Suppression of Survivin Expression Inhibits in Vivo Tumorigenicity and Angiogenesis in Gastric Cancer

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ABSTRACT

Survivin plays an important role in cancer development. We aim to show here that suppression of survivin expression or function by antisense and dominant-negative (DN) mutant can inhibit gastric cancer carcinogenesis and angiogenesis in vivo. Plasmid constructs expressing survivin antisense and DN mutant replacing the cysteine residue at amino acid 84 with alanine (Cys84Ala) were prepared and introduced into BCG-823 and MKN-45 gastric cancer cells to establish stable transfectants. We showed that both antisense and DN mutant stable transfectants exhibited abnormal morphology, with decreased cell growth and increased rate of spontaneous apoptosis and mitotic catastrophe. Furthermore, in nude mice xenografts, these cells exhibited decreased de novo gastric tumor formation and reduced development of angiogenesis. Results from these studies strongly suggest that survivin is a promising target for gastric cancer treatment.

INTRODUCTION

Survivin, a structurally unique IAP family protein, is expressed in mitosis in a cell cycle-dependent fashion and localized to components of the mitotic apparatus (1). It is potentially involved in both the inhibition of apoptosis and control of cell division (2, 3). Survivin is found in most human cancers but is either undetectable or expressed at a very low level in differentiated adult tissues (4). In most cancers, expression of survivin correlated with reduced apoptotic index, poor prognosis, and increased risk of recurrence (5–8). Antisense targeting of survivin results in the dysregulation of mitotic spindle checkpoint as well as defects in microtubule assembly and function that lead to mitotic catastrophe (9–12). Mitotic catastrophe is the form of cell death that results from aberrant mitosis, which is characterized by supernumerary centrosomes, failure of cytokinesis, and a significant increase in the percentage of abnormal nuclei, the appearance of which includes multiple multilobulated nuclei and abnormally large-sized nuclei (13). A survivin DN Thr34Ala mutant, with the threonine residue at amino acid 34 changed to alanine and the phosphorylation site for p34cdc2-cyclin B1 abolished, has also been shown to cause apoptosis and inhibit tumor formation and tumor growth (14–16). These unique features of survivin make it a promising target for cancer therapy. However, most of the studies regarding the role of survivin in apoptosis and cell division are performed using the transient expression method. Furthermore, the in vivo mechanism of targeting survivin in cancer treatment is not fully understood (17, 18).

It has been shown that survivin is up-regulated in angiogenically stimulated endothelium in vitro and in vivo but undetectable in quiescent endothelial cells (19, 20). Angiogenic agents, such as vascular endothelial growth factor and basic fibroblast growth factor, induce survivin expression in endothelial cells (21). Because tumor angiogenesis depends on endothelial viability, it is conceivable that targeting survivin may favor apoptotic involution of newly formed blood vessels and indirectly inhibit tumor formation. However, whether knock-down of survivin function in tumor cells will affect angiogenesis in vivo remains unknown.

Gastric cancer is one of the most common malignant tumors worldwide. Studies show that 35–82.6% of gastric cancers expressed survivin, and survivin expression correlated with poor survival of patients (22–24). Moreover, up-regulation of survivin is found in gastric cancer cell lines after treatment with cytotoxic drugs, indicating that survivin contributes to chemoresistance in gastric cancer (25). In this study, we aimed to study the effect of constitutive suppression of survivin on gastric cancer carcinogenesis using a DN mutant of survivin, replacing the cysteine residue at amino acid 84 with alanine (Cys84Ala), that binds to the mitotic apparatus and displaces wild-type survivin from polymerized microtubules (19). We established stable cell lines expressing Surv-AS cDNA or DN Surv-Mut (Cys84Ala) and investigated the effect of targeting survivin in gastric cancer treatment. Our results showed that suppression of survivin induced both apoptosis and mitotic catastrophe in vitro and in vivo in gastric cancer epithelial cells. The antisense or mutant (Cys84Ala)-mediated suppression of survivin function also inhibited tumor formation and angiogenesis in gastric cancer xenograft model in vivo.

