c-Jun NH$_2$-Terminal Kinase 1 Is a Critical Regulator for the Development of Gastric Cancer in Mice

Wataru Shibata, Shin Maeda, Yohko Hikiba, Ayako Yanai, Kei Sakamoto, Hayato Nakagawa, Keiji Ogura, Michael Karin, and Masao Omata

Abstract
c-Jun NH$_2$-terminal kinase (JNK) links several cellular processes, including proliferation and survival, and is believed to be involved in carcinogenesis. However, the role of JNK in gastric tumorigenesis is unknown. Immunohistochemical analysis reveals that JNK is frequently activated in human gastric cancer tissue. We investigated whether JNK1, a major JNK isozyme, is involved in chemically induced gastric cancer development. Mice lacking JNK1 exhibited a marked decrease in gastric carcinogenesis induced by N-methyl-N-nitrosourea, relative to their wild-type counterparts. Impaired tumor development correlated with decreased tumor initiation, which is associated with the production of reactive oxygen species. We also found that lower levels of tumorigenesis were correlated with the decreased expression of cyclin D and CDK as well as decreased cell proliferation. Taken together, JNK seems to be involved in both tumor initiation and promotion and may be an attractive target for the prevention of gastric carcinogenesis. [Cancer Res 2008;68(13):5031–9]

Introduction

The c-Jun NH$_2$-terminal kinase (JNK) belongs to a family of mitogen-activated protein kinases, together with extracellular regulated kinases and p38. The JNK subgroup of mitogen-activated protein kinases is encoded by three loci: Jnk1 and Jnk2 are ubiquitously expressed, and Jnk3 is expressed primarily in heart, testis, and brain (1–3). JNKs are activated by stress signals and proinflammatory stimuli.

Activated JNKs phosphorylate c-Jun, JunD, activating transcription factor, and other transcriptional factors, which are involved in the formation and activation of the activator-protein 1 (AP-1) complex (4). JNK activation has been shown to induce apoptosis, cell proliferation, or transformation, depending on the cell type and stimuli (5).

Using gene knockout mice, it was shown that JNKs are involved in many processes, including liver inflammation and proliferation, neuronal apoptosis, T cell activation, and insulin resistance (6–10). With regard to carcinogenesis, Tong and colleagues reported that JNK1 knockout mice spontaneously developed intestinal tumors, suggesting a role for JNK1 in the suppression of tumor formation in the intestine (11). In contrast, mice lacking JNK1 were much less susceptible to diethylnitrosamine-induced hepatocarcinogenesis, which was correlated with decreased cyclin D and vascular endothelial growth factor expression, diminished cell proliferation, and reduced tumor neovascularization (12). These findings suggest that JNKs can have tumor-promoting or tumor-suppressing functions, depending on the cell type or organ.

Gastric cancer is the second leading cause of cancer deaths worldwide. The incidence of gastric cancer has been declining in recent decades. A high-salt diet, smoked foods rich in nitrates, and water contaminated with Helicobacter pylori seem to be major environmental inducers of gastric cancer (13–16). A high-salt diet disrupts the gastric mucosal barrier, and N-nitroso mutagens formed from dietary nitrates in a hypochlorhydric stomach can damage DNA (13). The role of H. pylori in causing mucosal effects has been heavily investigated because of the possibility that some cancers could be prevented with antibiotics against H. pylori (16).

However, it remains unclear which molecular signals actually initiate the program of irreversible transformation.

N-nitroso mutagens, which are important gastric carcinogens, are potent inducers of cellular stress, leading to chromosomal aberrations, point mutations, cell death, and DNA damage. It has been reported that these agents induce a specific cellular stress response program, which includes the activation of JNKs and p38 mitogen-activated protein kinase (17).

