TSG101 Silencing Suppresses Hepatocellular Carcinoma Cell Growth by Inducing Cell Cycle Arrest and Autophagic Cell Death

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BD 1 Zhuo Shao*
DE 1 Weiping Ji*
CF 2 Anan Liu*
D 3 Ancheng Qin*
F 1 Li Shen
B 1 Gang Li
C 1 Yinqi Zhou
E 1 Xiangui Hu
A 1 Enda Yu
AG 1 Gang Jin

1 Department of General Surgery, Changhai Hospital, Second Military Medical University, Shanghai,P.R. China
2 Department of General Surgery, Changzheng Hospital, Second Military Medical University, Shanghai, P.R. China
3 Department of General Surgery, Suzhou Municipal Hospital, Suzhou, Jiangsu, P.R. China

* These authors contributed equally to this work

Corresponding Author: Gang Jin, e-mail: smmujg@163.com or Enda Yu, e-mail: smmuyed@163.com
Source of support: Departmental sources

Background: The tumor susceptibility gene 101 (TSG101) was originally identified as a tumor-suppressor gene that mediates many molecular and biological processes, such as ubiquitination, endosomal trafficking, cell survival, and virus budding, but its role in hepatocellular carcinoma (HCC) is currently unknown.

Material/Methods: We assessed the expression of TSG101 in HCC and paracancerous tissues using qPCR. Then, we used the TSG101-specific siRNA mix to disrupt the expression of TSG101 to investigate the subsequent effect on human hepatoma-7 (Huh7) cells. Western blot was used to detect the protein expression of TSG101 and other molecules. Cell growth assay was performed using CCK8. Transwell assay was used to investigate the migration and invasion ability of Huh7 cells after transfection with of TSG101 siRNA. Flow cytometry was used to estimate the effect of TSG101 knockdown on cell cycle and apoptosis. Confocal laser scanning microscopy was used to observe the actin filaments change and the formation of autophagy.

Results: TSG101 was over-expressed in HCC tissues. TSG101 silence was able to suppress Huh7 cell proliferation, migration, and invasion. Furthermore, silencing of TSG101 could induce cell cycle arrest at G1 phase and inhibit the expression of cyclin A and cyclin D, while up-regulating the expression of CDK2. The mechanism might be induction of autophagic cell death and inactivation of Akt and ERK1/2.

Conclusions: TSG101 plays an important role in the development of HCC and may be a target for molecular therapy.

MeSH Keywords: Autophagy • Carcinoma, Hepatocellular • Cell Cycle • Genes, Tumor Suppressor • RNA, Small Interfering

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/894447

📄 2865   ▦ —   📊 6   📖 30

Background

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, and due to its poor prognosis and high recurrence it has recently become the third leading cause of cancer-related death recently[1]. Although diagnostic and treatment strategies, such as surgical resection, radio-frequency ablation, transcatheter arterial chemoembolization, and radiation, have been improved, the postoperative prognosis of HCC remains unsatisfactory [2]. Therefore, novel therapeutic strategies are needed to improve the treatment of HCC; molecular targeted therapy may be such a strategy [3]. In fact, there have been many reports revealing that many molecules have showed great potential in the treatment of HCC. Yet, as different molecules may express and function differently in the development of HCC, to find more molecules and to get a better understand of the molecules may benefit treatment of HCC.

Tumor susceptibility gene 101 (TSG101), discovered in a screen for tumor susceptibility genes, was originally identified as a tumor-suppressor gene that encodes a multi-domain protein and mediates many molecular and biological processes, such as ubiquitination, endosomal trafficking, cell growth, and virus budding [4–6]. Many investigations showed that its role in cellular function is complex, and may be opposite in different cells [4,7]. The expression of TSG101 protein levels are normally tightly controlled within a narrow range, as both increased and suppressed expression of TSG101 will lead to abnormal cell growth in NIH 3T3 cells [4]. The deletion of TSG101 was expected to lead to increased tumor growth and perhaps increased tumor cell proliferation, as supported by early investigations [8]. However, recent studies have shown that the silencing of TSG101 *in vitro* resulted in impaired cell growth, and homozygous knockout of TSG101 in mice led to embryonic lethality [9,10]. In addition, the expression of TSG101 in human papillary thyroid carcinomas, ovarian cancer, gastrointestinal tumors, colorectal carcinoma, and gallbladder cancer tissues was higher than that in normal tissues [7,11–18]. This indicates that TSG101 may play an important role in cell growth and tumor development, but the role of TSG101 in HCC has not been investigated until now.

In the present study, we show that silencing of TSG101 decreased the proliferation, migration, and invasion of human hepatoma-7 (Huh7) cells. Silencing TSG101 was able to induce cell arrest at G1 phase and inhibit the expression of cyclin A and cyclin C, while increasing the expression of cyclin-dependent kinase 2 (CDK2). In addition, deletion of TSG101 could lead to the accumulation of GFP-LC3 and of Lamp1. Furthermore, silencing of TSG101 was able to up-regulate the expression of Beclin 1 and LC3 II and down-regulate the expression of p62, possibly due to the inhibited activation of ERK1/2 and AKT. Altogether, the data showed that TSG101 plays an important role in the development of HCC and might be a potential target in the treatment of HCC.

Material and Methods

Clinical specimens

The approval from the Ethics Committee of Changhai Hospital and written informed consent from each patient were obtained prior to the use of these materials. A total of 10 HCC and corresponding paracancerous tissues were acquired through surgery, which were kept in liquid nitrogen before the performance of the experiment.

