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Down-regulation of TSG101 by small interfering RNA inhibits the proliferation of breast cancer cells through the MAPK/ERK signal pathway

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Summary. We designed to investigate the effects of down-regulating the tumor susceptibility gene 101 (TSG101) on the proliferation and apoptosis of the human breast cancer MCF-7 cell line, and the role of the MAPK/ERK signal pathway in this process. The siRNA against TSG101 was transfected into the breast cancer MCF-7 cell line using Lipofectamine 2000. After TSG101 knockdown, the proliferation of MCF-7 cells was measured by the MTT assay. The cell cycle distribution and apoptosis were examined by using flow cytometry while cell migration was measured using a transwell assay. The protein level of p-ERK was further assessed by immunofluorescence and western blotting. Our results are as following, the MCF-7 cells transfected with TSG101 siRNA proliferated significantly slower and exhibited significantly increased rates of apoptosis compared to the control cells. In the TSG101 siRNA transfected cells, the percentage of cells in the G_0/G_1 and S phase of the cell cycle was significantly higher and lower, respectively, compared to the control cells. Moreover, the migration ability of TSG101 siRNA transfected cells was lower than the control groups. Lastly, the level of p-ERK protein in TSG101 siRNA transfected cells was significantly decreased compared with the control cells. In conclusion, TSG101 knockdown in breast cancer cells induces apoptosis and inhibits proliferation. The TSG101 depleted cells are arrested at the G_1/S transition of the cell cycle. The migration of breast cancer cells is also impaired by TSG101 siRNA. TSG101 may play a biological role through modulation of the MAPK/ERK signaling pathway in breast cancer.

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Introduction

The tumor susceptibility gene 101 (TSG101) was initially identified in NIH3T3 cells, a murine fibroblast cell line, in 1996 (Li and Cohen, 1996). TSG101 is involved in a variety of biological functions, such as vesicular transport and cell cycle control. The role TSG101 plays in the onset of tumors remains to be elucidated. Bennett et al. found that the declining expression of TSG101 protein is correlated with the occurrence of ovarian cancer or hysterocarcinoma (Bennett et al., 2001). Lo et al. studied 30 breast cancer tissue samples, and detected relatively slight expression of full-size 46 kDa TSG101 protein (Lo et al., 2000). All the findings above indicated that TSG101 might be a tumor suppressor gene, although, some researchers strongly challenged the above statement. Zhu et al. (2003) found that TSG101 in breast ductal carcinoma in situ and invasive breast cancer presented positive expression by immunofluorescence, by contrast, TSG101 in normal duct and lobule showed negative or slight staining occasionally. They also found that the expression of TSG101 in breast ductal carcinoma in situ and invasive breast cancer periphery tissue is less compared with that in central tissue. Oh et al. (2007) comparatively studied 16 cases of normal breast or benign breast tumor and 16 cases of invasive breast cancer by immunofluorescence, and found that the expression of TSG101 in normal and benign tissues is lower than that in invasive breast cancer tissue. However, compared with the findings by Oh et al. (2007) and Zhu et al. (2007) detected a stronger expression of TSH101 invaded into tumor cells in breast

cancer, suggesting that TSG may play a role in the progress and development of breast cancer.

It has been shown by Ma et al. that TSG101 is constitutively upregulated in colorectal cancers (Ma et al., 2008). In 2008, Young et al. showed that the expression of TSG101 is increased in human ovarian cancers (Young et al., 2007). Whether TSG101 is an oncogene or tumor suppressor gene remains to be elucidated. Moreover, there is a lack of understanding of its influence on cellular biological behavior. However, existing accumulated evidence has proved that the TSG101 gene plays a vital role within the organism.

The MAPK/ERK signaling pathway is one of the canonical mitogen-activated protein kinase (MAPK) pathways, which play a pivotal role in promoting cell proliferation and differentiation and the inhibition of apoptosis. The ERK signaling cascade is involved in the core signaling network that regulates cell growth, development, and division. Oh et al. (2007) revealed that in the transfected rat breast with overexpression of TSG101, the level of p-ERK increased, suggesting that the overexpression of TSG101 activates the MARK/ERK signaling pathway. However, for human breast cancer cells, the relationship between TSG101 and MARK/ERK signaling pathway needs further investigation.

Therefore, in this report, we used small interfering RNA (siRNA) targeting against TSG101 to study the effects of TSG101 down-regulation on the growth features of the breast cancer cell line MCF-7 and the relationship between TSG101 and MAPK/ERK signaling pathway. We find that TSG101 plays an important role in growth, survival and malignant biological behavior of MCF-7 cells. In MCF-7 cells, TSG101 and MARK/ERK are significantly correlated.

Materials and methods

Reagents and siRNAs

The reagents used in this study included: fetal bovine serum (FBS), RPMI-1640 Medium, trypsin, and Lipofectamine 2000 from Invitrogen Inc. (Carlsbad, CA, USA); antibodies against TSG101, phospho-ERK, and total ERK-1/2 from Santa Cruz (Santa Cruz, CA, USA); goat anti-human ß-actin antibody, HRP-secondary goat anti-mouse IgG, and FITC-labeled goat-anti mouse IgG from Zhongshan Goldenbridge Biotech. (Beijing, China); a PI staining kit and an annexin V-FITC kit from Jingmei Inc. (Shanghai, China); MTT from Sigma (NJ, USA); and transwell plates from Corning Inc. (USA).