To explore the role of JNK in gastric carcinogenesis, we used JNK1 knockout mice as an N-methyl-N-nitrosourea (MNU)–mediated gastric cancer model. Mice lacking JNK1 exhibited a marked decrease in gastric carcinogenesis caused by MNU, relative to wild-type controls. The impaired tumor development correlated with decreased tumor initiation, which is associated with reactive oxygen species (ROS) production. We also found that the reduced tumorigenesis correlated with decreased expression of cyclin D and CDK, resulting in decreased cell proliferation. JNK1 thus seems to be involved in both tumor initiation and promotion steps and may be an attractive target for the prevention of gastric carcinogenesis.

Materials and Methods

Mice. This study was approved by the Ethics Committee of the Institute for Adult Diseases, Asahi Life Foundation. Animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Institute for Adult Diseases, Asahi Life Foundation. C57BL/6 J mice were obtained from Tokyo Laboratory Animal Science (Tokyo, Japan). The JNK$^{+/−}$ mice have been previously described (18). All mice were maintained under pathogen-free conditions with a 12/12-h light/dark cycle.

Gastric cancer model. MNU (Sigma Chemical) was dissolved in distilled water at a concentration of 240 ppm and was freshly prepared twice per week for administration in drinking water in light-shielded bottles. The 6-week-old wild-type and Jnk1$^{−/−}$ mice were given drinking water.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Shin Maeda, Division of Gastroenterology, Institute for Adult Diseases, Asahi Life Foundation, 1-6-1 Marunouchi, Chiyoda-ku, Tokyo 100-0005, Japan. Phone: 81-3-3201-6781; Fax: 81-3-3201-6881; E-mail: shinmaeda2-g@umin.ac.jp.

©2008 American Association for Cancer Research. doi:10.1158/0008-5472.CAN-07-6332

www.aacrjournals.org 5031 Cancer Res 2008; 68: (13). July 1, 2008

Downloaded from cancerres.aacrjournals.org on June 9, 2017. © 2008 American Association for Cancer Research.
containing 240 ppm of MNU on alternate weeks for a total of 5 weeks of exposure, according to a protocol described in a previous report (19). The MNU-treated mice were sacrificed at 40 weeks after starting MNU administration.

Preparation of tissue samples for tumor counting and histologic analysis. The stomach was removed and opened along the greater curvature. The number and the long diameter of the tumors in the stomach were measured under a dissecting microscope (5× magnification). Tumors >1.0 mm in diameter were mapped and counted. After counting, all of the excised stomachs were fixed for 24 h in neutral-buffered 10% formalin, embedded in paraffin, sectioned at 4 μm, and stained with H&E.

Immunohistochemistry and terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling assay. Sections were deparaffinized, rehydrated, treated with 3% H2O2 in PBS, and incubated overnight at 4°C with anti–phosphorylated JNK1, anti-JNK1, anti–phosphorylated c-Jun (Cell Signaling Technology), and anti–proliferating cell nuclear antigen (PCNA; Santa Cruz Biotechnology) antibodies. The binding of the primary antibody was detected with biotin-labeled anti-rabbit IgG or anti-rat IgG antibodies (1:500 dilution; Vector Laboratories), followed by a streptavidin–horseradish peroxidase reaction, visualization with 3,3-diaminobenzidine (Sigma-Aldrich), and counterstaining with hematoxylin. No specific staining was observed in the negative control slides prepared without primary antibody. To evaluate epithelial cell apoptosis, a terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling assay was performed according to the manufacturer’s instructions (ApopAlert DNA fragmentation assay kit; TAKARA Bio, Inc., Japan).

**Figure 1.** Loss of JNK1 decreases MNU-induced tumor development. A, sections were stained with an anti–phosphorylated JNK antibody. A representative sample of positive (top left) or negative (top right) staining of a tumor. Sections were also stained with an anti-JNK1 antibody (bottom). B, stomachs of 11-month-old male wild-type (WT) and Jnk1−/− mice that were given MNU (240 ppm in the drinking water) every other week for a total of 5 wk. Arrowheads, tumor. C, number of tumors (>1.0 mm) and maximal tumor size (diameter) in stomachs of male wild-type (WT; n = 10) and Jnk1−/− (n = 9) mice 10 mo after MNU administration. Columns, mean; bars, SE. *, P < 0.05, versus wild-type mice.
**Human tissue array.** A human tissue array was purchased from Isu Abxis Co., Ltd. Immunohistochemistry for phosphorylated JNK1 or JNK1 was performed as above. We counted >100 cells in three microscopic fields (magnification, ×200). When >30% of the cells were positive for phosphorylated JNK, we defined the tumor as phosphorylated JNK-positive.

