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Hypoxia and inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) is a general term to describe inflammatory diseases of the gastrointestinal tract such as Crohn's disease and ulcerative colitis. IBD affects approximately 1 in 200 individuals and exerts a significant health and quality of life burden on patients. Surgical intervention can be curative in ulcerative colitis but there is currently no cure for Crohn's disease. Since this is the case, and the fact that patients are often diagnosed at a young age, IBD exerts a significant financial burden on the health care system, and society as a whole.

The underlying pathology of IBD is complex and involves a combination of genetic, environmental and microbial factors. Regardless of the underlying causes of the condition, this disease is universally characterized by disruption to the protective epithelial barrier separating the intestinal lumen above from the mucosal immune system below. Once this barrier becomes compromised a sequence of events ensues, that can occur in repetitive cycles to ensure long-term and serious damage to the gut.

The role of hypoxia and hypoxia-dependent signalling pathways are increasingly appreciated to play a role in the physiology and pathophysiology of the intestine. The intestinal epithelium normally exists in a state of physiological hypoxia, with additional tissue hypoxia a feature of active inflammatory disease. Furthermore, recent pre-clinical animal studies have clearly supported the rationale for pharmacologically manipulating the oxygen-sensitive hypoxia-inducible factor (HIF) pathway in models of IBD. Thus, this review will discuss the contribution of hypoxia sensitive pathways in the pathology of IBD. Finally we will discuss the emerging evidence for manipulation of hypoxia-sensitive pathways in the treatment of IBD.

Keywords: Hypoxia; Inflammatory bowel disease; Crohn's disease; Colitis; Hypoxia inducible factor; Prolyl hydroxylase inhibitors

1. Inflammatory bowel disease

Inflammatory bowel disease is an umbrella term encompassing Crohn's disease (CD) and ulcerative colitis (UC). Crohn's disease can affect any section of the gastrointestinal tract from the oesophagus to the anus while ulcerative colitis is predominantly restricted to the colon. As such it is a heterogenous disease with differing clinical phenotypes described by the Montreal and Paris classifications [1]. In particular, age of presentation with location and extent of the disease being key determinants of disease progression and prognosis [2].

Symptoms of IBD can range from mild to severe and include abdominal pain, diarrhoea, weight loss, fever and GI bleeding. UC is more prevalent than CD with the highest incidences in the northern Europe and North America. Incidence of UC in these regions is 9–20 cases per 100,000 person years and prevalence rates range from 156 to 291 cases per 100,000 people [3].

Several disease susceptibility loci are shared between CD, UC as well as other immune related disease (e.g. ankylosing spondylitis and psoriasis) [4]. Important disease susceptibility loci for IBD include NOD2, 3p21 (MST1) and the MHC [5]. Nucleotide-binding oligomerisation domain-containing protein 2 (NOD2) is an intracellular receptor involved in innate immune defence [6]. Macrophage stimulating 1 (MST1) regulates Foxp3 expression and consequently the expression and

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development of Tregs [7]. The Major Histocompatibility complex (MHC) is central to antigen presentation of peptides to T cells [8].

Genome wide association studies have provided powerful stratification of IBD patients, and identified a key genetic separation between ileal and colonic CD [5]. Importantly, the location of the disease appears to underpin much of the fundamental biology of IBD while the requirement for surgery is a marker of disease progression [5]. While there is significant evidence for a genetic component to IBD, the role of the environment is also of crucial importance with factors such as diet, smoking and the microbiota contributing. Interestingly, smoking has a paradoxically divergent effect on the two main disease types in IBD. Smoking is associated with a worse disease prognosis in CD, while smokers are less likely to develop UC than non-smokers [9]. The gut microbiota is increasingly appreciated to play a major role in IBD. Bacterial dysbiosis or imbalance is evident in IBD patients compared to healthy controls [10]. For example loss of Firmicutes (which includes anaerobic *Clostridia* (discussed below)) has been widely reported in UC patients [10]. Thus, loss of microbial richness and diversity is thought to disturb the balance that exists between commensal and pathogenic bacteria, which in turn elicits a pro-inflammatory response in genetically predisposed individuals. In summary IBD is a complex disease affecting an estimated 1–1.4 million people in the US [11,12]. The intricate interplay between genetic susceptibility, environment, and microbiome has led to limited success in treating this group of diseases. In this review we will discuss critical cells and pathways involved in IBD that are affected by hypoxia and hypoxia-induced transcription factors such as HIF.

