Role of *regenerating gene I* in claudin expression and barrier function in the small intestine

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We have recently shown that loss of the *regenerating gene (Reg) I* causes susceptibility to nonsteroidal anti-inflammatory drug–induced gastrointestinal damage. However, the mechanism by which Reg I plays a protective role against this pathophysiological condition is unclear. Here, we investigated whether Reg I plays roles in the induction of tight junction proteins and mucosal barrier function in the small intestine. The small-intestinal permeability was evaluated in *Reg I*–deficient mice by FITC-dextran and transepithelial electrical resistance (TEER) assay. The effect of REG Iα on TEER, claudins expression, and intracellular signaling was examined using Caco2 cells *in vitro*. Small-intestinal expression of claudins 3 and 4 was investigated in *Reg I*–deficient mice *in vivo*. *REG I* deficiency significantly decreased the expression of claudin 3 in the small-intestinal epithelium. When mice were treated with indomethacin, the serum level of FITC-dextran in *Reg I* knockout mice was significantly higher than that in wild-type (WT) mice. The level of small-intestinal TEER was significantly decreased in *Reg I* knockout mice compared with WT mice under normal condition. REG Iα stimulation significantly enhanced the level of TEER in Caco2 cells. Treatment with REG Iα enhanced the expression of claudins 3 and 4 and promoted Sp1, Akt, and ERK phosphorylation in Caco2 cells, whereas these effects were attenuated by treatment with anti-REG Iα antibody. Reg I may play a role in the maintenance of mucosal barrier function by inducing tight junction proteins such as claudins 3 and 4. (Translational Research 2016;173:92–100)

Abbreviations: GI = gastrointestinal; NSAID = nonsteroidal anti-inflammatory drug; Reg = regenerating gene; TEER = transepithelial electrical resistance

INTRODUCTION

The *regenerating gene I* (*Reg I*) was originally isolated from rat regenerating pancreatic islet cells, and its human homologue was named *REG Iα*.[1] We have previously reported that REG Iα is overexpressed in the gastrointestinal (GI) mucosa of patients with GI inflammatory diseases.[2-4] Furthermore, we have recently shown that Reg I is overexpressed in the

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Submitted for publication October 14, 2015; revision submitted February 17, 2016; accepted for publication March 12, 2016.

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1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2016.03.007

AT A GLANCE COMMENTARY

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Background

Nonsteroidal anti-inflammatory drugs (NSAIDs) are indispensable for patients with arthritis or cardiac and cerebrovascular diseases. However, gastrointestinal (GI) injury is clinically concerned as a diverse effect of NSAID, and the mechanism of NSAID–induced GI injury remains unclear.

Translational Significance

We clarified that Reg protein enhances the expression of claudins 3 and 4 via Sp1 phosphorylation in epithelial cells, and moreover, that loss of *Reg* gene promotes the permeability of GI epithelium. Thus, Reg protein is a key player in the maintenance of mucosal barrier by inducing tight junction proteins and a possible target to prevent NSAID–induced GI injury.

small-intestinal mucosa of mice treated with nonsteroidal anti-inflammatory drugs (NSAIDs).[5] Interestingly, we also found that *Reg I*–deficient mice are susceptible to NSAID–induced GI damage, suggesting that Reg I may have a protective effect against NSAID–induced GI injury.[5] However, the mechanism responsible for the protective role of Reg I under such pathophysiological conditions has remained unclear.

Although NSAIDs are an indispensable treatment for patients with arthritis or cardiac and cerebrovascular diseases, GI injury as an adverse effect is of clinical concern.[6] The involvement of various factors in the mechanism of NSAID–induced GI injury has been suggested, including lack of prostaglandins, dysfunction of the mucosal barrier, neutrophil activation, oxygen free radicals or microcirculatory disturbance.[7,8] Among them, mucosal barrier dysfunction appears to be especially significant as it results in invasion of bacteria or harmful antigens into the lamina propria.[7,8] Therefore, in the present study, we aimed to clarify whether susceptibility to NSAID–induced GI injury in *Reg I*–deficient mice is associated with the dysfunction of the mucosal barrier, focusing on the link between REG protein and tight junction molecules.

MATERIALS AND METHODS

Cell culture and reagents. Human intestinal epithelial cell line Caco2 cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, Calif) with 10% fetal bovine serum (Biowest, Nuaillé, France) in a humidified incubator at 37°C with an atmosphere of 5% CO_2. Anti-Sp1 and -β-actin antibodies were purchased from Cell Signaling Technology (Beverly, Mass). Anti-claudin 3, -claudin 4, and -phosphorylated Sp1 (p-Sp1) antibodies were purchased from Abcam (Cambridge, UK). Anti-claudin 4 antibody for immunohistochemistry was purchased from Invitrogen (Camarillo, Calif). Recombinant REG Iα protein was prepared as previously described.[9]

Animal. *Reg I* knockout (KO; *Reg* $I^{-/-}$) mice and wild-type (WT; *Reg* $I^{+/+}$) littermates were used in this study. The *Reg I* KO mice were generated on an Institute of Cancer Research (ICR) background as previously described.[10] All mice were maintained in cages on a 12 hour light-dark cycle under specific pathogen-free conditions. The following animal experiments were carried out with the approval of the Animal Use and Care Committee at Hyogo College of Medicine.

Mucosal integrity. *Reg I* KO and WT mice were given a subcutaneous injection of indomethacin (Sigma, St Louis, Mo; 10 mg/kg in 5% NaHCO3) and then administered fluorescein isothiocyanate (FITC) dextran (average molecular weight, 3000–5000; Sigma-Aldrich1) orally 24 hours later. After an additional period of 4 hours, the mice were killed, and samples of small-intestinal tissue and blood were collected from them. The tissues were snap-frozen, cut into sections 8-μm thick, and observed by fluorescence microscopy. The serum concentration of FITC-dextran was measured with a fluorometer (excitation, 480 nm; emission, 520 nm).

Measurement of transepithelial electrical resistance. Electrical resistance across the stratified epithelium was measured using a Millicell-ERS-2 instrument (Millipore, Bedford, Mass) with "chopstick" electrodes, as described previously.[11] The value obtained from a blank insert was subtracted to give the net resistance, which was then multiplied by the membrane area to give the resistance in area-corrected units ($\Omega \cdot cm^2$). Transepithelial electrical resistance (TEER) values were recorded at 24, 48, and 96 hours after stimulation. *Ex vivo* permeability assays were performed according to a previously reported method.[12] In brief, the small-intestinal tissues were removed from experimental mice under normal condition. The obtained tissues were opened longitudinally, placed apical side up on 0.4-μm pore size membrane (Costar Incorporated Corning, NY), and transferred into microsnapwell system. Electrical resistance was measured as described previously.

Real-time reverse transcription polymerase chain reaction. Total RNA was isolated from small-intestinal tissues with Trizol reagent (Invitrogen). Total RNA