Intranuclear Translocation Signaling of HB-EGF Carboxy-Terminal Fragment and Mucosal Defense through Cell Proliferation and Migration in Digestive Tracts

Satoshi Tanida Hiromi Kataoka Tsutomu Mizoshita Takaya Shimura
Takeshi Kamiya Takashi Joh

Department of Gastroenterology and Metabolism, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan

Key Words

Interleukin-8 • G-protein-coupled receptor • Heparin-binding EGF-like growth factor • Migration • Repair

Abstract

Mucosal integrity in the digestive tracts is maintained by defense mechanisms which comprise mucin production, microcirculatory blood flow, intercellular junctions, cell growth and mucosal repair. These physiological features are known to be regulated by prostaglandins, nitric oxides, growth factors, and cytokines. Mucosal injury and inflammation occur when cytotoxic attacks overwhelm cellular capacity for defense or repair. Interleukin-8 (IL-8) promotes tumor cell proliferation in colon cancer cells after binding to its receptors, which are members of the G-protein-coupled receptor (GPCR) family. Stimulation of GPCR can induce shedding of epidermal growth factor (EGF) ligands via activation of A disintegrin and metalloprotease (ADAM), with subsequent transactivation of the EGF receptor (EGFR). In parallel with EGF signaling through EGFR activation, heparin-binding (HB)-EGF carboxy-terminal fragment (CTF) signaling is also involved in cell proliferation through nuclear

export of promyelocytic leukemia zinc finger. IL-8 induces cell proliferation and migration by an ADAM-dependent intranuclear translocation pathway of HB-EGF-CTF. Here, we focus on the mechanisms of IL-8-induced HB-EGF-CTF signaling, which is involved in cytoprotection and cellular repair.

Copyright © 2010 S. Karger AG, Basel

Introduction

Mucosal injury and inflammation arise primarily when the effects of cytotoxic factors and conditions overwhelm cells' capacity for defense (i.e. cytoprotection) or repair. To date, most research in this area has focused on agents and processes involved in producing mucosal injury. Cytotoxic factors against mucosa include bacterial infection, impaired vascular perfusion and non-steroidal anti-inflammatory drugs. On the other hand, mucosal defense factors include mucin production, microcirculatory blood flow, intercellular junctions, cell growth and mucosal repair which are known to be regulated by prostaglandins, nitric oxides, epidermal growth factors (EGF) and other growth factors, cytokines and hormones. Less

consideration is currently given to the inherent mechanisms of cytoprotection and cellular repair on carboxy-terminal fragment (CTF) signaling after the ectodomain shedding of pro-heparin-binding-EGF (proHB-EGF), although EGF signaling through EGF receptor (EGFR) activation has been well studied to date. Here, we give an overview of the mechanisms of cytokine (interleukin-8)-induced HB-EGF CTF signaling involved in cytoprotection and cellular repair.

EGF as a Key Factor during Mucosal Repair and Regeneration

During healing from mucosal injuries and ulcerations, regenerated mucosal epithelia grow, migrate and finally cover mucosal defects from the ulcer margin with new vessel growth. EGF physiologically induces proliferation of epithelial and mesenchymal cells, mucous production and secretion, and ulcer healing [1]. Thus, EGF plays an important role in the process of mucosal protection and defense, in addition to basic fibroblast growth factor, hepatic growth factor, and platelet-derived growth factor.

EGFR and Its Ligand Variation

Binding of EGF to its receptor (EGFR/ErbB) is known to trigger biological responses such as proliferation and migration [1]. The ErbB receptor family has four members: EGFR/ErbB1/HER1, ErbB2/Neu/Her2, ErbB3/HER3, and ErbB4/HER4. All members have a common extracellular ligand-binding region, a single membrane-spanning region, and a cytoplasmic protein tyrosine kinase domain. Direct stimulation of the receptor by binding of a ligand to the receptor's extracellular domain leads to homo- and heterodimerization and subsequent autophosphorylation of the two receptor molecules, thereby creating phosphotyrosine docking sites to activate intracellular signaling cascades. The EGFR ligand family comprises seven members to date: EGF [2], heparin-binding EGF (HB-EGF) [3], amphiregulin [4], transforming growth factor- α (TGF- α) [5], betacellulin [6], epiregulin [7], and epigen [8]. Each ligand displays overlapping but distinct binding affinities toward ErbB receptors. No direct high-affinity ligands for ErbB2 have been identified, and some evidence has suggested that ErbB2 is the preferred heterodimerization partner for other ErbB family members.