MATERIALS AND METHODS

Cell Lines and Cell Culture. We selected two human gastric adenocarcinoma cell lines for this study. BCG-823 (Beijing Institute of Cancer Research, Beijing, China) is an adherent, poorly differentiated, human gastric adenocarcinoma cell line with mutant p53 (26–28), and the MKN-45 cell line (RIKEN, the Institute of Physical and Chemical Research, Cell Bank, Ibaraki, Japan) expresses wild-type p53; however, it has p21, p16 homozygous deletion, and p27 rearrangement (29). Both cell lines are gastric cancer epithelial cells and grow as adherent cells in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) containing 10% FCS (Life Technologies, Inc.), 2 mM L-glutamine (Bio-Whittaker, Walkersville, MD), 100 units/ml penicillin, and 100 μg/ml streptomycin (BioWhittaker). All cell lines were maintained at 37°C in a humidified incubator with an atmosphere of 5% CO2, Cisplatin and 5-fluorouracil (Pharmac & Upjohn, Ltd., Corp., Chatswood, Australia) were solubilized in DMSO to concentrations of 2 and 10 μg/ml, respectively, and stored at 4°C.

Construction of Surv-AS and DN Mutant Plasmids. Total RNA was extracted from MKN-45 cells using Trizol reagent (Life Technologies, Inc.). cDNA coding survivin was generated by reverse transcription-PCR using the survivin forward primer (5’-GGGAATTCTATGGGTTGCCGGCCACGTGGCC-3’) and reverse primer (5’-CTTCCAATCACTTGGCAGCCGCAGCT-3’). Genet Sense Biotech, Pre Ltd., Singapore). The PCR product was inserted into the EcoRI and Xhol sites of pcDNA3(+) and pcDNA3(−) (Invitrogen,

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The abbreviations used are: IAP, inhibitor of apoptosis protein; DN, dominant-negative; Surv-AS, survivin antisense; Surv-Mut, survivin mutant; MITT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL, terminal deoxynucleotidyltransferase-mediated nick end labeling; MVD, microvessel density; PI, propidium iodide.
Groningen, the Netherlands) to generate pcDNA3-surivin and pcDNA3(-)Sur-AS plasmids, respectively.

We used an overlap extension PCR to construct a DN mutant of survivin using pcDNA3-survivin plasmid as the template. The 5'-flanking forward primer and 3'-flanking reverse primer were as described above. For Sur-Mut Cys84Ala, the forward primer was 5'-ATTCGTCCGCGCGCTTTCCCT TCTG-3', and the reverse primer was 5'-CAGAAAGGAAAGCGGCGCGG- GAGCAAT-3', which produced a TGC-to-GCC substitution at nucleotide 251-253 and resulted in replacement of the cysteine residue at amino acid 84 with an alanine. The fragment containing Sur-Mut was cloned in the EcoRI and XhoI site of the pcDNA3 vector, generating pcDNA3-Sur-Mut (Cys84Ala) plasmid. The precision of all of the constructs was confirmed by sequencing.

Establishment of BCG-823 and MKN-45 Stable Transfectants Expressing Su-AS and Sur-Mut. For transfection experiments, BCG-823 and MKN-45 cells were plated into 6-well plates (3 × 10⁵ cells/well) 18 h before transfection. The cells were transfected with 4 μg/well of empty pcDNA3 vector, pcDNA3-Sur-AS, or pcDNA3-Sur-Mut (Cys84Ala) plasmid using LipofectAMINE 2000 (Life Technologies, Inc., Grand Island, NY) according to the manufacturer's instructions. Forty-eight h after transfection, the cells were passaged at 1:15 (v/v) and cultured in medium supplemented with Geneticin (G418) at 1000 μg/ml for 4 weeks. Stably transfected clones were picked and maintained in medium containing 400 μg/ml G418 for additional studies.

