PATHOBIOLOGY IN FOCUS

Hydroxylases as therapeutic targets in inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a common and debilitating clinical disorder comprising ulcerative colitis and Crohn's disease. IBD occurs when inappropriate immunological activity in the intestinal mucosa results in epithelial barrier dysfunction leading to exposure of the mucosal immune system to luminal antigenic material. This in turn results in the cycles of inflammation and further barrier dysfunction which underlie disease progression. Although significant therapeutic advances have been made over the last decade, current immunosuppressive and anti-inflammatory treatments for IBD have significant limitations due to lack of treatment response in some patients and adverse effects, including increased risk of infection and malignancy. Recent studies using experimental models of IBD have identified that intracellular hydroxylases, a group of enzymes responsible for oxygen sensing and activation of adaptive transcriptional responses to hypoxia may represent a new class of therapeutic targets in IBD. Hydroxylase inhibitors are effective in ameliorating symptoms of colitis at least in part through the promotion of intestinal epithelial barrier function. The mechanism of this protection is due to activation of hypoxia-sensitive transcription factors, including the hypoxia-inducible factor (HIF) and nuclear factor kappa-B (NF- κ B), which activate specific epithelial barrier-protective transcriptional programs. In this review, the mechanism(s) of action and the therapeutic potential of small molecule hydroxylase inhibitors for the treatment of IBD will be discussed.

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Inflammatory Bowel Disease (IBD)

IBD is an umbrella term used to describe a heterogeneous family of conditions, including ulcerative colitis and Crohn's disease. Ulcerative colitis, which is restricted to the colonic mucosa was first described in the mid 19th century and its worldwide prevalence has been steadily increasing since the middle of the 20th century.^{1,2} Crohn's disease, first described in 1932, is often transmural and can affect any portion of the gastro-intestinal tract with inflammation seen most commonly in the ileum and/or colon.^{3,4} Peak incidence of IBD occurs in early adulthood and its prevalence results in high individual and societal costs.⁵ Current incidence and prevalence rates of IBD are 8–14 per 100 000 persons and 120–200 per 100 000 persons, respectively.⁶ Furthermore, there has been a recently described increase in the incidence of IBD in developed countries.⁷ Experimental, genetic and clinical evidence indicates that IBD 'results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host'.⁴ However, this susceptibility has been associated with mutations at multiple chromosomal loci making the

relationship between genetics and IBD-susceptibility complex.^{4,8–10} To date, the greatest documented risk of IBD susceptibility occurs in individuals with a homozygous polymorphism in the NOD2 gene, which encodes a bacterial peptidoglycan receptor associated with activity of the nuclear factor kappa-B (NF- κ B) transcriptional pathway.¹¹ Other genes reported to affect susceptibility to IBD, include CARD9, IL23R and MUC19 which encode proteins associated with innate immune signaling, cytokine signaling and mucin production, respectively.^{10,12–14} CARD9 is involved in the regulation of hypoxia-sensitive pathways.¹⁵ While the underlying cause of IBD is complex and multifactorial involving the epithelial barrier, innate immune defenses and the intestinal microbiome, ultimately, it is a loss of intestinal epithelial barrier function with subsequent uncontrolled exposure of the mucosal immune system to the luminal microbiome with resultant amplification of mucosal inflammation, which allows the disease to progress.⁴ The symptoms of IBD can range from mild to severe and include abdominal pain, intestinal bleeding, weight loss, fever and diarrhea.¹⁶

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The therapeutic approach to IBD typically involves multi-disciplinary management, including medical management which employs the use of broad spectrum immunosuppressants to reduce mucosal inflammation.¹⁶ Traditionally, a step-up approach to the therapy of IBD has been used, which involves the use of 5-aminosalicylate-based compounds (eg mesalazine) as a first line approach followed by corticosteroids (eg prednisolone). Immunomodulators such as azathioprine, 6-mercaptopurine and methotrexate have been increasingly used as maintenance treatment (typically as a corticosteroid sparing strategy). The treatment of IBD has more recently been revolutionized by the development of targeted molecular agents with the use of therapeutic antibodies against TNF α (anti-TNF) to suppress mucosal inflammation.^{17,18} The step-up approach for the treatment of IBD has been challenged with the emergence of such 'biologic' agents and a 'top-down' therapeutic paradigm has been proposed, which advocates early aggressive use of therapeutic antibodies as a first line of treatment.¹⁹ However, early use of therapeutic antibodies, typically combined with immunomodulators to promote mucosal healing may also lead to increased risk of toxicity, infections and the rare risk of hepatosplenic T-cell lymphoma in young men.^{20–22}