Quantitative real-time PCR (qPCR)

Total RNA of the tissue was extracted using Trizol reagent and reverse transcribed to cDNA with the PrimeScript RT reagents Kit. Then the expression of TSG101 in the tissue was detected using SYBR Premix Ex Taq on Rotor Gene 3000A (Corbett Research, Australia) with GAPDH as the control. The following primers were used for quantitative real-time PCR: TSG101, GCCACCTCTA GAATGGCGGT GTCGGAGAGC C (forward), GGTGGCGTCG ACTCAGTAGA GGTCACTGAG ACC (reverse); GAPDH, GGGTGGAGCCAAACGGGTC (forward), GGAGTTGCTGTTGAAGTCGCA (reverse).

Cell lines and reagents

Human hepatoma-7 (Huh7) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS, Gibco) supplemented with penicillin and streptomycin. Antibodies to GAPDH, lamp1, Akt, phosphor-Akt, ERK1/2, and phosphor-ERK1/2 were purchased from Cell Signaling Technology (Beverly, MA). Antibodies to TSG101, LC3, p62, and Beclin1 were purchased from Abcam (Cambridge, USA). Alexa Fluor 555 Phalloidin, Alexa Fluor 555 secondary antibody, HRP-conjugated secondary antibodies, Trizol reagent, and lipofectamine 2000 were purchased from Invitrogen (Shanghai, China). siRNA targeting TSG101 was purchased from Dharmacon (GE Healthcare Life Sciences). PrimeScript RT Kit and SYBR Premix Ex Taq were obtained from TAKARA BIOTECHNOLOGY (Dalian, China). Propidium iodide and Annexin V kit were purchased from Sungene Biotech Company (Tianjin, China). Matrigel Matrix was obtained from BD Biosciences (San Jose, USA). The plasmid expressing EGFP-LC3 fusion protein was obtained from Addgene (plasmid 11546). The transwell chamber and other cell culture plates were purchased from Corning, Inc. (NY, USA).

Cell growth assay

Cell growth assay was performed using the Cell Counting Kit 8 (CCK8) assay according to the manufacturer's instructions. Briefly, 2000 cells were seeded in the 96-well plate to incubate overnight. Furthermore, the cells were transfected with siRNA

for 48 hours, and then the culture medium was changed to110 µl fresh medium containing 10 µl CCK8 and incubated for another 2 hours. Finally, the plate was read by a BioTek synergy Multi-Mode Microplate Reader.

Cell migration and invasion assay

Cell migration assay was performed using a Transwell chamber with 6.5 mm diameter polycarbonate filters with 8-µm pore size according to the manufacturer's instructions. We added 2×10^4 cells transfected with TSG101 siRNA for 48 hours in culture media containing 0.5% FBS to the inserts. The lower chambers were filled with culture media containing 1% FBS and the cells were allowed to migrate for 16 hours. For the cell invasion assay, the filters were pre-coated with Matrigel before invasion assay. We added 5×10^4 cells transfected with TSG101 siRNA for 48 hours in culture media containing 0.5% FBS to the inserts. The lower chambers were filled with culture media containing 5% FBS. Cells were allowed to invade for 24 hours and then cells were fixed with 4% paraformaldehyde. The cells still in the upper inserts were then removed using cotton swabs. The cells were stained with 0.5% crystal violet for 20 minutes and the images were captured with an inverted microscope (IX81, Olympus). The migrated and invaded cells were counted manually, and the experiments were repeated at least 3 times.

Cell cycle assay

Cells treated with TSG101 siRNA for 48 hours were harvested using trypsin and fixed in 70% ethanol overnight. Then the cells were washed with PBS twice and were re-suspended in PBS containing propidium iodide. The cells were incubated with propidium iodide for 30 minutes at room temperature. After 2 washes with PBS, the cells were analyzed using Cell Lab Quanta SC (BECKMAN COULTER, USA), and the data were analyzed using Modfit software.

Cell apoptosis assay

Cells treated with TSG101 siRNA for 48 hours were harvested using trypsin, and washed twice with PBS. Then, the cells were incubated with FITC Annexin V for 15 minutes at room temperature in the dark. The stained cells were then incubated with propidium iodide for 5 minutes at room temperature in the dark. The samples were analyzed using a Cell Lab Quanta SC (BECKMAN COULTER, USA), and the data were analyzed by FLOWJO v7.6 software.

Immunofluorescence staining

Cells grown on glass slides were fixed by 4% paraformaldehyde and washed twice with PBS. The cells were then blocked with 5% bovine serum albumin (BSA) for 1 hour at room temperature. Then the slides were washed twice with PBS and incubated with Alexa Fluor 555 Phalloidin diluted in 1% BSA for 1 hour at room temperature. The slides were then washed with PBS once and incubated with DAPI for 15 minutes. After washing with PBS once, the cells was analyzed with a Zeiss LSM-710 fluorescence microscope. For the detection of autophagy, cells were transiently transfected with GFP-LC3 plasmid using lipofectamine 200 for 48 hours. Then, the cells were harvested using trypsin and seeded to a glass slide. The cells were further treated with TSG101 siRNA for 48 hours and were then fixed by 4% paraformaldehyde. After being blocked with BSA, the slide was washed and incubated with lamp1 antibody for 2 hours at room temperature. Then, the slide was washed and incubated with Alexa Fluor 555 antibody for 1 hour at room temperature. After being stained with DAPI for 15 minutes, the slide was analyzed with a Zeiss LSM-710 fluorescence microscope.