Assay for Anchorage-Independent Cell Growth. Cells (1 × 10⁵) were plated in complete culture medium containing 0.3% agar on top of 0.6% agar in the same medium. All dishes were incubated at 37°C in a humidified atmosphere of 5% CO₂. Colonies were scored 16 days after plating, fixed with 70% ethanol, stained with Coomassie Blue, and counted under a dissection microscope. Colonies containing ≥50 cells were considered viable.

Acridine Orange and PI Staining. Cells were fixed with 4% formalin/ PBS, stained with 10 μg/ml acridine orange (Sigma, St. Louis, MO) or 200 μg/ml PI, and visualized under a fluorescence microscope. Apoptotic cells were defined as cells showing cytoplasmic and nuclear shrinkage, chromatin condensation, or fragmentation morphologically. At least 300 cells/field were counted to determine the apoptotic index or the number of aberrant nuclei.

Fig. 1. A, the expression of survivin protein in the parental vector transfectants, Sur-AS transfectants, and Sur-Mut (Cys84Ala) transfectants of BCG-823 and MKN-45 cells. B, the expression of other IAP family proteins in BCG-823 cells evaluated by immunoblotting assay. The blots were reacted with specific antibodies recognizing survivin, XIAP, c-IAP-1, c-IAP-2, livin, and β-actin, respectively. C, the BCG-823 transfectants were grown to confluence and either photographed with a phase-contrast microscope (top panels) or fixed with 4% paraformalin, stained with 4',6-diamidino-2-phenylindole, and photographed under fluorescence microscopy (bottom panels). Arrows indicate abnormal morphological cells (original magnification, ×200).
**MTT Assay.** Cytotoxicity was measured by MTT assay. Cells growing exponentially were plated onto 96-well plates at a density of 1000 cells/well for 24 h. The cells were then treated with different concentrations of drugs for 96 h. One hundred μl of MTT stock solution (1 mg/ml) were added to each well, and the cells were further incubated at 37°C for 4 h. The supernatant was replaced with isopropl alcohol to dissolve formazan production. The absorbance at wavelength 595 nm was measured with a micro-ELISA reader (Bio-Rad, Hercules, CA). The ratio of the absorbance of treated cells relative to that of the control cells was calculated and expressed as a percentage of cell death.

**Flow Cytometry.** Cells were collected and fixed with ice-cold 70% ethanol in PBS and stored at −4°C until use. After resuspension, cells were incubated with 100 μl of RNase I (1 mg/ml) and 100 μl of PI (400 μg/ml) at 37°C and analyzed by flow cytometry (Coulter, Luton, United Kingdom). The cell cycle phase distribution was calculated from the resultant DNA histogram using Multicycle AV software (Phoenix Flow Systems, San Diego, CA). The ratio of the absorbance of treated cells relative to that of the control cells was calculated and expressed as a percentage of cell death.

**Immunofluorescence.** Cells were grown on a 2-well glass chamber slide (Nunc, Naperville, IL) and transfected with plasmids, as described previously. Cells were fixed with 2% formaldehyde for 10 min and permeabilized with 0.5% NP40 in PBS (α-tubulin) or fixed and permeabilized in ice-cold methanol for 20 min (γ-tubulin). Antibodies to α-tubulin (clone DM1A) and γ-tubulin (clone GTU-88), and FITC-conjugated goat antirabbit antibodies were purchased from Sigma. Antibodies were used at a 1:100 dilution for tubulins. Nuclei were stained with 1 μg/ml Hoechst 22388, and cells were analyzed using a Zeiss Axioscop fluorescence microscope.

**In Situ Detection of Apoptotic Cells by TUNEL Assay.** Tumor tissues from xenografts were excised and formalin-fixed immediately after resection. TUNEL staining was carried out using the ApoAlert DNA fragmentation assay kit (Clontech Corp., Palo Alto, CA) according to the manufacturer’s instructions. Apoptotic cells exhibit strong nuclear green fluorescence. The percentage of apoptotic cells was assessed in 10 randomly selected fields viewed at ×40 magnification. The apoptotic index was calculated as the number of apoptotic cells/total number of nucleated cells ×100%.