According to the report by Shen et al. (2004), the siRNA against TSG101 was synthesized by Lianxing Biotech. (Shengyang, China). The non-targeting siRNA was used as a negative control and was named as siRNA NC (siRNA negative control). The sequences of the siRNA are TSG101 siRNA, sense: 5'-CCUCCAGUCU UCUCUCGUCtt-3'; antisense: 5'-GACGAGAGAAGA CUGGAGGtt-3'; siRNA NC, sense: 5'-UUCUCCGAA CGUGUCAUGUtt-3'; antisense: 5'-ACGUGACACG UUCGGAGAAtt-3'.

Cell culture and grouping

The breast cancer cell line MCF-7 was acquired from the Third Department, the Center of Experimental technology of our school. For the experiments, the MCF-7 cells were maintained in RPMI-1640 medium supplemented with 10% FBS and incubated at 37°C with 5% CO_2 and saturated humidity.

MCF-7 cells were randomly divided into 3 groups: the MCF-7-siRNA group receiving TSG101 siRNA transfection; the MCF-7-siRNA-NC group receiving siRNA NC transfection; and the untransfected MCF-7 group.

Cell transfection

MCF-7 cells were trypsinized, counted, and seeded in 6-well plates 24 hours before transfection, so that the cells reached 30-50% confluence at the time of transfection. The cells were washed with serum-free medium 3 times and were transfected with TSG101 siRNA or siRNA NC using Lipofectamine 2000, according to the manufacturer's protocol. The medium was changed to serum-containing medium after 6 hours and the cells were further cultured for 72 hours before protein extraction.

Total cellular protein extraction

To harvest cellular protein, the medium was removed and the cells were trypsinized and collected by centrifugation. After washing with ice-cold 0.01 M PBS twice, cells were centrifuged again to remove the PBS, resuspended in lysis buffer, and incubated on ice for 20 minutes. Then the cell lysates were sonicated and cleared by centrifugation at 12,000xg for 30 minutes at 4°C. The supernatant contained the total cellular protein extract and the protein concentration was determined using Coomassie brilliant blue staining.

Western blotting

The same amount of protein for each sample was loaded and separated by electrophoresis on SDS-polyacrylamide gels. After electrophoresis, the proteins were transferred onto PVDF membranes (Fluka Chemical, Switzerland). The PVDF membrane was then washed in TBS, blocked in blocking buffer for 1 hour, and incubated with antibodies against TSG101, phospho-ERK, ERK1/2, and ß-actin at 4°C overnight. During the second day of the procedure, the membrane was washed with Tris-buffered saline with Tween-20 (TBST), further incubated with an HRP-labeled secondary antibody at room temperature for 1 hour, washed again with TBST, and developed with the ECL reagent (Sigma) which consisted of mixing solutions A

and B. The level of ß-actin protein was used as the internal standard, or loading control. The intensity of each band was quantified utilizing the gel imagine analyzing system and normalized to the intensity of the ß-actin band.

MTT assay

The MCF-7 cells in each group were resuspended into single cells and seeded into a 96-well plate at a density of $1x10^3$ cells/well in triplicates. At 24, 48, 72, and 96 hours after transfection, 20 μl of 5 mg/ml MTT solution was added into each well. After incubation at 37°C for 4 hours, the medium was removed and each well was supplied with 150 μl of DMSO followed by 10 minutes of shaking. The OD at 570 nm was analyzed on a plate reader.

Fluorescence activated cell sorting (FACS) for cell cycle and apoptosis analysis

FACS was performed according to the manufacturer's protocol. For the detection of cell cycle distribution using PI staining, the cells were trypsinized, centrifuged, washed with PBS, and re-centrifuged. The supernatant was discarded and the cell pellets were suspended in 1 ml PI staining solution (0.05 g/L PI, 0.02 g/L RNase, 0.01 g/L triton X-100, 1 g/L Sodium Citrate, 5.85 g/L NaCl) and incubated for 30 minutes in the dark at 4°C. The cells were then subjected to a FACScalibur analysis.

Annexin V-FITC/PI double staining to detect the apoptosis rate of MCF-7 cells

The different groups of cells were harvested, centrifuged and washed with PBS twice, and re-suspended in binding buffer at a cell density of $1x10^6$ cells/ml. The 200 μl of cells were added into 10 μl of annexin V-FITC and 5 μl of PI staining solutions. The cells were mixed gently and incubated at room temperature for 15 minutes. Then, 300 μl of binding buffer was added and the cells were run through a FACScalibur analysis.