A Disintegrin and Metalloproteinase-Mediated EGFR Ligand Shedding and EGFR Signal Transactivation by GPCR Agonists

All EGFR ligands are synthesized as inactive membrane-anchored precursor forms that can undergo ectodomain proteolytic cleavage to release mature bioactive growth factors [1]. However, until recently, there has been little information on the identity of the proteases responsible for proteolytic ectodomain shedding of EGFR ligands. Several G-protein-coupled receptors (GPCRs), characterized by seven transmembrane α -helices, have been shown to activate EGFR (designated as transactivation), even if the GPCR agonists do not directly stimulate EGFR [9]. Using a chimeric receptor combined with a tyrosine kinase domain, the metalloproteinase-cleaved extracellular domain of the EGFR ligand, HB-EGF, is first involved in the GPCR-induced EGFR transactivation pathway [10]. Blocking both proHB-EGF function using the diphtheria toxin mutant Crm197 and metalloproteinase function using batimastat (BB94) abrogates GPCR-induced EGFR, Shc and MAPK phosphorylation. It has also been reported that there are numerous GPCR agonists, including thrombin, angiotensin II, endothelin-1, carbachol, bombesin, phenylephrine, lysophosphatidic acid, and interleukin-8 (IL-8). 12-O-tetradecanoylphorbol-13-acetate, calcium ionophores, and *Helicobacter pylori* are also responsible for the release of soluble EGFR ligands. GPCR agonists induce rapid transient EGFR phosphorylation and subsequent activation of downstream signaling events such as mitogen-activated protein kinase (MAPK) phosphorylation or *c-fos* gene expression [11].

The family of membrane-anchored A disintegrin and metalloproteinases (ADAMs) has been recently identified as a mediator required for EGFR transactivation by several GPCR agonists [12]. Moreover, recent studies with pharmaceutical ADAM inhibitors have confirmed an important role for ADAMs in the ectodomain shedding of EGFR ligand precursors to produce a mature soluble ligand under physiological and pathological conditions. ADAMs belong to the metzincin superfamily and 40 ADAM orthologues have been identified to date. Of the 21 human ADAMs, 12 have a consensus motif (HEXGHXXGXXHD) within the catalytic site of the metalloproteinase domain. Of these, proteinase activity that can cleave the mature soluble EGFR ligands has been shown in ADAM8, -9, -10, -12, -15, -17, -19, -28 and -33. Further detailed investigations have demonstrated that ADAM10 is involved in proHB-EGF shedding and sub-

sequent EGFR transactivation in the bacterial lipoteichoic acid-induced activation of the platelet-activating factor receptor in the lung carcinoma cell line NCI-H292 [13], and that ADAM10 is involved in EGFR transactivation in COS-7 and PC-3 cells through bombesin-induced proHB-EGF [14]. There is further evidence of the involvement of ADAM10 in the processing of proHB-EGF in IL-8-induced EGFR transactivation in gastric carcinoma cells, KATOIII [15] and of the involvement of ADAM9 in TPA-induced processing of HB-EGF in Vero-H cells during activation of protein kinase C δ [16]. Moreover, GPCR-induced cardiac hypertrophy is required for ADAM12-mediated processing of proHB-EGF in cardiomyocytes after stimulation with catecholamines, phenylephrine, angiotensin II or endothelin-1 [17]. Reintroducing ADAM17 into the immortalized fibroblasts derived from mice with a deleted ADAM17 zinc-binding domain resulted in increased processing of proHB-EGF [18]. Taken together, these results suggested that ADAM9, -10, -12, and -17 are involved in various signal-mediated processing of proHB-EGF.

