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Review Article

Tissue metabolism and host-microbial interactions in the intestinal mucosa

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ABSTRACT

In recent years, studies in the gastrointestinal (GI) mucosa have taught us a number of important lessons related to tissue oxygenation and metabolism in health and disease. The highly vascularized mucosa lies immediately adjacent to an anaerobic lumen containing trillions of metabolically active microbes (i.e. the microbiome) that results in one of the more austere tissue microenvironments in the body. These studies have also implicated a prominent role for oxygen metabolism and hypoxia in inflammation, so called "inflammatory hypoxia", that results from the activation of multiple oxygen consuming enzymes. Inflammation-associated shifts in the composition of the microbiome and microbial-derived metabolites have revealed a prominent role for the transcription factor hypoxia-inducible factor (HIF) in the regulation of key target genes that promote inflammatory resolution. Analyses of these pathways have provided a multitude of opportunities for understanding basic mechanisms of both homeostasis and disease and have defined new targets for intervention. Here, we review recent advances in our understanding of metabolic influences on host-microbe interactions in the GI mucosa.

1. Introduction

Recent investigations of the metabolic demands placed on the mucosa during homeostasis and disease have provided important insights into the biochemical pathways employed during host-microbe interactions. At the center of these metabolic pathways is molecular oxygen utilization. The gastrointestinal (GI) tract, for instance, is characterized by a particularly unique oxygenation profile, experiencing regular intervals of profound blood flow fluctuations [1]. Even at baseline, epithelial cells lining the mucosa exist at a relatively low oxygen tension environment, herein described as 'physiologic hypoxia'. Countercurrent oxygen exchange mechanisms in the small intestine have revealed that oxygen from arterial blood supply diffuses to adjacent venules, along the crypt villus axis, resulting in graded hypoxia [2]. A steep oxygen gradient has also been documented in distal, colonic regions of the GI tract, spanning from the anaerobic lumen, across the epithelium to the richly vascularized sub-epithelial mucosa [3]. Given the high-energy requirement of the gut and the integral role of the epithelium in maintaining intestinal homeostasis, it is not surprising that these cells have evolved a number of mechanisms to cope with this austere metabolic environment [4]. Here, we will discuss how such metabolic shifts are regulated, particularly as they relate to host-microbe interactions.

2. Oxygen metabolism in healthy and inflamed tissues

The oxygenation profile of the healthy mucosa is an area of significant interest. A comparison of the lung and intestine, for example, provides a number of stark contrasts. Breathable air at sea level contains a partial O_2 pressure (pO_2) of ~145 mmHg (approximately 21% O_2). Measurements of the healthy lung alveolus have revealed a pO_2 of 100–110 mmHg [5]. Conversely, the most luminal aspect of the healthy colon exists at a pO_2 of less than 10 mmHg [3,6]. This difference is attributed primarily to the source of O_2, active local metabolism and the anatomy of blood flow [4].

Tissue oxygenation, particularly at low O_2, has been tracked using 2-nitroimidazole dyes, a class of compounds known to undergo intracellular metabolism dependent on the level of tissue oxygenation [7]. These dyes were originally developed to image the low O_2 environment of growing tumors [8] and have subsequently been used as tools to monitor levels of tissue oxygenation. Nitroimidazoles form adducts with thiol groups in proteins, peptides and amino acids where all atoms of the ring and side-chain of the 2-nitroimidazole are retained at $pO_2 < 10$ mmHg. Intestinal mucosal localization of these nitroimidazole dyes has revealed two striking observations. First, in the normal GI mucosa, particularly in the colon, "physiologic hypoxia" predominates [6]. Recent studies have shown that these low O_2 conditions are critical for the constitutive expression of certain innate immune factors found within the mucosa (e.g. human β defensin-1) [9]. Second,

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inflammatory lesions within the mucosa are profoundly hypoxic or even anoxic, similar to that seen in some tumors [4]. It is likely that there are multiple contributing factors, i.e. vasculitis, vasoconstriction, edema, increased O_2 consumption, predisposing the inflamed intestinal epithelia to decreased oxygen delivery and hypoxia [6]. These nitroimidazole compounds have shown significant clinical utility in tumor imaging and in the identification of stroke regions within the brain of patients [10]. As opposed to other imaging techniques, these molecules have the advantage that they image only viable tissue, are independent of oxygen radical accumulation and are not active in apoptotic/necrotic tissue [11].