Cell motility analysis

The cell motility assay was carried out in a transwell chamber. The transwell chambers were placed in a well of a 24-well culture plate. The different groups of cells were switched into serum-free medium and cultured for 24 hours. The cells were trypsinized, and resuspended in RPMI-1640 medium containing 0.1% BSA at a final cell density of $4x10^5$ cells/ml. Then, 100 μl of cells were added into the upper chamber and 600 μl of RPMI-1640 media supplemented with 10% FCS was added to the bottom chamber. Every group was analyzed in triplicate. After incubation at 37°C in 5% CO_2 for 24 hours, the transwell chambers were removed, untransferred cells were washed out, and the rest of the cells were fixed in 95% ethanol for 20 minutes. The samples were dried, stained by trypan blue for 20 minutes, and examined microscopically. Five fields were randomly selected and the number of cells present was counted. The average number was subsequently calculated.

Immunofluorescence

The cells were transfected with siRNA against TSG101 or control siRNA for 48 hours. Then the cells were spread onto glass slides for 24 hours, washed with 0.5% triton X-100 in PBS, fixed with 4% formaldehyde for 15 minutes, treated with H_2O_2 for 10 minutes, and blocked in 5% BSA for 2 hours. Then the anti-p-ERK primary antibody was applied (1:200), incubated overnight for 4°C, and washed with PBS three times. A FITC-coupled secondary antibody was then added to the cells and incubated for 1 hour, washed with PBS and sealed with nail polish. The expression of p-ERK protein was examined under a laser confocal microscope.

Statistical Analysis

SPSS 13.0 software was used to perform data processing. The statistical data are expressed as mean ± SD. Differences were considered statistically significant when $P<0.05$.

Results

Detection of the knock down efficiency of TSG101 siRNA

A specific band with a molecular weight of 45 kDa was detected by immunostaining using the monoclonal anti-TSG101 antibody (Fig. 1). As shown in Figure 2, the data are calculated by gray value ratio of TSG101 and ß-actin. MCF-7 cells transfected with TSG101 siRNA for 72 hours (0.0708±0.0091) showed a remarkable reduction of TSG101 protein compared with MCF-7 cells (0.5460±0.0145) which received the negative control (NC) siRNA (0.4782±0.0678). There was no significant difference in the level of TSG101 between non-transfected and NC siRNA transfected

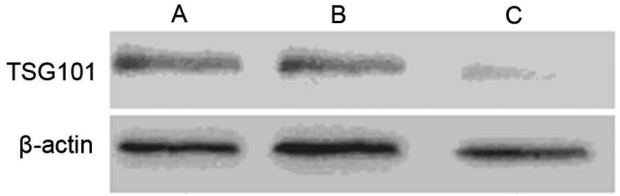

Fig. 1. Expression of TSG101 in MCF-7 cells. A: Non-transfected MCF-7 cells. B: MCF-7 cells transfected with the negative control siRNA. C: MCF-7 cells transfected with the TSG101 siRNA for 72 hours. The expression of TSG101 protein in MCF-7 cells transfected with the TSG101 siRNA for 72 hours was significantly lower compared with those of the other two groups.

MCF-7 cells.

Cell proliferation analyzed by the MTT assay

As shown in Table 1, the OD values of TSG101 siRNA transfected cells on day 1 to 4 were 0.175±0.007, 0.227±0.009, 0.381±0.005, 0.522±0.008, respectively, which were significantly lower compared with those in non-transfected cells (0.198±0.013, 0.315±0.006, 0.612±0.015, 0.619±0.011) and NC siRNA transfected cells(0.312±0.005, 0.312±0.005, 0.604±0.013, 0.710±0.011) (P<0.05).

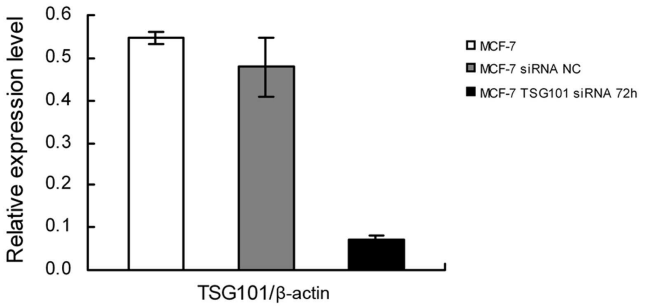

Fig 2. The relative expression amount of TSG101 protein in MCF-7 cells As shown in Fig. 2, the relative expression amount of TSG101 protein in MCF-7 cells transfected with the TSG101 siRNA for 72 hours was significantly lower than those of the other two groups.

Cell cycle analysis

As shown in Figure 3 and Table 2, TSG101 siRNA transfected MCF-7 cells exhibited an increased and decreased number of cells in G_0/G_1(70.51±0.23)and S phase(23.65±0.78) (P<0.01), respectively, when compared with non-transfected (59.15±0.77; 34.96±0.45) or NC siRNA-transfected MCF-7 cells (59.21±0.68; 34.89±0.30).

Apoptosis analysis

As shown in Figure 4 and Table 3, apoptosis was significantly increased in TSG101 siRNA transfected MCF-7 cells (13.71±1.31) compared to non-transfected (4.37±0.87) or NC siRNA-transfected MCF-7 cells (6.00±0.08) (P<0.01). As shown in Figure 4, the necrotic ratio of TSG101 siRNA transfected cells is higher compared with those of the other two groups.

Table 1. OD570 of MCF-7 cells with different treatments at different time points.

