p53 Negatively Regulates the Hepatoma Growth Factor HDGF

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Abstract

Hepatoma-derived growth factor (HDGF) is a secreted heparin-binding growth factor that has been implicated in cancer development and progression. Here, we report that HDGF is a critical target for transcriptional repression by the tumor suppressor p53. Endogenous HDGF expression was decreased in cancer cells with introduction of wild-type p53, which also downregulated HDGF expression after DNA damage. In support of the likelihood that HDGF is a critical driver of cancer cell growth, addition of neutralizing HDGF antibodies to culture media was sufficient to block cell growth, migration, and invasion. Similarly, these effects were elicited by conditioned culture medium from p53-expressing cells, and they could be reversed by the addition of recombinant human HDGF. Interestingly, we found that HDGF was overexpressed also in primary gastric, breast, and lung cancer tissues harboring mutant p53 genes. Mechanistic investigations revealed that p53 repressed HDGF transcription by altering HDAC-dependent chromatin remodeling. Taken together, our results reveal a new pathway in which loss of p53 function contributes to the aggressive pathobiological potential of human cancers by elevating HDGF expression. Cancer Res; 71(22); 7038–47. ©2011 AACR.

Introduction

Alterations of the p53 gene are among the most common and important events in human carcinogenesis. In response to DNA damage or other cellular stresses, p53 is activated and inhibits cell growth through the activation of both cell-cycle arrest and apoptosis, maintaining genome stability and preventing cancer development (1). p53 achieves these functions mainly through the transactivation of a large number of downstream target genes. However, p53 can also negatively regulate the transcription of several genes, including those involved in regulatory cascades mediating cell proliferation and tumorigenesis (2).

In an attempt to identify novel genes responsive to p53-induced growth inhibition, Saos-2 human osteosarcoma cells were profiled with the Affymetrix U95 gene chip (3) and a novel p53-repressed gene, HDGF, which encodes a heparin-binding protein, was revealed. Hepatoma-derived growth factor (HDGF) was originally purified from HuH-7 liver cancer cell line conditioned medium (CM; ref. 4). Although it is regarded as a secreted growth factor, the amino acid sequence lacks a signal peptide but includes a region homologous to the high-mobility group box of nuclear proteins (4). HDGF stimulates the proliferation of fibroblasts, endothelial cells, vascular smooth muscle cells, and some liver cancer cells. Recent studies have suggested the involvement of HDGF in the development of the liver, lung, kidney, gut, and cardiovascular systems (5–9). HDGF expression is increased in several types of carcinomas compared with adjacent nontumorous areas in mouse and human (10). Several findings suggest that HDGF overexpression is associated with aggressive phenotypes of cancer cells, such as proliferation, invasiveness, and metastasis (11–15). Therefore, HDGF may prove useful as a prognostic factor for patients with hepatocellular carcinoma (16), gastric (17), pancreatic (18) and non–small-cell lung cancers (19), and gastrointestinal stromal tumors (20).

In this study, HDGF expression was downregulated by the transduction of the p53 gene and by endogenous p53 activation induced by DNA damage. p53-induced HDGF repression is not mediated through direct binding to the p53 consensus sequences, but rather through the recruitment of histone deacetylase (HDAC) repressors. A neutralizing antibody against HDGF or HDGF-depleted medium conditioned by p53-expressing cells both strongly inhibited cancer cell growth, migration and invasion. Conversely, adding recombinant human HDGF abrogated the inhibitory effects of the CMs. In addition to the in vitro studies, significantly higher levels of HDGF protein were found in gastric, breast, and lung tumors harboring mutant p53 compared with those with wild-type p53. Together, these observations provide support for the
hypothesis that the loss of p53 function contributes to an aggressive clinical phenotype in cancer in part through elevated HDGF expression.

