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Review Article

Involvement of Cell Proliferation Induced by Dual Intracellular Signaling of HB-EGF in the Development of Colitis-Associated Cancer during Ulcerative Colitis

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

Department of Gastroenterology and Metabolism, Graduate School of Medical Sciences, Nagoya City University, 1 Kawasumi, Mizuho, Nagoya, Aichi 467-8601, Japan

Correspondence should be addressed to Satoshi Tanida, stanida@med.nagoya-cu.ac.jp

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In ulcerative colitis (UC), the duration and severity of inflammation are responsible for the development of colorectal cancer. Reactive oxygen species (ROS), reactive nitric metabolites (RNMs) and interleukin (IL)-8, released by epithelial and immune cells, are involved in the pathogenesis of colitis-associated cancer. Nitric oxide and peroxynitrite activate epidermal growth factor receptor (EGFR), and therapeutic agents targeted towards EGFR are currently used to treat advanced colorectal cancer. IL-8 (a G-protein coupled receptor (GPCR) agonist), which is involved in neutrophil recruitment and activation in persistent active colitis, also promotes cleavage of the proheparin-binding epidermal growth factor-like growth factor (proHB-EGF) through a disintegrin and metalloproteinase (ADAM). The cleaved HB-EGF and C-terminal fragments (intracellular CTF) regulate proliferation via EGFR activation and nuclear export of promyelocytic leukemia zinc finger, transcription repressor, respectively. Here, we focus on the mechanisms by which RNM- and IL-8-induced EGF signaling regulate cell proliferation during the development of colitis-associated cancer.

1. Introduction

The incidence of inflammatory bowel diseases such as UC has been increasing on an annual basis. Uncontrolled and excessive host immune responses, during which oxidative stress-like reactive oxygen species (ROS), reactive nitrogen metabolites (RNMs), and free radicals are produced from inflammatory cell infiltrates in the gut mucosa, are known to trigger mucosal injury and induce inflammation. Long-term high disease activity has been established as a potential risk factor for the development of colitis-associated cancer [1, 2]. Colorectal cancer represents the major cause of excess morbidity and mortality due to malignancy associated with UC. The cumulative incidence of such a malignancy is below 1% in the first 8–10 years of UC, but then rises in annual increments of 0.5–1.0% thereafter to reach 5–10% after 20 years and 15–20% after 30 years [3]. In addition to ROS and RNM, the perpetuation of active inflammation in the mucosa of patients with UC, which is mediated by inflammatory cytokines such as interleukin (IL)-8 and tumor necrotic factor- (TNF-) α, is also believed to increase the risk of colitis-associated cancer. Thus, ROS, RNM, and inflammatory cytokines play important roles in the perpetuation of inflammation in UC leading to the development of colorectal cancer.

There has been recent, widespread application of therapeutic treatments targeting key molecules that are involved in cell proliferation in malignant neoplasms [4]. Therapeutic treatments targeted towards advanced colorectal cancers are mainly focused on the design of therapeutic agents that target epidermal growth factor (EGF) signaling, such as the monoclonal antibody cetuximab which blocks the EGFR [5, 6]. However, less attention has been paid to the possibility of targeting the dual signaling of HB-EGF. This dual signaling arises from metalloproteinase cleavage of the precursor of HB-EGF into the cleaved extracellular fragments and C-terminal fragments (CTFs), respectively. The cleaved HB-EGF binds to and activates EGFR, whereas the

CTF fragments are translocated to and modulate signaling in, the nucleus. Here, we provide an overview of the mechanisms by which RNM, especially nitric oxide and peroxynitrite, and IL-8 modulate UC inflammation and their induced EGF signaling regulate cell proliferation.

2. Involvement of ROS and RNM as Noxious Factors in UC

Direct measurement of ROS in tissues is difficult because of their short biological half lives [7]. However, direct quantification of ROS levels in colon biopsy specimens from UC patients using chemiluminescence assays showed that ROS levels in these samples are increased compared to those in normal mucosa and positively correlate with disease activities [8–11]. There is mounting evidence, based on analysis of nitric oxide synthase activity, that there are increased levels of RNM such as NO in inflamed inflammatory bowel disease (IBD) mucosa [12–15]. Thus, increased levels of both ROS and RNM are closely correlated with the clinical development of IBD.

3. Oxidative Damage by ROS and Peroxynitrite, and EGFR Activation by Nitric Oxide and Peroxynitrite

Excessive levels of ROS attack and impair almost all cellular components, including cell membranes, proteins, enzymes, and DNA, and consequently cause apoptotic cell death.