Groups	A value			
	1d	2d	3d	4d
MCF-7	0.198±0.013	0.315±0.006	0.612±0.015	0.619±0.011
MCF-7 siRNA NC	0.192±0.005	0.312±0.005	0.604±0.013	0.710±0.011
MCF-7 TSG101 siRNA	0.175±0.007*	0.227±0.009*	0.381±0.005*	0.522±0.008*

*: MCF-7 TSG101 siRNA vs. MCF-7, MCF-7 TSG101 siRNA vs. MCF-7 siRNA NC, P<0.05. A is expressed as average value ± SD.

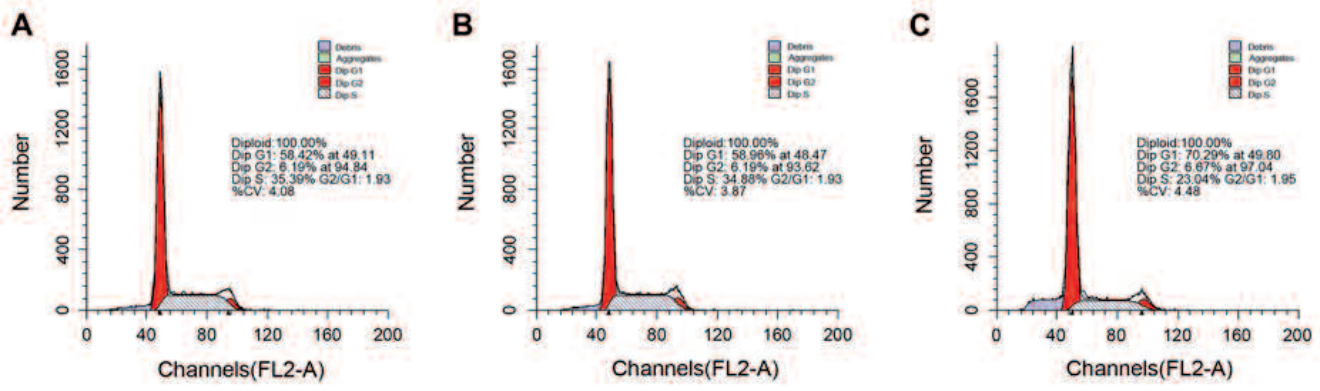

Fig. 3. Cell cycle analysis of MCF-7 cells transfected with TSG101 siRNA for 72 hours. Compared with the other two groups, the percentage of cells in the G_0/G_1 phase increased in TSG101 siRNA for 72 hours group, but that in S phase decreased in TSG101 siRNA for 72 hours group.

Cell motility analysis

As shown in Figure 5 and Table 4, mean cell count in each field was dramatically reduced in TSG101 siRNA transfected MCF-7 cells (4.60±0.80) compared to non-transfected (24.47±1.90) or NC siRNA-transfected MCF-7 cells (23.80±2.20) (P<0.01).

Immunofluorescence

The immunofluorescence results showed that the

Table 3. Apoptosis of the MCF-7 cells with different treatments.

Group	Apoptosis (%)
MCF-7	4.37±0.87
MCF-7 siRNA NC	6.00±0.08
MCF-7 TSG101 siRNA 72 h	13.71±1.31*

*: MCF-7 TSG101 siRNA vs. MCF-7, MCF-7 TSG101 siRNA vs. MCF-7 siRNA NC, P<0.01.

Table 2. Cell cycle analysis of TSG101 siRNA transfected MCF-7 cells for 72 hours, non-transfected MCF-7 cells, and NC siRNA-transfected MCF-7 cells.

Group	G_0/G_1 phase (%)	S phase (%)	G_2/M phase (%)
MCF-7	59.15±0.77	34.96±0.45	5.89±0.31
MCF-7 siRNA NC	59.21±0.68	34.89±0.30	5.90±0.42
MCF-7 TSG101 siRNA 72h	70.51±0.23*	23.65±0.78*	5.84±1.01

*: MCF-7 TSG101 siRNA vs. MCF-7, MCF-7 TSG101 siRNA vs. MCF-7 siRNA NC, P<0.01.

Table 4. Cell motility analysis of the MCF-7 cells receiving different treatments for 48 h.

Group	cells/field
MCF-7	24.47±1.90
MCF-7 siRNA NC	23.80±2.20
MCF-7 TSG101 siRNA 48 h	4.60±0.80*

*: MCF-7 TSG101 siRNA vs. MCF-7, MCF-7 TSG101 siRNA vs. MCF-7 siRNA NC, P<0.01.