Western blotting

Cells were lysed by RIPA lysis buffer purchased from Beyotime Biotechnology (Shanghai, China). The protein extracted from cells was separated by 10% SDS-PAGE gel and transferred to polyvinylidene fluoride membrane. The membranes were then incubated with indicated primary and secondary antibodies conjugated to horseradish peroxidase. Finally, the blots were incubated with Super Signal West Pico chemoluminescent substrate and visualized using the GenegGnome HR Image Capture System.

Statistical analysis

The data were obtained from at least 3 independent experiments and are expressed as mean ±SD. The expression of TSG101 in tissues was analyzed using Student's t test. The other statistical significance was computed using one-way ANOVA and Tukey test. The level of significance was set as $P<0.05$, which is marked by (*). When P value <0.01, the data is indicated by (**).

Results

TSG101 is over-expressed in HCC tissue

Although TSG101 plays an important role in the progress of many tumors, the function of TSG101 in HCC was largely unknown. To explore the potential role of TSG101 in HCC, we initially examined the expression level of TSG101 in HCC and corresponding paracancerous tissues. The data indicated that the expression of TSG101 in HCC tissue was much higher than that in paracancerous tissue (Figure 1A). The result was consistent with recent investigations reporting that the role of
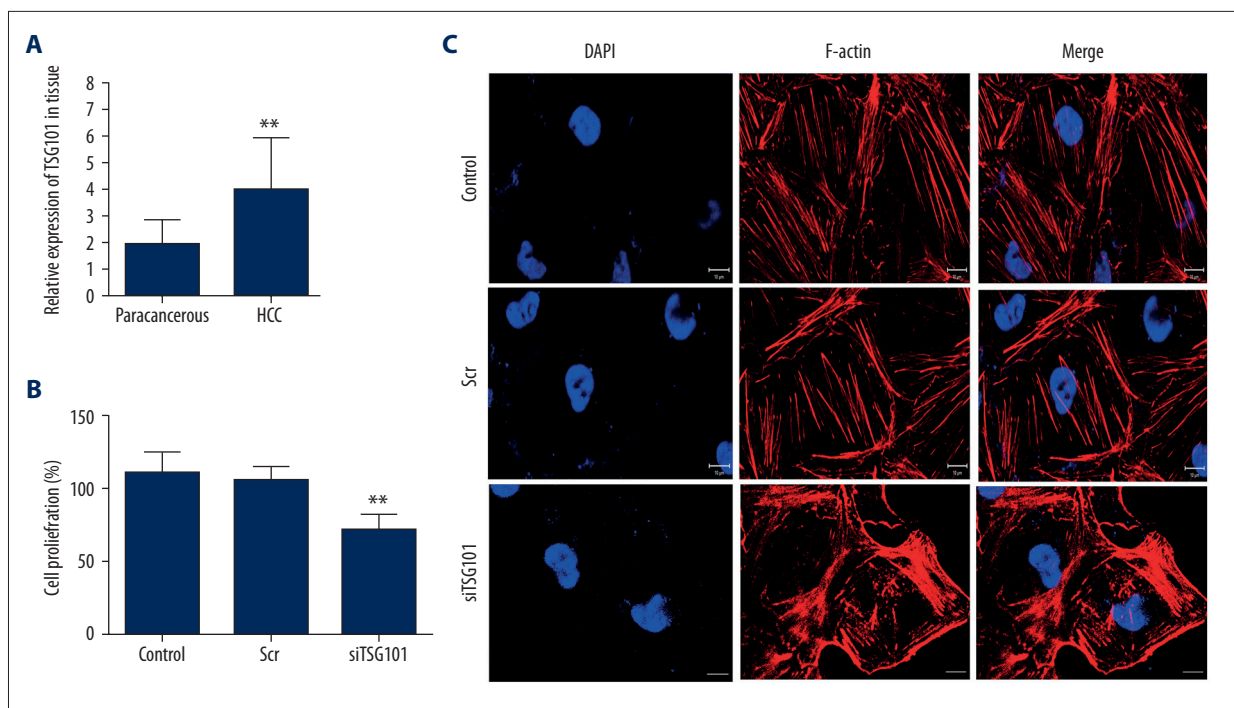
Figure 1. The expression of TSG101 in HCC tissues and the effect of silencing of TSG101 on cell proliferation and actin filaments. (**A**) The expression of TSG101 was much higher in HCC tissue than in paracancerous tissue. (**B**) Cell growth of different groups was examined by CCK-8 assay and presented in a histogram. (**C**) TSG101 knockdown resulted in obvious changes in the architecture of the cytoskeleton, with less bundling. ** p<0.01; all data are representative results of 3 independent experiments.

TSG101 was complex and could be opposite in different cancers [4,7,16–18].

