

RAPID COMMUNICATION

## Expression of T-STAR gene is associated with regulation of telomerase activity in human colon cancer cell line HCT-116

Ling Zhang, Lian Guo, Yong Peng, Bing Chen

Ling Zhang, Department of Disease Prevention and Health Protection, Southwest Hospital, The Third Military Medical University, Chongqing 400038, China

Lian Guo, Yong Peng, Bing Chen, Department of Endocrinology, Southwest Hospital, The Third Military Medical University, Chongqing 400038, China

Supported by the National Natural Science Foundation of China, No. 30271442, No. 39980010

Correspondence to: Dr. Bing Chen, Department of Endocrinology, Southwest Hospital, 30 Gaotanyan, Sapingba, Chongqing 400038, China. chenb@mail.tmmu.com.cn

Telephone: +86-23-68754138

Received: 2006-02-08

Accepted: 2006-03-27

### Abstract

**AIM:** To investigate the effects on telomerase activity of transfection of human T-STAR gene full-length sense cDNA or partial antisense cDNA into human colon cancer cell line HCT-116.

**METHODS:** mRNA and protein expression levels of T-STAR gene were determined by RT-PCR and western blot, and telomerase activity was measured by PCR-ELISA, after transfection of T-STAR sense or antisense gene into HCT-116 cells with lipofectamine.

**RESULTS:** T-STAR gene expression was enhanced or knocked down both at mRNA and protein levels, and telomerase activity was significantly increased or decreased.

**CONCLUSION:** The T-STAR gene may participate in regulation of telomerase activity in human colon cancer HCT-116 cells in a parallel fashion.

© 2006 The WJG Press. All rights reserved.

**Key words:** T-STAR; Telomerase; Human colon cancer cells; Cell transfection

Zhang L, Guo L, Peng Y, Chen B. Expression of T-STAR gene is associated with regulation of telomerase activity in human colon cancer cell line HCT-116. *World J Gastroenterol* 2006; 12(25): 4056-4060

<http://www.wjgnet.com/1007-9327/12/4056.asp>

### INTRODUCTION

T-STAR, a recently cloned member of STAR gene family, is highly expressed in testis, muscle, and brain and contains a STAR domain, an RNA-binding domain present in a growing family of proteins involved in developmental processes<sup>[1-3]</sup>. The protein can bind to a variety of signal-transducing proteins and may act as an adaptor in signal-transduction pathways. T-STAR was identified as a protein interacting with the protein RNA-binding motif (RBM) in spermatogenesis. Down-regulation of T-STAR expression was found in SV40-transformed immortal fibroblasts, and this protein may interact with telomerase<sup>[4]</sup>. In most progressive phase malignant tumor tissues and cancer cell lines, the telomerase activity maintained at a high level, whereas, at a lower or undetectable level in normal cells. The activation of telomerase is involved in tumorigenesis and progression<sup>[5-8]</sup>. In this study, we transfected the sense or antisense T-STAR gene into human colon cancer cell line HCT-116 to overexpress or inhibit T-STAR protein, and to further observe whether T-STAR gene participates in the regulation of telomerase activity in HCT-116 cells.

### MATERIALS AND METHODS

#### Materials

The eukaryotic expression plasmid, pcDNA3.1 was purchased from Invitrogen. Human T-STAR full length cDNA was a gift by Dr. Chew of Leicester University. The full length cDNA of T-STAR was inserted into pcDNA3.1 vector at *EcoRI* site in a sense orientation to generate a recombinant sense expression plasmid, pcDNA-STAR. The fragment of T-STAR cDNA digested by *ApaI* restriction enzyme was ligated in the *ApaI* site of pcDNA3.1 in an antisense orientation to construct a recombinant antisense plasmid, pcDNA-asSTAR. Human colon cancer cell line HCT-116 was provided by Department of Abdominal Surgery of Southwest Hospital, Chongqing. The transfection reagent of Lipofectamine2000 was purchased from Invitrogen. RPMI1640, fetal calf serum, and other reagents for cell culture were purchased from Hyclone. Monoclonal antibody against T-STAR, mAbT was purchased from Santa Cruz Biotech. Rabbit antigoat IgG polyclonal antibodies HRP-conjugated were purchased from Beijing Zhongshan Biotech (China). RNA PCR reagent kit was

from Dalianbo Biotech. Telomerase PCR ELISA reagent kit was from Roche.