ADAM17 is required for the processing of proamphiregulin in head and neck squamous cell carcinoma after stimulation with lysophosphatidic acid and carbacol, leading to EGFR phosphorylation, and activation of MAPKs and Akt. ADAM17-mediated cleavage of amphiregulin has been reported in response to stimulation of lung carcinoma NCI-H292 cells with cigarette smoke [19]. ADAM17 is also involved in angiotensin II-induced processing of TGF- α [20]. A recent study using fibroblasts derived from ADAM17-knockout mice confirmed a role for ADAM17 in the processing of TGF- α [21].

IL-8 as a Growth Factor under Pathophysiological Conditions

IL-8 was initially identified as a neutrophil chemoattractant and belongs to the CXC chemokine family [22]. It was demonstrated to promote angiogenesis. IL-8 receptor (GPCR) was then discovered to have two homologues (CXCR1 and CXCR2) in the membrane of melanoma cells. IL-8 has since been implicated in the promotion of cell proliferation by binding to its receptor in various types of cancer cell, thus confirming that IL-8 functions as a growth factor. Taken together, these results suggest that IL-8 promotes angiogenesis to vascular endothelial cells and cell proliferation to tumor cells. IL-8 is not constitutively expressed in non-cancerous cells, but is upregulated by stimuli such as LPS, TPA, tumor necrotic fac-

tor- α and/or cellular stress [23]. In contrast, constitutive expression of IL-8 is implicated in various human neoplasms.

EGFR Transactivation through GPCR in IL-8-Induced Cell Proliferation and Cell Migration

Wound healing assay has shown that keratinocyte proliferation and migration are mediated in an autocrine manner by EGFR-ligand interaction. EGFR ligands are expressed on the keratinocyte cell surface in membrane-anchored precursor forms, and wound fluid in skin contains soluble bioactive forms. In addition, the metalloproteinase inhibitor KB-R7785 abrogates wound-induced EGFR activation by blocking proHB-EGF shedding, thus suggesting that shedding of proHB-EGF plays an important physiological role in keratinocyte migration under the wound state [24]. Inhibition of metalloproteinase-mediated EGFR ligand shedding can reduce proliferation and migration in breast cancer cells, while IL-8, a GPCR agonist, promotes cell proliferation and migration in the colon cancer cell line HT-29 and Caco2 through ADAM-mediated cleavage of proHB-EGF on growth curve assay and wound healing assay, respectively [25]. Thus, IL-8-induced proHB-EGF shedding and transactivation is mediated by activation of ADAM10 [15].

HB-EGF CTF Signaling following Ectodomain Cleavage of proHB-EGF

The proteolytic cleavage of proHB-EGF by ADAM generates another type of mitogenic signal. Thus, it has recently been reported that proHB-EGF is cleaved to release the ectodomain HB-EGF by specific metalloproteinases, and that CTFs of HB-EGF (HB-EGF-CTF) move into nucleus, subsequently exert effects on regulation of cell proliferation by binding nuclear promyelocytic leukemia zinc finger (PLZF) protein, a transcriptional repressor, thereby causing its nuclear export [26]. PLZF exerts transcriptional repression through decreased expression of cyclin A, forming a repressor complex containing N-CoR, SMRT, Sin3a and histone deacetylases. IL-8 also induces trafficking of HB-EGF-CTF into the nucleus after ADAM10-mediated ectodomain processing of proHB-EGF. Moreover, suppressed trafficking of HB-EGF-C into nucleus by the ADAM inhibitor KB-R7785 decelerates mitogenic signaling through delayed entry into S-phase of the cell cycle. As

Fig. 1. EGFR transactivation and intranuclear translocation of HB-EGF-CTF. IL-8 induces ADAM10-mediated proteolytic ectodomain processing of proHB-EGF. Processed soluble HB-EGF induces rapid transient EGFR phosphorylation and subsequent activation of downstream signaling events such as MAPK phosphorylation, leading to transcription of various genes. Simultaneously, HB-EGF-CTF is transported into the nucleus after ADAM10-mediated ectodomain processing of proHB-EGF, thus inducing nuclear export of PLZF and leading to cell cycle progression. P stands for phosphorylation.

