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Helicobacter pylori CagA protein targets the c-Met receptor and enhances the motogenic response

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Infection with the human microbial pathogen *Helicobacter pylori* is assumed to lead to invasive gastric cancer. We find that *H. pylori* activates the hepatocyte growth factor/scatter factor receptor c-Met, which is involved in invasive growth of tumor cells. The *H. pylori* effector protein CagA intracellularly targets the c-Met receptor and promotes cellular processes leading to a forceful motogenic response. CagA could represent a bacterial adaptor protein that associates with phospholipase Cγ but not Grb2-associated binder 1 or growth factor receptor–bound protein 2. The *H. pylori*–induced motogenic response is suppressed and blocked by the inhibition of PLCγ and of MAPK, respectively. Thus, upon translocation, CagA modulates cellular functions by deregulating c-Met receptor signaling. The activation of the motogenic response in *H. pylori*–infected epithelial cells suggests that CagA could be involved in tumor progression.

Introduction

The gram-negative bacterium *Helicobacter pylori* colonizes the stomach of at least half of the world's population and could induce peptic ulcers, mucosa-associated lymphoid tissue lymphoma of the stomach, and gastric atrophy as well as distal gastric adenocarcinoma (Peek and Blaser, 2002). The presence of a pathogenicity island (PAI)* in *H. pylori* is connected with an increased risk of developing the aforementioned diseases. Several PAI genes are homologous to genes that encode type IV secretion system proteins (Covacci et al., 1999). After *H. pylori* adherence to epithelial cells, the bacterial PAI-encoded CagA protein is translocated into the host cell (Segal et al., 1999; Asahi et al., 2000; Backert et al., 2000; Odenbreit et al., 2000; Stein et al., 2000), where it undergoes tyrosine phosphorylation at different sites (Higashi et al., 2002b). *H. pylori* infection also triggers morphological changes and motility in host cells similar to those induced by hepatocyte growth factor (HGF; Segal et al., 1999; Churin et al., 2001). Cell motility is a critical rate-limiting step in the invasive growth program under physiological and pathophysiological conditions. Little is known about the mechanisms that underlie the process of *H. pylori*–induced cell motility and its putative role in tumor progression.

Here, we demonstrate that *H. pylori* activates the HGF/scatter factor receptor c-Met in host cells. *H. pylori* protein CagA binds c-Met and could represent an adaptor protein, which associates with phospholipase Cγ (PLCγ). Thus, upon translocation, CagA modulates cellular functions by deregulating c-Met receptor signaling.

Results and discussion

In vitro, HGF promotes epithelial cell growth and survival, as well as epithelial–mesenchymal transition, where it stimulates the dissociation and dispersal of colonies of epithelial cells and the acquisition of a fibroblastic morphology. This results in increased cellular motility and invasiveness (Thiery, 2002). Hence, we tested whether epithelial cell clusters become migratory after infection with *H. pylori*. Comparison of the same AGS cell colony before and 4 h after *H. pylori* infection demonstrated the strong stimulation of AGS cell motility (Fig. 1 A) but HGF does not induce motility in AGS cells (not depicted). *H. pylori* could also stimulate the motility of MDCK cells, which was similar in HGF-treated cells (Fig. 1 A).

Activation of signal transduction pathways in response to HGF stimulation is mediated by autophosphorylation of specific tyrosine residues within the intracellular region of c-Met that form multisubstrate docking sites (Naldini et al.,

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*Abbreviations used in this paper: EGFR, EGF receptor; ERK, extracellular-regulated kinase; HGF, hepatocyte growth factor; PAI, pathogenicity island; PI3-K, phosphatidylinositol 3-OH kinase; PKB, protein kinase B; PLCγ, phospholipase Cγ; siRNA, small interfering RNA.

Key words: epithelial–mesenchymal transition; hepatocyte growth factor; motility; tumor invasion; motogenic response; PLCγ

