Targeting Hypoxia-Inducible Factor-1 (HIF-1) Signaling in Therapeutics: Implications for the Treatment of Inflammatory Bowel Disease

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Abstract: In response to hypoxia, adaptive hypoxia-inducible factor-1 (HIF-1) signaling events are activated to increase oxygen transport, anaerobic energy production and protective pathways to minimize ischemic tissue damage. Although the activation and subsequent induction of gene transcription by HIF-1 is normally associated with hypoxia, it is now established that HIF-1 signaling can be triggered under inflammatory conditions. HIF-1 has been implicated in a number of inflammatory diseases including rheumatoid arthritis, allergic asthma, psoriasis and inflammatory bowel disease (IBD). In the gastrointestinal tract, HIF-1-regulated gene products, such as vascular endothelial growth factor, intestinal trefoil factor and CD73, have been shown to provide protection in animal models of intestinal inflammation. Given the importance of HIF-1 signaling in the aforementioned diseases, there exists considerable interest in the development of methods to modulate HIF-1 expression as well as down-stream signaling events. This review examines HIF-1 signaling with a special focus on the gastrointestinal tract. The patents pertaining to the modulation of HIF-1 signaling are summarized, and their relevance to the treatment of inflammatory bowel disease is discussed.

Keywords: Hypoxia-inducible factor, inflammatory bowel disease, inflammation, colitis, Crohn's disease, prolyl hydroxylase, intestinal trefoil factor, vascular endothelial growth factor, CD73.

INTRODUCTION

Numerous physiological and pathophysiological conditions can cause an imbalance in oxygen supply and demand. In response to a reduction in oxygen tension, tissues initiate signaling events that trigger the up-regulation of genes to allow for short- and long-term adaptation to hypoxia. Many of these events are initiated following the activation of the hypoxia-inducible factor (HIF) family of transcription factors. The activation of HIF can trigger the transcription of genes that can increase blood flow to stressed tissue and optimize oxygen delivery, through vasodilation, angiogenesis and increase in the blood's oxygen carrying capacity [1-4]. Additionally, HIF activation can drive the expression of molecules involved in cellular metabolism and energy production [5-7]. Given the propensity of HIF signaling to enhance tissue survival under hypoxic conditions, much of the interest in its regulation surrounds its role in tumour growth and survival (reviewed in [8,9]). This is reflected in the vast number of patents involving methods aimed at inhibiting HIF signaling with the hope of preventing angiogenesis and tumour survival. However, recent reports suggest a very dynamic role for HIF-1 signaling in the regulation of inflammation and maintenance of barrier function within the gastrointestinal tract [10-14]. In some instances, patents have been filed that directly focus on therapies that inhibit HIF signaling in an attempt to dampen HIF-mediated inflammation in conditions such as arthritis and inflammatory eye diseases. This review will discuss HIF-1 signaling, its activation under inflammatory conditions and its protective effects with a specific focus on the gastrointestinal tract. We will then summarize the patents concerning HIF-1 regulation for the treatment of inflammatory conditions with a specific focus on gastrointestinal disease states, including: inflammatory bowel disease (IBD), infectious colitis, intestinal ischemia and colorectal neoplasia. Lastly, we will conclude with a summary of potential targets for HIF-1 regulation and an analysis of the potential pitfalls for the development of therapeutics that target systemic HIF-1 signaling.

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HIF REGULATION AND SIGNALING

The HIFs are a family of heterodimeric transcription factors composed of an oxygen-sensitive α-subunit and a constitutively expressed β-subunit. The α-subunit has three isoforms all of which contain basic helix-loop-helix (bHLH) and Per-Arnt-Sim (PAS) domains, transactivation domains as well as an oxygen-dependent degradation (ODD) domain that provides oxygen-sensitivity. HIF-1α and HIF-2α display some non-redundant functions with both isoforms being able to heterodimerize with HIF-1β to form functional HIF-1 transcription factor complexes. HIF-3α lacks a C-terminal transactivation domain and may act to inhibit HIF-1α and HIF-2α signaling through sequestration of β-subunit. The β-subunit, also termed the aryl hydrocarbon receptor nuclear translocator (ARNT), has a number of splice variants, but unlike the α-subunit, its stability is insensitive to reduced oxygen tension.

The overall cellular activity of HIF-1 is dependent upon the intracellular levels of the HIF α isoforms. The HIF-1α subunit has been most studied, and its regulation under changing oxygen conditions has been well characterized. HIF-1α is subject to constitutive transcription and translation; however, under normoxic conditions the HIF-1α

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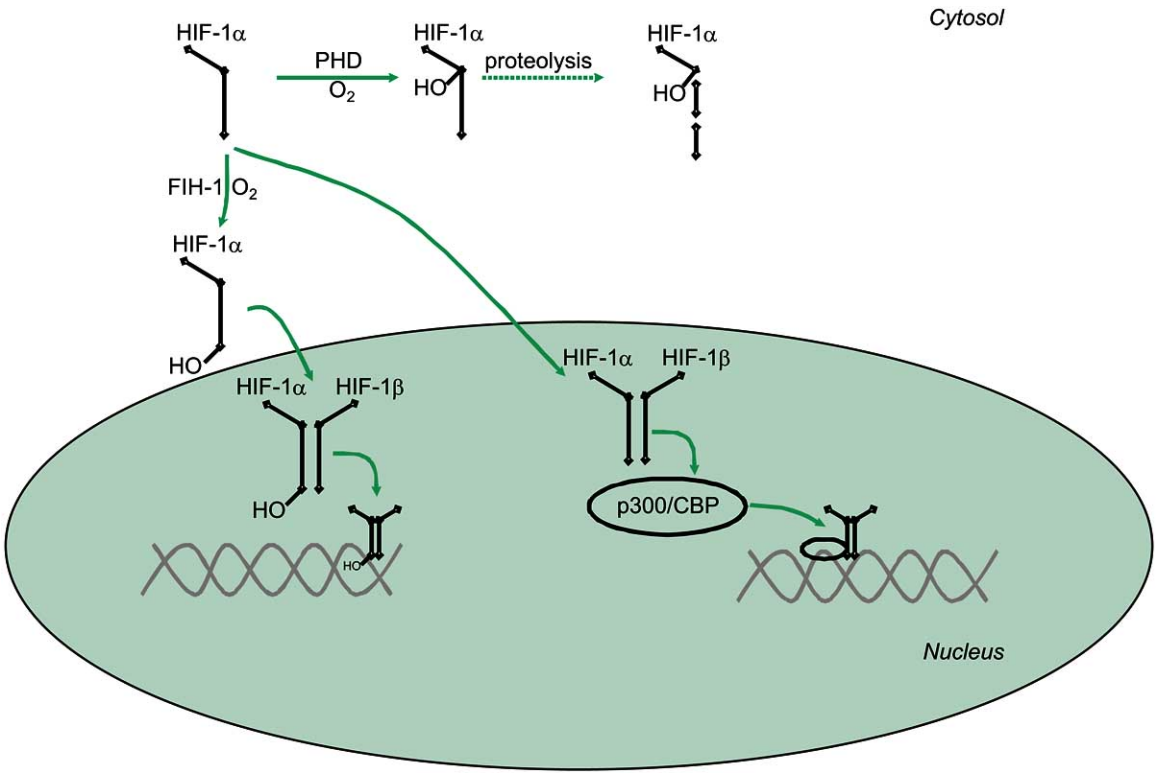

Fig. (1). **The regulation of HIF-1 signaling.** During normoxia, cytosolic HIF-1α is hydroxylated by prolyl-4-hydroxylase (PHD) which leads to polyubiquitination and eventual proteasomal degradation. Furthermore, factor-inhibiting HIF-1 (FIH-1) can hydroxylate an asparagine residue in the C-terminal transactivation domain of HIF-1α and inhibit the interaction of the HIF-1 complex with its co-activators p300 and CREB-binding protein (CBP). During hypoxia the lack of oxygen reduces the activity of PHD and FIH-1, resulting in the migration of HIF-1α to the nucleus where it binds to its partner HIF-1β to form the HIF-1 complex. The subsequent binding of the HIF-1 complex to p300/CBP leads to the induction of gene transcription.

protein is targeted for degradation and thus has a very short half-life. The brunt of HIF-1α regulation is driven by the oxygen-dependent hydroxylation of the α-subunit by prolyl hydroxlyases (PHD) that target this subunit for proteolysis via a von Hippel-Lindau tumour suppressor protein (pVHL)-dependent ubiquitin-proteasome pathway. In this process, normal oxygen tension, along with the presence of iron and 2-oxoglutarate, allows for PHD-mediated hydroxylation of α-subunit at two proline residues (402 and 564) within the ODD domain. Hydroxylation of the α-subunit acts as a recognition signal for pVHL leading to ubiquitination and subsequent degradation. Under hypoxic conditions, PHD hydroxylase activity is reduced and the stability and half-life of the HIF-1α protein is increased. Upon stabilization, HIF-1α translocates to the nucleus where it associates with the HIF-1β/ARNT subunit. This dimerization is essential for DNA binding and is mediated by the bHLH and PAS domains in each subunit. The binding of HIF-1 heterodimer to hypoxia-response elements (HRE) ultimately culminates in gene transcription. To date, approximately 100 genes involved angiogenesis, metabolic adaptation, apoptosis and metastasis have been identified as direct targets of HIF-1.

