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ST13, a proliferation regulator, inhibits growth and migration of colorectal cancer cell lines*

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Abstract: Background and objective: *ST13*, is the gene encoding the HSP70 interacting protein (HIP). Previous research has shown that *ST13* mRNA and protein levels are down-regulated in colorectal cancer (CRC) tissues compared with adjacent normal tissues. This study aims at the role of *ST13* in the proliferation and migration of CRC cells. Methods: The transcript level of *ST13* in different CRC cell lines was evaluated by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). *ST13*-overexpressed and *ST13*-knockdown CRC cells were constructed respectively by lentiviral transduction, followed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay, plate colony formation, cell-cycle analysis, and migration assays to evaluate the influence of *ST13* on proliferation and migration in vitro. Moreover, a mouse xenograft study was performed to test in vivo tumorigenicity of *ST13*-knockdown CRC cells. Results: Lentivirus-mediated overexpression of *ST13* in CRC cells inhibited cell proliferation, colony formation, and cell migration in vitro. In contrast, down-regulation of *ST13* by lentiviral-based short hairpin RNA (shRNA) interference in CRC cells significantly increased cell proliferation and cloning efficiency in vitro. In addition, down-regulation of *ST13* expression significantly increased the tumorigenicity of CRC cells in vivo. Conclusions: *ST13* gene is a proliferation regulator that inhibits tumor growth in CRC and may affect cell migration.

Key words: Colorectal cancer, *ST13*, Proliferation, Colony formation, Cell cycle, Migration
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1 Introduction

Heat-shock proteins (HSPs) are a highly conserved molecular chaperone family, some of which are induced by sublethal cellular stresses including temperature elevation, oxidative damage, and hypoxia (Young *et al.*, 2004; Mahalingam *et al.*, 2009). In addition, they are known to facilitate folding of nascent polypeptides, induce solubilisation of loose protein aggregates, afford protection against protein aggregation, participate in refolding of proteins which have been damaged, and sequester damaged proteins and target them for degradation (Hartl, 1996; Csermely, 1997; Söti and Csermely, 2002; Soo *et al.*, 2008). HSPs are generally classified by their molecular sizes, e.g., HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs (sHSPs) (Powers and Workman, 2007). They have been found to be overexpressed in many human cancers, including both solid tumours and haematological malignancies (Ciocca *et al.*, 1993; Kimura *et al.*, 1993; Chant *et al.*,

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1995; Ralhan and Kaur, 1995; Takayama *et al.*, 2003; Whitesell and Lindquist, 2005; Neckers, 2007) and play important roles in tumor initiation and progression, such as rendering cancer cells refractory to anti-proliferative signals (Hollstein *et al.*, 1991; Lane *et al.*, 1993) and avoiding apoptosis (Lanneau *et al.*, 2008). HSPs are potentially ideal therapeutic targets in cancer treatment.

ST13 is the gene encoding the HSP70 interacting protein (HIP), a co-chaperone of the 70-kDa HSPs (HSC/HSP70) (Höhfeld *et al.*, 1995; Shi *et al.*, 2007). We originally identified the *ST13* gene by subtraction hybridization with normal mucosal tissue and colorectal cancers (Zheng *et al.*, 1997). The *ST13* gene, which is located on chromosome 22q13 (Zhang *et al.*, 1998), has shown frequent loss of heterozygosity in colorectal, ovarian, and breast cancers. However, the precise location of *ST13* (22q13.2) is apparently outside the minimal region of deletion that is common for both colorectal and breast cancers according to a detailed human chromosome map, the Human Genome Project (Castells *et al.*, 2000). ST13 protein (HIP) is composed of an N-terminal region, a central tetratricopeptide repeat (TPR) domain followed by a highly charged region, and a C-terminal region containing glycine-glycine-methionine-proline (GGMP) repeats and a Sti1 motif (Prapapanich *et al.*, 1996a; 1998; Irmer and Höhfeld, 1997). HIP may facilitate the chaperone function of HSC/HSP70 in controlling the activities of regulatory proteins such as steroid receptors and regulators of proliferation or apoptosis, and in protein folding and repair (Prapapanich *et al.*, 1996a; 1996b; Höhfeld and Jentsch, 1997; Irmer and Höhfeld, 1997; Shi *et al.*, 2007).

In previous studies, we have proved that *ST13* mRNA and protein levels were lower in colorectal cancer tissues compared with adjacent normal tissues (Mo *et al.*, 1996; Zheng *et al.*, 1997; Dong *et al.*, 2005; Wang *et al.*, 2005). Moreover, increased ST13 protein expression suppressed proliferation of colorectal cancer cells and induced apoptosis in colorectal cancer cell lines (Yang *et al.*, 2008; Yu *et al.*, 2009). In the present study, we employed a lentiviral system to overexpress or knock down *ST13* gene in colorectal cancer cells and examined the roles of *ST13* in colorectal cancer cell growth both in vitro and in vivo. We also tested functions of *ST13* in migration.

2 Materials and methods

2.1 Cell lines and cell culture

Human colorectal cancer cell lines (RKO, HT29, SW480, SW620, LOVO, LS174T, and HEK293) were purchased from the American Type Culture Collection (Manassas, VA) and cultured under recommended conditions.

2.2 Isolation of RNA and real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA from cell line was extracted from subconfluent cells in the exponential phase of growth using Trizol reagent (Invitrogen, Carlsbad, CA), according to the manufacture's instructions. Real-time PCR primers were *ST13* (5′-CGGAGAAAGTA TGAGCGAAAA-3′ and 5′-AAGCCACCTGGAAA AGAGCC-3′) and β-actin (5′-TTCCAGCCTTCCTTC CTGGG-3′ and 5′-TTGCGCTCAGGAGGAGCAA T-3′). Transcript level of *ST13* was determined by real-time PCR using the Applied Biosystems StepOne Real-Time PCR system (Applied Biosystems, Carlsbad, CA). The real-time PCR was carried out in a total volume of 20 μl per well containing SYBR® master mix reagent kit (Applied Biosystems, Carlsbad, CA) in triplicate. Human β-actin was amplified as an endogenous control. The relative gene expression levels were calculated using the comparative threshold cycle C_t ($\Delta\Delta C_t$) method (according to Applied Biosystems), where the relative expression is calculated as $2^{-\Delta\Delta C_t}$.