**Western Blot Analysis.** Cells were lysed with lysis buffer [50 mM Tris-HCl (pH 7.5), 250 mM NaCl, 0.1% NP40, and 5 mM EGTA containing 50 mM sodium fluoride, 60 mM β-glycerol-phosphate, 0.5 mM sodium vanadate, 0.1 mM phenylmethylsulfonfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin]. Protein samples were electrophoresed in a 10% denaturing SDS gel and transferred to Immobilon-P membrane (Millipore, Bedford, MA). The blots were incubated with specific primary antibodies, reacted with a peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA), and finally visualized by enhanced chemiluminescence (Amersham, Piscataway, NJ). Polyclonal antibodies recognizing survivin (2 μg/ml) and livin (2 μg/ml) were purchased from Alpha Diagnostic International, Inc. (San Antonio, TX); XIAP (2 μg/ml) monoclonal antibody was obtained from StressGen Biotechnologies Corp. (Victoria, British Columbia, Canada); and
**RESULTS**

Characterization of Stable Transfectants Expressing Sur-AS and Sur-Mut. We established BCG-823 and MKN-45 stable transfectants with either control vector pcDNA3, Sur-AS plasmid, or Sur-Mut plasmid. Eight clones from each transfection were selected and analyzed by Western blot to determine the survivin protein expression, and three clones were selected for expansion and additional studies. As shown in Fig. 1A, the level of survivin protein in pooled BCG-823 Sur-AS transfectants was reduced by >90%, although pooled Sur-Mut (Cys84Ala) transfectants expressed 2.53-fold survivin protein compared with that expressed by BCG-823 parental cells (Fig. 1A). In contrast, no change in the protein level of other IAP...
family genes was detected in all of the stable clones isolated from either BCG-823 (Fig. 1B) or MKN-45 (data not shown) cells. Furthermore, the BCG-823/Sur-AS and BCG-823/Sur-Mut (Cys84Ala) transfectants exhibit aberrant morphology with larger and flatter cells observed under phase-contrast microscope (Fig. 1C, top panels). As demonstrated by 4′,6-diamidino-2-phenylindole staining, BCG-823/Sur-AS and BCG-823/Sur-Mut (Cys84Ala) transfectants exhibited flattened and giant nuclei, whereas parental cells and BCG-823/vector-transfected cells showed normal morphology (Fig. 1C, bottom panels). Similar results were also obtained from the MKN-45 stable transfectant (data not shown).

Inhibition of Cell Growth in Stable Transfectants. As shown in Fig. 2, single transfectant BCG-823/Sur-AS1, BCG-823/Sur-AS2, and BCG-823/Sur-AS3 and pooled BCG-823/Sur-AS-P transfectants or single transfectant BCG-823/Sur-Mut1, BCG-823/Sur-Mut2, and BCG-823/Sur-Mut3 and pooled BCG-823/Sur-Mut (Cys84Ala) cells had significant decreases in cell number compared with BCG-823/vector-transfected and parental cells 5 days after plating (P < 0.01). By day 7, the number of cells was reduced by 64 ± 7% and 78 ± 9% in BCG-823/Sur-AS-P and BCG-823/Sur-Mut (Cys84Ala)-P transfectants compared with parental cells (P < 0.001; Fig. 2, A and B). Similar results were obtained in MKN-45/Sur-AS and MKN-45/Sur-Mut transfectants (data not shown). Furthermore, single and pooled BCG-823/Sur-AS and BCG-823/Sur-Mut (Cys84Ala) transfectants formed significantly fewer and smaller colonies in soft agar (Fig. 2, C and D). Colony forming efficiencies in BCG-823/Sur-AS-P and BCG-823/Sur-Mut (Cys84Ala)-P cells were reduced by 80 ± 5% and 90 ± 5%, respectively, as compared with parental cells (P < 0.001; Fig. 2B). Similar results were obtained in single and pooled MKN-45/Sur-AS and MKN-45/Sur-Mut transfectants (Fig. 2, E and F).