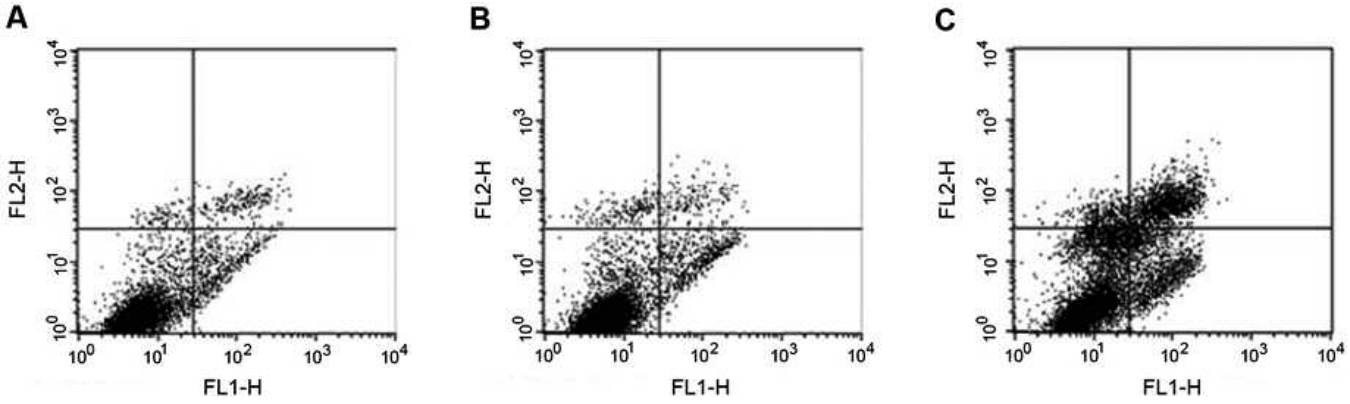

Fig. 4. Apoptosis of TSG101 siRNA-transfected MCF-7 cells for 72 h. The apoptosis rate in TSG101 siRNA for 72 hour group was higher compared with those of the other two groups (Note: FL1-H instead of annexin V-FITC; FL2-H instead of PI).

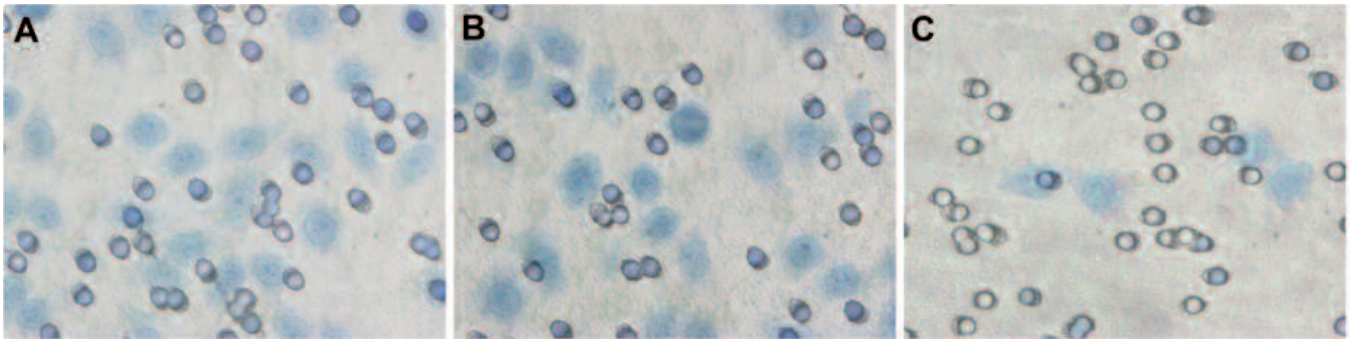

Fig. 5. Cell motility analysis of the MCF-7 cells receiving different treatments for 48 h. **A.** Non-transfected MCF-7 cells. **B.** MCF-7 cells transfected with the negative control siRNA. **C.** MCF-7 cells transfected with the TSG101 siRNA for 48 hours. According to the figure, mean cell count per field in TSG101 siRNA-transfected MCF-7 cells significantly decreased compared with those of the other two groups.

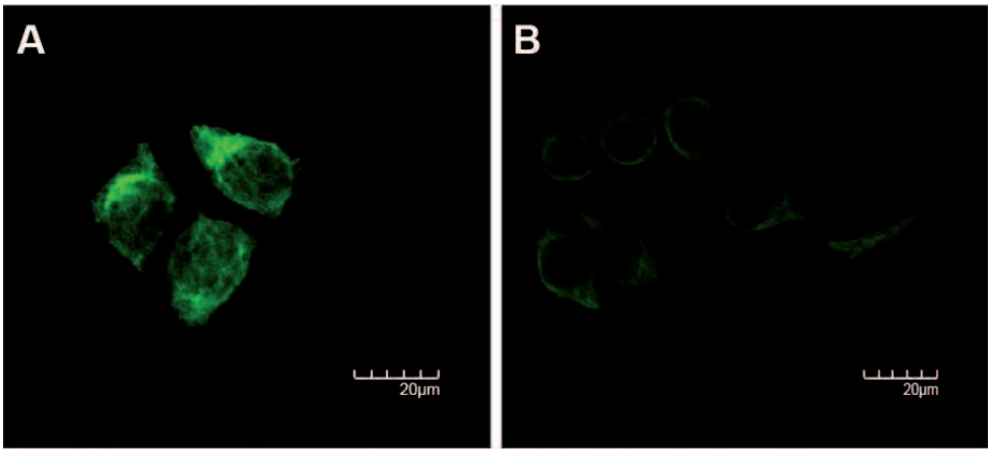

Fig. 6. Expression of p-ERK in TSG101 siRNA-transfected MCF-7 cells The immunofluorescence results showed that the expression of p-ERK was dramatically decreased in TSG101 siRNA-transfected MCF-7 cells compared with those in siRNA NC cells.

