**Advances in Brief**

*Helicobacter pylori* VacA Toxin Up-Regulates Vascular Endothelial Growth Factor Expression in MKN 28 Gastric Cells through an Epidermal Growth Factor Receptor-, Cyclooxygenase-2-dependent Mechanism

Rosa Caputo,1 Concetta Tuccillo,2 Barbara A. Manzo, Raffaele Zarrilli, Giampaolo Tortora, Camillo Del Vecchio Blanco, Vittorio Ricci, Fortunato Ciardiello,3 and Marco Romano3


**Abstract**

**Purpose:** *Helicobacter pylori* causes gastric damage and is involved in gastric carcinogenesis. Vascular endothelial growth factor (VEGF) plays a major role in gastric mucosa is involved in gastric carcinogenesis. We investigated: (a) whether *H. pylori*, and in particular *H. pylori* VacA toxin, affected VEGF expression in gastric epithelial cells in culture; and (b) the signal transduction pathway involved in any effect exerted by *H. pylori*.

**Experimental Design:** MKN-28 cells were incubated with unincoculated BCF (control) or with BCF obtained from *VacA*-producing wild-type *H. pylori* 60190 strain or from its isogenic mutant 60190:v1, specifically lacking vacA gene in the presence or absence of *ZD 1839*, a selective inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase, PD098059, a selective inhibitor of mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase, the kinase responsible for ERK phosphorylation, or SC-236, a selective inhibitor of cyclooxygenase (COX)-2 for 24–48 h.

**Results:** (a) Toxigenic *H. pylori* up-regulated VEGF mRNA and protein expression and caused a 2.5-fold increase in VEGF release compared with control, whereas nontoxigenic *H. pylori* did not; (b) *H. pylori* VacA toxin-induced up-regulation of VEGF was counteracted by selective inhibition of EGFR tyrosine kinase; (c) toxigenic *H. pylori* activated the ERK/MAP kinase cascade, and inhibition of MAP kinase activation counteracted *H. pylori*-induced VEGF up-regulation; (d) toxigenic *H. pylori*-regulated COX-2 expression, and this effect was counteracted by blockade of EGFR tyrosine kinase; and (e) COX-2 selective inhibition counteracted *H. pylori*-induced up-regulation of VEGF.

**Conclusion:** (a) *H. pylori* up-regulates VEGF expression in gastric epithelial cells; and (b) this effect is specifically related to VacA toxin and seems to depend on the activation of an EGFR-, MAP kinase-, and COX-2-mediated pathway.

**Introduction**

*Helicobacter pylori* is the major causative agent of chronic superficial gastritis and peptic ulcer disease in humans (1). Moreover, epidemiological and interventional studies in humans, as well as experimental studies in rodents, strongly indicate that *H. pylori* infection increases the risk for developing adenocarcinoma of the distal stomach (2–4).

*H. pylori*-related gastroduodenal disease depends on the inflammatory response of the host and release of a number of bacterial virulence factors (5). In particular, a *M. pylori* strain producing vacuole forming in epithelial cells in vitro is produced by 60% of wild-type isolates of *H. pylori* (6). Essentially, all *H. pylori* strains possess the vacA gene, and nearly all strains secrete a vacA product, but those that have the cytotoxin phenotype (encoded by vacA alleles belonging to the type s1 family) are referred to as Tox+. An increasing body of evidence indicates that, in western countries, *H. pylori* strains producing a vacuolating VacA are preferentially associated with the development of peptic ulcer disease or gastric cancer (7).

VEGF,4 the most well-characterized angiogenic factor (8), plays a major role in the multistep process leading to the reconstruction of normal mucosa architecture by stimulating the

---

1 Supported in part by grants from Ministero della Università e Ricerca Scientifica, Consiglio Nazionale delle Ricerche, and Centro Interuniversitario per Ricerche su Alimenti, Nutrizione e Apparato Digerente, Seconda Università di Napoli.
2 R. C. and C. T. equally contributed to this work.
3 To whom requests for reprints should be addressed, at Dipartimento di Internistica Clinica e Sperimentale “F. Magrassi,” Seconda Università di Napoli, C/O II Policlinico, Edificio 3, Secondo piano, Via Pansini 5, 80131 Napoli, Italy. Phone: 39-0815666713; Fax: 39-0815666713; E-mail: marco.romano@unina2.it, or fortunatociardiello@yahoo.com.
4 The abbreviations used are: VEGF, vascular endothelial growth factor; COX, cyclooxygenase; BCF, broth culture filtrate; MAP, mitogen-activated protein; RT-PCR, reverse transcription-PCR; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; RT, reverse transcriptase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EGFR, epidermal growth factor receptor.
process of angiogenesis, which ensures that healing tissues receive an adequate supply of nutrients (9). Moreover, VEGF plays a pivotal role in tumor-associated microvascular angio-
genesis (10) and has been demonstrated to be overexpressed in human gastric carcinomas (11–13).