Given the substantial shifts in metabolism and oxygen availability during inflammation, a number of studies have shown that stabilization of transcription factor hypoxia-inducible factor (HIF) in low oxygen environments triggers the expression of genes that are essential to epithelial barrier function [12–15]. Additionally, HIF is one of the central regulators of overall tissue metabolism [16] and has profound influences on the inflammatory response [4]. Its activity is dependent on stabilization of an O_2-dependent degradation (ODD) domain expressed on the α-subunit and subsequent nuclear translocation to form a functional complex with HIF-1β [17]. In normally oxygenated tissues, iron, alpha-ketoglutarate and O_2-dependent hydroxylation of two prolines (Pro564 and Pro402 of HIF-1α) within the ODD of the alpha subunit initiates the association with the von Hippel-Lindau tumor suppressor protein (pVHL) and rapid degradation via ubiquitin-E3 ligase proteasomal targeting [18,19].

Intestinal epithelial cells express both HIF-1α and HIF-2α [20] and genetic studies in mice indicate that these proteins have non-redundant roles [21]. It has been suggested that distinct transcriptional responses mediated by HIF-1α and HIF-2α may have evolved as particular adaptations to hypoxia. For example, the transcriptional responses that coordinate metabolic adaptation through glycolytic pathways are selective for the HIF-1α over the HIF-2α isoform [22]. Conversely, studies addressing selectivity of the two isoforms for increased perfusion and oxygen carrying capacity (e.g. erythropoietin induction) have indicated a more prominent role for HIF-2α [23]. Iron absorption through intestinal epithelial cells also appears to be selective for HIF-2α [24]. While this specificity for individual gene regulation is not well understood, some evidence has shown that binding of HIF-1α or HIF-2α to gene promoters is dependent on interactions with STAT3 and USF2, respectively [25].

The spectrum of basal oxygenation within individual tissues is immense. Given the steep oxygen gradient due to countercurrent blood flow, colonic epithelia exist at very low pO_2 [26]. These cells have proven to be remarkably resistant to hypoxia, where even very low levels of oxygenation allow these cells to function normally [27,28]. The importance of HIF to epithelial function was originally shown by microarray analysis of intestinal epithelial cells cultured in low O_2 conditions (pO_2 ~20 mmHg) [12]. These studies were subsequently validated in murine models of colitis [6,29–33] and in diseased human tissues [34–36]. Notably, the cluster of functional proteins regulated by HIF localizes prominently to the most luminal aspect of polarized epithelia and is composed of proteins important for increased mucin production, [37] molecules that modify mucin function [38], antimicrobial defense [9], barrier function [39], xenobiotic clearance [13] and nucleotide metabolism/signaling (by ecto-5'-nucleotidase and CD73) [14,15] (see Fig. 1). Molecular studies of these hypoxia-regulated pathway(s) have shown a dependence on HIF-mediated transcriptional responses. Original studies by Karhausen, et al.. generated mice expressing either mutant Hif-1α (causing constitutive repression of *Hif-1α*) or mutant von Hippel-Lindau (causing constitutive overexpression of HIF) targeted to the intestinal epithelial cells revealed a more severe colitic phenotype in which increased intestinal permeability was a prominent feature [6]. These findings were somewhat model-dependent, since epithelial HIF-based signaling has also been shown to promote inflammation in another study [33]. Further examination of these pathways revealed that active colonic inflammation, defined by the influx of large numbers of neutrophils, depleted local oxygen levels sufficient enough to stabilize intestinal epithelial cell HIF and imprinted a transcriptional phenotype that strongly reflected HIF stabilization [40]. This phenotype was dependent on neutrophil NADPH oxidase implicated epithelial HIF stabilization in goblet cell differentiation and the production of mucins and mucin-binding elements, which has been suggested by other studies in the colon [12,37]. Overall, these findings confirm that intestinal epithelial cells can adapt to hypoxia and that HIF may play a key role in such an adaptation.

