Published in final edited form as:
J Mol Med (Berl). 2013 February ; 91(2): 183–193. doi:10.1007/s00109-012-0988-7.

Transcriptional control of adenosine signaling by hypoxia-inducible transcription factors during ischemic or inflammatory disease

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Abstract

Inflammatory lesions, ischemic tissues or solid tumors are characterized by the occurrence of severe tissue hypoxia within the diseased tissue. Subsequent stabilization of hypoxia-inducible transcription factors – particularly of hypoxia-inducible factor 1α (HIF1A) - results in significant alterations of gene expression of resident cells or inflammatory cells that have been recruited into such lesions. Interestingly, studies of hypoxia-induced changes of gene expression identified a transcriptional program that promotes extracellular adenosine signaling. Adenosine is a signaling molecule that functions through the activation of four distinct adenosine receptors - the ADORA1, ADORA2A, ADORA2B and ADORA3 receptor. Extracellular adenosine is predominantly derived from the phosphohydrolysis of precursor nucleotides such as ATP or AMP. HIF1A-elicited alterations in gene expression enhance the enzymatic capacity within inflamed tissues to produce extracellular adenosine. Moreover, hypoxia-elicited induction of adenosine receptors – particularly of the ADORA2B – results in increased signal transduction. Functional studies in genetic models for HIF1A or adenosine receptors implicate this pathway in an endogenous feedback loop that dampens excessive inflammation and promotes injury resolution, while at the same time enhancing ischemia-tolerance. Therefore, pharmacological strategies to enhance HIF-elicited adenosine production or to promote adenosine signaling through adenosine receptors are being investigated for the treatment of acute inflammatory or ischemic diseases characterized by tissue hypoxia.

Keywords

adenosine; A1; A2A; A2B; A3; ischemia; cancer; hypoxia-inducible factor; HIF1; HIF2; equilibrative nucleoside transporters; ENT1; ENT2; adenosine kinase; adenosine deaminase; CD73; ecto-nucleotidase; CD39; apyrase; AMP; ATP; acute lung injury; colitis; inflammatory bowel disease; ischemia; sepsis

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DISCLOSURE

The authors declare that they have no conflict of interests.

Introduction

The field of hypoxia changed its direction dramatically, when Gregg Semenza performed studies of the erythropoietin promoter that led to the subsequent identification of hypoxia-inducible factor HIF1A – the key transcription factor of hypoxia adaptation [1-5]. It turned out that this transcription factor controls numerous hypoxia-inducible genes, and has been implicated in a number of physiological and pathological changes that are closely associated with hypoxic conditions such as occur during ischemic or inflammatory diseases [6]. Subsequently, it has been appreciated that tissue hypoxia is a distinct feature of a wide variety of diseases, including ischemia, inflammatory diseases and cancer [7, 8], and that the induction of hypoxia-inducible transcription factors under these disease conditions is not simply a bystander effect, but significantly impacts the inflammatory or ischemic micro-environment [9-12]. One of the important functions of HIF1A-dependent alterations of gene expression during hypoxia is its transcriptional effect on extracellular adenosine signaling. In addition, there are also other transcription factors than HIF involved in the adaptive response to hypoxia (for example the Sp1 transcription factor) [13, 14]. In the present review, we discuss the mechanisms of how HIF-dependent changes in gene expression are associated with enhanced adenosine responses. Moreover, we give examples of how hypoxia-elicited increases in extracellular adenosine provide an endogenous feedback signal that dampens excessive inflammation and promotes tissue repair and healing. This pathway has been implicated in tissue protection during acute lung injury, inflammatory bowel disease or during ischemic disorders (discussed and referenced below).

Tissue hypoxia occurs in a wide range of clinical disorders

Many clinically relevant diseases are characterized by significant tissue hypoxia [3, 4, 8]. For example, ischemic tissue injury – such as occurs in the context of myocardial infarction [15, 16], acute kidney injury [17] or stroke [18] – is characterized by a profound increase in tissue hypoxia due to lack of arterial supply with oxygen from the blood [7, 19]. Experimental evidence for the existence of tissue hypoxia within ischemic tissues comes from histologic staining studies [17]. Indeed, tissue hypoxia can be made visible on a histologic level by utilizing nitroimidazole compounds that are retained in hypoxic cells. Figure 1 describes how these compounds are retained in hypoxic cells when intracellular oxygen levels are low [20]. As such, studies utilizing hypoxia staining have shown dramatic increases of tissue hypoxia in ischemic organs [17]. Interestingly, the occurrence of tissue hypoxia (i.e., positive hypoxia staining) in an ischemic organ can persist beyond the actual ischemic period, even if the vascular supply is restored. In some instances, this can be due to a post-ischemic no-reflow phenomenon [17, 21-23]. Other studies indicate that inflammatory diseases are characterized by significant tissue hypoxia. For example, studies from the laboratory of Sean Colgan have revealed that the inflamed intestinal mucosa becomes severely hypoxic during experimentally induced colitis – an animal model for inflammatory bowel disease [10, 24-26]. The cyclic occurrence of intermittent hypoxia has been implicated in the pathogenesis of sleep apnea and leads to subsequent inflammatory activation [27]. Other diseases characterized by tissue hypoxia include infections with pathogens [28-31], cancer [32-34] and obesity [35]. The interdependent relationship between hypoxia and inflammation within a tumor leads to primarily hypoxic areas that become infiltrated by inflammatory cells, while cancer inflammation further exacerbates tissue hypoxia within tumors by causing alterations in the supply and demand for metabolites, particularly for oxygen [8]. Figure 2 demonstrates how inflammation and hypoxia, as well as oxygen supply and demand are interconnected in several different clinical disorders.