We have shown previously that \textit{H. pylori} up-regulates the expression of EGF-related growth factors and COX-2, the inducible isoform of the enzyme responsible for prostaglandin production (14), in human gastric epithelial cells \textit{in vitro} (15, 16), as well as in human gastric mucosa \textit{in vivo} (17, 18). In addition, COX-2 products (i.e., prostaglandins) are known to stimulate VEGF expression (19). Whether \textit{H. pylori} alters VEGF expression in gastric epithelial cells is not known.

This study was therefore designed to evaluate whether \textit{H. pylori} alters VEGF expression in gastric epithelial cells \textit{in vitro} and to assess the role of \textit{H. pylori} vaculocating cytotoxin VacA in any such effect. In addition, we sought to dissect out the action of this effect and to assess the role of \textit{H. pylori} vacA in the up-regulation of VEGF expression in gastric epithelial cells and that this effect depends on the expression of VacA because it was not observed with an isogenic mutant specifically lacking vacA. Additionally, \textit{H. pylori} VacA-induced up-regulation of VEGF seems to be mediated by the activation of the EGFR and to depend on COX-2 activity because it is counteracted by selective inhibition of EGFR tyrosine kinase or COX-2. We postulate that VEGF is involved in the reparative response of human gastric mucosa to \textit{H. pylori} infection and that VEGF overexpression might participate in the pathogenesis of \textit{H. pylori}-related gastric carcinogenesis.

Materials and Methods

\textbf{Materials.} Clinical grade ZD1839 (Iressa) was provided by AstraZeneca Pharmaceuticals (Macclesfield, United Kingdom). MAP kinase inhibitor PD098059 was from Calbiochem (La Jolla, CA). SC-236 was from Pharmacia (Upsala, Sweden).

\textbf{Bacterial Strains and Growth Conditions.} We used the urease-positive \textit{Tox} \textsuperscript{+} \textit{type s1a/m1 vacA allele} CagA\textsuperscript{+} wild-type \textit{H. pylori} strain 60190 (ATCC 49503) and its isogenic mutant 60190:v1 in which vacA gene was disrupted by insertional mutagenesis (Ref. 20; both strains kindly provided by T. L. Cover and M. J. Blaser, Nashville, TN). Bacteria were grown in brucella broth (DIFCO Laboratories, Detroit, MI) supplemented with 1% Vitox (Oxoid, Basingstoke, United Kingdom) and 5% FCS (Life Technologies, Inc., Paisley, United Kingdom) at 37°C in a humidified atmosphere of 5% CO\textsubscript{2}.

\textbf{VEGF Concentration in the Conditioned Media.} The concentration of VEGF in the conditioned media from MKN 28 cells was measured using a commercially available sandwich ELISA kit (R&D Systems, Inc., Minneapolis, MN), according to the manufacturer’s instructions, as described previously (24). Assays were performed on serum-free medium collected after 24 or 48 h of cell incubation. Results were normalized for the number of producing cells and reported as pg/10\textsuperscript{6} cells.

\textbf{Western Blot Analysis.} Total cell protein extracts (50 pg of total proteins/lane) were resolved by electrophoresis using 7.5 or 12.5% SDS-PAGE precast gels (Bio-Rad Laboratories, Milan, Italy) as appropriate, transferred to BA 85 0.45 μm PROTRAN nitrocellulose filters (Schleicher & Schuell, Inc., Dassel, Germany), blocked with 5% nonfat dry milk (Bio-Rad Laboratories, Hercules, CA) in phosphate buffer solution 1X with 0.1% Tween and incubated with appropriate primary antibodies. Peroxidase-conjugated antirabbit or antimouse or antigoat secondary antibodies (Amersham International, Buckinghamshire, United Kingdom) were incubated at the dilution of 1:2000. Immunoreactive proteins were visualized by enhanced chemiluminescence (Amersham International) as described previously (25). Immunoblot analysis using anti-β-actin antisera was performed as a control for protein loading. The primary antibodies (all from Santa Cruz Biotechnology Inc., Santa Cruz, CA) include: (a) antihuman VEGF (mouse monoclonal, sc-7269, used at the dilution of 1:1000); (b) antihuman COX-2 (rabbit polyclonal sc-7951, used at the dilution of 1:1000); (c) antihuman actin (goat polyclonal sc-1615, used at the dilution of 1:1000); and (d) anti-ERK-2 (mouse monoclonal sc-1647, used at the dilution of 1:1000).

