ORIGINAL ARTICLE

Silencing of tripartite motif (TRIM) 29 inhibits proliferation and invasion and increases chemosensitivity to cisplatin in human lung squamous cancer NCI-H520 cells

Chunxiao Liu[1], Xiaoxi Huang[2], Shengcai Hou[1], Bin Hu[1] & Hui Li[1]

1 Department of Thoracic Surgery, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China
2 Department of Medical Research Center, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China

Abstract

Background: TRIM29 belongs to the tripartite motif (TRIM) protein family. It has been reported to be a tumor suppressor or have oncogenic function in many cancer types. The aim of this study was to investigate whether downregulation of TRIM29 by small interfering ribonucleic acid (siRNA) could inhibit cell proliferation and invasion and increase chemosensitivity to cisplatin in human lung squamous cancer NCI-H520 cells in vitro.

Methods: We transformed TRIM29 siRNA into NCI-H520 cells. Real time reverse transcriptase polymerase chain reaction and Western blotting assay were employed to determine TRIM29 messenger (m)RNA and protein expressions. MTT assay was used to determine the cell proliferation. Transwell invasion assay was used to determine the cell invasion. An Annexin V-propidium iodide (AnnV/PI) staining apoptosis test was used for detecting apoptosis.

Results: TRIM29 siRNA could specifically and efficiently suppress TRIM29 expression at both mRNA and protein levels. Silencing of the TRIM29 by siRNA in NCI-H520 cells inhibited cell proliferation and invasion in vitro. TRIM29 knockdown resulted in chemosensitivity enhancement in NCI-H520 cells.

Conclusion: Downregulation of TRIM29 can lead to potent antitumor activity and chemosensitizing effect in human lung squamous cancer NCI-H520 cells.

Introduction

Lung cancer is the leading cause of cancer-associated death worldwide.[1] Although treatment with surgery and chemotherapy has improved dramatically, there has been no great improvement in the rate of survival over the past years.[2] Therefore, a better understanding of the molecular mechanisms underlying lung cancer formation and progression should be helpful in developing more effective treatments for this disease.

TRIM29 is a member of the tripartite motif (TRIM) family. This family consists of at least 37 member proteins and is characterized by its unique structure: a RING (R), a B-box type 1 (B1) and B-box type 2 (B2), followed by a coiled-coil (CC) region. While some of the domains may be absent or present, TRIM29 contains the B1-B2-CC domains, but lacks the R domain.[3] The TRIM family has been implicated in a variety of cellular processes, including development and growth. TRIM29 has been reported to be upregulated and downregulated in some cancer types. This suggests that the function of TRIM29 may be dependent on different cellular context and molecular signaling pathways.[4]

The involvement of TRIM29 with proliferation and tumoral progression has been reported in many cancer cells, such as multiple myeloma, colorectal, endometrial, breast, prostate, gastric, and pancreatic cancers.[5]

TRIM29 is involved in a variety of cancers, however, its function can change, depending on the cell type, level of expression, posttranslational modification, and compartmentalization. Although TRIM29 has been implicated as a tumor suppressor in some types of breast and bone cancers,[6] it is also known to have oncogenic effects in gastric and pancreatic cancers.[4] Transfection of wildtype TRIM29 into osteosarcoma and breast cancer cell lines lacking detectable messenger ribonucleic acid (mRNA) and protein expression of TRIM29 results in suppression of colony-forming efficiency in soft agar, suggesting that TRIM29 can cause reversion of a malignant phenotype. In contrast, in vitro and in

vivo studies in pancreatic cancer revealed that TRIM29 can increase proliferation and invasion through stabilization β-catenin and activation of Disheveled 2.[4]

Although TRIM29 was shown to be overexpressed in lung cancer cells and has been implicated as playing important roles in the development of lung cancer,[7] the exact function of TRIM29 in lung cancer cell growth and progression is not fully understood.

RNA interference (RNAi) has been recently developed as a powerful tool to suppress the expression of specific gene products.[8] We used RNAi methods to suppress TRIM29 expression in NCI-H520 cells. Our results suggest that knockdown of TRIM29 reduced the proliferative and metastatic capacity of the human lung squamous cancer cell line NCI-H520, and could make NCI-H520 cells more sensitive to cisplatin therapy. Thus, TRIM29 may be a good molecular target in the treatment of human lung squamous cancer.

