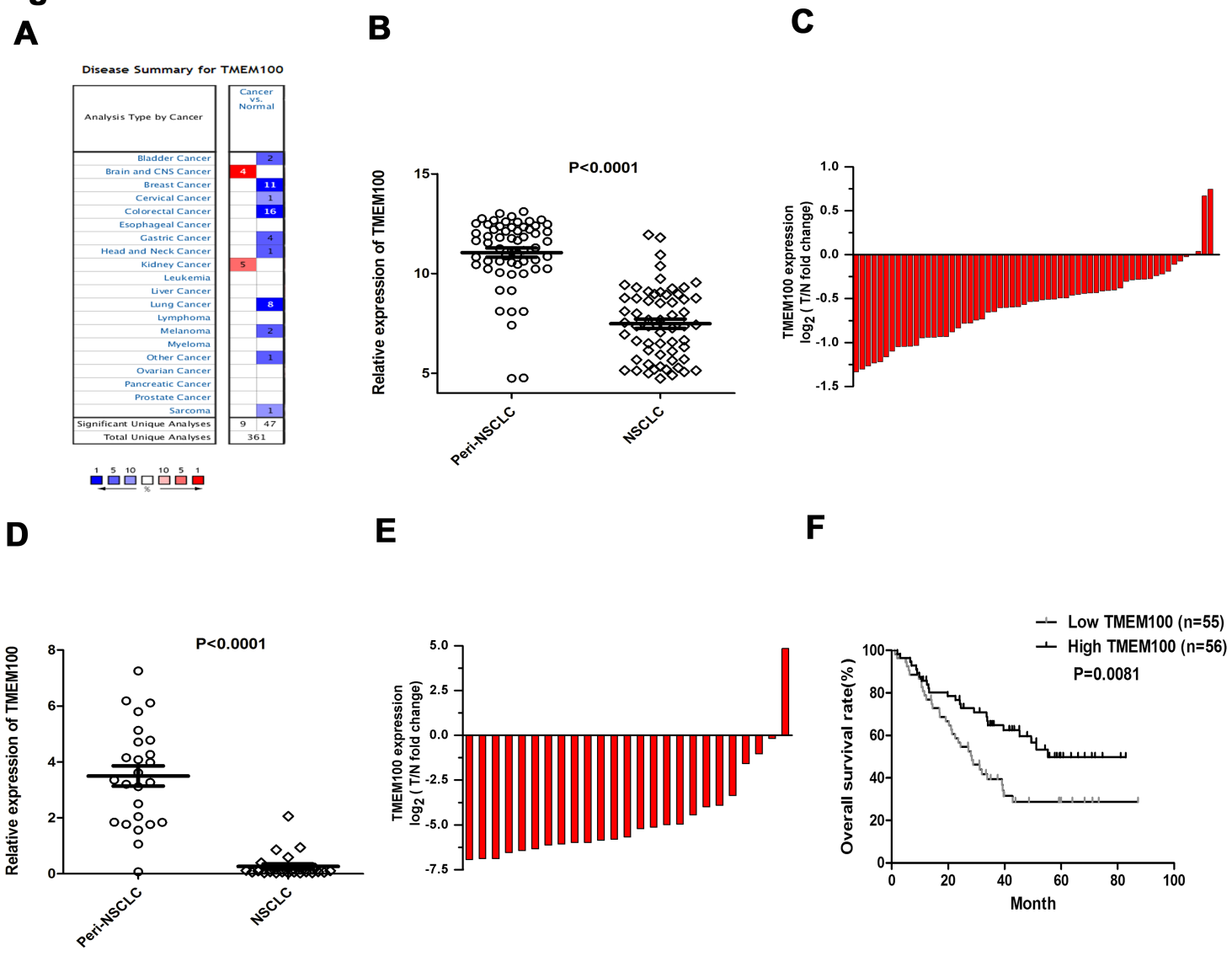

Figure 1

TMEM100 is frequently downregulated in human NSCLC. A: Expression of TMEM100 in different types of cancer tissues in Oncomine. B: mRNA levels of TMEM100 were obviously decreased in 60 cases of NSCLC tissues compared with paired peritumoral tissues. C: Expression data of TMEM100 in the paired samples of NSCLC are presented as the fold change (log2 (T/N)). D: Expression of TMEM100 was significantly downregulated in 25 cases of NSCLC tissues. E: TMEM100 expression in the paired samples of NSCLC is presented as the fold change (log2 (T/N)). F: Kaplan-Meier analysis of overall survival in NSCLC patients based on TMEM100 expression. The results showed that low expression, rather than high expression, of TMEM100 was associated with poorer overall survival.