### Methods

Human colon cancer cell line HCT-116 cells were maintained in RPMI1640 medium, supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL of penicillin and 100 µg/mL of streptomycin and were grown at 37°C, in a humidified incubator containing 5 mL/L CO<sub>2</sub>. Transfection was performed according to the LipofectAMINE2000 method (Invitrogen) using 2 µg of each plasmid DNA (empty vector pcDNA3.1 as control, antisense plasmid pcDNA-asSTAR and sense plasmid pcDNA-STAR as experimental group). Briefly, adherent cells were seeded in six-well plates (5 × 10<sup>5</sup> cells per well) grown overnight to 50%-60% confluency and were washed three times with phosphate-buffered saline, and medium was replaced with 500 µL of OPTI-MEM medium before a mixture of LipofectAMINE and DNA was added to the well for 8 h. And then the medium containing transfection mixture was replaced with 2 mL fresh RPMI1640. After 24 h of transfection, the cells were selected in RPMI1640 supplemented with 500 µg/mL G418 (Geneticin, GIBCO) for 10 d. Furthermore, the cells of selected single colonies were grown in same medium with 200 µg/mL G418 for another two weeks. Total RNA was extracted from each group of transfected cells using TRIzol reagent (Invitrogen Life Technologies). Concentration and purity were confirmed by spectrophotometry and electrophoresis on the denaturing formaldehyde-agarose gel. Reverse transcription reactions were performed on 5 µg of the isolated total RNA using the ProSTAR RT-PCR kit (Stratagene, La Jolla, CA). The following specific primers were used for PCR assay, for human T-STAR gene: P1-5'CAGGATGGGACATGCTTTG3', P2-5'TCTGTAGACGCCCTTTGCT3', for inner control β-actin: P1-5'GTGGGC CGCTCTAGGCACCAA3', P2-5'CTCTTTGATGTCACGCACGATTTC3'. All PCR reactions were incubated at 95°C for 5 min, and then cycled at 95°C for 45 s, 48°C for 30 s and 72°C for 1 min for 35 cycles and, finally, at 72°C for 10 min for extension. The PCR products were electrophoresed on 10 g/L agarose gel to calculate the amount of T-STAR mRNA expression. The densities of target DNA bands of each group on the electrophoresis were analysed by software of Quantity One, and was normalized to inner control β-actin. The relative quantities are given as mean ± SD. The experiment was done in triplicates. Total cellular proteins were isolated from each group of transfected cells. Briefly, 90% confluency live cells were washed twice with 10 mmol/L PBS, and then were completely lysed with 200 µL 1 × SDS loading buffer. The lysate was transferred to a new 1.5 mL eppendorf tube, and heated to 100°C for 10 min. Then cellular genomic DNA was broken by sonication. About 10 µL protease inhibitor of PMSF per mL extract was mixed together. Protein concentration was determined according to Bradford, and samples were stored at -20°C. For western blot analysis, 40 µg total cellular proteins were separated on a 4%-15% sodium dodecyl sulfate-polyacrylamide gel. After electrotransfer to nitrocellulose membranes (BioRad),

uniformity of loading and transfer was confirmed by Poncea staining. The membranes were blocked overnight in TS complete (20 mol/L Tris-HCl, 150 mol/L NaCl, 5% non-fat dry milk, and 0.1% Tween 20) at 4°C. Blots were incubated for 1 h at room temperature with T-STAR mAb (Santa Cruz Biotech.) antibodies. Blots were washed and then incubated with horseradish peroxidase-conjugated secondary antibody (1:2000) (Sigma, St. Louis, MO) at room temperature for 2 h. The immune complexes were detected by the automated image documentation systems and bands density was analyzed by Quantity One software. The telomerase activity was measured with PCR ELISA kit<sup>[9]</sup>.