Materials and methods

Cell culture and small interfering ribonucleic acid (siRNA) transfection

Human lung squamous cancer cell line NCI-H520 was obtained from the Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing, China. Cells were cultured in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories Inc, Logan, UT, USA) in a humidified 5% CO_2 atmosphere. The human TRIM29 specific small interfering ribonucleic acid (siRNA) was purchased from GenePharma Co Ltd (Shanghai, China). The sequences of the siRNA used were as follows: siRNA1: sense, 5′-GAGCUGC GCAAGUCCAUUUTT-3′, siRNA2: sense,5′-ACGGAGCU GUCAUUGCAAATT-3′, siRNA3:sense, 5′-GUGCAUUG AUGAGCAAUUATT-3′, Negative control siRNA: sense, 5′-UUCUCCGAACGUGUCACGUTT-3′. The siRNA transfection was performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in Opti-MEM (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The transfection reagent was removed after eight hours and the cells were harvested after 48 hours.

Real-time polymerase chain reaction (PCR) analysis

Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA, USA) reagent according to the manufacturer's instructions. RNA was reverse-transcribed using an Omniscript RT kit (Tiangen, Beijing, China). Real time polymerase chain reaction (PCR) was performed with an ABI PRISM 7500 instrument (Applied Biosystems, Foster City, CA, USA) using SYBRGreen PCR reagents (Tiangen, Beijing, China); 18S was applied as an internal control. For each group, TRIM29 mRNA expression was corrected against 18S mRNA levels and the comparative threshold cycle number method was used to assess the relative quantification of gene expression.

Western blotting analysis

NCI-H520 cells were lysed with a radioimmunoprecipitation assay (RIPA, Pierce, Rockford, IL, USA) buffer that was supplemented with complete proteinase and phosphatase inhibitor cocktails (Roche, Basel, Switzerland). After centrifugation at 4°C, the supernatant was collected and protein concentration was measured using the bicinchoninic acid assay kit (Pierce, Rockford, IL, USA). Protein extracts (30 μg) were separated using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto nitrocellulose membranes (Schleicher & Schuell, Keene, NH, USA). The membranes were then blocked with five percent skim milk and incubated with specific primary antibodies overnight at 4°C followed by species-specific secondary antibody. Primary antibodies for TRIM29 (1:200 dilution) and β-actin (1:2000 dilution) were from Cell Signaling Technology (Beverly, MA, USA). Immunoreactive material was detected by the enhanced chemiluminescence technique (Amersham, Little Chalfont, Buckinghamshire, UK).

MTT assays for cell proliferation

Cell proliferation ability was assessed by the MTT assay. Cells treated with TRIM29 siRNA, scrambled control siRNA or with medium alone were plated at 5×10^3 cells per well on a 96-well plate. At various time-points, 20 μl of sterile MTT was added to each well at a final concentration of 5 mg/mL. Cells were further incubated at 37°C for four hours. Dimethyl sulfoxide (DMSO) (150 μl) was then added to stop the reaction. The absorbance of each well was determined at 490 nm. Each experiment was performed independently three times.

Annexin V-propidium iodide (AnnV/PI) staining apoptosis test

Cells in each well were harvested after siRNA transfection and cisplatin (5 μg/mL) treatment. Flowcytometric analysis was performed to detect apoptosis using a FACScan flow cytometer (BD, San Jose, CA, USA) in accordance with the manufacturer's protocol.

