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Original Article

The oncogenic role of MST3 in human gastric cancer

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Abstract: MST3 (mammalian STE20-like kinase) is one of the protein kinase of the GCK III subfamily STE 20, and is known to play a role in cell growth and apoptosis. Our laboratory has demonstrated that MST3 promotes tumorigenicity through the VAV2/Rac1 signal axis in breast cancer. In this report, we further investigated the potential oncogenic role of MST3 in gastric cancer. Examination of tissue samples from 101 gastric cancer patients revealed that higher expression of MST3 was observed in tumor part with immunohistochemistry. Furthermore, high expression of MST3 predicts poor prognosis in gastric cancer patients. To investigate the function of MST3 in vitro, MKN45 and NCI-N87 cell lines were transfected with the MST3 shRNA and stable clones were established. Downregulation of MST3 inhibited cell proliferation. The p21 expression was enhanced by MST3 shRNA in MKN45 gastric cancer cell line. Finally, downregulation of MST3 attenuated the anchorage-independent growth in soft agar and tumor growth in NOD/SCID mice. Altogether, our results indicate that MST3 potentially plays an oncogenic role in gastric cancer.

Keywords: MST3, gastric cancer, anchorage-independent growth, p21

Introduction

Sterile 20 (ste20) is a serine/threonine protein kinase locating upstream of MAPK kinase (a yeast mitogen-activated protein kinase kinase kinase kinase (MAP4K), and they are involved in the distinct signaling pathway in Saccharomyces cerevisiae [1]. These pathways include pseudohyphal development, invasive growth, cell integrity, sporulation, and response to high extracellular osmolality [2]. Based on the regulation and structure, the mammalian Ste20 consists of two subfamilies, including the p-21 activated kinase family (PAK) and the germinal center kinase (GCK) family. The Ste20-related kinases are characterized by the presence of a kinase domain and a non-catalytic region with a great structural diversity. PAK has two subfamily, PAK I and PAK II. GCK has eight subfamily, GCK I-VIII [3]. Four mammalian ste20-related (MST) kinases have been identified. MST1 and MST2 belong to GCK II subfamily. MST3 and MST4 are members of the GCK III subfamily [5]. In Drosophila, hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with Salvador and warts. Hippo pathway regulates both cell proliferation and cell death [3]. MST1 is induced by oxidative stress, and activates the caspase-mediated pathway and cell apoptosis in human BJAB (B lymphoma cell) and 293T (Human embryonic kidney cell) cell lines [6]. MST1 and MST2 double-knockout mice would cause early embryonic death. Phenotype of the MST-1/2 conditional knock mice demonstrates the aberrant organ overgrowth including liver, intestine, pancreas and heart, and even presents cancer cell development. MST1/MST2 is considered to be a tumor suppressor gene [7].

MST4 promotes cell growth and transformation via modulation of the ERK pathway [8]. In zebrafish, MST4 binds to CCM3 (cerebral cavernous malformation 3) to regulate the cardiovascular development and cavernous malformation. MST4 could form a stable complex with CCM3 to promote cell proliferation and migration. CCM is a common human vascular disease that will result in stroke, seizure, and cerebral hemorrhage [9, 10]. MST3 was identified to express widely in different brain regions of human brain, and it was regulated by cAMP-dependent protein kinase [11]. MST3 contrib-

uted the caspase-mediated proteolytic activation in apoptosis [12]. The first identified substrate for MST3 is NDR protein kinase [13]. NDR protein kinase are involved in the regulation of cell cycle progression and morphology. MST3 could affect cell cycle by regulating NDR protein. NDR protein could promote G1 progression by stabilizing c-myc and preventing p21 accumulating, indicating a potential oncogenic role for MST3 [14]. On the other hand, MST3 inhibit cell migration by regulating paxillin phosphorylation through tyrosine phosphatase PTP-PEST [15]. We further demonstrated that MST3 promotes proliferation and tumorigenicity through the VAV2/Rac1 signal axis in breast cancer. MST3 is overexpressed in human breast tumors, and overexpression of MST3 in human breast cancer predicts poor prognosis in human breast cancer patients as demonstrated by Kaplan-Meier plotter analysis [16]. Recently, MST3 inhibitor was identified and capable of inhibiting cell migration through inhibition of MST3 in the study [17]. Altogether, the function of MST3 on growth and migration appears to be cell-type dependent.