### Statistical analysis

Data are expressed as mean ± SD. Differences between experimental groups were assessed for significance by using the two-tailed unpaired Student's *t* test. A *P* value of < 0.05 was considered to be statistically significant.

## RESULTS

### Cellular T-STAR mRNA expression level

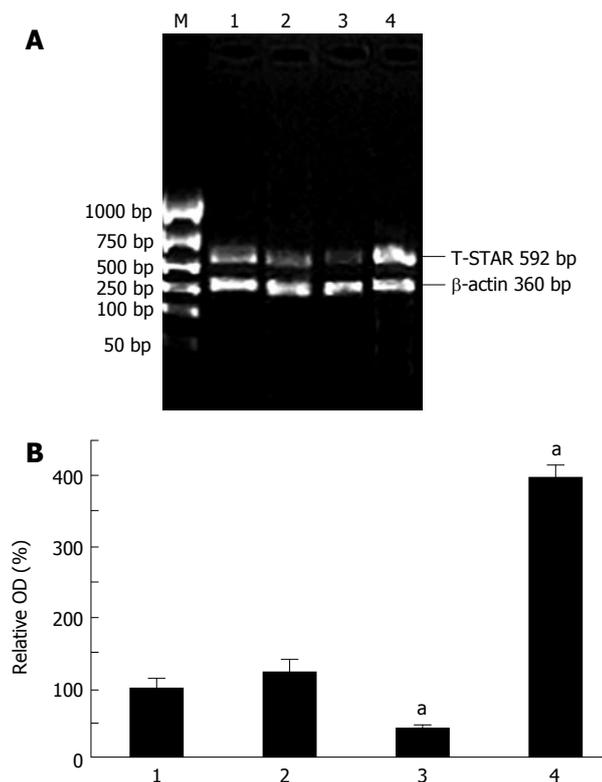
Three clear bands were present at 5S, 18S and 28S on the electrophoresis of total RNA of each group of cells. The brightness of the band at 28S was about 2-fold compared with that at 18S. There was no significant degradation with the mRNA, which could be applied in further experiments (data not shown). Both cDNA of T-STAR gene and β-actin gene simultaneously being reverse transcribed from the mRNA were electrophoresed on an agarose gel (Figure 1A). The semi-quantitative analysis was applied to the bands of T-STAR cDNA (Figure 1B). Compared with the average expression level of HCT-pcDNA transfected control cell group, T-STAR mRNA expression was increased to 296% in HCT-STAR group (*P* < 0.01), decreased to 59% in HCT-asSTAR group (*P* < 0.01), and remained slightly changed in HCT-116 group (*P* < 0.01).

### T-STAR protein expression level

The T-STAR protein could be specifically detected at the molecular weight *Mr*55 on the immuno-blots in all the HCT-116 cell groups transfected with different constructs (Figure 2A). The T-STAR protein amount was enhanced in HCT-STAR group, reduced in HCT-asSTAR group, and not significantly changed in HCT-116 group in contrast to HCT-pcDNA group. Densitometry analysis showed that the T-STAR protein expression was increased about 180.8% in HCT-STAR cells (*P* < 0.01), decreased about 83.80% in HCT-asSTAR cells (*P* < 0.01) compared with HCT-pcDNA cells (Figure 2B).

### Telomerase activity

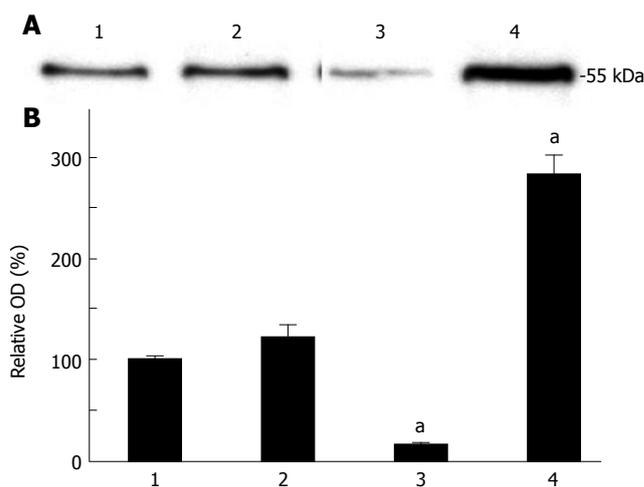
No significant changes of cellular telomerase activity were observed between HCT-116 cell group and HCT-pcDNA transfected cell group (Figure 3). Interestingly, the telomerase activities were significantly decreased in HCT-asSTAR group (*P* < 0.01), in which the expression of T-STAR gene was inhibited; whereas, the telomerase activities were significantly increased in the HCT-STAR group (*P* < 0.05) with up-regulation of T-STAR gene expression.