Transwell invasion assay

BioCoat transwell chambers (Corning Costar, Cambridge, MA, USA) with uncoated porous inserts (pore size 8 μm) were used in cell invasion assay. The upper surface of the
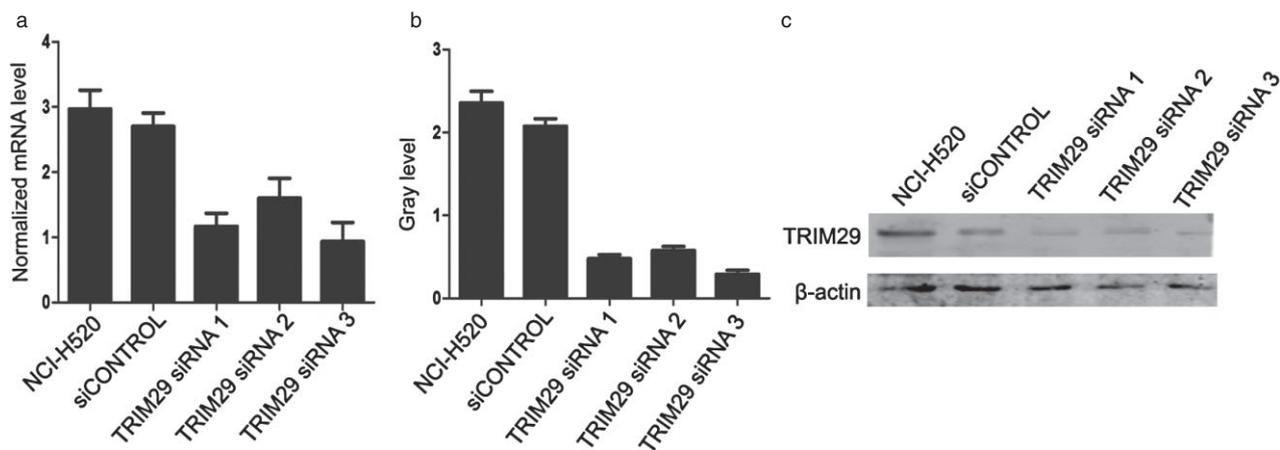
Figure 1 Specific downregulation of tripartite motif (TRIM)29 messenger ribonucleic acid (mRNA) and protein expression by TRIM29 small interfering (si)RNA. Cells were transfected with TRIM29 siRNA or scrambled siRNA (siCONTROL) for 48 hours. (**a**) The relative mRNA level of TRIM29 was quantified by real-time polymerase chain reaction analysis, and 18S was used as an internal standard. (**b**) The relative TRIM29 protein level was determined by Western blotting, and data was normalized using β-actin as an internal standard. Compared with TRIM29 siRNA1 and 2, siRNA3 mostly silenced TRIM29 expression. *P* < 0.01 versus siCONTROL.

transwell filters was coated with Matrige Matrix (BD Biosciences, San Jose, CA, USA). Cells were harvested in 200 μl of serum free RPMI-1640 medium and added to the upper compartment of the chamber. After 24 hours incubation at 37°C in a five percent CO_2 incubator, cells that had migrated across the matrigel into the pores of the inserted filter were fixed with 90% methanol, stained with hematoxylin, mounted, and dried at 80°C for 30 minutes. The number of cells was counted under the microscope from three randomly selected visual fields (magnification ×200). The experiments were performed independently three times.

Statistical analysis

SPSS 13.0 (SPSS, Chicago, IL, USA) software was used for analyses. Results are expressed as mean ± standard deviation (SD). The statistical significance of the results was determined using the Student's t-test. *P* < 0.01 indicated a statistically significant difference.

Results

siRNA targeting tripartite motif (TRIM)29 knocks down TRIM29 expression

After siRNA interference TRIM29 for 48 hours, TRIM29 mRNA and protein were measured by quantitative real-time PCR and Western blotting, respectively. In our assay, three TRIM29 siRNAs (TRIM29 siRNA1, TRIM29 siRNA2, TRIM29 siRNA3) were used to suppress TRIM29 expression, Compared with TRIM29 siRNA1 and 2, TRIM29 siRNA3 could mostly specifically and efficiently suppress TRIM29

expression at both mRNA and protein levels (Fig 1). Therefore, we chose TRIM29 siRNA3 to fulfil our assay.