TSG101 deletion inhibits cell growth and causes remodeling of actin filaments

Although it is known that TSG101 plays important roles in the progress of different tumors, there is no report on the role of TSG101 in HCC. In this study, we used TSG101-specific siRNA to inhibit the expression of TSG101 in Huh7 cells. We found that the TSG101 siRNA was able to clearly decrease the expression of TSG101 (Supplementary Figure 1). The cells transfected with TSG101 siRNA for 48 hours showed suppressed growth ability by about 30% compared with the control groups (Figure 1B). Furthermore, TSG101 knockdown resulted in obvious changes in the architecture of the cytoskeleton. The phalloidin-based fluorescent staining of F-actin of TSG101 siRNA-treated cells showed a clearly abnormal reorganization of actin filaments with less bundling compared with control groups (Figure 1C).
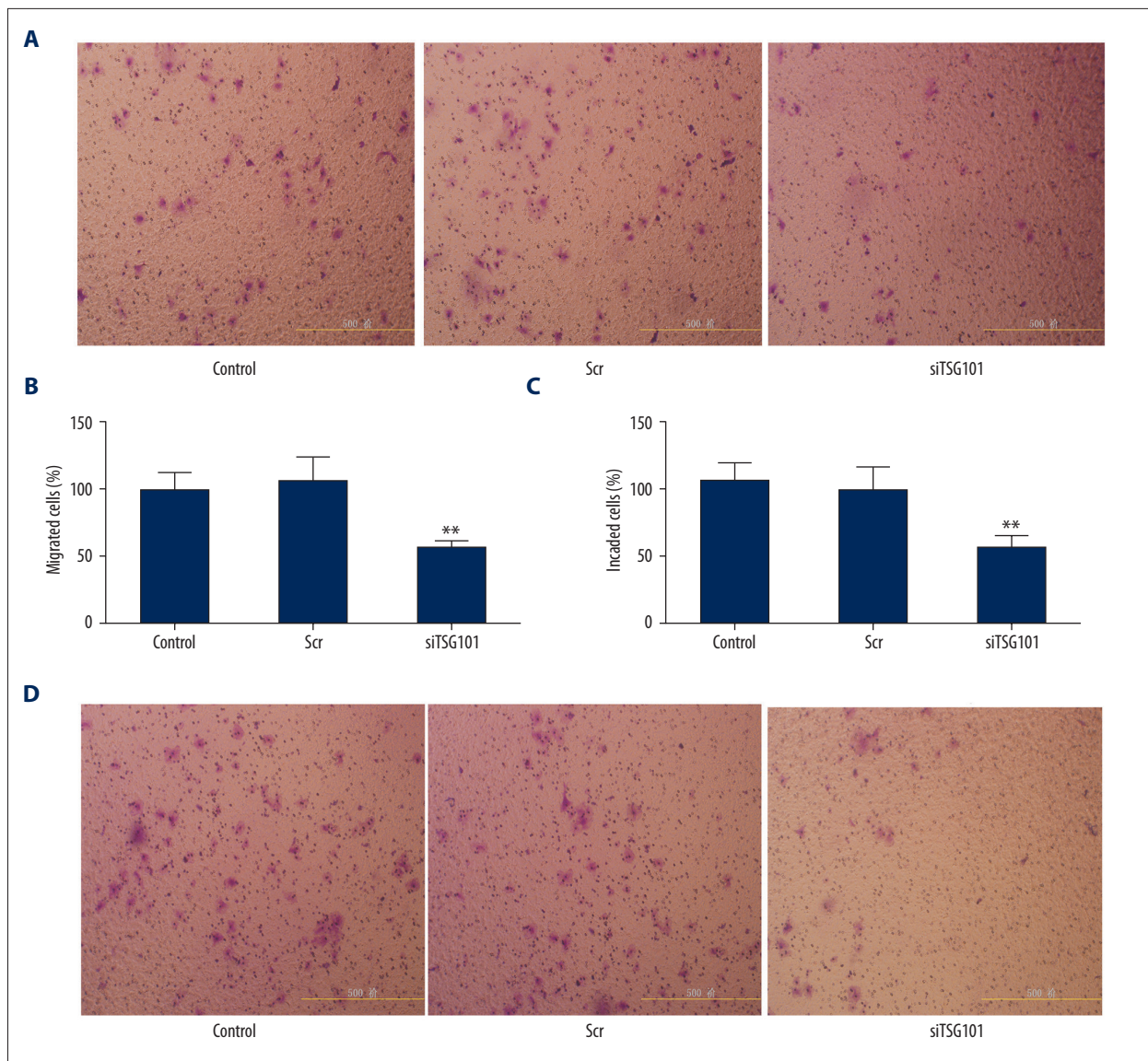
TSG101 deletion inhibits cell migration and invasion

The high rate of intrahepatic and extrahepatic metastases may be the key factor which led to the poor prognosis and high recurrence of HCC [19]. Metastasis is largely dependent on the
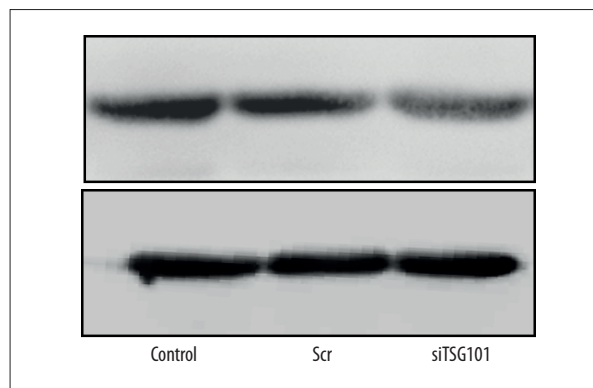
Supplementary Figure 1. TSG101 expression in control, scramble control (Scr), and TSG101 siRNA (siTSG101)-treated groups was examined by Western blotting.

ability of cell migration and invasion, which is influenced by many cell-intrinsic identities and extrinsic microenvironment molecule. Therefore, we examined the effect of TSG101 silencing on cell migration and invasion *in vitro*. Compared to the control groups, TSG101 deletion was able to reduce the migration ability of Huh7 cells by 45%, as is shown in Figure 2A, 2B. Next, when we evaluated cell invasion ability in TSG101 knockdown cells and control cells, we found that TSG101 knockdown cells

Figure 2. Silencing of TSG101 in Huh7 cells inhibits cell migration and invasion. (**A**) Cells treated with siTSG101 for 48 hours were collected and seeded in the transwell chamber, which were then cultured for 16 hours in 0.5% FBS. Representative images of migration are shown. (**B**) Quantitative data of migrated cells in cell migration assay. (**C**) Cells in different groups were collected and seeded in transwells covered with Matrigel and cultured for 20 hours. Representative images of cell invasion assay are shown. (**D**) Quantitative data of invasive cells in cell invasion assay. ** *p*<0.01; all data are representative results of 3 independent experiments.

displayed significantly reduced (by 50%) cell invasion ability, as shown in Figure 2C, 2D. Altogether, these data suggest an important role of TSG101 in Huh7 cell migration and invasion.