Materials and Methods

Cell culture, plasmids, and recombinant adenoviruses

The cell lines used in this study were purchased from American Type Culture Collection or the Japanese Collection of Research Bioresources. The status of the endogenous p53 gene in these lines is as follows: HEK293 (human embryonic kidney) and HeLa (cervical cancer) have wild-type p53; U373 (glioma) have mutant p53; and Saos-2 (osteosarcoma) and H1299 (lung cancer) are p53 null. HCT116 (p53 wild type) cell line and its derivative HCT116-p53(−/−) lacking p53 were kindly provided by Dr. Bert Vogelstein (Howard Hughes Medical Institute, Johns Hopkins University, Baltimore, MD). The construction, purification, and infection procedures of replication-deficient recombinant adenoviruses containing p53 (Ad-p53), TAp73α (Ad-p73α), TAp73β (Ad-p73β), TAp63α (Ad-p63α), and the bacterial lacZ gene (Ad-lacZ) were described previously (21–24). To determine the relative efficiency of adenovirus-mediated gene transfer, cells were infected with adenovirus containing the bacterial lacZ gene (Ad-lacZ). Several human cancer cell lines that showed highly efficient gene transfer were used, with 90% to 100% of the cells staining for β-galactosidase activity at a multiplicity of infection (MOI) of 25 to 100. Wild-type p53 and p53 mutant constructs encoding 8 p53 mutants commonly seen in human cancers (R175H, G245S, R248Q, R248W, R249S, R273C, R273H, and R282W) were generated by subcloning into the pcDNA3.2 plasmid with an N-terminal FLAG epitope tag (Invitrogen). Adriamycin and trichostatin A (TSA) were purchased from Sigma.

Clinical samples

Primary gastric carcinoma samples with genomic sequence analysis of p53 exons were from previously reported cases (25). They (a total of 57) were obtained with informed consent at surgery from Sapporo Keiyukai Hospital and Sapporo Medical University Hospital. HDGF mRNA levels in a publicly available breast cancer dataset, GSE3494, which includes gene expression data on a total of 251 cases with mutation status, was also investigated (26). Statistical analyses were done with the 2-sided Student t tests.

Other methods were mainly previously published, and the modifications of procedures are given in detail in the Supplementary Methods.

Results

p53 family downregulates expression of the HDGF mRNA and protein

Microarray expression profiling revealed almost a 3-fold reduction in HDGF mRNA observed in p53− or p63−transfected Saos-2 cells compared with lacZ-transfected cells. Using the HDGF cDNA as a probe, Northern blot analysis was done on human cancer cell lines, Saos-2 and U373. As shown in Fig. 1, HDGF mRNA was clearly decreased by the exogenous expression of p53, p73α, p73β, and p63ß in both cells. HDGF reduction by p63α was less pronounced in both cells. We then determine whether the decrease in HDGF mRNA was accompanied by a decrease in protein expression. The level of HDGF protein was examined by immunoblot (middle panel) shows that total cellular HDGF protein was decreased in p53−, p73− and p63−transfected cells, consistent with the Northern blot analysis. In contrast, p53 family members induced p21 significantly in all cell lines tested (Fig. 1). A similar reduction in HDGF mRNA and protein was also observed in HCT116-p53(−/−) and HCT116-p53(−/−) cells (Supplementary Fig. S1). HDGF is a heparin-binding proliferation factor originally identified in the conditioned medium (CM) of liver cancer cells. HDGF protein levels in the CM were also decreased after the overexpression of p53 family members (Fig. 1, bottom panel), which suggests an inhibitory effect of p53 family in HDGF secretion from human cancer cells.
HDGF has been reported to be highly expressed in fetal tissues, and an association with the aggressive biological potential of cancer cells, such as proliferation, has been proposed (5, 6, 11, 12). Whether cell growth arrest resulted in a decrease in HDGF expression was, therefore, tested. Similar to p53 family, the transduction of cdk inhibitors p21 and p16 induced cell-cycle arrest and growth inhibition in Saos-2 and HCT116 cells (Supplementary Fig. S2). We found that neither p21 nor p16 significantly repressed HDGF mRNA (Supplementary Fig. S3), which suggests that the cell-cycle arrest itself does not mediate HDGF downregulation.