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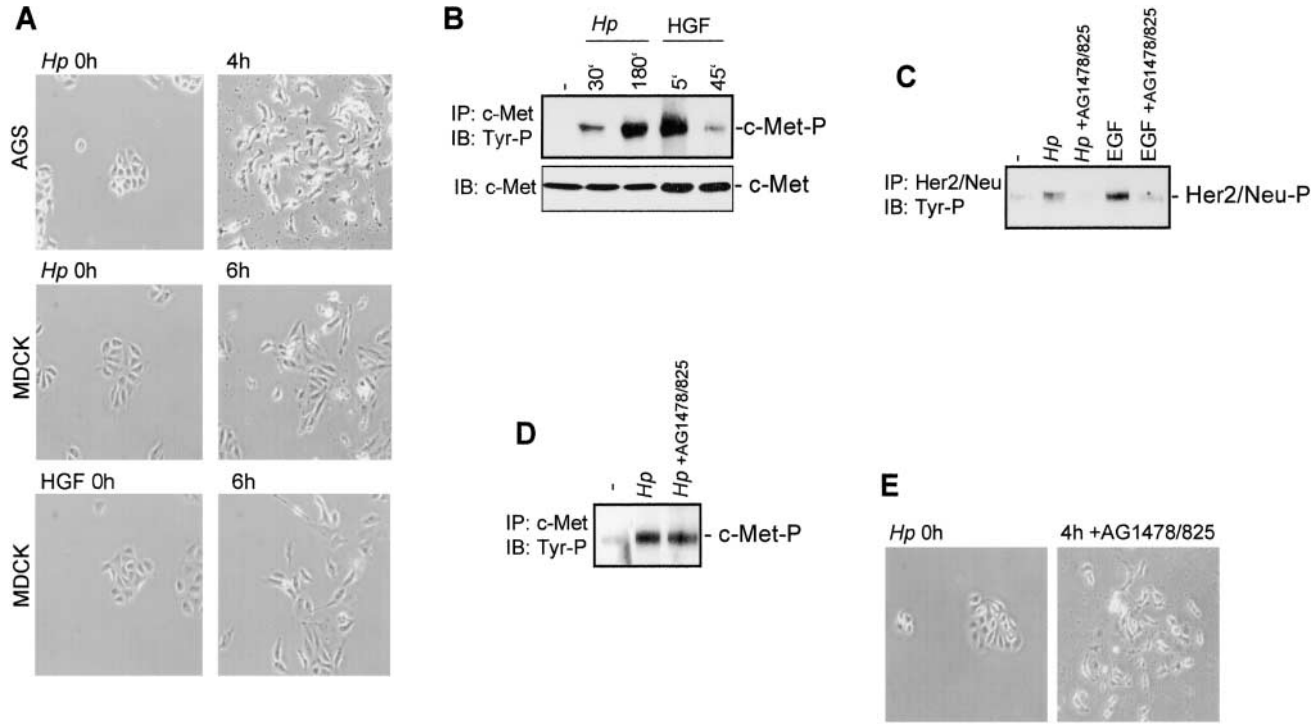

Figure 1. ***H. pylori* activates c-Met receptor tyrosine kinase and induces the motogenic response.** (A) *H. pylori* infection induces motility of AGS and MDCK cells. AGS and MDCK cells were infected with *H. pylori*, or MDCK cells were treated with 50 U/ml HGF. Phase-contrast microscopy was performed at the indicated time points. (B) *H. pylori* activates the c-Met receptor in AGS cells. AGS cells were infected with *H. pylori* or treated with HGF. c-Met was immunoprecipitated from lysates prepared at the indicated time points. Immunoprecipitates (IP) were subjected to SDS-PAGE and immunoblot (IB) analysis with antiphosphotyrosine (top) or anti–c-Met (bottom) antibodies. (C) *H. pylori* infection activates HER2/Neu. AGS cells were pretreated with or without AG1478 and AG825, and either infected with *H. pylori* for 90 min or treated with 10 ng/ml EGF for 5 min. Cell lysates were prepared, and HER2/Neu was immunoprecipitated and subjected to Western blot analysis using antiphosphotyrosine antibody. (D) AG1478 and AG825 have no effect on c-Met activation. AGS cells were pretreated with or without AG1478 and AG825 and infected with *H. pylori* for 180 min, and c-Met was immunoprecipitated and subjected to Western blot analysis using antiphosphotyrosine antibody. (E) The inhibitors of EGFR and HER2/Neu had no effect on the motility of AGS cells. AGS cells were treated with the inhibitors of EGFR (AG1478) and HER2/Neu (AG825) and infected with *H. pylori*. Phase-contrast microscopy was performed 4 h after infection.

1991; Furge et al., 2000). Therefore, we next examined whether *H. pylori* infection could activate c-Met in AGS cells. Host cells were infected with *H. pylori* and c-Met was immunoprecipitated from AGS cell lysates prepared at different time points after infection. Western blot analysis of the immunoprecipitated proteins using the phosphotyrosine-specific antibody PY99 demonstrated the stimulation of c-Met tyrosine phosphorylation 30 min immediately after infection (Fig. 1 B).

The activation of EGF receptor (EGFR) in epithelial cells by *H. pylori* was observed recently (Keates et al., 2001; Wallasch et al., 2002). One of the biological responses to EGFR activation is the stimulation of cell motility (Xie et al., 1998). Therefore, we used inhibitors of EGFR (AG1478) and of the closely related HER2/Neu receptor (AG825) to investigate the role of these receptors in stimulation of AGS cell motility. HER2/Neu was immunoprecipitated from AGS cell lysates infected with *H. pylori* or treated with EGF. Western blot analysis of the immunoprecipitates using anti-PY antibody revealed that HER2/Neu was activated by *H. pylori* infection and EGF treatment in AGS cells. This activation was strongly reduced after treatment with the inhibitors (Fig. 1 C), whereas both inhibitors had no effect on the activation of c-Met by *H. pylori* (Fig. 1 D). In spite of the presence of inhibitors, AGS cells became migratory after infection (Fig. 1 E). These observations indicated that *H. pylori* induced the sustained activation of c-Met in AGS cells that could lead to the stimulation of host cell motogenic response.