\textbf{RNA Extraction.} Total RNA was isolated from MKN-28 cells using an RNA extraction reagent, TRizol (Invitrogen Life Technologies, Inc., Grand Island, NY), according to the standard acid-guanidium-phenol-chloroform method (26).

\textbf{RT-PCR.} RT-PCR analysis was performed on total RNA as described previously (17, 27). First-strand complementary DNA was prepared using 200 units of RT (Supertranscript RT, Life Technologies, Inc., Gaithersburg, MD). 1 μg of total RNA as template, and 10 pmol/liter random hexamers in the presence of 0.1 mmol/liter DTT, 0.5 mmol/liter deoxynucleotides triphosphate-lithium salt (Pharmacia, Milan, Italy), and 20 units of RNase inhibitor (Promega, Madison, WI). The reaction profile was 37°C × 10 min, followed by 42°C × 60 min. To control for contamination by genomic DNA, all RNA samples were run in duplicate with or without the addition of RT. RT-PCR coamplification of VEGF/GAPDH transcript was performed using VEGF (sense, TGGATCATGAACTTTCTGTC; antisense, TCACCAGCTTGCTGGTGC; GAPDH (sense, CACCATCTTCCAGGACCG; antisense, TCCACC-CACAGTTTCCCCGGA)-specific primers (provided by PRIMM Srl, Milano, Italy), and used at the final concentration of 2 μmol/liter.
mmol/liter). Primers were placed on different exons. GAPDH primers were as described previously (15, 17). VEGF primers were designed on the basis of the coding sequences of the human VEGF mRNA (28). These primers were chosen based on their ability to recognize all known VEGF splice variants. PCR amplifications were performed using 50 ng of cDNA in the presence of 0.2 mm deoxynucleotide triphosphate (Boehringer Mannheim, Mannheim, Germany) and 0.3 μl of AmpliTaq DNA polymerase (Perkin-Elmer, Branchburg, NJ). MgCl₂ was added at the final concentration of 1.5 mmol/liter. After an initial denaturation step, 95°C × 5 min, the PCR amplification was performed using the following profile: 94°C × 40 s, 57°C × 1 min, and 72°C × 1 min for 35 cycles. GAPDH primers were placed after seven cycles, and the final extension was at 72°C × 10 min. PCR products were separated on 1.8% agarose gel electrophoresis and visualized by ethidium bromide staining. Sizes of the amplified fragments were estimated from migration of the 1-kb ladder molecular weight marker (Life Technologies, Inc.), and identity was assessed by restriction enzyme digestion. To test for contamination by genomic DNA, samples were run in duplicate with (+RT) or without (−RT) the addition of RT. Three kinds of PCR product of 656, 584, and 452 bp were obtained, which encoded VEGF isoforms VEGF₁₁₈, VEGF₁₆₅, and VEGF₁₂₁, respectively.

Results

**H. pylori Stimulates VEGF Release from MKN 28—Role of VacA Cytotoxin and of EGFR Activation.** After 24- or 48-h incubation with uninoculated BCF (control) or BCFs obtained from Tox⁺ H. pylori 60190 strain, we observed a 2.5-fold increase in VEGF concentration in the conditioned media of MKN 28 cells (Fig. 1). This effect was not observed after incubation with the Tox⁻ isogenic mutant of the bacterium specifically lacking vacA, thus implying that the effect is related to the production of the VacA cytotoxin (Fig. 1). To assess whether H. pylori-induced increase in VEGF release might be contributed to by the activation of the EGFR, we incubated MKN 28 cells with Tox⁺ H. pylori BCF in the presence or absence of the selective EGFR tyrosine kinase inhibitor ZD1839 (1 μM). At the end of the incubation time, cells were lysed, and immunoblot analysis was performed using anti-VEGF monoclonal antibody. Immunoblot analysis using anti-β-actin antiserum was performed as a control for protein loading. Arrows, VEGF and β-actin immunoreactive bands. Representative Western blots of three independent experiments are shown.

