

Three novel *NEIL1* promoter polymorphisms in gastric cancer patients

Masanori Goto, Kazuya Shinmura, Hong Tao, Shoichiro Tsugane, Haruhiko Sugimura

Masanori Goto, Kazuya Shinmura, Hong Tao, Haruhiko Sugimura, First Department of Pathology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi Ward, Hamamatsu, Shizuoka 431-3192, Japan

Shoichiro Tsugane, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan

Author contributions: Goto M performed the majority of the experiments; Shinmura K and Sugimura H designed the study and wrote the manuscript; Tao H performed the statistical analysis; Tsugane S coordinated the collection of all of the human materials and provided them.

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Correspondence to: Haruhiko Sugimura, PhD, First Department of Pathology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi Ward, Hamamatsu, Shizuoka 431-3192, Japan. hsugimur@hama-med.ac.jp

Telephone: +81-53-4352220 Fax: +81-53-4352225

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frequency of 0.6%, 9.4%, and 4.4%, respectively, in Japanese gastric cancer patients.

CONCLUSION: Three *NEIL1* promoter polymorphisms detected in this study may be of importance in gastric carcinogenesis.

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Key words: Gastric cancer; *NEIL1*; Base excision repair; Genetic polymorphism

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Abstract

AIM: To identify genetic polymorphisms in the promoter region of the human base excision repair gene *NEIL1* in gastric cancer patients.

METHODS: The *NEIL1* promoter region in DNA from 80 Japanese patients with gastric cancer was searched for genetic polymorphisms by polymerase chain reaction-single-strand conformation polymorphism and subsequent sequencing analyses.

RESULTS: Three novel genetic polymorphisms, i.e. c.-3769C>T, c.-3170T>G, and c.-2681TA[8], were identified in the *NEIL1* promoter region at an allele

INTRODUCTION

Stomach tissue is exposed to oxidative stress, including inflammation induced by *Helicobacter pylori* infection, sodium chloride, and smoking^[1-5]. Since severe oxidative stress leads to accumulation of huge amounts of damaged bases^[6-9], maintenance of a system to repair damaged bases in the stomach is thought to be important. The base excision repair protein NEIL1 has activity that is capable of removing oxidatively damaged bases, including thymine glycol, 5-hydroxyuracil, urea, formamidopyrimidine-A, and formamidopyrimidine-G, which have been shown to cause mutagenesis and cell death^[10-14]. We have recently demonstrated somatic inactivating *NEIL1* mutations and reduced NEIL1 expression in a subset of gastric cancers, suggesting that reduced NEIL1 activity is involved in gastric carcinogenesis^[15]. In a recent investigation of the NEIL1 expression system

an approximately 1.2 kb sequence upstream of the transcriptional initiation site of the *NEIL1* gene was shown to have promoter activity by a luciferase reporter assay in human cells^[16]. However, since no genetic polymorphisms have been reported in the promoter region thus far and genetic polymorphisms in the region may be of importance in gastric carcinogenesis, we tried searching DNA extracted from the blood of 80 gastric cancer patients for *NEIL1* promoter polymorphisms.

MATERIALS AND METHODS

Samples

Blood samples from 80 gastric cancer patients were obtained from hospitals in Nagano Prefecture, Japan, and genomic DNA was extracted from them with a DNA Extractor WB Kit (Wako, Osaka, Japan)^[17]. The baseline characteristics of the patients have been described previously^[17]. This study was approved by the Institutional Review Boards of Hamamatsu University School of Medicine and the National Cancer Center.

Polymerase chain reaction (PCR)-single-strand conformation polymorphism (SSCP) and sequencing analyses

PCR-SSCP analysis was used to examine the DNA samples for genetic polymorphisms in the *NEIL1* promoter region. An approximately 1.2 kb 5' upstream sequence that was shown to have promoter activity in a previous study^[16] was divided into 8 regions (Figure 1A), and each region was amplified by PCR with HotStarTaq DNA polymerase (QIAGEN, Valencia, CA, USA). The primer sets used were: 5'-CAAATATTGCAGTCTGA AAGGGG-3' and 5'-GAAACTGATCAAGACAGGG GC-3' for region 1, 5'-GTTTCTCTAATGCAGAGGTC TGG-3' and 5'-TACAGGGGATAAGCCACTA CGC-3' for region 2, 5'-CCTCCTGATATGATGCAATTC-3' and 5'-CACTCCCAGCTGATTTTGTG-3' for region 3, 5'-ATGGTGAAACCCCGTCTCTAC-3' and 5'-T GCTGGGAATTAGATCTAAAGGC-3' for region 4, 5'-AGCACCTAGGAAGTATCCCTG-3' and 5'-GTCTC AGCCAGTTGTGTGTTTG-3' for region 5, 5'-CAAATT GAGAATGTGATGCAGC-3' and 5'-CAGATTTCCCC AATTGTCCC-3' for region 6, 5'-TGACCCATGATTG TAGCCTG-3' and 5'-GAGGTTTCGCCT TGTTGG-3' for region 7, and 5'-GAGGCGGGCAGATTACTT G-3' and 5'-CTCACTGCAGCC TCCACTTC-3' for region 8. The PCR products of regions 4 and 6 were digested with restriction enzymes *MvaI* (New England Biolabs, Beverly, MA, USA) and *AvaI* (New England Biolabs), respectively, in order to adjust their size to < 230 bp before SSCP. The PCR products of all regions were diluted with two volumes of loading solution, and after applying them to 8% polyacrylamide gels in the presence or absence of 5% glycerol, the products were electrophoresed at room temperature and 4°C and detected by silver staining. PCR products exhibiting an abnormally shifted band in the SSCP analysis were directly sequenced with a BigDye Terminator Cycle