Regarding the effect of ROS on the cell membrane, it is known that the polyunsaturated fatty acids in the cell membrane lipid bilayer have two or more carbon double bonds within their structure that are susceptible to oxidative attack [16]. Sequential attack against these bonds by hydroxyl radicals ($^{\bullet}OH$) converts these membrane lipids into oxidized phospholipids (lipid peroxidation). The accumulation of peroxidized lipids accelerates the disruption of cell membrane integrity that occurs when the ability of the cell to remove excessive products of hydroxyl radicals and their precursors, particularly the products of hydrogen peroxide (H_2O_2), fails. This failure and the subsequent increase in ROS results in decreased function of transmembrane enzymes, transporters, receptors, and other membrane proteins, which are consequently degraded [17, 18]. Moreover, colonic epithelia disintegrate because of the ROS-induced increase in mucosal permeability [19, 20].

Proteins and enzymes, which are the predominant constituents of cells, are also targets of ROS and oxidative stress. Thus, the $^{\bullet}OH$ radical also attacks, and results in the degradation or inhibition of the function of many proteins and enzymes. The toxic oxidative effects of $^{\bullet}OH$ include the induction of protein conformational change, which is a major cause of the partial or complete loss of protein function [21].

In addition, nuclear DNA [22] is also known to be a target of oxidative attack, particularly from $^{\bullet}OH$ and peroxynitrite ($ONOO^-$), which cause base and sugar hydroxylation [23] as well as breaks in the double strand, leading to adenosine triphosphate depletion and gene mutations [24, 25]. These changes ultimately induce malignant transformation and apoptotic cell death. $ONOO^-$ is a potent oxidant and nitrating species that is formed from a rapid reaction between the superoxide anion ($O_2^{\bullet -}$) and nitric oxide (NO) [26]. $ONOO^-$ easily crosses biological membranes, and, despite a relatively short half life (within 10 ms), it can interact with target molecules in an adjacent cell within one or two cell diameters [27]. Interestingly, exposure to $ONOO^-$ promotes the conversion of tyrosine residues into 3-nitrotyrosine, which cannot be readily phosphorylated. $ONOO^-$ thus interferes with cellular signaling that is dependent on tyrosine phosphorylation by protein tyrosine kinases [28]. Tyrosine nitration by $ONOO^-$ can either prevent a protein from functioning as the phosphorylated form or can mimic the structural change induced by phosphorylation and thereby imitate the consequences of phosphorylation [29]. In contrast to $^{\bullet}OH$, $ONOO^-$ can up- or downregulate signaling cascades by controlling the activities of protein kinases. Regarding the particular effect of $ONOO^-$ on EGF signaling, $ONOO^-$ is known to promote EGFR dimerization and phosphorylation, followed by activation of extracellular-regulated kinases (ERK) 1 and 2, via activation of Ras and mitogen-activated protein kinase kinase (MKK) 1 or 2 and ultimately induces cell proliferation [30].

NO also promotes EGFR autophosphorylation following activation of the p21Ras-ERK1/2 signaling cascade and subsequently induces cell proliferation (Figure 1) [31].

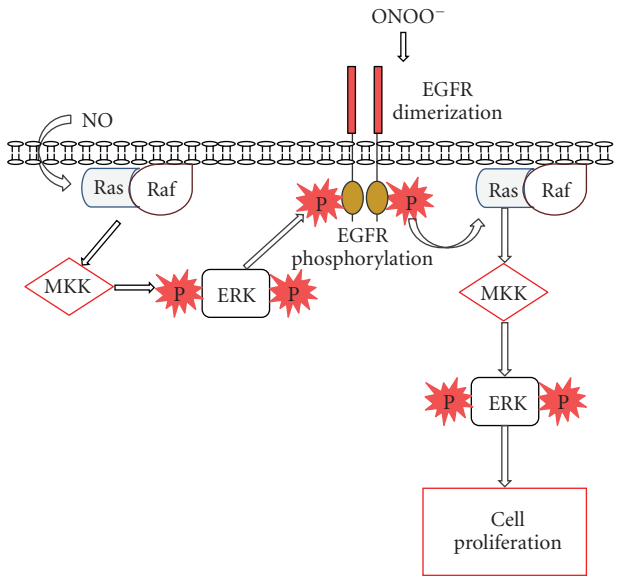

Figure 1: *EGFR activation induced by nitric oxide and peroxynitrite.* Quoted from [32] and modified. Peroxynitrite, $ONOO^-$ promotes EGFR dimerization and phosphorylation, which is followed by activation of extracellular-regulated kinases (ERK) 1 and 2, via activation of Ras and mitogen-activated protein kinase kinase (MKK) 1 or 2. Nitric oxide, NO promotes EGFR autophosphorylation through activation of ERK. P indicates phosphorylation.