To test whether c-Met is directly involved in the stimulation of host cell motogenic response by *H. pylori* infection, we used small interfering RNA (siRNA) to silence the expression of the c-Met receptor by RNA interference in epithelial cells. An siRNA to c-Met efficiently and specifically silenced c-Met receptor expression, whereas EGFR expression was not affected. Furthermore, the silencing of c-Met receptor expression had no effect on CagA tyrosine phosphorylation (Fig. 2 A). Epithelial cells transfected with siRNA to c-Met did not express c-Met and were resistant to the induction of motility by *H. pylori* (Fig. 2, B and C). This effect could not be attributed to manipulations required to introduce siRNA into cells because the inhibition of EGFP expression by siRNA had no effect on *H. pylori*–induced cell motility (Fig. 2 C). Transfection of siRNA, which blocks c-Met expression, also inhibits *H. pylori*–induced scattering in AGS cells. Experimental data are shown for HeLa cells be-

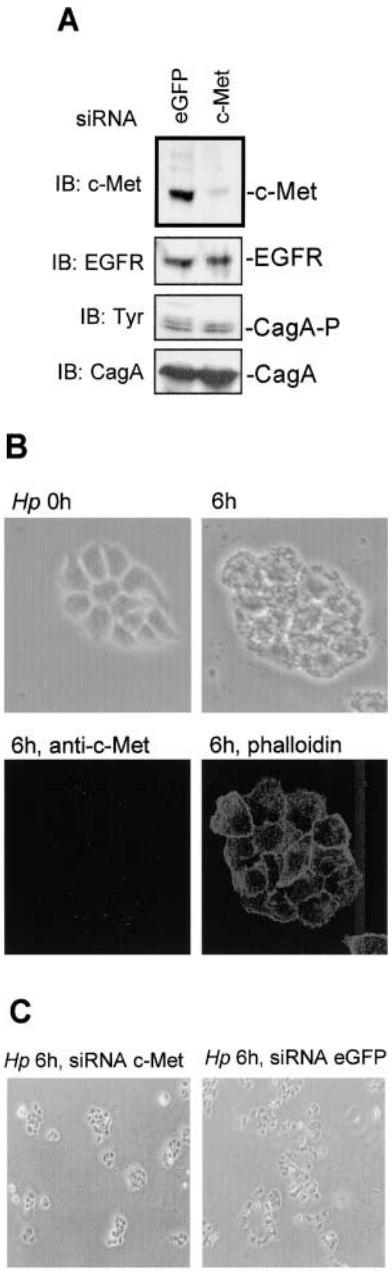

Figure 2. **c-Met receptor expression is essential for *H. pylori*–induced motogenic response in epithelial cells.** HeLa cells were transfected with siRNA to c-Met or with siRNA to EGFP (as a control for the effect of transfection). After culturing for 72 h, cells were infected with the *H. pylori* strain *P1* for 6 h. The siRNA to c-Met efficiently silenced c-Met receptor expression analyzed in a Western blot (A, top). Silencing of c-Met expression had no effect on EGFR expression (second panel) and phosphorylation (third panel) of translocated CagA protein (bottom). (B) Cells were transfected with c-Met siRNA and infected with the wild-type *H. pylori* strain *P1*. Cells are shown by phase-contrast microscopy (top two panels) or stained with immunofluorescence using c-Met antibody. Actin filaments were visualized with rhodamine-conjugated phalloidin. (C) HeLa cells transfected with c-Met siRNA are resistant to the induction of the motogenic response by *H. pylori*. Phase-contrast of cells transfected with siRNA to c-Met or siRNA to EGFP.

cause these cells were transfectable with high efficiency. We conclude that c-Met expression is necessary for *H. pylori*–induced motility in epithelial cells.

We have previously shown that the *H. pylori* mutant strain *PAI* failed to stimulate AGS cell motility, in contrast to the isogenic wild-type *H. pylori* strain (Churin et al., 2001). Therefore, we examined in more detail whether the induction of the motogenic response and c-Met activation depended on the CagA protein and a functional type IV secretion system. Compared with the wild-type strain, the isogenic *H. pylori cagA* mutant strain induced only a weak motogenic response in AGS cells. The *virB11* mutant strain lacking the functional type IV secretion system failed to promote the motogenic response (Fig. 3 A). Furthermore, overexpression of CagA in AGS cells did not induce motility, indicating that *H. pylori* infection and translocation of the CagA protein are required for the motogenic response (unpublished data).

Western blot analysis of c-Met tyrosine phosphorylation revealed that all three *H. pylori* strains induced activation of c-Met (Fig. 3 B). However, the wild-type strain activated c-Met stronger than the mutant strains. Thus, *H. pylori* moderately activated c-Met independent of the functional type IV secretion system. This finding, together with the observation that the *cagA* mutant strain induced a less strong motogenic response, indicates that CagA translocation is necessary for increased AGS cell motogenic response.