Sequencing Reaction Kit (Applied Biosystems, Tokyo, Japan) and an ABI 3100 Genetic Analyzer (Applied Biosystems). A PCR product of region 7 was also sequenced after subcloning into a pGEM-T Easy vector (Promega, Madison, WI, USA). The reference nucleotide sequence is accession number NM_024608. Deviation of the genotype distribution from Hardy-Weinberg equilibrium (HWE) was tested by using SNPalyze software (Dynacom, Yokohama, Japan).

RESULTS

We searched for genetic polymorphisms in the region containing *NEIL1* promoter activity by PCR-SSCP analysis using blood samples derived from 80 gastric cancer patients. Three genetic polymorphisms, c.-3769C>T, c.-3170T>G, and c.-2681TA[8], were identified in the *NEIL1* promoter region at an allele frequency of 0.6%, 9.4%, and 4.4%, respectively (Figure 1B). The distribution of the genotypes of these polymorphisms was in HWE. Examination of the frequency of the polymorphisms revealed a homozygote for the variant allele of only one of the three polymorphisms, c.-3170T>G, and in only one patient, indicating that the three polymorphisms in the *NEIL1* promoter are rare genetic polymorphisms.

DISCUSSION

In this study, we found three novel promoter polymorphisms, c.-3769C>T, c.-3170T>G, and c.-2681TA[8]. None of these polymorphisms has previously been reported or registered in the database of the single nucleotide polymorphism (dbSNP) homepage of the National Center for Biotechnology Information web site (web site : <http://www.ncbi.nlm.nih.gov/SNP/>) or the database of the Japanese single nucleotide polymorphism homepage (<http://snp.ims.u-tokyo.ac.jp/>), indicating that they are novel genetic polymorphisms.

Interestingly, when we used Genomatix software (<http://www.genomatix.de/matinspector.html>) to search for transcription factors that putatively bind to the sequence containing these polymorphism sites, a sequence containing c.-3170T was predicted to bind to GATA binding factors and a sequence containing c.-2681TA[7] was predicted to bind to a GZF1, a TATA-binding protein and LIM homeodomain factors. The change from c.-3170T to c.-3170G eliminates the binding site for GATA binding factors. On the other hand, although the change from c.-2681TA[7] to c.-2681TA[8] would appear to retain the sequence of binding sites for the GZF1, TATA-binding protein, and LIM homeodomain factors, there are examples of a change in the number of repetitive sequences in a promoter being associated with a difference in the expression level^[18]. Thus, these nucleotide changes may be associated with a difference in the *NEIL1* expression level. Moreover, since some factors involved in the regulation of the transcription level in human cells remain unknown,