2.3 Establishment of stable *ST13* knockdown SW620 cell clones

Small hairpin RNA (shRNA) lentiviral particles used for *ST13* knockdown (sc-40684-v) and Mock knockdown (sc-108080) were purchased from Santa Cruz, CA, USA. SW620 cells were infected with shRNA over 48 h in the presence of polybrene (4 μg/ml) in a 6-well plate as described previously (Shi *et al.*, 2012). The shRNA-Mock group was transfected by control shRNA. Stable colonies were selected and isolated in the presence of puromycin at the concentration of 3 μg/ml and evaluated for *ST13* mRNA expression by quantitative RT-PCR (qRT-PCR).

2.4 Lentiviral vector construction, virus production and transduction

pcDNA3.1-*ST13* was constructed in previous experiments by Prof. Shu ZHENG's group (Yu *et al.*, 2009). All constructs were made by standard DNA recombination techniques. Briefly, *ST13* insert was isolated by PCR amplification from pcDNA3.1-*ST13* with two pairs of restriction primers. PCR products were sequenced (ABI Prism 3100 DNA Sequencer, Applied Biosystems, Foster City, CA) and confirmed to contain the entire *ST13* coding sequence. The insert was then cloned into the pLenti6.3-MCS-IRES2-EGFP plasmid (Invitrogen), which was co-transfected with Packaging plasmids (Invitrogen) into HEK293T cells. The viral supernatant was harvested, filtered, and concentrated by centrifugation. Lenti-Mock constructs were constructed similarly, only without the *ST13* insert. The viral concentrate was diluted in polybrene to infect SW620 cells. A successful transduction was confirmed by visualizing enhanced green fluorescent protein (EGFP; included in the pLenti6.3-IRES2-EGFP vector) and sustained *ST13* expression was confirmed at least every two weeks by qRT-PCR. Virus-infected cells were selected with 8 μg/ml blasticidin (Invitrogen). The antibiotic-resistant clones were pooled and used for subsequent assay.

2.5 Cell proliferation assay

Cells were cultured at a density of 5×10^3 cells/well in triplicate in 96-well plates with 10% fetal bovine serum (FBS) L-15 at 37 °C and 5% CO_2 for varying periods and exposed to fresh media every other day. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay was performed every day for up to 5 d. Briefly, 20 μl of 5 mg/ml MTT (Sigma, St. Louis, MO, USA) was added to each well; plates were incubated at 37 °C for 4 h. The generated formazan was dissolved in 150 μl dimethyl sulfoxide (DMSO) and measured with a microplate reader (BioRad, Hercules, CA) at optical density at 570 nm (OD_{570}) for determining the cell viability.

2.6 Plate colony formation assay

Cell colony formation rate was measured by plate colony formation assay. About 200 cells were added to each well of a 6-well plate. Plates were incubated at 37 °C for 14 d, and then were gently washed and stained with crystal violet. Viable colonies containing at least 50 cells were counted.

2.7 Cell cycling analysis

Cells were washed with ice-cold phosphate buffered saline (PBS) twice and fixed with 70% ethanol overnight at 4 °C. The cells were digested with 50 μg/ml RNase A in 100 μl of PBS for 30 min at room temperature and stained with 20 μg/ml propidium iodide (PI; 300 μl) for 20 min. The cell cycling was analyzed on an FACScanner (Coulter Biosciences). All assays were carried out in triplicate (Li *et al.*, 2009).

2.8 Mouse xenograft study

Our animal protocol was approved and performed strictly in accordance with the related ethics regulations of Zhejiang Chinese Medical University. SW620 Mock-knockdown cells (shRNA-Mock) and SW620 *ST13*-knockdown cells (shRNA-*ST13*) were cultured until 80%–90% confluence before harvesting. Cells were trypsinized, washed with PBS twice, and resuspended in serum-free L-15 (Leibovitz's) medium to a concentration of 5×10^6 cells per 200 μl. Then, 200 μl cells were injected subcutaneously into the dorsal flanks of 5-week-old female nude mice. Tumor sizes were measured in two dimensions with calipers twice a week and tumor volumes (V, mm^3) were calculated as $V=L\times W^2/2$, where L is tumor length and W is tumor width.

2.9 Protein extraction and Western blotting

Cells were harvested in the exponential phase of growth and whole-cell lysates were prepared using the Mammalian Protein Extraction Reagent (Merck, Germany) in accordance with the manufacturer's instructions. Protein concentrations of samples were determined by the bicinchoninic acid (BCA) protein assay (Pierce, USA). Protein samples (40 μg of each protein) were boiled for 5 min, and Western blot analysis was performed as described previously (Ge *et al.*, 2006). Three independent Western blot assays were performed for all samples. The primary antibodies used were polyclonal antibodies against ST13 (Cell Signaling Technology, USA) and β-actin (Sigma-Aldrich, USA) (Ye *et al.*, 2011).

2.10 Migration assays

Unless specified otherwise, cells (1×10^5 cells/well) were suspended in 150 μl FBS-free L-15 and dispensed into the upper chambers of transwells (8 μm pore size), with 800 μl of 10% FBS-containing L-15 in the lower chamber. After 48 h, cells on the upper surface were removed with a cotton swab, and cells migrating to the lower membrane surface were fixed with 90% alcohol, stained with crystal violet, and examined under a microscope. A total of six random high-power microscopic fields (400×) per filter were photographed and the numbers of cells were directly counted.

2.11 Statistical analysis

For continuous variables, data were expressed as mean±standard error of the mean (SEM). The difference among groups was determined by analysis of variance (ANOVA) analysis and comparison between two groups was analyzed by the Student's *t*-test using the GraphPad Prism software version 4.0 (GraphPad Software Inc., San Diego, CA).

