Regulators of the hypoxic response: a growing family

“Deregulation of the hypoxic response is widely accepted as a key step in cellular transformation and subsequent tumorigenesis and metastasis.”

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In recent years there has been a significant increase in the identification of previously unrecognized protein regulators of the hypoxic response. In this editorial, we highlight this growing family, including our recent identification of the Ajuba family of LIM domain-containing proteins. Such proteins are now considered key regulators of the hypoxic response, an adaptive cellular signaling pathway that regulates the cellular and physiological response to changes in oxygen levels by controlling degradation and transcriptional activity of hypoxia-inducible transcription factors (HIFs). Deregulation of the hypoxic response is widely accepted as a key step in cellular transformation and subsequent tumorigenesis and metastasis.

Responding to hypoxia

In order to survive in a given environment, optimum levels of oxygen are required throughout cells and tissues, as defined by cellular function and tissue type. Oxygen homeostasis is therefore tightly regulated in metazoans to ensure that tissues are sufficiently perfused. While partial pressure of oxygen (pO_2) within the human body ranges from 150 mmHg in the upper airway to an average of 40 mmHg in most organs, each tissue type has a basal requirement for oxygen under which essential cellular processes such as mitochondrial respiration, protein synthesis and ion transport become impaired [1–4]. Therefore, rapid reaction and adaptation of a cell or tissue to low pO_2 (hypoxia) can enable it to remain viable, thus reducing potential damage to the organism.

The hypoxic response signaling pathway is activated by HIFs, initiating a cascade of transcriptional events that result in an increase in levels of proteins such as VEGF and EPO [5,6]. These and other hypoxia-responsive gene products promote survival in low O_2 conditions

through increasing oxygen delivery to the cell and adapting intracellular regulatory pathways. Regulation of the hypoxic response is a tightly coordinated process. HIF is a heterodimer comprised of HIF- α and HIF- β subunits, of which HIF- α is degraded in an oxygen-dependent process whereby it is hydroxylated with available molecular oxygen by PHD1–3 proteins, and once hydroxylated engaged by the VHL protein, the recognition component of an E3 ubiquitin ligase [7–10]. HIF- α is subsequently ubiquitinated and degraded. The hypoxic response is frequently deregulated in transformed cells due to mutation or deletion of key hypoxic regulatory genes such as *VHL* and *EGLN1* (PHD2), resulting in irregular activation of hypoxia-responsive genes under nonhypoxic conditions.

The hypoxic paradox

Within the hypoxic response exists a fundamental paradox of how its ubiquitous nature can be translated into specificity of signaling across many different tissue types and transcriptional targets. A degree of specificity is enabled by the isoforms of key proteins within the hypoxic response, including the PHD, VHL, and HIF proteins. The three PHD isoforms (PHD1–3) have different expression across tissue types, HIF- α hydroxylation site specificities and sensitivities to inhibition at low pO_2 [11,12]. A further hydroxylase enzyme FIH catalyzes the asparaginyl hydroxylation of HIF- α to inhibit transcriptional coactivator p300 recruitment [13]. Three HIF- α isoforms exist; HIF-1 α and HIF-2 α have distinct as well as overlapping tissue expression and transcriptional targets, while HIF-3 α is localized to the cytoplasm and dimerizes with HIF-1 α /2 α to inhibit their nuclear localization and transcriptional activities [14–16]. VHL has p19 and p30 isoforms, which although reported to exert the same effect upon HIF- α , have some

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distinct binding partners and functions [17,18]. Therefore, with such combinations we begin to see how different permutations of the hydroxylase enzymes, VHL isoforms and HIFs across different tissue types and under differing pO_2 can begin to confer complexity within this signaling pathway at the level of HIF stability within the cytoplasm.