While current therapeutic approaches reduce disease activity and improve symptoms, the degree to which sustained mucosal healing has been achieved has been disappointing in randomized-controlled trials in both ulcerative colitis and Crohn's disease.²³ A number of key limitations still exist with current therapeutic options. These include non-responsiveness of patients to 5-ASA, side-effects associated with high-level and prolonged corticosteroid usage and non-response/loss of response, high cost and increased risk of infection and malignancy with the use of biologic agents, especially when combined with immunomodulators.^{17,24} Indeed, despite therapeutic developments, the numbers of patients ultimately requiring surgical intervention remains high.²⁵ With this in mind, there is a significant need for new approaches to the treatment of IBD, which may be used in addition to or in combination with existing approaches to alleviate symptoms in patients.

Tissue Hypoxia in IBD

Physiologic perfusion of the gastro-intestinal tract fluctuates according to whether or not food has been recently consumed and increased tissue perfusion is required to facilitate the necessary oxygen delivery and nutrient absorption.²⁶ In the normal state, the epithelium of the richly perfused mucosal bed of the intestine is in direct contact with the anoxic lumen of the gut which houses the intestinal microbiome. As a result of this juxtaposition, there normally exists a uniquely steep trans-mucosal oxygen gradient ranging from relatively high levels of oxygen in vascular bed to the anoxic conditions at the luminal-epithelial interface.^{26–28} Whether oxygen consumption by the intestinal microbiome contributes to this steep physiologic oxygen gradient currently

remains unknown, however, there is evidence for bacteria-associated oxygen consumption and activation of hypoxia-sensitive pathways in the context of pathogen infection.²⁹

When inflamed, the microenvironment of the intestinal mucosa demonstrates a high degree of hypoxia, likely reflective of vascular disruption due to chronic inflammation in combination with increased oxygen demand by highly metabolically active infiltrating immune and inflamed resident cells.^{28,30} Furthermore, it is likely that the high oxygen requirements of infiltrating neutrophils, used to generate reactive oxygen species in the oxidative burst, likely contributes to a large increase in oxygen consumption. The existence of mucosal hypoxia during chronic inflammation is supported by studies demonstrating elevated levels of hypoxia-dependent proteins such as hypoxia-inducible factor (HIF) (see below) in biopsies taken from regions of active IBD.³¹ Therefore, significant evidence now supports the existence of prominent areas of focal hypoxia in inflamed regions of the colon in active IBD. Interestingly, the coexistence of hypoxia and inflammation is not restricted to IBD as other chronically inflamed tissues such as arthritic joints also demonstrate tissue hypoxia.^{32,33}

Hypoxia-Sensitive Transcriptional Pathways

Hypoxia arises when the cellular demand for oxygen exceeds the vascular supply and is associated with a wide range of physiologic and pathophysiologic conditions where either oxygen delivery is decreased (eg fetal development, ischemia, cancer) or where oxygen demand is increased (eg exercise, inflammation). Because the energy provided by the chemical reduction of molecular oxygen by the mitochondrial electron transport chain is the primary source of metabolic energy used to fuel all biochemical reactions, it is perhaps not surprising that eukaryotes have evolved the capacity to deal with hypoxia at the transcriptional level with the induction of a range of hypoxia-sensitive genes.^{34,35} Primary among the hypoxia responsive transcriptional regulators is the HIF a dimeric transcription factor, the abundance of which is regulated by oxygen availability across the physiologic range in cells, and which controls the expression of over 300 genes responsible for regaining oxygen homeostasis in hypoxic tissues. HIF-dependent genes include factors affecting angiogenesis, metabolism, vascular tone and cell survival. As well as having a central role in the adaptive response to hypoxia, HIF has more recently been appreciated as a key regulator of innate immunity and inflammation.^{28,36,37} Indeed, conditional knockout of the HIF-1 α isoform has revealed a key role for HIF in mediating functional immune responses in macrophages, dendritic cells, T-cells and neutrophils.^{38–42} The expression of hypoxia-inducible transcription factors such as HIF in mucosal tissue has been demonstrated to be increased in diseases of the GI tract including celiac disease⁴³ and IBD.³¹ Although most studies support a protective role for HIF activation in inflammatory

conditions of the gut,^{27,44,45} its activation has also been proposed to be detrimental.⁴⁶