3 Results

3.1 *ST13* expression in colorectal cancer cell lines

The expression of *ST13* mRNA was quantified by qRT-PCR in six human colorectal cancer cells and human embryonic kidney cells (HEK293 cells), and as Fig. 1a indicates, utilizing $\Delta\Delta C_t$ method, *ST13* expression in mRNA levels was lower in SW620 cells as compared to other five human colorectal cancer cells (RKO, HT29, SW480, LOVO, and LS174T). SW620 easily forms tumors in xenograft mouse. Therefore, SW620 cells were chosen to do a series of function experiments.

As shown in Figs. 1b and 1c, *ST13* mRNA expression in the Lenti-*ST13* group remarkably increased compared to that in the Lenti-Mock group and wild-type SW620 group, while *ST13* mRNA expression in the shRNA-*ST13* group remarkably decreased by about 90% compared to that in the shRNA-Mock group and wild-type SW620 group. Furthermore, we performed Western blot to verify the level of ST13 protein in the Lenti-*ST13*, Lenti-Mock, SW620, shRNA-*ST13*, and shRNA-Mock groups. The levels of ST13 protein in the Lenti-Mock, wild-type SW620, and shRNA-Mock groups were almost identical, while the level in the Lenti-*ST13* group was much higher and the level in the shRNA-*ST13* group was almost none (Fig. 1d). These Western blot results were consistent with the results of qRT-PCR.

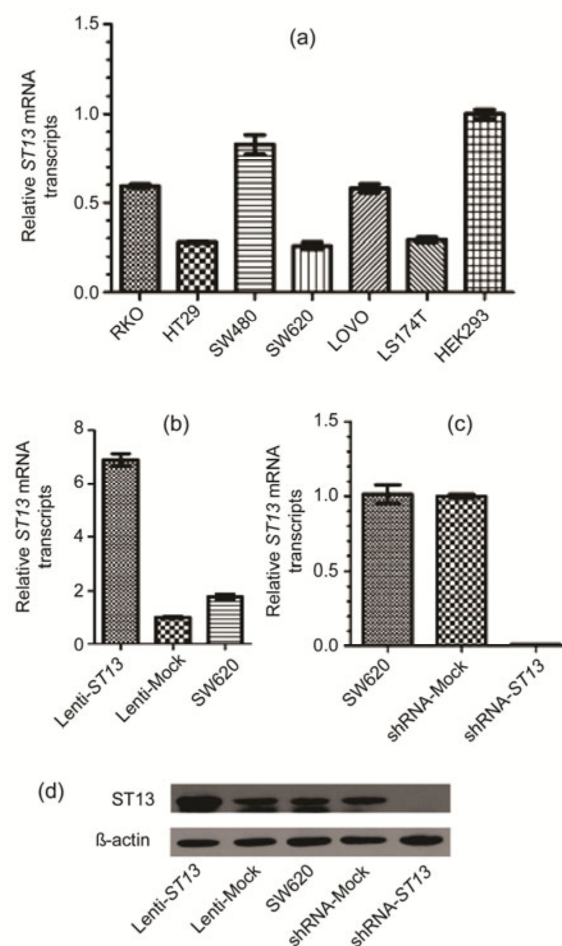

Fig. 1 *ST13* expression in colorectal cancer cell lines
(a) qRT-PCR shows relative transcript levels of *ST13* in six colorectal cancer cell lines, including RKO, HT29, SW480, SW620, LOVO, and LS174T, and HEK293. The relative quantity was normalized to HEK293 cells, and β-actin was used as an internal control. (b) qRT-PCR shows relative transcript levels of *ST13* in *ST13*-lentivirus-infected Lenti-*ST13*, control lentivirus-infected Lenti-Mock, and SW620. The relative quantity was normalized to Lenti-Mock cells, and β-actin was used as an internal control. (c) qRT-PCR shows relative transcript levels of *ST13* in SW620, control-shRNA-lentivirus-infected shRNA-Mock, and *ST13*-shRNA-lentivirus-infected shRNA-*ST13*. The relative quantity was normalized to shRNA-Mock cells, and β-actin was used as an internal control. (d) Western blot results show that ST13 was expressed in SW620 cells with different groups, including Lenti-*ST13*, Lenti-Mock, SW620, shRNA-Mock, and shRNA-*ST13*. β-actin was served as loading control. Error bars indicate SEM (*n*=3 experiments)

3.2 Effects of *ST13* expression on proliferation ability of SW620 cells in vitro

As seen in Fig. 2a, overexpression of *ST13* caused a dramatic reduction in the proliferation of the Lenti-*ST13* group when compared with that in the Lenti-Mock or SW620 group, especially from the 4th to 5th day after MTT detection ($P<0.01$, one way ANOVA). As expected, the Lenti-Mock group had the similar growth ability as the SW620 group.

Moreover, a significant decrease was observed in the colony formation of the Lenti-*ST13* group when compared to the Lenti-Mock or SW620 group ($P<0.05$, one way ANOVA) (Figs. 2b and 2c).

Knockdown of *ST13* remarkably increased the proliferation ability of the shRNA-*ST13* group when compared with that of the shRNA-Mock group, especially from the 3rd to 5th day after MTT detection ($P<0.01$, Student's *t*-test) (Fig. 2d). Moreover, a significant increase was observed in the colony formation of the shRNA-*ST13* group when compared to the shRNA-Mock group ($P<0.05$, Student's *t*-test) (Figs. 2e and 2f).

3.3 Effects of *ST13* expression on the distribution of cell cycle in SW620 cells

To explore the potential mechanism(s) underlying the action of *ST13* in the growth of SW620 cells, cell cyclings of the Lenti-*ST13*, Lenti-Mock, SW620, shRNA-*ST13* and shRNA-Mock groups were characterized by fluorescence-activated cell sorting (FACS) analysis.