In addition to the PHD-mediated regulation of HIF-1 stability, another oxygen-dependent modification can occur that can alter HIF-1 signaling. Once HIF-1 binds to the HRE, transcriptional co-activators are recruited to form an initiation complex. This process is mediated by two distinct transactivation domains present within HIF-1α: an oxygen-regulated C-terminal transactivation domain (CAD) and a centrally located transactivation domain termed the N-terminal transactivation domain (NAD). Both the NAD and CAD function to recruit transcriptional co-activators. The factor-inhibiting HIF-1 (FIH-1) can hydroxylate an asparagine residue in the C-terminal transactivation domain of HIF-1α Like the PHDs, FIH-1 is a hydroxylase with 2-oxoglutarate- and iron-dependency. Hydroxylation of the asparagine residue within the CAD is sufficient to prevent the interaction of HIF-1α with the essential transcriptional co-activator p300/CBP (cAMP-response-element-binding (CREB)-binding protein), reduce transactivational potential and thus inhibits HIF-1-mediated transcriptional events Fig. (**1**).

NORMOXIC INDUCTION OF HIF-1 SIGNALING DURING INFLAMMATION

Although the activation and subsequent induction of gene transcription by HIF-1 is normally associated with a decrease in oxygen availability, it is now well established that HIF-1α accumulation and transcriptional activation can be triggered under normoxic and inflammatory conditions [15]. In fact cytokine-induced stimulation of HIF-1 signaling has been implicated as a link between inflammation and cancer (review in [16-18]). In addition to cytokines, viral and

bacterial interactions can induce HIF-1 signaling in some cells. For example, human T-cell leukaemia virus (HTLV)-1 [19], Epstein-Barr virus [20,21], Hepatitis B virus [22-26] and Kaposi's sarcoma-associated herpes virus [27-29] use a variety of molecular strategies to increase HIF-1α expression and/or transcriptional activity. In addition to viruses, a variety of bacteria and their products, especially lipopolysaccharide (LPS), up-regulate HIF-1α expression in a number of cell types [30-34] Fig. (**2**).

Mechanistically, HIF-1 signaling can be potentiated by inflammatory cytokines and chemokines through an increase in HIF-1α transcript production or direct stabilization of the HIF-1α subunit. Cytokines, such as interferon-alpha (IFN-α), can increase the transcription of the HIF-1α subunit leading to an increase in HIF-1α protein [35]. In terms of protein stabilization, a series of reports suggest that HIF-1α protein can accumulate and trigger HIF-1-related signaling events through the activation of the PI3-kinase (PI3K) pathway [12,36-43], production of nitric oxide (NO) [44-48] and up-regulation of cyclooxygenase-2 (COX-2) [49-51].

Due to the high-energy demands and reduced perfusion associated with the induction of an inflammatory response and subsequent tissue repair, sites of inflammation are characterized by low oxygen tension and reduced glucose levels [52, 53]. This environment alone is sufficient to induce HIF-1 signaling through the traditional oxygen-dependent mechanisms. However, the presence of inflammatory mediators and reactive oxygen species can also trigger HIF-1-dependent gene transcription and may augment HIF-1α accumulation induced by inflammation-associated hypoxia [15, 54-57]. In fact, the low levels of constant inflammation in the intestinal mucosa in conjunction with a transmural hypoxia gradient are associated with what has been termed a 'physiological' hypoxia accompanied by HIF-1α accumulation (reviewed in [58]). This is especially, evident in the amplification of HIF-1α transcription that can occur following HIF-1-induced up-regulation of NF-κB and Ικκα [59]. The activation of HIF-1 signaling under inflammatory conditions appears necessary for the proper function of cells of the myeloid lineage such as macrophages and neutrophils [59-65]. The induction of HIF-1 signaling during inflammation can occur via regulation of HIF-1α production, during transcription and translation or by post-translational modification that increases stability and activity. Furthermore, cytokines can increase the HIF-1 DNA binding resulting in increased HIF-1-dependent signaling.

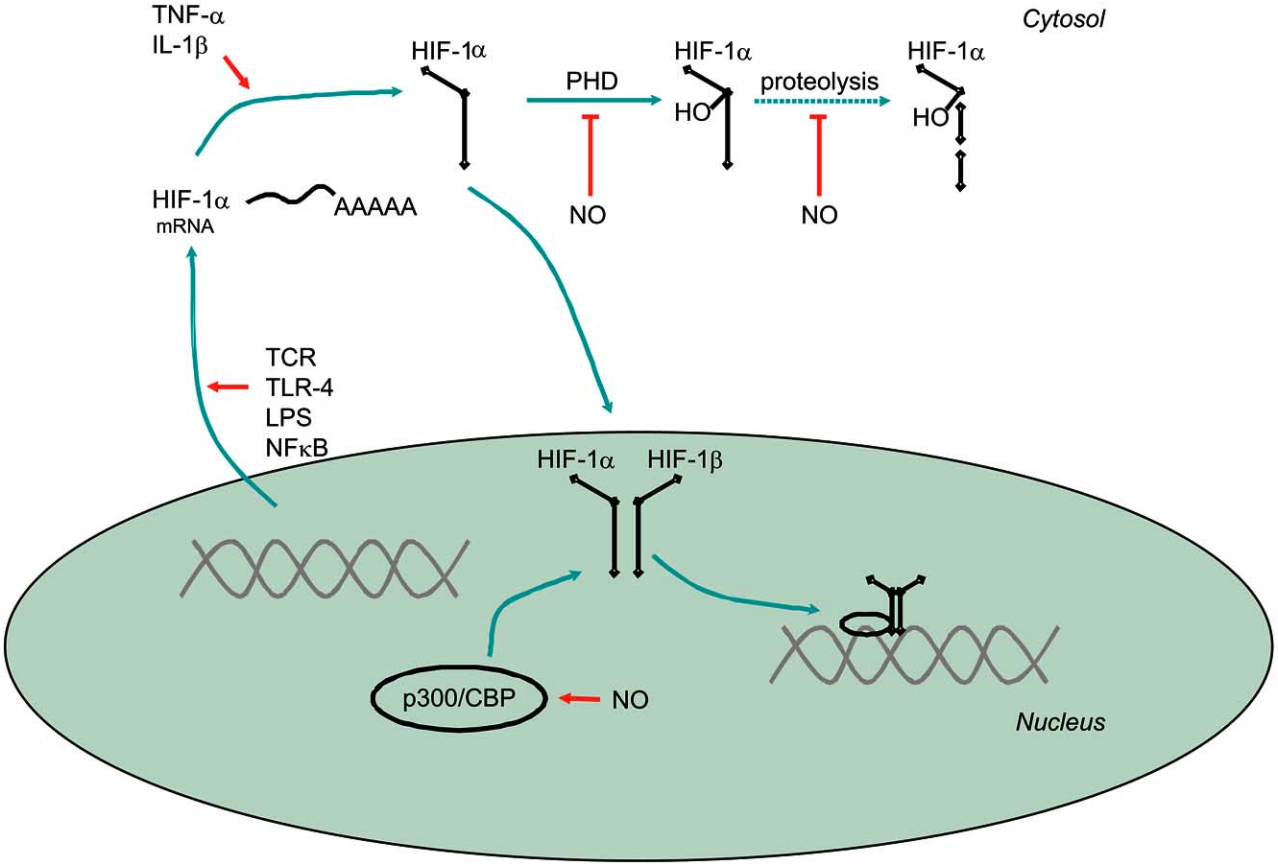

Fig. (2). The regulation of HIF-1 signaling during inflammation. HIF-1α transcription can be induced by the activation of the T-cell receptor (TCR). Bacterial lipopolysaccharide (LPS) can trigger the transcription of HIF-1α in part through the activation of the Toll-like receptor 4 (TLR-4) and nuclear factor kappa B (NFκB). Translation of HIF-1α mRNA can be increased by cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β). Nitrosylation of prolyl-4-hydroxylase (PHD) reduces its ability to hydroxylate HIF-1α. NO can also directly stabilize HIF-1α preventing its degradation and increase the interaction of the HIF-1 complex with its co-activators p300 and CREB-binding protein (CBP).