Regulators of the hypoxic response signaling pathway

There is now an increasing group of regulatory proteins of the hypoxic response that, in addition to PHD and VHL proteins (or in combination with them) reveal additional layers of multifaceted regulation within the HIF- α degradation pathway that may enable further understanding of the long-standing hypoxic paradox discussed above. These proteins include Siah1/2, which act to stabilize HIF-1 α in hypoxia by enhancing degradation of PHD1 and 3 [19]. A number of groups have reported that SUMOylation of HIF-1 α can both positively and negatively affect its stability and transcriptional activity [20,21]. Some HIF-1 α regulators function in an oxygen-independent manner, including the competitive binding of RACK1 or HSP90 to the PAS-A domain of HIF-1 α , resulting in its degradation via Elongin C/CUL-2/RBX1 or stabilization, respectively [22]. A further group of regulators exists that interact with HIF-1 α , VHL and Elongin C to enhance ubiquitination of prolyl-hydroxylated HIF-1 α , including SSAT2 and MCM7 [23,24].

The LAW family of HIF regulators

We have recently identified the LIM domain-containing proteins LIMD1, Ajuba and WTIP (LAW) as a unique family of regulators of the hypoxic response [25]. Unlike other regulators identified to date, the LAW proteins are able to interact simultaneously with PHDs and VHLs in a multimeric complex. This enables optimum HIF-1 α degradation efficiency due to the increased proximity of the hydroxylation and ubiquitination enzymes compared with LAW-independent PHD/VHL degradation. The identification of LAW gives further insight into the ability of hypoxic regulatory proteins to confer specificity within the hypoxic response. Our recent discoveries demonstrated that LAW LIM domain family members are able to interact with one or more of the PHD and VHL isoforms *in vivo*. LIMD1 is able to bind to PHD1, 2 and 3 and simultaneously interact with the p30 isoform of VHL, while Ajuba and WTIP interact with

both p19 and p30 VHL isoforms, but cannot bind to PHD2.

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The existence of these protein complex variants raises interesting questions about their function; for example, would a PHD1-WTIP-p19 VHL complex confer a different effect upon HIF-1 α degradation than a PHD2-LIMD1-p30 VHL complex? It is possible that these complexes are cell-type specific, or that they enable specificity for PHD substrate recognition (HIF-1/2 α) and hence specificity for inhibition of HIF transcriptional targets. Our study demonstrated that depletion of LIMD1 using RNAi resulted in enhanced transcription of a number of HIF target genes. Interestingly, the effect of LIMD1 depletion upon *BNIP3* (BCL2/adenovirus E1B 19 kDa interacting protein 3) and other HIF target genes was dependent on hypoxic conditions, increased transcription of *JMJD1A* and *ALDOC* was observed irrespective of pO_2 . This indicates that LIMD1 may have gene-specific or O_2 tension-specific responses, and hence its role in the degradation of HIF-1 α may enable inhibition of specific HIF target genes.

Targeting the regulator proteins of HIFs may enhance specificity in cancer treatments

The increasing identification of novel regulators of the hypoxic response may represent a possible explanation of how the simple biochemical or kinetic properties of the hydroxylase-ubiquitin ligase couple may be adapted to provide tissue specificity, length of response and specification of target genes necessary for physiological oxygen homeostasis, and may also provide us with increasingly precise targets for drug development and cancer treatment. A substantial number of antitumorigenic HIF-1-inhibiting drugs have been identified, including the antioxidant *N*-acetyl cysteine, which enhances HIF-1 α degradation via the PHD2-VHL pathway, inhibiting lymphomagenesis [26,27]. It is therefore conceivable that such cancer therapeutics could also target the precise interfaces within hypoxic regulator protein-PHD-VHL complexes. This could potentially enhance HIF-1 α degradation in a manner

specific for both the tumor tissue type and, if these complexes can in fact render specificity for inhibiting precise HIF target genes, even the genes that are upregulated in the tumor. As such, it is essential that the molecular mechanisms of regulatory proteins of the hypoxic response remain an important focus in molecular oncology research, as they hold the potential to enhance targeting and improve treatment efficacy of HIF-induced cancers.

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