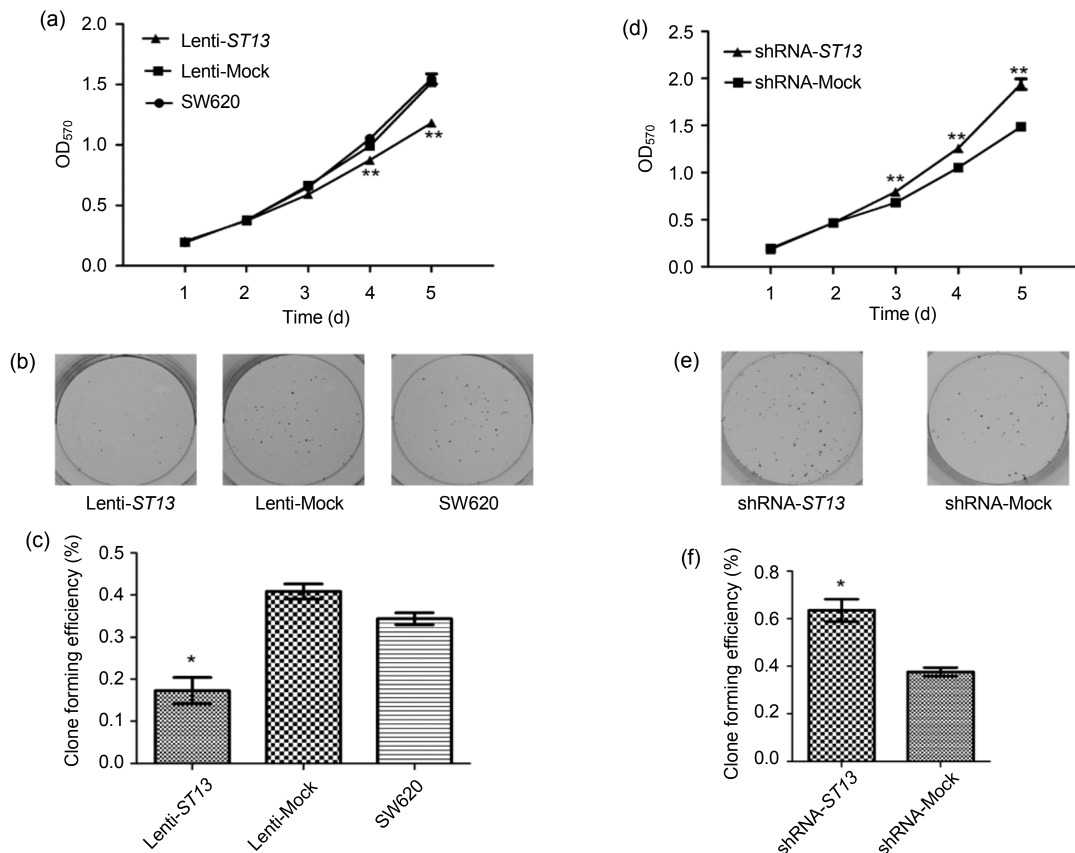

Fig. 2 Effect of *ST13* expression on SW620 cell growth in vitro

(a) Effect of *ST13* overexpression on SW620 cell proliferation determined by MTT assay. Error bars indicate SEM (n=3 experiments). ** $P<0.01$, Lenti-*ST13* vs. Lenti-Mock and SW620 (one way ANOVA and Dunnett's test). (b) Colony formations of Lenti-*ST13*, Lenti-Mock, and SW620 cells in vitro. (c) Quantitative analyses of colony formations of Lenti-*ST13*, Lenti-Mock, and SW620 groups. Error bars indicate SEM (n=3 experiments). * $P<0.05$, Lenti-*ST13* vs. Lenti-Mock and SW620 (one way ANOVA and Dunnett's test). (d) Effect of *ST13* knockdown on SW620 cell proliferation determined by MTT assay. Error bars indicate SEM (n=3 experiments). ** $P<0.01$, shRNA-*ST13* vs. shRNA-Mock (Student's *t*-test). (e) Colony formations of shRNA-*ST13* and shRNA-Mock cells. (f) Quantitative analyses of colony formations of shRNA-*ST13* and shRNA-Mock groups. Error bars indicate SEM (n=3 experiments). * $P<0.05$ shRNA-*ST13* vs. shRNA-Mock (Student's *t*-test)

As shown in Figs. 3a–3c and 3f, there was no significant difference between the Lenti-Mock and SW620 (P>0.05, one way ANOVA). However, the frequency of the Lenti-*ST13* group cells at the G_0/G_1 phase was significantly higher compared with that in the controls (P<0.01, one way ANOVA) while that in G_2/M stage remarkably decreased as compared to the controls (P<0.05, one way ANOVA). The data indicate that up-regulation of *ST13* expression arrested Lenti-*ST13* cell cycling at the G_0/G_1 phase, which might inhibit the growth of SW620 cells.

In Figs. 3d–3e and 3g, the frequency of the shRNA-*ST13* group cells at the G_0/G_1 phase was significantly lower compared with that in the shRNA-Mock group (P<0.05, Student's t-test). The frequency of the shRNA-*ST13* group cells at the S and G_2/M phases increased compared to that of the shRNA-Mock group, although the difference was not statistically significant. These data indicate that down-regulation of *ST13* expression promoted shRNA-*ST13* cell cycling, which might promote the growth of SW620 cells in vitro.

3.4 Effect of *ST13* expression on SW620 cell migration in vitro

We examined the impact of *ST13* expression on the migration of SW620 cells by transwell migration assay (Fig. 4). While an average of 56.5±3.9 Lenti-Mock and 58.7±4.7 SW620 cells per high power field had migrated onto the filter surface, only 8.7±1.6 Lenti-*ST13* cells reached the filter (P<0.01, one way ANOVA).

