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Heparin-binding EGF-like growth factor (HB-EGF) promotes cell migration and adhesion via focal adhesion kinase

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ABSTRACT

Background: Cell migration and adhesion are essential in intestinal epithelial wound healing and recovery from injury. Focal adhesion kinase (FAK) plays an important role in cell–extracellular matrix signal transduction. We have previously shown that heparin-binding EGF-like growth factor (HB-EGF) promotes intestinal epithelial cell (IEC) migration and adhesion *in vitro*. The present study was designed to determine whether FAK is involved in HB-EGF–induced IEC migration and adhesion.

Materials and methods: A scrape wound healing model of rat IECs was used to examine the effect of HB-EGF on FAK-dependent cell migration *in vitro*. Immunofluorescence and Western blot analyses were performed to evaluate the effect of HB-EGF on the expression of phosphorylated FAK (p-FAK). Cell adhesion assays were performed to determine the role of FAK in HB-EGF–induced cell adhesion on fibronectin (FN).

Results: HB-EGF significantly increased healing after scrape wounding, an effect that was reversed in the presence of an FAK inhibitor 14 (both with $P < 0.05$). HB-EGF increased p-FAK expression and induced p-FAK redistribution and actin reorganization in migrating rat IECs. Cell adhesion and spreading on FN were significantly increased by HB-EGF ($P < 0.05$). FAK inhibitor 14 significantly inhibited both intrinsic and HB-EGF–induced cell adhesion and spreading on FN (both with $P < 0.05$).

Conclusions: FAK phosphorylation and FAK-mediated signal transduction play essential roles in HB-EGF–mediated IEC migration and adhesion.

1. Introduction

The gastrointestinal epithelium functions as an important barrier that separates luminal contents from the underlying tissue compartment and is vital in maintaining mucosal homeostasis [1–3]. Mucosal wounds associated with inflammatory disorders compromise this critical epithelial barrier, undermine gut barrier function, and allow bacterial

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translocation and absorption of toxins, antigens, proteases, and other macromolecules, leading to local infection followed by distant organ pathology. In response to injury, rapid reestablishment of epithelial barrier function by intestinal epithelial cells (IECs) depends on migration of uninjured IEC to cover denuded sections of the basement membrane [1,4,5]. This process is known as intestinal restitution [6,7].

IEC migration is a mitosis independent process that starts as early as a few minutes after injury and is completed within 6–12 h [8]. To initiate migration, cells polarize, extend protrusions (lamellopodia) in a particular direction, and form adhesions in the direction of migration. Focal adhesions (FAs) are composed of various structural proteins and represent sites, where a number of intra- and extra-cellular signaling events regulating cell migration take place [9,10]. A key player involved in the control of the interaction between cells and the extracellular matrix (ECM) is focal adhesion kinase (FAK), a tyrosine kinase that is localized to cellular focal contact sites, and is the pivotal molecule that controls FA formation [11–13]. On stimulation, FAK is phosphorylated at Tyr-397, a step that is necessary for the full activation of the kinase domain [14]. Activated FAK partners with cell membrane integrins with the assistance of other proteins, such as paxillin and vinculin, resulting in FA formation, cell adhesion, and cell migration [10,15].

Heparin-binding EGF-like growth factor (HB-EGF) is a member of the epidermal growth factor family that was initially identified in the conditioned medium of cultured human macrophages [16] and then found to be a member of the epidermal growth factor family [17]. During the past 20 years, we have investigated the roles of HB-EGF in protecting the intestines in various models of intestinal injury, including necrotizing enterocolitis [18–22], ischemia-reperfusion injury [23,24], and hemorrhagic shock and resuscitation [25–27]. Our previous studies have demonstrated that HB-EGF promotes intestinal restitution in murine necrotizing enterocolitis and intestinal ischemia-reperfusion models *in vivo* and in an IEC scrape wound healing model *in vitro* [24,28,29]. We have also demonstrated that HB-EGF promotes enterocyte migration, in part, by affecting integrin–ECM interactions and intercellular adhesions [28]. Numerous studies in the last 20 years have established FAK as a central mediator of integrin signaling and as an important component of signaling by other cell surface receptors in the regulation of cell migration in many cell types [10,14,30,31]. The present study was designed to examine the effect of HB-EGF on FAK in IEC and explore the role of FAK in HB-EGF–induced IEC migration and adhesion.

2. Materials and methods

2.1. Materials

Tyr-397–phosphorylated FAK (p-FAK) antibody and the FAK inhibitor 14 (FAK I-14) were from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Rhodamine phalloidin was from Cytoskeleton Inc (Denver, CO). horse radish peroxidase-conjugated goat anti-rabbit antibody, Cy3, and Alexa-488 secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA). Human plasma fibronectin (FN) was from Millipore (Billerica, MA). Mounting medium for fluorescence with 4,6-diamidino-2-phenylindole was from Vector Laboratories Inc (Burlingame, CA). Radioimmunoprecipitation assay buffer, protease inhibitor cocktail, and the bicinchoninic acid protein assay kit were from Thermo Scientific (Rockford, IL). Phosphatase inhibitor cocktail was Sigma–Aldrich Corp (St Louis, MO). The ECL Plus system was from Amersham Biosciences (Piscataway, NJ). All experiments used human recombinant HB-EGF corresponding to amino acids 74–148 of the mature HB-EGF protein that was produced using a *Pichia pastoris* expression system (Trillium Therapeutics, Toronto, Canada). The epidermal growth factor receptor (EGFR) inhibitor AG1478 (4-[3-chloroanilino]-6, 7-dimethoxyquinazoline) was from Calbiochem (San Diego, CA). The doses of HB-EGF and AG1478 chosen were based on our previous studies [24,28,29,32].

2.2. Cell culture

The rat intestinal epithelial (RIE) 1 cell line was kindly provided by Dr John Barnard (Columbus, OH). Cells were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37°C in 5% CO_2. All experiments were performed on cells between passages 4 and 10.

2.3. Scrape wound healing assay

The scrape wound healing assay was performed as we have previously described [24,28,29]. Briefly, cells were grown in 24-well plates until confluent and then serum starved overnight. The bottom of each plate was marked by drawing two crossed lines across the plate diameter and then a 100-μL sterile pipette tip was used to create a scrape wound perpendicular to the marked lines. After scraping, cells were immediately treated with either (1) regular control culture medium, (2) HB-EGF (10 ng/mL), (3) the EGFR inhibitor AG1478 (500 nmol/L), or (4) AG1478 followed 30 min later by HB-EGF. To assess the effect of FAK activation on HB-EGF–induced cell migration, scraped cells were treated with FAK I-14 (2 μM), with some cells receiving HB-EGF (10 ng/mL) 60 min later. Nontreated cells served as a negative control. Cell migration was quantified at various time points (6, 12, 18, 24, and 28 h). Photographs were taken immediately after wounding (0 h) or at the indicated time points after wounding. The width of the wound was measured across each of the two marked lines in each well (two fields per well) using Axiovision 3.1 software (Carl Zeiss Inc, Thornwood, NY). Each experiment was performed three times in duplicates. The percent of wound healing was calculated as follows: Wound healing % = (W0h) − (WIT), where W0h and WIT represent the average wound width per well (measured across the premarked lines) at 0 h and at the indicated time points, respectively. The cells were then fixed for immunofluorescent staining or harvested for protein analysis.

2.4. Western blot analysis

Cells were lysed in radioimmunoprecipitation assay buffer supplemented with phosphatase inhibitor cocktail and protease inhibitor cocktail. Protein concentration was measured using a bicinchoninic acid protein assay kit (Thermo