After activation, the multifunctional docking site mediates the binding of several adaptor proteins that, in turn, recruit several signal transducing proteins (Furge et al., 2000). Disruption of the multifunctional docking site abrogates the capability of c-Met to induce oncogenic transformation and invasive growth of tumor cells (Bardelli et al., 1998). Thus, we examined whether CagA could interact with the cytoplasmic part of c-Met. AGS cells were infected with *H. pylori* and lysates were prepared at different time points after infection. The c-Met receptor was immunoprecipitated using anti–c-Met antibody, and immunoprecipitates were analyzed by Western blot analysis using anti-CagA antibody. We found that CagA was coimmunoprecipitated with c-Met in AGS cells during *H. pylori* infection (Fig. 3 C, top). The level of CagA phosphorylation increased during infection (Fig. 3 C, bottom). Interaction of CagA and c-Met was confirmed by coimmunoprecipitation using anti-CagA antibody (Fig. 3 D). Next, we investigated whether CagA–c-Met interaction depended on tyrosine phosphorylation of the interactive partners. AGS cells were transfected with HA-tagged wild-type CagA or the HA-tagged phosphorylation-resistant CagA. To induce the c-Met phosphorylation, the cells were treated with HGF or infected with the *H. pylori cagA* mutant strain before lysis. Western blot analysis of the HA immunoprecipitates using anti–c-Met and antiphosphotyrosine antibodies revealed that CagA only interacted with phosphorylated c-Metl and this interaction was independent of CagA phosphorylation (Fig. 3 E). Furthermore, CagA tyrosine phosphorylation was not affected in epithelial cells, which were silenced of c-Met receptor expression using siRNA to c-Met, indicating that the c-Met receptor is not required for CagA tyrosine phosphorylation (Fig. 2 A).

AGS cells grow on plastic as a nonpolarized monolayer. For effective migration, cells must establish an asymmetry in cell–substratum biophysical interactions permitting cellular protrusive and contractile motive forces to produce a net cell body translocation-polarized cell shape. Attachment of *H.*