When HIF-1α was first discovered, most believed that its transcription was constitutive and its accumulation mainly involved post-translational modification rendering it insensitive to pVHL-mediated breakdown. However, it is now clear that the activation of inflammatory signaling cascades can evoke HIF-1α mRNA production via the activation of NFκB and AP-1 [33,66]. Activation of macrophages through the binding of bacterial LPS to the Toll-like receptor (TLR) 4 can trigger the transcription of HIF-1α mRNA and accumulation of HIF-1α protein under normoxic conditions [31-33,67]. This process can be blocked by transcriptional inhibitors and selective blockade of NFκB, suggesting that the accumulation of HIF-1α following LPS challenge was not due to a post-translational modification [33]. In addition to LPS, macrophages stimulated with thrombin and angiotensin II exhibit an up-regulation of HIF-1α in a transcription-dependent process [67,68]. Activated T lymphocytes also display an increase in HIF-1α mRNA following stimulation of the T cell receptor (TCR) [69]. Lukashev *et al.* (2001) reported that cross-linking of the TCR led to an actinomycin D-sensitive increase in HIF-1α mRNA expression. HIF-1α appears to be necessary for appropriate TCR signaling as targeted deletion of HIF-1α in T lymphocytes results in a reduction in calcium responses following TCR activation [70]. Altogether, these data suggest that the up-regulation of HIF-1α, through the induction of gene transcription, may play an integral role inflammatory responses.

In addition to the induction of transcription, inflammatory mediators can also increase the translation of HIF-1α mRNA resulting in an accumulation of HIF-1α protein. The increase in HIF-1α mRNA can often off-set the pVHL-dependent degradation that occurs during normoxia. Cytokines such as TNF-α and IL-1β can increase the HIF-1α translation and stability in a mechanism that is independent of changes in HIF-1α-hydroxylation [71,72]. In this process, inhibition of translation attenuated the IL-1β-induced increase in HIF-1α translation and HIF-1 activity [71]. Cytokine-induced translation is induced in both CAP-dependent and internal ribosomal entry site (IRES)-mediated mechanisms. Bert *et al.* (2006) reported that the up-regulation of HIF-1α induced by TNF-α in HEK cells occurred in an IRES-mediated fashion [72]. Similarly, TNF-α led to an accumulation of HIF-1α protein in tubular LLC-PK cells under normoxic conditions [73]. This effect was sensitive to inhibitors of mitogen-activated protein kinases (MAPK), NFκB and PI3K [73]. Interestingly, the PI3K/mTOR and MAPK signaling pathways are associated with the initiation of CAP-dependent translation through the activation of p70S6 kinase (p70S6K) or eukaryotic initiation factor-4E (eIF-4E). Both p70S6K and eIF-4E have been implicated in the accumulation of HIF-1α in response to growth factors, hormones or cytokines [74]. Indeed thioredoxin 1, a cytosolic mediator that plays a key role in the regulation of apoptosis and protection from oxidative stress, increases HIF-1α translation through a PI3K/mTOR/Akt-p70S6K- and eIF-4E-dependent process [75]. In addition to directly regulating CAP-dependent translation through the activation of p70S6K or eIF-4E, virus-induced up-regulation of p42/44 MAPKs led to an increase in HIF-1α translation that was dependent on the generation of hydrogen peroxide [21].

Several post-translational modifications have been identified that stabilize HIF-1α and increase HIF-1-dependent gene transcription during normoxia. A number of signaling pathways can converge to prevent oxygen-dependent HIF-1α breakdown and promote HIF-1-mediated transcription. Phosphorylation of HIF-1α by casein kinase II at Thr-796 in the CAD domain prevents hydroxylation by FIH-1 and leads to an increased association with p300/CBP. MAPKs can also phosphorylate HIF-1α to promote transactivation with p300/CBP. It has also been reported that MAPK-dependent phosphorylation can block nuclear translocation of HIF-1α. NO can reduce PHD hydroxylase activity to effectively halt the breakdown of HIF-1α [76]. Furthermore, NO can directly modify HIF-1α through a process of nitrosylation to render the protein insensitive to PHD-mediated hydroxylation and increase its transactivation potential [48, 77, 78] Fig. (**2**). NO donors can also stabilize HIF-1α resulting in increased transcriptional activity (i.e., increased binding affinity to p300/CBP, [78]); however, their effects appear to be dependent on the NO-donor and the relative oxygen tension, in that NO up-regulates HIF-1α during normoxia but triggers down-regulation during hypoxia [44,45,77-80].

One of the key mechanisms by which HIF-1α is stabilized during normoxia is through *S*-nitrosylation of a cysteine residue (Cys-533) within the ODD domain [77]. This modification renders HIF-1α insensitive to PHD-dependent hydroxylation. The up-regulation of HIF-1 signaling in airway epithelial cells following respiratory syncytial virus (RSV) involves NO-mediated stabilization of HIF-1α [44]. RSV-induced NO release resulted in an increase in HIF-1α protein and HIF-1 signaling, as measured by the production of vascular endothelial growth factor (VEGF) [44]. This effect on HIF-1α stabilization was nearly abolished by the administration of an NO scavenger (i.e. carboxy-PTIO). In addition to an autocrine effect, NO can act in a paracrine fashion to stabilize HIF-1α protein. Radiation can stimulate iNOS-dependent NO release from tumour-associated macrophages; this NO can then stabilize HIF-1α in tumours to increase HIF-1-dependent gene transcription [77]. Moreover, Sandau *et al.* (2001) reported that over-expression of iNOS promoted HIF-1α accumulation in tubular LLC_1-PK cells. In a co-culture model, LPS-induced NO production in RAW 264.7 macrophages led to an up-regulation of HIF-1α in wild-type LLC1-PK cells, an effect that was attenuated by inhibitors of NO synthases and the NO scavenger carboxy-PTIO [78]. Interestingly, iNOS has also been implicated in HIF-1α stabilization in endothelial cells [46] and appears to induce HIF-1 signaling in the colon [81].

In addition to directly modifying the α-subunit, NO has been implicated in the inhibition of HIF-1α breakdown through the pVHL-dependent pathway [76]. NO donors have been reported to inhibit PHD activity, thereby protecting HIF-1α from proteasomal targeting and breakdown. Metzen *et al.* (2003) reported that an NO-donor, S-nitrosoglutathione, increased HIF-1α stability and signaling, independent of transcriptional or translational events. This effect was associated with an NO-dependent inhibition of PHD activity, without a change in the ability of pVHL to interact with a recombinant substrate that mimicked the hydroxylated ODD

domain [76]. Another NO donor (±)-S-nitroso-N-acetylpenicillamine (SNAP), increased HIF-1α accumulation and transactivation in Hela cells by inhibiting the interaction of HIF-1α with pVHL [76]. This effect was independent of changes in PHD-mediated hydroxylation of HIF-1α. However, SNAP also inhibited FIH-1-dependent asparagine hydroxylation, allowing for full interaction of HIF-1α with its co-activators CBP/p300 [76]. These data recapitulated an earlier report by Yaminska & Sumbayev (2003), suggesting that S-nitrosylation of a cysteine residue of HIF-1α could increase its interaction with p300 and stimulate transcriptional activity in HEK293 cells [82]. Thus, the presence of NO can inhibit pVHL-mediated breakdown of HIF-1α and concurrently increase its transcriptional activity through enhancing interactions with it co-activators CBP/p300.

