Epithelial Barrier Regulation by Hypoxia-Inducible Factor

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Abstract

Mucosal tissues represent surfaces that are exposed to the outside world and provide a conduit for internal and external communication. Tissues such as the intestine and the lung are lined by layer(s) of epithelial cells that, when organized in three dimensions, provide a critical barrier to the flux of luminal contents. This selective barrier is provided through the regulated expression of junctional proteins and mucins. Tissue oxygen metabolism is central to the maintenance of homeostasis in the mucosa. In some organs (e.g., the colon), low baseline P_{O_2} determines tissue metabolism and results in basal expression of the transcription factor, hypoxia-inducible factor (HIF), which is enhanced after ischemia/inflammation. Recent studies have indicated that HIF contributes fundamentally to the expression of barrier-related genes and in the regulation of barrier-adaptive responses within the mucosa. Here, we briefly review recent literature on the topic of hypoxia and HIF regulation of barrier in mucosal health and during disease.

Keywords: metabolism; cell–cell junction; inflammation; creatine; creatine kinase

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Surfaces lined by epithelial cells provide a selective barrier between biologic surfaces, preventing the free mixing of luminal antigenic material with the underlying tissues. The maintenance of this selectively permeable barrier occurs through interactions of multiple transmembrane proteins found in select domains of the plasma membrane (e.g., adherens junctions [AJs] and tight junctions [TJs]) and between the epithelium and the extracellular matrix (1). These membrane domains define the three-dimensional structure of the tissue and establish the polarized protein and lipid organization within the plasma membrane (i.e., the "fence" function of the epithelium).

Mucosal tissues also maintain a rich and complex vasculature. The gastrointestinal tract, for example, adapts to profound fluctuations in blood perfusion on a daily basis (2). At baseline, epithelial cells lining the gastrointestinal mucosa experience P_{O_2} levels approaching anoxia, a homeostatic state termed "physiologic hypoxia" (3). Analysis of oxygen exchange within the intestine has revealed that arterial blood–derived O_2 diffuses across the villus to parallel venules, resulting in graded regions of significant hypoxia (4). In the colon, a gradient of O_2 emanating from the submucosa toward the anaerobic lumen establishes one of the more complex microenvironments found in mammals (5). In these settings, even small perturbations in blood flow can result in relatively large decreases in O_2 delivery with resultant ischemia/hypoxia. These changes in blood flow can be particularly profound during injury. The high-energy requirements of the mucosa, in combination with the role of the epithelium in maintaining homeostasis, has driven the evolution of a number of mechanisms to cope with this low oxygen state. Here, we discuss how the mucosa adapts to changes in blood flow in health and disease.

Oxygen Utilization in the Mucosa

A comparison of the various mucosal tissues reveals marked difference in oxygen distribution. The healthy lung alveolus, for example, supports a surface P_{O_2} of 100–110 mm Hg (6). Conversely, the tip of villi in healthy colon exits at a P_{O_2} of less than 10 mm Hg (5, 7). Such differences reflect a combination of O_2 sources, the anatomy of blood flow, and the presence of large numbers of commensal microbes in the colon (8). Inflammatory lesions are profoundly hypoxic (or even anoxic), comparable to levels observed in some large tumors (7). Although there are multiple contributing factors (i.e., increased O_2 consumption, vasoconstriction, edema) that result in decreased O_2 delivery and resultant hypoxia (7), it was recently shown that a major component of deep-tissue hypoxia in active inflammation is derived

from nicotinamide adenine dinucleotide phosphate oxidase–dependent O_2 consumption by activated leukocytes, particularly neutrophils (9).

Numerous studies have shown that the transcription factor, hypoxia-inducible factor (HIF), regulates the expression of target genes that enable the epithelium to function as an effective barrier (10, 11). The two α isoforms of HIF, namely HIF-1 and HIF-2, represent period–aryl hydrocarbon receptor nuclear translocator–single-minded members of the basic helix-loop-helix family of transcription factors, and function as central regulators of tissue O_2 metabolism (12). HIF stabilization in the cytoplasm depends on modifications to the O_2-dependent degradation domain expressed on the HIF-1α and HIF-2α subunits with subsequent nuclear localization to form a functional complex with the common β subunit HIF-1β, also called the aryl hydrocarbon receptor nuclear translocator (13). When O_2 supply is sufficient, O_2- and iron-dependent hydroxylation of two proline motifs within the O_2-dependent degradation domain of the α subunit of HIF initiates von Hippel-Lindau tumor suppressor protein–dependent ubiquitylation and degradation by the proteasome (14).