Stable Transfectants Were More Susceptible to Proapoptotic Stimuli. To avoid unintended differences related to gene insertion rather than to the expression of Sur-AS or Sur-Mut, we chose pooled clones to perform further study. We treated BCG-823/Sur-AS and

![Fig. 4. Mitotic catastrophe in gastric cancer cells. A, nuclear morphology and centrosomes in parental cells and stable transfectants. Cells were stained with an antibody to α-tubulin for spindle components or γ-tubulin for centrosomes. Cells were analyzed by fluorescence microscopy (B, C, and top panels of A) or confocal laser-scanning microscope (D, E, and middle and bottom panels of A). Photographs were from a representative experiment. B, representative normal nuclei. C, representative abnormal nuclei and multilobed nuclei and aberrant mitoses. D, representative chromosome fusions. E, representative DNA bridges. Original magnification: ×200 in top panels of A and ×250 in middle panels of A. Scale bar, bottom panels of A and B. 10 μm. F, quantification of cells in aberrant nuclei in stable transfectants. Data represent the mean of three independent experiments. An average of 150–300 cells were counted for each determination. *, P < 0.001 compared with control vector transfectants.](https://cancerres.aacrjournals.org/cm/84/3/7728/F1A.png)
BCG-823/Sur-Mut transfectants with serum deprivation and cytotoxic drugs. As shown in Fig. 3, MTT assay showed that BCG-823 cells transfected with Sur-AS or Sur-Mut plasmid were more sensitive to cisplatin and 5-fluorouracil than parental and vector-transfected cells (Fig. 3, A and B). The number of apoptotic cells induced by cisplatin and 5-fluorouracil increased to about 2.5–3-fold in BCG-823/Sur-AS and BCG-823/Sur-Mut compared with their control cells (P < 0.01; Fig. 3C). Similar results were obtained in MKN-45/Sur-AS and MKN-45/Sur-Mut stable transfectants (Fig. 3D). Furthermore, BCG-823/Sur-AS and BCG-823/Sur-Mut transfectants underwent spontaneous apoptosis more readily than parental and control vector cells in the presence or absence of serum (P < 0.01; Fig. 3E). Similar results were obtained in MKN-45/Sur-AS and MKN-45/Sur-Mut stable transfectants (Fig. 3F).

Stable Transfectants Encountered Mitotic Catastrophe. We selected the BCG-823/Sur-AS and BCG-823/Sur-Mut transfectants to evaluate the morphological changes. Nuclei and centrosomes were stained with an antibody recognizing α-tubulin for spindle components or γ-tubulin for centrosomes. Cells were then analyzed by fluorescence microscopy. As shown in Fig. 4, BCG-823/Sur-AS and BCG-823/Sur-Mut cells showed abnormal nuclei; in terms of morphology, they were micronucleated, multilobulated, and of abnormally large size as compared with parent and BCG-823/vector transfectants. Furthermore, BCG-823/Sur-AS and BCG-823/Sur-Mut cells had significant increases in the percentage of aberrant chromosomes, such as chromatin bridges (Fig. 4, A and E, arrowhead) and chromosome fusion (Fig. 4, A and D, small arrow), which characterized the exit from mitosis without completing chromosome segregation or cytokinesis. Percentages of aberrant nuclei in BCG-823/Sur-AS and BCG-823/Sur-Mut cells were 28.9% and 30.8%, respectively (Fig. 4F). Percentages of abnormal mitoses in BCG-823/Sur-AS and BCG-823/Sur-Mut cells were 17 ± 3% and 19 ± 4%, respectively (Fig. 4G).

Constitutive Suppression of Survivin Inhibited Tumor Formation in Vivo in Nude Mice Xenografts. We then asked whether stable transfectants in which the function of survivin has been...
knocked down could have reduced tumorigenicity in nude mice xenografts. We selected the BCG-823/Sur-AS and BCG-823/Sur-Mut transfectants to perform the in vivo study because BCG-823 cells have stronger tumorigenicity in nude mice than the other gastric cancer cell lines used.5 In all mice that received injection of control vector-transfected cells, the cells readily formed localized palpable tumors comprised of malignant cells that stained positively for survivin (Fig. 5, B and C). Tumors appeared within 1 week in all of the animals of the control group (Fig. 5A). Two of eight (75%) mice that received injection of the BCG-823/Sur-AS cells developed palpable tumors with considerably smaller size starting at day 22 (Fig. 5A). No tumor developed in mice that received injection with BCG-823/Sur-Mut transfectants (Fig. 5B). All of these animals remained tumor free for an additional 2 months.

