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New horizons in hypoxia signaling pathways

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ABSTRACT

Investigation into the regulation of the erythropoietin gene by oxygen led to the discovery of a process of direct oxygen sensing that transduces many cellular and systemic responses to hypoxia. The oxygen-sensitive signal is generated through the catalytic action of a series of 2-oxoglutarate-dependent oxygenases that regulate the transcription factor hypoxia-inducible factor (HIF) by the post-translational hydroxylation of specific amino acid residues. Here we review the implications of the unforeseen complexity of the HIF transcriptional cascade for the physiology and pathophysiology of hypoxia, and consider the origins of post-translational hydroxylation as a signaling process.

1. Introduction

Work over the last two to three decades has revealed a system of direct oxygen sensing that operates in essentially all animal cells, generating a new field of research into the biology of hypoxia, one to which Lorenz Poellinger made a range of important contributions. In this review we provide a brief background and, in the spirit of Lorenz's enigmatic enquiring mind, highlight some areas of ongoing intrigue.

Precise co-ordination of oxygen supply with demand is essential to meet the needs of metabolism and avoid toxicity. But despite intensive study of the physiology of oxygen delivery systems (the heart, lungs and blood circulation) for most of the twentieth century, the possibility of direct oxygen sensing was overlooked in favour of systems that respond to the products of energy metabolism. Remarkably it was the observation that exposure to cobalt induced erythrocytosis [1] that led to the concept of a specific oxygen sensor whose function was perturbed by cobaltous ions, but it was believed that this process was restricted to erythropoietin producing cells in liver and kidney. Initial clues that this system might operate more generally came from the observation that very small quantities of erythropoietin mRNA were expressed outside the liver and kidneys, but also induced by physiological hypoxia [2]. However, the first clear evidence that the same molecular pathways underlying erythropoietin regulation operated widely across mammalian cells came from transfection studies which revealed that oxygen-regulated control sequences derived from the erythropoietin gene operated widely in non-erythropoietin producing cells [3]. This work was closely followed by demonstration of the general operation of the key transcription factor hypoxiainducible factor (HIF) [4] and by the identification of genes encoding glycolytic enzymes as the first genes other than erythropoietin regulated through this pathway [5,6]. Rapidly, it became clear that the repertoire of HIF target genes was very large. Successive advances in genetic and genomic analysis revealed yet greater complexity and in complete contrast to the entry point through erythropoietin, hundreds of genes are now known to respond directly or indirectly to hypoxia via HIF.

HIF binds to DNA as a heterodimer of one alpha isoform with one beta isoform, each of which is a basic-helix-loop-helix PAS (Per-Ahr/ARNT-Sim) protein [7]. Elucidation of the mechanism of oxygen sensing revealed a remarkably direct process which again was entirely unanticipated. In the presence of oxygen, hydroxylation of two specific prolyl residues in HIF-alpha chains promotes interaction with the von Hippel-Lindau ubiquitin (VHL) E3 ligase leading to ubiquitylation and hence proteasomal destruction [8–10]. In human cells, three closely related enzymes PHD (prolyl hydroxylase domain) 1, 2 and 3 catalyse this reaction [11,12]. In a second oxygen-sensitive system asparaginyl hydroxylation at a site within the C-terminal activation domain of HIF-alpha [13], catalyzed by a more distantly related enzyme FIH (factor inhibiting HIF), impairs the recruitment of co-activators and down-regulates HIF transcriptional activity (reviewed in [14]). These enzymes are all 2-oxoglutarate-dependent oxygenases that split dioxygen and incorporate one atom of oxygen into the HIF substrate, coupling this reaction to the oxidative decarboxylation of 2-oxoglutarate, yielding succinate and carbon dioxide. In hypoxia, these reactions are suppressed leading HIF-alpha to escape destruction and assemble into an active transcriptional complex. The hydroxylation reaction is also powerfully inhibited by cobaltous ions, accounting for the founding

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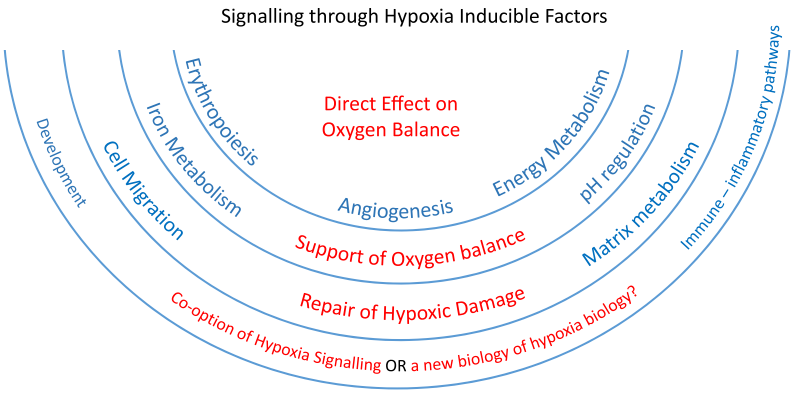

Fig. 1. Schematic illustrating the operation of the HIF pathway on processes both directly and indirectly related to the maintenance of oxygen balance. The HIF targets first identified were genes with direct effects on oxygen balance but as the number of identified HIF targets has increased the links of many to oxygen homeostasis have become increasingly indirect as exemplified by those involved in development and immune-inflammatory pathways.

toxicological observations [11].