Hypoxia-induced transcription factors

Research stimulated by the discovery of HIF1A subsequently identified the molecular pathway that promotes hypoxia-elicited alterations of gene expression [3-5, 8, 36]. HIF is a heterodimeric transcription factor. It consists of a constitutively expressed beta-subunit (HIF1B) and an alpha-subunit (expressed as one of two isoforms – HIF1A or HIF2A), which is highly regulated on a post-translational level. When oxygen levels are high, HIF1A and HIF2A are subjected to immediate proteasomal degradation involving two hydroxylation steps (Figure 3). Factor inhibiting HIF (FIH) hydroxylates an asparagine residue in the C-terminal activation domain (CAD) of HIF [37]. This first step prevents co-activator binding, and thereby functionally inhibits HIF activity. A second hydroxylation step is mediated by a group of enzymes that function as prolyl-hydroxylases PHD1, PHD2 or PHD3 [38-40]. PHDs hydroxylate conserved proline residues within the N-terminal activation domain of the HIF1A or HIF2A protein. This second hydroxylation step facilitates binding of the Von Hippel-Lindau gene product (VHL), thereby promoting ubiquitination and subsequent proteasomal degradation of HIF1A or HIF2A [41-44]. Key observations on the pathway of normoxic HIF degradation via proline hydroxylation and proteasomal degradation were made in the research laboratories of William Kaelin and Peter Ratcliff.

Since PHDs and FIH require oxygen as co-factor for their individual hydroxylation reactions, hypoxia is associated with a functional inhibition of FIH and PHDs, respectively. Therefore, hypoxic conditions are associated with the post-translational stabilization of HIF1A and HIF2A. It should be noted that the levels of other co-substrates and products can also modulate FIH and PHD activities (e.g., 2-oxoglutarate, succinate) [45]. While HIF1A is expressed ubiquitously, HIF2A expression is limited to certain tissues (e.g. vascular endothelia). Understanding the differential effects of HIF1A versus HIF2A is currently an area of intense investigation. Indeed, there is emerging evidence that a specific hypoxia-elicited response is predominantly mediated by either HIF1A or HIF2A [46]. For example, a study in patients with familial erythrocytosis discovered a gain-of-function mutation in the HIF2A gene as cause for the disease [47].

Following stabilization of HIF1A/HIF2A and co-activator binding, the alpha-subunit forms a heterodimer with the HIF1B, translocates into the nucleus and binds to promoter regions within HIF target genets. This promoter region is conserved and referred to as hypoxia response element (HRE). The consensus core sequence for an HRE is RCGTG (where R is A or G) [48]. In most instances, binding of the HIF heterodimer to an HRE causes transcriptional induction of the gene [7, 8, 49]. However, there are also examples for HIF-mediated gene repression either by HIF-dependent induction of a gene repressor – such as microRNAs [50] or other inhibitor pathways – or by the same mechanism that also induces expression: direct binding of HIF to an HRE within the promoter region of the gene [17, 51-54]. It is presently not understood why direct binding of HIF to an HRE can in some instances function as gene repressor. HIF-dependent alteration in gene expression have been implicated in many hypoxia-adaptive responses, such as the induction of erythropoietin during anemia, or the induction of vascular endothelial growth factor (VEGF) in hypoxic tissues. In addition to hypoxia-dependent stabilization of HIF, there are also examples of hypoxia-independent HIF stabilization during inflammatory or infectious diseases [28, 29]. In the present review, we discuss how HIF functions increase extracellular adenosine signaling events and concomitant tissue protection from hypoxia, ischemia and inflammation.

