Loss of Krüppel-Like Factor 4 Expression Contributes to Sp1 Overexpression and Human Gastric Cancer Development and Progression

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Abstract

Purpose: Increasing evidence indicates that the transcription factor, Sp1, regulates the expression of multiple genes involved in tumor development and progression. We have recently reported that Sp1 overexpression is directly correlated with the angiogenic potential of and poor prognosis for human gastric cancer. However, the underlying mechanisms that result in Sp1 overexpression remain unclear.

Experimental Design: The expression of Sp1 and Krüppel-like factor 4 (KLF4), a potential tumor suppressor gene, in gastric cancer tissue was analyzed by immunohistochemistry and Western blot analysis. Alterations of Sp1 and KLF4 expression were achieved by gene transfer and verified by Northern and Western blot analyses. Furthermore, Sp1 promoter activity assay, electrophoretic mobility shift assay, and chromatin immunoprecipitation assay were done to identify the KLF4 binding sites on the Sp1 promoter.

Results: Mutually exclusive expression of Sp1 and KLF4 was evident in gastric cancer and non-cancerous tissue. Specifically, strong Sp1 expression but loss of KLF4 expression was found in cancer tissue, whereas the adjacent noncancerous tissue showed negative Sp1 expression but strong KLF4 expression. Enforced KLF4 expression repressed Sp1 expression at the promoter activity, mRNA, and protein levels. Moreover, a region within the proximal Sp1 promoter was identified to have overlapping KLF4- and Sp1-binding sites, to which KLF4 and Sp1 compete for binding. Sp1 positively regulated its own promoter, whereas KLF4 did the opposite.

Conclusions: Our data suggests that disruption of KLF4-mediated negative regulation contributes to the molecular events of Sp1 overexpression and to the development and progression of human gastric cancer.

Although the incidence of gastric cancer declined in the west from the 1940s to the 1980s, it remains a major public health problem throughout the world (1). In Asia and parts of South America in particular, it is the most common epithelial malignancy and is a leading cause of cancer-related death. In fact, gastric cancer remains the fourth most frequently diagnosed malignancy worldwide and is the cause of 12% of all cancer-related deaths annually (1). Advances in the treatment of this disease are likely to come from a fuller understanding of its biology and behavior. Although various genetic and molecular alterations have been found to be associated with the malignant transformation of gastric cancer, they may represent only the pathogenesis of this disease, and they have not been identified as a specific sequence of changes leading to gastric carcinoma (2, 3). Therefore, the role and detailed mechanisms of genetic and epigenetic changes in gastric cancer development and progression remain unclear.

Sp1 is a zinc finger transcription factor that is important to the transcription of many cellular and viral genes that contain GC boxes in their promoter. Additional transcription factors that are similar to Sp1 in their structural and transcriptional properties (Sp2, Sp3, and Sp4) have been cloned, thus forming the Sp1 multigene family (4). Although Sp1 has been perceived as a basal transcription factor since its discovery, increasing evidence suggests that it regulates a variety of biological functions, including cell survival, growth and differentiation, and tumor development and progression (5). For example, Sp1 expression is increased in squamous cell carcinoma and colorectal cancer when compared with that in skin papillomas and normal colorectal tissue, respectively (6, 7). Also, interference of Sp1 activity has been shown to suppress tumor cell growth (8), and small interfering RNA (siRNA) duplexes of Sp1 mRNA block cell cycle progression or tumor formation in athymic mice (9, 10). Furthermore, we have reported that Sp1...
overexpression is directly correlated with the angiogenic potential and poor prognosis of human gastric cancer (11, 12).

However, the underlying mechanisms that result in Sp1 overexpression in tumors remain unknown. Although a number of studies have reported gene regulation by Sp1, few have reported the transcriptional regulation of Sp1 itself. One recent study showed that the Sp1 promoter contains a number of putative binding sites for several transcription factors, including Sp1, Sp3, nuclear factor Y, activator protein 2, and CCAAT/enhancer-binding protein (13, 14). Because the Sp1 promoter contains putative Sp1 binding sites, Sp1 overexpression may result from autotransactivation of its own promoter. On the other hand, Krüppel-like factor 4 (KLF4) is a zinc finger transcription factor that binds to a consensus sequence 5’-(G/A)(G/A)GG(C/T)G(C/T)-3’. It is a member of the KLF family and is highly expressed in the gastrointestinal tract and other epithelial tissues (15). KLF4 can either activate or repress the transcription of several genes (16–19), and its alterations lead to aberrant proliferation and differentiation in gastric and colonic epithelium (20, 21). Additionally, accumulating clinical evidence shows that KLF4 is a potential tumor suppressor. Reduced KLF4 expression has been reported in various tumors including esophagus, stomach, colon, bladder, lung, and adult T cell leukemia cells (22–27), and restored KLF4 expression induces growth arrest in colon cancer cells (28) or apoptosis in bladder, gastric, and leukemia cells (23, 24, 27). In fact, genetic or epigenetic alterations of the KLF4 gene have been found in both gastric cancer and colorectal cancer (23, 25).