4. IL-8 Mediates the Activation of Neutrophils during Inflammation in UC

Intestinal inflammation that occurs in UC is characterized by mucosal and submucosal infiltration of numerous neutrophils and lymphocytes in both moderate and severe UC cases. In patients with UC, the circulating levels of neutrophils are also reported to be up to three times as high as the level in healthy controls [33]. Since granulocyte apheresis is therapeutic for active UC, clearly, circulating and infiltrating neutrophils are strongly involved in the development of UC [34].

Increased levels of IL-8 protein production and IL-8 mRNA expression are observed in the inflamed mucosa of patients with UC, especially in macrophages, neutrophils, and colonic epithelial cells [35, 36]. Moreover, luminol-dependent chemiluminescence and myeloperoxidase (MPO) activities of neutrophils in the mucosa are markedly increased in active UC compared with their levels in inactive UC and in controls. Thus, there is a close correlation between the levels of IL-8, the intensity of chemiluminescence and MPO levels [37]. These results suggested that most neutrophils that infiltrate in the inflamed mucosa in UC are activated by IL-8.

5. IL-8 as a Growth Factor in Transformed States

IL-8 was initially identified as a neutrophil chemoattractant and belongs to the CXC chemokine family [38]. IL-8 was first demonstrated to promote angiogenesis. The 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 mediated by binding to its receptor in various types of cancer cells, thus confirming that IL-8 functions as a growth factor. The combined results suggest that IL-8 promotes both the angiogenesis of vascular endothelial cells and the proliferation of tumor cells. IL-8 is not constitutively expressed in noncancerous cells, but is upregulated by stimuli such as lipopolysaccharide (LPS) and TNF-α [39]. In contrast, IL-8 is constitutively expressed in various human neoplasms.

Thus, IL-8 acts not only as a chemoattractant but also as a growth factor in inflamed mucosa in UC, suggesting that IL-8 play a critical role in the development of colitis-associated cancer during UC.

6. EGFR Transactivation through GPCRs in IL-8-Induced Cell Proliferation

Several GPCRs have been demonstrated to activate the EGFR (an event known as transactivation), even though GPCR agonists do not directly interact with the EGFR. EGFR transactivation by GPCR agonists was first discovered to mediate several critical downstream EGFR signals and activities such as ERK activation, c-fos induction, and cell proliferation. EGFR transactivation involves a disintegrin and metalloproteinase (ADAM) that convert proform of EGFR ligands including EGF, HB-EGF, amphiregulin, betacellulin, epiregulin, and epigen to bioactive forms by inducing their ectodomain shedding. The metalloproteinase-cleaved extracellular domain of the EGFR ligand, HB-EGF, was first involved in the GPCR-induced EGFR transactivation pathway by using a chimeric receptor composed of the EGFR extracellular domain and the platelet-derived growth factor (PDGF) receptor transmembrane and tyrosine kinase domain [40]. Blocking of proHB-EGF function using the diphtheria toxin mutant Crm197 and of metalloproteinase activity using the inhibitor batimastat (BB94), both abrogate GPCR-induced EGFR, Shc, and MAPK phosphorylation. Inhibition of metalloproteinase-mediated EGFR ligand shedding can reduce proliferation in breast cancer cells. Numerous GPCR agonists, including angiotensin II, endothelin-1, carbachol, bombesin, phenylephrine, and IL-8 have been reported. IL-8, which is the major neutrophil chemoattractant in UC, and is involved in activities and perpetuation of inflammation in UC, promotes cell proliferation in the colon cancer cell line Caco2 through ADAM-mediated cleavage of proHB-EGF, as assessed using the incorporation of [^{3}H] thymidine into DNA [41]. Thus, IL-8-induced proHB-EGF shedding and EGFR transactivation is mediated by activation of ADAM10 [42].

Interestingly, defective regeneration of colon epithelial cells and breakdown of the intestinal barrier was observed because of impaired shedding of EGFR ligands followed by failure to phosphorylate STAT3 via EGFR during dextran sulfate sodium-induced experimental colitis in mice with dramatically reduced expression level of ADAM 17 with the exon-induced translational stop method. Thus, ADAM 17-mediated cleavage of EGFR ligands is also involved in cell proliferation and tissue regeneration [43].