Adenosine

Adenosine belongs to the family of purine nucleosides and is composed of the nucleobase adenine attached to a single ribose sugar molecule [24, 38-40, 55, 56]. It is well known for its biological function as molecular building block of the universal energy currency ATP [57]. In contrast, extracellular adenosine is known for its function as signaling molecule. It can activate four known adenosine receptors, the ADORA1, ADORA2A, ADORA2B or the ADORA3. These are G-protein coupled receptors with intracellular second messenger systems, one of which is cAMP. While signaling through ADORA1 and ADORA3 decreases intracellular cAMP, activation of the ADORA2A and ADORA2B increases intracellular cAMP levels [32, 33, 49, 58]. Many biological functions have been attributed to adenosine signaling (Figure 4). For example, the heart-rate slowing effects of intravenous adenosine that is used for patient treatment of supraventricular tachycardia are mediated through the ADORA1A receptor [59]. The ADORA2A receptor is expressed on inflammatory cells: pharmacologic studies from the laboratory of Bruce Cronstein provided critical evidence that activation of ADORA2A on neutrophils attenuates inflammatory responses [60]. Moreover, a landmark paper from the laboratory of Michail Sitkovsky was the first to provide genetic in vivo evidence for the anti-inflammatory functions of ADORA2A signaling by functioning as endogenous feedback loop in dampening acute inflammatory responses [61]. Other studies from the laboratories of Joel Linden and of Mark Okusa confirmed the anti-inflammatory role of ADORA2A signaling and provided insight into its tissue-specific origin. Indeed, these studies indicated a functional role of ADORA2A signaling on inflammatory cells, including T-cells [62-64] or dendritic cells [65]. Studies from the laboratory of Sean Colgan and our research group implicated the ADORA2B receptor in hypoxia-adaptive responses [66-68], for example during myocardial ischemia [15, 16, 69], acute kidney injury [17, 38, 70] or intestinal inflammation [55, 71-74] – such as occurs during inflammatory bowel disease . The ADORA3A has been suggested to play a functional role in histamine release from rodent mast cells [75].

Influence of hypoxia on extracellular adenosine signaling events

The magnitude of extracellular adenosine signaling events is determined by the concentration of adenosine in the extracellular space – available to activate the receptor – and the concentration of an individual adenosine receptor. This is a highly dynamic process and many different controls contribute to this process. During conditions of hypoxia or inflammation, extracellular adenosine signaling is a reflection of (1) adenosine production from precursor molecules, (2) expression of adenosine receptors and (3) adenosine breakdown (Figures 4 and 5).

Extracellular adenosine production

During injurious conditions, many cell types release adenosine precursor molecules into the extracellular compartment [40, 76]. This occurs predominantly in the form of the adenosine precursor nucleotides ATP and ADP. Since intracellular ATP concentrations are generally high, many cells release ATP upon stimulation. For example, inflammatory cells or vascular endothelia release ATP during inflammation or hypoxia [68, 76-78]. Other sources for extracellular nucleotides include platelets that release ADP via granular release [7]. ATP can function as a signaling molecule itself, via activation of ATP receptors [79]. In many instances, ATP signaling drives pro-inflammatory responses [80], and hypoxia-dependent enhancement of ATP conversion to adenosine functions as an endogenous feedback loop to dampen excessive inflammatory injury [6, 24]. Extracellular ATP and ADP are rapidly converted to adenosine through a two-step enzymatic process, including CD39-dependent conversion of ATP/ADP to AMP, and CD73-dependent conversion of AMP to adenosine [72, 81-83]. Studies on the transcriptional effects of hypoxia revealed that CD39 is

transcriptionally induced by hypoxia through an SP1-dependent transcriptional pathway [13, 14]. Similarly, CD73 is transcriptionally induced by HIF1A binding to the CD73 promoter and subsequent induction of CD73 transcript and protein levels [67, 68, 84, 85] (Figure 5A). As such, hypoxia and inflammation are associated with increased production of adenosine from its precursor molecules, thereby shifting the balance from pro-inflammatory ATP signaling towards anti-inflammatory adenosine signaling.

Adenosine receptor expression during hypoxia

Comparative studies of adenosine receptor expression revealed that the ADORA2B is selectively induced in different tissues during inflammation or hypoxia, including vascular endothelia [68], intestinal epithelia [66, 72-74], the kidneys [17, 70], the heart [15, 16, 69] and the lungs [86]. Similarly, studies from human tissues – e.g. biopsies from ischemic myocardial tissues - revealed a selective induction of ADORA2B transcript levels [15]. HIF1A binding to an HRE within the ADORA2B promoter results in increased ADORA2B transcription, protein expression and function during conditions of hypoxia [66, 68]. Other studies identified a transcriptional pathway for the ADORA2A, indicating that this receptor is transcriptionally induced in pulmonary endothelial cells during hypoxic conditions via a transcriptional pathway under the control of HIF2A [87]. Taken together, these studies demonstrate that hypoxia drives extracellular adenosine signaling events on the receptor level by HIF-dependent induction of the ADORA2A and the ADORA2B [38-40].