In the present study, we found mutually exclusive expression of Sp1 and KLF4 in human gastric cancer. Enforced KLF4 expression repressed Sp1 expression at the promoter activity, mRNA, and protein levels. A region within the proximal Sp1 promoter was identified to have overlapping KLF4- and Sp1-binding sites, to which KLF4 and Sp1 compete for binding. Sp1 positively regulated its own promoter, whereas KLF4 did the opposite. Collectively, our data suggests that disruption of KLF4-mediated negative regulation contributes to the molecular events of Sp1 overexpression and to the development and progression of human gastric cancer.

Materials and Methods

Cell lines and culture conditions. The human gastric cancer cell lines, AGS, HTB103, HTB135, N87, SNL-1, and TMK1, and FG human pancreatic cancer cell line, were purchased from the American Type Culture Collection (Manassas, VA), and the human gastric cancer cell line, SK-GT5, was obtained from Dr. Gary K. Schwartz (Memorial Sloan-Kettering Cancer Center). All of the cell lines were maintained in plastic culture dishes and transduced with a recombinant adenovirus containing enhanced green fluorescent protein (Ad-EGFP) at a multiplicity of infection (MOI) of 10. Twenty-four hours later, the cells were collected and nuclear extracts were prepared as previously described (31). Electrophoretic mobility shift assays (EMSA) were done by using the Gel Shift Assay System (Promega) according to the manufacturer’s instructions. Briefly, the oligonucleotides as indicated in each experiments were annealed and 5’-end-labeled with [32P]ATP by using T4 polynucleotide kinase (Amersham Life Sciences) according to standard procedures. The nuclear extracts (10 μg each) were preincubated in binding buffer for 10 minutes at room temperature; this was followed by the addition of the [32P]ATP-labeled double-strand oligonucleotide and a second incubation at room temperature for 25 minutes. For the supershift assay, specific Sp1 (PEP2), KLF4 (H180, sc-20691 X), and FLAG (M2; Sigma Aldrich, St. Louis, MO) antibodies were added with a final concentration of 0.1 μg/μL and incubated for 15 minutes at room temperature before the addition of a labeled probe. Samples were then loaded on a 4.5% polyacrylamide gel, and electrophoresis was done at 4°C for 4 hours at 200 V. The gel was then dried for 40 minutes at 80°C and exposed to Kodak film (Eastman Kodak, Rochester, NY) at −70°C.

Western blot analysis. Fresh gastric cancer and corresponding noncancerous gastric tissue specimens were obtained from patients who underwent gastrectomy at The University of Texas M.D. Anderson Cancer Center. Both the cancerous and noncancerous specimens were macroscopically identified and excised by experienced pathologists, and the presence or absence of cancer was further confirmed by histopathologic examination. Additionally, whole cell lysates were prepared from gastric cancer tissue and adjacent noncancerous specimens or cell cultures. Standard Western blotting was done with an anti-Sp1 antibody (PEP2; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-KLF4 antibody (H180; Santa Cruz Biotechnology). Two bands were detected following KLF4 transfection, as reported previously (16, 19, 23), and the low molecular weight protein may result from a rapid proteolysis of KLF4 protein (30). Equal protein sample loading was monitored by incubating the same membrane filter with an anti–glyceraldehyde-3-phosphate dehydrogenase antibody (FL-335; Santa Cruz Biotechnology). The probe proteins were detected by using the enhanced chemiluminescence system (Amersham Life Sciences, Piscataway, NJ) according to the manufacturer’s instructions.