TSG101 deletion induces cell cycle arrest at G1 phase

Cell cycle, consisting 4 stages – G1 phase, S phase, G2 phase, and M phase – is a complicated process, which the cell must undergo to proliferate [20]. The cancer cell usually has abnormal cell cycle distribution, the inhibition of which may be a

promising treatment. To evaluate whether TSG101 deletion affects the cell cycle distribution, we performed flow cytometry after the cells had been transfected with TSG101 siRNA for 48 hours. The data show that TSG101 deletion led to an increase of cell population in G1 phase (Figure 3A, 3B). Furthermore, we studied the effect of TSG101 deletion on the expression of cyclins. We found that the expression of cyclin A and cyclin D was down-regulated and the expression of CDK2 was up-regulated significantly (Figure 3C). TSG101 deletion had no effect on Huh7 cell apoptosis (Supplementary Figure 2). These data
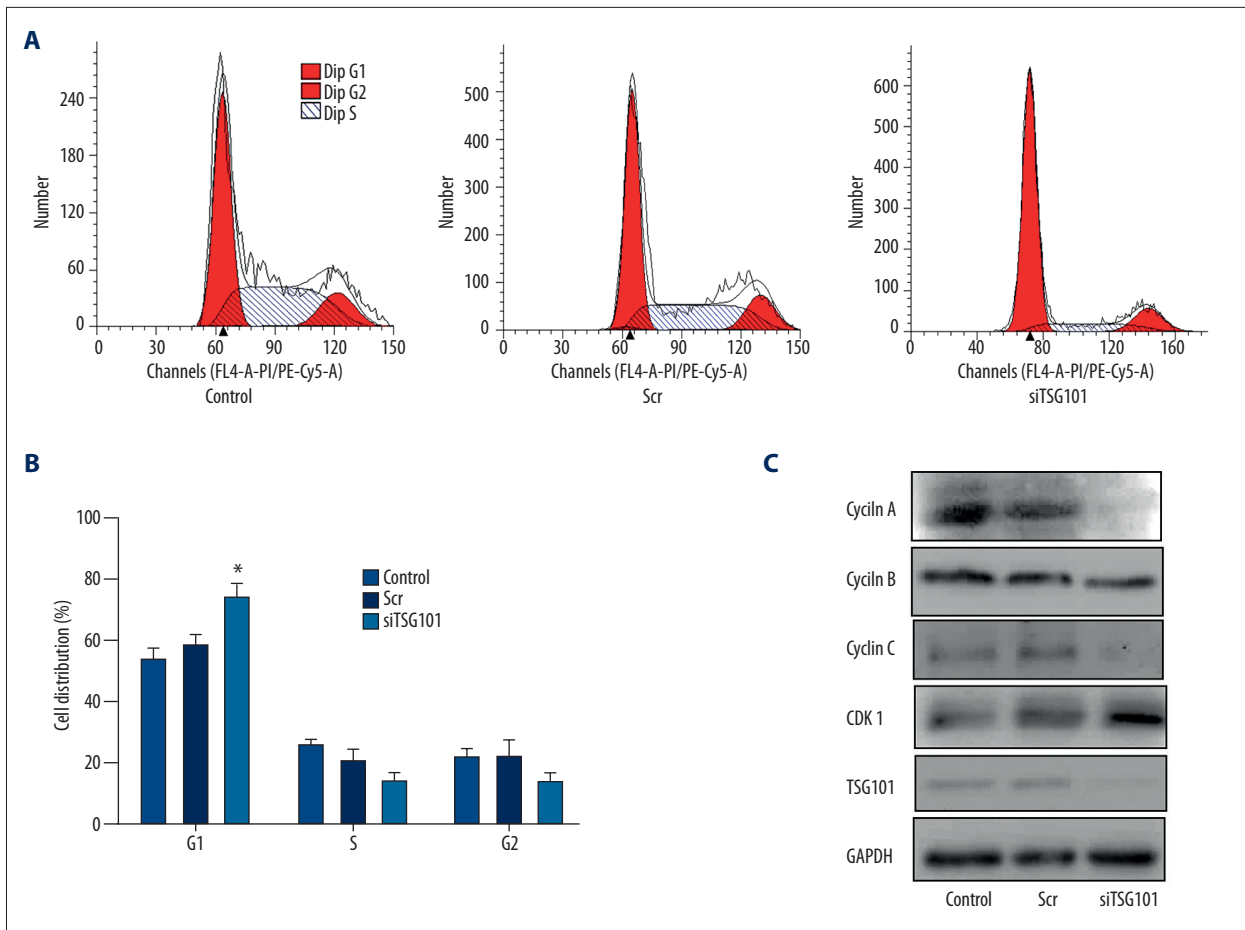