Endogenous p53 downregulates HDGF

To examine whether endogenous p53 would have a similar effect, HCT116 cells expressing wild-type p53 [HCT116-p53 (+/+) and isogenic mutant cells lacking p53 [HCT116-p53 (−/−)] were exposed to adriamycin (ADR). The treatment of HCT116-p53 (+/+) cells with ADR (0.5 μg/mL) resulted in the upregulation of p53 and its target p21 with a decrease in HDGF mRNA and protein expression (Fig. 2). However, a decrease in HDGF expression was not observed in HCT116-p53 (−/−) cells following the same ADR treatment. In addition, ADR treatment resulted in the reduction of secreted HDGF protein in the CM of HCT116-p53 (+/+) cells but not HCT116-p53 (−/−) cells (Fig. 2B). In contrast, PAI-1, a secreted protein encoded by a p53-inducible gene, was increased in the CM after ADR treatment in a p53-dependent manner.

The levels of HDGF in HCT116-p53 (+/+) and HCT116-p53 (−/−) cells after treatment with the Mdm2 inhibitor Nutlin-3 were then examined. Because Mdm2 is a negative regulator of p53, inhibition of Mdm2/p53 interaction by Nutlin-3 led to p53 activation (Supplementary Fig. S4). In HCT116-p53 (−/−) cells, Nutlin-3 treatment had no effect on p53 accumulation and p21 induction. The levels of HDGF mRNA and protein were decreased in HCT116-p53 (+/+) but not in HCT116-p53 (−/−) cells (Supplementary Fig. S4A and B). Similarly, when various human gastric and breast cancer cells were treated with ADR or Nutlin-3, a decrease in HDGF expression was found in a wild-type p53-dependent manner (Supplementary Fig. S5). Moreover, HDGF expression was reduced in mouse and rat fibroblasts in a wild-type p53–dependent manner (Supplementary Fig. S6). These results indicated that HDGF is downregulated by activating endogenous p53 in cancer and normal cells.

Repression of HDGF promoter activity by p53

Because p53 mainly functions as a transcription factor, the regulation of the HDGF promoter was examined with a reporter assay. Human genomic DNA upstream of the HDGF gene was amplified by PCR, and a 911-bp fragment was cloned into a firefly luciferase-expressing reporter plasmid (pGL3-HDGF-pro). Cotransfections of the reporter pGL3-HDGF-pro along with a range of concentrations of a vector expressing wild-type p53 were done in H1299 (p53 null) and HEK293 (wild-type p53) cells. The HDGF reporter activity was reduced accordingly with increasing amounts of wild-type p53 vector (Supplementary Fig. S7). In addition, similar experiments were done with mutant p53 expression plasmids. As expected, wild-type p53 downregulated the HDGF promoter and upregulated the p53AIP1 promoter activity in HCT116-p53 (+/+) and HCT116-p53 (−/−) cells (Fig. 3A). Of the 8 tumor-derived p53 mutants examined, 7 mutants (R175H, R248Q, R248W, R249S, R273C, R273H, and R282W) lost this activity almost completely. An intriguing finding was that the mutant G245S retained partially the wt-p53 activity (Fig. 3A). In addition, we generated replication-deficient recombinant adenoviral vectors containing these p53 mutants and examined the HDGF protein levels following transfection of HCT116-p53 (−/−) cells with wild-type p53– or mutant p53-expressing adenovirus vectors. Supplementary Figure S8 shows that wild-type, but not 8 p53 mutants, repressed HDGF protein expression in HCT116-p53 (−/−) cells.