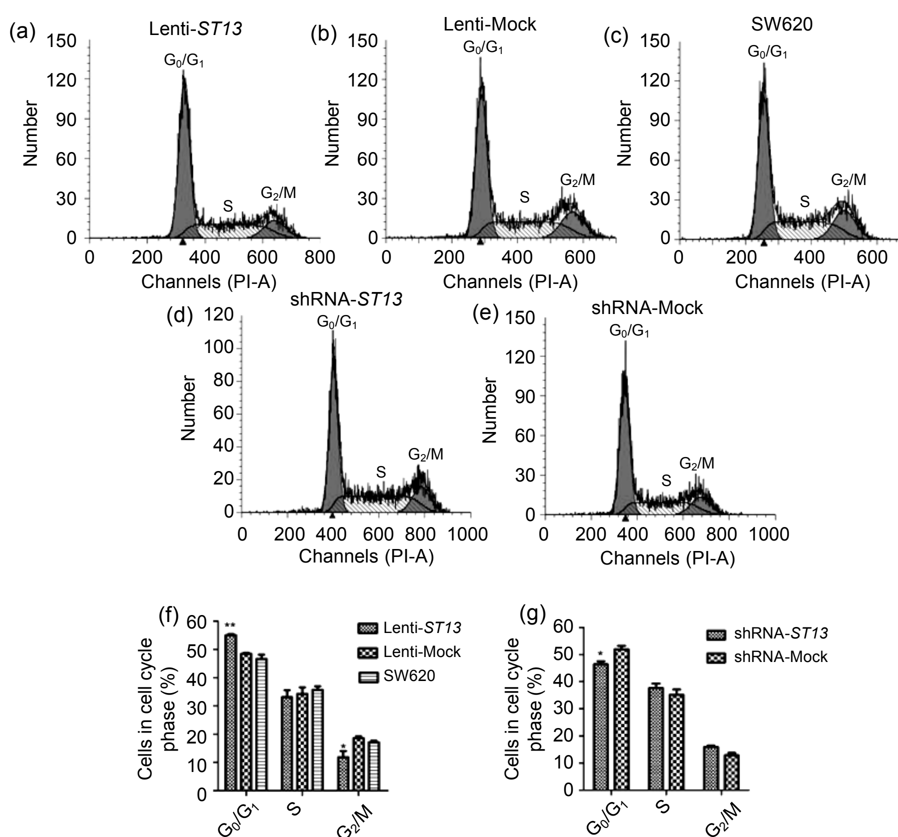

Fig. 3 Effect of *ST13* expression on SW620 cell cycling

(a–e) Cell cycle progressions of Lenti-*ST13*, Lenti-Mock, SW620 cells, shRNA-*ST13*, and shRNA-Mock, respectively. (f) The relative frequency of different phases of Lenti-*ST13*, Lenti-Mock, and SW620 cells. Error bars indicate SEM (n=3 experiments). * P<0.05, Lenti-*ST13* vs. Lenti-Mock and SW620; ** P<0.01, Lenti-*ST13* vs. Lenti-Mock and SW620 (one way ANOVA and Dunnett's test). (g) The relative frequency of different phases of shRNA-*ST13* and shRNA-Mock cells. Error bars indicate SEM (n=3 experiments). * P<0.05, shRNA-*ST13* vs. shRNA-Mock (Student's t-test)

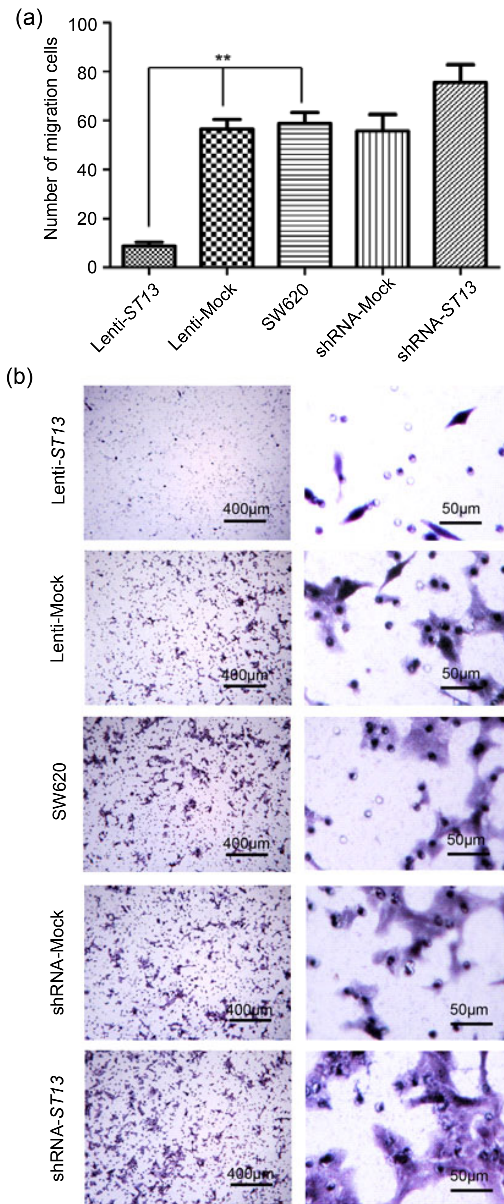

Fig. 4 Effect of *ST13* expression on SW620 cells migration in vitro

Lenti-*ST13*, Lenti-Mock, SW620, shRNA-Mock, and shRNA-*ST13* cells were cultured in the top well of a transwell system and migration of SW620 cells was measured by counting the number of SW620 cells migrating to the filter surface. (a) Quantitative measurement of invaded cells. Data are representatives of each group and expressed as mean±SEM of cells per six high power fields from three separated experiments. ** $P<0.01$, Lenti-*ST13* vs. Lenti-Mock and SW620 (one way ANOVA and Dunnett's test). (b) Images of Lenti-*ST13*, Lenti-Mock, SW620, shRNA-Mock, and shRNA-*ST13* cells on the filter surface

In contrast, an average of 55.7±6.8 shRNA-Mock and 58.7±4.7 SW620 cells per high power field had migrated onto the filter surface, while an average of 75.6±7.3 shRNA-*ST13* cells reached the filter; however, this difference was not significant (P=0.077, one way ANOVA).