**Cells and MNU treatment.** Fibroblasts derived from wild-type and Jnk1−/− mouse embryos were cultured in DMEM plus 10% FCS (20). Rat gastric epithelia cell line, RGM1, and human gastric cancer cell lines, AGS, SH101, and MKN1, were maintained in RPMI 1640 or DMEM supplemented with 10% fetal bovine serum, L-glutamine, 100 units of penicillin-G, and 100 μg/mL of streptomycin. For transformation analysis, cells were exposed to MNU (100 μg/mL) for 1 h, once a week for up to 6 weeks. Cell numbers were determined with a cell counting kit, according to the manufacturer’s protocol (Dojindo Laboratories). For other in vitro analyses, cells were exposed to MNU (200 μg/mL).

**Immunoblot and kinase assay.** Protein lysates were prepared from cultured mouse embryonic fibroblasts (MEF), separated by SDS-PAGE, transferred to polyvinylidene difluoride membrane (Millipore), and analyzed by immunoblotting with the indicated antibodies. The antibodies used were anti-JNK1, anti-PCNA, anti-Cdc2, anti-Cdk2, anti–cyclin-A, anti–cyclin-D1, and anti–cyclin-E (all from Santa Cruz Biotechnology); and anti–phosphorylated JNK1, anti–phosphorylated c-Jun, anti–c-Jun, anti-tubulin, and anti–phosphorylated p53 (all from Cell Signaling). A JNK immune complex kinase assay was performed as described elsewhere (21), using anti-JNK1 antibody (PharMingen) and glutathione S-transferase c-Jun (1–79) as a substrate. The basal JNK1 level was monitored by immunoblotting.

**DNA damage detection.** DNA damage induced by MNU treatment was analyzed using a DNA damage quantification kit (Oxford Biomedical Research, Inc.). In brief, DNA was isolated from MNU-stimulated and control MEFs using a DNeasy isolation kit (Qiagen). Apurinic/apyrimidinic (AP) sites were detected in isolated DNA using an aldehyde-reactive probe reagent. After treating the DNA with the aldehyde-reactive probe reagent, AP sites were tagged with biotin, and the DNA was bound to a microtiter plate, along with aldehyde-reactive probe–tagged DNA standards. The number of AP sites in the sample DNAs was determined by absorbance at 450 nm and comparison with a standard curve.

**Xenograft tumor model.** MEFs (1 × 10^5) with or without MNU treatment were injected s.c. into the flanks of 6-week-old male/female nude mice. The sizes of the resulting tumors were measured with calipers at each time point for 28 days.

**Small interfering RNA, transfection, and reagents.** RNA oligonucleotides for small interfering RNA (siRNA) were synthesized by Dharmacon Inc. All siRNA transfections were performed with Lipofectamine RNAiMAX reagent (Invitrogen Life Technologies). All siRNAs were tested and verified as reducing expression, leading to 80% reduction in protein in AGS and SH101 cells as determined by immunoblot analysis. The JNK inhibitor SP600215 and the MEK inhibitor PD98059 were from Calbiochem.

**Quantitative real-time reverse transcription-PCR.** Gastric tissues from MNU-treated mice were examined for mRNA expression by quantitative real-time reverse transcription-PCR. RNA was extracted using ISOGEN reagents (Nippon Gene), according to the manufacturer’s protocol. The cDNA was generated using SuperScript II (Invitrogen Life Technologies), and the amounts of the different mRNAs were measured by real-time...
PCR, using glyceraldehyde-3-phosphate dehydrogenase mRNA for normalization. Primer sequences are available on request.