Whether ADR treatment downregulated the HDGF promoter in a p53-dependent manner was then investigated. When pGL3-HDGF-pro was transiently transfected into HCT116-p53 (+/+) cells, treatment with ADR significantly reduced luciferase expression from this construct (Fig. 3B). The basal activity of the HDGF promoter was higher in HCT116-p53 (−/−) cells than in HCT116-p53 (+/+) cells (Fig. 3B, compare bar 4 with bar 1). Furthermore, ADR treatment did not substantially reduce HDGF promoter activity in HCT116-p53 (−/−) cells (Fig. 3B, bar 6). These data suggest that, in response to DNA-damaging agents, the HDGF promoter responds in a similar manner to the endogenous HDGF gene. Like wild-type p53, p63 and p73β were also shown to inhibit the HDGF promoter activity in H1299 and Saos-2 cells (Supplementary Fig. S9). These results are consistent with the ability of the p53 gene family to strongly repress endogenous HDGF.

Figure 2. Endogenous p53 downregulates HDGF mRNA and protein levels. A, downregulation of HDGF mRNA and protein by DNA damage in HCT116-p53 (+/+) but not in HCT116-p53 (−/−) cells. Top, Northern blot analysis of HDGF and p21 in HCT116-p53 (+/+) and HCT116-p53 (−/−) cells after treatment with 0.5 μg/mL ADR for the indicated time points (hours). Bottom, immunoblot analysis of HDGF, p53, p21, HDAC1, and β-actin in HCT116-p53 (+/+) and HCT116-p53 (−/−) cells after ADR treatment. B, secreted HDGF protein in CM of HCT116-p53 (+/+) and HCT116-p53 (−/−) cells. Cells were seeded and incubated for 24 hours. The cells were rinsed twice with serum-free medium and were incubated with or without 0.5 μg/mL ADR for 12 or 24 hours. The media were collected and concentrated 20-fold, electrophoresed, and immunoblotted with anti-HDGF and PAI-1 Abs.
Transcriptional activation by p53 is generally associated with the binding of p53 protein to the response elements. The classical p53-binding sequence consists of 2 palindromic pentamers, PuPuPuCPuPuPuPyPyPy (27). In some cases of transcriptional repression, however, the direct binding of the p53 protein to its response element in the downregulated promoter has been observed (2). Because the activity of pGL3-HDGF-pro was reduced in transient transfection assays by wild-type p53 (Fig. 3), the existence of consensus p53-binding sequences in the human HDGF gene (GenBank Accession Number NT004487) was explored. Three putative p53-binding sites were found within 10 kb in and around exon 1 of the HDGF gene, but not within the HDGF promoter. These 3 candidate sequences were identified at nucleotide position +1,551, and +8,250 (intron 5), where +1 represents the transcription start site. ChIP assays were then done to determine whether the p53 protein could bind to these candidate sequences in vivo. As shown in Supplementary Fig. S10, these 3 candidates were amplified by PCR in the input positive control, but not in the samples immunoprecipitated with an Ab against p53. The p21 and MDM2 promoters served as positive controls for p53 binding. Although a p53 consensus sequence was not found in the HDGF core promoter, direct or indirect binding of p53 protein to the HDGF promoter was tested by ChIP assay. With an Ab directed against p53, no DNA fragment corresponding to the HDGF promoter could be coprecipitated and subsequently PCR amplified. Taken together, ChIP analyses indicate that p53 protein does not bind to the putative p53-binding sites in the HDGF gene, which suggests that the regulation of HDGF expression is independent of sequence-specific binding of p53 protein.