In addition to the direct modulation of HIF-1 signaling through the actions of NO and other cytokines, inflammation can also up-regulate HIF-1α levels through the generation of reactive oxygen species (ROS) (reviewed in [54]). Indeed a number of cytokines, including TNF-α and IL-1β, trigger the accumulation of HIF-1α, in part, through the activation of NADPH oxidase and the subsequent generation of ROS [83-85]. Furthermore, enzymes involved in the generation of inflammatory mediators, such as those involved in the metabolism of arachidonic acid and the generation of NO, can act as a source of superoxide anions and hydrogen peroxide, the precursor to ROS production [86, 87]. Interestingly, the ROS-dependent accumulation of HIF-1α appears to be concentration dependent. *in vitro* studies suggest that low levels of ROS favour HIF-1α accumulation whereas high levels reduce HIF-1α stability and signaling [88, 89]. ROS-induced NFκB activation has been suggested as a mechanism by which oxidative stress can increase the transcription of HIF-1α [89]. Others have reported that ROS-dependent up-regulation of HIF-1α is due to an increase in PI3K-dependent mRNA translation [68]. It has been suggested that MAPKs can also influence HIF-1α activity in a ROS-dependent manner [90-93]. Interestingly, ERK1/2-dependent HIF-1α accumulation is induced by a number of non-hypoxic stimuli, such as IL -1β [94] and PGE_2 [95], that can induce ROS production.

HIF-1 PLAYS A CRITICAL ROLE IN INTESTINAL INFLAMMATION AND HOMEOSTASIS

It is apparent that HIF-1 signaling can be initiated by inflammation since these sites are characterized by low oxygen tension and reduced glucose levels [52,53] or through the direct actions of cytokines such as TNF-α, IL-1β and NO [18, 37, 44, 77, 96]. The induction of HIF-1 signaling during inflammation may be necessary for the proper function of inflammatory cells. Indeed macrophage phagocytic activity is heavily reliant on an up-regulation of HIF-1α following stimulation by bacterial LPS [60, 64, 65]. Hypoxia induces a significant increase in macrophage phagocytosis, an effect that was attenuated in the presence of siRNA to HIF-1α. In contrast, macrophage activity was enhanced when cells were forced to over-express HIF-1α [97]. Furthermore, monocyte chemoattractive agents can also be up-regulated in a HIF-1α-dependent fashion [98]. HIF-signaling may also play a role in the restitution of inflammation. Louis *et al.* (2005) reported that HIF-1-dependent expression of epithelial CD55 was necessary for the clearance of neutrophils following an inflammatory response [99].

Chronic inflammatory conditions, such as arthritis [42,100-104], asthma [105-108] and psoriasis [109,110], are often characterized by increased angiogenesis and HIF-1 signaling. Given this paradigm, therapeutics for the inhibition of HIF-1 signaling and downstream transcriptional events are being considered for the treatment of a broad spectrum of chronic inflammatory disease states. Several patent disclosures suggest that inhibition of HIF-1 signaling may prove effective in the treatment of inflammatory bowel disease. However, data from the literature suggests that HIF-1α plays a protective and regulatory role in the gastrointestinal tract through maintenance of intestinal barrier function and colonic inflammation [10-14,111,112]. Utilizing transgenic mice, Karhausen *et al.* (2004) reported that targeted deletion of HIF-1α in the intestinal epithelium rendered these animals more susceptible to trinitrobenzene sulfonic acid (TNBS)induced colitis. In contrast, targeted deletion of pVHL in the intestinal epithelium, generating a constitutively active HIF-1 signaling complex, provided protection in the same model of colitis [13]. However, others have shown that chronic HIF-1 signaling, triggered through targeted deletion of pVHL, rendered mice more susceptible to DSS-induced colitis [113]. The contradictory findings of the aforementioned studies may reflect differences in the chemical agents used to induce intestinal inflammation (i.e. TNBS versus DSS) or different promoters employed in the targeted deletion of pVHL. Karhausen *et al.* (2004) utilized the fatty acid-binding protein promoter to induce targeted deletion of pVHL, whereas Shah *et al.* (2008) utilized the villin promoter [13,113]. Nevertheless, two recent studies, published simultaneously, reported that pharmacological stabilization of HIF-1α protected mice from intestinal inflammation and damage in two different experimental models of colitis. Robinson *et al.* (2008) reported that pre-treatment with novel PHD inhibitor FG-4497 up-regulated HIF-1α and protected mice in a TNBS model of colitis. FG-4497 treatment reduced weight loss, attenuated colonic damage and reduced the levels of inflammatory cytokines in response to TNBS [10]. These effects were attributed to the retention of intestinal barrier function in response to HIF-1α stabilization with FG-4497 treatment [10]. Cummins *et al.* (2008) examined the effects of dimethyloxalylglycine (DMOG), a pan-hydroxylase inhibitor, on dextran sulfate sodium-(DSS) induced intestinal inflammation and damage. Treating intestinal epithelial cells with DMOG up-regulated HIF-1α and downstream signaling events, resulting in a significant increase cell survival [11]. Interestingly, DMOG also up-regulated NFκB signaling, suggesting a role for this transcription factor in the maintenance of barrier function. Treating mice with DMOG significantly attenuated DSS-induced intestinal inflammation and the associated damage [11]. Taken together, these data suggest a role for HIF-1α in maintenance of barrier function in models of intestinal inflammation.

The intestinal mucosa has unique metabolic demands. The intestinal epithelium is exposed to numerous noxious stimuli within the luminal contents, and thus these cells have a short half-life and a high mitotic rate. Intestinal epithelial

cells and a network of tight junctions that join them form the barrier against the luminal contents. The cells of this mucosal layer are in a constant state of growth and proliferation. Epithelial cells proliferate in the colonic crypts and migrate to the luminal surface where differentiation occurs [114,115]. Following these steps of proliferation, migration and differentiation the epithelial cells have a short life span and undergo controlled apoptosis. This entire process is repeated such that the entire epithelial surface is regenerated every 24 to 96 hours [116,117]. This process also enhances rapid wound repair following injury or damage to the epithelial barrier. Regeneration of the barrier, through epithelial repair and regeneration, is an absolute requirement for maintenance of intestinal homeostasis. In the absence of a competent mucosal barrier, constant stimulation of the mucosal immune cells by luminal contents can lead to the chronic inflammation found in inflammatory bowel disease.

The rapid mitotic rates, the ongoing processes of wound repair and regeneration, as well as the fact that epithelial cells are directly in contact with the hypoxic environment of the colonic lumen puts these cells at risk for hypoxic insults. Although the intestinal vasculature usually provides adequate perfusion, the mucosa experiences considerable hypoxia, even in the absence of abnormal physiological conditions [58]. Furthermore, intestinal mucosa oxygen demands are dramatically increased with injury and inflammation [13]. This has been reported utilizing specialized dyes that reveal a hypoxic gradient that spans the mucosal layer [13]. Utilizing 2-nitroimidazole dyes to detect sites of low oxygen, Karhausen *et al.* (2004) reported the existence of a 'physiological' hypoxia as determined by the dye localization in the epithelial cells lining the intestinal lumen. Furthermore, the accumulation of dye increased in cells overlying mucosal lesions and areas of inflammation [13].

Given the role for HIF-1 signaling in cell survival and adaptation to low oxygen levels, it was hypothesized that HIF-1α may play a role in the response of the intestinal epithelium to hypoxia. Interestingly, cultured intestinal epithelial cells appear resistant to hypoxia-induced loss of barrier function, as measured by changes in trans-epithelial resistance [14]. Furthermore, HIF-1 signaling has been implicated in wound healing and restitution [118-120]. In addition to the activation of HIF-1 signaling in the intestinal epithelium in response to hypoxia, cytokines and immune mediators have also been reported to modulate HIF-1α activity in intestinal epithelial cells [37, 95, 96]. Prostaglandin E_2 can induce VEGF synthesis in a HIF-1α-dependent fashion in an immortalized intestinal epithelial line [95]. A mixture of proinflammatory cytokines (TNF, IFN-γ and IL-1β) increased the transcription of HIF-1-related genes in IEC-6 cells, a non-transformed rat intestinal epithelial cell line [96]. Observations derived from studies that have examined the effect of hypoxia on intestinal epithelial barrier function may reflect a conserved mechanism by which hypoxia, in combination with inflammatory mediators, may stimulate protective HIF-1 signaling Fig. (**3**).