**Statistical analysis.** Data are expressed as means ± SE. Differences were analyzed by Student's t test. P ≤ 0.05 was deemed statistically significant.

**Results**

**JNK activation in human gastric cancer tissues.** To determine whether JNK was activated in human gastric cancer tissues, tissue sections from 40 gastric cancers, including adenocarcinomas and signet ring cell carcinomas, were immunohistochemically stained with anti–phosphorylated JNK or anti-JNK1 antibody (Fig. 1A). JNK activation was seen in 40% of adenocarcinomas and in 23% of signet ring cell carcinomas, whereas noncancerous tissues showed no apparent activation. There was no association between JNK activation and either the intestinal and diffuse subtypes of gastric cancer (data not shown). Intestinal metaplasia and gastritis tissues showed a small number of epithelial cells with JNK activation (Supplementary Fig. S1). Total JNK1 was expressed in all cancerous and noncancerous tissues (Fig. 1A). These results indicate that JNK is frequently activated in gastric cancer tissues and suggest that this activation may play a role in cancer development.

**Loss of JNK1 attenuates gastric tumor development.** To explore the role of JNK in gastric cancer development, we used a mouse model of chemically induced gastric carcinogenesis. The administration of MNU to mice results in efficient gastric cancer induction (22). Of the two JNK loci expressed in the stomach, Jnk1 and Jnk2, Jnk1-encoded isoforms were primarily responsible for cell proliferation (7) and cell death (23). Wild-type and Jnk1−/− male mice, both from a C57BL/6 background, were given drinking water containing MNU in light-shielded bottles on alternate weeks (total exposure was 5 weeks) and then normal tap water until week 40. All wild-type mice (100%) given MNU developed gastric tumors, but gastric tumors were induced in only four of nine (44%) Jnk1−/− mice (P < 0.05; Fig. 1B). In addition, the number of detectable gastric tumors was significantly reduced in Jnk1−/− mice, as were maximal tumor diameters (Fig. 1C). These data suggest that JNK1 is required for efficient gastric tumor induction in response to MNU administration.

**JNK-deficient tumors exhibited decreased cell proliferation.** A representative adenocarcinoma lesion is shown in Fig. 2A. Immunohistochemical analysis revealed many PCNA-positive cells in wild-type tumors (Fig. 2A). Cells positive for phosphorylated c-Jun and JNK were also found in tumors (Fig. 2A) but not in background tissues (data not shown). Jnk1−/− tumors contained a few cells with phosphorylated c-Jun and phosphorylated JNK, and fewer PCNA-positive cells than wild-type cells (Fig. 2A), but there was no significant difference in the number of apoptotic cells or cells positive for PCNA between the background tissues (Fig. 2C; further data not shown). We also examined the expression of known cell cycle regulators in tumors of both genotypes. The levels of cyclin D1, PCNA, and CDK2 in the average size of tumors of both genotypes were substantially higher in wild-type tumors than in Jnk1−/− tumors (Fig. 2B).

**JNK1 deficiency decreased cellular transformation.** A single oral administration of MNU induced JNK activation in the gastric epithelium at 4 hours (Fig. 3A). To investigate whether JNK1 contributed to tumor initiation, we analyzed the effect of MNU treatment on MEF. JNK activation was observed after 15 minutes of MNU administration, and this activation declined to almost basal levels at 120 minutes. With longer observations, however, the activity was elevated again at 4 to 12 hours (Fig. 3B). We also

---

**Figure 3.** MNU induces JNK activation in ROS-dependent manner. **A,** MNU-treated gastric sections were stained with phosphorylated JNK antibody. A representative sample of positive (left) or negative (right) staining (original magnification, ×100). **B,** time course of MNU-mediated JNK1 activity in wild-type mouse fibroblasts. Kinase activities were measured by immune complex kinase assays. **C,** analysis of JNK activation after treatment with MNU (100 μg/mL) with or without BHA (100 μmol/L) in MEFs, RGM1, and MKN1 cells.