Gastric cancer is the 5th most common cancer in the world. It is the third leading causes of cancer mortality with widely varying incidence worldwide. Until now, surgery is the main therapeutic approach. There is no definite effective medical therapy for late gastric cancer. Early detection is difficult in most countries. Advanced disease carries a poor prognosis and treatment remains challenging with a 5-year OS rate < 5% [18]. Therefore, more understanding the molecular profiling of gastric cancer may be an aid for diagnosis and treatment. Since the biological function of MST3 is unclear in gastric cancer, we aimed to identify the biological function of MST3 by studying the pathological samples and the gastric cancer cell lines. Higher expression of MST3 predicts poor prognosis and downregulation of MST3 leads to decrease of anchorage-independent growth. Our results indicated that MST3 plays an oncogenic role in gastric cancer. MST3 inhibition may be a feasible cancer therapy in the future.

Material and methods

Study participants

A total 101 patients who were diagnosed with gastric cancer at the National Cheng Kung University Hospital from Jan 1998 to Dec 2006 were enrolled after obtaining a formal written informed consent. Patient demographics, histopathological findings, and outcomes were recorded from retrospective chart review. All patients received follow-up annually. The overall survival rate was defined as the period from surgery until death. These patient's formalin-fixed, paraffin-embedded tissue samples were obtained from surgical procedure. This study was approved by the Institutional Review Board of the National Cheng Kung University Hospital (NCKUH IRB number: ER-97-175).

Immunohistochemistry

Serial 5 μm-thick sections were cut from formalin-fixed paraffin-embedded samples. The sections were de-paraffinized in xylene and rehydrated in graded alcohol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. For heat-induced epitope retrieval, the sections were immersed in 10 mM citrate buffer and heated under pressure. The sections were next incubated overnight at 4°C with an anti-MST3 monoclonal antibody (1:200, EP1468Y, abcam, United Kingdom). The sections were next incubated with the avidin-biotin complex reagent (DAKO, California, United States) and final color development was achieved with 3-amino-9-ethyl carbazole (Zymed, California, United States). The sections were counterstained with Mayer's hematoxylin and then mounted.

Calculating H-score

Our immunohistochemistry results of gastric cancer were further evaluated by a semi-quantitative approach to assign an H-score (or "Histo" score) to tumor samples. First, membrane-staining intensity (0, 1+, 2+, 3+) is determined for each cell in a fixed field. The H-score was based on a predominant staining intensity, or more complexly, included the sum of individual H-scores for each intensity level seen. By the method, an H-score is assigned using the following formula: [1 × (% cell 1+) + 2 × (% cell 2+) + 3 × (% cell 3+)].

The final score, ranging from 0 to 300, give more relative weight to higher-intensity membrane staining in given tumor sample [19, 20].

We examined all 101 tumor specimens, and got an H-score in every specimen. We obtained overall survival curve according to MST3 expression using the Kaplan-Meier method.

Table 1. Basic Demographic and Clinical pathological Data of 101 patients with gastric cancer

Clinical Variables	No. (n = 101)	Percentage
Sex		
Male	63	62.4%
Female	38	37.6%
Age		
≤ 40 y/o	3	3%
41-50 y/o	21	20.8%
51-60 y/o	10	9.9%
61-70 y/o	32	31.7%
> 70 y/o	35	34.7%
Number of lesions		
Single	98	97.1%
Two	3	2.9%
Tumor site		
Cardia	16	15.8%
Body	25	24.8%
Antrum	43	42.6%
Pylorus	7	6.9%
Angularis	4	4%
Others	6	5.9%
Tumor configuration		
Early	16	15.8%
Advanced	85	84.2%
Histologic type		
Tubular/well-differentiated	8	7.9%
Tubular/moderately-differentiated	35	34.7%
Tubular/poorly-differentiated	31	30.7%
Signet ring	16	15.8%
Mucinous	4	4%
Mixed	7	6.9%
Stage		
I	29	28.7%
II	23	22.8%
III	27	26.7%
IV	22	21.8%
Lymph node metastasis		
Yes	61	60.4%
No	40	39.6%
Perineural invasion		
Yes	46	45.5%
No	55	54.5%
Depth of invasion: (serosa invasion)		
Yes	46	45.5%
No	55	54.5%
Tumor size (n = 101)	Size (cm)	
Average	4.76	
Median	4.2	
Max	14	
Min	0.5	

n = number of patients.