p53-mediated repression of HDGF promoter is inhibited by TSA

It is generally thought that transcriptional repressors are often associated with HDACs because deacetylases have been shown to be involved in several gene repression systems (28). HDAC activity in particular is required for HDAC1-mediated repression of target genes (29). TSA is a nonspecific inhibitor of HDACs (28). First, whether HDAC activity is required for the repression of HDGF by p53 was investigated. H1299 and HEK293 cells were transiently cotransfected with pGL3-HDGF-pro and wild-type p53 in the absence or presence of TSA.
TSA. Although wild-type p53 repressed HDGF promoter activity (Fig. 4A, bars 2 and 6), the repression by p53 seemed to be alleviated in the presence of TSA (bars 4 and 8). The HDGF promoter activity increased 2-fold with the addition of 300 nmol/L TSA to the culture medium (compare bars 2 and 6 with bars 4 and 8, respectively). Although the transcriptional repression by p53 could not be completely abrogated by TSA, the recovery of HDGF promoter activity by TSA in the presence of p53 is significant. Western blot analysis showed that p53 protein levels were relatively comparable in the absence and presence of TSA (Fig. 4B), which rules out the possibility that the increase in promoter activity was due to the reduction of p53 protein expression. In addition, there was no apparent increase in HDGF promoter activity when cells were treated with TSA alone (Fig. 4A, compare bars 1 and 5 with bars 3 and 7). HCT116-p53(+/+) and HCT116-p53(−/−) cells were incubated either with or without ADR treatment in the presence or absence of TSA. TSA treatment alone did not result in any significant change in the transcript levels of HDGF in both cells (Fig. 4B). The decreased HDGF mRNA level following DNA damage was restored to some extent by TSA in HCT116-p53(+/+) cells (Fig. 4B; compare lane 2 with lane 4), which is consistent with the heterogeneous results from the reporter assay shown in Fig. 4A. This demonstration of the ability of TSA to revert the repression by p53 suggests that histone acetylation is involved in the mechanism of p53-mediated transcriptional repression.

The above results indicate that p53-mediated transcriptional repression of the HDGF gene may be involved in chromatin remodeling mediated by HDAC transcriptional repressors. Thus, the status of histone acetylation at the HDGF promoter was examined by ChIP assays using Abs against acetylated histones H3 and H4. Levels of histone acetylation were reduced after ADR treatment in HCT116-p53(+/+) cells but not in

Figure 4. Decreased HDGF mRNA following DNA damage is recovered by HDAC inhibitor TSA. A, TSA partially recovers the repression of HDGF promoter mediated by p53. H1299 and HEK293 cells were cotransfected with pGL3-HDGF-pro with a wild-type p53-expressing (+) or empty vector (−). At 4 hours after transfection, cells were left untreated (−) or treated (+) with 300 nmol/L TSA for 24 hours and subjected to the dual luciferase assay. Control promoter activity was set to 100%. B, HCT116-p53(+/+) and HCT116-p53(−/−) cells were not treated (−) or treated (+) with ADR (0.5 mg/mL), TSA (300 nmol/L), or both for 24 hours. Total RNA was extracted and HDGF mRNA levels were analyzed by Northern blot and quantified by densitometry. HDGF mRNA levels are shown as a percentage of that expressed in untreated HCT116-p53(+/+) cells. The last 3 rows show p53, p21, and β-actin immunoblot analysis of cell lysates. C, ChIP assay of acetylated histones H3 and H4, and HDAC1 in HCT116 cells. ADR treatment decreases the acetylation of histone proteins bound to the HDGF promoter in a p53-dependent manner. HCT116-p53(+/+) and HCT116-p53(−/−) cells were left untreated (−) or treated (+) with ADR (0.5 μg/mL), TSA (300 nmol/L), or both for 24 hours. ChIP assays were carried out using Abs against acetylated histones H3 and H4 and HDAC1, or as a negative control without Ab. PCR amplification following ChIP was done using primer pairs corresponding to the HDGF gene (promoter, −8,000, and +3,000) and to the MDM2 promoter region, as indicated.
HCT116-p53(−/−) cells (Fig. 4C), which implies that the histone acetylation at the HDGF promoter correlated with p53 expression. In contrast, the treatment of cells with ADR did not affect histone acetylation at a distal region of the promoter (−8,000) or in intron 1 (+3,000; Fig. 4C). In contrast, histone acetylation levels at the MDM2 promoter in HCT116-p53(+/+) cells were enhanced in response to DNA damage (Fig. 4C). Whether temporal recruitment of HDAC1 protein occurred following DNA damage was then tested. Strikingly, HDAC1 protein was recruited to the HDGF promoter 24 hours after ADR treatment only in the presence of p53 (Fig. 4C). Because ADR treatment had no effect on HDAC1 expression (Fig. 2A), these observations raise the possibility that p53 may repress HDGF transcription by the recruitment of chromatin modifying factors to the HDGF promoter.