7. Trafficking of the C-Terminal Fragment of HB-EGF following Ectodomain Cleavage of proHB-EGF

It has recently been reported that carboxy-terminal fragment of HB-EGF (HB-EGF-CTF) is translocated into nucleus following ectodomain cleavage of HB-EGF by specific metalloproteinases, subsequently exerts effects on the regulation of cell proliferation by binding nuclear promyelocytic leukemia zinc finger (PLZF) protein, a transcriptional repressor, thereby causing its nuclear export [44]. IL-8 also induces trafficking of HB-EGF-CTF into the nucleus after ADAM10-mediated ectodomain processing of proHB-EGF. Moreover, suppression of HB-EGF-CTF trafficking into the nucleus by use of the ADAM inhibitor KB-R7785 decelerates mitogenic phenomena through delayed entry into the S-phase of the cell cycle. Inhibition of *metalloproteinase* activity abrogates this process, suggesting that ectodomain processing triggers trafficking of HB-EGF-CTF into the nucleus [44, 45]. In parallel with HB-EGF-CTF signaling, the shed ectodomain of HB-EGF through binding to and activation of the EGFR, promotes G1-phase progression in the cell cycle by upregulating the expression of cyclin D through the Ras-MAPK signaling cascade including MKK1/2 and ERK [44,

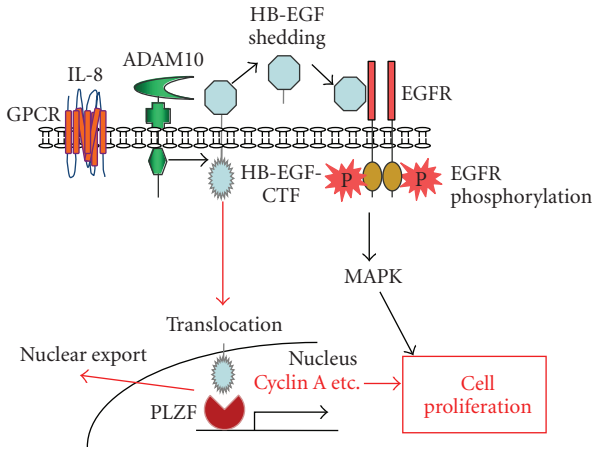

Figure 2: *Dual HB-EGF signaling induced by IL-8*. Quoted from [46] and modified. IL-8 binds to the GPCR, thereby inducing ADAM10-mediated cleavage of proHB-EGF, resulting in ectodomain shedding of its N-terminus and the generation of an intracellular C-terminal fragment (HB-EGF-CTF). The shed soluble HB-EGF binds to the EGFR and induces rapid transient EGFR phosphorylation and subsequent activation of downstream signaling events such as mitogen-activated protein kinase (MAPK) phosphorylation, leading to the transcription of various genes. In parallel, HB-EGF-CTF is transported into the nucleus, where it subsequently induces nuclear export of PLZF, which leads to cell cycle progression. P indicates phosphorylation.

45]. Therefore, processing of proHB-EGF induced by IL-8 generates two types of mitogenic signals: EGFR transactivation followed by downstream Ras-MAPK activation, and the intracellular HB-EGF-CTF generation followed by nuclear export of PLZF. Thus, IL-8 modulates cell proliferation through dual intracellular signaling pathways (Figure 2).

8. Conclusion and Perspectives

It has been established that ROS, RNM, and IL-8 play important roles in perpetuation of inflammation in UC leading to the development of colorectal cancer through EGF signaling. Future development and study of neutralizing or blocking antibodies that target molecules involved in the development of UC and colorectal cancer will ensure that the relationship between ROS, RNM, and IL-8 signaling, and EGF signaling as well as the detailed molecular mechanisms that underlie the role of these signaling pathways in cancer development in UC will be elucidated in the future.

Conflict of Interests

Authors declare that no financial or other conflict of interests exists in relation to the content of the paper.

Abbreviations

UC: Ulcerative colitis
ROS: Reactive oxygen species
RNM: Reactive nitrogen metabolites
IL: Interleukin
GPCR: G-protein-coupled receptor
HB-EGF: Heparin-binding EGF-like growth factor
ADAM: A disintegrin and metalloproteinase
CTF: C-terminal fragment
IBD: Inflammatory bowel disease
TNF: Tumor necrotic factor
ERK: Extracellular-regulated kinase
MKK: Mitogen-activated protein kinase kinase
MPO: Myeloperoxidase
LPS: Lipopolysaccharide
EGFR: Epidermal growth factor receptor
MAPK: Mitogen-activated protein kinase
PDGF: Platelet-derived growth factor
PLZF: Promyelocytic leukemia zinc finger.

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