---
observed JNK activation by MNU administration in RGM1, rat gastric epithelial cells, and MKN1, gastric cancer cells (Fig. 3C). MNU is a multisystem teratogen that damages proliferating cells through macromolecule alkylation and generation of ROS (24). Increased ROS production is a major factor in JNK activation and acute injury, at least in the liver, and both of these can be prevented by antioxidants such as butylated hydroxyanisole (BHA; ref. 25).

Thus, we next assessed whether MNU-mediated JNK activation was ROS-dependent. MNU increased ROS production in both wild-type and Jnk1−/− MEFs at almost the same level (data not shown). As in the liver, pretreatment with BHA significantly blocked activation of JNK by MNU in MEFs, RGM1 and MKN1 (Fig. 3C).

To investigate whether JNK activation resulted in cellular transformation after MNU administration, we analyzed MNU-mediated DNA damage in both wild-type and Jnk1−/− MEFs, using a DNA damage detection kit. We found that MNU, an alkylating agent, strongly induced DNA damage in wild-type MEFs but not in Jnk1−/− cells (Fig. 4A). Next, to assess whether MNU induced transformation in MEFs, we treated wild-type and Jnk1−/− cells with MNU for 1 h, once a week for up to 6 weeks. We counted cell numbers until 8 weeks after the initial treatment. Wild-type cells started to die at 12 hours after the initial MNU treatment, and the number of cells was decreased by up to 50% at 72 hours. In contrast, cell viability of Jnk1−/− cells was unchanged by this treatment. Wild-type cells were also considerably more sensitive to MNU compared with Jnk1−/− cells at the final treatment. Finally, both cells started to grow 48 hours after the final treatment (Fig. 4B). We analyzed cell cycle–associated proteins by Western blotting after 8 weeks and found that cdc2 and cyclin A were increased in MNU-treated wild-type cells but not in Jnk1−/− cells (Fig. 4C). c-Jun was detected and increased only in wild-type cells (Fig. 4C). We also analyzed the potential of cells growing in soft agar and found that wild-type cells yielded a number of colonies, whereas Jnk1−/− cells did not (data not shown). Finally, we implanted the cells into immunodeficient nude mice and found that MNU-treated wild-type cells, but not Jnk1−/− cells, grew (Fig. 4D). Untreated cells did not grow in nude mice. These results suggest that JNK1 plays an important role in MNU-mediated transformation.

Loss of JNK1 attenuates MNU-mediated apoptosis. Cell injury is an important process for compensatory proliferation and is linked to carcinogenesis (12, 25). In vitro, MNU induced lower levels of cell death in Jnk1−/− cells, compared with wild-type cells, as above. We investigated apoptotic cell death, which is strongly associated with JNK activation by certain stimuli such as tumor necrosis factor-α and MNU treatment. Oral MNU administration caused more gastric apoptosis in wild-type mice than in Jnk1−/− mice (Supplementary Fig. S2). MNU-treated wild-type cells showed...
more apoptosis than similarly treated \( Jnk1^{-/-} \) cells, whereas no obvious difference was found between wild-type and \( Jnk1^{-/-} \) cells without MNU treatment (Fig. 5A). MNU-treated MKN1 also showed more apoptosis than similarly treated JNK1 knockdown cells of MKN1 (JNK1 siRNA), whereas no obvious difference was found without MNU treatment (Fig. 5A).

In wild-type cells, MNU treatment resulted in the phosphorylation of p53, one of the most important molecules in inducing apoptosis owing to DNA-damaging agents such as MNU, and this response was markedly attenuated in \( Jnk1^{-/-} \) cells (Fig. 5B). We also observed that phosphorylation of p53 by MNU was attenuated in JNK1 knockdown cells (Fig. 5B). The activation of p53 by MNU was also inhibited by treatment with the antioxidant BHA, suggesting that p53 activation was ROS-dependent and JNK-dependent (Fig. 5C). The mRNA levels of Noxa, an apoptotic mediator induced by p53 activation, was significantly lower in MNU-treated \( Jnk1^{-/-} \) mice than in similarly treated wild-type controls (Fig. 5D). These results suggest that JNK1 contributed to MNU-induced apoptosis by promoting the ROS-dependent activation of p53. The mechanism of p53 modulation by JNK1 remains to be elucidated.

