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Expression of matrix metalloprotease-2, -7 and -9 on human colon, liver and bile duct cell lines by enteric and gastric *Helicobacter* species

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Abstract

Gastric and enteric *Helicobacter* species have been associated with malignant and inflammatory diseases of the stomach, liver, gall bladder and intestine. Matrix metalloproteinases (MMPs) participate in degradation of extracellular matrix, which allows bacteria to come in contact with and interact with the cells. Enhanced level of MMPs facilitates metastasis and cell invasion of tumor cells by removal of physical barriers, as well as modulation of biologic activities of the proteins residing in the extracellular matrix. The aim of this study was to evaluate the effect of gastric and enteric *Helicobacter* on induction of MMPs in hepatocytes and epithelial cells of gall bladder and colon. Human hepatocytes HepG2, gall bladder epithelial cells TFK-1, and colon epithelial cells HT29 were infected with strains of *H. pylori cagA+*, *cagE+*, *H. pylori cagA-*, *cagE−*, *H. pullorum*, *H. cholecystus*, *H. bilis* and *H. hepaticus*. Protein levels of MMPs were analyzed by enzyme-linked immunosorbent assay and immunohistochemistry. Reverse transcription-quantitative polymerase chain reaction was used to study mRNA levels. Increased expression of MMP-2 and MMP-9 was observed on HepG2, TFK-1 and HT29 infected with *H. pylori cagA+*, *cagE+* and *H. cholecystus* strains. *H. pylori cagA+*, *cagE+*, *H. cholecystus*, *H. pullorum*, *H. bilis* and *H. hepaticus* strains increased expression of MMP-7 on HT29, compared to uninfected control cells. The effect of MMP upregulation on HepG2, TFK-1 and HT29 was bacterial dose dependent. *H. pylori cagA−*, *cagE−* strain did not increase expression of MMPs. Inducible MMPs on colon and bile duct epithelial cells as well as hepatocytes may play an important role in facilitating invasion and progression of cancer by *Helicobacter* species colonizing the hepatobiliary and gastrointestinal tract.

Keywords: *Helicobacter*; Matrix metalloproteinase; TFK-1; HT29; HepG2

1. Introduction

The *Helicobacter* genus are gram-negative bacteria, and includes more than 20 adequately characterized species to date [1]. These species have been isolated from a number of hosts, such as humans and other primates, pigs, cats, dogs, poultry, and rodents. Moreover, within their hosts, *Helicobacter* species have been identified from both the gastric and the enterohepatic niches of the gastrointestinal tract. *H. pullorum*, first detected in the liver and intestinal contents of poultry [2], has since been isolated from humans with gastroenteritis [3]. *H. bilis* and *H. hepaticus* have been isolated from the liver and the intestinal mucosa from mice, and has been implicated to be associated with rodent chronic hepatitis and liver cancer [4,5]. *H. cholecystus* has been identified from the gall bladder of hamsters [6]. *H. pylori* is the most widely studied species and is associated with human gastric pathology [7].

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The matrix metalloproteinases (MMPs) comprise a family of over 25 members, which are secreted from a variety of cells, and are normally found as pro-enzymes in the extracellular matrix (ECM). When these molecules are activated, they participate in degradation of ECM proteins [8], and allows bacteria to come into contact with and interact with the cells. An enhanced level of MMPs also facilitates metastasis and cell invasion of tumour cells by removal of physical barriers, as well as modulation of cell adhesion and biologic activities of proteins residing in the ECM [9]. MMP-9 (gelatinase B) digests denatured collagens, such as the major component of the basement membrane type IV collagen, and has three repeats of a type II fibronectin domain in the catalytic site, which bind to the substrates including collagens, gelatine and laminin [8]. MMP-2 (gelatinase A) is structurally related to MMP-9 and cleaves ECM proteins, such as collagen types I and IV [8]. MMP-7 (matrilysin) is the smallest of all the MMPs consisting of a pro-peptide domain and a catalytic domain [8]. It degrades various matrix substrates, including proteoglycans, elastine, and gelatine, and cleaves non-matrix proteins from the cell surface, including E-cadherin, pro-tumour necrosis factor α and Fas ligand [8]. MMPs can be activated by proteinases, or in vitro, by chemical agents, low pH and heat treatment [8].