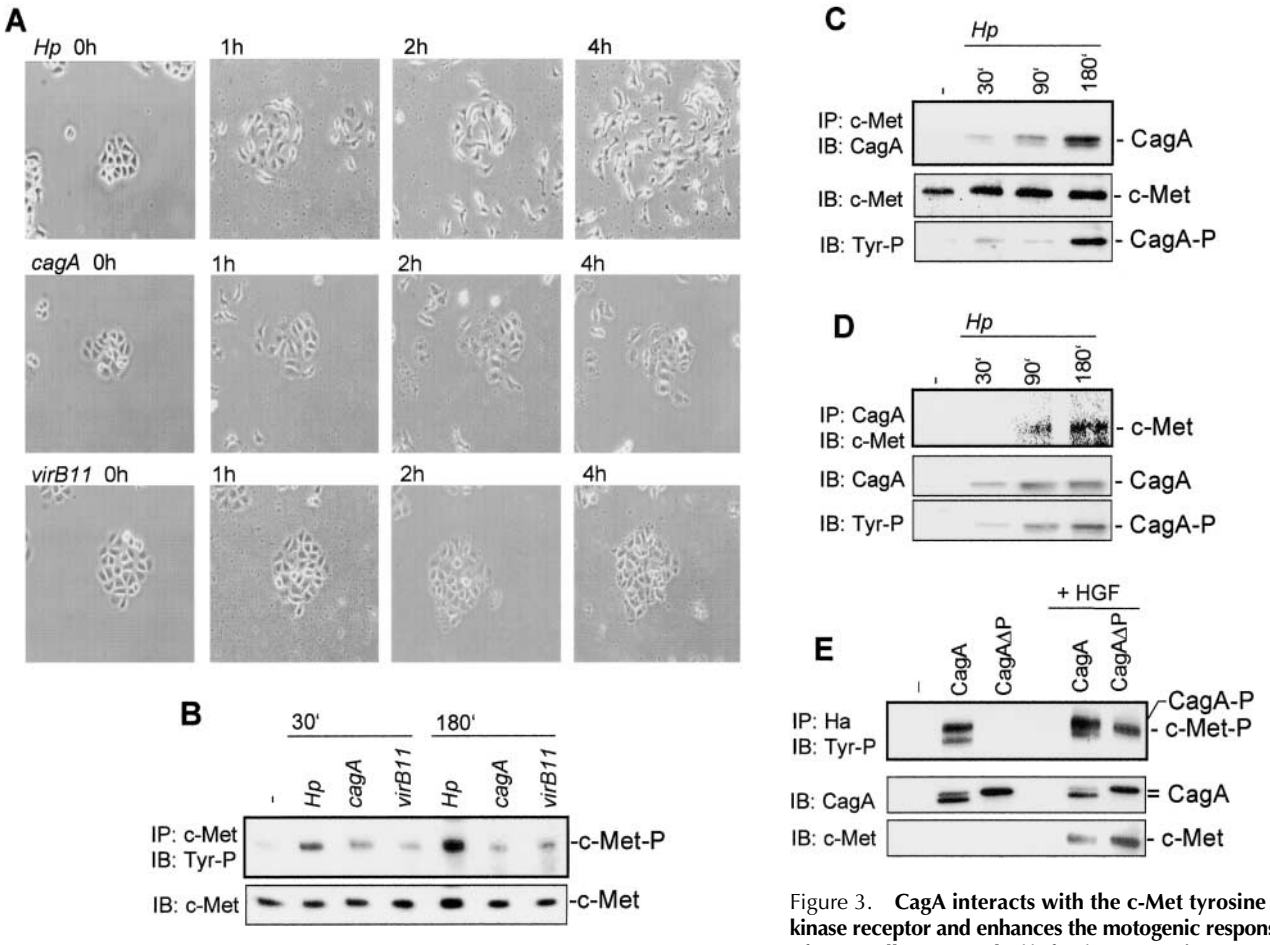

Figure 3. **CagA interacts with the c-Met tyrosine kinase receptor and enhances the motogenic response of AGS cells to *H. pylori* infection.** (A and B) AGS cells were infected with the wild-type *H. pylori* strain or isogenic mutant strains *cagA* and *virB11*. Phase-contrast microscopy was performed at the indicated time points. (B) Different *H. pylori* strains activate c-Met. Cells were harvested at the indicated time points after infection. c-Met was immunoprecipitated (IP) with anti–c-Met antibody and analyzed by immunoblotting (IB) using antiphosphotyrosine antibody. (C and D) CagA interacts with the c-Met receptor. AGS cells were infected with *H. pylori*. Cell lysates were prepared at the indicated time points. c-Met (C) or CagA (D) were immunoprecipitated with the corresponding specific antibodies and subjected to immunoblot analysis using anti-CagA (C) and anti–c-Met (D) antibodies. (E) CagA–c-Met interaction depends on c-Met tyrosine phosphorylation. AGS cells were transiently transfected with plasmids expressing either HA-tagged wild-type CagA (CagA) or HA-tagged phosphorylation-resistant CagA (CagAΔP) and were treated with HGF for 5 min. CagA was precipitated with anti-HA antibody, and immunoprecipitates were analyzed by Western blot analysis using anti–c-Met (top), antiphosphotyrosine (middle) antibodies, and anti–c-Met (bottom) antibodies.

pylori to, and translocation of, CagA in the host cell could promote such asymmetry. PLCγ signaling is linked to cytoskeletal alterations and promotes cell migration by increasing the fraction of cells in a motility-permissive morphology (Wells et al., 1999). Therefore, we tested whether CagA could interact with PLCγ. AGS cells were infected with *H. pylori* and CagA was immunoprecipitated with anti-CagA antibody. Western blot analysis of immunoprecipitates, performed using antibody against PLCγ, showed the physical interaction of CagA and PLCγ (Fig. 4 A). PLCγ tyrosine phosphorylation, which is provoked by nearly all growth factor receptors, is necessary to achieve maximal enzymatic activity (Carpenter and Ji, 1999). Upon activation, PLCγ cleaves its membrane-bound substrate, phosphatidylinositol bisphophate (PIP2). PIP2 releases bound actin-modifying proteins such as gelsolin, profilin, and cofilin, which then interact with the submembrane actin cytoskeleton (Chen et al., 1996). We next tested whether *H. pylori* could stimulate tyrosine phosphorylation of PLCγ. *H. pylori* induced PLCγ phosphorylation, whereas both mutant strains *cagA* and *virB11* failed to activate PLCγ (Fig. 4 B). Inhibition of the PLCγ signaling pathway blocks growth factor–induced cell motility (Kassis et al., 2001). In our work, we were able to suppress the motogenic response of AGS cells after *H. pylori* infection by using the pharmacological agent U73122 (Fig. 4 C). The motogenic response of AGS cells in the presence of U73122 was weak and resembled that after the infection of AGS cells with the *H. pylori* mutant strain *cagA* (Fig. 3 A). We have previously shown that wild-type *H. pylori* strains and the *cagA* mutant strain could activate Rho GTPases Rac1 and Cdc42 in AGS cells. Furthermore, Rac1 and Cdc42 were recruited to the site of bacterial attachment (Churin et al., 2001). Rho GTPases control polarity, protrusion, and adhesion during cell movement (Nobes and Hall, 1999). Thus, a weak motogenic response of AGS cells to the infection with the *cagA* mutant strain could be explained by