The cellular transcriptional response to hypoxia is not restricted to regulation by HIF and multiple other transcriptional pathways demonstrate hypoxic sensitivity.⁴⁷ Principal among the HIF-independent transcription factors, which display hypoxic sensitivity is NF- κ B, a master regulator of innate immune and inflammatory gene expression.^{48,49} NF- κ B -dependent genes include cytokines, adhesion molecules and regulators of cellular apoptosis.^{50,51} Indeed, NF- κ B signaling in intestinal epithelial cells has been shown to be protective against the development of colitis.⁵² However, while hypoxia represents a strong and primary stimulus for HIF, it is a more moderate stimulus for NF- κ B signaling, which may favor the expression of an antiapoptotic rather than proinflammatory pattern of NF- κ B dependent gene expression. Thus, in response to hypoxic stress, cells are capable of switching on protective transcriptional responses, which are mediated by hypoxia-sensitive transcription factors such as HIF and NF- κ B.

Hydroxylases as Intracellular Oxygen Sensors

The mechanism underpinning the hypoxic-sensitivity of HIF was discovered in 2001.^{53,54} This elegant yet surprisingly simple and highly conserved mechanism has recently been expertly reviewed.^{55,56} In brief, under conditions where mitochondrial oxygen demand is satisfied and 'spare' non-mitochondrial oxygen is available, a family of three prolyl hydroxylases (PHD1–3) and a single asparagine hydroxylase (FIH) are active and are responsible for the hydroxylation-dependent targeting of the HIF α subunit for ubiquitylation by the von Hippel Lindau E3 ligase complex leading to proteasomal degradation. Under conditions of hypoxia, where oxygen availability is limiting, the HIF hydroxylases are inhibited allowing the stabilization of a transcriptionally active HIF α / β dimer and the subsequent expression of HIF-dependent genes. Subsequent studies revealed that the mechanism by which hypoxia leads to activation of NF- κ B is also largely dependent upon altered HIF-hydroxylase activity.^{57–59}

Hydroxylase Inhibitors in Models of IBD

As outlined above, hydroxylases are key oxygen sensors, which confer hypoxic sensitivity to transcriptional regulators including HIF and NF- κ B, both of which are protective against colitis when activated in intestinal epithelial cells. As HIF and NF- κ B are key regulators of both immune and inflammatory processes^{35,37} it was initially proposed that pharmacologic hydroxylase inhibition, leading to enhanced HIF and NF- κ B activity may be of therapeutic benefit in chronic inflammatory conditions such as IBD. Indeed, several studies provided evidence that pharmacologic hydroxylase inhibition with compounds such as DMOG or FG-4497 is protective in murine models of colitis.^{60,61} While these initial studies utilized chemical models of acute colitis, subsequent

Figure 1 Protective mechanisms of hydroxylase inhibitors in IBD. Pharmacologic hydroxylase inhibition protects against colitis through two principal mechanisms; reduced inflammatory activity and enhanced epithelial barrier function. (a) Increased basal NF- κ B activity in intestinal epithelial cells leads to decreased apoptosis resulting in enhanced epithelial barrier function. (b) Increased HIF activity leads to the activation of a program of barrier protective genes in intestinal epithelial cells. (c) Inhibition of PHD3 in neutrophils leads to enhanced neutrophil apoptosis and decreased inflammatory activity.

studies using genetic-, ischemic- and toxin-based acute and chronic models support a protective role for hydroxylase inhibition in colitis.^{44,45,62,63} More recently, the mechanisms underpinning the protective effects of hydroxylase inhibitors have become clearer (Figure 1). These mechanisms will be described below.