2. More than oxygen homeostasis or a new physiology of hypoxia?

Many of the earliest HIF targets genes to be identified, encoding erythropoietin, angiogenic growth factors, and enzymes catalysing pathways of energy metabolism, had clear functions in maintaining oxygen balance. Others, such as those encoding molecules involved in iron transport or pH regulation had functions that could be connected indirectly with oxygen metabolism, and HIF became known as the master regulator of oxygen homeostasis. Interestingly, as the number of identified targets of HIF has expanded the links of many to oxygen homeostasis have become increasingly indirect (Fig. 1).

An important inflection point was the discovery, using genetic inactivation of HIF-1alpha in myeloid cells, of effects in inflammatory systems that extend well beyond the conventional boundaries of oxygen physiology [15]. Most recently pan-genomic studies have identified hundreds of direct HIF transcriptional targets whose products operate in an extraordinarily wide range of cellular processes [16]. Together with the operation of HIF on microRNA [17] and long non-coding RNA networks [18] and on multiple epigenetic and secondary transcriptional cascades [14], these studies suggest that the pathway impinges on almost every area of cell biology and extends into numerous areas that were previously unconnected with the physiology of hypoxia. In parallel it has become clear that HIF can be induced by a range of non-hypoxic stimuli, including growth, differentiation, metabolic, inflammatory and immune signals.

These unanticipated findings raise new questions. Do they represent a new physiology of hypoxia, in which a wide range of processes are dynamically controlled by oxygen availability, do they reflect co-option of non-hypoxia HIF signaling for different purposes, or do they reflect a permissive dependence on the integrity of the pathway? These possibilities are of course not mutually exclusive. Intriguingly, many of the newly defined processes are anatomically localised to areas of physiological hypoxia or occur in zones of pathological hypoxia. For instance, stem cell populations often localise to hypoxic micro-environments (reviewed in [19]). Marked zonal hypoxia is observed within the thymus and lymphoid tissues [20]. Dense cellular infiltrates and impaired vascular delivery create profound hypoxia in inflamed tissues (reviewed in [21]). It is likely that micro-environmental hypoxia itself contributes to the activation of HIF in many of these settings. However, an important, and largely unanswered question concerns the extent to which these processes are affected by systemic hypoxia, such as occurs in diseases that affect oxygen delivery, or at altitude. Attempts to date to address this question have yielded conflicting answers. On the one hand, studies using direct measurement of tissue pO2 have suggested that many local zones of physiological hypoxia are little influenced by systemic hypoxia [22]. Other studies have measured HIF directly and revealed biologically important effects [23]. For instance, systemic hypoxia clearly induces HIF in the renal papilla where the normal 'physiological' pO2 is already so low that a further reduction must necessarily be small. This suggests, at least in this setting, that very small changes in local pO2, which may be difficult to measure physically, have the potential to exert biological effects through the HIF pathway [23].

Given the role of HIF signaling in so many processes, this question of interplay between systemic and local hypoxia is relevant to many aspects of physiology and medicine. Does developmental hypoxia constrain development itself, if so does systemic hypoxia impinge on development? Does sojourn at altitude affect the function of immune/inflammatory pathways in ways that could affect disease susceptibility or (as was once popular) recovery from disease? The relevance of these questions is further underscored by the development of therapy that aims to modulate these pathways. The most advanced clinical application is that of HIF prolyl hydroxylase inhibitors that aim to activate HIF in the treatment of anaemia associated with deficient erythropoietin production in kidney disease (reviewed in [24]). The pharmacokinetic concentration of drugs in the liver and kidneys, together with the intrinsically high sensitivity of the erythropoietin gene to this system, might permit relatively selective action on erythropoiesis. Nevertheless, it is likely that clinical exposure to such agents in the setting of renal anaemia will impinge, for better or worse, on inflammatory disease, at least within the kidneys. Thus, a better understanding of whether and in what way small dose-dependent systemic activation of HIF has the potential to modulate the many new targets of the HIF system, is urgently required.

3. Un-physiological 'switching' of the HIF pathway in cancer

The massive interconnectivity of HIF pathways also has important implications in pathology. Much has been written about the constraints of highly connected molecular systems on species evolution. At the level of the somatic cell massive pathway connectivity has related implications which have received much less attention.

In general, it would be expected that responses to common