Influence of hypoxia on the termination of adenosine signaling

Extracellular adenosine signaling is terminated by transport of adenosine from the extracellular towards the intracellular compartment through equilibrative nucleoside transporters (ENT1 and ENT2) [17, 51, 53, 54], followed by intracellular metabolism to inosine (via enzymatic activity of intracellular adenosine deaminase (ADA)) [88] or to AMP (via enzymatic activity of adenosine kinase (ADK)) [52]. Conditions of hypoxia or inflammation result in a functional attenuation of adenosine breakdown by repressing adenosine transporter activity and metabolism. Indeed, studies on the transcriptional control of ENTs demonstrate a HIF1A-dependent pathway of ENT1 [17, 54] and ENT2 repression [51, 53, 89], resulting in attenuated adenosine uptake and prolonged signaling effects during conditions of hypoxia. In addition, intracellular adenosine metabolism via ADK is attenuated, due to HIF1A-dependent repression of ADK transcript, protein and function [52] (Figure 5B). Taken together, hypoxia-elicited repression of adenosine transporters (particularly ENT1 and ENT2) and ADK result in enhanced extracellular adenosine levels and signaling events.

Hypoxia enhances adenosine signaling through netrin-1

Several reports indicate that during conditions of hypoxia, additional molecular signals can influence adenosine signaling. For example, hypoxia-elicited induction of netrin-1 has been implicated in enhancing extracellular adenosine signaling during inflammatory hypoxia [71, 90, 91]. Netrin-1 (NTN1) was originally described as neuronal guidance molecule. Recent studies, however, also discovered an anti-inflammatory role of NTN1 in different diseases (e.g., inflammatory bowel disease, acute lung injury) [55, 71, 91-94]. Just like hypoxia increases levels of extracellular adenosine, so it increases NTN1 levels, albeit by direct transcriptional induction of gene expression [91]. Surprisingly, several studies indicate that NTN1 exerts its immunological effects by enhancing signaling events through the ADORA2B receptor [91]. As such, migration of leukocytes is inhibited, making NTN1 a strong chemorepulsive molecule, which can dampen the influx of inflammatory cells during conditions of limited oxygen availability. The mechanism by which netrin-1 signaling enhances extracellular adenosine signaling remains unclear (e.g. direct activation of the

receptor, indirect enhancement of adenosine levels or allosteric enhancement of Adora2b signaling).

Functional role of hypoxia-elicited adenosine elevations during acute inflammatory diseases

Inflammatory bowel disease

Many studies have implicated hypoxia-elicited elevations of extracellular adenosine levels in an endogenous feed-back loop to dampen intestinal inflammation as it occurs during inflammatory bowel disease [24]. The intestinal mucosa is already under physiologic conditions among the more “hypoxic” organs of the body. This phenomenon is referred to as “physiological hypoxia” and relates to the fact that the intestinal lumen is anaerobic, thereby causing an extremely steep oxygen gradient across the intestinal epithelial monolayer covering the mucosal surface. Due to alterations in oxygen supply and demand caused by intestinal inflammation, the degree of hypoxia becomes far more severe during intestinal inflammation [25]. Interestingly, genetic studies on the role of HIF uncovered that stabilization of HIF provides a protective and anti-inflammatory pathway during intestinal inflammation [10, 11, 25, 95-97]. Moreover, studies with pharmacologic compounds that achieve HIF stabilization (PHD inhibitors) demonstrate robust protection from intestinal inflammation. As discussed above, HIF stabilization results in enhanced extracellular adenosine production and signaling. As such, several studies show that mice with failure to produce extracellular adenosine (*CD39*$^{-/-}$ or *CD73*$^{-/-}$ mice) develop a more severe course of disease when exposed to experimentally induced inflammatory bowel disease [98, 99]. Moreover, extracellular adenosine signaling through ADORA2B or ADORA2B has been shown to protect from intestinal inflammation [71, 74, 100-102].

Intestinal ischemia

Similar to intestinal inflammation, intestinal ischemia is characterized by profound intestinal tissue hypoxia in the context of intermittent loss of perfusion to the gut, resulting in dramatic increases in morbidity and mortality [103]. In this context, different studies have provided evidence for an anti-inflammatory role for HIF1A-elicited enhancement of extracellular adenosine production via CD73 [81] and signaling through the ADORA2B [73].). In addition, a recent study targeted HIF1A during intestinal ischemia using pharmacological or genetic approaches. Initial studies with the pharmacological HIF activator (PHD inhibitor) dimethyloxallyl glycine (DMOG) indicated attenuation of intestinal injury with DMOG treatment in intestinal ischemia and reperfusion injury (IR) [72]. In this murine model, DMOG treatment was associated with induction of CD73 and ADORA2B transcript and protein levels, while DMOG protection was abolished in *CD73*$^{-/-}$ or *Adora2b*$^{-/-}$ mice. Finally, studies of mice with tissue-specific deletion of Hif1a in intestinal epithelia or pharmacological inhibition of Hif1a with 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin revealed enhanced tissue injury during IR, thereby providing strong evidence for the Hif-adenosine pathway in gut protection from ischemia [72].