Figure 2

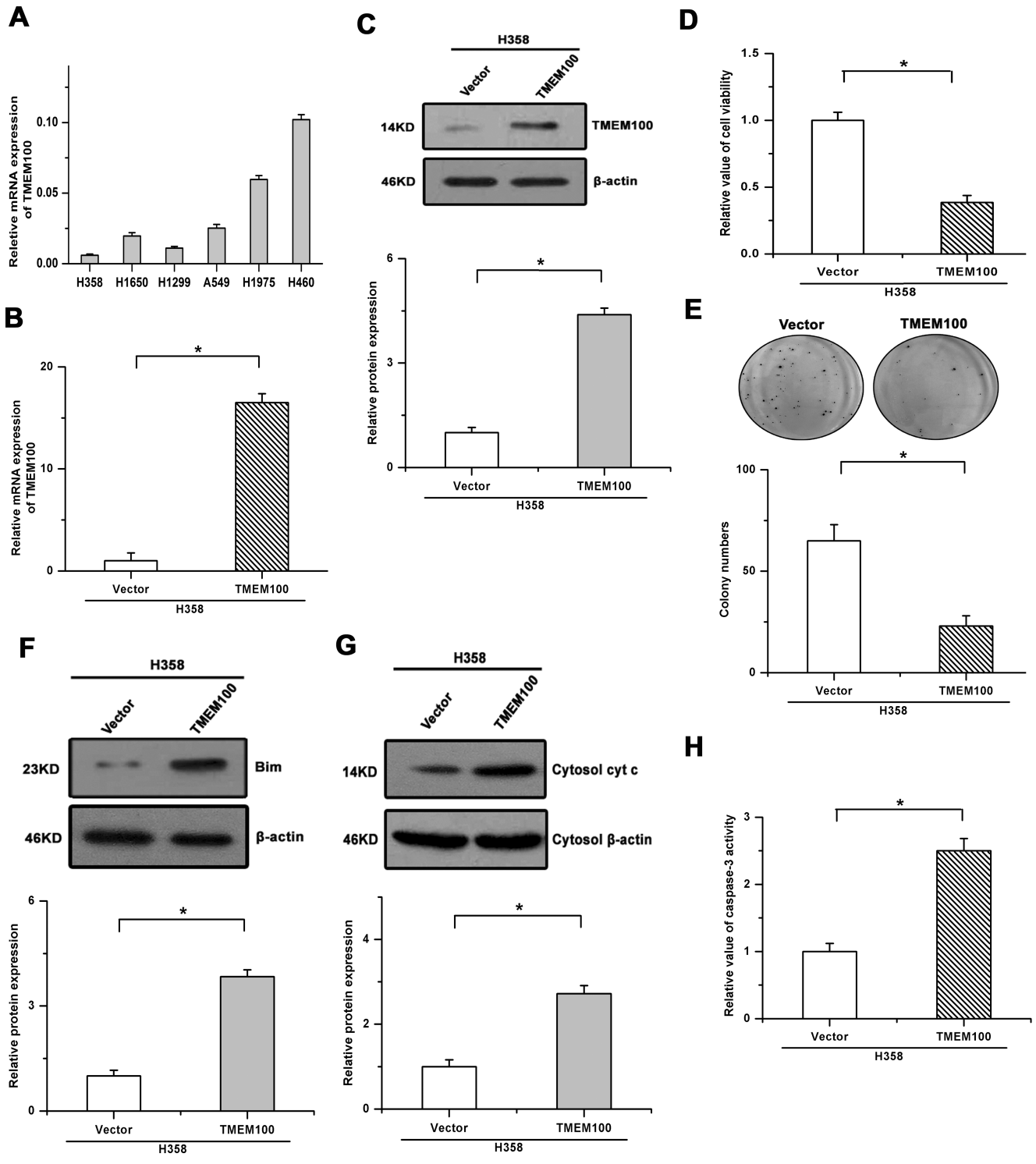

Figure 2

Overexpression of TMEM100 mitigates cell growth and induces cell apoptosis. A: Endogenous expression of TMEM100 in six NSCLC cell lines. B and C: Overexpression efficiency was examined by real-time PCR (B) and western blot (C) analyses. D: TMEM100 overexpression repressed cell viability and inhibited growth in H358 cells. E: Colony formation was mitigated by the overexpression of TMEM100. F and G: Overexpression of TMEM100 facilitated Bim expression (F) and induced the release of cytochrome c into

the cytoplasm (G). H: Caspase-3 activity was significantly increased by TMEM100 overexpression in H358 cells.