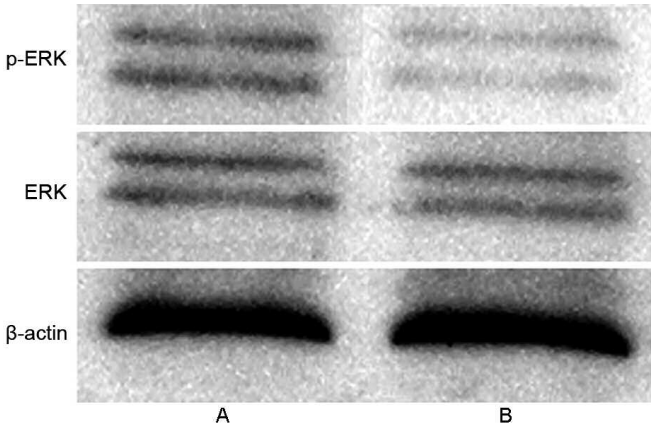

Fig. 7. Effect of TSG101 siRNA transfection on the expression of p-ERK and ERK1/2 in MCF-7 cells. A: MCF-7 siRNA NC 72 hours; B: MCF-7 TSG101 siRNA 72 h. Compared with the other two groups, the expression of p-ERK protein in MCF-7 cells transfected with the TSG101 siRNA for 72 hours group was significantly lower , but the expression of ERK1/2 protein showed no significant difference.

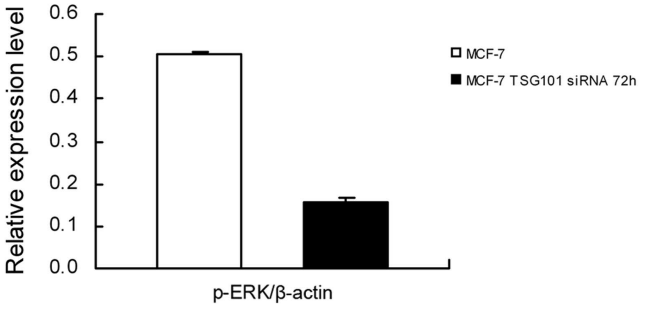

Fig. 8. The relative expression of p-ERK protein in MCF-7 cells.

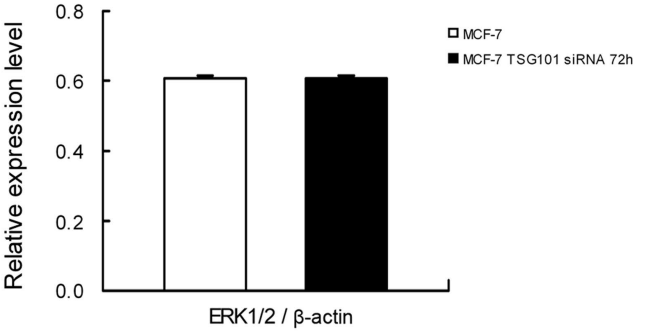

Fig. 9. The relative expression of ERK1/2 protein in MCF-7 cells.

expression of p-ERK was dramatically decreased in TSG101 siRNA-transfected MCF-7 cells (Fig. 6).

Analysis of p-ERK expression by western blot

The western blot results showed that the expression of p-ERK in TSG101 siRNA-transfected MCF-7 cells (0.1582±0.0093) was reduced by 68% compared with negative control cells (0.5078±0.0036). However, the expression of ERK1/2 was not altered (Figs. 7-9).

Discussion

It has been established that chromosome 11p15 and the adjacent region is often lost heterogeneously in many kinds of human tumors, indicating that the short arm of chromosome 11 may contain one or more tumor suppressor genes. Thus, TSG101, a gene in this region, has been described as a candidate tumor suppressor gene. TSG101 loss-of-function causes the metastasis of mouse fibroblasts. After injection into nude mice, these transformed cells can form tumors and induce metastatic neoplasia of the lung. Wild type TSG101 can partially

rescue the metastatic phenotype (Li and Cohen, 1996), suggesting that loss of TSG101 could lead to tumor formation. The human TSG101 gene and mouse TSG101 gene share 86% similarity, and encode a protein with a relative molecular weight of 46 kDa. The similarity between the human TSG101 protein and mouse TSG101 protein is 94%. The TSG101 protein contains a N-ubiquitin conjugating enzyme (E2)-like domain, a proline-rich domain, a C-coiled-coil domain, and a stable-box domain controlling its stability (Feng et al., 2000). Recent studies show that TSG101 plays important roles in a number of biological processes. There is abnormal expression of TSG101 in many human malignant tumors. However, the relationship between TSG101 and tumors is not yet fully understood.