this process is abrogated by inhibition of metalloprotease activity, ectodomain processing is required for trafficking of HB-EGF-CTF into the nucleus [26, 27]. Simultaneously, the processed ectodomain of HB-EGF, a soluble ligand of HB-EGF, promotes G1-phase progression in the cell cycle by upregulating the expression of cyclin D through the Ras-MAPK signaling cascade [26, 27]. Therefore, processing of proHB-EGF induced by IL-8 generates two types of mitogenic signals: EGFR transactivation followed by downstream Ras-MAPK, and the internalized HB-EGF-CTF pathway followed by nuclear export of PLZF plays important roles in cellular repair of the impaired mucosa in the digestive tract promoting cell proliferation and migration through dual intracellular signaling (fig. 1).

Conclusion and Perspectives

It has been established that IL-8 plays a critical role in repair of mucosal injury and ulceration through EGF signaling. Selective ADAM inhibitors have recently been investigated and applied clinically. Moreover, selective inhibitors targeting HB-EGF-CTF trafficking into the nucleus are also going to be developed and studied, thereby ensuring that detailed molecular mechanisms for mucosal repair and EGF signaling will be elucidated in the future.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

References

- Holbro T, Hynes NE: ErbB receptors: directing key signaling networks throughout life. *Annu Rev Pharmacol Toxicol* 2004;44:195–217.
- Cohen S: Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. *J Biol Chem* 1962;237:1555–1562.
- Higashiyama S, Abraham JA, Miller J, Fiddes JC, Klagsbrun M: A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science* 1991;251:936–939.
- Shoyab M, Plowman GD, McDonald VL, Bradley JG, Todaro GJ: Structure and function of human amphiregulin: a member of the epidermal growth factor family. *Science* 1989;243:1074–1076.
- Derynck R, Roberts AB, Winkler ME, Chen EY, Goeddel DV: Human transforming growth factor- α : precursor structure and expression in *Escherichia coli*. *Cell* 1984;38:287–297.
- Shing Y, Christofori G, Hanahan D, Ono Y, Sasada R, Igarashi K, Folkman J: Betacellulin: a mitogen from pancreatic β -cell tumors. *Science* 1993;259:1604–1607.
- Toyoda H, Komurasaki T, Uchida D, Takayama Y, Isobe T, Okuyama T, Hanada K: Epi-regulin. A novel epidermal growth factor with mitogenic activity for rat primary hepatocytes. *J Biol Chem* 1995;270:7495–7500.
- Strachan L, Murison JG, Prestidge RL, Sleeman MA, Watson JD, Kumble KD: Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily. *J Biol Chem* 2001;276:18265–18271.