Figure 3

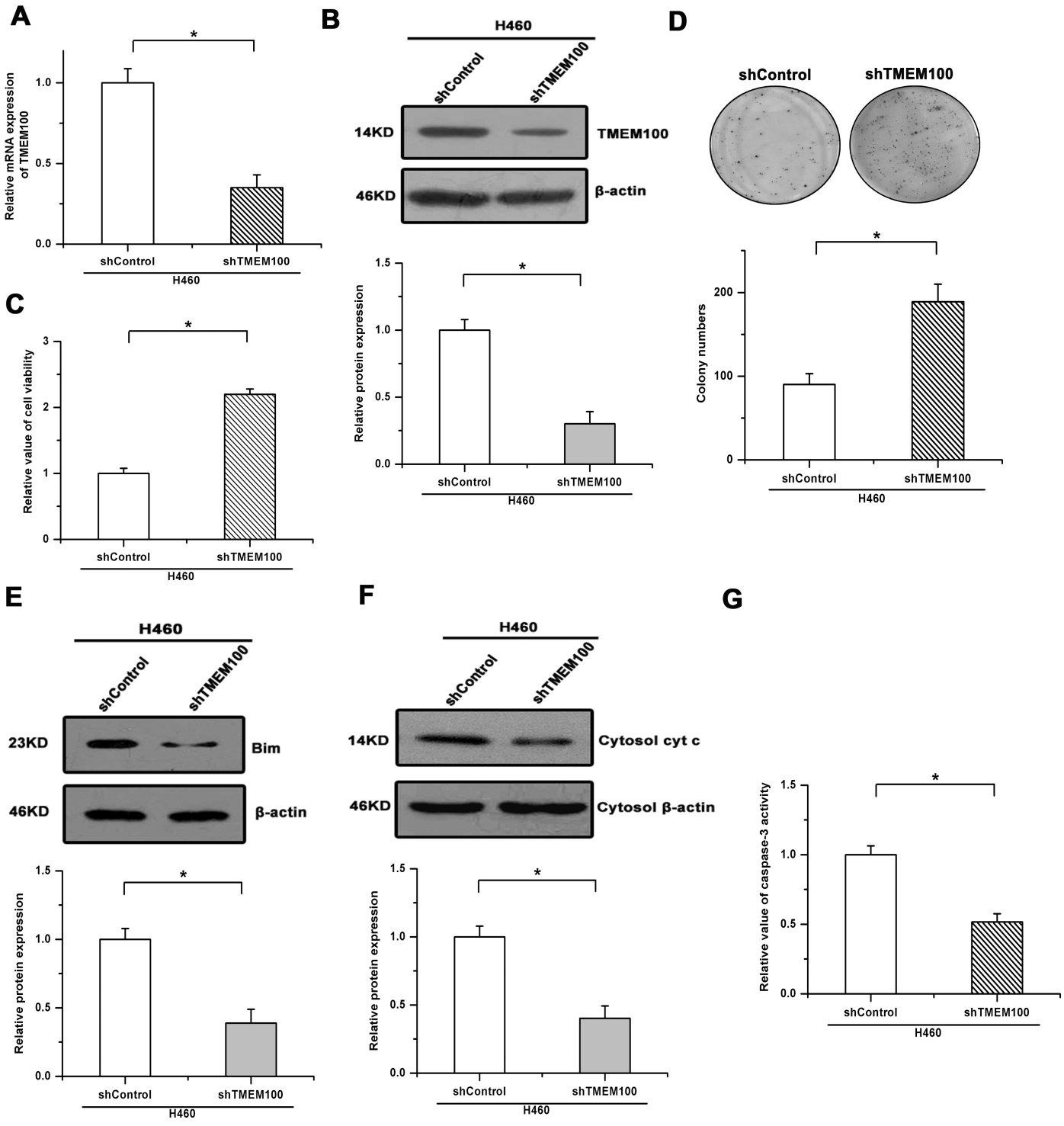

Figure 3

Knockdown of TMEM100 inhibits cell apoptosis. A and B: Real-time PCR (A) and western blot (B) analyses were used to verify the knockdown efficiency. C and D: Cell viability (C) and colony formation (D) were facilitated by the knockdown of TMEM100 after treatment with SD in H460 cells. E and F:

TMEM100 knockdown repressed the protein levels of Bim (E) and the release of cytochrome c into the cytoplasm (F) under conditions of SD. G: The activity of caspase-3 was decreased by the knockdown of TMEM100 in starved H460 cells.