PROTECTIVE MECHANISMS OF HYDROXYLASE INHIBITORS IN IBD

Inhibition of Intestinal Epithelial Cell Apoptosis

One mechanism which may contribute to the protective effects of pharmacologic hydroxylase inhibition in colitis is through the promotion of intestinal barrier function through inhibition of epithelial cell apoptosis. Intestinal epithelial cells have a relatively short lifespan lasting 48–72 h *in vivo* during which they have an important role in epithelial barrier function as they migrate up the intestinal crypt and villus before undergoing apoptosis and being shed into the intestinal lumen. Consequently, the rate of intestinal epithelial cell apoptosis is central to the maintenance of an intact intestinal barrier.⁶⁴ The rate of intestinal epithelial cell apoptosis is increased in IBD leading to a loss of intestinal barrier function with subsequent exposure of the mucosal immune system to the microbiota.⁶⁵ Initial studies into the protective effects of pharmacologic hydroxylase inhibition revealed an antiapoptotic role for the pan-hydroxylase inhibitor DMOG in DSS colitis.⁶⁰ This was associated with elevated levels of the antiapoptotic protein cIAP-2 in DMOG-treated mice compared with controls when treated with DSS. Further investigations implicated the PHD1 isoform as the

key hydroxylase isoform regulating intestinal epithelial cell apoptosis.⁶⁶ In this study, mice homozygous deficient for PHD1 were protected in a model of DSS-induced colitis while PHD2-deficient heterozygotes and PHD3-deficient homozygotes were equally as susceptible as wild-type controls.⁶⁶ In summary, inhibition of intestinal epithelial cell apoptosis with a resultant increase in intestinal barrier function is a key mechanism by which pharmacologic hydroxylase inhibition is protective in experimental models of colitis.

Enhanced Expression of Barrier Protective Genes

Recent work has identified a 'barrier protective' transcriptional signature in intestinal epithelial cells exposed to hypoxia or pharmacologic hydroxylase inhibition. The primary regulator of this transcriptional signature is HIF-1 α . Initially it was demonstrated that conditional knockout of HIF-1 α in mouse intestinal epithelial cells results in enhanced susceptibility to the development of colitis.²⁷ Further studies demonstrated that among the HIF-dependent epithelial genes involved in the resolution of inflammatory events were CD55 which is involved in the clearance of neutrophils,⁶⁷ ecto-5' nucleotidase and the A2B receptor, which promote enhanced adenosine signaling contributing to the resolution of mucosal inflammation,^{68,69} MUC-3 and intestinal trefoil factor which lead to the formation of a protective mucous layer,^{70,71} P-glycoprotein which is involved in intestinal epithelial cell homeostasis and host-bacteria interactions.^{28,30,72} Therefore, a third mechanism whereby pharmacologic hydroxylase inhibition may provide protection in murine models of colitis is through enhancing the expression of a battery of epithelial-specific barrier protective genes via the HIF pathway.

Promotion of Neutrophil Apoptosis

A third mechanism whereby pharmacologic hydroxylase inhibition may have anti-inflammatory effects in colitis is via the regulation of neutrophil apoptosis.⁷³ Neutrophil infiltration and the formation of colonic crypt abscesses is a hallmark of IBD and the regulation of neutrophil lifespan is consequently a key event in determining the extent and duration of inflammation. Hypoxia-dependent pathways have been previously demonstrated to have an important role in the regulation of neutrophil apoptosis. PHD3 loss results in decreased-neutrophil survival under hypoxic conditions.⁷³ Furthermore, in mice which are homozygous deficient in PHD3, there is a reduction in neutrophilic inflammation in DSS-induced colitis.⁷³ Therefore, enhanced neutrophil apoptosis is another mechanism by which pharmacologic hydroxylase inhibition may be protective in murine models of colitis. While HIF-1 α has been shown to be important in regulating the immunological function of other immune cells such as macrophages, T-cells and dendritic cells, how this contributes to the

anti-inflammatory effects of hydroxylase inhibitors in IBD currently remains unknown.

Potential Risks Associated with the Use of Hydroxylase Inhibitors

Because hydroxylase inhibition leads to the activation of HIF, NF- κ B and likely other transcriptional pathways, a number of possible risks may be associated with their use. Firstly, a possible unwanted side effect of systemic hydroxylase inhibitor administration is elevated Epo levels with associated erythropoiesis. While this may be desirable in the treatment of anemic patients, it may increase the risk of cardiovascular events in non-anemic patients due to overstimulation of erythropoiesis. Furthermore, because HIF regulates the expression of glycolytic enzymes and components of the mitochondrial electron transport chain, it is possible that systemic hydroxylase inhibition could impact upon metabolic processes, however the *in vivo* implications for this currently remain unknown. Secondly, at least theoretically, there could be increased risk of the promotion of growth of pre-existing tumors through the promotion of HIF-dependent angiogenesis. Currently, little data exists relating to the real possibility of such risks in patients. However, in the context of treating IBD, an attractive therapeutic option would be the directed delivery of hydroxylase inhibitors to inflamed regions of the intestine over short therapeutic windows using formulated preparations to enhance drug delivery to the site of inflammation and minimize systemic exposure.