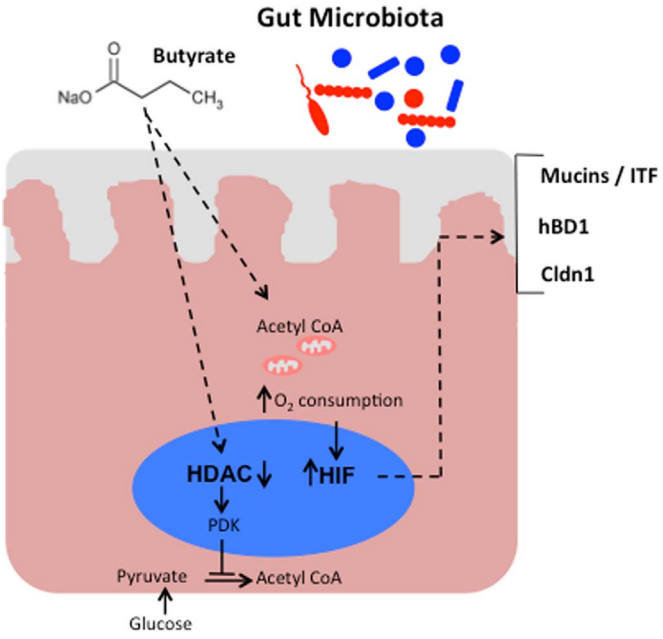

Fig. 1. *Preferential oxidation of butyrate by intestinal epithelium stabilizes HIF and contributes to barrier function.* Microbiota-derived butyrate is absorbed apically and acts to inhibit histone deacetylation. One consequence is increased expression of pyruvate dehydrogenase kinase (PDK), which inactivates pyruvate dehydrogenase. This precludes oxidative metabolism of pyruvate, allowing for the majority of acetyl CoA that is used for oxidative metabolism to be derived from β-oxidation of butyrate. Oxidative respiration leads to increased oxygen consumption, stabilization of HIF, and expression of HIF target genes important in barrier regulation (Muc3/ITF, hBD1, Cldn1).

3. Host-microbial metabolism and tissue hypoxia

The gastrointestinal tract of mammals is host to trillions of bacteria. This finely tuned host-microbe relationship exists on the surface of the intestinal mucosa, where microbes are essential for host health, but can also initiate and perpetuate disease [41]. These microbes, in addition to aiding in digestion, produce a number of vitamins and benefit the host through the local synthesis of short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate.

SCFAs are end products of bacterial fermentation, primarily derived from resistant starches, dietary fibers and undigested proteins [42]. Anaerobic bacteria in the colon, particularly members of the Phyla *Firmicutes* [43], produce butyrate through the conversion of microbial acetyl-CoA to the butyryl-CoA via β-oxidation of fatty acids. The final conversion from butyryl-CoA to butyrate is either catalyzed by butyryl-CoA: acetate CoA transferase or butyrate kinase. Due the presence of highly conserved regions these enzymes can be used for the identification of butyrate-producing bacterial communities in molecular analyses [44–46]. Amino acids can also serve as a substrate for SCFA production. Acetate can be produced through microbial metabolism of several amino acids, including glycine, alanine, threonine, glutamate, lysine and aspartate [42]. Propionate can be synthesized from alanine and threonine and butyrate from lysine and glutamate. Acetate and