Sp1 promoter constructs, site-specific mutagenesis, and analysis of Sp1 promoter activity. The full-length and minimal Sp1 promoter reporters in pGL3 luciferase constructs were used as described previously (13). Site-specific mutagenesis of the minimal Sp1 promoter was done by using a QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. The primers used to generate the mutant vector were: sense, 5’-gagaattagggggtcctaggtggtctg-3’ and antisense, 5’-caacccggctggatacgcggctc-3’ (the mutated sites are shown in boldface). Mutation was confirmed by DNA sequencing. To examine the transcriptional regulation of the Sp1 promoter by KLF4, SK-GT5 and N87 cells were seeded to ~80% confluence in six-well plates (in triplicate) and transiently transfected with 0.6 μg of full-length or minimum Sp1 reporter plasmid and 0.3 μg of effector expression plasmids as indicated in each experiment by using LipofectAMINE (Invitrogen) according to the manufacturer’s instructions. The reporter luciferase activity was determined 48 hours later with a luciferase assay kit (Promega, Madison, WI). Promoter activity was normalized according to the protein concentration as described previously (13).

Electrophoretic mobility shift assay. SK-GT5, N87, and FG cells were seeded to ~80% confluence in 15 cm culture dishes and transduced with Ad-KLF4 or Ad-EGFP at a MOI of 10. Twenty-four hours later, the cells were collected and nuclear extracts were prepared as previously described (31). Electrophoretic mobility shift assays (EMSA) were done by using the Gel Shift Assay System (Promega) according to the manufacturer’s instructions. Briefly, the oligonucleotides as indicated in each experiments were annealed and 5’-end-labeled with [32P]ATP by using T4 polynucleotide kinase (Amersham Life Sciences) according to standard procedures. The nuclear extracts (10 μg each) were preincubated in binding buffer for 10 minutes at room temperature; this was followed by the addition of the [32P]ATP-labeled double-strand oligonucleotide and a second incubation at room temperature for 25 minutes. For the supershift assay, specific Sp1 (PEP2), KLF4 (H180, sc-20691 X), and FLAG (M2; Sigma Aldrich, St. Louis, MO) antibodies were added with a final concentration of 0.1 μg/μL and incubated for 15 minutes at room temperature before the addition of a labeled probe. Samples were then loaded on a 4.5% polyacrylamide gel, and electrophoresis was done at 4°C for 4 hours at 200 V. The gel was then dried for 40 minutes at 80°C and exposed to Kodak film (Eastman Kodak, Rochester, NY) at −70°C.

Generation of recombinant adenoviruses and transduction of tumor cells. The full-length KLF4 and nucleus localization–deficient mutant KLF4 in pcDNA3 plasmid were provided by Dr. Chichuan Tseng. Adenoviral vectors (Ad-KLF4 and Ad-EGFP) were generated with the use of the AdEasy Adenoviral Vector System (Stratagene). The recombinant adenoviruses were expanded in HEK293 cells and purified by double CsCl gradient centrifugation to achieve a titer of ~1010 plaque-forming units/mL as previously described (23).
Chromatin immunoprecipitation. SK-GT5 cells were seeded to ~80% confluence in 10 cm culture dishes and transfected with Ad-KLF4 or Ad-EGFP at a MOI of 10. Twenty-four hours later, chromatin immunoprecipitation was done by using the Chromatin Immunoprecipitation Assay Kit (Upstate Cell Signaling Solutions, Lake Placid, NY) according to the manufacturer’s instructions. Briefly, DNA cross-linking proteins were cross-linked with DNA and lysed in SDS lysis buffer. The lysate was sonicated to shear DNA to 200 to 500 bp. After preclearing with a salmon sperm DNA/protein A agarose-50% slurry for 30 minutes at 4°C, chromatin samples were immunoprecipitated overnight with no antibody, an anti-Sp1 antibody (PEP2), an anti-KLF4 antibody (H180), and an anti-FLAG antibody (M2). The region between −224 and −53 bp of the Sp1 promoter was amplified by using the following primers: sense, 5’-cagggcgcaacttctg-3’, and antisense, 5’-tgagggaggagggacg-3’. The PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light.

siRNA. RNA interference was done by using the TransSilent Human Sp1 siRNA (Pamonitor, Inc., Redwood, CA) or Human KLF4 siRNA (Ambion, Austin, TX) following the manufacturer’s instructions. Briefly, SK-GT5 cells were seeded to ~80% confluence in six-well plates in triplicate and transiently transfected with 2.5 μg of a Sp1 or KLF4 siRNA mix and 0.6 μg of minimal Sp1 reporter plasmid with the use of Lipofectamine 2000 (Invitrogen). The reporter luciferase activity was determined 48 hours later by using a luciferase assay kit (Promega). The promoter activity was normalized according to the protein concentration, and the efficiencies of Sp1 and KLF4 siRNA, which were 90% and 80%, respectively, were confirmed by Western blot analyses.