Our studies show that cell proliferation is dramatically decreased when TSG101 is knocked down by siRNA. Cell cycle analysis shows that TSG101 siRNA-treated cells exhibit an increase and decrease in the number of cells in the G_0/G_1 and S phase of the cell cycle, indicating that TSG101 may be required for the G_1/S transition during cell cycle progression. Meanwhile, when TSG101 is knocked down, apoptosis increases and cell motility decreases. Our results are partially consistent with previous reports. Using mouse embryonic fibroblasts, Krempler et al. found that a conditional knockout of TSG101 caused the cells to arrest at the G_1/S phase and then subsequently die (Krempler et al., 2002). Homozygous deletion of the TSG101 locus causes abnormal development in which the embryos cannot survive for more than 6.5 days (Ruland et al., 2001), suggesting that TSG101 is required for rapid cell proliferation during the early stages of development. Wagner et al. showed that TSG101 is required for growth, proliferation and survival of primary cultured mammary epithelial cells (Wagner et al., 2003). They also showed that mouse embryos could not develop normally when TSG101 is knocked out. Travis et al. used siRNA to reduce the expression of TSG101 in human ovary carcinoma SKOV-3 cells and found that cell growth was inhibited (Young et al., 2007). When TSG101 depleted SKOV-3 cells were injected into nude mice, they resulted in much smaller tumors, which was confirmed by Young et al. (2007). Zhu et al. previously down regulated the expression of TSG101 in MDA-MB-231 breast cancer cells, and found that downregulation of TSG101 inhibited cell proliferation, suppressed the cell cycle, enhanced cell apoptosis, and also blocked the migration (Zhu et al., 2004). Both MCF-7 and MDA-MB-231 cells are representatives of breast epithelial carcinoma cells, and their biological behavior distinctively differed from each other. For instance, estrogen receptor (ER) and progesterone receptor (PR) both showed positive expressions, Cyclooxygenase (COX-2) was not detectable, P53 gene was wild type, in MCF-7 cells. However, in MDA-MB-231 cells, ER and PR were both negative, COX-2 was significantly expressed, and P53 gene was mutant type (Ostrakhovitch and Cherian, 2004). In this study, we used MCF-7 cells and adopted slightly modified experimental procedure, although our findings are basically consistent with theirs. Our investigations provide supportive evidence towards the viewpoint that TSG101 plays an important role in breast cancer.

MAPK signaling is an important pathway that transduces signals from the cell surface to the nucleus and plays essential roles in cell proliferation and apoptosis. ERK is the most important kinase pathway, and includes two isoforms: ERK1 and ERK2. After stimulation by cytokines, growth factor hormones, mitogen receptors and hypoxia, ERK is phosphorylated and translocated into the nucleus where it activates transcription of many oncogenes and causes metastasis (Aebersold et al., 2001). The current study analyzed the expression of p-ERK in MCF-7 cells after TSG101 knock down by using immunofluorescence and western blot. We found that down-regulation of TSG101 inhibits the expression of p-ERK in mammary carcinoma MCF-7 cells, suggesting that TSG101 normally functions through activation of the MAPK/ERK signaling pathway. This conclusion is consistent with a study by Oh et al. (2007), in which they constructed TSG101 transgenic mice with TSG101 specifically overexpressed in the mammary glands. They then detected the expression of p-ERK protein in the mammary gland in different developmental stages of the transgenic mice. Their results showed that TSG101 transgenic mice exhibited a significant increase in MAPK signaling activity during the gestation and nursing phases, except for the first day of the nursing phase. However, the expression of p-ERK was barely detected during the gestation phase or the 4^{th} and 10^{th} day of the nursing phase in wild type mice. The expression of TSG101 was significantly increased (3 fold) in transgenic female mice compared with wild type female mice.

Young et al. used siRNA to inhibit TSG101 in ovary carcinoma cells and they found that inhibiting TSG101 delayed cell growth, arrested the cell cycle, increased apoptosis and increased the expression of both p21 mRNA and protein (Young et al., 2007). They also found that TSG101 and p21 were negatively correlated in ovary carcinoma tissues, indicating that TSG101 plays a biological role in down-regulating p21. Two previous studies (Boucher et al., 2000; Kang et al., 2000) indicated that the MARK/ERK signaling pathway plays a vital role in regulating cell proliferation, cell cycle, and cell apoptosis. Shen et al. found that external stimulus, which induce cell migration such as serum, allow the phosphorylation of Nudel by activating protein kinase Erk. The phosphorylated Nudel accumulated at the motion frontier when cells migrated, where Nudel competed with Cdc42 to combine with a GAP protein of Cfc42, namely Cdc42GAP. Therefore, Nudel regulates the ability of cell migration by maintaining the active status of GEF-activated Cdc42 (Shen et al., 2008). Our study found that reducing TSG101 expression inhibited cell proliferation and cell motility in MCF-7 cells.

Meanwhile, we observed cell cycle arrest, apoptosis and the decreased expression of p-ERK as a result of reduced TSG101 expression. These results suggest that the effects of TSG101 siRNA treatment on MCF-7 cells may be related to MAPK/ERK signaling. Further studies are needed to confirm this conclusion.

We believe that understanding the roles of the TSG101 gene in carcinoma and metastasis, and its regulation of the MAPK/ERK pathway will reveal the mechanisms of human carcinogenesis. This information will also provide important clues for early diagnosis and detection of breast tumors.

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Competing Interests. The authors declare that they have no competing interests.

References

Aebersold D.M., Burri P., Beer K.T., Laissue J., Djonov V., Greiner R.H. and Semenza G.L. (2001). Expression of hypoxia-inducible factor-1alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res. 61, 2911-2916.

