Expression of inducible nitric oxide synthase in human gastric cancer

Jun Yu, Fei Guo, Matthias P.A. Ebert and Peter Malfertheiner

**Subject headings** stomach neoplasms; nitric oxide synthase; nitric oxide

**INTRODUCTION**

Inducible nitric oxide synthase (iNOS) is an enzyme that catalyzes the formation of nitric oxide (NO) from L-arginine. iNOS expression and activity results in the production of high levels of NO[1]. The generation of physiological levels of NO is important for mucosal function and it also exerts a cytoprotective effect on the gastrointestinal mucosa. However, increased iNOS expression has been observed in patients with chronic inflammatory diseases of the gastrointestinal tract, such as ulcerative colitis[2,3], and gastritis[4] and it has been speculated that increased NO may induce DNA damage[5,6] and angiogenesis[7]. Nonetheless, the role of iNOS in human GI neoplasia is largely unknown. Previous studies have demonstrated increased iNOS expression in breast cancer[8,9], and increased iNOS activity and protein levels have been demonstrated in colorectal cancer[10] and adenocarcinoma of the esophagus[11]. However, to date, the role of iNOS in gastric carcinogenesis has not been elucidated.

**MATERIALS AND METHODS**

Gastric biopsies were obtained from individuals undergoing gastric endoscopy. Two or three mucosal biopsies were endoscopically obtained for histological study. One or two additional biopsies were obtained for mRNA isolation. The biopsies were snap frozen in liquid nitrogen and stored at -80 °C. The samples used in this study were collected from tumor and a tumor free location in 6 gastric cancer patients, and 7 biopsies were obtained from the histologically normal gastric mucosa in corpus and/or antrum from healthy subjects. RNA was extracted using the RNA-zol B procedure. After completion of this extraction, RNA was separated on a 1.5% agarose gel and RNA was visualized by ethidium bromide staining. cDNAs were generated from one microgram of total RNA; it was denatured at 65 °C for 10 min and cooled on ice for 2 min. The RNA was reversely transcribed in a 20 µL final volume of 5x AMV RT buffer, MgCl₂, dNTPs, random primers, 16 U of Rnasin and 1.5 U AMV Reverse Transcriptase. The reaction mixture was incubated for 1 hour at 37 °C, and for 5 min at 96 °C. For confirmation of cDNA integrity, a RT-PCR analysis using β-actin primers was also performed. The sequence of the primers were as follows: sense primer (s-iNOS), 5’ TAGAGGAACATCTGGGCCAGG-3’; antisense primer (as iNOS), 5’-TGGCAGGTTCCCTCTGATG-3’, generating a 372 bp fragment of the iNOS transcript. PCR was performed under the following conditions: 94 °C for 5 min, 60°C for 45 sec, 72 °C for 1 min; which was repeated for 35 cycles. Ten µL of the PCR reaction was separated on a 1.5% agarose gel and cDNA was visualized by ethidium bromide staining.

**RESULTS**

RT-PCR analysis using primers specific for human iNOS mRNA generated a 372 bp fragment of the predicted size. Using this RT-PCR analysis iNOS mRNA was detected in 3 of 6 tumor tissues, and in one of the adjacent tumor free gastric tissues obtained from gastric cancer patients (Table 1). In addition, a fragment of iNOS mRNA was amplified in one of 7 normal gastric tissues obtained from four healthy individuals undergoing endoscopy (Table 2). *H. pylori* infection was detected histologically in 5 of 6 cancer patients and in the stomach of two of the four healthy individuals. In two of the *H. pylori* infected indivi duals iNOS mRNA was detected in the non-cancerous mucosa, whereas all individuals without *H. pylori* infection did not exhibit iNOS mRNA.

**Table 1** iNOS expression in gastric cancer patients

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<tr>
<th>Patient</th>
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<th>Sex</th>
<th>Cancer type</th>
<th>Hp status</th>
<th>iNOS expression</th>
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<td>m</td>
<td>Diffuse</td>
<td>+</td>
<td>+</td>
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<td>3</td>
<td>69</td>
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<td>+</td>
<td>+</td>
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<tr>
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from the Land Sachsen-Anhalt (2775A/0087H) awarded to M.P.A. Ebert.

REFERENCES


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Table 2 iNOS expression in healthy individuals

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<thead>
<tr>
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<th>Gastritis</th>
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