SIGNALING

Histone modifiers are oxygen-sensors

Hypoxia signals directly to chromatin via Histone demethylases to alter gene expression

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Approximately 2.6 billion years ago during the Proterozoic period, the evolution of photosynthesis in cyanobacteria led to the introduction of the by-product of this reaction, oxygen, into the Earths atmosphere (*1*). This great oxidative event heralded the rise of multicellular organisms, which are almost totally dependent on oxygen as an efficient fuel for metabolism and a cofactor in many critical physiological enzymatic reactions. Central to this adaptation, and to allow cellular physiology across a wide range of oxygen concentrations (tensions), metazoans have evolved the highly conserved hypoxia inducible factor (HIF) pathway (*2*). This is important for both physiological and pathological processes that occur in a hypoxic microenvironment, including embryogenesis, stem cell homeostasis, cancer and cardiovascular disease. It has long been observed that hypoxia induces histone lysine hypermethylation, a form of epigenetic chromatin modification. However, whether this represents a direct sensing of oxygen tension or an indirect effect, perhaps through the HIF pathway, has not been established (*4*). On page XXX and YYY of this issue, Batie *et al.* (*5*) and Chakraborty *et al.* (*6*) resolve this question, demonstrating in different cellular systems that the activity of the lysine-specific demethylases (KDM), KDM5A and KDM6A, is oxygen sensitive, identifying them as oxygen sensors (*5, 6*).

In ambient normoxic conditions HIF-1α, the DNA-binding component of the heterodimeric transcription factor complex, HIF, is targeted for ubiquitylation and destruction. This occurs through hydroxylation on proline residues in HIF-1α by the EglN family of prolyl hydroxylases (PHDs), which are 2-oxoglutarate and oxygen dependent dioxygenase enzymes that sense physiological changes in oxygen tension, being activated in normoxia. However, in hypoxic conditions, PHD activity is lost and so HIF-1α is stabilised so it can bind to its partner ARNT (also called HIF-1β). The HIF complex translocates to the nucleus and induces hypoxia-specific gene expression programmes that mediate alter cellular metabolism and survival, through binding to specialised hypoxia response elements (HRE) in gene promoters. The family of 2-oxoglutarate and oxygen dependent dioxygenases is large with more than 60 members (*3*), and also includes the TET and JmjC KDMs families of epigenetic regulators.

Using biochemical analysis of recombinant proteins, Batie *et al.* and Chakraborty *et al.* add KDM5A and KDM6A to the list of dioxygenases that have low oxygen affinities (K_M values) comparable to the EglN PHD family. Batie *et al.* then used time course experiments to show that histone methylation changes following the induction of hypoxia were rapid and preceded, although predictive of subsequent transcriptional events, preceded these changes. Importantly, in cellular systems expressing loss-of-function and gain-of-function mutant proteins in the HIF pathway and through documenting the speed of HIF-1α stabilisation following induction of hypoxia, histone methylation changes were found to be independent of HIF and also not dependent on other known hypoxia-inducible inhibitors of KDM activity, such as reactive oxygen species and 2-hydroxyglutarate. Linking direct histone hypermethylation to cellular function, Chakraborty *et al.* demonstrated that hypermethylation of lysine 27 of histone H3 (H3K27), a histone change that is associated with gene repression, prevented differentiation in different cell line model systems . Conversely, these effects could be antagonised by inhibition of the reciprocal histone methyltransferase, enhancer of zeste homolog 2 (EZH2). Batie *et al.* focussed on histone methylation modifications associated with gene activation, lysine 36 of histone H3 (H3K36) and particularly trimethylation of lysine 4 of histone 3 (H3K4me3). They identified KDM5A as responsible for the hypermethylation of H3K4 in their HeLa, human cervical cancer cell line system and linked H3K4me3 to the induction of enhancer (long range promoters of transcription) activity and both HIF-dependent and –independent promoter function. Both studies report preliminary data regarding the structural basis of differences in oxygen affinity between these two KDMs.

These two complementary studies further clarify the regulatory mechanisms of histone demethylases and specifically how these directly, rather than through the HIF pathway or via the influence of metabolic intermediates coordinate a range of epigenetic alterations, transcriptional outputs and cell fate decisions in response to external environmental changes (see the figure). However, these studies raise as many important questions as they answer. The alteration of a large number of histone modifications evident in the multiplexed mass spectrometric assay upon hypoxia and described in the literature (*5, 7, 8*) suggests that the full identity of oxygen-sensitive JmjC KDMs is not yet known. For example, the increased H3K36me3 in response to hypoxia, is not linked to loss of KDM5A or KDM6A activity and so the responsible KDM needs to be identified. Further studies are therefore warranted, with this knowledge not only informing biology but also facilitating elucidation of the structural basis of oxygen affinity in KDMs.

Additionally, the HIF pathway has been implicated in an array of physiological and pathological cellular processes and it is likely that the direct oxygen-sensing KDM pathways are similarly implicated in a number of these processes. However, the exact nature of the pathways involved and whether they function independently and/or cooperatively with HIF-mediated transcriptional programmes remains to be determined. Malignancies often develop and/or metastasise to hypoxic environments and activation of the HIF pathway is frequently observed in cancer (*9, 10*). Moreover, multiple mutations of epigenetic regulators are described in malignancies and both loss-of-function mutations in many histone methyltransferases and KDMs (*11, 12*), potentially mimicking hypoxia are observed . Moreover, therapeutics that target chromatin modifiers are being evaluated for treating certain cancers in clinical trials (*13*). We speculate that the direct oxygen-sensing KDM pathways are also aberrant in malignancy and other pathological states, such as cardiovascular disease. Targeting the HIF pathway with small molecule inhibitors is currently being explored in cancer therapy and other non-malignant conditions such as renal disease. These studies suggest that oxygen sensing in KDMs might also be specifically therapeutically targeted, if the mechanistic basis for this sensing is determined.

Interestingly, Batie *et al.* and Chakraborty *et al.* speculate that based on phylogenetic sequence conservation the direct oxygen-sensing KDM pathways may evolutionarily pre-date the HIF pathway. It is likely that

both pathways have more recently co-evolved and function in a coordinated and temporally defined manner to modulate the cellular response to low oxygen tensions. This is suggested by the immediate hypermethylation of H3K4 at the promoters of HIF-target genes,. However, further delineating the interaction between these pathways and how this might be modulated will be important to understand. Together, these observations have profound implications for our understanding of how microenvironmental changes, and specifically oxygen concentrations, might affect both physiological and pathological cell fate decisions and phenotypes through direct effects on chromatin structure.

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Hypoxia-mediated alterations in transcription via direct (HDM-mediated) and indirect (HIF-mediated) mechanisms

In hypoxic conditions, prolyly hydroxylases (PHDs) are inactivated, allowing HIF-1α to dimerize with ARNT, translocate to the nucleus and activate HIF target genes. Chakraborty *et al*. and Batie *et al*. find that the lysine demethylases, KDM6A and KDM5A, are also direct oxygen sensors that are inactivated during hypoxia. This allows both immediate hypermethylation of H3K27 (KDM6A target) and gene repression and hypermethylation of H3K4 (KDM5A target) and gene activation. Under normoxic conditions, the HIF-1α subunit is targeted for destruction through hydroxylation by PHDs. This allows ubiquitylation by the Von-Hippel Lindau tumor suppressor protein (VHL), and subsequent destruction in the proteasome.