![Fig. 2 Tox⁺ H. pylori BCF up-regulates VEGF protein expression in MKN 28 cells through an EGFR-mediated pathway. A, time course of Tox⁺ H. pylori-induced up-regulation of VEGF; cells were incubated for 6, 12, 24, or 48 h with uninoculated BCF (control) or BCF from Tox⁺ H. pylori. B, effect of the selective EGFR tyrosine kinase inhibitor ZD1839 on Tox⁺ H. pylori-induced up-regulation of VEGF protein expression; cells were incubated for 48 h with uninoculated BCF (control), Tox⁺ H. pylori BCF, or Tox⁻ H. pylori BCF in the presence or absence of ZD1839 (1 μM). At the end of the incubation time, cells were lysed, and immunoblot analysis was performed using an anti-VEGF monoclonal antibody. Immunoblot analysis using anti-β-actin antiserum was performed as a control for protein loading. Arrows, VEGF and β-actin immunoreactive bands. Representative Western blots of three independent experiments are shown.](image-url)
observed with *Tox*+ *H. pylori* BCFs and whether selective inhibition of EGFR tyrosine kinase affected *H. pylori* stimulation of VEGF expression. Fig. 2B shows that 48-h incubation with *Tox*+ but not *Tox*− *H. pylori* BCFs up-regulates VEGF protein expression in MKN 28 cells. In addition, selective EGFR tyrosine kinase inhibition counteracts *Tox*+ *H. pylori*-induced increase in VEGF protein expression. We next asked whether the effect of *H. pylori* on VEGF production was at the RNA level by determining VEGF mRNA expression in *H. pylori*-treated or untreated (control) MKN 28 cells through RT-PCR. We found that *Tox*+ *H. pylori*, but not *Tox*− *H. pylori*, BCF up-regulated VEGF mRNA expression (Fig. 3), causing an increase in VEGF mRNA levels, which peaked after 48-h incubation. Treatment with the EGFR tyrosine kinase inhibitor ZD1839 counteracted *Tox*+ *H. pylori*-induced up-regulation of VEGF mRNA expression (Fig. 3).

**Inhibition of MAP Kinase Cascade Activation Counteracts *Tox*+ *H. pylori*-induced Increase in VEGF Expression in MKN 28 Gastric Epithelial Cells.** Increased expression of VEGF is mediated by the activation of the MAP kinase cascade (29). We therefore asked whether *H. pylori* activated the MAP kinase cascade in MKN 28 cells and, if so, whether inhibition of MAP kinase counteracted *H. pylori*-induced up-regulation of VEGF expression. MKN 28 cells were incubated with *Tox*+ *H. pylori* BCF or un inoculated BCF (control) for ≤60 min in the presence or absence of ZD1839 (a selective EGFR tyrosine kinase inhibitor) or PD098059 (a selective inhibitor of MAP/ERK kinase, the kinase responsible for ERK phosphorylation). As illustrated in Fig. 4A, *Tox*+ *H. pylori* time dependently activated ERK-2 phosphorylation with maximal effect exerted at 10 and 30 min, and this was efficiently counteracted by either ZD1839 or PD98059 (Fig. 4A). Subsequently, MKN 28 cells were incubated with *Tox*+ *H. pylori* BCF or uninoculated BCF (control) for 48 h in the presence or absence of the selective inhibitor of MAP/ERK kinase, PD98059. Inhibition of MAP kinase activation counteracted *H. pylori*-induced up-regulation of VEGF expression (Fig. 4B).

Selective COX-2 Blockade Counteracts *Tox*+ *H. pylori*-induced Up-Regulation of VEGF Expression in MKN 28 Cells. COX-2 is involved in the up-regulation of VEGF expression in a number of experimental models (19, 30, 31). We have shown previously that *H. pylori* up-regulates COX-2 expression in MKN 28 cells and human gastric mucosa in vivo (16, 18). In addition, exposure of MKN 28 cells to *H. pylori* is associated with up-regulation of the expression of heparin-binding EGF-like growth factor and amphiregulin, members of the EGFR family of ligands, which, in turn, are able to up-regulate COX-2 mRNA expression (15, 17). We therefore hypothesized that *H. pylori*-induced up-regulation of VEGF might be mediated by an increase in COX-2 expression through the activation of the EGFR. To this end, we first investigated COX-2 expression in MKN 28 cells exposed to uninoculated BCF (control) or *Tox*+ *H. pylori* BCF in the absence or presence of the EGFR tyrosine kinase inhibitor ZD1839. Blockade of EGFR activation efficiently counteracted *Tox*+ *H. pylori*-induced increase in COX-2 protein expression (Fig. 5A). We then examined VEGF expression in MKN 28 cells treated with uninoculated BCF (control) or *Tox*+ *H. pylori* BCF in the absence or presence of the COX-2 selective inhibitor SC-236 (10 μM) for 48 h. Our data show that selective COX-2 inhibition efficiently counteracted *H. pylori*-induced up-regulation of VEGF expression (Fig. 5B).