Acute kidney injury

Similar to intestinal IR, several studies have shown a protective role of hypoxia-elicited increases in extracellular adenosine production and signaling during IR in other organs, including the heart [7, 14-16, 69, 104] and the liver [13, 82, 89]. Particularly during ischemic tissue injury of the kidneys - a life-threatening condition that frequently complicates the care of hospitalized patients - hypoxia-elicited increases in adenosine production have been shown to protect from acute kidney injury (AKI). Studies in mice deficient in the pathway for extracellular adenosine production or deficient for signaling

through the Adora2b experience more severe kidney injury [70, 105-107]. Additional studies on the termination of extracellular adenosine signaling revealed a functional role for hypoxia-elicited elevations of extracellular adenosine in preventing a no-reflow phenomenon [17]. Indeed, a complex biologic network regulates kidney perfusion under physiologic conditions. This system is profoundly perturbed during ischemic AKI [17]. Due to its role in adapting tissues to hypoxia, one of our recent studies pursued the hypothesis that extracellular adenosine has a regulatory function in the post-ischemic control of renal perfusion [17]. Consistent with the notion that ENTs terminate adenosine signaling, this study found that pharmacologic ENT inhibition in mice elevated renal adenosine levels and dampened AKI. Deletion of the ENTs resulted in selective protection in *Ent1*$^{-/-}$ mice. Comprehensive examination of adenosine receptor-knockout mice exposed to AKI demonstrated that renal protection by ENT inhibitors involves the Adora2b. Subsequent studies in mice with tissue specific deletion of the Adora2b and Adora2b reporter mice revealed a crosstalk between renal Ent1 and Adora2b expressed on vascular endothelia to effectively prevent a post-ischemic noreflow phenomenon. Together, these studies identify ENT1 and adenosine receptors as key to the process of re-establishing renal perfusion following ischemic AKI [17].

Acute lung injury

Acute lung injury (ALI) is characterized by alveolar injury and uncontrolled inflammation [108, 109]. Several studies have provided evidence for a protective role for the hypoxia-adenosine pathway also during acute lung injury (ALI) [86, 110-112]. Very compelling evidence comes from the laboratory of Michail Sitkovsky and his team [32, 33, 113]. The group examined the consequences of different levels of oxygenation, and concomitant alterations of the adenosine pathway on outcome parameters during murine ALI [113]. ALI frequently requires symptomatic supportive therapy by intubation and mechanical ventilation with the supplemental use of high oxygen concentrations. Although oxygen therapy represents a life-saving measure, the discovery of hypoxia-elicited adenosine production and concomitant lung protection would predict that administration of oxygen to ALI patients with uncontrolled pulmonary inflammation could have dangerous side effects [113]. As such, oxygenation could weaken tissue hypoxia-driven adenosine production and signaling, and thereby dampen the hypoxia-mediated anti-inflammatory pathway and further exacerbate lung injury. To examine this hypothesis, the authors used a mouse model of ALI induced by bacterial infection. Thiel et al. exposed one group of mice to 100% oxygen, mimicking therapeutic oxygenation, and left another group at normal ambient levels (21% oxygen). Five times more mice died after receiving 100% oxygen than died breathing normal oxygen levels [113, 114]. Mice given 60% oxygen—considered clinically safe - developed severe pneumonia, but didn't die [114]. Indeed, the authors went on to demonstrate that hypoxia-dependent lung protection during ALI involves enhancement of extracellular adenosine signaling through the Adora2a.

Other studies from the laboratory of Sean Colgan have implicated extracellular adenosine production and signaling through the ADORA2B in lung protection during hypoxia – as occurs in the setting of hypoxic preconditioning. They found that ADORA2B-dependent adenosine signaling helps to protect the lungs via inhibition of the pro-inflammatory transcription factor nuclear factor NF-κB [115]. Indeed, adenosine-dependent inhibition of NF-κB involved the proteasomal pathway and was mediated by adenosine-mediated cullin-1 deneddylation [115]. As such, these findings implicate hypoxia-elicited elevations of extracellular adenosine and ADORA2B-dependent adenosine signaling in lung protection from excessive inflammation [115, 116].

Conclusions

Research over the past decade indicates that several disease conditions such as ischemia and reperfusion, inflammatory bowel disease or acute lung injury are associated with the stabilization of hypoxia-inducible transcription factors. One of the key outcomes of HIF activation during these disease conditions includes the activation of extracellular adenosine signaling – particularly through the ADORA2B. Many functional studies demonstrate that this pathway resembles an endogenous feedback loop to dampen hypoxia-induced inflammation and to provide organ protection during conditions of limited oxygen availability. It will be critical for the next years to translate these basic research findings from the bench towards patient treatment. For example, this could be achieved by pharmacological means of HIF activation (such as PHD inhibitors) or adenosine receptor agonists. We anticipate that targeting hypoxia-induced increases in extracellular adenosine signaling will provide novel therapies for a wide range of acute inflammatory diseases.