siRNA TRIM29 inhibits NCI-H520 cell proliferation

We used an MTT assay kit to evaluate the effect of TRIM29-RNAi on cell growth. As shown in Figure 2, the growth curves
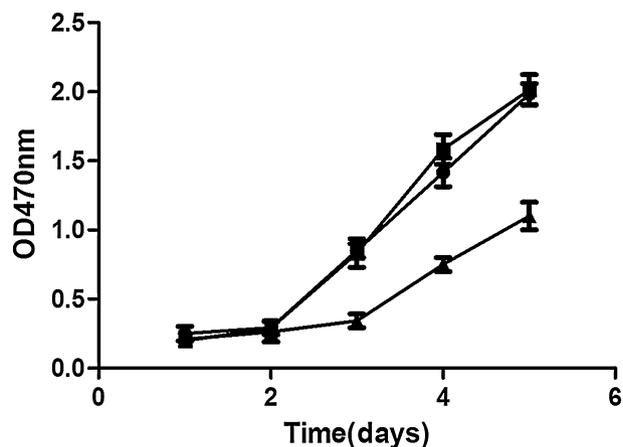
Figure 2 RNA interference (RNAi)-mediated downregulation of tripartite motif (TRIM)29 reduces NCI-H520 cell proliferation. Cells treated with TRIM29 small interfering (si)RNA, scrambled control siRNA or with medium alone were plated on a 96-well plate. Cells were evaluated for proliferation at 24-hour intervals by MTT assay. Data were from three independent experiments. *P* < 0.01 versus siCONTROL. ●, NCI-H520; ■, siCONTROL; ▲, TRIM29 siRNA.
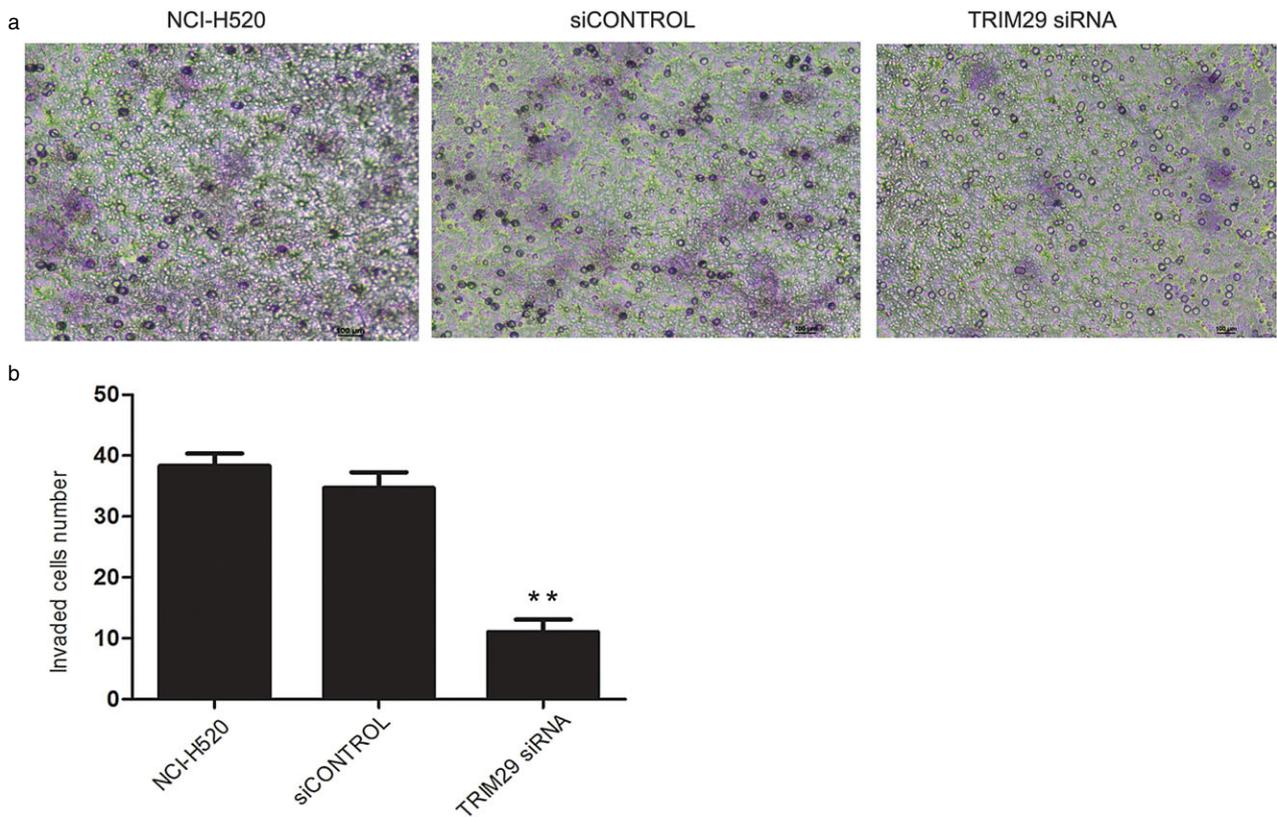
Figure 3 Downregulation of tripartite motif (TRIM)29 inhibits invasion of NCI-H520 cells. (**a**) NCI-H520, siCONTROL and TRIM29 small interfering ribonucleic acid (siRNA) cells were seeded in the upper chamber in medium supplemented with five percent fetal bovine serum (FBS. After 24 hours, cells were fixed, stained, and counted (bar shows 100 μm). (**b**) The number of invasive cells in the TRIM29 siRNA treated group were significantly lower than in the NCI-H520 and siCONTROL groups ($P < 0.01$).

of TRIM29 knockdown cells were significantly lower than those of the control cells for the five days of incubation.