Although the majority of studies in the literature have focused on hypoxia as the key regulator of HIF activity, nonhypoxic HIF stabilizers have also been described. Inflammatory cytokines, such as IL-1 and tumor necrosis factor, have long been known to regulate the activity of HIF-1 in both normoxia and hypoxia (15). Such regulation can occur through increased HIF-1 mRNA, increased protein stability, and increased HIF-1 DNA binding (15). Parallel studies have shown that pathways upstream and downstream of HIF stabilization can impact HIF activity, including phosphoinositide-3-kinase (16), cullin-2 neddylation (17), nuclear factor-κB (18), and sestrin-2 (19). Likewise, microbial-derived products found within the mucosa, including butyrate (20) and siderophores (21, 22), can also stabilize HIF. It is notable that the activation of some intracellular pathways, such as nitric oxide synthase (23), can result in the redistribution of intracellular oxygen to the extent that HIF is stabilized. Thus, it remains to be determined to what extent the nonhypoxic pathways may be mediated by shifts in cellular metabolism that can be "sensed" as hypoxia by the proly hydroxylase enzymes.

HIF Targets of Barrier Regulation

A major function of the mucosa is the provision of a physical barrier between the inside and outside world. This barrier is tightly regulated in the healthy mucosa and barrier dysfunction is associated with a plethora of mucosal diseases. Here, we provide examples of barrier components regulated by HIF.

Mucin Expression and HIF

A number of mucosal surfaces extend the barrier apically through formation of a mucus layer. Goblet cells produce and secrete mucins that form mixture of glycoproteins at the epithelial surface that prevents the direct exposure of the mucosa to luminal contents. Depending on the tissue origin, goblet cells secrete up to 10 distinct surface-localized and gel-forming mucins (24), which, in the healthy mucosa, consist of an adherent mucus layer that is devoid of bacteria and a superficial layer that is many times the depth of the epithelium (25, 26). Hypoxia and HIF regulate several components of the mucus layer. For example, the promoter of mammalian mucin 5AC contains evolutionarily conserved regions proximal to the mRNA coding region that bind functional sma-mothers against decapentaplegic 4 and HIF-1α binding regions (27). In the colon, both mucin-3 and intestinal trefoil factor-3 are prominent HIF-1α target genes that function in concert to provide epithelial protection and to promote wound healing (28, 29).

Mucus also provides a reservoir for secreted epithelial factors, such as antimicrobial peptides (AMPs) (30). Defensins, for example, are a class of cysteine-rich AMPs that possess broad antimicrobial activity (31, 32). Human β defensin-1 (hBD1) is an example of an AMP secreted by the intestinal epithelium in a constitutive manner, as opposed to other defensin-like AMPs that are released only in response to inflammatory mediators (8). Homeostatic expression of hBD1 was demonstrated to depend on HIF-1α signaling in multiple cells, where hBD1 expression correlated with other HIF target genes in human tissues (33). A notable feature of hBD1 is that the full spectrum of its antimicrobial activity is most prominently revealed with reduced disulfide bonds (34). Thus, at multiple levels, hypoxia and HIF appear to provide important regulatory roles for the expression and function of the mucus layer.

Tight Junctions and HIF

TJs form the backbone to the structural integrity of the barrier and provide the physical basis for permeability to solutes and ions (35). TJs also prevent lipid diffusion between apical and basolateral membrane domains, the so-called "fence function" (35). The TJ is composed of both transmembrane and peripheral membrane proteins tightly linked to the actin-based cytoskeleton (36). The assembly of TJ structure and function within the membrane is regulated by a variety of physiological and pathophysiological stimuli (1). Hypoxia, for example, dramatically influences the integrity of TJ, and can result in loss of barrier function. These results have been observed using a number of approaches, including chemical depletion of ATP (37) and *in vitro* hypoxia (28, 38, 39).

Claudins are a large family of tetraspannin integral membrane proteins that function to provide the selective permeability of TJs (35). Functionally, claudins are categorized as "tight" or "leaky" with regard to their influence on barrier properties (40). Claudin-1 (*CLDN1*) is a "tight" claudin that has been shown to be dysregulated in a variety of human diseases, including inflammatory bowel disease (35). In a screen of TJ targets, CLDN1 was identified to explain an aberrant junctional morphology of HIF deficient intestinal epithelial cell lines (41) (*see* Figure 1). Using loss- and gain-of-function approaches, this work showed that HIF signaling maintains CLDN1 expression through binding HIF-responsive element sequences in the gene promoter. The reintroduction of CLDN1 expression in HIF-deficient epithelial cells restored barrier function and reversed the morphologic abnormalities. Furthermore, *in vivo* analysis revealed an importance for HIF-mediated CLDN1 expression during mucosal insult. These results identify a critical link between HIF and TJ structure/function, providing important insight into mechanisms of HIF-regulated epithelial homeostasis (41).