Figure 3. TSG101 deletion induces cell cycle arrest at G1 phase. (**A**) Representative images of cell cycle assay, which shows an increase at G1 phase in the TSG101 siRNA-treated group. (**B**) Quantitative data of cell cycle distribution are shown. (**C**) The protein level of cyclin A, cyclin B, cyclin D, and CDK2 were analyzed by immunoblotting with specific antibodies. * $p<0.05$; all data are representative results of 3 independent experiments.
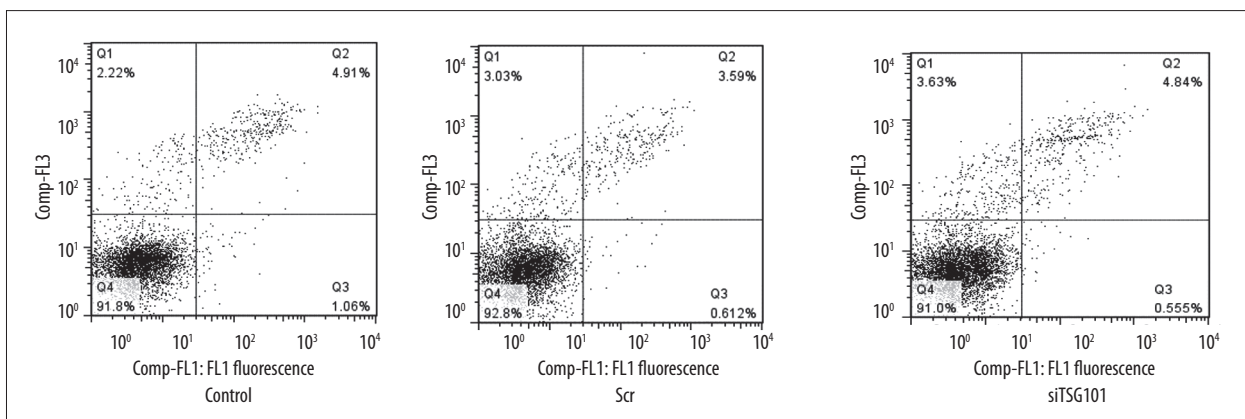
Supplementary Figure 2. TSG101 deletion had no effect on cell apoptosis. Representative images of the cell apoptosis assay. Q1, Q2, Q3, and Q4 indicate the dead cells, apoptotic cells in late stage, apoptotic cells in early stage, and live cells, respectively.
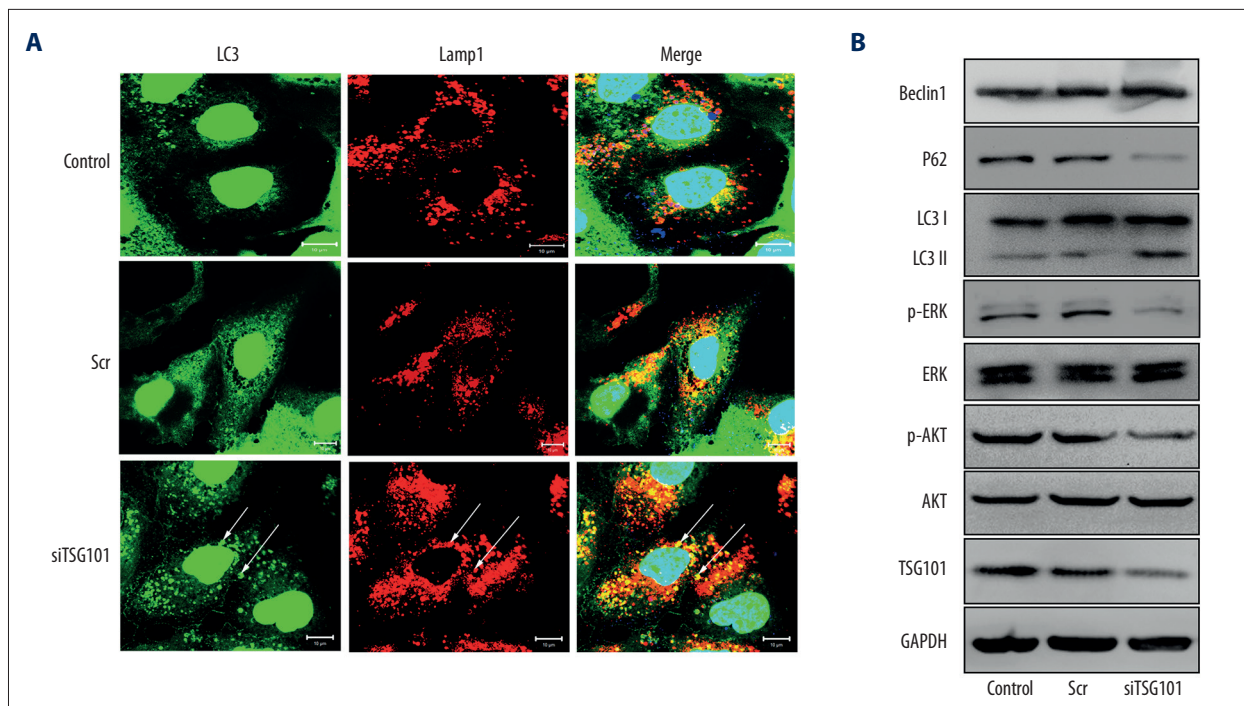
Figure 4. TSG101 deletion induces autophagy and inhibits the activation of ERK1/2 and AKT. (**A**) Immunofluorescence staining of cells in different groups shows increased GFP-LC3 dots (Green) and lamp1 (Red). Nuclei were stained with DAPI (Blue). (**B**) The protein level of Beclin 1, p62, LC3, p-ERK1/2, ERK1/2, p-AKT, and AKT were assessed by immunoblotting with specific antibodies.

indicate that TSG101 may affect cell growth though disrupting the cell cycle process.