Figure 4

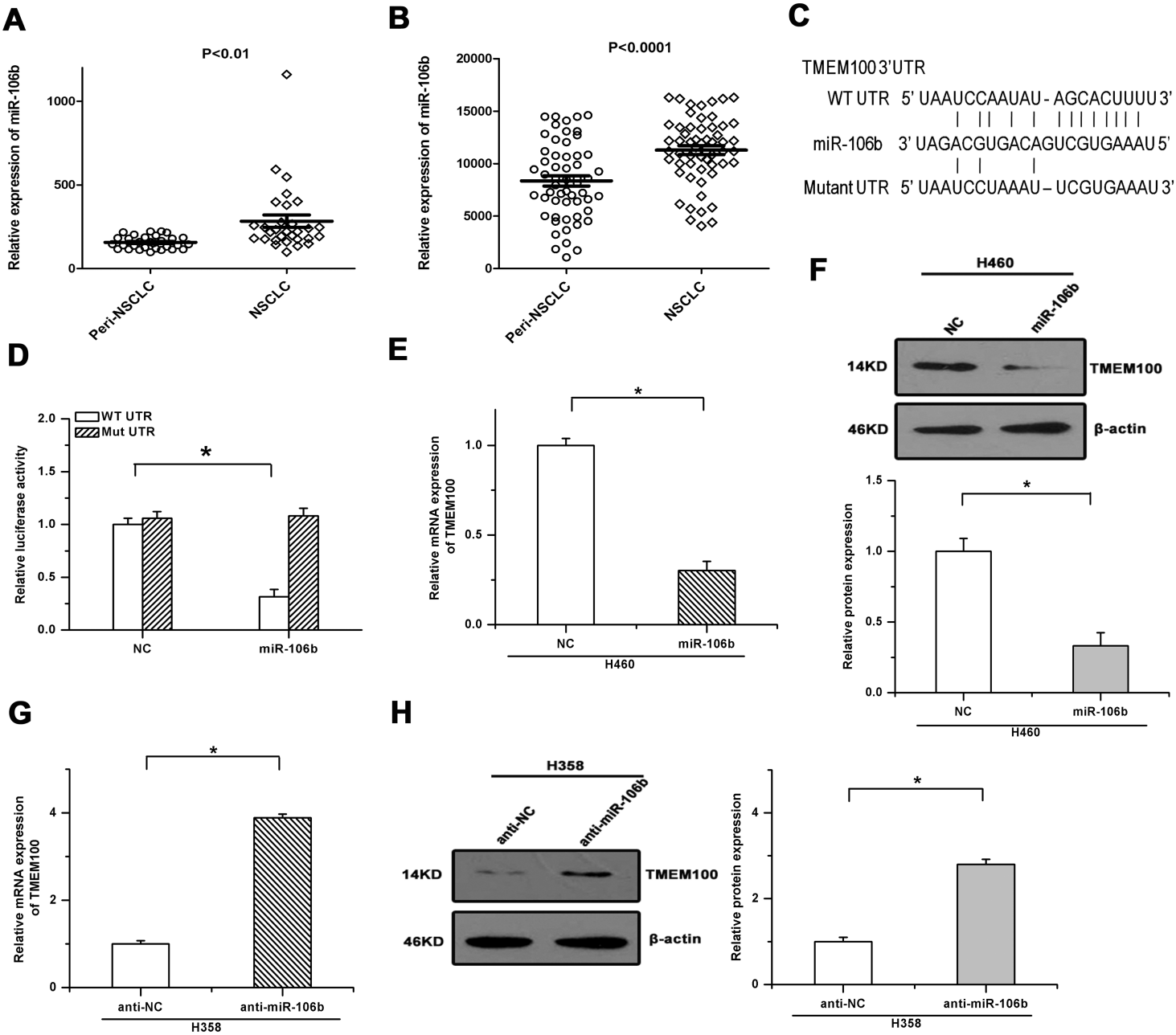

Figure 4

miR-106b downregulated TMEM100 expression in NSCLC. A and B: miR-106b was significantly upregulated in NSCLC tissues compared with paired peritumoral tissues. C: miR-106b was predicted to integrate with the sequence conserved in the 3'UTR of TMEM100 mRNA. D: Luciferase activity of the WT plasmid was significantly repressed by miR-106b, while the luciferase activity of the MT plasmid was not affected by miR-106b. E and F: The mRNA (E) and protein expression (F) of TMEM100 was significantly

downregulated by miR-106b. G and H: Inhibition of miR-106b led to the increased expression of TMEM100 at the mRNA (G) and protein (H) levels.