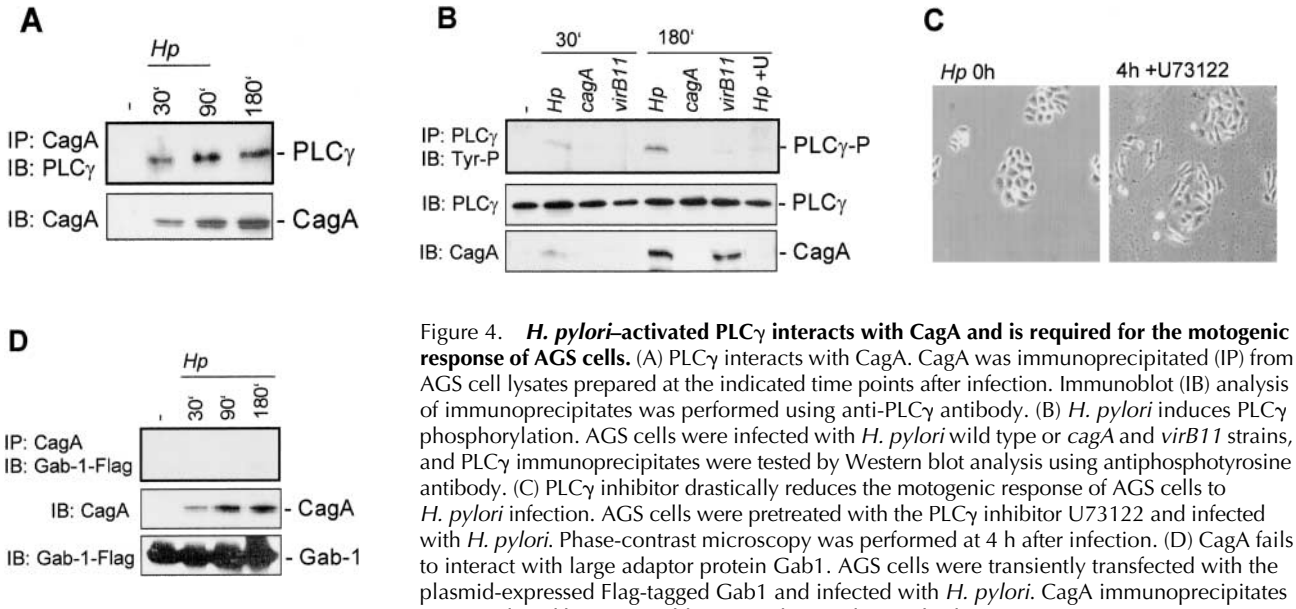

Figure 4. ***H. pylori*–activated PLCγ interacts with CagA and is required for the motogenic response of AGS cells.** (A) PLCγ interacts with CagA. CagA was immunoprecipitated (IP) from AGS cell lysates prepared at the indicated time points after infection. Immunoblot (IB) analysis of immunoprecipitates was performed using anti-PLCγ antibody. (B) *H. pylori* induces PLCγ phosphorylation. AGS cells were infected with *H. pylori* wild type or *cagA* and *virB11* strains, and PLCγ immunoprecipitates were tested by Western blot analysis using antiphosphotyrosine antibody. (C) PLCγ inhibitor drastically reduces the motogenic response of AGS cells to *H. pylori* infection. AGS cells were pretreated with the PLCγ inhibitor U73122 and infected with *H. pylori*. Phase-contrast microscopy was performed at 4 h after infection. (D) CagA fails to interact with large adaptor protein Gab1. AGS cells were transiently transfected with the plasmid-expressed Flag-tagged Gab1 and infected with *H. pylori*. CagA immunoprecipitates were analyzed by immunoblotting with anti-Flag antibody.

activation of Rho GTPases that leads to the transient polarization of the host cells. Together, CagA–PLCγ physical interaction is necessary to produce the complete motogenic response of AGS cells after *H. pylori* infection.

After binding to the multisubstrate docking site of c-Met, adaptor proteins recruit several SH2 domain–containing proteins to form an intricate signaling complex (Furge et al., 2000). One of the proteins that plays an important role in c-Met signaling is the large adaptor protein Gab1 (Weidner et al., 1996). Growth factor treatment can induce Gab1 tyrosine phosphorylation and its direct association with SH2 domains of several signal transducers including phosphatidylinositol 3-OH kinase (PI3-K), PLCγ, and SHP-2 phosphatase (Weidner et al., 1996). However, the large adaptor protein Gab1 could mediate the interaction of CagA with signal transducers. To test whether CagA interacted with Gab1, AGS cells were transfected with a Flag-tagged Gab1 expression plasmid before infection with *H. pylori*. However, CagA failed to coimmunoprecipitate with Gab1 (Fig. 4 D). Furthermore, another adaptor protein, Grb2 (Ponzetto et al., 1994), also failed to bind CagA (unpublished data). The interaction of tyrosine phosphatase SHP-2 and CagA has been described recently (Higashi et al., 2002a). However, this interaction was demonstrated in AGS cells transfected with the plasmid encoding CagA. We have shown that the CagA protein translocated into the host cell during infection interacts with PLCγ. Thus, CagA directly interacts with signal transducing proteins, and may play a role as adaptor protein in growth factor receptor signaling.