3.5 Impact of reduced *ST13* expression on the tumor development of inoculated SW620 cells in vivo

SW620 cells have high tumorigenicity and following inoculation they develop solid tumors in immunocompromised nude mice. To determine the role of *ST13* in the tumorigenicity of SW620 cells and development of solid SW620 tumors, shRNA-*ST13* or shRNA-Mock cells were injected subcutaneously into nude mice. The development of solid SW620 tumors was monitored for 32 d. As shown in Fig. 5a, the solid tumors were first visible at about 8 d post inoculation and rapidly grew later in the shRNA-*ST13*

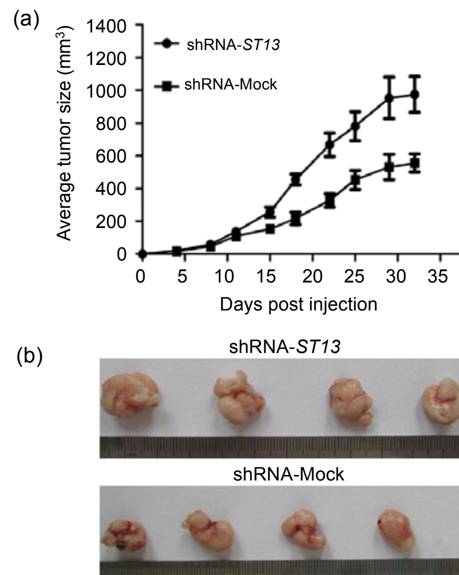

Fig. 5 Effect of *ST13* expression on the development and growth of inoculated SW620 cells

BALB/c nude mice (n=4 per group) were inoculated with 5×10^6 shRNA-*ST13* or shRNA-Mock cells and the development of solid SW620 tumors was monitored every 3–4 d. The mice were sacrificed 32 d post-inoculation and the tumors were taken at the same time. (a) The dynamics of shRNA-*ST13* and shRNA-Mock tumor growth. Data are expressed as mean±SEM for each group. (b) The images of individual tumors

groups. In contrast, the development of shRNA-Mock solid tumors grew slowly and the mean volume of solid SW620 tumors in shRNA-*ST13* group increased by about 75%, as compared with that in control groups (Fig. 5b). Collectively, these data indicate that down-regulation of *ST13* expression increased the tumorigenicity of SW620 cells in vivo.

4 Discussion

ST13 is the gene encoding the HIP (Höhfeld *et al.*, 1995). In our previous study, *ST13* expression was lower in colorectal cancer tissues compared with the tumor-adjacent normal mucosa specimens (Wang *et al.*, 2005). The previous study showed that *ST13* re-expression mediated by oncolytic adenovirus within colorectal cancer cells can induce cell apoptosis and exert potent antitumour efficacy in colorectal cancer xenografts in nude mice. Adenovirus-*ST13* regulated apoptosis by interacting with ASK1 and inducing JNK activation (Yang *et al.*, 2008; 2010; Yu *et al.*, 2009).

In order to better understand the roles of *ST13* in colorectal cancer, we performed lentivirus-mediated overexpression of *ST13* and lentivirus-mediated shRNA down-regulation of *ST13* expression to make stable transfection cell lines. High expression of *ST13* decreased proliferation, while knockdown of *ST13* enhanced proliferation ability of SW620 colorectal cancer cells in vitro. We also proved that down-regulation of *ST13* expression increased the tumorigenicity of SW620 cells in vivo. Our findings were consistent with previous research that *ST13* re-expression mediated by oncolytic adenovirus inhibited tumor cells growth both in vitro and in vivo (Yang *et al.*, 2008; Yu *et al.*, 2009). To explore the potential mechanism(s) underlying the action of *ST13* in the growth of SW620 cells with stable transfection, cell cycling was characterized by FACS analysis. The data indicates that up-regulation of *ST13* expression arrests Lenti-*ST13* cell cycling at the G_0/G_1 phase and down-regulation of *ST13* expression activates shRNA-*ST13* cell cycling. This supports that *ST13* inhibited SW620 proliferation in the stable transfection situation through cell cycle regulation.

Colorectal cancer is a highly metastatic malignancy. However, little has been reported on the impact of *ST13* in migration. We examined the impact of *ST13* expression on the migration of SW620 cells by transwell migration assay (Fig. 4). While many control Lenti-Mock and SW620 cells migrated to the filter surface, the numbers of Lenti-*ST13* cells on the filter surface decreased by 83%. Therefore, overexpression of *ST13* in SW620 cells remarkably reduced the migration capacity of SW620 cells in vitro. We also showed that in the shRNA-*ST13* group there were more cells per high power field that had migrated onto the filter surface compared to the shRNA-Mock and SW620 groups, although this difference was not significant (P=0.077, one way ANOVA). SW620 is a very high migration cell line, and knocked-down *ST13* did not change the migration of SW620 too much. If another low migration cell line was used, there might have been significant differences of migration between the knocked-down *ST13* and Mock groups.

Transforming growth factor-β (TGF-β) is the prototype of a large family of secreted polypeptide growth factors that regulate a multitude of cellular processes affecting proliferation, differentiation, apoptosis, and epithelial-mesenchymal transition (EMT) (Roberts and Sporn, 1993; Roberts, 1998). The tumor suppression of TGF-β is caused by potent inhibition of cell proliferation due to cell cycle arrest in the G_1 phase. Such anti-proliferative responses are mediated by a signaling system that includes two types of cell surface receptors and intracellular signal transducers, the SMAD proteins, including SMAD2 (Itoh *et al.*, 2000; Schiffer *et al.*, 2000). Many carcinoma cells are no longer sensitive to growth inhibition by TGF-β. These carcinoma cells at this stage retain the TbR-I/TbR-II/SMAD2/3/4 signaling cascade, oversecrete TGF-β, exhibit EMT (induced by autocrine TGF-β), and become more metastatic and invasive (Derynck *et al.*, 2001; Piek and Roberts, 2001). In previous studies, we used CO-IP to find that ST13 can pull down the HSP70 (data not shown). Yang *et al.* (2008) also confirmed that transient transfection of *ST13* can increase the expression of HSP70. Recent data have shown that HSP70 prevents receptor-dependent phosphorylation and nuclear translocation of SMAD2, and blocks TGF-β-induced EMT (Li *et al.*, 2011). So we speculated that the effects of *ST13* in migration might, through increasing HSP70, prevent SMAD2 phosphorylation, block TGF-β-induced

EMT and inhibit SW620 migration. Conceivably, overexpression of *ST13* may be used as a new strategy for the inhibition of colorectal cancer metastasis.