Figure 5

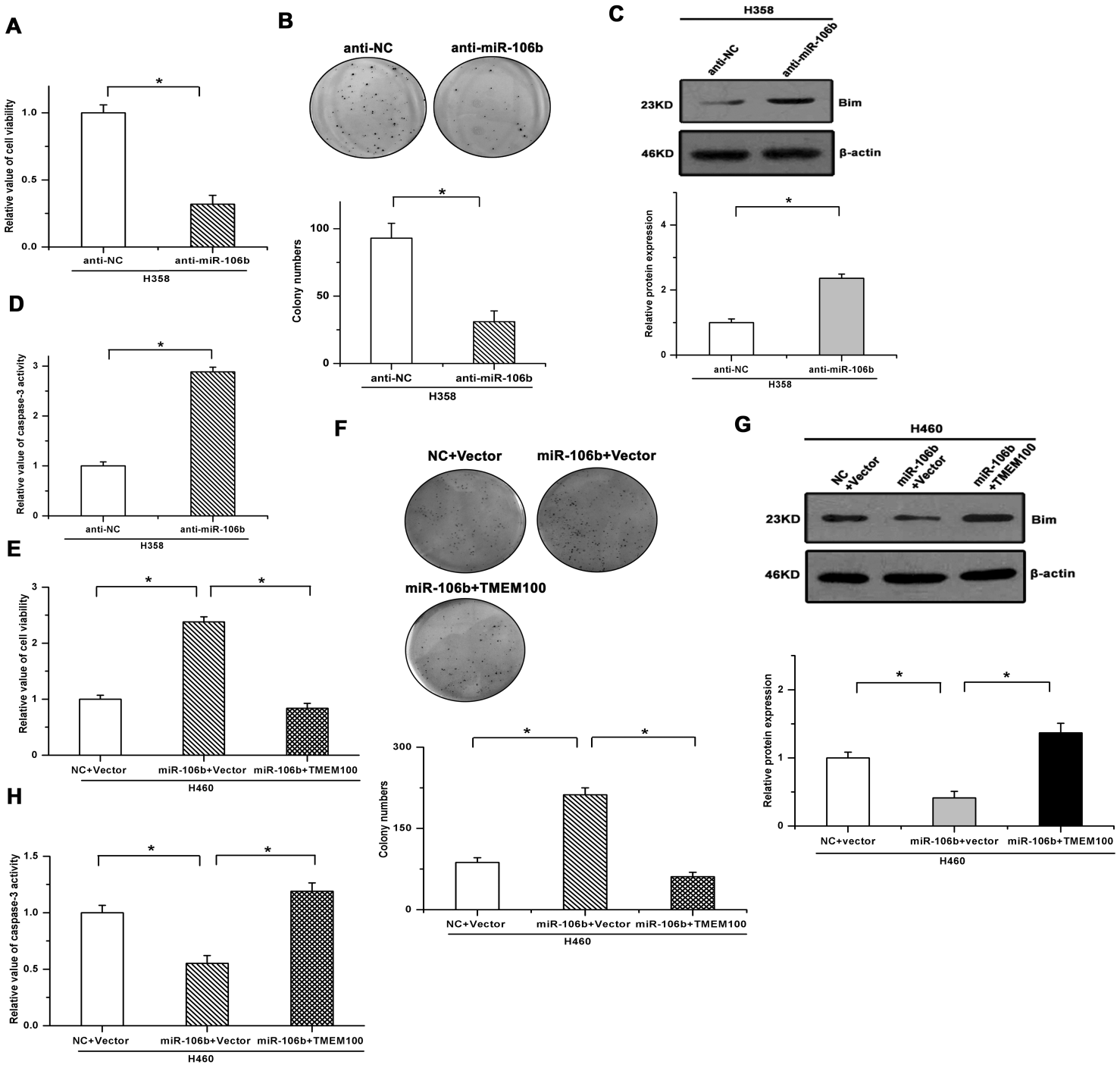

Figure 5

miR-106b inhibits apoptosis by repressing TMEM100 expression in NSCLC cells. A and B: Cell viability (A) and colony formation (B) were attenuated by miR-106b inhibition in H358 cells. C and D: Treatment with anti-miR-106b induced the protein expression of Bim (C) and enhanced the activation of caspase-3 (D). E and F: Cell viability (E) and colony formation (F) increased by miR-106b were antagonized by the

reintroduction of TMEM100 after treatment with SD in H460 cells. G and H: miR-106b-mitigated Bim expression (G) and caspase-3 activity (H) were eliminated by the restoration of TMEM100 in starved H460 cells.