The dual protein/phospholipid kinase PI3-K has been shown to be activated during growth factor signaling (Comoglio and Boccaccio, 2001; Kassis et al., 2001). Therefore, we tested next whether PI3-K is involved in stimulation of cell motility by *H. pylori*. AGS cells were treated with Ly294002, an inhibitor of PI3-K before infection with *H. pylori*. We assayed the activity of PI3-K by monitoring the phosphorylation state of the PI3-K downstream target protein kinase B (PKB). Recruitment of this serine-threonine kinase to the cellular membrane and subsequent phosphorylation at Thr308 and Ser473 residues is dependent on the production of the PI3-K lipid product, PIP3 (Marte and Downward, 1997). *H. pylori* infection activated PI3-K in AGS cells and Ly294002 strongly inhibited the PI3-K activation (Fig. 5 A). However, in spite of the presence of the PI3-K inhibitor, AGS cells were motile (Fig. 5 B). These observations indicated that the induction of AGS motogenic response by *H. pylori* is independent of PI3-K. In contrast to AGS and HeLa cells, MDCK cells treated with a specific PI3-K inhibitor and infected with *H. pylori* does not show scattering (unpublished data). AGS and HeLa cells are gastric and cervix cancer cell lines, whereas MDCK cells represent polarized primary canine kidney cells, thus, the observed difference in PI3-K requirement is due to cell type specificity.

Studies using MAPK-signaling pathway inhibitors have established a role for the MAPK-signaling pathway in regulating cell motility (Klemke et al., 1997). Within the family of MAPK, the extracellular-regulated kinases (ERKs) promote cell motility in a transcription-independent manner (Klemke et al., 1997). It has been previously reported that *H. pylori* activates ERKs in AGS cells (Keates et al., 1999; Wessler et al., 2000). Therefore, we tested whether ERKs are also involved in the regulation of *H. pylori*–induced motogenic response in AGS cells. Inhibition of MAPKs with PD98059 completely blocked ERK activation (Fig. 5 C) and *H. pylori*–induced motogenic response (Fig. 5 D). These observations demonstrate that MAPK-signaling events are critical for the induction of the motogenic response in *H. pylori*–infected epithelial cells.

The induction of the motogenic response by *H. pylori* in epithelial cells represents an example of how human microbial pathogens could activate growth factor receptor tyrosine kinases, and modify signal transduction in the cell using translocated bacterial proteins. *H. pylori* effector protein CagA targets intracellularly the c-Met receptor and enhances the motogenic response, which suggests that dysregulation of growth factor receptor signaling could play a role