Statistical analysis

All statistical analyses were performed using SPSS (Version 20) for Windows. The contingency tables were analyzed using Fisher's exact probability test or the Chi square test. Correlation between the continuous and categorical variables was evaluated using the Mann-Whitney U test for two groups and the Kruskal-Wallis for more than three groups. The overall survival (OS) times were calculated from the date of surgery to the date of a patient's death. OS probabilities were calculated using the Kaplan-Meier product limit method and intergroup differences were determined using log-rank test. A P value < 0.05 was considered statistically significant.

Cell culture, transfections, and treatments

All the gastric cancer cell lines (MKN45 and NCI-N87) used in this study were maintained in RPMI1640 Medium supplemented with 10% fetal bovine serum (FBS). Cells were incubated under 5% CO_2 in air atmosphere in a suitable 37°C incubator. The stable clones MKN45 or NCI-N87 (pLKO.1-shLuc), MKN45 or NCI-N87 (shMST3)-1 and -2 were transfected using TurboFect (Thermo, USA) and maintained in the same cultural medium containing 1 μg/mL puromycin (Sigma, USA). To monitor the efficacy of shMST3, the expression of MST3 in MKN45 and NCI-N87 stable transfectants was analyzed by Western blotting. shMST3 is obtained from RNAi core in Academic Sinica (Clone ID. TRCN 0000000645, NM_003576).

Immunoblot analysis

Cells lysate was prepared by incubating MKN45 or NCI-N87 cells (about 2×10^6) with 50 μL RIPA buffer (1% Triton X-100, 0.1% SDS, 50 mM Tris, pH8.0, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Na-deoxycholate, 5 μg/mL leupeptin, and 5 μg/mL aprotinin) at 4°C for 30 min. The particulates were removed from cell lysates by centrifugation at 4°C and a speed of 16,100 × g for 20 min. Cell lysates or tissue crude extracts (40 μg each) were loaded on a 12% SDS-polyacrylamide electrophoresis gel (SDS-PAGE). Following electrophoresis, the protein bands were transferred to a polyvinylidine difluoride (PVDF) membrane (Millipore) by electroblotting. Immunoblot analysis was perfo-

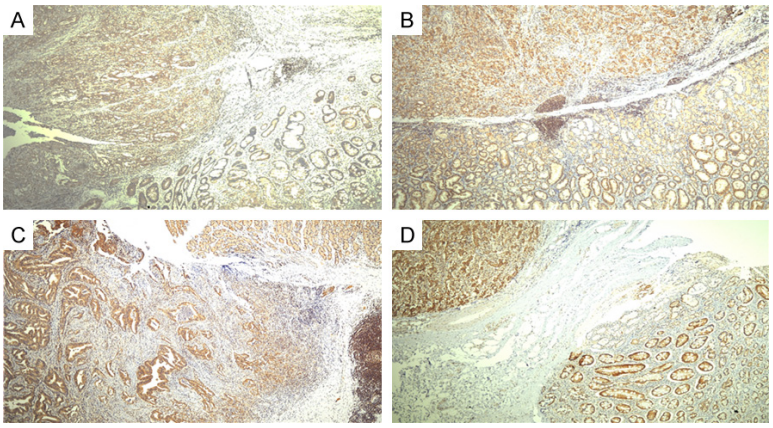

Figure 1. MST-3 expressions among normal and cancer cells from human gastric cancer patients. (A-D) Immunohistochemistry revealed MST-3 expressions in normal or tumor part. (A-D) from the different gastric cancer patient.

rmed using antibodies specifically against MST3 (1:1000 dilution; BD #611057, USA), p21 Waf1/Cip1 (DCS60) (1:2000 dilution, Cell Signaling #2946, USA), GAPDH (1:5000 dilution; #GTX 100118, GeneTex, USA), Cyclin B1 (C-term) (1:10000 dilution; EPITOMICS #14-95-1, USA), cyclin A (C-19) (1:1000 dilution; Santa Cruz #sc-596, USA), Cyclin E1 (1:1000 dilution; EPITOMICS #1655-1, USA), Cyclin D1 (DCS6) (1:2000 dilution; Cell Signaling #29-26, USA), p27 Kip1 (1:1000 dilution; Cell Signaling #2552, USA) Protein bands were visualized by using ECL Chemiluminescence kit.