Statistical analysis. The two-tailed χ² test was used to determine the significance of the difference between the expressions of KLF4 and Sp1 in human gastric cancer. Other statistical differences were evaluated by using the two-tailed Student’s t test. Differences were considered statistically significant at P < 0.05. All experiments were done at least twice, and one representative of at least two experiments with similar results was presented.

Results

**Mutually exclusive expression of Sp1 and KLF4 protein in human gastric cancer.** Sections of paraffin-embedded gastric cancer tissue specimens were immunostained with antibodies against Sp1 and KLF4 protein (11, 23). After the analysis of Sp1 and KLF4 expression scores in 86 primary gastric tumor tissue specimens using previously published data (11, 23), we found a significant inverse correlation between Sp1 expression and KLF4 expression (Fig. 1A; P = 0.006). Moreover, we found KLF4 expression only in residual noncancerous epithelial cells (Fig. 1B1), but strong Sp1 expression in the majority of the tumor epithelial cells (Fig. 1B2). In most of the gastric cancer specimens, KLF4 expression was significantly decreased or lost (Fig. 1B3), whereas Sp1 overexpression was strikingly evident (Fig. 1B4). To further confirm these findings, we did Western blot analyses to examine the expression of KLF4 in two paired human gastric tumor and adjacent noncancerous gastric tissue specimens, two tumor tissue specimens exhibiting strong Sp1 expression, and two tumor tissue specimens exhibiting weak Sp1 expression. As shown in Fig. 1C1, the loss of KLF4 expression correlated with increased Sp1 expression. Additionally, we determined the level of both Sp1 and KLF4 expression in various human gastric cancer cell lines. As compared with normal human gastric tissue, all of the gastric cancer cell lines had significantly reduced or lost KLF4 but exhibited Sp1 overexpression (Fig. 1C2). These data suggested that the expression of Sp1 and KLF4 was mutually exclusive.

**Inhibition of Sp1 expression by enforced KLF4 overexpression.** To determine whether KLF4 represses Sp1 expression, we examined the effects of KLF4 overexpression on Sp1 expression using Northern and Western blot analyses. We found that
restored KLF4 expression repressed Sp1 expression at both the mRNA (Fig. 2A) and protein (Fig. 2B) levels in SK-GT5 and N87 cells. Transduction with Ad-EGFP did not affect the KLF4 and Sp1 expression, suggesting that the repressed Sp1 expression was not mediated by adenoviral transduction. To determine the mechanisms by which KLF4 inhibits Sp1 expression, we examined the effect of KLF4 on Sp1 promoter activity using a full-length Sp1 promoter-driven pGL3 luciferase reporter. In both SK-GT5 and N87 cells, cotransfection of a KLF4 expression vector inhibited Sp1 promoter activity, whereas cotransfection of a mutant KLF4 expression vector, which lacked the nuclear localization signal (32), did not inhibit but rather slightly increased Sp1 promoter activity (Fig. 2C). A previous study (32) localized signal (32), did not inhibit but rather slightly increased Sp1 promoter activity (Fig. 2C). A previous study (32) reported that a minimal Sp1 promoter (−217 bp upstream of the transcription initiation site) contains binding sites for various transcription factors, including Sp1, nuclear factor Y, activator protein 2, and CCAAT/enhancer-binding protein (13, 14). We then tested the effect of KLF4 expression on this minimal Sp1 promoter luciferase reporter. Consistent with the results of the use of the full-length Sp1 promoter construct, KLF4 inhibited the minimal Sp1 promoter activity, but mutant KLF4 did not (Fig. 2D). These results suggested that the minimal Sp1 promoter contains KLF4-responsive element(s) that mediate transcriptional repression of Sp1 gene expression.