**Discussion**

*H. pylori* infection causes gastritis and gastroduodenal mucosal ulceration and is associated with an increased risk for development of adenocarcinoma of the distal stomach in humans (32, 33). Specific virulence factors produced by the bacterium, such as the vacuolating toxin VacA, contribute to gastroduodenal mucosal injury and impair the healing process of the damaged mucosa (5, 22). In addition, host response to the infection and environmental factors are thought to be involved in the pathogenesis of *H. pylori*-related gastroduodenal disease (32, 33). In particular, an increase in the proliferative activity of gastric epithelial cells without a corresponding increase in apoptosis has been suggested to be implicated in *H. pylori*-related gastric carcinogenesis (34, 35). Polypeptide growth factors play a crucial role in the maintenance of gastric homeostasis (36). They are involved in the protection and healing of the gastric mucosa (37–39) and modulate the balance between proliferation and apoptosis in normal
ically lacking vacA found that only toxigenic that observed with its toxigenic wild-type parental strain. We studied whether an isogenic mutant H. pylori up-regulated VEGF mRNA and protein expression in MKN 28 cells, causing a 2.5-fold increase in the concentration of VEGF in the conditioned media. In addition, H. pylori-mediated up-regulation of VEGF expression, and this is inhibited by EGFR tyrosine kinase inhibition. MKN 28 cells were incubated for 48 h with uninoculated BCF (control) or Tox H. pylori BCF in the presence or absence of ZD1839 (1 μM). At the end of the incubation time, cells were lysed, and immunoblot analysis was performed using anti-VEGF antisera. Representative Western blots of three independent experiments are shown. C, control (i.e., MKN 28 cells incubated with uninoculated BCF); ZD, ZD1839; PD, PD098059.

and damaged gastrointestinal mucosa (36, 40). In particular, VEGF contributes to the restoration of normal mucosal architecture after injury through stimulation of angiogenesis in the granulation tissue at the ulcer bed (9). Furthermore, VEGF has been found to be overexpressed in gastric cancer (11–13).

H. pylori infection causes up-regulation of a number of EGF-related growth factors in vitro and in vivo (15, 17). Additionally, we have recently demonstrated that H. pylori up-regulates the expression of COX-2 in gastric mucosal cells in vitro and in human gastric mucosa in vivo (16, 18). Whether H. pylori alters the expression of VEGF is not known. We found that H. pylori bacterial suspension or BCF increased the release of VEGF from MKN 28 cells, causing a 2.5-fold increase in the concentration of VEGF in the conditioned media. In addition, H. pylori up-regulated VEGF mRNA and protein expression in MKN 28 gastric mucosal cells. To assess the role of the vacuolating toxin VacA in H. pylori-induced up-regulation of VEGF, we studied whether an isogenic mutant H. pylori strain specifically lacking vacA (i.e., nontoxicigenic) exerted similar effect to that observed with its toxigenic wild-type parental strain. We found that only toxigenic H. pylori strain up-regulated VEGF expression, whereas its tox H. pylori isogenic mutant did not, thus implying that VEGF up-regulation strictly depends on the integrity of a cytototoxic phenotype.