Cell proliferation assay

Premixed WST-1 Cell Proliferation assays were performed according to the manufacturer's instructions (Clontech). A density of 2×10^3 MKN45 (4×10^3 for NCI-N87) cells/well were seeded in 100 µl culture medium in flat bottom 96-well tissue culture plates. Reserved one well without cells for a background control (culture medium only). Cells were cultured for 24 hr at 37°C in a humidified atmosphere maintained at 5% CO_2. 10 µl of Premixed WST-1 Cell Proliferation Reagent was added to each well. The cells were incubated for 2 hr at 37°C and 5% CO_2. The absorbance of the samples were measured at 450 nm against the background control, using a multiwell plate reader.

Soft agar colony formation assay

1.5 mL of mixture of serum-supplemented medium and 0.6% agar at 40°C were added in 6-well plates and allowed to solidify (base agar). Next, a mixture of serum supplemented medium and 0.3% agar (total of 1 mL) containing 10^4 cells at 40°C was added on top of the base layer and allowed to solidify (top agar). Subsequently, the dishes were kept in tissue culture incubator maintained at 37°C and 5% CO_2 for two or three weeks to allow for colony growth. All assays were done in triplicate. The colony assay was terminated at day 14 for MKN45 cell (day 21 for NCI-N87 cell). The plates were stained with 0.5% crystal violet solution in 25% methanol overnight at 4°C. Colonies were counted and photographed under microscope (40 ×), and the colony size was determined by ImageJ software.

Xenograft models

To assess the effect of MST3 on tumor growth, five NOD-SCID mice (8 to 10 week old, male) were s.c. injected with MKN45/shLuc, MKN45/shMST3-1, and MKN45/shMST3-2 (10^6 cells in 100 µL of serum free RPMI1640 Medium) in the ventral region of mice. Tumor growth was monitored and recorded twice a week by measuring the perpendicular tumor diameters, length (L) and width (W), with a vernier caliper. The volume of tumor (V) was calculated by the formula $V = (L \times W^2) \times 0.52$. Mice were sacrificed under anesthesia four week after implantation of tumor cells.

Results

Higher expression of MST3 in gastric cancer patients

A total of 101 tissue samples were obtained from gastric cancer patients who underwent a gastrectomy in National Cheng Kung University hospital. Patient characteristics are presented in **Table 1**. The average age of the study objects was 64 years (range between 22-91 years), and the ratio of men to women was 1.7:1. Ninety-eight patients had single tumor, and three patients had two tumors synchronously. The tumor site was located at the cardia in 16 patients, the body in 25 patients, the

Table 2. Association of MST3 expression level with clinical pathological variables

Clinical Variables	MST3 < 150 (n = 58)	MST3 ≥ 150 (n = 43)	*P* value
Sex			
Male	32	31	0.08
Female	26	12	
Age			
< 65	29	14	0.08
≥ 65	29	29	
Number of Lesion			
Single	58	40	0.041*
Two	0	3	
Number of Tumor Site			
Single	57	38	0.037*
Multiple	1	5	
Tumor Configuration			
Early	13	3	0.036*
Advanced	45	40	
Histologic type			
Tubular/well-differentiated	4	4	
Tubular/moderately-differentiated	17	18	0.476
Tubular/poorly-differentiated	18	13	
Signet ring	11	5	
Mucinous	2	2	
Mixed	6	1	
Stage			
I	19	10	0.479
II	11	12	
III	17	10	
IV	11	11	
Lymph node metastasis			
Yes	32	29	0.213
No	26	14	
Perineural invasion			
Yes	25	21	0.567
No	33	22	
Depth of invasion: serosa invasion			
Yes	28	18	0.522
No	30	25	
Tumor size (cm)			
Average	4.907	4.572	0.541
Median	4.5	4	
Max	14	9	
Min	0.5	1	

n = number of patients; *P value < 0.05.

antrum in 43 patients, the pylorus in 7 patients, the incisura angularis in 4 patients, and the other sites in 6 patients. Sixteen patients are early type, and 85 patients are advanced type in tumor configuration. There were 8 patients with tubular carcinoma/well-differentiated, 35 patients with tubular carcinoma/moderately-differentiated, 31 patients with tubular carcinoma/poorly-differentiated, 16 patients with signet ring cell, 4 patients with mucinous carcinoma, and 7 patients with mixed-type carcinoma. According to AJCC cancer staging manual, there were 29 patients with stage I, 23 patients with stage II, 27 patients with stage III, and 22 patients with stage IV disease. MST3 expression in the normal, intestinal metaplasia, and tumor part was examined. MST3 expression was observed in the cytoplasm of gastric cancer cell. MST3 expression were higher and more heterogeneous in the tumor part than that in non-tumor part (**Figure 1**).