- 9 Gschwind A, Zwick E, Prenzel N, Leserer M, Ullrich A: Cell communication networks: epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission. *Oncogene* 2001;20:1594–1600.
- 10 Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, Ullrich A: EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* 1999;402:884–888.
- 11 Pierce KL, Luttrell LM, Lefkowitz RJ: New mechanisms in heptahelical receptor signaling to mitogen activated protein kinase cascades. *Oncogene* 2001;20:1532–1539.
- 12 Dempsey PJ, Garton K, Raines EW: Emerging roles of TACE as a key protease in ErbB ligand shedding. *Mol Interv* 2002;2:136–141.
- 13 Lemjabbar H, Basbaum C: Platelet-activating factor receptor and ADAM10 mediate responses to *Staphylococcus aureus* in epithelial cells. *Nat Med* 2002;8:41–46.
- 14 Yan Y, Shirakabe K, Werb Z: The metalloprotease Kuzbanian (ADAM10) mediates the transactivation of EGF receptor by G-protein-coupled receptors. *J Cell Biol* 2002;158:221–226.
- 15 Tanida S, Joh T, Itoh K, Kataoka H, Sasaki M, Ohara H, Nakazawa T, Nomura T, Kinugasa Y, Ohmoto H, Ishiguro H, Yoshino K, Higashiyama S, Itoh M: The mechanism of cleavage of EGFR ligands induced by inflammatory cytokines in gastric cancer cells. *Gastroenterology* 2004;127:559–569.
- 16 Izumi Y, Hirata M, Hasuwa H, Iwamoto R, Umata T, Miyado K, Tamai Y, Kurisaki T, Sehara-Fujisawa A, Ohno S, Mekada E: A metalloprotease-disintegrin, MDC9/meltrin- γ /ADAM9 and PKC δ are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *EMBO J* 1998;17:7260–7272.
- 17 Asakura M, Kitakaze M, Takashima S, Liao Y, Ishikura F, Yoshinaka T, Ohmoto H, Node K, Yoshino K, Ishiguro H, Asanuma H, Sana-da S, Matsumura Y, Takeda H, Beppu S, Tada M, Hori M, Higashiyama S: Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat Med* 2002;8:35–40.
- 18 Sunnarborg SW, Hinkle CL, Stevenson M, Russell WE, Raska CS, Peschon JJ, Castner BJ, Gerhart MJ, Paxton RJ, Black RA, Lee DC: Tumor necrosis factor- α converting enzyme (TACE) regulates epidermal growth factor receptor ligand availability. *J Biol Chem* 2002;277:12838–12845.
- 19 Lemjabbar H, Li D, Gallup M, Sidhu S, Drori E, Basbaum C: Tobacco smoke-induced lung cell proliferation mediated by tumor necrosis factor- α -converting enzyme and amphiregulin. *J Biol Chem* 2003;278:26202–26207.
- 20 Lautrette A, Li S, Alili R, Sunnarborg SW, Burtin M, Lee DC, Friedlander G, Terzi F: Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. *Nat Med* 2005;11:867–874.
- 21 Peschon JJ, Slack JL, Reddy P, Stocking KL, Sunnarborg SW, Lee DC, Russell WE, Castner BJ, Johnson RS, Fitzner JN, Boyce RW, Nelson N, Kozlosky CJ, Wolfson MF, Rauch CT, Cerretti DP, Paxton RJ, March CJ, Black RA: An essential role for ectodomain shedding in mammalian development. *Science* 1998;282:1281–1284.
- 22 Matsushima K, Morishita K, Yoshimura T, Lavu S, Kobayashi Y, Lew W, Appella E, Kung HF, Leonard EJ, Oppenheim JJ: Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin-1 and tumor necrosis factor. *J Exp Med* 1988;167:1883–1893.
- 23 Mukaida N: Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. *Am J Physiol* 2003;284:L566–L577.
- 24 Inui S, Higashiyama S, Hashimoto K, Higashiyama M, Yoshikawa K, Taniguchi N: Possible role of coexpression of CD9 with membrane-anchored heparin-binding EGF-like growth factor and amphiregulin in cultured human keratinocyte growth. *J Cell Physiol* 1997;171:291–298.
- 25 Itoh Y, Joh T, Tanida S, Sasaki M, Kataoka H, Itoh K, Oshima T, Ogasawara N, Togawa S, Wada T, Kubota H, Mori Y, Ohara H, Nomura T, Higashiyama S, Itoh M: IL-8 promotes cell proliferation and migration through metalloproteinase-cleavage proHB-EGF in human colon carcinoma cells. *Cytokine* 2005;29:275–282.
- 26 Nanba D, Mammoto A, Hashimoto K, Higashiyama S: Proteolytic release of the carboxy-terminal fragment of proHB-EGF causes nuclear export of PLZF. *J Cell Biol* 2003;163:489–502.
- 27 Shimura T, Kataoka H, Ogasawara N, Kubota E, Sasaki M, Tanida S, Joh T: Suppression of proHB-EGF carboxy-terminal fragment nuclear translocation: a new molecular target therapy for gastric cancer. *Clin Cancer Res* 2008;14:3956–3965.