Adherens Junctions and HIF

Polarization and intercellular junctions depend, in large part, on cell–cell contact mediated by cadherin–catenin interactions and, subsequent, assembly of the AJ (42).

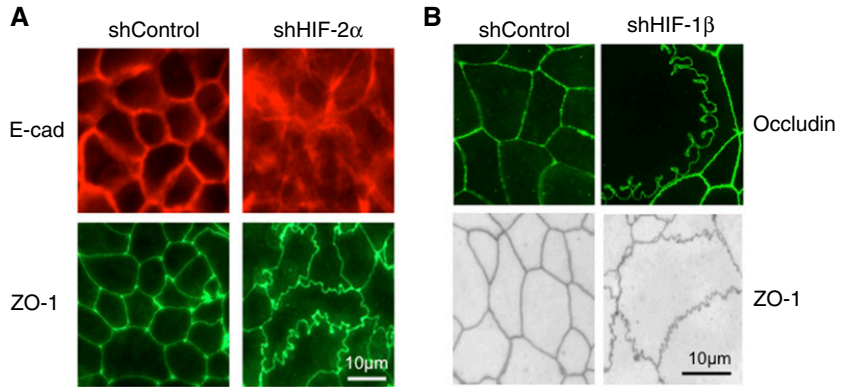

Figure 1. Epithelial junctional defects in cells lacking hypoxia-inducible factor (HIF) expression. (*A*) The localization of the adherens junction protein E-cadherin (E-cad) and the tight junction (TJ) protein zonula occludens (ZO)-1 in epithelial cells deficient in HIF-2α. Cells were depleted of HIF-2α by transduction of lentiviral short hairpin RNA. Shown here is a nontargeting control (shControl) and shHIF-2α. Note defective expression of both E-cad and ZO-1 in shHIF-2α cells. (*B*) Defective expression of the TJ proteins occludin and ZO-1 in cells lacking the HIF-1 and HIF-2 dimeric partner, HIF-1β. Adapted from References 41 and 47. sh = short hairpin.

Evidence from both ATP depletion models and ischemia described the dissolution of AJ complexes (43), likely initiated by the hyperphosphorylation of the catenins (44). Because the AJ plays a crucial role for the establishment and maintenance of polarized epithelia, these changes constitute a critical lesion in epithelial hypoxia (45). During restitution, epithelia depend on reassembly and reformation of the AJ proteins. Within the AJ, β-catenin signaling through T cell factor/lymphoid enhancer factor transcription factors regulates barrier restitution during active mucosal inflammation (46). These studies in the lung revealed that neutrophil migration across epithelial monolayers elicits an epithelial gene programming that results in activated β-catenin signaling and the restitution of epithelial barrier function (46).

Original studies using ATP depletion models revealed an important role for high-energy phosphates in the regulation of barrier function (47). These studies have prompted further analysis of hypoxia adaptation to such conditions. Notable is the observation that cytosolic creatine (Cr) kinase genes are HIF-2–selective targets expressed within the AJ of confluent intestinal epithelia. Studies in the mucosa revealed that each of the Cr kinase subunits (i.e., muscle, brain, and mitochondrial) is expressed in cultured intestinal epithelial cell lines, murine colonic epithelia, and in human colonic epithelia (47). During high–energy demand states, Cr and phospho-Cr levels are regulated to near equilibrium, providing a buffering capacity for ATP and ADP, allowing for the proper functioning of a numerous of cellular ATPases (48). Active inflammation represents a high-energy state accounted for by functions such as cell migration, proliferation, and the restitution of epithelial cells after insult. Under such conditions, energy expenditure at epithelial cell–cell junctions is tightly linked to the circumferential F-actin belt (36). Thus, it would appear that Cr:phospho-Cr ratios may serve as functional biomarkers of cellular energy demand that could be targetable in ways to promote tissue barrier function and epithelial wound healing in the mucosa.

Conclusions

Epithelial cells that line mucosal surface normally function in diverse and often harsh conditions. A major function of the epithelium is the provision of a barrier as a selectively permeable membrane that prevents the free mixing of luminal and serosal constituents. The stark differences in tissue O_2 tension between mucosal tissues (e.g., compare oxygenation profiles of lung and colon) and the potential shifts in energy requirements during injury have unveiled important lessons about tissue metabolism in health and disease. In particular, HIF-target pathways have revealed potential targets with the tissue barrier that could serve as templates for new therapies. A more precise understanding of the gene targets and functional components of the HIF pathway provides ample opportunities for the development of therapies directed at promoting mucosal wound healing. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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