Downregulation of TRIM29 expression inhibits cell invasion

We studied the effect of TRIM29 knockdown on the invasion of NCI-H520 cells. The results showed that invasion was significantly inhibited in the TRIM29 knockdown cells compared with the untreated group, or the siCONTROL group (Fig 3).

Knockdown of TRIM29 expression increases sensitivity of NCI-H520 cells to chemotherapy

We examined the effect of TRIM29 suppression on NCI-H520 cell apoptosis with flow cytometric analysis. As shown in Figure 4a and c, after transfection with TRIM29 siRNA at 48 hours, cell apoptosis was induced. Furthermore, cell apoptosis was increased when cells were treated with 5 μg/mL cisplatin (Fig 4b and d).

Discussion

Lung cancer is one of the most common human cancers worldwide. According to the World Health Organization GLOBOCAN project, 1.6 million new cases of lung cancer, accounting for 12.7% of the world's total cancer incidence, were diagnosed in 2008.[9] Despite advances in surgical and chemotherapy techniques, the survival rate of lung cancer is far from satisfactory. To further improve the clinical outcome of lung cancer patients, identifying novel and effective antitumor strategies, such as targeted therapy, will be crucial.

TRIM29, located at chromosome 11q22–23, encodes a 588 amino acid protein with multiple zinc-finger motifs and an adjacent leucine-zipper motif. TRIM29 has been reported to be overexpressed in variety of different cancers, including lung, bladder, colorectal, ovarian and endometrial cancers, and multiple myeloma.[10–16] Conversely, it has been reported to be diminished in some cancers.[17–20] These results suggest that the function of TRIM29 may vary depending upon the tissue origin of the neoplasm. A recent study found TRIM29
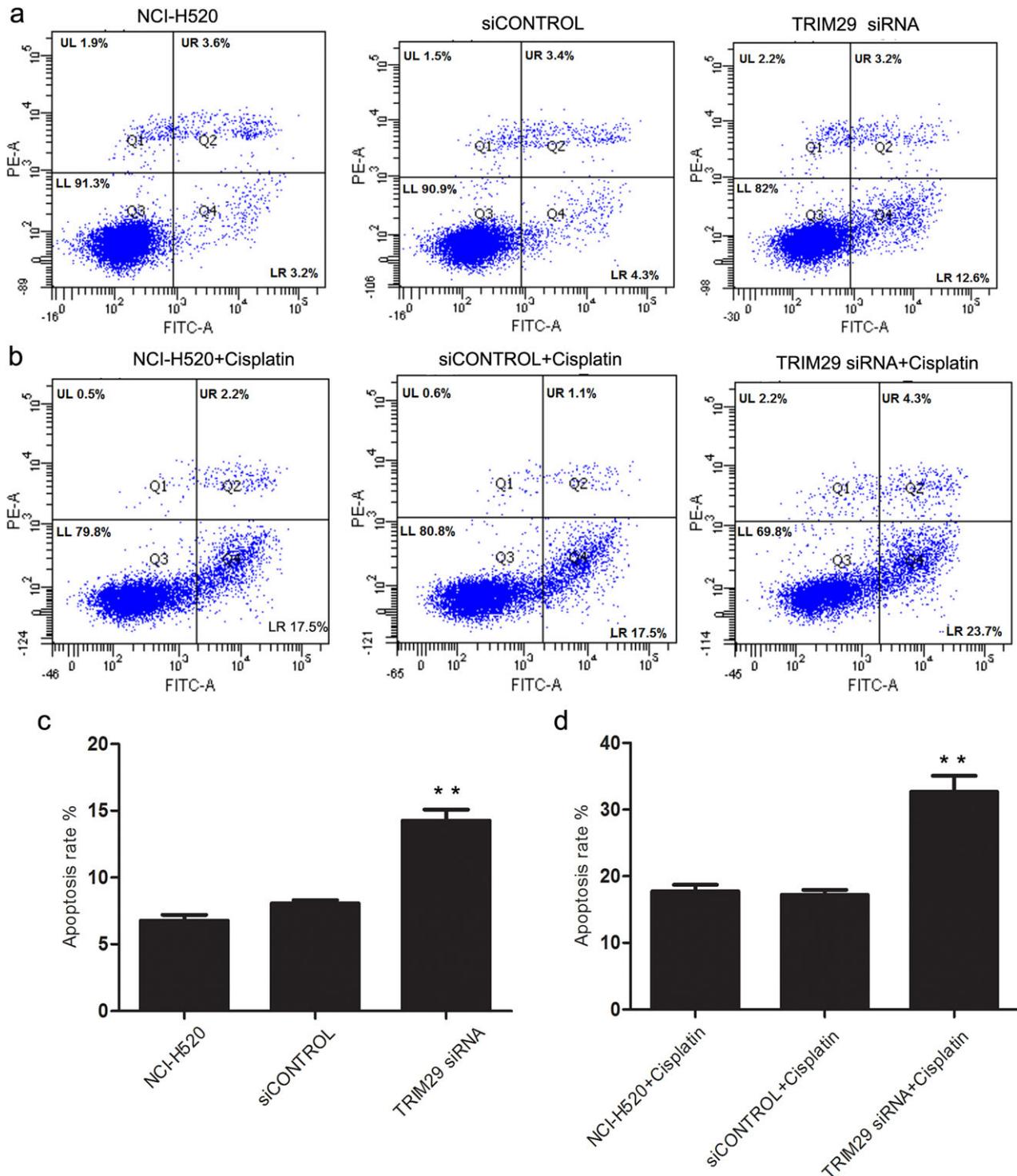
Figure 4 Small interfering ribonucleic acid (siRNA) for tripartite motif (TRIM29) induces apoptosis and enhances chemosensitivity. (**a** and **c**) TRIM29 inhibition by siRNA induced apoptosis in NCI-H520 cells. (**b** and **d**) After treating with 5 µg/mL cisplatin, cell apoptosis (including early and late apoptosis) was further enhanced. Combining TRIM29 inhibition with cisplatin increased the incidence of apoptosis. After 48 hours transfecting with siRNA, cells were treated with 5 µg/mL cisplatin for 24 hours, and then cells were double stained with Annexin V-FITC and propidium iodide (PI) followed by FACS analysis. FACS analysis scatter-grams of Annexin V/PI staining display four different cell populations marked as: double negative (unstained) cells showing live cell population (lower left), Annexin V positive and PI negative stained cells showing early apoptosis (lower right), Annexin V/PI double-stained cells showing late apoptosis (upper right), and, finally, PI positive and Annexin V negative stained cells showing dead cells (upper left). $P < 0.01$ versus siCONTROL.