5 Conclusions

We provide a report assessing the role of *ST13* in the tumorigenesis and progression of colorectal cancer. Our findings demonstrate that high expression of *ST13* decreased proliferation ability, arrested cell cycle, and inhibited migration while cells knockdown of *ST13* expression led to enhanced proliferation ability and activated cell cycle in SW620 colorectal cancer cells. Although the mechanism in which *ST13* interacts with other proliferation and migration regulators is poorly understood, *ST13* might serve as a novel diagnosis and prognosis biomarker as well as a potential therapeutic target in colorectal cancer.

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References

Castells, A., Gusella, J.F., Ramesh, V., Rustgi, A.K., 2000. A region of deletion on chromosome 22q13 is common to human breast and colorectal cancers. *Cancer Res.*, **60**(11): 2836-2839.

Chant, I.D., Rose, P.E., Morris, A.G., 1995. Analysis of heat-shock protein expression in myeloid leukaemia cells by flow cytometry. *Br. J. Haematol.*, **90**(1):163-168. [doi:10.1111/j.1365-2141.1995.tb03395.x]

Ciocca, D.R., Clark, G.M., Tandon, A.K., Fuqua, S.A., Welch, W.J., McGuire, W.L., 1993. Heat shock protein hsp70 in patients with axillary lymph node-negative breast cancer: prognostic implications. *J. Natl. Cancer Inst.*, **85**(7): 570-574. [doi:10.1093/jnci/85.7.570]

Csermely, P., 1997. Proteins, RNAs and chaperones in enzyme evolution: a folding perspective. *Trends Biochem. Sci.*, **22**(5):147-149. [doi:10.1016/S0968-0004(97)01026-8]

Derynck, R., Akhurst, R.J., Balmain, A., 2001. TGF-β signaling in tumor suppression and cancer progression. *Nat. Genet.*, **29**(2):117-129. [doi:10.1038/ng1001-117]

Dong, Q.H., Zheng, S., Hu, Y., Chen, G.X., Ding, J.Y., 2005. Evaluation of *ST13* gene expression in colorectal cancer patients. *J. Zhejiang Univ.-Sci. B.*, **6**(12):1170-1175. [doi:10.1631/jzus.2005.B1170]

Ge, W., Hu, H., Ding, K., Sun, L., Zheng, S., 2006. Protein interaction analysis of ST14 domains and their point and deletion mutants. *J. Biol. Chem.*, **281**(11):7406-7412. [doi:10.1074/jbc.M510687200]

Hartl, F.U., 1996. Molecular chaperones in cellular protein folding. *Nature*, **381**(6583):571-579. [doi:10.1038/381571a0]

Höhfeld, J., Jentsch, S., 1997. GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. *EMBO J.*, **16**(20):6209-6216. [doi:10.1093/emboj/16.20.6209]

Höhfeld, J., Minami, Y., Hartl, F.U., 1995. Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. *Cell*, **83**(4):589-598. [doi:10.1016/0092-8674(95)90099-3]

Hollstein, M., Sidransky, D., Vogelstein, B., Harris, C.C., 1991. p53 mutations in human cancers. *Science*, **253**(5015):49-53. [doi:10.1126/science.1905840]

Irmer, H., Höhfeld, J., 1997. Characterization of functional domains of the eukaryotic co-chaperone Hip. *J. Biol. Chem.*, **272**(4):2230-2235. [doi:10.1074/jbc.272.4.2230]

Itoh, S., Itoh, F., Goumans, M.J., Ten Dijke, P., 2000. Signaling of transforming growth factor-β family members through Smad proteins. *Eur. J. Biochem.*, **267**(24):6954-6967. [doi:10.1046/j.1432-1327.2000.01828.x]

Kimura, E., Enns, R.E., Alcaraz, J.E., Arboleda, J., Slamon, D.J., Howell, S.B., 1993. Correlation of the survival of ovarian cancer patients with mRNA expression of the 60-kD heat-shock protein HSP-60. *J. Clin. Oncol.*, **11**(5): 891-898.

Lane, D.P., Midgley, C., Hupp, T., 1993. Tumour suppressor genes and molecular chaperones. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **339**(1289):369-372, discussion 372-373. [doi:10.1098/rstb.1993.0036]

Lanneau, D., Brunet, M., Frisan, E., Solary, E., Fontenay, M., Garrido, C., 2008. Heat shock proteins: essential proteins for apoptosis regulation. *J. Cell. Mol. Med.*, **12**(3):743-761. [doi:10.1111/j.1582-4934.2008.00273.x]

Li, S., Chai, Z., Li, Y., Liu, D., Bai, Z., Li, Y., Li, Y., Situ, Z., 2009. *BZW1*, a novel proliferation regulator that promotes growth of salivary muocepodermoid carcinoma. *Cancer Lett.*, **284**(1):86-94. [doi:10.1016/j.canlet.2009.04.019]

Li, Y., Kang, X., Wang, Q., 2011. HSP70 decreases receptor-dependent phosphorylation of Smad2 and blocks TGF-beta-induced epithelial-mesenchymal transition. *J. Genet. Genomics*, **38**(3):111-116. [doi:10.1016/j.jgg.2011.02.001]

Mahalingam, D., Swords, R., Carew, J.S., Nawrocki, S.T., Bhalla, K., Giles, F.J., 2009. Targeting HSP90 for cancer therapy. *Br. J. Cancer*, **100**(10):1523-1529. [doi:10.1038/sj.bjc.6605066]

Mo, Y., Zheng, S., Shen, D., 1996. Differential expression of *HSU17714* gene in colorectal cancer and normal colonic mucosa. *Chin. J. Oncol.*, **18**(4):241-243 (in Chinese).

