Epigenomics

Epigenetic regulation by histone demethylases in hypoxia

The response to hypoxia is primarily mediated by the hypoxia-inducible transcription factor (HIF). Levels of HIF are regulated by the oxygen-sensing HIF hydroxylases, members of the 2-oxoglutarate (2OG) dependent oxygenase family. JmjC-domain containing histone lysine demethylases (JmjC-KDMs), also members of the 2OG oxygenase family, are key epigenetic regulators that modulate the methylation levels of histone tails. Kinetic studies of the JmjC-KDMs indicate they could also act in an oxygen-sensitive manner. This may have important implications for epigenetic regulation in hypoxia. In this review we examine evidence that the levels and activity of JmjC-KDMs are sensitive to oxygen availability, and consider how this may influence their roles in early development and hypoxic disease states including cancer and cardiovascular disease.

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Oxygen is essential for eukaryotic cellular respiration, and cells cannot survive for prolonged periods without it. Although the air we breathe is at an ambient oxygen concentration of 21%, this drops as oxygen is transported to the tissues of the body, where 'normoxic' levels are variable: oxygen concentrations in the brain, lungs and kidneys are 4.4, 5.6 and 9.5%, respectively (Figure 1) [1]. Oxygen diffuses down a concentration gradient and thus tissues with high perfusion, for example, the kidneys have higher oxygen concentrations. Low oxygen levels, termed 'hypoxia', can disrupt normal cellular processes.

Hypoxia is usually defined as an oxygen concentration of ≤2% [4], and may have a number of causes. These include environmental exposure, such as high altitude and vigorous exercise, or a number of disease states. Hypoxia is a key pathological feature of cancer and cardiovascular diseases, predominantly due to localized reduction in tissue perfusion. Conversely, low oxygen conditions during early embryogenesis are essential to normal embryonic development [5].

Hypoxia, HIF & the HIF hydroxylases

Hypoxic conditions induce an adaptive response, which occurs on both a cellular and physiological level. Cellular responses include changes in metabolism (oxidative phosphorylation to glycolysis), autophagy, and partial shut-down in high-energy consumption processes, while physiological outcomes include erythropoiesis and angiogenesis. In animals, these responses are primarily mediated by the Hypoxia Inducible transcription Factor, HIF. The HIF-mediated hypoxic response has been well characterized and recent reviews include those by Kaelin and Ratcliffe [6], Myllyharju [7] and Semenza [8].

Briefly, HIF is an α/β dimer consisting of a DNA-binding basic helix-loop-helix (bHLH) region, a PAS (Per-ARNT-Sim) domain that mediates dimerization, an oxygen-dependent degradation domain (in HIFα) and a C-terminal transactivation domain. A stable dimer
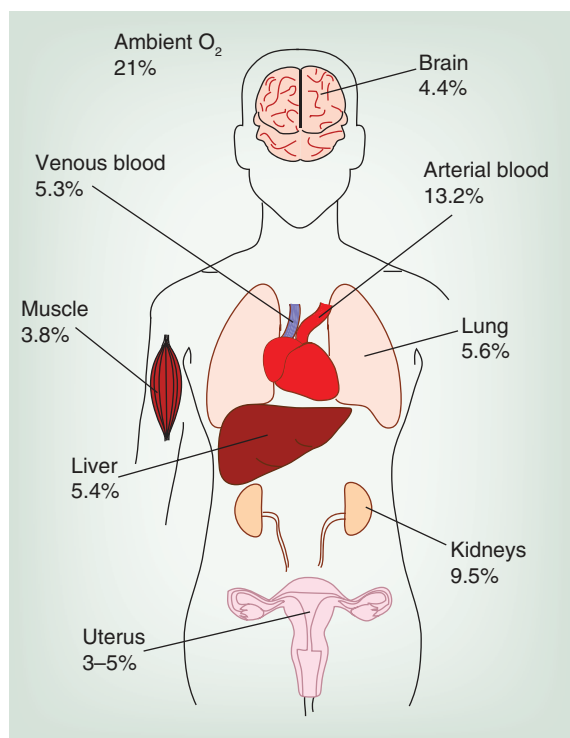
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Figure 1. Oxygen concentrations as measured in selected mammalian tissues. Most tissues exist at oxygen concentrations well below ambient levels, typically 3–5% [1–3]. The definition of hypoxia is an oxygen concentration of ≤2%, while concentrations of ≤0.02% oxygen are defined as severe hypoxia or anoxia [4].

binds to hypoxia response elements (HREs) upstream of an array of genes that enable the hypoxic response (Figure 2A). There are several hundred known genes with HIF-binding HREs in their promoter regions, with functions as diverse as cell proliferation, angiogenic signaling and altered metabolism [9]. Responses are cell-type specific and can be mediated by HIF isomers HIF-1 or HIF-2, further tailoring targeted gene upregulation [10]. HIF is therefore referred to as the master regulator of the hypoxic response [11].