SUMMARY

In summary, metazoan cells poses an evolutionarily conserved oxygen sensing pathway, which is governed by a family of oxygen dependent hydroxylases that control the activity of transcription factors such as HIF and NF- κ B. A number of recently published studies support the possible use of hydroxylase inhibitors in the treatment of IBD. Furthermore, targeted administration of such agents specifically to inflamed regions of the intestine over a short therapeutic timeframe to promote epithelial restitution would reduce the risk of any potential side effects and decrease the pharmaceutical burden imposed.

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DISCLOSURE/CONFLICT OF INTEREST

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1. Wilks S. Morbid appearances in the intestines of Miss Banks. *Med Times Gazette* 1859;2:264–265.
2. Danese S, Fiocchi C. Ulcerative colitis. *N Engl J Med* 2011;365:1713–1725.
3. Crohn BB, Ginzburg L, Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. 1932. *Mt Sinai J Med* 2000;67:263–268.

4. Abraham C, Cho J. Inflammatory bowel disease. *N Engl J Med* 2009;361:2066–2078.
5. Latella G, Papi C. Crucial steps in the natural history of inflammatory bowel disease. *World J Gastroenterol* 2012;18:3790–3799.
6. Cosnes J, Gower-Rousseau C, Seksik P, *et al*. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* 2011;140:1785–1794.
7. Molodecky NA, Soon IS, Rabi DM, *et al*. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012;142:46–54 e42, quiz e30.
8. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. *Gastroenterology* 2011;140:1704–1712.
9. Kenny EE, Pe'er I, Karban A, *et al*. A genome-wide scan of Ashkenazi Jewish Crohn's disease suggests novel susceptibility loci. *PLoS Genet* 2012;8:e1002559.
10. Rivas MA, Beaudoin M, Gardet A, *et al*. Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. *Nat Genet* 2011;43:1066–1073.
11. Hugot JP, Zaccaria I, Cavanaugh J, *et al*. Prevalence of CARD15/NOD2 mutations in Caucasian healthy people. *Am J Gastroenterol* 2007;102:1259–1267.
12. Ruland J. CARD9 signaling in the innate immune response. *Ann N Y Acad Sci* 2008;1143:35–44.
13. Zhu L, Lee P, Yu D, *et al*. Cloning and characterization of human MUC19 gene. *Am J Respir Cell Mol Biol* 2011;45:348–358.
14. Abraham C, Cho J. Interleukin-23/Th17 pathways and inflammatory bowel disease. *Inflamm Bowel Dis* 2009;15:1090–1100.
15. Yang H, Minamishima YA, Yan Q, *et al*. pVHL acts as an adaptor to promote the inhibitory phosphorylation of the NF-kappaB agonist Card9 by CK2. *Mol Cell* 2007;28:15–27.
16. Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002;347:417–429.
17. Blonski W, Buchner AM, Lichtenstein GR. Inflammatory bowel disease therapy: current state-of-the-art. *Curr Opin Gastroenterol* 2011;27:346–357.
18. Perrier C, Rutgeerts P. Cytokine blockade in inflammatory bowel diseases. *Immunotherapy* 2011;3:1341–1352.
19. Devlin SM, Panaccione R. Evolving inflammatory bowel disease treatment paradigms: top-down *versus* step-up. *Gastroenterol Clin North Am* 2009;38:577–594.
20. Bongartz T, Sutton AJ, Sweeting MJ, *et al*. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* 2006;295:2275–2285.
21. Lichtenstein GR, Feagan BG, Cohen RD, *et al*. Serious infection and mortality in patients with Crohn's disease: more than 5 years of follow-up in the TREAT registry. *Am J Gastroenterol* 2012;107:1409–1422.
22. Kotlyar DS, Osterman MT, Diamond RH, *et al*. A systematic review of factors that contribute to hepatosplenic T-cell lymphoma in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2011;9:36–41 e1.
23. Neurath MF, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. *Gut* 2012;61:1619–1635.
24. Cohen BL, Torres J, Colombel JF. Immunosuppression in inflammatory bowel disease: how much is too much? *Curr Opin Gastroenterol* 2012;28:341–348.
25. Cosnes J, Nion-Larmurier I, Beaugerie L, *et al*. Impact of the increasing use of immunosuppressants in Crohn's disease on the need for intestinal surgery. *Gut* 2005;54:237–241.
26. Glover LE, Colgan SP. Hypoxia and metabolic factors that influence inflammatory bowel disease pathogenesis. *Gastroenterology* 2011;140:1748–1755.
27. Karhausen J, Furuta GT, Tomaszewski JE, *et al*. Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis. *J Clin Invest* 2004;114:1098–1106.