Suppression of cancer cell growth by p53-mediated repression of HDGF

Because HDGF possesses proliferative and angiogenic and activity (10, 30–32), the functional implication of HDGF in cancer cell growth was investigated. Colony formation assays revealed that the growth of HCT116 cells and HeLa cells was significantly enhanced in response to recombinant HDGF protein in a dose-dependent manner (Fig. 5A). This growth enhancing effect was neutralized by the addition of anti-HDGF Ab, which suggests that HDGF stimulates cancer cell growth. Whether the negative regulation of HDGF mediated by p53 could affect cancer cell growth was then tested. CM from Saos-2 cells transfected with Ad-p53 (p53-CM) or Ad-lacZ (lacZ-CM) was prepared (see Fig. 1), and HeLa cells were subsequently cultured with the CM. When HeLa cells were cultured with p53-CM, their ability to form colonies was significantly inhibited compared with those in culture with lacZ-CM. When anti-HDGF Ab was added to HeLa cells cultured in lacZ-CM, cell growth was suppressed comparable with cells cultured in p53-CM. Furthermore, the growth-suppressing effect of p53-CM was reduced by the addition of recombinant HDGF (Fig. 5B). Taken together, these results strongly support the notion that HDGF, a secretory protein negatively regulated by p53, functions as a paracrine growth factor that is important for the proliferation of tumor cells.

Inhibition of cancer cell migration and invasion by p53-mediated repression of HDGF

It was recently suggested that HDGF is involved in smooth muscle cell migration (30) and anchorage-independent growth and invasion of lung cancer cells (15). Whether HDGF expression could stimulate cancer cell migration was therefore tested. HeLa cells were allowed to migrate in lacZ-CM, and wound closure resulted after 8 hours (Fig. 5C, first row; lacZ-CM); this migration was inhibited by the addition of anti-HDGF Ab (Fig. 5C, second row: lacZ-CM + HDGF Ab). Conversely, p53-CM completely suppressed the migration of HeLa cells in scratch-wound cultures at 8 hours (Fig. 5C: compare the first to the third row). The inhibitory activity of p53-CM on cancer cell migration was partially abrogated by the addition of recombinant human HDGF (Fig. 5C, bottom row). Moreover, similar patterns were observed in cell invasion assays (Supplementary Fig. S11). These results suggest that p53 inhibits cancer cell migration and invasion mediated in part by downregulating HDGF.

Expression of HDGF and p53 mutation in human cancer tissue

Overexpression of HDGF has been seen previously in cancer (17–19); therefore, to investigate the p53-mediated downregulation of HDGF in human cancer cells, an immunohistochemical analysis of HDGF expression in a panel of primary gastric cancers was examined. Representative results of immunohistochemical analysis of HDGF in primary gastric cancer tissues are shown in Fig. 6A. Staining for the gene product was localized to the nucleus of tumor cells. Of the 57 specimens tested, 52 expressed higher levels of HDGF than adjacent nongastric tumoral stomach tissue. This result suggests that the overexpression of HDGF is a frequent event in gastric cancer, which is consistent with results reported previously (10–13). The relationship between HDGF expression and p53 mutational status was then investigated. Surprisingly, the HDGF expression level showed a marked correlation with p53 mutational status (Fig. 6A). HDGF was clearly more frequently overexpressed in primary tumor tissues with mutant p53 status, as compared with overexpression in those with wild-type status (P < 0.0001). Importantly, all of the 7 samples with the highest HDGF expression (grade 3) contained a p53 mutation. In addition, a large published dataset that includes 251 clinically annotated cases of breast cancer with RNA microarray and p53 mutation data was interrogated (26). HDGF mRNA expression in these breast cancers was significantly correlated with mutant p53 status (Fig. 6B, P = 1.1 × 10−77). Another dataset of 117 lung cancers showed a weaker but significant association between p53 mutations and HDGF mRNA expression (Supplementary Fig. S12, P = 0.018). In summary, high expression levels of HDGF were found in tumors with mutant p53. Taken together, these data suggested that HDGF expression is increased at least partially in a mutant p53-dependent manner in human cancers.