Identification of a KLF4-binding site within the minimal Sp1 promoter. Initially, we used a software program to identify KLF4-binding sites on the Sp1 promoter and found a putative KLF4-binding site overlapping a proximal Sp1-binding site (Fig. 3A). To confirm that KLF4 and Sp1 bind to this sequence, we generated an oligonucleotide probe (Fig. 3A). For all in vitro binding assays, N87 (Fig. 3B1), SK-GT5 (Fig. 3B2), and FG cells (Fig. 3B3) were transduced for 24 hours with Ad-KLF4 or Ad-EGFP at a MOI of 10. The nuclear proteins were extracted for EMSA. Both Sp1 and KLF4 bound to the same oligonucleotide probe as confirmed by supershifts (Fig. 3B, lanes 2 and 6 for Sp1 and lanes 3, 4, 7, and 8 for KLF4), and restored KLF4 expression repressed Sp1 binding (Fig. 3B, lane 1 versus lane 5). Mutant oligonucleotide probes completely abolished both Sp1 and KLF4 binding (Fig. 3B, lanes 9 and 10). These data suggested that KLF4 and Sp1 competitively bind to an overlapping region of the Sp1 promoter.

Next, we sought to determine whether Sp1 and KLF4 bind to the same region of the Sp1 promoter in vivo using a chromatin immunoprecipitation assay. The schematic structure of the proximal Sp1 promoter and the nucleotide positions and sequences of the PCR forward and reverse primers are shown in Fig. 4A. For chromatin immunoprecipitation assays, SK-GT5 cells were transduced for 24 hours with Ad-KLF4 or Ad-EGFP at a MOI of 10 and chromatin fragments were immunoprecipitated overnight with or without specific anti-FLAG antibody. As compared with EGFP-transduced cells, restored KLF4 expression clearly bound to the target sequence of the Sp1 promoter (Fig. 4B, lane 6). To determine whether overexpression of KLF4 affects Sp1 binding to its own promoter, chromatin fragments were immunoprecipitated overnight with or without specific anti-Sp1, Sp, and KLF4 antibodies. Increased KLF4 binding clearly reduced the binding of Sp1 and Sp3 to the same region (Fig. 4C, lane 3 versus lane 7 for Sp3; lane 4 versus lane 8 for Sp1). The experiment was repeated thrice and the relative bindings of Sp1 and Sp3 (band intensity relative to that of input) were calculated and their reductions were significant (P < 0.01). These data supported the idea that KLF4 and Sp1 bind to the same region of the Sp1 promoter in vivo in a competitive manner.

Antagonizing effects of Sp1 and KLF4 on Sp1 promoter. To determine whether Sp1 and KLF4 have antagonizing effects on
the Sp1 promoter activity, we generated a minimal Sp1 promoter reporter containing the same mutation applied to EMSA probe (Fig. 5A). Mutation of the KLF4-binding site increased the minimal Sp1 promoter activity, suggesting that this site was important for the negative regulation of Sp1 promoter activity and made SK-GT5 cells less sensitive to the repressive effect of KLF4 restoration (Fig. 5B). Finally, we tested the effect of interference with endogenous Sp1 expression on the minimal Sp1 promoter activity using a siRNA. Treatment with Sp1 siRNA significantly knocked down the endogenous Sp1 protein expression (Fig. 5C, inset), which was accompanied by a reduced minimal Sp1 promoter activity (Fig. 5C). In contrast, KLF4 knockdown increased Sp1 expression (Fig. 5C, inset), which was consistent with increased Sp1 promoter activity (Fig. 5C). These data suggested that endogenous Sp1 is positively involved in Sp1 promoter activity, whereas KLF4 does the opposite.

Discussion

In this study, we found mutually exclusive expression of KLF4 and Sp1 in human gastric cancer and noncancerous tissues. This observation is consistent with our previous reports showing that the majority of human gastric cancer cases exhibit Sp1 overexpression but significantly reduced or lost KLF4 expression. Restored expression of KLF4 inhibited Sp1 expression at both the mRNA and protein level in human gastric cancer cell lines (23). Furthermore, we located the KLF4 binding site within the proximal Sp1 promoter sequence that overlaps the Sp1 binding site and competition with Sp1, which is a positive regulator of its own transcription. These findings suggest that loss of KLF4 expression contributes to Sp1 overexpression in human gastric cancer.