TSG101 deletion induces autophagy and inhibits the activation of ERK1/2 and AKT

To assess whether TSG101 deletion leads to autophagy, we first used immunofluorescence staining to examine the intracellular localization of GFP-LC3 and lamp1, which are the constituents of autophagosome and lysosome, separately. The increased dots and co-staining of GFP-LC3 and lamp1 showed that autophagy in TSG101-deficient cells was elevated (Figure 4A). Furthermore, we estimated the expression of LC3 II, p62, and Beclin 1 in TSG101 siRNA-treated cells. We found that the expression of LC3 II and Beclin 1 had been up-regulated and the expression of p62 had been down-regulated (Figure 4B), which indicated the occurrence of autophagy in another way. To better understand the effect of TSG101 on autophagy, we then investigated the effect of TSG101 deletion on phosphorylation of Akt and ERK1/2. The data showed that silence of TSG101 resulted in significantly decreased activation of Akt and ERK1/2 (Figure 4B). Altogether, the results suggested that TSG101 silence was able to inactivate the phosphorylation of Akt and ERK1/2, which accelerate the occurrence of autophagy and thus increase autophagic cell death.

Discussion

Although TSG101 was initially identified as a tumor-suppressor gene, recent investigations reveal that it may function as an oncogene [11,15,16]. An early study showed that TSG101 deficiency is able to cause the metastasis of mouse fibroblasts and that these transformed cells can form tumors and induce neoplasia of the lung after they have been injected into nude mice, while re-acquisition of TSG101 can partially rescue the metastatic phenotype [4]. However, recent research reveal that TSG101 is overexpressed in many kinds of malignant tumors, and silencing of TSG101 is able to inhibit cancer cell growth [7,16,18,21]. However, until now there has been no report investigating the role of TSG101 in the development of HCC.

Our data showed that TSG101 was over-expressed in HCC tissue, which indicated that TSG101 might be an oncogene in the process of HCC. As the hallmarks of malignant cancer are characterized by abnormal cell growth and metastases, we performed cell growth assay, cell migration assay, and cell invasion assay to estimate the effect of TSG101 deletion in Huh7 cells [22]. The data showed that silencing of TSG101 clearly suppressed Huh7 cell growth, migration, and invasion (Figures 1B, 2A–2D), which indicates an important role of TSG101 in the occurrence and development of HCC. It

is known that dynamic actin cytoskeletal reorganization is indispensable for cell motility; therefore, we examined the impact of silencing of TSG101 on the cell cytoskeleton [23]. The data suggest that TSG101 deletion is able to impair the bundling of actin filaments, which decreases the ability of cell migration and invasion.

To better understand the role of TSG101 in cell growth of Huh7 cells, we performed flow cytometry to examine cell cycle distribution and found that the silencing of TSG101 resulted in cell cycle arrest at G1 phase, which indicates a role of TSG101 in regulation of cyclins. Then, we found TSG101 deficiency was able to suppress the expression of cyclin A and cyclin D while increasing the expression of CDK2. Cyclin A and cyclin D are normally up-regulated during G1/S transition, which is regulated by CDK2 [24]. The inhibited expression of cyclin A and cyclin D might be responsible for the cell cycle arrest at G1 phase in TSG101-silenced Huh7 cells.

Autophagy was initially found to be a strategy by which cells increase material and energy recycling by digestion of aged organelles with autophagy-specific lysosomes, which is regarded as strengthening the ability of cancer cells to survive stress or nutrient-insufficient microenvironments [25]. However, researchers found that increased autophagy could cause cell death as another type of programmed cell death besides apoptosis, which was called autophagic cell death [26,27]. Our data showed that silencing of TSG101 had no effect on apoptosis of Huh7 cells, but might induce autophagic cell death. Compared

to apoptosis, autophagic cell death has different features, such as massive autophagic vacuolization, overexpression of Beclin 1, conversion of LC I to LC II, and depletion of p62 [28]. The results indicate that autophagic cell death might participate in the inhibition of cell growth. AKT and ERK1/2 signal pathways are known to be involved in the induction of autophagy; therefore, we estimated the activation of AKT and ERK1/2 [29,30]. The data are consistent with previous investigations reporting that silencing of TSG101 was able to inhibit phosphorylation, which might account for autophagic cell death in TSG101-deleted cells.

Conclusions

We demonstrated that TSG101 was over-expressed in HCC tissues and that TSG101deletion was able to suppress Huh7 cell growth, migration, and invasion. Furthermore, silencing of TSG101 could induce cell cycle arrest at G1 phase and inhibit the expression of cyclin A and cyclin D, while up-regulating the expression of CDK2. The possible mechanism might be induction of autophagic cell death and inactivation of Akt and ERK1/2. The data indicate an important role of TSG101 in the development of HCC and it may be a potential target for molecular therapy.

Conflict of interest

The authors declare no conflict of interest.