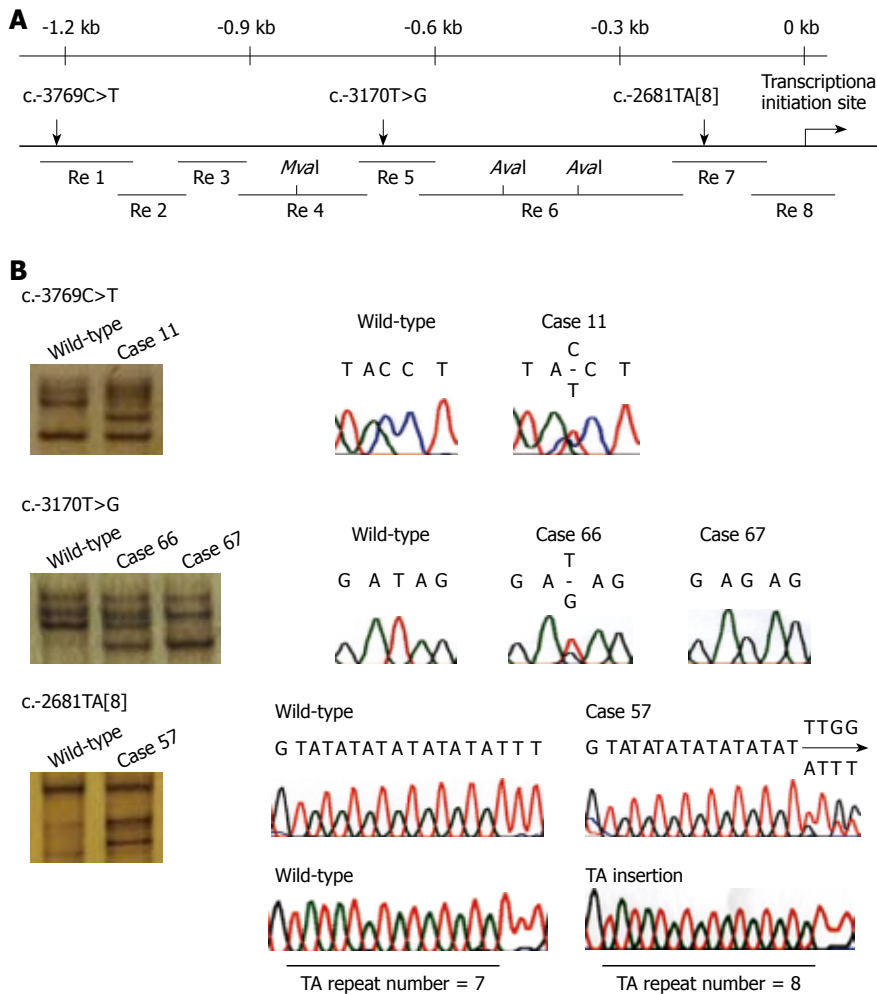


Figure 1 Identification of novel *NEIL1* promoter polymorphisms by PCR-SSCP and subsequent sequencing analyses. **A:** Schematic map of the *NEIL1* promoter region. The three genetic polymorphisms identified in this study are mapped, and the 8 PCR-amplified regions (Re 1-Re 8) and restriction enzyme sites are shown; **B:** Identification of c.-3769C>T, c.-3170T>G, and c.-2681TA[8] polymorphisms in the *NEIL1* promoter. The panels on the left show representative results of the PCR-SSCP analysis. The electropherograms on the right show the results of the sequencing analysis. Only the lowermost panels show the results of sequencing after subcloning the PCR product; the others show the results of direct sequencing.

the three *NEIL1* promoter polymorphisms may be associated with differences in the *NEIL1* expression level by binding to factors that have yet to be identified.

Most common genetic polymorphisms have been registered in various genetic polymorphism databases, such as dbSNP. However, as shown in this study, there appear to be many genetic polymorphisms that still have not been registered in databases, the reason being that many unregistered genetic polymorphisms are rare. Since finding rare and novel polymorphisms requires many human samples and repeating this kind of study, the *NEIL1* data presented in this study are very valuable for future studies, such as searches for alleles that increase the risk of diseases and allele-specific expression analyses. Furthermore, *NEIL1* protein plays a very important role in excision repair of oxidatively damaged bases, which have been implicated in a wide variety of human cancer. Promoter polymorphisms in some DNA repair genes, including *XRCC1*, *MLH1*, and *MSH2*, have recently been reported to be associated with an increased risk of cancer^[19-21]. Like these examples, the novel *NEIL1* promoter polymorphisms identified by screening gastric cancer patients in this study may be associated with increased risk of gastric cancer. If so, this information should be of value in management to prevent the development of gastric cancer in individuals with the risk allele. We are therefore planning to examine

the *NEIL1* promoter polymorphisms in the framework of a gastric cancer case-control study in the future, and we are also planning to investigate the effect of the polymorphisms on *NEIL1* promoter activity.

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COMMENTS

Background

The human base excision repair protein *NEIL1* has activity that is capable of removing oxidatively damaged bases, such as thymine glycol and 5-hydroxyuracil. We have recently demonstrated somatic inactivating *NEIL1* mutations and reduced *NEIL1* expression in a subset of gastric cancers, suggesting that reduced *NEIL1* activity is involved in gastric carcinogenesis. In the present study, we searched for genetic polymorphisms in the promoter

region of the human *NEIL1* gene in gastric cancer patients and succeeded in identifying three novel genetic polymorphisms.

Research frontiers

Oxidized-DNA-base lesions have been implicated in carcinogenesis, and base excision repair proteins are involved in the repair of such lesions. The research frontier in the area of studying the relationship between the base excision repair genes and carcinogenesis lies in the discovery of genetic variants in the genes that are associated with increased cancer risk.

Innovations and breakthroughs

Stomach tissue is exposed to oxidative stress, including inflammation induced by *Helicobacter pylori* infection, sodium chloride, and smoking, and promoter polymorphisms in some DNA repair genes have been reported to be associated with increased cancer risk. However, there have been no reports of studies that have examined associations between *NEIL1* promoter polymorphisms and gastric cancer risk. The identification of three novel *NEIL1* promoter polymorphisms in this study should be of value for future research in this field.

Applications

The *NEIL1* data presented in this study will be useful for various future studies, such as studies that evaluate the effects of *NEIL1* polymorphisms on the risk of disease, haplotype analyses, and allele-specific expression analyses.

Peer review

This study investigated genetic polymorphisms in the human *NEIL1* gene and identified three polymorphisms in the promoter region of the gene. Although the study did not determine whether the polymorphisms are specific for gastric cancer patients, and no functional analysis of the polymorphisms was performed, the polymorphisms identified are indeed novel, and the results may facilitate future research in this field. In conclusion, the results of this study are somewhat valuable, and the paper appears to be worth publishing as a brief communication.

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