protein was upregulated in NSCLC tissues compared to the adjacent normal tissues and correlated with clinic pathological factors and prognosis.[7] In our study, we found that TRIM29 mRNA and protein were highly expressed in NCI-H520 cells. These results are consistent with previous reports. Inhibiting specific gene expression by RNAi has become an important method of cancer treatment.[21,22] We employed RNAi technology to downregulate TRIM29 expression in this cell line. After transfection with the TRIM29 siRNA, the mRNA and protein expressions decreased significantly compared with control group. These results indicated that TRIM29 siRNA could suppress the expression of TRIM29 effectively and specifically in NCI-H520 cells.

Previous studies have found that TRIM29 expression was associated with cell proliferation and tumor growth. Wang *et al.* discovered that TRIM29 promoted pancreatic cancer cell proliferation in vitro and enhanced tumor growth and metastasis in vivo.[4] Yuan *et al.* reported that TRIM29 increases cancer cell proliferation via inhibition of p53 nuclear activities.[23] Sho *et al.* proved that TRIM29 functions as an oncogene that promotes tumor growth.[24] Jiang *et al.* found that TRIM29 plays an important role in promoting colorectal cancer progression and downregulation of TRIM29 inhibited tumor cell proliferation in vitro.[25] To further investigate the functions of TRIM29 in lung cancer, we examined the NCI-H520 cells transfected with TRIM29 siRNA. The proliferation rate was analyzed by MTT assay. We found that suppressing the expression of TRIM29 in NCI-H520 cells would suppress cell proliferation compared with control group. This result suggests that TRIM29 expression influences proliferation rates in NCI-H520 cells.