Correlation of MST3 IHC scores with clinicopathological variables

We observed a significant correlation of MST3 IHC score with number of lesion and tumor configuration (**Table 2**). There was no correlation of MST3 IHC scores with other clinicopathological variables. However, there was a trend to express higher MST3 IHC score in male and old-aged patients (P = 0.08).

Patients with higher MST3 IHC scores had poor survival rates

We then examined overall survival curve according to MST3 expression using the Kaplan-Meier method (**Figure 2**). Patients with higher MST3 expression (IHC score ≥ 150) had a significantly poorer overall survival than did patients with expressions level below the cut-off values (P = 0.025, < 0.05).

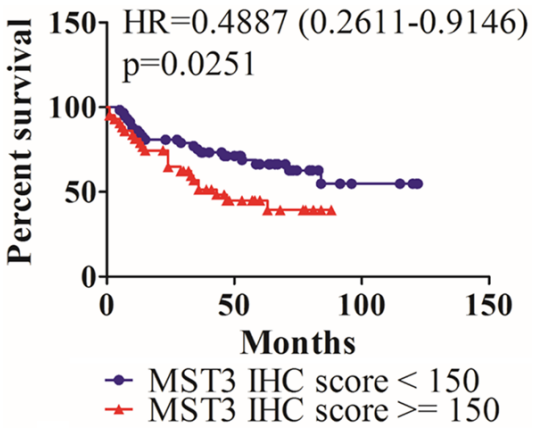

Figure 2. Kaplan-Meir survival analysis demonstrated overall survival curves according to MST3 expressions. Patients with higher MST3 expression (H score ≥ 150, n = 43) had significantly poorer prognosis than lower MST3 expression (H score < 150, n = 58) (Log rank test P = 0.025).

Knock down of MST3 inhibited cell proliferation and growth

In order to analyze the influence of MST3 in human gastric cancer, we first determined the mRNA and protein amount of MST3 in human gastric cancer cell lines, MKN45 and NCIN87 (**Figure 3**). The MST3 expression was effectively downregulated in MKN45 and NCIN87 cell lines (**Figure 3B**). MST3 shRNA inhibited the cell growth of MKN45 and NCIN87 cell lines. Two stable transfectants of each cell line displayed low growth profile (**Figure 3C**). It has been reported that p21 is a MST3 downstream effector. It was interesting to note that p21 was significantly upregulated by MST3 shRNA in MKN45, but the expression of p21 was not altered in NCIN87 cell line (**Figure 3B**). p21 may play an important role in MST3-mediated growth inhibition in MKN45, but not in NCIN87.

The effects of MST3 knockdown on cell regulators

Since p21 alone cannot explain the decrease of cell growth, we determined the influences of MST3 on cell regulators, including cyclin B1, A, E1, D1, and p27 by western blotting. MKN-45 and NCI-N87 cells were transfected with control shRNA or shRNA against MST3, and cell lysates of stable transfectants were used for the western blotting. Cyclin D1 is weakly increased in the MKN45 MST3 knockdown clone, but consistent changes of other cell cycle regulators were not observed (**Figure 4**).

Knockdown of MST3 suppressed cell proliferation in vitro and tumor growth in vivo

We then determined the effects of MST3 knockdown on anchorage-independent growth. Downregulation of MST3 in MKN45 and NCI-N87 (**Figure 5A**) inhibited the cell growth in soft agar. The MST3 stable knockdown transfectants display attenuated anchorage-independent growth. Finally, we also investigated the influence of MST3 knockdown on the tumor growth rate in NOD-SCID mice. The mice were implanted with MST3-knockdown MKN45 cells or control transfectants. Two MST3 knockdown transfectants exhibited smaller tumor volume when compared with control transfectants (**Figure 5B**).