**Discussion**

In the current study, we showed that JNK1 activation was important in gastric cancer development induced by the DNA-alkylating agent MNU. Loss of JNK1 attenuates cancer cell growth. Proliferation is also an important step in carcinogenesis. Not only tumor number but also tumor size were decreased in \( Jnk1^{-/-} \) mice. We thus investigated the possible role of JNK in tumor cell proliferation. Gastric cancer cells, SH101 and AGS, were cultured with or without a JNK inhibitor, SP600125, or a MEK inhibitor, PD98059. Cell numbers were significantly inhibited by treatment with the JNK inhibitor, whereas the effect of the MEK inhibitor was modest (Fig. 6A). Western blot analysis of the proliferation-associated and cell cycle–associated proteins PCNA, cdc2, cyclin D1, and c-Jun showed that they were also decreased by the JNK inhibitor (Fig. 6B). We used JNK1 siRNA in SH101 and MKN1 cells and found that proliferation-regulated and cell cycle–regulated genes were decreased by JNK1 inhibition (Fig. 6C and D). These results suggest that not only tumor initiation, which is defined as transformation, but also tumor proliferation were dependent on JNK activation.

**Figure 5.** Loss of JNK1 attenuates MNU-mediated apoptosis. A, apoptosis in MNU-treated MEFs and MKN1 was analyzed by the apoptotic cell death assay. B, phosphorylated p53 and phosphorylated JNK in MNU-treated MEFs and MKN1 were analyzed by immunoblotting. C, analysis of early phase p53 phosphorylation in MEFs, RGM1, and MKN1 treated with MNU with or without BHA (100 \( \mu \text{mol} \)) \( \text{L}^{-1} \)). D, RNA was isolated from MEFs treated with MNU for 4 h, untreated wild-type MEFs, or \( Jnk1^{-/-} \) MEFs, and RNA levels were determined by real-time PCR.
MNU. Moreover, we also showed that it is important in both initiation and promotion steps.

Regarding the initiation step, a role for JNKs in oncogenic transformation has been previously suggested (26, 27). Originally, JNKs were identified as protein kinases that phosphorylated c-Jun on serine residues (28). c-Jun is a well-characterized oncogene, especially in liver (29), and its phosphorylation by JNK may be of relevance in hepatocellular carcinogenesis (12). In addition, Rennefahrt and colleagues reported that constitutive JNK activation induced transformation without oncogenic induction (30). We showed that MNU activated JNK for a relatively long time and strongly induced c-Jun phosphorylation, dependent on JNK activation. We also showed that JNK was involved in transformation by MNU in vitro and in vivo. To our knowledge, this is the first report in which JNK activation is involved in transformation by extracellular stimuli and not by intracellular oncogenic overexpression.

The pathogenesis of human gastric cancer is a multistep and multifactorial process (31). Free radicals or ROS are low molecular weight metabolites sufficiently reactive to damage essential biological molecules. The release of ROS inside the nuclear membrane of a cell can damage DNA and induce mutations, which is a basis for carcinogenesis (32). In this study, we showed that ROS were necessary for JNK activation by MNU. This indicates that ROS are an important factor in MNU-mediated transformation through JNK and are involved in the transformation of gastric epithelial cells. It remains unknown whether other carcinogens for gastric cancer, such as *H. pylori*, also involve ROS-mediated carcinogenesis through JNK activation.