A recent report described a correlation between TRIM29 expression with poor histological grade, tumor node metastasis (TNM) stage, and lymph node metastasis in lung squamous cell carcinoma tissues.[7] We further studied the effect of TRIM29 suppression on the metastatic capacity of NCI-H520 cells. The assays showed that downregulation of TRIM29 significantly reduced NCI-H520 cell invasiveness compared with the control group. Therefore, there is a relationship between TRIM29 and the invasion ability in human lung squamous cancer cells. TRIM29 has previously been reported to be involved in cancer metastasis in other cell lines. Lai *et al.* discovered that both TRIM29 mRNA and protein expression in esophageal squamous-cell carcinoma (ESCC) tissues were significantly correlated with tumor differentiation, stage, and lymph node metastasis.[26] Jiang *et al.* reported that the expression of TRIM29 was significantly associated with the depth of tumor invasion, lymph node metastasis, and distant metastasis in colorectal cancer.[25] Early in 2007, Kosaka *et al.* proved that increased TRIM29 expression was markedly associated with tumor invasion and lymph node metastasis in gastric cancer.[11] Our results are consistent with previous research that has shown that TRIM29 expression

can influence the extent of tumor invasion and lymph node metastasis in some cancers.

Cisplatin is usually used for the treatment of several types of cancer and it primarily acts by causing DNA damage. However, the ability of cancer cells to become resistant to cisplatin remains a significant impediment to successful chemotherapy.[9] Previous studies have found a number of mechanisms for cisplatin resistance.[27] However, exact mechanisms are needed to find new targets to prevent drug resistance.

Early in 1989, TRIM29 was found to suppress the sensitivity to ionizing radiation (IR).[28] Yuan *et al.* discovered that TRIM29 expression leads to resistance to ionizing radiation, whereas silencing of TRIM29 expression increases sensitivity to ionizing radiation in cell cultures.[23] Another study reported that TRIM29 participates in the survival of differentiating keratinocytes, which are more and more exposed to ultraviolet (UV)B and pro-death stimuli.[29] Cisplatin, ionizing radiation, and UVB could induce the DNA damage response process by causing cell apoptosis. Based on this mechanism and previous research, we postulate that inhibition of TRIM29 expression with siRNA could increase chemosensitivity to cisplatin. As expected, in our study we found that knockdown of TRIM29 enhanced apoptosis induced by cisplatin and made cells more sensitive to cisplatin treatment.

Conclusion

Our data have shown that downregulation of TRIM29 by siRNA inhibited the proliferation and invasion of NCI-H520 cells and increased chemosensitivity to cisplatin. These findings suggest that TRIM29 may have a wide therapeutic application in the treatment of human lung squamous cancer.

Acknowledgments

Disclosure

No authors report any conflict of interest.

References

1 Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. (Published erratum appears in *CA Cancer J Clin* 2011; **61**: 133–4) *CA Cancer J Clin* 2010; **60**: 277–300.
2 Min XJ, Li H, Hou SC *et al.* Dysfunction of volume-sensitive chloride channels contributes to cisplatin resistance in human lung adenocarcinoma cells. *Exp Biol Med (Maywood)* 2011; **236**: 483–91.

3 Reymond A, Meroni G, Fantozzi A *et al.* The tripartite motif family identifies cell compartments. *EMBO J* 2001; **20**: 2140–51.

4 Wang L, Heidt DG, Lee CJ *et al.* Oncogenic function of ATDC in pancreatic cancer through Wnt pathway activation and beta-catenin stabilization. *Cancer Cell* 2009; **15**: 207–19.

5 Tang ZP, Dong QZ, Cui QZ, Papavassiliou P, Wang ED, Wang EH. Ataxia-telangiectasia group D complementing gene (ATDC) promotes lung cancer cell proliferation by activating NF-kappaB pathway. *PLoS ONE* 2013; **8**: e63676.

6 Liu J, Welm B, Boucher KM, Ebbert MT, Bernard PS. TRIM29 functions as a tumor suppressor in nontumorigenic breast cells and invasive ER+ breast cancer. *Am J Pathol* 2012; **180**: 839–47.

7 Zhou ZY, Yang GY, Zhou J, Yu MH. Significance of TRIM29 and beta-catenin expression in non-small-cell lung cancer. *J Chin Med Assoc* 2012; **75**: 269–74.

8 Sagiv E, Starr A, Rozovski U *et al.* Targeting CD24 for treatment of colorectal and pancreatic cancer by monoclonal antibodies or small interfering RNA. *Cancer Res* 2008; **68**: 2803–12.

9 Yang Y, Li H, Hou S, Hu B, Liu J, Wang J. The noncoding RNA expression profile and the effect of lncRNA AK126698 on cisplatin resistance in non-small-cell lung cancer cell. *PLoS ONE* 2013; **8**: e65309.