Discussion

In this report, we explored the biological effects of MST3 in human gastric cancer. First, we identified MST3 expression in human gastric normal and cancer cells. We further found that the patients with higher MST3 expressions had advanced disease, more tumor numbers, and higher occurrence sites. We demonstrated that patients with higher IHC score (≥ 150) of MST3 would have poor survival rates. MST3 expression is a negative prognostic factor in human gastric cancer. We further tried to identify the oncologic mechanism about MST3 expression in human gastric cancer. Downregulation of MST3 in gastric cell line induced p21 overexpression in MKN45 cell. Therefore, we propose that MST3 may promote cell proliferation through cell cycle by activating cyclin-dependent kinase enzymes. In addition, we also demonstrated that knockdown MST3 decreased anchorage-independent growth. It was interesting to note that knockdown of MST3 in another gastric cancer cell line NCI-N87 also inhibited the cell growth and anchorage-independent growth. However, examination of many cell cycle markers did not reveal the intrinsic mechanism. Further study is awaited to reveal the possible mechanism. Finally, MKN45 control transfectants exhibits higher tumor growth than MST3 knockdown transfectant in NOD-SCID mice. Our study suggests that MST3 may regulate different pathways to control cell growth in gastric cancer cells. To our best knowledge, this is the first study to demonstrate the biological functions of MST3 in human gastric cancer.

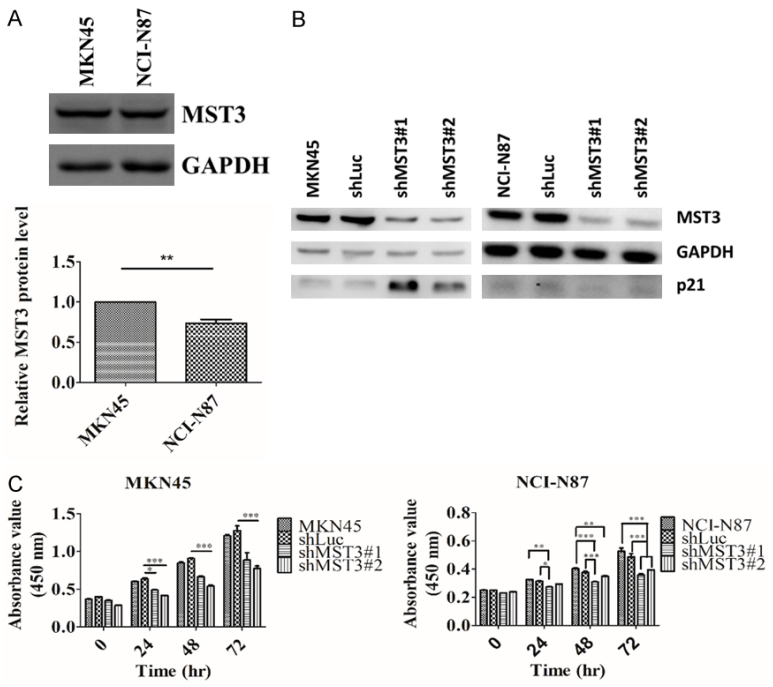

Figure 3. MST3 shRNA attenuated the cell growth of gastric cancer cell lines. A. Protein expression level of the MST3 in gastric cancer cell lines (MKN45 and NCI-N87) by western blotting. B. shRNA against MST3 downregulates MST3 expression and induces p21 upregulation in MKN45. MKN45 and NCI-N87 were transfected with MST3 shRNA or control Lucifrease shRNA and the stable clones were selected. The cell lysates were analyzed with western blotting probed by anti-MST3 Ab and anti-p21 Ab. GAPDH was used as control. C. The cell growth rates of parental MKN45 and NCI-N87, control transfectants, and MST3 shRNA transfectants were determined with WST-1 Cell Proliferation assays at different time intervals ranging from 0 to 96 h. Mean ± SEM of absorbance from each group (n = 3) is shown. All *P* values were obtained by two-way ANOVA.

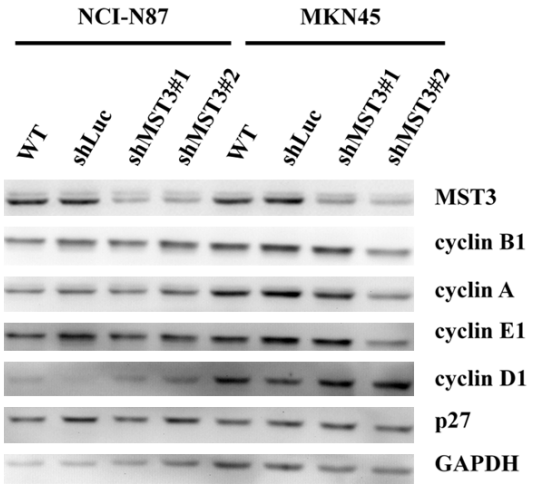

Figure 4. The effects of MST3 knockdown on the cell cycle regulators. The cell lysates of MKN45 and NCI-N87 cells, control transfectants, MST3 shRNA transfectants were analyzed with western blotting and probed with different antibodies include anti-cyclin A, B1, D1, E1 and p27 Abs.