Acknowledgments

The present research work was supported by Grant D/10/52531 from the German Academic Exchange Service (DAAD) to J. M. P., a German Research Foundation (DFG) Grant (EH401/1-1) to H.E., an American Heart Association Grant to A. G. and National Heart Institute Grants R01-HL0921, R01-DK083385, R01-HL098294 and a grant by the Crohn's and Colitis Foundation of America (CCFA) to H. K. E.

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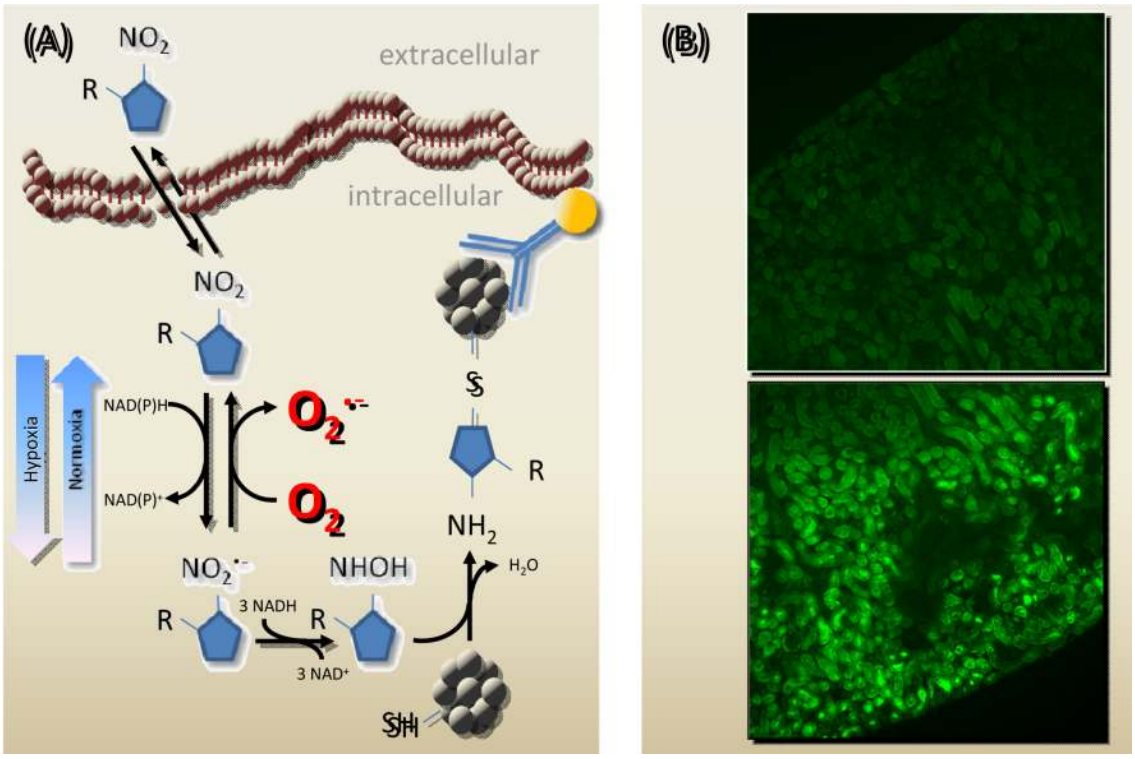

Figure 1. 2-Nitroimidazole compounds (NO_2-R) stain hypoxic tissues in vivo

(A) Schematic of NO_2-R metabolism in the cell: After passive uptake (top left) NO_2-R undergoes a single-electron reduction to a nitro radical anion intermediate (lower left). Oxygen regenerates the native compound by electron-uptake (marked in red) and subsequent reaction to H_2O_2 (not shown). In the absence of oxygen, the activated compound intermediate is processed to a hydroxylamine intermediate (bottom middle) which then stably binds SH-containing molecules such as proteins (bottom right). These adducts accumulate in the cell and can be visualized using labeled antibodies (right). **(B)** C57bl/6 mice were subjected to sham procedure (upper panel) or kidney ischemia (occlusion of the renal artery for 30 minutes; lower panel). After 5 minutes of reperfusion, mice were injected with pimonidazole. Antibody staining was performed after additional 15 minutes.