In the context to the intestinal epithelium, the resistance to hypoxia-induced changes in permeability is associated with HIF-1-dependent activation of a number of protective

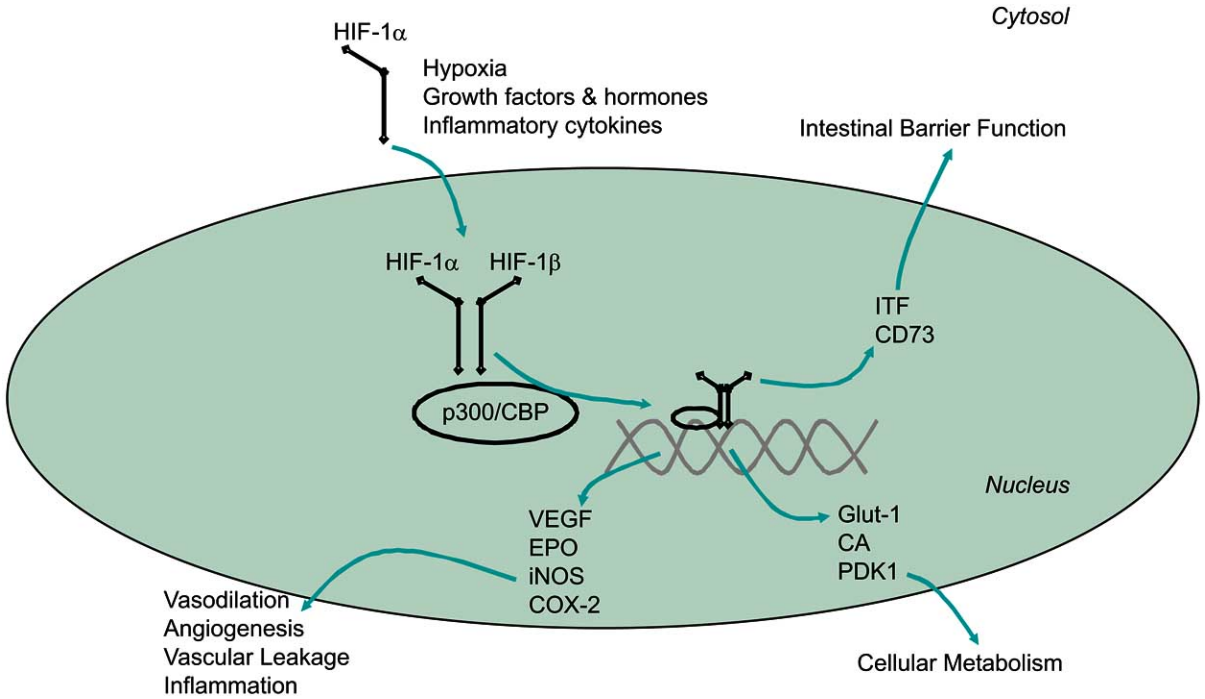

Fig. (3). **Protective mechanisms induced by HIF-1.** Stabilization of HIF-1α leads to nuclear migration and association with its partner HIF-1β. The active HIF-1 complex binds its co-activators p300/CBP leading to gene transcription to increase in oxygen transport (vascular endothelial growth factor - VEGF; erythropoietin - EPO; inducible nitric oxide synthase - iNOS; cyclooxygenase-2 - COX-2), cellular metabolism (glucose transporter-1 - Glut-1; carbonic anhydrase - CA; pyruvate dehydrogenase kinase-1 - PDK1) and intestinal barrier function (intestinal trefoil factor - ITF; ecto-5'-nucleotidase - CD73).

genes. One such gene product that is induced by hypoxia is intestinal trefoil factor (ITF, also known as TFF-3). ITF is produced by goblets cells within in the intestinal epithelium and plays an essential role in protecting the colonic epithelium from injury and enhancing wound repair [14,111]. HIF-1α plays a critical role in regulating the hypoxia-ITF mediated events in that antisense oligonucleotides to HIF-1α can block hypoxia-induced up-regulation of ITF [14]. Moreover, ITF-deficient mice displayed significant hypoxia-induced increases in intestinal permeability [14]. Louis *et al.* (2006) reported that hypoxia-induced up-regulation of ITF is associated with an increase in the expression of mucin 3 (MUC3) in intestinal colonocytes [111]. Mucins are glycosylated epithelial glycoproteins that form a protective layer lining the intestinal mucosal surface where they act as a physical barrier to bacterial invasion [121-124]. Interestingly, HIF-1α could bind to both the mouse and human MUC3 promoter. Furthermore, confocal microscopy suggested that ITF and MUC3 were co-localized. This was reinforced by the observation that antibodies to MUC3 could co-immunoprecipitate ITF in lysates from intestinal colonocytes exposed to hypoxic challenges [111]. Interestingly, the effects of ITF can be augmented by keratinocyte growth factor (KGF) [125] in part by stimulating proliferation and differentiation of intestinal epithelial cells including ITF and mucin producing goblet cells [126,127].

In addition to the protective effects of ITF, HIF-1-signaling in the gastrointestinal tract has also been linked to the up-regulation of an enzyme that is involved in adenosine metabolism [13,112]. Ecto-5´-nucleotidase (CD73) is an enzyme that is expressed on the cell surface and functions to hydrolyze adenosine-monophosphate (AMP) to adenosine in the extracellular domain [128]. Adenosine has been reported to play a regulatory role in intestinal homeostasis by influencing epithelial ion transport and mucosal hydration (reviewed in [129]). Activation of the adenosine A_{2B} receptor plays a role in neutrophil-dependent enhancement of barrier function in endothelial cells [130]. Furthermore, selective inhibition of CD73 hinders resealing of endothelial and epithelial barriers [131]. During intestinal inflammation, adenosine may control the immune cell infiltrate by regulating barrier function (endothelial and epithelial) and directly modulating neutrophil-endothelial interactions [128, 131,132]. During hypoxia or periods of inflammation, CD73 is up-regulated in a HIF-1α-dependent fashion in the intestinal mucosa [13,112]. Targeted deletion of CD73 renders mutant mice significantly more susceptible to experimental models of colitis [133]. This effect can be mimicked in wild-type mice by pharmacological inhibition of CD73 [112]. Hypoxia-induced up-regulation of CD73 and the resulting increase in the metabolism of AMP to adenosine confers protection to intestinal epithelial cells [112,133]. In culture, epithelial monolayers exposed to hypoxia display a significant increase in trans-epithelial resistance, compared to cells kept at normal oxygen levels. This effect was abolished in the presence of a selective adenosine A_{2B} receptor antagonist [112]. Furthermore, the protective effect of CD73 activity *in vitro* may be linked to an up-regulation of protective cytokines such as IL-10 and IFNαA [133].

Although basal, 'physiological' inflammation may prove beneficial in the maintenance of barrier function and control of the mucosal immune system, the pathogenesis of inflammatory bowel disease is thought to involve a breakdown in barrier function which triggers an inappropriate activation of the mucosal immune system resulting in overt tissue inflammation. It has become apparent that HIF-1 signaling plays an integral role in regulation of intestinal homeostasis through the maintenance of barrier function, control of inflammation and response to tissue injury. Data from animal models of intestinal inflammation suggest that enhancing HIF-1 signaling may prove beneficial in the treatment of inflammatory bowel disease.

HIF-1 SIGNALING IN INFLAMMATION: CURRENT PATENTS

As described in detail in the previous sections, the HIF-1 signaling cascade activates downstream events that allow for adaptation to low oxygen levels and metabolic stress. The accumulation of HIF-1α can also occur during normoxia, especially during inflammation, and results in HIF-1-mediated gene transcription. It has been suggested that the etiology of many chronic inflammatory disorders involves aberrant HIF-1 signaling. In rheumatoid arthritis, HIF-1α is elevated in the cells of the synovial fluid and increased angiogenesis is observed in the early phases of the disease. Pro-angiogenic signaling can provide the necessary vascular adaptation to accommodate the energy requirements of the chronic inflammatory state. Increased HIF-1 signaling contributes to the over-activation of myeloid cells that can drive the inflammation [52,60] and increase the expression of matrix metalloproteinases (MMPs) that contribute to the destruction of the cartilage and bone invasion in arthritis [100]. The induction of HIF-1-mediated angiogenesis, a prerequisite for tissue remodelling, has also been observed in allergic asthma. Increased levels of VEGF have been measured in the bronchiolar lavage of patients with asthma as compared to healthy individuals [134-137]. Furthermore, biopsies from asthmatic patients displayed significant levels of HIF-1α immunoreactivity [136]. Interestingly, inhibition of HIF-1 signaling reduced the severity of airway inflammation and remodelling in a mouse model of chronic airway inflammation and hyperresponsiveness [106]. HIF-1 signaling has also been implicated in the pathogenesis of inflammatory eye diseases. Angiogenesis, immune cell infiltration and cytokine release associated with acute endotoxin-induced uveitis are driven by HIF-1 signaling [138]. Furthermore, inhibition of NFκB and HIF-1α activation attenuates LPS-induced ocular inflammation.