Figure 6

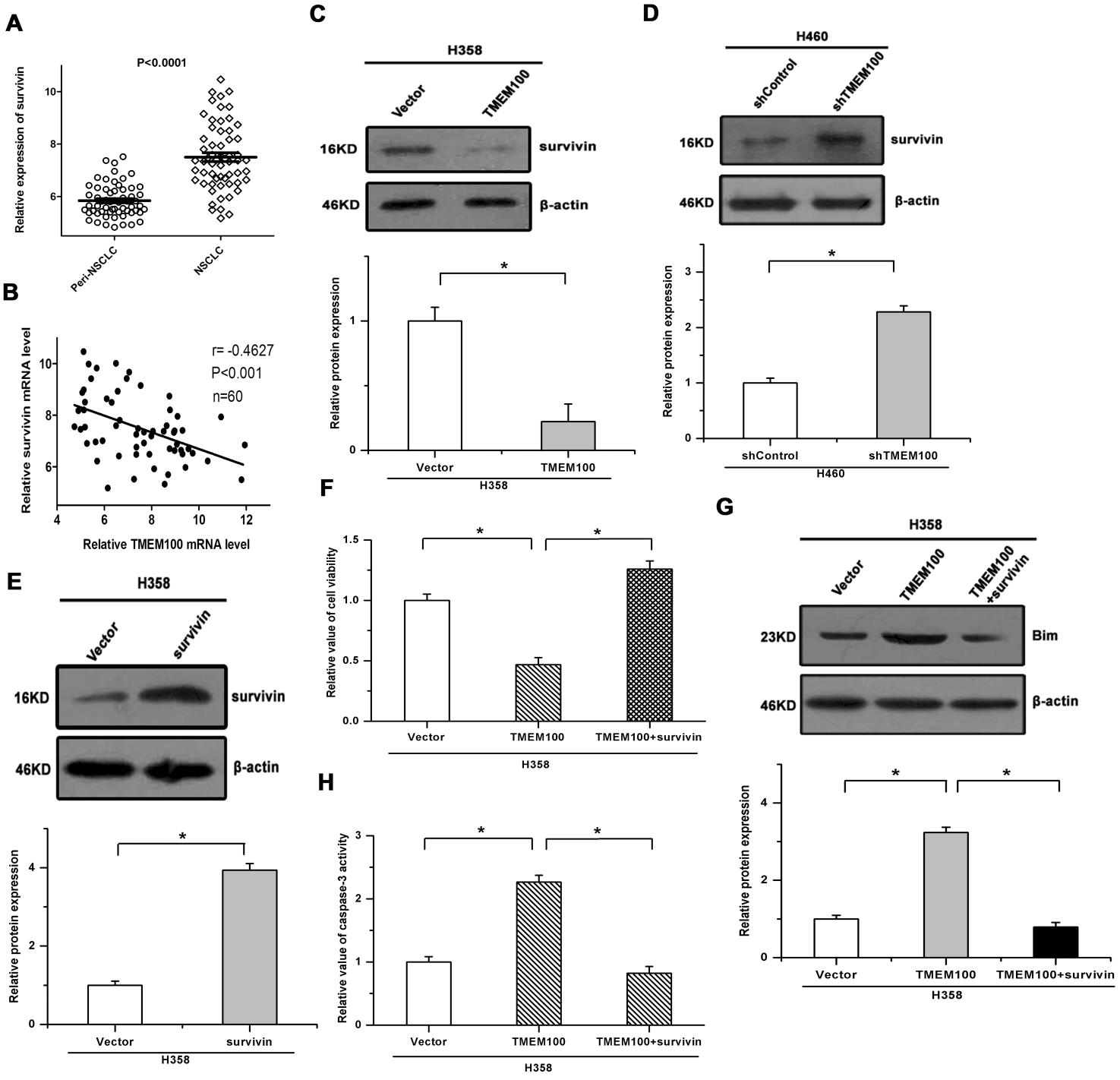

Figure 6

The effects of TMEM100 on cell apoptosis were attenuated by the reintroduction of survivin. A: Expression of survivin was significantly upregulated in 60 cases of tumor tissues compared with paired peritumoral tissues. B: TMEM100 expression was negatively correlated with the expression of survivin in

the same NSCLC tissues. C and D: Overexpression of TMEM100 repressed the protein levels of survivin (C), while the expression of survivin was facilitated by TMEM100 knockdown (D). E: Expression of survivin was significantly increased by the transfection of a recombinant plasmid in H358 cells. F: TMEM100 overexpression-inhibited cell growth was mitigated by the increased expression of survivin. G and H: The increased expression of Bim (G) and activation of caspase-3 (H) induced by TMEM100 overexpression were antagonized by the restoration of survivin protein.