Bennett N.A., Pattillo R.A., Lin R.S., Hsieh C.Y., Murphy T. and Lyn D. (2001). TSG101 expression in gynecological tumors: relationship to cyclin D1, cyclin E, p53 and p16 proteins. Cell. Mol. Biol. (Noisy-le-grand) 47, 1187-1193.

Boucher M.J., Morisset J., Vachon P.H., Reed J.C., Laine J. and Rivard N. (2000). MEK/ERK signaling pathway regulates the expression of Bcl-2, Bcl-X(L), and Mcl-1 and promotes survival of human pancreatic cancer cells. J. Cell. Biochem. 79, 355-369.

Feng G.H., Lih C.J. and Cohen S.N. (2000). TSG101 protein steady-state level is regulated posttranslationally by an evolutionarily conserved COOH-terminal sequence. Cancer Res. 60, 1736-1741.

Kang C.D., Yoo S.D., Hwang B.W., Kim K.W., Kim D.W., Kim C.M., Kim S.H. and Chung B.S. (2000). The inhibition of ERK/MAPK not the activation of JNK/SAPK is primarily required to induce apoptosis in chronic myelogenous leukemic K562 cells. Leuk. Res. 24, 527-534.

Krempler A., Henry M.D., Triplett A.A. and Wagner K.U. (2002). Targeted deletion of the Tsg101 gene results in cell cycle arrest at G1/S and p53-independent cell death. J. Biol. Chem. 277, 43216-43223.

Li L. and Cohen S.N. (1996). Tsg101: a novel tumor susceptibility gene isolated by controlled homozygous functional knockout of allelic loci in mammalian cells. Cell 85, 319-329.

Lo Y.F., Chen T.C., Chen S.C. and Chao C.C. (2000). Aberrant expression of TSG101 in Taiwan Chinese breast cancer. Breast Cancer Res. Treat. 60, 259-266.

Ma X.R., Edmund Sim U.H., Pauline B., Patricia L. and Rahman J. (2008). Overexpression of WNT2 and TSG101 genes in colorectal carcinoma. Trop. Biomed. 25, 46-57.

Oh K.B., Stanton M.J., West W.W., Todd G.L. and Wagner K.U. (2007). Tsg101 is upregulated in a subset of invasive human breast cancers and its targeted overexpression in transgenic mice reveals weak oncogenic properties for mammary cancer initiation. Oncogene 26, 5950-5959.

Ostrakhovitch E.A. and Cherian M.G. (2004). Differential regulation of signal transduction pathways in wild type and mutated p53 breast cancer epithelial cells by copper and zinc. Arch. Biochem. Biophys. 423, 351-361.

Ruland J., Sirard C., Elia A., MacPherson D., Wakeham A., Li L., de la Pompa J.L., Cohen S.N. and Mak T.W. (2001). p53 accumulation, defective cell proliferation, and early embryonic lethality in mice lacking tsg101. Proc. Natl. Acad. Sci. USA 98, 1859-1864.

Shen H.Q., Wang Q., Han Z.Y., Xie H.H. and Fan D.M. (2004). Construction and identification of TSG101 siRNA eukaryotic expression vector. J. Shanxi Med. Univ. 24, 515-518.

Shen Y., Li N., Wu S., Zhou Y., Shan Y., Zhang Q., Ding C., Yuan Q., Zhao F., Zeng R. and Zhu X. (2008). Nudel binds Cdc42GAP to modulate Cdc42 activity at the leading edge of migrating cells. Dev. Cell 14, 342-353.

Wagner K.U., Krempler A., Qi Y., Park K., Henry M.D., Triplett A.A., Riedlinger G., Rucker I.E. and Hennighausen L. (2003). Tsg101 is essential for cell growth, proliferation, and cell survival of embryonic and adult tissues. Mol. Cell. Biol. 23, 150-162.

Young T.W., Mei F.C., Rosen D.G., Yang G., Li N., Liu J. and Cheng X. (2007). Up-regulation of tumor susceptibility gene 101 protein in ovarian carcinomas revealed by proteomics analyses. Mol. Cell. Proteomics 6, 294-304.

Young T.W., Rosen D.G., Mei F.C., Li N., Liu J., Wang X.F. and Cheng X. (2007). Up-regulation of tumor susceptibility gene 101 conveys poor prognosis through suppression of p21 expression in ovarian cancer. Clin. Cancer Res. 13, 3848-3854.

Zhu G., Reynolds L., Crnogorac-Jurcevic T., Gillett C.E., Dublin E.A., Marshall J.F., Barnes D., D'Arrigo C., Van Trappen P.O., Lemoine N.R. and Hart I.R. (2003). Combination of microdissection and microarray analysis to identify gene expression changes between differentially located tumour cells in breast cancer. Oncogene 22, 3742-3748.

Zhu G., Gilchrist R., Borley N., Chng H.W., Morgan M., Marshall J.F., Camplejohn R.S., Muir G.H. and Hart I.R. (2004). Reduction of TSG101 protein has a negative impact on tumor cell growth. Int. J. Cancer 109, 541-547.

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