References:

1. Jemal A, Bray F, Center MM et al: Global cancer statistics. Cancer J Clin, 2011; 61(2): 69–90

2. Maluccio M, Covey A: Recent progress in understanding, diagnosing, and treating hepatocellular carcinoma. Cancer J Clin, 2012; 62(6): 394–99

3. Kudo M: Treatment of advanced hepatocellular carcinoma with emphasis on hepatic arterial infusion chemotherapy and molecular targeted therapy. Liver Cancer, 2012; 1(2): 62

4. Li L, Cohen SN: Tsg101: a novel tumor susceptibility gene isolated by controlled homozygous functional knockout of allelic *loci* in mammalian cells. Cell, 1996; 85(3): 319–29

5. Ponting C, Cai Y, Bork P: The breast cancer gene product TSG101: a regulator of ubiquitination? J Mol Med, 1997; 75(7): 467–69

6. Garrus JE et al: Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. Cell, 2001; 107(1): 55–65

7. Zhang Y, Song M, Cui ZS et al: Down-regulation of TSG101 by small interfering RNA inhibits the proliferation of breast cancer cells through the MAPK/ERK signal pathway. Histol Histopathol, 2011; 26(1): 87

8. Watanabe M, Yanagi Y, Masuhiro Y et al: A putative tumor suppressor, TSG101, acts as a transcriptional suppressor through its coiled-coil domain. Biochem Biophys Res Commun, 1998; 245(3): 900–5

9. Wagner KU, Krempler A, Qi Y et al: Tsg101 is essential for cell growth, proliferation, and cell survival of embryonic and adult tissues. Mol Cell Biol, 2003. 23(1): 150–62

10. Ruland J, Sirard C, Elia A et al: p53 accumulation, defective cell proliferation, and early embryonic lethality in mice lacking tsg101. Proc Natl Acad Sci USA, 2001; 98(4): 1859–64

11. Young TW, Rosen DG, Mei FC et al: Up-regulation of tumor susceptibility gene 101 conveys poor prognosis through suppression of p21 expression in ovarian cancer. Clin Cancer Res, 2007; 13(13): 3848–54

12. Liu RT, Huang CC, You HL et al: Overexpression of tumor susceptibility gene TSG101 in human papillary thyroid carcinomas. Oncogene, 2002; 21(31): 4830–37

13. Koon N, Schneider-Stock R, Sarlomo-Rikala M et al: Molecular targets for tumour progression in gastrointestinal stromal tumours. Gut, 2004; 53(2): 235–40

14. Zhang M, Wang Q, Ren XJ et al: [Changing of TSG101 expression on the invasion and metastasis of gastric cancer cells]. Zhonghua Yi Xue Za Zhi, 2013; 93(16): 1219–23 [in Chinese]

15. Broniarczyk JK, Warowicka A, Kwaśniewska A et al: Expression of TSG101 protein and LSF transcription factor in HPV-positive cervical cancer cells. Oncol Lett, 2014; 7(5): 1409–13

16. Liu Z, Yang Z, Liu D et al: TSG101 and PEG10 are prognostic markers in squamous cell/adenosquamous carcinomas and adenocarcinoma of the gallbladder. Oncol Lett, 2014; 7(4): 1128–38

17. Ma XR, Edmund Sim UH, Pauline B et al: Overexpression of WNT2 and TSG101 genes in colorectal carcinoma. Trop Biomed, 2008; 25(1): 46–57

18. Zhu G, Gilchrist R, Borley N et al: Reduction of TSG101 protein has a negative impact on tumor cell growth. Int J Cancer, 2004; 109(4): 541–47

19. Bruix J, Gores GJ, Mazzaferro V: Hepatocellular carcinoma: clinical frontiers and perspectives. Gut, 2014; 63(5): 844–55

20. Williams GH, Stoeber K: The cell cycle and cancer. J Pathol, 2012; 226(2): 352–64

21. Morris CR, Stanton MJ, Manthey KC et al: A knockout of the Tsg101 gene leads to decreased expression of ErbB receptor tyrosine kinases and induction of autophagy prior to cell death. PloS One, 2012; 7(3): e34308

22. Hanahan D, Weinberg RA: Hallmarks of cancer: the next generation. Cell, 2011; 144(5): 646–74

23. Wu MH, Hong TM, Cheng HW et al: Galectin-1-mediated tumor invasion and metastasis, up-regulated matrix metalloproteinase expression, and reorganized actin cytoskeletons. Mol Cancer Res, 2009; 7(3): 311–18

24. Casimiro MC, Crosariol M, Loro E et al: Cyclins and cell cycle control in cancer and disease. Genes Cancer, 2012; 3(11–12): 649–57

25. Cuervo AM, Bergamini E, Brunk UT et al: Autophagy and aging: the importance of maintaining" clean" cells. Autophagy, 2005; 1(3): 131–40

26. Kroemer G, Levine B: Autophagic cell death: the story of a misnomer. Nat Rev Mol Cell Biol, 2008; 9(12): 1004–10

27. Ouyang L, Shi Z, Zhao S et al: Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. Cell Prolif, 2012; 45(6): 487–98

28. Shen S, Kepp O, Kroemer G: The end of autophagic cell death? Autophagy, 2012; 8(1): 1–3

29. Owen KA, Meyer CB, Bouton AH, Casanova JE: Activation of focal adhesion kinase by Salmonella suppresses autophagy via an Akt/mTOR signaling pathway and promotes bacterial survival in macrophages. PLoS Pathog, 2014; 10(6): e1004159

30. Liu Y, Yang Y, Ye YC et al: Activation of ERK–p53 and ERK-mediated phosphorylation of Bcl-2 are involved in autophagic cell death induced by the c-Met iInhibitor SU11274 in human lung cancer A549 cells. J Pharmacol Sci, 2012; 118(4): 423–32