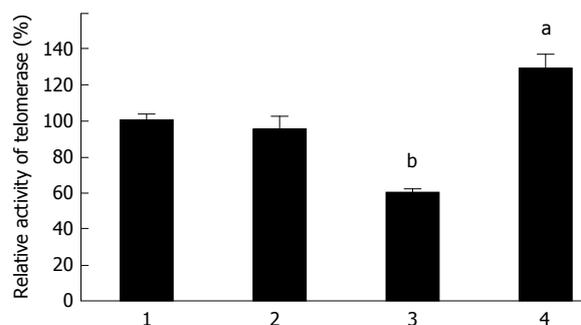
**Figure 1** A: The quantitation of T-STAR gene cDNA by RT-PCR from mRNA on electrophoresis of agarose gel; B: The relative expression levels of T-STAR mRNA of each group on electrophoresis of agarose gel, <sup>a</sup>*P* < 0.01 vs 2. M: Marker; 1: HCT; 2: HCT-pcDNA; 3: HCT-asSTAR; 4: HCT-STAR.

## DISCUSSION

T-STAR is a 55 kDa protein, one of three members of the SAM68 family of RNA-binding proteins with proline and tyrosine-rich regions that have been shown to be involved in various gene expression pathways including the developmental processes and/or extracellular signals to control the cellular fate of mRNA<sup>[10,11]</sup>. T-STAR gene is localized to chromosome 8q24.3, and the full length cDNA ranged in size from 1.2 kb to 1.9 kb. T-STAR is highly expressed in testis, muscle, and brain<sup>[10]</sup>. The STAR proteins have multiple functions in pre-mRNA splicing, signalling and cell cycle control. T-STAR generally acts as a growth suppressor, which is down-regulated upon immortalization of many cell lines<sup>[9-11]</sup>. Our previous study found that T-STAR may interact with hTERT, participating in processes of cell growth and proliferation. In this study, we successfully established stable colon cancer cell lines in which T-STAR gene was specially up-regulated or down-regulated both at protein and mRNA levels in HCT-STAR cells or HCT-asSTAR cells. Meanwhile, the changes of telomerase activities had a parallel fashion with the expression level of T-STAR in those two cell lines, which was enhanced in HCT-STAR and reduced in HCT-asSTAR. Similar results have been found in our previous study in other tumor tissues or cell lines (unpublished data). The current data further demonstrated that the expression of T-STAR is positively correlated with hTERT and telomerase activity. In other words, it is possible that up-regulation of T-STAR may lead to hTERT and



**Figure 2** A: Western blot analysis of the T-STAR protein expression level of each group of cells; B: Densitometry analysis of the amount of T-STAR protein expression in each group of cells, <sup>a</sup>*P* < 0.01 vs 2. 1: HCT; 2: HCT-pcDNA; 3: HCT-asSTAR; 4: HCT-STAR.



**Figure 3** Quantitation analysis of telomerase activity of each HCT cell groups, <sup>a</sup>*P* < 0.05 vs 2, <sup>b</sup>*P* < 0.01 vs 2. 1: HCT; 2: HCT-pcDNA; 3: HCT-asSTAR; 4: HCT-STAR.

telomerase activity increase in tumor cells, and vice versa.