Tumor development and progression require cell growth, apoptosis resistance, cell invasion, and angiogenesis. Increasing evidence shows that Sp1 regulates multiple genes that are important for tumor development and progression (33). These genes include epidermal growth factor receptor (34), hepatocyte growth factor receptor (35), transforming growth factor-α (36), and thymidine kinase (37), which are key to cell proliferation and growth; Bcl-2 (38), Bcl-x (39), and survivin (40), which are key to cell survival and apoptosis resistance; matrix metalloproteinase-2 (41, 42) and urokinase-type plasminogen activator (43), which are key to cell migration and invasion; and basic fibroblast growth factor and vascular endothelial growth factor (44–47), which are key to angiogenesis. Consistent with these findings, we recently reported that Sp1 overexpression is associated with vascular endothelial...
growth factor expression, advanced stage, and poor prognosis in patients with resected gastric cancer (11, 12), and increased angiogenic potential of human pancreatic cancer (48). Thus, Sp1 may play a central regulatory role in many such pathways of tumor development and progression and may be an attractive target for cancer treatment. In fact, we recently showed that the cyclooxygenase-2 inhibitor, celecoxib, inhibits angiogenesis and metastasis of human pancreatic cancer in part by suppression of Sp1 activity (31).

On the other hand, accumulating evidence indicates that KLF4 is a putative tumor suppressor gene. Reduced and/or lost KLF4 expression has been reported in various tumors including esophagus, stomach, colon, bladder, lung, and adult T cell leukemia (22–27). We recently reported that genetic or epigenetic loss of KLF4 expression in human gastric cancer and SK-GT5 cells used in the present study carry hemizygous deletion of the KLF4 gene (23). Functionally, KLF4 up-regulates differentiation marker genes such as keratin 4 or intestine alkaline phosphatase (16, 17), whereas KLF4 down-regulates positive cell cycle–regulatory genes such as cyclin D1 and ornithine decarboxylase (18, 19). Interestingly, several reports have indicated that KLF4 represses the expression of several genes by competing with Sp1 (49, 50). However, whether KLF4 is involved in Sp1 regulation and tumor development and progression in gastric cancer is unknown.

The Sp1 promoter contains several putative Sp1-binding sites, suggesting that Sp1 regulates its own expression (13). This notion of positive autoregulation is substantiated by our results, showing that knocking down Sp1 expression with a siRNA significantly decreased Sp1 promoter activity. Conversely, a putative KLF4-binding site within the proximal Sp1 promoter sequence overlaps the proximal Sp1-binding site, to which both Sp1 and KLF4 compete for binding as shown by in vitro and in vivo functional analyses. Moreover, Sp1 promoter activity was increased upon inactivation of the KLF4-binding site. Therefore, this region plays an important role in the positive and negative regulation of Sp1 by Sp1 and KLF4, respectively. However, this mutation did not completely abrogate the inhibitory effects of KLF4, suggesting that KLF4 may interfere with other Sp1 binding sites, Sp1 protein stability, or other mechanisms which require further investigation.

Several previous studies showed that Sp1 activity is regulated by posttranslational modification, degradation, and interaction with other transcription factors (5). The present study provided a novel mechanism for the regulation of Sp1 expression, i.e., Sp1 expression itself is negatively regulated by KLF4. Physiologically, the balance between Sp1 and KLF4 expression may play a critical role in the homeostasis of gastrointestinal mucosa. However, during gastric cancer development and progression, alteration of KLF4 expression may lead to aberrant...
Sp1 expression. This notion is supported by the recent report that conditional KLF4 knockout mice showed altered proliferation and differentiation and precancerous changes in the adult stomach (20). This mechanism may not be specific to gastric cancer but rather a common mechanism in the progression of gastrointestinal cancer, because Sp1 overexpression and KLF4 down-regulation have also been reported in colorectal cancer (7, 25).

In summary, we showed that KLF4 inhibited Sp1 expression at the Sp1 promoter activity, mRNA, and protein levels, in part through its binding site overlapping a Sp1-binding site. A proper Sp1/KLF4 ratio may control Sp1 transcription at the physiologic level (Fig. 6A). However, reduced or lost Sp1 expression because of genetic or epigenetic changes in the tumor may trigger Sp1 overexpression because of attenuated negative regulation of KLF4 and augmented positive autoregulation by Sp1. The resulting Sp1 overexpression may promote tumor development and progression by altering the expression of multiple genes key to the regulation of cell growth, apoptosis resistance, angiogenesis, and cell invasion (Fig. 6B). Therefore, negative regulation of Sp1 expression by KLF4 may play an important role in the homeostasis of normal gastrointestinal tissue, and loss of KLF4 expression and Sp1 overexpression may contribute to tumor development and progression. Finally, because of the structural and functional similarity among members of the Sp1 family, KLF4 might also affect the expression and function of Sp2, Sp3, and Sp4, and the underlying mechanisms need further investigation (33, 47).

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References


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