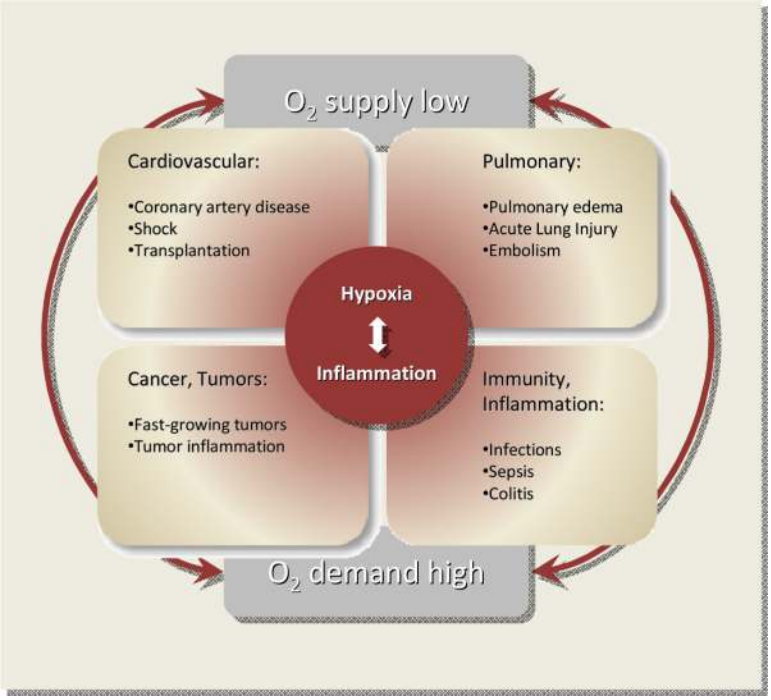

Figure 2. Clinical examples for diseases characterized by hypoxia

In the schematic, disorders and diseases in which either increased consumption or decreased supply of oxygen dominate exemplify the interdependence of hypoxia and inflammation.

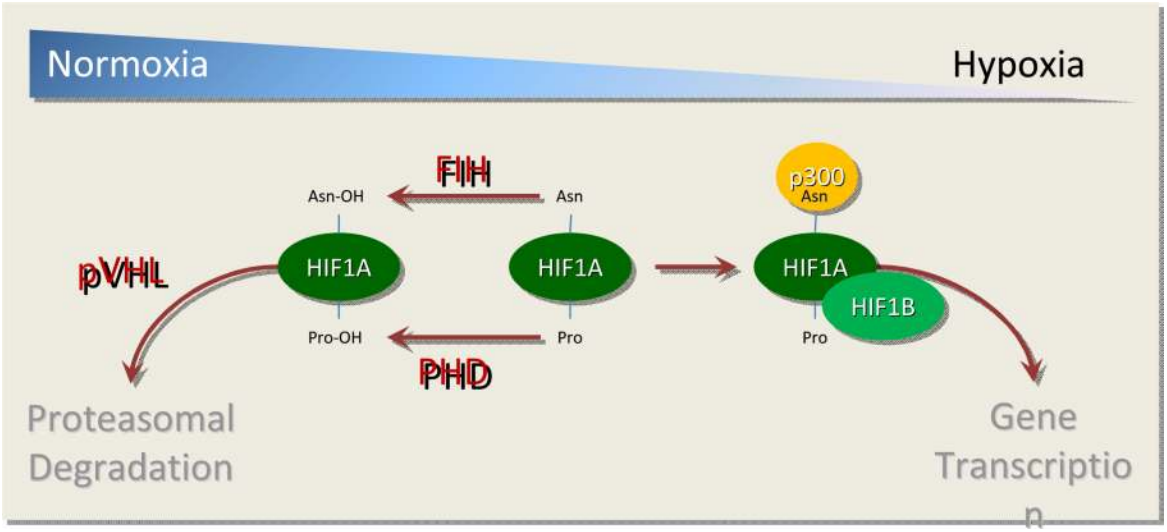

Figure 3. Hypoxia-dependent stabilization of the transcription factor hypoxia-inducible factor HIF

In normoxic conditions (left side of schematic), hydroxylases inactivate HIFA-subunits. FIH hydroxylates an asparaginyl residue in the carboxy-terminal activation domain (CAD), preventing co-activator (p300) recruitment. PHDs hydroxylate a proline residues in the N-terminal activation domain (in the oxygen dependent degradation domain (ODD)), facilitating pVHL-dependent ubiquitination and proteasomal degradation. In hypoxia, PHDs and FIH are inhibited and the co-activator p300 is recruited to the HIFα-subunit, which forms a heterodimer with HIFβ. This complex is transcriptionally active.