Neckers, L., 2007. Heat shock protein 90: the cancer

chaperone. *J. Biosci.*, **32**(3):517-530. [doi:10.1007/s12038-007-0051-y]

Piek, E., Roberts, A.B., 2001. Suppressor and oncogenic roles of transforming growth factor-β and its signaling pathways in tumorigenesis. *Adv. Cancer Res.*, **83**:1-54. [doi:10.1016/S0065-230X(01)83001-3]

Powers, M.V., Workman, P., 2007. Inhibitors of the heat shock response: biology and pharmacology. *FEBS Lett.*, **581**(19):3758-3769. [doi:10.1016/j.febslet.2007.05.040]

Prapapanich, V., Chen, S., Nair, S.C., Rimerman, R.A., Smith, D.F., 1996a. Molecular cloning of human p48, a transient component of progesterone receptor complexes and an Hsp70-binding protein. *Mol. Endocrinol.*, **10**(4):420-431. [doi:10.1210/me.10.4.420]

Prapapanich, V., Chen, S., Toran, E.J., Rimerman, R.A., Smith, D.F., 1996b. Mutational analysis of the hsp70-interacting protein Hip. *Mol. Cell Biol.*, **16**(11):6200-6207.

Prapapanich, V., Chen, S., Smith, D.F., 1998. Mutation of Hip's carboxy-terminal region inhibits a transitional stage of progesterone receptor assembly. *Mol. Cell Biol.*, **18**(2): 944-952.

Ralhan, R., Kaur, J., 1995. Differential expression of Mr 70000 heat shock protein in normal, premalignant, and malignant human uterine cervix. *Clin. Cancer Res.*, **1**(10): 1217-1222.

Roberts, A.B., 1998. Molecular and cell biology of TGF-β. *Miner. Electrolyte Metab.*, **24**(2-3):111-119. [doi:10.1159/000057358]

Roberts, A.B., Sporn, M.B., 1993. Physiological actions and clinical applications of transforming growth factor-β (TGF-β). *Growth Factors*, **8**(1):1-9. [doi:10.3109/08977199309029129]

Schiffer, M., von Gersdorff, G., Bitzer, M., Susztak, K., Böttinger, E.P., 2000. Smad proteins and transforming growth factor-β signaling. *Kidney Int.*, **58**(S77):45-52. [doi:10.1046/j.1523-1755.2000.07708.x]

Shi, Z., Bai, R., Fu, Z.X., Zhu, Y.L., Wang, R.F., Zheng, S., 2012. Induced pluripotent stem cell-related genes influence biological behavior and 5-fluorouracil sensitivity of colorectal cancer cells. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **13**(1):11-19. [doi:10.1631/jzus.B1100154]

Shi, Z.Z., Zhang, J.W., Zheng, S., 2007. What we know about ST13, a co-factor of heat shock protein, or a tumor suppressor? *J. Zhejiang Univ.-Sci. B*, **8**(3):170-176. [doi:10.1631/jzus.2007.B0170]

Soo, E.T., Yip, G.W., Lwin, Z.M., Kumar, S.D., Bay, B.H., 2008. Heat shock proteins as novel therapeutic targets in cancer. *In. Vivo*, **22**(3):311-315.

Söti, C., Csermely, P., 2002. Chaperones and aging: role in neurodegeneration and in other civilizational diseases. *Neurochem. Int.*, **41**(6):383-389. [doi:10.1016/S0197-0186(02)00043-8]

Takayama, S., Reed, J.C., Homma, S., 2003. Heat-shock proteins as regulators of apoptosis. *Oncogene*, **22**(56): 9041-9047. [doi:10.1038/sj.onc.1207114]

Wang, L.B., Zheng, S., Zhang, S.Z., Peng, J.P., Ye, F., Fang, S.C., Wu, J.M., 2005. Expression of ST13 in colorectal cancer and adjacent normal tissues. *World J. Gastroenterol.*, **11**(3):336-339.

Whitesell, L., Lindquist, S.L., 2005. HSP90 and the chaperoning of cancer. *Nat. Rev. Cancer*, **5**(10):761-772. [doi:10.1038/nrc1716]

Yang, M., Cao, X., Yu, M.C., Gu, J.F., Shen, Z.H., Ding, M., Yu, D.B., Zheng, S., Liu, X.Y., 2008. Potent antitumor efficacy of *ST13* for colorectal cancer mediated by oncolytic adenovirus via mitochondrial apoptotic cell death. *Hum. Gene Ther.*, **19**(4):343-353. [doi:10.1089/hum.2007.0137]

Yang, M., Yu, M., Guan, D., Gu, J., Cao, X., Wang, W., Zheng, S., Xu, Y., Shen, Z., Liu, X., 2010. ASK1-JNK signaling cascade mediates Ad-ST13-induced apoptosis in colorectal HCT116 cells. *J. Cell. Biochem.*, **110**(3): 581-588. [doi:10.1002/jcb.22551]

Ye, Y.W., Wu, J.H., Wang, C.M., Zhou, Y., Du, C.Y., Zheng, B.Q., Cao, X., Zhou, X.Y., Sun, M.H., Shi, Y.Q., 2011. Sox17 regulates proliferation and cell cycle during gastric cancer progression. *Cancer Lett.*, **307**(2):124-131. [doi:10.1016/j.canlet.2011.03.024]

Young, J.C., Agashe, V.R., Siegers, K., Hartl, F.U., 2004. Pathways of chaperone-mediated protein folding in the cytosol. *Nat. Rev. Mol. Cell Biol.*, **5**(10):781-791. [doi:10.1038/nrm1492]

Yu, D.B., Zhong, S.Y., Yang, M., Wang, Y.G., Qian, Q.J., Zheng, S., Liu, X.Y., 2009. Potent antitumor activity of double-regulated oncolytic adenovirus-mediated ST13 for colorectal cancer. *Cancer Sci.*, **100**(4):678-683. [doi:10.1111/j.1349-7006.2009.01110.x]

Zhang, Y., Cai, X., Schlegelberger, B., Zheng, S., 1998. Assignment of human putative tumor suppressor genes *ST13* (alias SNC6) and *ST14* (alias SNC19) to human chromosome bands 22q13 and 11q24→q25 by in situ hybridization. *Cytogenet. Cell Genet.*, **83**(1-2):56-57. [doi:10.1159/000015125]

Zheng, S., Cai, X., Cao, J., 1997. Application of subtractive hybridization in screening for colorectal cancer negatively related genes. *Natl. Med. J. China*, **77**(4): 256-259 (in Chinese).