Given the evidence supporting a role for HIF-1 signaling in inflammatory conditions, a number of patents have been filed to protect intellectual property that encompass methods to inhibit HIF-1 signaling (Table **1**). Some have explicitly claimed their inventions apt for the treatment of inflammatory disorders, whereas others have designed therapies to target HIF-1 signaling in conditions associated with aberrant HIF-1-dependent angiogenesis mediated through the increased production of VEGF.

siRNA Targeting HIF-1α Expression

The HIF-1 signaling pathway can be inhibited in a variety of ways that are reflected in the diversity of HIF-1-related patents. The most direct means for the inhibition of

Table 1. Methods to Down-Regulate HIF-1 Related Signaling

Patent#	Title	Inventors	Claims
US2003034826	COMPOSITIONS AND METHODS FOR siRNA INHIBITION OF HIF-1 ALPHA	Reich, S., Surace, E.M., Tolentino, M.J.	-siRNA to HIF-1α -treatment of diseases associated with increased angiogenesis including inflammatory disorders (e.g. rheumatoid arthritis)
US2004002344	ANTISENSE OLIGONUCLEOTIDES THAT INHIBIT EXPRESSION OF HIF-1	Yoon, H., Mao, L., Lee, Y.B., Ahn, C.H., Jiang, X.	-antisense oligonucleotide sequences to inhibit the expression of HIF-1α -inhibition of angiogenesis -reduction in cell viability
IB2003001758	OLIGOMERIC COMPOUNDS FOR THE MODULATION HIF-1ALPHA EXPRESSION	Thrue, C., A., Høg, A.M., Kristjansen, P. E.G.	-oligonucleotide sequences to inhibit expression or transcription of HIF-1α -inhibition of angiogenesis -treatment of IBD
US2003037383	MODULATION OF HIF1α; AND HIF2α; EXPRESSION	Ward, D.T., Dobie, K.W., Freier, S.M., Marcusson, E.G.	-oligonucleotides designed to target the nucleic acids that encode HIF-1α and HIF-2α -inhibition of angiogenesis -treatment of inflammation
US2004040704	HIF OLIGONUCLEOTIDE DECOY MOLECULES	McEvoy, L.M., Powell, L., Zhang, J., Morris, K.	-specific nucleotide sequences that bind to active HIF-1 signaling complex -inhibition of HIF-1-dependent gene transcription -treatment of inflammation & IBD
SE2003000030	HIF-1 ALPHA PROTEINS MUTATED IN THE C-TERMINAL TRANSACTIVATION DOMAIN	Ruas, J.L., Poellinger, L., Pereira, T.	-mutant HIF-1α protein with no C-terminal transactivational domain -reduces HIF-1 signaling -no specific disease claims
US2004022656	REGULATION OF HIF PROTEIN LEVELS VIA DEUBIQUITINATION PATHWAYS	Kirkpatrick, L., Powis, G., Welsh, S.J.	-agents to increase ubiquitination of HIF-1α -increase breakdown of HIF-1α -treatment of inflammation
US2005038317	METHODS FOR LOWERING HIF-1 MEDIATED GENE EXPRESSION	Verma, A., Lu, H.	-synthetic peptides to inhibit inactivation of hydroxylating enzymes -treatment of diseases associated with aberrant angiogenesis -treatment of arthritis
US2007008034	INHIBITION OF HIF-1 ACTIVATION FOR ANTI-TUMOR AND ANTI-INFLAMMATORY RESPONSES	Li, C.Y., Li, F., Sonveaux, P., Dewhirst, M.W.	-methods to decrease NO-mediated stabilization of HIF-1α -inhibition of angiogenesis -treatment of inflammation
US2004039484	TREATMENT OF RHEUMATOID ARTHRITIS WITH HYPOXIA-INDUCIBLE FACTOR 1α ANTAGONISTS	Defranoux, N., Hurez, V.J., Michelson, S., G., Shoda, L.K., Wennerberg, L.G.	-variety of agents to inhibit HIF-1-dependent gene transcription -treatment of inflammatory diseases (e.g. rheumatoid arthritis & inflammatory bowel disease)

HIF-1 signaling is through the targeted down-regulation of HIF-1α. Small interfering RNAs (siRNA) against HIF-1α (US2003034826; US2004002344) have been designed to attenuate HIF-1α protein production resulting in an inhibition of disease-associated angiogenesis, often a contributing factor in chronic inflammatory disorders [139,140]. Reich *et al.* (US2003034826) and Thrue *et al.* (IB2003001758) have developed specific siRNA sequences that are designed to target HIF-1α mRNA for destruction in the treatment of HIF-1 and VEGF-related disorders driven by aberrant angiogenesis [139,141]. The claims made by Reich *et al.* include the use of HIF-1α targeting siRNA in the treatment of diabetic retinopathy, macular degeneration and inflammatory disorders, including rheumatoid arthritis [139].

Thrue *et al.* have similar claims including the treatment of a variety of cancers, pre-eclampsia and inflammatory bowel disease [141]. Ward *et al.* (US2003037383) have also designed siRNA sequences that target the transcripts of both HIF-1α and HIF-2α. In addition to the treatment of cancer, the patent claims that the specific oligo-nucleotide sequences designed to decrease HIF-1α protein levels may be effective in the treatment of inflammatory disorders, including inflammatory bowel disease [142]. In contrast to targeting existing HIF-1α transcripts, Yoon *et al.* (US2004002344) have designed specific antisense oligonucleotide sequences that bind to the HIF-1α gene and prevent its transcription [140]. These oligonucleotide sequences have proven effective in the reduction in cancer cell viability but have not yet been assessed for effectiveness in the treatment of HIF-1-related inflammatory disorders.

Inhibition of HIF-1-Dependent Gene Transcription

In addition to using oligonucleotides for the purpose of interfering with HIF-1α protein production, McEvoy *et al.* (US2004040704) have designed specific sequences that act to bind the activated HIF-1 signaling complex and effectively sequester it, preventing its interaction with HREs and thus inhibiting gene transcription [143]. These oligonucleotides may be effective in treating some HIF-1-related immune disorders and other disease states with aberrant HIF-1 signaling. McEvoy *et al.* also suggest this invention to be effective in the treatment of inflammatory bowel disorders including Crohn's disease and ulcerative colitis [143].

Diseases associated with aberrant HIF-1 signaling are characterized by an increase in the transcription of genes that contain HREs within promoter binding regions. In addition to targeting HIF-1α for breakdown or eliminating its production, methods have been developed to inhibit HIF-1-dependent transactivation. Ruas *et al.* (SE2003000030) submit that the transfection of targets cells or tissue with a recombinant HIF-1α protein lacking the CAD domain is a potential method to reduce HIF-1 related gene transcription [144]. By preventing the interaction of the active nuclear HIF-1 complex with its co-activators p300/CBP and SRC-1, the mutant HIF-1α subunit competitively reduces HIF-1 dependent gene transcription. This technique might prove effective in the treatment of inflammatory disorders that result from increased HIF-1-dependent gene transcription; however, no specific claims were made to this regard.