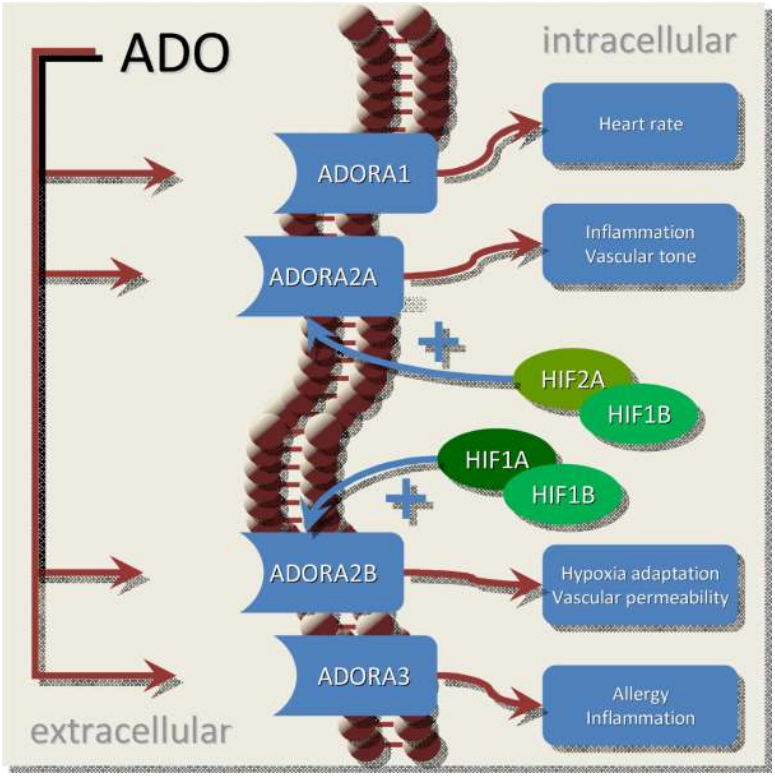

Figure 4. Hypoxia induces signaling through the ADORA2A and ADORA2B adenosine receptors

Four adenosine receptors (AR) have been described, mediating the effects of extracellular adenosine: ADORA1, ADORA2A, ADORA2B and ADORA3. All of these receptors modulate intracellular cAMP levels. ADORA1 and ADORA3 signaling lower cAMP concentrations, signaling through ADORA2A and ADORA2B – which are both transcriptionally induced in hypoxia – increases cAMP level. The schematic gives examples for the biological effects of AR signaling.

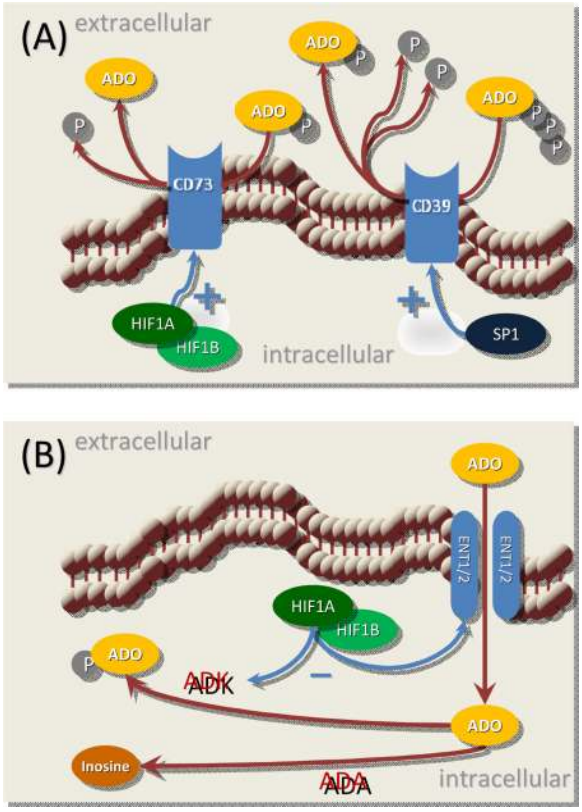

Figure 5. Hypoxia attenuates adenosine transport and metabolism, thereby enhancing extracellular adenosine signaling

(A) Extracellular ATP and ADP are released from different cell types upon stimulation (e.g., thrombocytes, neutrophils) or when they undergo necrosis or pyroptosis (danger associated molecular pattern molecules, DAMPs). The ecto-apyrase CD39 – which is expressed on epithelia, endothelia and immune cells – converts ATP and ADP to AMP. Ecto-5'-nucleotidase (CD73) rapidly converts extracellular AMP to ADO. Both enzymes are transcriptionally upregulated in hypoxic conditions, thereby promoting extracellular adenosine production during hypoxia. **(B)** Breakdown of extracellular adenosine is initiated by its uptake into the cell by equilibrative nucleoside transporters (ENT1 and ENT2). Intracellular ADO is either phosphorylated by adenosine kinase (ADK) or processed to inosine by adenosine deaminase (ADA). ENTs and AK are transcriptionally repressed during hypoxia, thereby prolonging adenosine signaling effects during conditions of hypoxia.