Discussion

HDGF function in cell proliferation and tumor formation has been extensively investigated (31). Recent studies have shown that HDGF is also involved in tumor angiogenesis through VEGF induction (32). Although the overexpression of HDGF has been implicated in a number of malignant tumors, the mechanism of HDGF regulation is largely unknown. The elevated expression of HDGF in human cancer cells may be explained by the inactivation of negative regulators of HDGF. In this study, HDGF expression was decreased in several cancer cells when p53 family was introduced. We found in human and rodent cells that the expression of HDGF is downregulated by DNA damage in a p53-dependent manner. Consistently, HDGF promoter activity was diminished when cells were transduced with p53 in a dose-dependent manner, which shows that p53 downregulates the HDGF gene at the transcriptional level (Fig. 3). Our present results suggest that the tumor suppressor p53 is a good candidate for a negative regulator of HDGF.

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Figure A: Comparative colony formation assays for HCT116 and HeLa cells with different HDGF concentrations and anti-HDGF Ab levels. Colony number is expressed relative to control conditions.

Figure B: Effect of HDGF and anti-HDGF Ab on lacZ and p53 CMs. Colony number is expressed relative to control conditions.

Figure C: Time-course analysis of Ad-lacZ CM, Ad-lacZ CM + HDGF Ab, Ad-p53 CM, and Ad-p53 CM + HDGF. Images show cell morphology and distribution at 0, 8, and 20 hours post-infection.
p53 Represses HDGF Expression

The ability of p53 to transactivate target genes containing specific binding sites is thought to be central to its role as a tumor suppressor (1, 33–35). The other two p53 family members, p73 and p63, can also bind to p53 response elements to transactivate a subset of p53 target genes, which suggests that they might overlap functionally with p53 itself (2, 36–38). In general, TA isoforms of p53 family members containing the transactivation domain might be expected to have a role in tumor suppression. Our results showed that TAp63 and TAp73, as well as p53, can act as transcriptional repressors of HDGF, which suggests an anticancerogenic role for TAp63 and TAp73 in human epithelial cells. It would also be of interest to determine whether TAp63/p73 prevents the malignant transformation of epithelial cells by repressing HDGF gene expression.

To the best of our knowledge, this study has provided a novel link between a prognostic marker HDGF and the tumor suppressor p53, a critical signaling component involved in multiple aspects of oncogenesis. Tumor-derived expression of HDGF has been reported to be a critical factor in tumor expansion in human liver cancer cells (39). Here, we showed that p53 activation resulted in a decrease in the secretion of HDGF protein. This decrease in HDGF secretion was associated with an inhibition of cancer cell growth and migration in vitro. Importantly, the growth-suppressive effect of p53-CM was partially recovered by the addition of recombinant HDGF (Fig. 5B). We also showed the inhibition of cancer cell invasion by either p53-CM or an exogenous supply of anti-HDGF Ab in the Matrigel invasion assay. These studies show an essential role for p53 in cellular growth, migration, and invasion that is mediated in part by HDGF downregulation. Replacement of the wild-type p53 gene has been pursued as a potential gene therapy strategy in several types of cancer (40). This strategy potentially relies on p53-mediated apoptosis, which in turn depends on the induction of a distinct class of transcriptional target genes, including BAX, NOXA, PUMA, and p33AIP1 (41).