Methods to Modify HIF-1α Stability

In contrast to these mechanisms to reduce the overall production of HIF-1α, compounds have also been developed to reduce the stability of HIF-1α. Under normoxic conditions, HIF-1α is degraded by the ubiquitin-proteasome pathway. Agents that can inhibit the enzymes involved in HIF-1α de-ubiquitination can augment its breakdown reducing HIF-1-dependent gene transcription. Kirkpatrick *et al.* (US2004022656) have developed a compound that augments the endogenous polyubiquitination of HIF-1α to significantly increase its rate of degradation [145]. This effect appears specific to HIF-1α since other proteins that are degraded in a proteasome-dependent fashion are unaffected. The primary use of these compounds appears related to inhibiting HIF-1-related angiogenesis associated with cancer; however, Kirkpatrick *et al.* do suggest that this invention may prove effective in the treatment of inflammatory disorders [145]. In a similar fashion to the mechanism developed by Kirkpatrick *et al.*, two patents have developed methods to decrease HIF-1α stability and activation under hypoxic or inflammatory conditions. Under normoxia, the hydroxylation of the ODD domain of HIF-1α by PHDs targets it for proteasomal breakdown. During normoxia or in the absence of various co-factors, PHD-mediated hydroxylation was significantly reduced to result in the stabilization of HIF-1α and activation of HIF-1 signaling. Furthermore, oxygen-dependent hydroxylation of the CAD domain of HIF-1α by FIH-1 significantly reduced the ability of the HIF-1 complex to initiate gene transcription in the nucleus. Verma and Lu (US2005038317) have designed synthetic peptides to prevent the inactivation of the aforementioned HIF-1α hydroxylating enzymes (PHD and FIH-1) [146]. These peptides augment the breakdown of HIF-1α and can effectively reduce HIF-1-dependent gene transcription. Claims for this technique include the treatment of disorders that are associated with aberrant angiogenesis including cancer and arthritis [146]. Furthermore, Verma and Lu highlight the importance of HIF-1α in myeloid cell function and suggest that their invention may be effective in reducing the activity of T cells, neutrophils and macrophages as method to decrease the inflammatory responses associated with various chronic inflammatory disorders. Li *et al.* (US2007008034) have designed a method to inhibit the activation of HIF-1 by NO released during inflammation. This method involves treating target tissues with NO scavengers or nitric oxide synthase inhibitors to reduce S-nitrosylation of HIF-1α during inflammation [147]. Their patent claims surround the treatment of radiation resistant tumours, tumour neovascularization and the inhibition of inflammatory responses.

HIF-1 Antagonists

Aside from directly modifying the expression or stability of HIF-1α, small molecule inhibitors of HIF-1 signaling have been developed to effectively inhibit angiogenesis and inflammation associated with rheumatoid arthritis. Defranoux *et al.* (US2004039484) have developed or licensed a series of agents that include nucleic acid sequences, peptides and other small molecule inhibitors to inhibit HIF-1-dependent gene transcription [148]. In the context of rheumatoid arthritis, Defranoux *et al.* claim these agents to be effective in reducing the quantity of synovial immune cells and cytokine levels (e.g., IL-6) to effectively reduce the destruction of joint cartilage. These agents can also be combined with other anti-inflammatory agents including, but not limited to, corticosteroids, non-steroidal anti-inflammatory compounds and cytokine receptor antagonists (e.g., IL-1 and TNF-α). In addition to the treatment of rheumatoid arthritis the inventors claim that their methods may prove effective for the treatment of a variety of inflammatory disorders, including lupus, rheumatic fever and inflammatory bowel diseases (i.e., Crohn's disease and ulcerative colitis).

The patents described in this section deal with methods to inhibit HIF-1 signaling and reduce inflammatory responses that are associated with increased levels of HIF-1-related gene products. Inflammatory conditions, such as rheumatoid

arthritis, exemplify this etiology since they prove very responsive to anti-HIF modalities. However, it is apparent that HIF-1 signaling is protective in the gastrointestinal tract and is integral in the proper function of myeloid immune cells in the context of bacterial infection. Indeed, targeted mutation of HIF-1α in the intestinal epithelium renders mice more susceptible to conditions that induce intestinal inflammation [13]. Methods designed to inhibit HIF-1-dependent gene transcription will not be effective in the treatment of disorders associated with intestinal inflammation including bacterial-induced gastroenteritis, Crohn's disease and ulcerative colitis. Treatment with anti-HIF-1 therapies may instead exacerbate these conditions. According to the HIF-1 signaling paradigm within the gastrointestinal tract, methods that up-regulate mucosal HIF-1 signaling to enhance intestinal barrier function and expedite wound healing may prove effective in the treatment of inflammatory diseases of the gastrointestinal tract.

UP-REGULATION OF HIF-1 SIGNALING: CURRENT PATENTS AND IMPLICATIONS IN GASTRO-INTESTINAL DISEASE STATES

As described in detail in the previous sections, HIF-1 signaling is integral to the maintenance of intestinal barrier function. Pharmacological agents that inhibit PHDs and lead to the stabilization of HIF-1α are effective in the prevention of mucosal damage in experimental models of intestinal inflammation [10,11]. Thus, methods that target the up-regulation of HIF-1 may prove effective in the treatment of inflammatory bowel diseases Table **2**.

The maintenance of an intact intestinal epithelium is essential for proper mucosal barrier function. Any breach of the barrier can result in the migration of luminal contents into the mucosal region and initiation of an inflammatory response. Thus, pharmacological augmentation of wound repair and restitution may prove effective in the attenuation of the mucosal immune system. Furthermore, the activation of macrophages and neutrophils through the stabilization of HIF-1α may augment the clearance of foreign agents that the breach the mucosal layer and aid in the overall restitution following an immune response. Arbeit (US2002030222) has invented a family of therapeutics designed to augment HIF-1-dependent wound restitution through the delivery of a stable recombinant HIF-1α peptide or nucleotide sequence encoding a mutant HIF-1α. Although, this patent makes no specific claims regarding the treatment of gastrointestinal inflammation, the applicant does suggest that the stable HIF-1α entities may be effective in the treatment of a variety of wounds and ulcers [149]. These agents can be delivered in a variety of ways including topically in a gel form, parenterally in a suspension with stabilizers or in capsule formulated for delayed release.

Pharmacological stabilizers of HIF-1α have been developed to treat a number of conditions that may improve gastrointestinal outcomes. Verma *et al.* (US2004037045) have designed compounds to augment HIF-1-dependent wound healing. These compounds, 2-oxoacid derivatives, are designed to stabilize HIF-1α by selective inhibition of PHD-mediated ODD domain hydroxylation [150]. In addition to accelerating wound healing through the induction of angiogenesis, Verma *et al.* also suggested that these compounds could improve immune function; however, no specific claims were indicated [150]. Klaus *et al*. (US2004017772) have also claimed the use of chemical stabilizers of HIF-1α as method to increase the production of erythropoietin in the treatment of anaemia associated with chronic inflammatory conditions such as Crohn's disease and colitis; however, they have made no claims suggesting these agents would be able to modulate the inflammation in inflammatory bowel disease [151]. They also claim that HIF-1α stabilization may help to reduce cytokine-induced expression of endothelial adhesion molecules (e.g., vascular cell adhesion molecule) resulting in decreased immune cell adhesion and rolling as well as the eventual migration from the circulation to the site of inflammation. From the claims made in this patent and from the information we have presented in the previous sections regarding HIF-1 signaling in intestinal inflammation, it could be hypothesized that these compounds may provide effective restitution of mucosal integrity through the acceleration of wound healing and the up-regulation of protective factors such as CD73, ITF and VEGF [13,14,111,112,133,152].

Another approach to enhance HIF-1 signaling is to target pathways that provide inhibitory input. Kim and Jeong (KR2003002577) have developed siRNA to modulate HIF-1α stability through the expression of arrest-defective protein 1 (ARD1). ARD1 is an N-acetyltransferase that binds to the ODD domain and targets HIF-1α for degradation upon acetylation. Cells transfected with ARD1 antisense oligonucleotides displayed significantly more HIF-1α accumulation in response to hypoxia [153]. However, HIF-1α levels were similar in ARD1 antisense and control cells when examined under normal oxygen conditions. Jeong *et al.* (2002) reported that acetylation of HIF-1α by arrest-defective-1 protein (ARD1), an acetyltransferase enhanced HIF-1α ubiquitination and degradation by increasing HIF-1α-pVHL interactions [154]. Furthermore, ARD1 was found to be decreased in hypoxia, suggesting it may play a role in oxygen-dependent regulation of HIF-1 signaling [154]. However, subsequent reports call into question the role of ARD1 in the regulation of HIF-1α stability during hypoxia [155-157]. The current disparity in the literature calls into question the idea of targeting ARD1 expression to selectively augment HIF-1 signaling in hypoxic or inflamed tissues (e.g. gastrointestinal mucosa). An alternative target for interfering RNA technology is the iron-only hydrogenase-like protein 1 (IOP1), a recently discovered regulator of HIF-1. Huang *et al.* (2007) reported that targeted knockdown of IOP1 by siRNA increased HIF-1α mRNA and protein as well as augmented HIF-1-dependent gene transcription [158]. Lee (US2006025329) incorporated these findings into an invention that targets IOP1 expression as a method to modulate HIF-1α expression and HIF-1-dependent signaling. The selective knock-down of IOP1 was provided as a mechanism to up-regulate HIF-1-dependent angiogenesis and treat diseases associated with hypoxia, with no claims made regarding inflammation.