We next examined whether the decreased tumor growth rate was associated with increased apoptosis and/or decreased proliferation in vivo. Consistent with the results in vitro, tumors derived from BCG-823/Sur-AS showed decreased expression of survivin protein compared with tumors derived from BCG-823/vector and parental cells (Fig. 5C). The tumors derived from BCG-823/Sur-AS had a higher apoptotic index (9 ± 1% compared with control transfectants) than tumors derived from BCG-823/vector and parental cells (4.0 ± 1% and 3 ± 1%, respectively; P < 0.01; Fig. 6A). The proportion of Ki67-positive cells in tumors derived from BCG-823/Sur-AS had a higher apoptotic index (9 ± 1%) than tumors derived from BCG-823/vector and parental cells (4.0 ± 1% and 3 ± 1%, respectively; P < 0.01; Fig. 6B). These results suggested that inhibition of tumor growth was attributable to increased spontaneous apoptosis and decreased cell proliferation in vivo. We further examined whether inhibition of survivin caused mitotic catastrophe in vivo. Our results showed that tumors from BCG-823/Sur-AS cells knocked down could have reduced tumorigenicity in nude mice xenografts. We selected the BCG-823/Sur-AS and BCG-823/Sur-Mut transfectants to perform the in vivo study because BCG-823 cells have stronger tumorigenicity in nude mice than the other gastric cancer cell lines used.5 In all mice that received injection of control vector-transfected cells, the cells readily formed localized palpable tumors comprised of malignant cells that stained positively for survivin (Fig. 5, B and C). Tumors appeared within 1 week in all of the animals of the control group (Fig. 5A). Two of eight (75%) mice that received injection of the BCG-823/Sur-AS cells developed palpable tumors with considerably smaller size starting at day 22 (Fig. 5A). No tumor developed in mice that received injection with BCG-823/Sur-Mut transfectants (Fig. 5B). All of these animals remained tumor free for an additional 2 months.

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exhibited a higher incidence of aberrant nuclei (7 ± 2%) compared with tumors from parental cells and BCG-823/vector (2% and 3%, respectively; P < 0.01; Fig. 6C).

Stable Suppression of Survivin Reduced Tumor Angiogenesis in Vivo. To further investigate the effect of survivin inhibition on angiogenesis, we determined the expression of PECAM-1/CD31, a well-established endothelial cell marker (30), in tumors of the same size derived from parental cells, BCG-823/vector, and BCG/Sur-AS transfectants. As shown in Fig. 7A, tumors derived from parental cells and BCG-823/vector transfectants contained thick vessels predominating over occasional discrete endothelial cells. In contrast, tumors derived from BCG-823/Sur-AS transfectants contained mainly discrete endothelial cells, with occasional small and thin vessels. Tumor-associated neovascularization as indicated by MVD was quantified. A significant reduction of MVD in tumors derived from BCG-823/Sur-AS transfectant was found as compared with tumors derived from BCG-823/vector transfectants and parental cells (P < 0.05; Fig. 7B). These results suggest that suppression of survivin reduces tumor angiogenesis in vivo.

DISCUSSION

In this study, we show that suppressing survivin expression or function causes spontaneous apoptosis and mitotic catastrophe in vivo in gastric cancer nude mice xenografts. Furthermore, our results indicate that suppression of survivin also inhibits de novo gastric cancer formation and angiogenesis in vivo.