28. Colgan SP, Taylor CT. Hypoxia: an alarm signal during intestinal inflammation. *Nat Rev Gastroenterol Hepatol* 2010;7:281–287.
29. Werth N, Beerlage C, Rosenberger C, *et al*. Activation of hypoxia inducible factor 1 is a general phenomenon in infections with human pathogens. *PLoS One* 2010;5:e11576.
30. Taylor CT, Colgan SP. Hypoxia and gastrointestinal disease. *J Mol Med (Berl)* 2007;85:1295–1300.
31. Giatromanolaki A, Sivridis E, Maltezos E, *et al*. Hypoxia inducible factor 1alpha and 2alpha overexpression in inflammatory bowel disease. *J Clin Pathol* 2003;56:209–213.
32. Kennedy A, Ng CT, Biniecka M, *et al*. Angiogenesis and blood vessel stability in inflammatory arthritis. *Arthritis Rheum* 2010;62:711–721.
33. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. *N Engl J Med* 2011;364:656–665.
34. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell* 2012;148:399–408.
35. Taylor CT. Mitochondria and cellular oxygen sensing in the HIF pathway. *Biochem J* 2008;409:19–26.
36. Zinkernagel AS, Johnson RS, Nizet V. Hypoxia inducible factor (HIF) function in innate immunity and infection. *J Mol Med (Berl)* 2007;85:1339–1346.
37. Nizet V, Johnson RS. Interdependence of hypoxic and innate immune responses. *Nat Rev Immunol* 2009;9:609–617.
38. Cramer T, Yamanishi Y, Clausen BE, *et al*. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* 2003;112:645–657.
39. Walmsley SR, Print C, Farahi N, *et al*. Hypoxia-induced neutrophil survival is mediated by HIF-1alpha-dependent NF-kappaB activity. *J Exp Med* 2005;201:105–115.
40. Dang EV, Barbi J, Yang HY, *et al*. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* 2011;146:772–784.
41. Jantsch J, Chakravorty D, Turza N, *et al*. Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. *J Immunol* 2008;180:4697–4705.
42. Naldini A, Morena E, Pucci A, *et al*. Hypoxia affects dendritic cell survival: role of the hypoxia-inducible factor-1alpha and lipopolysaccharide. *J Cell Physiol* 2012;227:587–595.
43. Vannay A, Sziksz E, Prokai A, *et al*. Increased expression of hypoxia-inducible factor 1alpha in coeliac disease. *Pediatr Res* 2010;68:118–122.
44. Hirota SA, Fines K, Ng J, *et al*. Hypoxia-inducible factor signaling provides protection in Clostridium difficile-induced intestinal injury. *Gastroenterology* 2010;139:259–69 e3.
45. Hart ML, Grenz A, Gorzolla IC, *et al*. Hypoxia-inducible factor-1alpha-dependent protection from intestinal ischemia/reperfusion injury involves ecto-5'-nucleotidase (CD73) and the A2B adenosine receptor. *J Immunol* 2011;186:4367–4374.
46. Shah YM, Ito S, Morimura K, *et al*. Hypoxia-inducible factor augments experimental colitis through an MIF-dependent inflammatory signaling cascade. *Gastroenterology* 2008;134:2036–2048, 48 e1–3.
47. Cummins EP, Taylor CT. Hypoxia-responsive transcription factors. *Pflugers Arch* 2005;450:363–371.
48. Eltzschig H, Carmeliet P. Hypoxia and inflammation. *N Engl J Med*, 364:656–665.
49. Taylor CT, Cummins EP. The role of NF-kappaB in hypoxia-induced gene expression. *Ann N Y Acad Sci* 2009;1177:178–184.
50. Luo JL, Kamata H, Karin M. The anti-death machinery in IKK/NF-kappaB signaling. *J Clin Immunol* 2005;25:541–550.
51. Hayden MS, NF-kappaB GhoshS. The first quarter-century: remarkable progress and outstanding questions. *Genes Dev* 2012;26:203–234.
52. Greten FR, Eckmann L, Greten TF, *et al*. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 2004;118:285–296.
53. Jaakkola P, Mole DR, Tian YM, *et al*. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* 2001;292:468–472.
54. Ivan M, Kondo K, Yang H, *et al*. HIF1alpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* 2001;292:464–468.
55. Kaelin Jr WG, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* 2008;30:393–402.
56. Greer SN, Metcalf JL, Wang Y, *et al*. The updated biology of hypoxia-inducible factor. *EMBO J* 2012;31:2448–2460.
57. Cummins EP, Berra E, Comerford KM, *et al*. Prolyl hydroxylase-1 negatively regulates IkkappaB kinase-beta, giving insight into hypoxia-induced NFkappaB activity. *Proc Natl Acad Sci USA*. 2006;103:18154–18159.
58. Takeda Y, Costa S, Delamarre E, *et al*. Macrophage skewing by Phd2 haploinsufficiency prevents ischaemia by inducing arteriogenesis. *Nature* 2011;479:122–126.