Table 2. Methods to Up-Regulate HIF-1 Related Signaling

Patent#	Title	Inventors	Claims
US2002030222	USE OF HIF-1α VARIANTS TO ACCELERATE WOUND HEALING	Arbeit, J.M.	-introduction of stable HIF-1α variant -increase HIF-1 signaling -acceleration of wound and ulcer healing -no specific disease claims
US2004037045	ACTIVATION OF HYPOXIA-INDUCIBLE GENE EXPRESSION	Verma, A., Lu, H., Forbes, R., Dalgard, C., Mohyeldin, A., Zenk, R.	-compounds that increase HIF-1-dependent gene transcription -agents that stabilize HIF-1α -acceleration of wound healing -improvement of immune function
US2004017772	USE OF HIF ALPHA STABILIZERS FOR ENHANCING ERYTHROPOIESIS	Klaus, S.J., Molineaux, C.J., Neff, T.B., Guenzler-Pukall, V., Lansetmo, P. I., Seeley, T.W., Stephenson, C.	-chemical agents to increase stability of HIF-1α -increase HIF-1-dependent production of erythropoietin -treatment of anemia associated with inflammatory conditions
KR2003002577	PHARMACEUTICAL COMPOSITION FOR CONTROLING THE STABILITY OF HIF-1α	Kim, K.W., Jeong, J.W.	-siRNA to inhibit the expression of ARD1 -reduce HIF-1α degradation -increase HIF-1 signaling
US2006025329	HIF-1 MODULATOR	Lee, F.S.	-siRNA to decrease expression of IOP1 -increase stability of HIF-1α -increase HIF-1 signaling
US2006004004	HIF MODULATING COMPOUNDS AND METHODS OF USE THEREOF	Johnson, R.S., Peyssonnaux, C., Nizet, V., Theodorakis, E.	-agents to stabilize HIF-1α -increase the function of cells of the innate immune system -increase clearance of necrotic tissue -accelerate wound healing
US2005042552	ENHANCING TREATMENT OF HIF-1 MEDIATED DISORDERS WITH ADENOSINE A_3 RECEPTOR AGONISTS	Borea, P.A., Baraldi, P.G., Merighi, S., Maclennan, S., Leung, E., Moorman, A.	-selective adenosine A_3 receptor agonists to increase HIF-1α levels -treatment of tissue damage associated with ischemia or inflammation

Increasing HIF-1 signaling in myeloid cells may also prove effective in the treatment of gastrointestinal inflammation. As mentioned previously, HIF-1-dependent up-regulation of CD73 is associated with proper neutrophil migration and function and the maintenance of epithelial and endothelial barrier function [112,131,132,159]. Loss of CD73 significantly increased the severity of inflammation in experimental models [133]. Johnson *et al*. (US2006004004) have claimed that the pharmacological stabilization of HIF-1α would be an effective method to increase the activity of the innate immune system, to accelerate the clearance of necrotic tissue, to improve wound healing and to reduce infections. These effects may prove beneficial in treatment of inflammatory bowel disease by increasing the phagocytic and bactericidal potential of macrophages with an effective reduction in the overall invasion of luminal antigens into the mucosal layer. Furthermore, HIF-1-mediated augmentation of CD73 may act to regulate neutrophilic infiltrate and ultimately to reduce mucosal inflammation through the actions of adenosine [132,160]. Interestingly, Borea *et al*. (US2005042552) claim that selective activation of the adenosine A_3 receptor could augment HIF-1α and function to increase tissue repair and protection in response to hypoxic or inflammatory events. In addition, agents that activate the adenosine A_3 receptor could be used in conjunction with agents that activate other adenosine receptors (e.g., A_{2B})

involved in the control of inflammation [131,132,160]. Interestingly, methods designed to increase HIF-1-mediated adenosine metabolism through the up-regulation of CD73 may activate a positive feedback loop whereby the activation of the adenosine receptors would result in further up-regulation of HIF-1α. The ultimate outcome would be augmentation of innate immune function and control of neutrophilic infiltration during inflammatory responses [59,64,65,132,160].

ENHANCING HIF-1 SIGNALLING: THE CANCER CAVEAT

While current data provides compelling evidence that the stabilization of HIF-1α can maintain colonic mucosal integrity to offer protection against intestinal injury during increased local inflammation [10,11,13], the functional consequences of HIF-1α stimulation as a therapeutic strategy for IBD and other conditions of intestinal damage remains speculative. Furthermore, it remains unclear how the induction of intestinal HIF-1α by pharmacological effectors for the treatment of IBD might affect the development of colorectal cancer. Several lines of evidence suggest that HIF plays an important role in colorectal cancer [161-165], and HIF-1α is being considered as a potential target for gene therapy as well as a biomarker of metastatic potential [161,166].

HIF-1 is strongly linked to the growth and survival of tumours (reviewed in [167]) since HIF-1 transcriptional targets have substantive roles in tumour biology. Accordingly, HIF-1α is over-expressed in many human cancers where it can activate transcription of genes involved in angiogenesis, cell survival and proliferation, tumour metabolism, invasion and metastasis. These changes efficiently counteract the characteristic decrease in oxygen tension that is distributed heterogeneously within the solid tumour mass. Indeed, hypoxia within the tumour is thought to regulate growth and favour the survival of the most aggressive malignant cells [168]. There is interest in the role of HIF-1α in cancer cell proliferation. Dang *et al.* (2006) reported that HIF-1α could promote hypoxia-independent cell proliferation in a subset of colon cancer cells [169]. In contrast, Cannito *et al.* (2008) recently examined the contribution of HIF-1α to the process of epithelial-mesenchymal transition (EMT) under hypoxic conditions and found that EMT-related events induced by hypoxia were dependent upon the increase in mitochondrial-derived ROS whereas late migration and invasiveness were sustained by HIF-1α and VEGF-dependent mechanisms [168]. The cell-specific mechanisms that make the survival of a colorectal cancer cell dependent on HIF-1 signaling have not been established. Currently, it remains to be determined what the functional consequence of HIF-1α induction by hypoxia-independent factors or pharmacological therapies would be on the proliferative characteristics of colorectal cancer cells.

CURRENT & FUTURE DEVELOPMENTS

It is clear from the current data that HIF-1 occupies an eminent role in the maintenance of intestinal mucosal barrier function. The activation of HIF-1 plays a role in the response to intestinal injury by up-regulating genes that govern intestinal epithelial cell growth and differentiation. Moreover, data from *in vitro* studies reveal that HIF-1 signaling can prevent intestinal damage and inflammation in experimental models of colitis. According to this paradigm, therapeutics that target HIF-1 signalling through the acute stabilization of HIF-1α protein may prove valuable in the treatment of IBD. A recent review by Taylor and Colgan (2007) describes a paradigm where inhibiting PHD activity could be therapeutically advantageous in the treatment of IBD. In this approach, compounds that target PHDs would up-regulate HIF-1 and NFκB signaling, both of which favour epithelial cell survival and increase mucosal barrier function [58]. Currently, there is a paucity of patents describing inventions designed to augment HIF-1 signaling in the context of intestinal disease. Although, the published literature suggest that increasing HIF-1 signaling can accelerate wound healing, no patents exist to specifically target HIF-1α stabilization for the treatment of intestinal diseases associated with compromised intestinal mucosal barrier function. Thus, it is apparent that more work is required to determine whether patented mechanisms to increase HIF-1 signaling will prove applicable for the treatment of inflammatory bowel disease.

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