Ste20 plays an important role in regulating the pheromone response pathway in budding yeast [21]. Ste20 is the upper stream kinase of MAP kinase pathway. Ste20 can be categorized into two subfamilies, PAK (p21-Rac/Cdc-42 activated kinase) and GCK (germinal center kinase), based on their amino acid sequences and the physical location of their kinase domains [22]. MST3 was belonged to GCK family. In the early study, MST3 was shown to play a role in caspase-mediated proteolytic cleavage during apoptosis [12]. MST3 could promote apoptotic cell death. However, MST3 could function as an NDR upstream kinase by phosphorylating NDR [13]. NDR protein kinase is known to be involved in the regulation of cell cycle progression and morphology. Therefore, MST3 seems to have multifaceted functions in regulating cell growth.

MST3 was found to cause tumorigenesis on human cancer cell. MST3 would interact with striatin protein to form a large STRIPAK complex. The STRIPAK complex is a regulator of the actomyosin cortex. The STRIPAK components determine mode of cancer cell migration and metastasis in breast cancer model [23]. In the previous study, we have found that MST3 could promote proliferation and tumorigenicity through the VAV2/Rac1 signal axis in breast cancer [16]. MST3 induces cyclin D1 by binding VAV2 and enhancing Rac1 activation to promote the tumorigenicity of breast cancer. Downregulating of MST3 inhibits proliferation and tumorigenicity in triple-negative breast cancer cell lines. These evidences supports MST3 plays an oncological role in human cancers.

Herein, we further identify the oncological roles in human gastric cancer. Based on analysis on clinical samples, we found more MST3 expression in the gastric patients with two lesions in