59. Chan DA, Kawahara TL, Sutphin PD, *et al*. Tumor vasculature is regulated by PHD2-mediated angiogenesis and bone marrow-derived cell recruitment. *Cancer Cell* 2009;15:527–538.
60. Cummins EP, Seeballuck F, Keely SJ, *et al*. The hydroxylase inhibitor dimethyloxallylglycine is protective in a murine model of colitis. *Gastroenterology* 2008;134:156–165.
61. Robinson A, Keely S, Karhausen J, *et al*. Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition. *Gastroenterology* 2008;134:145–155.
62. Hindryckx P, De Vos M, Jacques P, *et al*. Hydroxylase inhibition abrogates TNF- α -induced intestinal epithelial damage by hypoxia-inducible factor-1-dependent repression of FADD. *J Immunol* 2010;185:6306–6316.
63. Hindryckx P, Staelens S, Devisscher L, *et al*. Longitudinal quantification of inflammation in the murine dextran sodium sulfate-induced colitis model using muPET/CT. *Inflamm Bowel Dis* 2010;17:2058–2064.
64. Edelblum KL, Yan F, Yamaoka T, *et al*. Regulation of apoptosis during homeostasis and disease in the intestinal epithelium. *Inflamm Bowel Dis* 2006;12:413–424.
65. Salim SY, Soderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflamm Bowel Dis* 2011;17:362–381.
66. Tambuwala MM, Cummins EP, Lenihan CR, *et al*. Loss of prolyl hydroxylase-1 protects against colitis through reduced epithelial cell apoptosis and increased barrier function. *Gastroenterology* 2010;139:2093–2101.
67. Louis NA, Hamilton KE, Kong T, *et al*. HIF-dependent induction of apical CD55 coordinates epithelial clearance of neutrophils. *FASEB J* 2005;19:950–959.
68. Synnestvedt K, Furuta GT, Comerford KM, *et al*. Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *J Clin Invest* 2002;110:993–1002.
69. Kong T, Westerman KA, Faigle M, *et al*. HIF-dependent induction of adenosine A2B receptor in hypoxia. *FASEB J* 2006;20:2242–2250.
70. Louis NA, Hamilton KE, Canny G, *et al*. Selective induction of mucin-3 by hypoxia in intestinal epithelia. *J Cell Biochem* 2006;99:1616–1627.
71. Furuta GT, Turner JR, Taylor CT, *et al*. Hypoxia-inducible factor 1-dependent induction of intestinal trefoil factor protects barrier function during hypoxia. *J Exp Med* 2001;193:1027–1034.
72. Comerford KM, Wallace TJ, Karhausen J, *et al*. Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. *Cancer Res* 2002;62:3387–3394.
73. Walmsley SR, Chilvers ER, Thompson AA, *et al*. Prolyl hydroxylase 3 (PHD3) is essential for hypoxic regulation of neutrophilic inflammation in humans and mice. *J Clin Invest* 2011;121:1053–1063.