Telomeres are thought to play a critical role in cellular immortalization and carcinogenesis<sup>[12]</sup>. Our study on different pathologic types of tumor tissues found that T-STAR mainly expressed in adenoma cells. T-STAR may also be involved in cellular immortalization which is one of the basic characteristics of tumor cells<sup>[2,4,13]</sup>. Kool *et al*<sup>[4]</sup> have found that T-STAR is down-regulated in SV40-transformed immortalized fibroblasts (VH10/SV) compared with the corresponding nonimmortal SV40-transformed cells. In normal cells, T-STAR expression is clearly present and comparable with expression levels in SV40-transformed precrisis cells. No changes are observed in T-STAR expression as cells progress toward senescence. The down-regulation in the immortalized cells was not restricted to VH10/SV, but was also observed in other fibroblast cell lines transformed by SV40 large T<sup>[15,14]</sup>. Interestingly, the common feature of the SV40-transformed cell lines showing down-regulation of T-STAR is that they displayed a distinct proliferative crisis before immortal clones arose. In contrast, both immortal cell lines that do not show abrogation of T-STAR expression, arose without a clear crisis. These data suggest

that loss of T-STAR expression might be a prerequisite to escape from crisis. If immortalization occurs in earlier stages after SV40 transformation, these immortal cells will gradually overgrow the population before crisis and apparently do not need the downregulation of T-STAR. This could indicate that T-STAR plays a role during crisis, and that immortalization cannot occur unless T-STAR expression is down-regulated. T-STAR is likely one of important components in proliferative crisis or immortalization pathway<sup>[15,16]</sup>. So far, there is rarely experimental data to investigate the T-STAR expression in cell lines characterized with high telomerase activity and that immortalization has already been completed. Recent observation has shown that the mature male germ cells, as one kind of immortalized cells, with high telomerase activities also maintained abounding T-STAR protein<sup>[2]</sup>, which is consistent with our results. That is T-STAR expression might be positively correlated with telomerase activity in some kinds of immortalized cells, and T-STAR might be one component in the systems of telomerase activity regulation. Although the exact mechanism of this is unclear yet, from the analysis of T-STAR known functions, it can be speculated that T-STAR might play a role in phosphorylation and pre-mRNA splicing of hTERT.

Telomerase consists of an RNA component, telomerase RNA (TER), which includes the template for synthesis of telomere DNA, and a protein catalytic component, the telomerase reverse transcriptase (TERT), which mediates RNA template-dependent synthesis of telomere DNA. The phosphorylation<sup>[17,18]</sup> by PKC, AKT and PTK or dephosphorylation by PP2A for hTERT, the key component of the telomerase complex, can modulate telomerase activity<sup>[19-21]</sup>. The interaction of hTERT with c-Ab1 tyrosine kinase binding domain SH3 may result in phosphorylation and inhibit telomerase activity *in vitro* or *in vivo*<sup>[21-23]</sup>. Tyrosine phosphorylation of Sam68, the homologues of T-STAR and as an adaptor protein in signal transduction, promotes its interaction with SH2 containing proteins. The association of Sam68 with SH3 domain-containing proteins, and its tyrosine phosphorylation may negatively regulate its RNA binding activity<sup>[24,25]</sup>. T-STAR can form hetero-multimers<sup>[4,9,13]</sup> with Sam68 to inhibit the interaction of Sam68 with its target protein. So T-STAR might interfere with Sam68 binding with Ab1 SH3 by reverse phosphorylation, and lead to impairment of Ab1 SH3 function of increasing telomerase activity. Another possible mechanism is that T-STAR may take part in the splicing of hTERT pre-mRNA. Different length of hTERT transcripts by alternative-splicing sites exists in cells<sup>[26-28]</sup>, but only the full length transcripts for hTERT is functional, which is associated with telomerase activity. The selective-splicing for hTERT may be one of the modes that regulate telomerase activity. The 3'-UTR of T-STAR is the binding site for target mRNA<sup>[29]</sup>. T-STAR may play a role in processing of target pre-mRNA and selection of splicing sites<sup>[30]</sup>, and also interact with the protein involved in the selective splicing<sup>[30,31]</sup>. We assume that T-STAR might control pre-mRNA splicing for hTERT. The enhancement of full length transcripts for hTERT may up-regulate telomerase activity. However, the exact mechanism needs to be further investigated.

