

SUPPLEMENTARY MATERIALS

The supplementary information contains Supplementary materials and methods, Supplementary reference 1, and the legend for Supplementary Figure 1.

MATERIALS AND METHODS

Cell culture. MKN28 gastric epithelial cells were cultured at 37°C under 5% CO₂/95% O₂ in RPMI medium 1640 (GIBCO/BRL) supplemented with 10% FBS (Sigma) and gentamycin (20 µg/ml, Sigma). For co-culture experiments with *H. pylori*, RPMI medium 1640 supplemented with 10% FBS without gentamycin was used.

Western blot analysis. For analysis of total cellular protein, MKN28 cells were cultured for 72 hours and then co-cultured with or without *H. pylori* for 48 hours. Cells were lysed in RIPA buffer (50mM Tris, 150mM NaCl, 1% Triton X-100, 0.1% SDS) for 10 minutes at 4°C, centrifuged at 13,000 rpm for 10 minutes and supernatants were collected. To determine the effects of *H. pylori* on claudin-7 localization, detergent soluble and insoluble MKN28 cell fractions were separated by differential detergent extraction as previously described¹. Cells were lysed in lysis buffer (0.5% Triton X-100, 150mM NaCl, 50mM Tris-HCl) for 30 minutes at 4°C with rotation. Cells were centrifuged (14,000 rpm, 10 minutes) and supernatants were collected as the detergent soluble fraction. Pellets were then resuspended in lysis buffer containing 0.02% SDS, incubated for 30 minutes at 4°C, centrifuged (14,000 rpm, 10 minutes) and the supernatant was collected as the detergent insoluble fraction. Protein concentrations were quantified by the BCA assay

(Pierce). Proteins (20 μ g) were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Claudin-7 was detected using an anti-claudin-7 antibody (1:200, Life Technologies) in combination with goat anti-rabbit IgG (1:5000, Sigma) HRP-conjugated secondary antibody. Snail was detected using an anti-snail antibody (1:100, ABGENT) in combination with goat anti-rabbit IgG (1:5000, Sigma) HRP-conjugated secondary antibody. Samples were re-probed using a mouse anti-GAPDH antibody (Santa Cruz Biotechnology) as an additional control for equal loading. Primary antibodies were visualized by Western Lightning Chemiluminescence Reagent Plus according to the manufacturer's instructions.

Transfections and Luciferase Assays. MKN28 cells in 12-well plates were transfected with 3 μ l of Lipofectamine 2000 (Life Technologies) according to the manufacturer's instructions, and 1 μ g/ml Topflash or Fopflash (kind gifts from K. Kinzler and B. Vogelstein, Johns Hopkins University) with 1 μ g/ml Renilla vector in Opti-MEM (Life Technologies) for 24 hours. Transfection mixtures were then replaced with complete medium containing *H. pylori* or medium alone. After 24 hours, cells were harvested in 1x Passive Lysis Buffer (Promega). Luciferase activity was determined using a luminometer and normalized to Renilla luciferase using the Dual-Luciferase assay kit (Promega).

Transient transfection of siRNA. MKN28 cells in 12-well plates were transiently transfected using Lipofectamine 2000 transfection reagent (Life Technologies) according to the manufacturer's instructions. Briefly, transfection reagent (2.0 μ l/well) was mixed

with siRNA oligonucleotides (5 μ l of 20 μ M solution/well) in 200 μ l Opti-MEM (Life Technologies). Cells were incubated with the transfection mixture for 24 hours, fresh medium was added, and bacterial co-cultures were performed 24 hours later.

Immunofluorescence. Gastric epithelial cells were cultured on four-well chamber-slides (Nalge Nunc), and gastroids were cultured on MatTek dishes (MATTEK Corporation), washed twice in 1X PBS, and formalin-fixed. Cells were permeabilized using 1X PBS containing 0.1% Triton X-100 (30 minutes, room temperature), washed three times in 1X PBS, and non-specific binding was blocked by incubation in 1% BSA. Phalloidin was used to stain F-actin (Molecular Probes). Specific cell types in formalin-fixed gastroids were identified with antibodies raised against mucin (Sigma), or H⁺K⁺ATPase, gastrin, or chromogranin A (kind gifts from J. Goldenring, Vanderbilt University). Gastroids were stained with mouse anti-occludin (Life Technologies) followed by Alexa fluor 594-conjugated anti-mouse IgG antibody, rabbit anti-cleaved caspase 3 (Cell Signaling) followed by Alexa Fluor 488-conjugated anti-rabbit IgG antibody (1:200, Molecular Probes), or rabbit anti-snail (Abcam) followed by Alexa Fluor 488-conjugated anti-rabbit IgG antibody (1:200, Molecular Probes) and Hoechst 33342. MKN28 cells were stained with rabbit anti-claudin-7 antibody (1:200, Life Technologies) and Alexa Fluor 488-conjugated anti-rabbit IgG antibody (1:200, Molecular Probes). Images were captured using an Olympus FV-1000 Inverted Confocal Microscope. Image acquisition was performed using Fluoview FV10-ASW 1.7 software.

REFERENCES

1. Wroblewski LE, Shen L, Ogden S, et al. *Helicobacter pylori* dysregulation of gastric epithelial tight junctions by urease-mediated myosin II activation. *Gastroenterology* 2009;136;1:236-46.

Legend for Supplementary Figure 1.

β -catenin, but not apoptosis, is increased in treated gastroids. (A) Quantification of β -catenin demonstrating that the number of cells with cytoplasmic β -catenin is significantly increased in gastroids following treatment with LiCl. (B) Gastroids were uninfected, treated with LiCl, or infected with *H. pylori* strain 7.13 and stained for cleaved caspase 3 (green), a marker of apoptosis; nuclei are labeled with Hoechst (blue). (C) Cleaved caspase 3 quantification demonstrating no significant differences in apoptosis in uninfected, LiCl-treated, or *H. pylori* strain 7.13-infected gastroids. Data are expressed as means \pm s.e.m, n=3 independent replicates.