10 Hawthorn L, Stein L, Panzarella J, Loewen GM, Baumann H. Characterization of cell-type specific profiles in tissues and isolated cells from squamous cell carcinomas of the lung. *Lung Cancer* 2006; **53**: 129–42.

11 Kosaka Y, Inoue H, Ohmachi T *et al.* Tripartite motif-containing 29 (TRIM29) is a novel marker for lymph node metastasis in gastric cancer. *Ann Surg Oncol* 2007; **14**: 2543–9.

12 Dyrskjøt L, Kruhøffer M, Thykjaer T *et al.* Gene expression in the urinary bladder: a common carcinoma in situ gene expression signature exists disregarding histopathological classification. *Cancer Res* 2004; **64**: 4040–8.

13 Glebov OK, Rodriguez LM, Soballe P *et al.* Gene expression patterns distinguish colonoscopically isolated human aberrant crypt foci from normal colonic mucosa. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 2253–62.

14 Ohmachi T, Tanaka F, Mimori K, Inoue H, Yanaga K, Mori M. Clinical significance of TROP2 expression in colorectal cancer. *Clin Cancer Res* 2006; **12**: 3057–63.

15 Santin AD, Zhan F, Bellone S *et al.* Gene expression profiles in primary ovarian serous papillary tumors and normal ovarian epithelium: identification of candidate molecular markers for ovarian cancer diagnosis and therapy. *Int J Cancer* 2004; **112**: 14–25.

16 Zhan F, Hardin J, Kordsmeier B *et al.* Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. *Blood* 2002; **99**: 1745–57.

17 Luo J, Duggan DJ, Chen Y *et al.* Human prostate cancer and benign prostatic hyperplasia: molecular dissection by gene expression profiling. *Cancer Res* 2001; **61**: 4683–8.

18 Zhang P, Zhang Z, Zhou X, Qiu W, Chen F, Chen W. Identification of genes associated with cisplatin resistance in human oral squamous cell carcinoma cell line. *BMC Cancer* 2006; **6**: 224.

19 Nacht M, Ferguson AT, Zhang W *et al.* Combining serial analysis of gene expression and array technologies to identify genes differentially expressed in breast cancer. *Cancer Res* 1999; **59**: 5464–70.

20 Smith AP, Hoek K, Becker D. Whole-genome expression profiling of the melanoma progression pathway reveals marked molecular differences between nevi/melanoma in situ and advanced-stage melanomas. *Cancer Biol Ther* 2005; **4**: 1018–29.

21 Lapteva N, Yang AG, Sanders DE, Strube RW, Chen SY. CXCR4 knockdown by small interfering RNA abrogates breast tumor growth in vivo. *Cancer Gene Ther* 2005; **12**: 84–9.

22 Susa M, Iyer AK, Ryu K *et al.* Inhibition of ABCB1 (MDR1) expression by an siRNA nanoparticulate delivery system to overcome drug resistance in osteosarcoma. *PLoS ONE* 2010; **5**: e10764.

23 Yuan Z, Villagra A, Peng L *et al.* The ATDC (TRIM29) protein binds p53 and antagonizes p53-mediated functions. *Mol Cell Biol* 2010; **30**: 3004–15.

24 Sho T, Tsukiyama T, Sato T *et al.* TRIM29 negatively regulates p53 via inhibition of Tip60. *Biochim Biophys Acta* 2011; **1813**: 1245–53.

25 Jiang T, Tang HM, Lu S, Yan DW, Yang YX, Peng ZH. Up-regulation of tripartite motif-containing 29 promotes cancer cell proliferation and predicts poor survival in colorectal cancer. *Med Oncol* 2013; **30**: 715.

26 Lai W, Zhao J, Zhang C *et al.* Upregulated ataxia-telangiectasia group D complementing gene correlates with poor prognosis in patients with esophageal squamous cell carcinoma. *Dis Esophagus* 2013; **26**: 817–22.

27 Stewart DJ. Mechanisms of resistance to cisplatin and carboplatin. *Crit Rev Oncol Hematol* 2007; **63**: 12–31.

28 Kapp LN, Painter RB. Stable radioresistance in ataxia-telangiectasia cells containing DNA from normal human cells. *Int J Radiat Biol* 1989; **56**: 667–75.

29 Bertrand-Vallery V, Belot N, Dieu M *et al.* Proteomic profiling of human keratinocytes undergoing UVB-induced alternative differentiation reveals TRIpartite Motif Protein 29 as a survival factor. *PLoS ONE* 2010; **5**: e10462.