However, the death of tumor cells modified with the wild-type p53 gene may also lead to the killing of uninfected neighboring tumor cells due to a bystander effect (42–45). Although the mechanism of this bystander effect has not been fully established, it is possible that p53-mediated downregulation of HDGF and the resultant inhibition of cancer cell growth and migration may contribute to a more generalized therapeutic effect after transduction of a fraction of tumor cells with wild-type p53.

The data from promoter analyses using reporter and ChIP assays strongly implicate p53 as a transcriptional regulator of...
HDGF. However, the precise mechanism of p53-mediated inhibition of HDGF expression is not yet completely known. Several mechanisms have been proposed to explain p53-mediated repression (2). First, transcriptional repression can occur through interference with the functions of transcriptional activators by the binding of p53 protein to the transcription factor itself or by the direct binding of p53 protein to the target DNA, thus preventing the binding of another transcription factor. Such a mechanism has been described for α-fetoprotein, polymerase δ catalytic subunit, and survivin promoters (2). p53 protein can also interfere with the basal transcriptional machinery without an apparent requirement to act through gene-specific activators. Instead, p53 may interact with TATA-binding protein and certain transcription factors leading to a decrease in transcription (2). In another case, p53-mediated repression was dependent on binding to a novel p53-binding motif in which the two copies of the p53-binding site PuPuPuPuC(A/T) are arranged in head-to-tail orientation. Such alternative binding sites have been found in the promoter of MDR1, cyclin B1, cyclin A, and ARF genes (46). In this study, neither site was found within the HDGF promoter. Three putative p53 binding sites, however, were found within the HDGF gene, but ChIP analysis revealed that the p53 protein did not bind directly to these putative sites or to the HDGF promoter. Thus, it is likely that p53 represses HDGF indirectly by transactivating 1 or more p53 target genes. Interestingly, several tumor cell-derived mutants of p53, which are unable to bind to the p53-binding sites, had lost this inhibitory property. To identify the promoter region responsible for wild-type p53–mediated suppression of HDGF promoter activity, additional experiments with a series of deletion constructs of the HDGF promoter are needed.

It is generally thought that transcriptional repressors are often associated with HDACs because deacetylases have been shown to be involved in several gene repression systems (27). One proposed mechanism by which p53 represses gene transcription is through the interaction with and recruitment of HDAC complexes. Repression of Map4, α-tubulin, survivin, and cyclin B2 occurs through this mechanism (2, 47, 48). In this study, HDAC1 bound to the HDGF promoter only in the presence of wild-type p53, and when these proteins were bound, the endogenous HDGF promoter showed decreased acetylated histones H3 and H4. In addition, the inhibition of HDAC activity by TSA was shown to attenuate the ability of p53 to repress both the HDGF promoter activity and the expression of the endogenous HDGF gene.

Reports have shown that HDGF is expressed in certain primary tumors and cell lines at high levels (10–13, 16–20). These results led to us to question the relationship between HDGF and p53 mutation in clinical samples, and we have found that patients with mutant p53 show significantly higher expression of HDGF than those with wild-type p53. This result supports an inverse correlation between HDGF expression and wild-type p53. Taken together, these data suggest that wild-type p53 negatively regulates the tumor promoting growth factor HDGF in vivo and that HDGF is a target for downregulation in the p53 pathway. In conclusion, we have shown for the first time that HDGF is a transcriptional target repressed by p53 family members and that p53 regulates cancer cell growth and invasion in part via HDGF repression. HDGF is known to play an important role in vascular growth and formation by regulating endothelial cell proliferation and migration (49). Thus, HDGF derived from tumors may promote tumor growth and angiogenesis via paracrine mechanisms. The data presented suggest that molecular based therapies targeting HDGF may lead to a new effective strategy for controlling the progression of human cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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