2. HIF – hypoxia inducible factor

Metazoans have evolved the capacity to rapidly adapt to the situation of hypoxia. Hypoxia ensues when the cellular demand for oxygen in order to produce sufficient levels of ATP for physiologic function exceeds the supply. In order to deal with this relative shortage of oxygen, organisms have evolved a complex repertoire of transcriptional changes that are designed to reduce oxygen consumption and to promote additional oxygen delivery to a cell or tissue. The hypoxia-inducible factor (HIF) transcription factor is central to the regulation of this transcriptional response to decreased oxygen availability. HIF has been expertly reviewed elsewhere [13,14]. Briefly, HIF is comprised of an oxygen sensitive α-subunit (HIF-1α, HIF-2α, HIF-3α) and a constitutive co-activator HIF-1β. HIF-1α and HIF-2α can regulate distinct genes, common genes and can even have opposing effects on gene expression [15]. Under normoxic conditions HIF-α mRNA is constitutively expressed, but HIF protein is rapidly and efficiently degraded by the activity of a family of 2-oxyglutarate-dependent dioxygenases, the prolyl hydroxylases (PHDs). In the presence of sufficient levels of oxygen, the PHDs hydroxylate specific proline residues in the oxygen dependent degradation domain of HIF-α [16,17], targeting the protein for VHL-dependent ubiquitylation and proteasomal degradation [18]. Thus, oxygen exerts a repressive role on HIF-α protein stabilisation in normoxia. In hypoxia, oxygen is limiting, and the ability of the PHDs to hydroxylate HIF-α is compromised. HIF-α can then accumulate, translocate to the nucleus and activate gene expression with HIF-1β and p300. HIF is responsible for the up-regulation of over 200 genes involved in erythropoiesis, angiogenesis, intestinal barrier integrity, iron homoeostasis and glycolysis [19]. Recently, a number of pharmacological 'hypoxia-mimetics' have been developed to try and exploit the activation of the HIF pathway for therapeutic benefit. Many of these drugs to date are non-specific hydroxylase inhibitors structurally related to 2-oxyglutarate (discussed below).

3. Intestinal epithelial cells

Intestinal epithelial cells reside at the interface between the internal and external environments of the body, separating physiologically and ecologically distinct environments. In the small intestine, tall columnar epithelial cells line the surface of the intestine in order to provide an efficient means of absorbing digested nutrients across the epithelial barrier. The intestinal circulation is key for the efficient delivery of nutrients from the lumen to the blood [20]. In the colon, tall columnar epithelial cells are more frequently interspersed with mucous producing goblet cells, with the colon playing a more important role in the absorption of water and electrolytes. The epithelial barrier is efficiently sealed in the healthy gut by the presence of tight junctions, which securely block the passage of most molecules from crossing the epithelium paracellularly [21]. The epithelium is additionally coated in mucous-rich secretions that provide protection against physical and chemical damage. Finally, the colon epithelium in particular has an intimate relationship with commensal bacteria that have the ability to modify and shape intestinal physiology in health and in disease. Several of these characteristics of the intestinal epithelium contribute to a state of 'physiologic hypoxia' which influences cellular function [22]. Fig. 1 displays the normal physiology within the intestine (including oxygen gradients) and the function of key factors discussed throughout this review.

4. Epithelial microcirculation

The intestine receives oxygenated blood from the celiac, superior and inferior mesenteric arteries which amount to 20–25% of cardiac output in the unfed state but increases dramatically in response to a meal. Nutrients such as glucose, peptides and lipids can increase total intestinal blood flow by >200%. However, the perivascular PO_2 at the villus tip can decrease by approximately half under the same conditions [23]. The intestinal mucosa is densely vascularised, receiving a disproportionate amount of blood flow (60% in the villi of the small intestine, 40% in the crypts of the colon) [20]. The villus is perfused by afferent and efferent vessels which can form a hair-pin capillary loop at the tip of the villus before returning blood to a venule. Given the size of the villus