In two gastric epithelial cell lines, MKN45 and MKN28, MMP-9 expression has been shown to be upregulated by *H. pylori* possessing the cytotoxin-associated gene pathogenicity island (*cag* PAI), though independent of the vacuolating cytotoxin *vac* A locus [10]. The *cag* PAI has also been implicated to associate with upregulation of MMP-7 in colon epithelial cell line HT29 stimulated by *H. pylori* [11]. However, whether the non-gastric enteric species of the *Helicobacter* genus is associated with MMP stimulation has not been investigated, though the bacteria have been linked to inflammatory and ulcerative diseases as well as cancer of the enterohepatic tissue [1]. The object of this study was to elucidate the effect of enteric and gastric *Helicobacter* species on expression of MMP-2, MMP-7 and MMP-9 at protein as well as gene levels.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Five *Helicobacter* strains were obtained from Culture Collection University of Göteborg (CCUG), including four enteric *Helicobacter* strains: *H. pullorum* CCUG 33838, *H. cholecystus* CCUG 38167, *H. bilis* CCUG 38995 and *H. hepaticus* CCUG 33837. *H. pylori* CCUG 17874 *cagA*+, *cagE*+, *vacA s1m1*, and a chronic gastritis isolate *H. pylori* 15/96 *cagA*−, *cagE*−, *vacA s2m2*, were typed by PCR. Bacteria were grown on Blood-Brucella agar with 0.1% activated charcoal in microaerophilic milieu, generated with Anoxomat® (MART Microbiology BV, Licthenvoorde, Netherlands) in vented GasPak jars at 37 °C. Bacteria were sub-cultured every second day. For infection experiments, bacteria were harvested from agar plates, re-suspended in warm cell culture media, and were immediately incubated with the cell lines. Gram stain of bacteria was accomplished before sub-cultivation and inoculation with the cells to exclude contamination and to detect the rate of bacterial spiral and coccoid forms.

2.2. Cell lines and culture conditions

Human cell line cultures were maintained on 75 cm^2 cell culture flasks (TPP AG, Trasadihen, Switzerland). Liver cell line HepG2 and biliary tract epithelial cell line TFK-1 were cultured in RPMI 1640 (ICN, Inc., OH) supplemented with 10% fetal bovine serum (FBS, Invitrogen, Lidingö, Sweden) and 2 mM L-glutamine (ICN, Inc., OH). Colon epithelial cell line HT29 was grown in McCoy media (Invitrogen, Lidingö, Sweden) with 10% FBS and 2 mM L-glutamine (ICN, Inc., OH). Cells were incubated at 37 °C with 5% CO_2 and were split once a week. Twenty-four hours before infection, cells were transferred to 96-well tissue culture plates (TTP AG, Trasadihen, Switzerland), 100 μl per well, or to chamber glass slides (Nunc Lab-Tek™, Roskilde, Denmark), 2 ml per slide.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Bacterial doses of 10^5, 10^6 and 10^7 colony forming units (CFU) were inoculated to 2×10^4 cells in order to evaluate the dose dependency of bacterial stimulation for MMP expression. Infected cells on 96-well plates were cultured for 3, 6 or 24 h at 37 °C to evaluate the time course of MMP induction by bacteria. The wells were washed twice with 0.02 M phosphate buffered saline pH 7.2 containing 0.15 M NaCl (PBS), and fixed with 100% methanol for 10 min at −20 °C. Endogenous peroxidase activity was blocked with 0.75% H_2O_2 and 0.1% NaN_3 in PBS for 20 min at 37 °C. Cells were then washed, and primary monoclonal antibodies diluted in 1% bovine serum albumin in PBS were added (MMP-2 and MMP-9, final concentrations 0.2 μg ml^{-1}, Santa Cruz Biotechnology, Inc., CA; MMP-7 final concentration 0.3 μg ml^{-1}, Oncogene Research Products, Cambridge, MA). The plates were incubated for 1 h and 30 min at 37 °C and then washed. Secondary horseradish peroxidase (HRP)-conjugated antibody (final concentration 0.6 μg ml^{-1}) was incubated for 1 h and 15 min at 37 °C and then washed. Substrate for peroxidase *o*-phenylenediamine (Sigma–Aldrich, St. Louis, MO) was incubated for approximately 15 min in dark, and the reaction was stopped by addition of 1 M H_2SO_4. The absorbance was measured on a