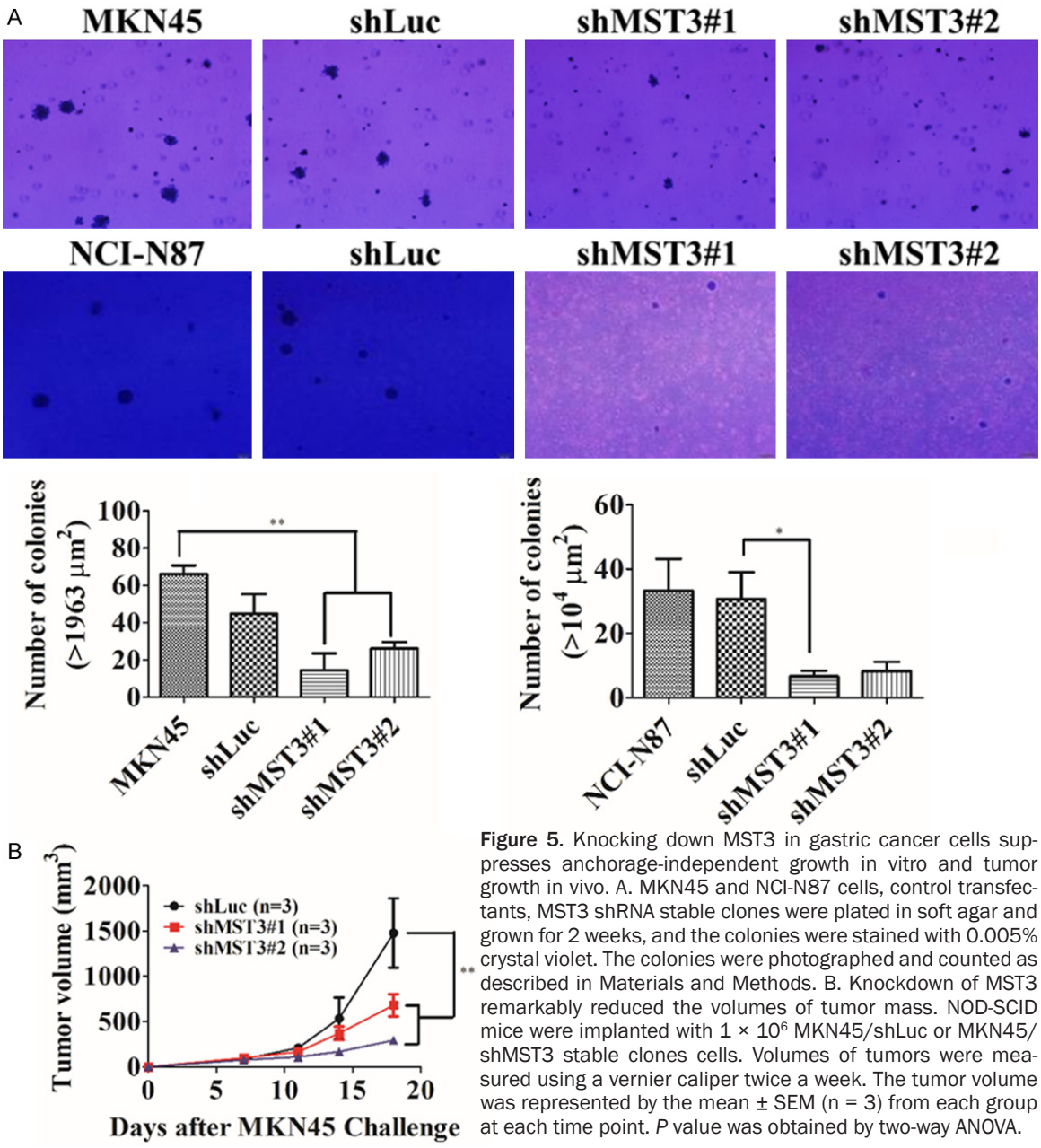

Figure 5. Knocking down MST3 in gastric cancer cells suppresses anchorage-independent growth in vitro and tumor growth in vivo. A. MKN45 and NCI-N87 cells, control transfectants, MST3 shRNA stable clones were plated in soft agar and grown for 2 weeks, and the colonies were stained with 0.005% crystal violet. The colonies were photographed and counted as described in Materials and Methods. B. Knockdown of MST3 remarkably reduced the volumes of tumor mass. NOD-SCID mice were implanted with 1×10^6 MKN45/shLuc or MKN45/shMST3 stable clones cells. Volumes of tumors were measured using a vernier caliper twice a week. The tumor volume was represented by the mean ± SEM (n = 3) from each group at each time point. *P* value was obtained by two-way ANOVA.

surgery, multiple occurrence sites, and advance tumor figuration. These findings indicated that MST3 was a negative prognostic factor. Kaplan-Meier plotter analysis indicated that the gastric cancer patients with higher MST3 expression would have poor survival rates. More MST3 expression in cancer cells than that in normal gastric tissues by immunohistological staining.

In a previous study, MST3 is an upstream kinase of NDR protein. MST3 could regulate p21 stability by phosphorylating NDR kinase. We demonstrated that knockdown of MST3 expression in human gastric cancer cell (MKN45) upregulated p21. In assaying anchorage-independent growth, knockdown of MST3 expression in human gastric cancer cell would inhibit cell growth in soft agar. These experiments demonstrated MST3 could affect cell growth in human gastric cancer cell.

There are some limitations in our study. We did not explore the impact of MST3 on apoptosis in human gastric cancer. MST3 may have dual biological functions in cell progression. MST3 may inhibit cell growth by caspase pathway, but promote cell growth by NDR pathway. In this study, we postulate that tumor cell growth path-

way was dominant over cell apoptosis pathway in gastric cancer cell progression. Recently, there are small MST3-targeted molecules available for study on treating cancers [17]. Human gastric cancer is a major cancer disease. Most of gastric cancer patients presented late-staged disease, and poor survival rates. Novel and effective treatments are required for these patients. The MST3 inhibition may be a feasible treatment option. In the future, we can test the treatment effects of MST3 inhibitor in human gastric cancer cell.

In summary, we demonstrated that MST3 is a tumor oncogene in human gastric cancer. The patients with higher MST3 expression will have poor clinical outcomes. MST3 promote growth and proliferation of gastric cancer cell in vitro and in vivo. The MST3 pathway may employ as a therapeutic target for gastric cancer.

Acknowledgements

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Disclosure of conflict of interest

None.

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