## REFERENCES

- 1 **Haegebarth A**, Heap D, Bie W, Derry JJ, Richard S, Tyner AL. The nuclear tyrosine kinase BRK/Sik phosphorylates and inhibits the RNA-binding activities of the Sam68-like mammalian proteins SLM-1 and SLM-2. *J Biol Chem* 2004; **279**: 54398-54404
- 2 **Elliott DJ**. The role of potential splicing factors including RBMY, RBMX, hnRNPG-T and STAR proteins in spermatogenesis. *Int J Androl* 2004; **27**: 328-334
- 3 **Wang L**, Xu J, Zeng L, Ye X, Wu Q, Dai J, Ji C, Gu S, Zhao C, Xie Y, Mao Y. Cloning and characterization of a novel human STAR domain containing cDNA KHDRBS2. *Mol Biol Rep* 2002; **29**: 369-375
- 4 **Kool J**, van Zaane W, van der Eb AJ, Terleth C. Down-regulation of T-STAR, a growth inhibitory protein, after SV40-mediated immortalization. *Cell Growth Differ* 2001; **12**: 535-541
- 5 **Gudjonsson T**, Villadsen R, Rønnev-Jessen L, Petersen OW. Immortalization protocols used in cell culture models of human breast morphogenesis. *Cell Mol Life Sci* 2004; **61**: 2523-2534
- 6 **Wai LK**. Telomeres, telomerase, and tumorigenesis—a review. *MedGenMed* 2004; **6**: 19
- 7 **Bocchetta M**, Carbone M. Epidemiology and molecular pathology at crossroads to establish causation: molecular mechanisms of malignant transformation. *Oncogene* 2004; **23**: 6484-6491
- 8 **Chang JY**. Telomerase: a potential molecular marker and therapeutic target for cancer. *J Surg Oncol* 2004; **87**: 1-3
- 9 **Venables JP**, Vernet C, Chew SL, Elliott DJ, Cowmeadow RB, Wu J, Cooke HJ, Artzt K, Eperon IC. T-STAR/ETOILE: a novel relative of SAM68 that interacts with an RNA-binding protein implicated in spermatogenesis. *Hum Mol Genet* 1999; **8**: 959-969
- 10 **Chen K**, Song Y, Zhang YY. [STAR family proteins and QKI: structure and function]. *Sheng Li Ke Xue Jin Zhan* 2003; **34**: 347-349
- 11 **Di Fruscio M**, Chen T, Richard S. Characterization of Sam68-like mammalian proteins SLM-1 and SLM-2: SLM-1 is a Src substrate during mitosis. *Proc Natl Acad Sci U S A* 1999; **96**: 2710-2715
- 12 **Kundra V**, Krane JF, Nikolaidis P, Green DS, Zou KH, Tuncali K, Vansonnenberg E, Silverman SG. Telomerase activity predicts malignancy in percutaneous image-guided needle biopsy specimens of the abdomen and pelvis. *Radiology* 2005; **234**: 941-947
- 13 **Venables JP**, Dalglish C, Paronetto MP, Skitt L, Thornton JK, Saunders PT, Sette C, Jones KT, Elliott DJ. SIAH1 targets the alternative splicing factor T-STAR for degradation by the proteasome. *Hum Mol Genet* 2004; **13**: 1525-1534
- 14 **Yaswen P**, Stampfer MR. Molecular changes accompanying senescence and immortalization of cultured human mammary epithelial cells. *Int J Biochem Cell Biol* 2002; **34**: 1382-1394
- 15 **Reddel RR**. The role of senescence and immortalization in carcinogenesis. *Carcinogenesis* 2000; **21**: 477-484
- 16 **Reddel RR**. Genes involved in the control of cellular proliferative potential. *Ann N Y Acad Sci* 1998; **854**: 8-19
- 17 **Oguchi K**, Tamura K, Takahashi H. Characterization of *Oryza sativa* telomerase reverse transcriptase and possible role of its phosphorylation in the control of telomerase activity. *Gene* 2004; **342**: 57-66
- 18 **Hao LY**, Strong MA, Greider CW. Phosphorylation of H2AX at short telomeres in T cells and fibroblasts. *J Biol Chem* 2004; **279**: 45148-45154
- 19 **Kimura A**, Ohmichi M, Kawagoe J, Kyo S, Mabuchi S, Takahashi T, Ohshima C, Arimoto-Ishida E, Nishio Y, Inoue M, Kurachi H, Tasaka K, Murata Y. Induction of hTERT expression and phosphorylation by estrogen via Akt cascade in human ovarian cancer cell lines. *Oncogene* 2004; **23**: 4505-4515
- 20 **Endoh T**, Tsuji N, Asanuma K, Yagihashi A, Watanabe N. Survivin enhances telomerase activity via up-regulation of specificity protein 1- and c-Myc-mediated human telomerase reverse transcriptase gene transcription. *Exp Cell Res* 2005; **305**:

- 300-311
- 21 **Mori S**, Cao Y, Yamada T, Sogawa K, Kondo K, Hino N, Miyazaki T, Kawaguchi Y, Oka S, Kawasaki K, Mashiba T, Norimatsu H. Enhanced expression of PP1gamma1, a catalytic subunit isoform of protein phosphatase type1 and expression of telomerase activity in Ewing's sarcoma cells. *Res Commun Mol Pathol Pharmacol* 2003; **113-114**: 269-274
- 22 **Bakalova R**, Ohba H, Zhelev Z, Kubo T, Fujii M, Ishikawa M, Shinohara Y, Baba Y. Antisense inhibition of Bcr-Abl/c-Abl synthesis promotes telomerase activity and upregulates tankyrase in human leukemia cells. *FEBS Lett* 2004; **564**: 73-84
- 23 **Haendeler J**, Hoffmann J, Brandes RP, Zeiher AM, Dimmeler S. Hydrogen peroxide triggers nuclear export of telomerase reverse transcriptase via Src kinase family-dependent phosphorylation of tyrosine 707. *Mol Cell Biol* 2003; **23**: 4598-4610
- 24 **Najib S**, Martín-Romero C, González-Yanes C, Sánchez-Margalet V. Role of Sam68 as an adaptor protein in signal transduction. *Cell Mol Life Sci* 2005; **62**: 36-43
- 25 **Paronetto MP**, Venables JP, Elliott DJ, Geremia R, Rossi P, Sette C. Tr-kit promotes the formation of a multimolecular complex composed by Fyn, PLCgamma1 and Sam68. *Oncogene* 2003; **22**: 8707-8715
- 26 **Zaffaroni N**, Villa R, Pastorino U, Cirincione R, Incarbone M, Alloisio M, Curto M, Pilotti S, Daidone MG. Lack of telomerase activity in lung carcinoids is dependent on human telomerase reverse transcriptase transcription and alternative splicing and is associated with long telomeres. *Clin Cancer Res* 2005; **11**: 2832-2839
- 27 **Barclay JY**, Morris A, Nwokolo CU. Telomerase, hTERT and splice variants in Barrett's oesophagus and oesophageal adenocarcinoma. *Eur J Gastroenterol Hepatol* 2005; **17**: 221-227
- 28 **Liu WJ**, Zhang YW, Zhang ZX, Ding J. Alternative splicing of human telomerase reverse transcriptase may not be involved in telomerase regulation during all-trans-retinoic acid-induced HL-60 cell differentiation. *J Pharmacol Sci* 2004; **96**: 106-114
- 29 **Itoh M**, Haga I, Li QH, Fujisawa J. Identification of cellular mRNA targets for RNA-binding protein Sam68. *Nucleic Acids Res* 2002; **30**: 5452-5464
- 30 **Stoss O**, Olbrich M, Hartmann AM, König H, Memmott J, Andreadis A, Stamm S. The STAR/GSG family protein rSLM-2 regulates the selection of alternative splice sites. *J Biol Chem* 2001; **276**: 8665-8673
- 31 **Venables JP**, Elliott DJ, Makarova OV, Makarov EM, Cooke HJ, Eperon IC. RBMY, a probable human spermatogenesis factor, and other hnRNP G proteins interact with Tra2beta and affect splicing. *Hum Mol Genet* 2000; **9**: 685-694

S- Editor Wang J L- Editor Zhu LH E- Editor Liu WF