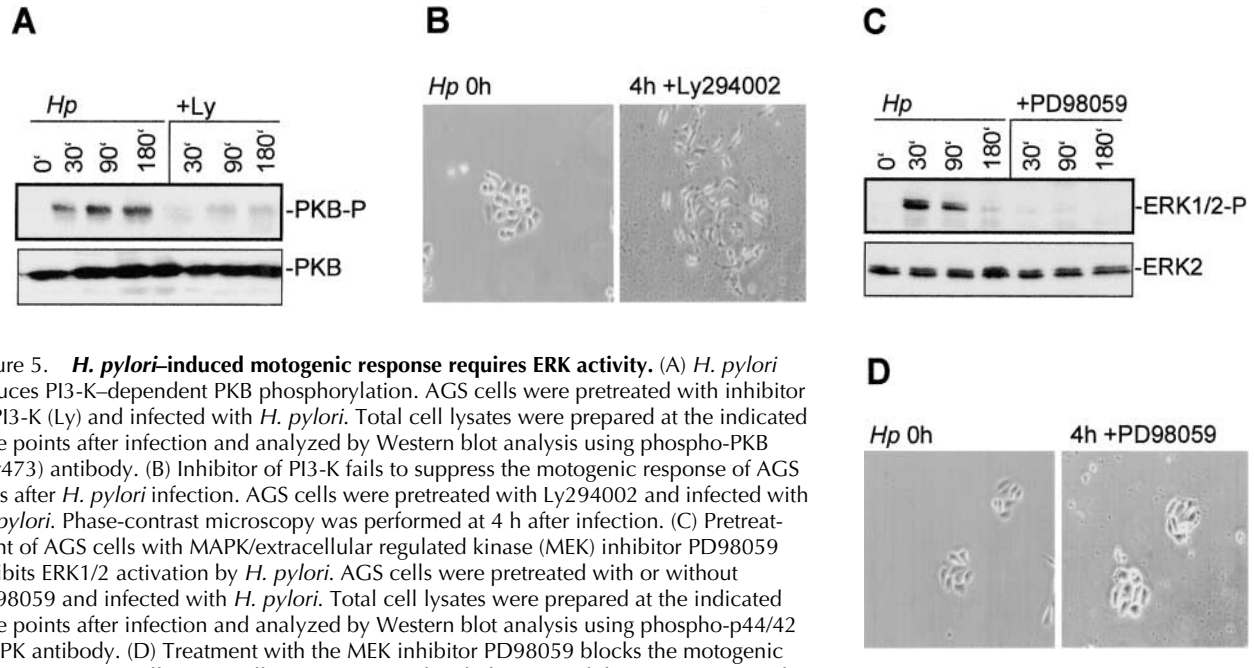

Figure 5. ***H. pylori*–induced motogenic response requires ERK activity.** (A) *H. pylori* induces PI3-K–dependent PKB phosphorylation. AGS cells were pretreated with inhibitor of PI3-K (Ly) and infected with *H. pylori*. Total cell lysates were prepared at the indicated time points after infection and analyzed by Western blot analysis using phospho-PKB (Ser473) antibody. (B) Inhibitor of PI3-K fails to suppress the motogenic response of AGS cells after *H. pylori* infection. AGS cells were pretreated with Ly294002 and infected with *H. pylori*. Phase-contrast microscopy was performed at 4 h after infection. (C) Pretreatment of AGS cells with MAPK/extracellular regulated kinase (MEK) inhibitor PD98059 inhibits ERK1/2 activation by *H. pylori*. AGS cells were pretreated with or without PD98059 and infected with *H. pylori*. Total cell lysates were prepared at the indicated time points after infection and analyzed by Western blot analysis using phospho-p44/42 MAPK antibody. (D) Treatment with the MEK inhibitor PD98059 blocks the motogenic response in AGS cells. AGS cells were pretreated with the MEK inhibitor PD98059 and infected with *H. pylori*. Phase-contrast microscopy was performed 4 h after infection.

in mobility and invasiveness of cells. Numerous experimental and clinical data indicate a particular role of HGF and the proto-oncogene c-Met in tumor invasive growth. The main challenge is to unravel how bacterial effectors interfere with cellular components and direct alterations in growth factor receptor signaling. Our results suggest that *H. pylori* modulates c-Met receptor signal transduction pathways, which could be responsible for cancer onset and tumor progression. Moreover, this work suggests that *H. pylori* colonization could not only be associated with stomach cancer development, but could also promote tumor invasion through stimulation of the motogenic response in infected cells.

Materials and methods

Cell culture and *H. pylori* infection

AGS cells were grown in RPMI 1640 medium containing 4 mM glutamin (Invitrogen), 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, and 10% FCS (Invitrogen) in a humidified 5% CO_2 atmosphere. The cells were seeded in tissue culture plates for 48 h before infection. 16 h before infection, the medium was replaced by fresh RPMI 1640 without serum. *H. pylori* strains were cultured on agar plates containing 10% horse serum under microaerophilic conditions at 37°C for 48 h. For the infection, bacteria were harvested in PBS, pH 7.4, and added to the host cells at a multiplicity of infection of 100. *H. pylori* strains used for infection were wild-type strain *P1* and isogenic mutant strains *cagA* and *virB11*. Inhibitors AG825 (5 μM), AG1478 (5 μM), U73122 (4 μM), Ly294002 (25 μM), and PD98059 (50 μM) were added to the cells 30 min before infection.

RNA interference

siRNAs were designed according to the method described previously (Elbashir et al., 2001). The siRNAs targeting c-Met (GenBank/EMBL/DDBJ accession no. NM_000245, position 311–331 relative to the start codon, 5′-AAGCCAATTTATCAGGAGGTG-3′; Xeragon) and EGFP (GenBank/EMBL/DDBJ accession no. U55762, position 802–822, 5′-AAGCUGACCCUGAAGUUCAUC-3′; Larova) were synthesized, purified, and duplexed. Transient transfection of AGS or HeLa cells with siRNA was performed using TransMessenger™ transfection reagent (QIAGEN) according to manufacturer's instructions.

Transfection of cells and immunoprecipitation

AGS cells (2.0×10^6 cells) were transfected with expression plasmids using DAC-30 reagent (Eurogentec). CagA cDNAs were described recently (Higashi et al., 2002a; cDNAs for CagA provided by M. Hatakeyama, Hokkaido University, Sapporo, Japan). For immunoprecipitation, AGS cells were harvested at different time points after infection in lysis buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 100 mM NaCl, 1% Triton X-100, and 10% glycerol) containing 2 mM Na_3VO_4, 1 mM PMSF, 1 mg/ml aprotinin, and 1 mg/ml pepstatin. The lysates were incubated with appropriate antibodies and the immune complexes were trapped on protein A– or G–Sepharose beads (Amersham Biosciences). The immunoprecipitates were subjected to SDS-PAGE. Antibodies used in this work were anti-Met, clone DO-24, and clone DQ-13 (Upstate Biotechnology); antiphosphotyrosine (PY99), anti-HA (Y-11), anti-Gab1 (H-198), anti-ERK 2 (C-14), and anti-Neu (9G6) (Santa Cruz Biotechnology); anti-PLCγ, anti-PKB, and phospho-PKB (P-Ser473) (BD Transduction Laboratories); and phospho-p44/p42 MAPK (Thr202/Tyr204) antibody (Cell Signaling).

Phase-contrast and immunofluorescence microscopy

AGS cells were grown in a 6-well tissue culture test plate in complete RPMI 1640 medium to form separate colonies. Cells were serum-starved for 16 h and infected with *H. pylori* at a multiplicity of infection of 100. Phase-contrast microscopy was performed using an inverted microscope (model IX50-S8F; Olympus). Immunofluorescence staining of HeLa cells was performed as previously described (Churin et al., 2001). To reveal c-Met, we used a rabbit polyclonal Met (C-28) antibody (Santa Cruz Biotechnology, Inc.). The samples were viewed with a confocal microscope (Leica) equipped with an argon–krypton mixed gas laser. The images were processed digitally with Photoshop 6.0 (Adobe Systems).

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