With regard to the role of c-Jun in carcinogenesis involving JNK activation, it has been reported that c-Jun, phosphorylated by JNK, interacts with the HMG-box transcription factor TCF4 to form a ternary complex containing c-Jun, TCF4, and β-catenin. In the Apc(Min) mouse model of intestinal cancer, genetic abrogation of c-Jun NH2-terminal phosphorylation or gut-specific conditional c-Jun inactivation reduced tumor number and size and prolonged life span (33). This mechanism may also be involved in MNU-induced carcinogenesis and needs examination. Consistent with this, the expression of cyclin D, which is regulated by the WNT and
AP-1 pathways, decreased in the JNK1 knockout group in this study. Similarly, in liver tumor development, disruption of JNK1 decreased chemically induced hepatic carcinogenesis and chemical-induced AP-1 activity, producing a modest decrease in c-Jun protein levels. The absence of JNK1 resulted in decreased expression of cyclin D proteins, which are important for hepatocyte and hepatoma cell proliferation (12).

In contrast to these findings, JNK can also act as a tumor suppressor. Mice deficient in JNK1 exhibited enhanced sensitivity for skin tumorigenesis (34). The upstream activator of JNKs, MKK4, has been identified as a metastasis suppressor in human cancers. Regarding gastrointestinal organs, it is reported that JNK1 plays a critical role in the suppression of tumor formation in the intestine, which has been linked to the altered expression of p21 (11). In contrast, we have checked the stomachs of more than 30 mice and detected no spontaneous tumors. In addition, p21 expression was not changed between wild-type and JNK1−/− mice. These results suggest that JNK1 seems to play a tumor-promoting role in the stomach. However, because of the opposing roles of JNKs in cancer, it is important to define the dominant role of JNKs in a specific cancer before JNK inhibitors can be widely employed as a cancer chemotherapeutic.

We focused on the transformation and proliferation of gastric epithelial cells in particular. However, it is necessary to examine the environment of the cells, including the levels of growth factors and inflammatory cytokines. It has been reported that JNK affects the expression of various factors through activation of c-Jun/AP-1, which is downstream of JNK. For example, angiogenic factors such as vascular endothelial growth factor or interleukin-8 are transcribed by an AP-1–dependent pathway (35, 36). Consistent with this, it has been reported that the expression of vascular endothelial growth factor in a liver cancer model was decreased in JNK1 knockout mice (12). We also analyzed the gene expression profiles of MNU-treated mouse stomachs and found that the expression of several inflammatory cytokines such as tumor necrosis factor-α and interleukin-6 was slightly decreased in JNK knockout mice, compared with wild-type controls.

DNA damage by MNU was decreased by a deficiency in JNK1, showing that DNA damage and subsequent immortality apparently involve the activation of JNK. On the other hand, through phosphorylation of p53, cells with DNA damage are removed by apoptosis (37). The attenuation of JNK activation by MNU in JNK1-deficient cells, which we showed here, may result in both decreased immortality and cell death attenuation and, as a result, decreased carcinogenesis. It is believed that increased cell death activates inflammation in neighboring cells, and much of the observed compensatory proliferation depends on cytokines produced by inflammatory cells (25). It would be very difficult to prove this hypothesis in vivo, but gastric mucosal injury and inflammatory cell infiltration were readily observed after MNU administration.

In conclusion, mice lacking JNK1 exhibited a marked decrease in gastric carcinogenesis caused by MNU, relative to wild-type controls. The impaired tumor development correlated with decreased transformation, which is associated with ROS production, and decreased cell proliferation. JNK1, therefore, is involved in both tumor initiation and promotion and may be an attractive target for the prevention of gastric carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 11/20/2007; revised 4/1/2008; accepted 4/14/2008.

Grant support: Grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (no. 17029026 and no. 19390025; S. Maeda and M. Omata), and the NIH and AACR (M. Karin). M. Karin is an American Cancer Society Research Professor. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

c-Jun NH2-Terminal Kinase 1 Is a Critical Regulator for the Development of Gastric Cancer in Mice

Wataru Shibata, Shin Maeda, Yohko Hikiba, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/13/5031

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2008/06/20/68.13.5031.DC1

Cited articles
This article cites 37 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/13/5031.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/68/13/5031.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.