H. pylori infection transactivates EGFR through up-regulation of EGF-related growth factor expression (41). Activation of the EGFR then initiates a cascade of intracellular signaling pathways, including activation of ERK/MAP kinase cascade, ultimately leading to the activation of the transcription factor activator-protein 1, which plays a crucial role in cell proliferation and transformation (42). Moreover, activation of the MAP kinase cascade is known to be associated with up-regulation of VEGF expression (29, 43). We therefore evaluated whether H. pylori was able to activate the ERK/MAP kinase cascade in MKN 28 cells and whether the inhibition of H. pylori-mediated ERK/MAP kinase activation had any effect on H. pylori-induced up-regulation of VEGF. Tox H. pylori BCF activated in a time-dependent manner ERK-2 phosphorylation, and this effect was counteracted by specific inhibition of EGFR tyrosine kinase inhibition. COX-2 plays a major role in regulating the balance between cell proliferation and apoptosis (44) and activates angiogenesis through up-regulation of VEGF expression (19, 30, 31). We have recently shown that H. pylori up-regulates COX-2 expression in MKN 28 cells (17). In addition, we have shown...
previously that \( H. pylori \) up-regulates the expression of heparin-binding EGF-like growth factor and amphiregulin, members of the EGFR family of ligands (15), which in turn up-regulate COX-2 expression in MKN 28 cells (17). We therefore sought to investigate first whether EGFR blockade counteracted \( H. pylori \)-induced up-regulation of COX-2 expression and, then, whether specific COX-2 inhibition had any effect on \( H. pylori \)-dependent up-regulation of VEGF. We found that the EGFR tyrosine kinase inhibitor ZD1839 inhibited \( H. pylori \)-induced increase in COX-2 protein levels in MKN 28 cells, thus indicating that \( H. pylori \)-induced up-regulation of COX-2 expression depends on the activation of an EGFR-related pathway. Our finding is in agreement with a recent report showing that bile acids up-regulate COX-2 expression in cholangiocarcinoma cells through activation of the EGFR and MAP kinase cascade (45). Moreover, SC-236, a selective COX-2 inhibitor (46, 47), almost completely abolished \( H. pylori \)-induced up-regulation of VEGF expression, thus suggesting that COX-2 expression is necessary for \( H. pylori \) to increase VEGF expression in gastric mucosal cells.

In conclusion, this study shows for the first time that \( H. pylori \) increases the expression of VEGF in gastric mucosa cells in \textit{vitro}. This effect is strictly VacA dependent because it was observed with the parental toxigenic wild-type \( H. pylori \) strain but not with its isogenic mutant specifically lacking the cytotoxin VacA. That EGFR tyrosine kinase and MAP kinase inhibition counteracted \( H. pylori \)-related increase in VEGF expression indicates that activation of the EGFR and MAP kinase cascade are necessary events for \( H. pylori \) to up-regulate VEGF expression. Finally, that \( H. pylori \)-induced COX-2 up-regulation was counteracted by inhibition of EGFR activation and that blockade of COX-2 activity abrogated \( H. pylori \)-induced VEGF up-regulation suggests that \( H. pylori \)-induced up-regulation of VEGF in MKN 28 cells might be mediated by COX-2 through the activation of EGFR-related events.

The identification of \( H. pylori \)-specific signaling pathways leading to the induction of cell cycle and/or angiogenesis mediators may be relevant as to the understanding of the mechanism of \( H. pylori \)-associated gastric carcinogenesis and might be of interest for therapeutic intervention to overcome \( H. pylori \)-associated gastric cancer. We postulate that \( H. pylori \)-induced gastric mucosal injury triggers the compensatory overexpression of EGFR-related polypeptide growth factors, which, through the activation of the MAP kinase cascade, stimulate cell prolifera-
tion, inhibit apoptosis, and stimulate COX-2 expression. This, through the increased production of prostaglandins, leads to further activation of the EGFR (48) and to increased expression of angiogenic VEGF. All these events may ultimately favor the progression from chronic gastritis to adenocarcinoma in the multistep model of gastric carcinogenesis (49). One may therefore envision the chemopreventive use of a specific EGFR tyrosine kinase inhibitor alone or in combination with a selective COX-2 inhibitor (50), in those \( H. pylori \)-infected subjects who are resistant to conventional therapeutic regimens and potentially at risk for developing adenocarcinoma of the distal stomach.

\begin{thebibliography}{99}

\end{thebibliography}


Helicobacter pylori VacA Toxin Up-Regulates Vascular Endothelial Growth Factor Expression in MKN 28 Gastric Cells through an Epidermal Growth Factor Receptor-, Cyclooxygenase-2-dependent Mechanism

Rosa Caputo, Concetta Tuccillo, Barbara A. Manzo, et al.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/9/6/2015

Cited articles  This article cites 46 articles, 10 of which you can access for free at: http://clincancerres.aacrjournals.org/content/9/6/2015.full#ref-list-1

Citing articles  This article has been cited by 9 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/9/6/2015.full#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, use this link http://clincancerres.aacrjournals.org/content/9/6/2015. Click on “Request Permissions” which will take you to the Copyright Clearance Center's (CCC) Rightslink site.