Functional studies have demonstrated that suppression of survivin levels in HeLa cells causes spindle defects and promotes apoptosis (1, 11). It is conceivable that the suppression of survivin function in gastric cancer cells will also block the association of survivin to the mitotic spindle, which will ultimately result in mitosis-initiated apoptosis. Gastric cancer in general is highly resistant to chemoradiotherapy and moderately resistant to apoptosis (31). A study has shown that gastric cancer cells expressing a high level of survivin are more chemoresistant (25). This may be attributable to the effect of survivin in facilitating gastric cancer cells to overcome both G0-G1 and G2-M checkpoint controls (25). These reports are consistent with our findings that suppression of survivin significantly increased the sensitivity of gastric cancer cells to both cytotoxic drugs and serum starvation.

Apart from apoptosis, we showed, for the first time, that stable suppression of survivin by antisense and mutant (Cys84Ala) caused mitotic catastrophe in gastric cancer in vitro and in vivo. The molecular mechanism of mitotic catastrophe remains unclear, although it has been shown that cell cycle checkpoint deficiencies, including the G1 checkpoint, G2 checkpoint, prophase checkpoint, and mitotic spindle checkpoint, promote mitotic catastrophe (13). Thus, antisense targeting of survivin may result in dysregulation of mitotic spindle checkpoint as well as defects in microtubule assembly and function that lead to mitotic catastrophe as suggested in other studies (18). Nevertheless, there are limited studies showing that transient expression of Sur-Mut (Cys84Ala) causes aberrant cell mitosis (11). A previous study showed that synchronized cells expressing survivin Thr34Ala mutant exhibited a relatively normal cell cycle (14). However, a more recent study shows that stable expression of survivin Thr34Ala mutant causes aberrant cytokinesis rather than apoptosis (32). Our results support the notion that stable suppression of survivin causes mitotic catastrophe, which leads to cell death. An increase in the frequency of micronucleated tumor cells after radiotherapy and chemotherapy was suggested to be a positive prognostic marker of treatment response. The mitotic catastrophe in vivo usually leads to necrosis, thereby causing local inflammation that may be beneficial to the antitumor effect (13, 33). In contrast, the process of apoptosis is noninflammatory and therefore does not recruit the resources of the immune system. With this consideration, inducing mitotic catastrophe appears in general to be a desirable goal in cancer treatment.

We also demonstrated, for the first time, that suppression of survivin in gastric cancer epithelial cells caused reduced development of angiogenesis in association with tumor growth in vivo. The critical role of microvesSEL angiogenesis in tumor formation and tumor migration has been widely recognized (34–35). In gastric carcinoma, the
incidence of metastasis correlated with the number and density of blood vessels (36, 37). Our study showed that blockade of survivin function inhibited angiogenesis in gastric cancer xenografts in nude mice. This is consistent with the previous reports that survivin plays a role in vascular endothelial growth factor-mediated endothelial cell protection (19, 20, 38). Survivin up-regulation is one of the important mechanisms for drug “resistance” in both tumor cells and tumor-associated blood vessel endothelial cells (39). It is suggested that suppression of survivin during angiogenesis removed the cytoprotective effect of vascular endothelial growth factor, resulting in endothelial apoptosis and promoting rapid involution of three-dimensional capillary-like vessels in vitro (38). Our observations provide the first in vivo evidence showing that, besides inducing apoptosis, targeting survivin exerts an additional antitumor effect by inhibition of the development of angiogenesis. Thus, targeting survivin expression therapeutically would not only compromise survival of the tumor cell but could also indirectly affect activated endothelial cells of the tumor vasculature. In the present study, we showed that stable expression of Sur-Mut Cys84Ala completely prevented gastric cancer formation with reduced development of angiogenesis in vivo.

In summary, our results showed that stable inhibition of survivin expression and function resulted in spontaneous apoptosis and mitotic catastrophe, enhanced sensitivity to cytotoxic drugs, and suppression of de novo tumor formation with reduced development of angiogenesis in gastric cancer xenografts in nude mice. Because of the preferential expression of survivin in gastric cancer but not in normal tissues (22, 23), these data suggest that targeting the survivin pathway alone or with cytotoxic drugs may be useful in the treatment of gastric cancer.

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