HIF levels are not directly regulated by oxygen but instead HIFα levels are regulated by oxygen-dependent enzymes which post-translationally hydroxylate specific residues in HIF (Figure 2A & Figure 3B). Hydroxylation of one or both of two prolyl residues in HIFα (Pro-402 or Pro-564 in HIF-1α) within the *N*- and *C*-terminal oxygen dependent degradation domains (NODD and CODD, respectively) targets HIFα for ubiquitination and degradation by the proteasome. Hydroxylation of an asparaginyl residue in the *C*-terminal transactivation domain (CAD) of HIFα (Asn-803 in HIF-1α) prevents its interaction with cotranscriptional activators. These post-translational modifications are catalyzed by the prolyl hydroxylases (PHD1–3) and Factor

Inhibiting HIF (FIH) respectively, collectively termed the HIF hydroxylases. In hypoxic conditions, the activity of these oxygen-dependent enzymes is reduced so HIFα levels and activity are maintained. This enables it to dimerize with HIFβ and promote the hypoxic response. The dependence of the HIF hydroxylases on oxygen has led to their classification as cellular oxygen sensors.

The HIF hydroxylases are members of the Fe(II)/2-oxoglutarate (2OG) dependent oxygenase family of enzymes. This enzyme family is widespread in nature, catalyzing a range of oxidative reactions including hydroxylation, demethylation, desaturation, ring closure, ring cleavage, epimerization, rearrangement and halogenation [17]. There are more than 60 known 2OG oxygenases in humans, with roles in carnitine biosynthesis, epigenetic regulation, signaling and many others [18]. Structural, kinetic and spectroscopic evidence over the past two decades has revealed a conserved structure and mechanism within the 2OG oxygenases [17]. The core structural feature of these enzymes is a double-stranded β helix (DSBH) jelly roll motif supporting an active site catalytic triad of His-X-Asp/Glu-X_n-His that coordinates Fe(II) (Figure 3C). 2OG coordinates to the active site Fe(II) via its carboxylate and ketone oxygen atoms [19]. The 2OG oxygenases act via a conserved mechanism (Figure 3A): Prime substrate binds to an Enz.Fe(II).2OG complex causing a conformational change that weakens H_2O binding to the sixth coordination position on the active site Fe(II), thus enabling oxygen to bind. Oxidative decarboxylation of 2OG to succinate and carbon dioxide results in formation of a highly reactive Fe(IV)=O species which carries out the oxidation step on an unactivated C-H bond [17,19].

To date, the HIF hydroxlases are the only members of the 2OG oxygenase family whose known prime physiological function is to act as cellular oxygen sensors. In order to effectively fulfill this role, the HIF hydroxylases need to sensitively respond to changes in oxygen concentration. This has been observed for the PHDs both biochemically and in cells. NODD and CODD hydroxylation in RCC4 cells [21,22] was shown to reduce over a gradient from normoxia to severe hypoxia (<0.02%). *In vitro* K_M values for oxygen for recombinant PHD2 have been reported to range between 230 μM and 1.7 mM, the highest of all reported 2OG oxygenases (Table 1) [23–28], while pre-steady state kinetic investigations revealed a slow reaction of PHD2 with respect to oxygen [27,29]. These results are indicative that PHD2 activity is highly dependent on oxygen. Of note, PHD2 is considered the most important of the HIF hydroxylases in normal conditions [30], and PHD2-/- mice are embryonic lethal [31].

Despite being a HIF hydroxylase, FIH does not appear to be as sensitive to oxygen as PHD2; its K_M value for oxygen is reported to be lower at 90–150 μM (Table 1) [24,25], and CAD hydroxylation is maintained in RCC4 cells exposed to an environment of 0.25% oxygen [22]. Further, FIH also functions as an ankyrin repeat domain (ARD) hydroxylase [33,34], and has been reported as having higher affinities for these alternative substrates [35], with a higher affinity for oxygen in reactions with ARDs than HIF substrates [32]. The significance of FIH in the HIF-mediated hypoxic response is therefore yet to be fully ascertained, particularly as

minimal HIF-related effects were observed in FIH null mice [36].

While some studies have reported on the oxygen kinetics of other 2OG oxygenases (Table 1), including K_M(oxygen) values for the collagen prolyl hydroxylase C-P4H and the mature phytanoyl-CoA hydroxylase mPAHX of 40 μM [23] and 93 μM [24] respectively, and fast pre-steady state kinetic activation by oxygen of TauD [37] and a viral collagen prolyl hydroxylase [38], detailed kinetic studies of other human 2OG oxygenases with respect to oxygen have so far been limited. Nevertheless, the dependence of this enzyme
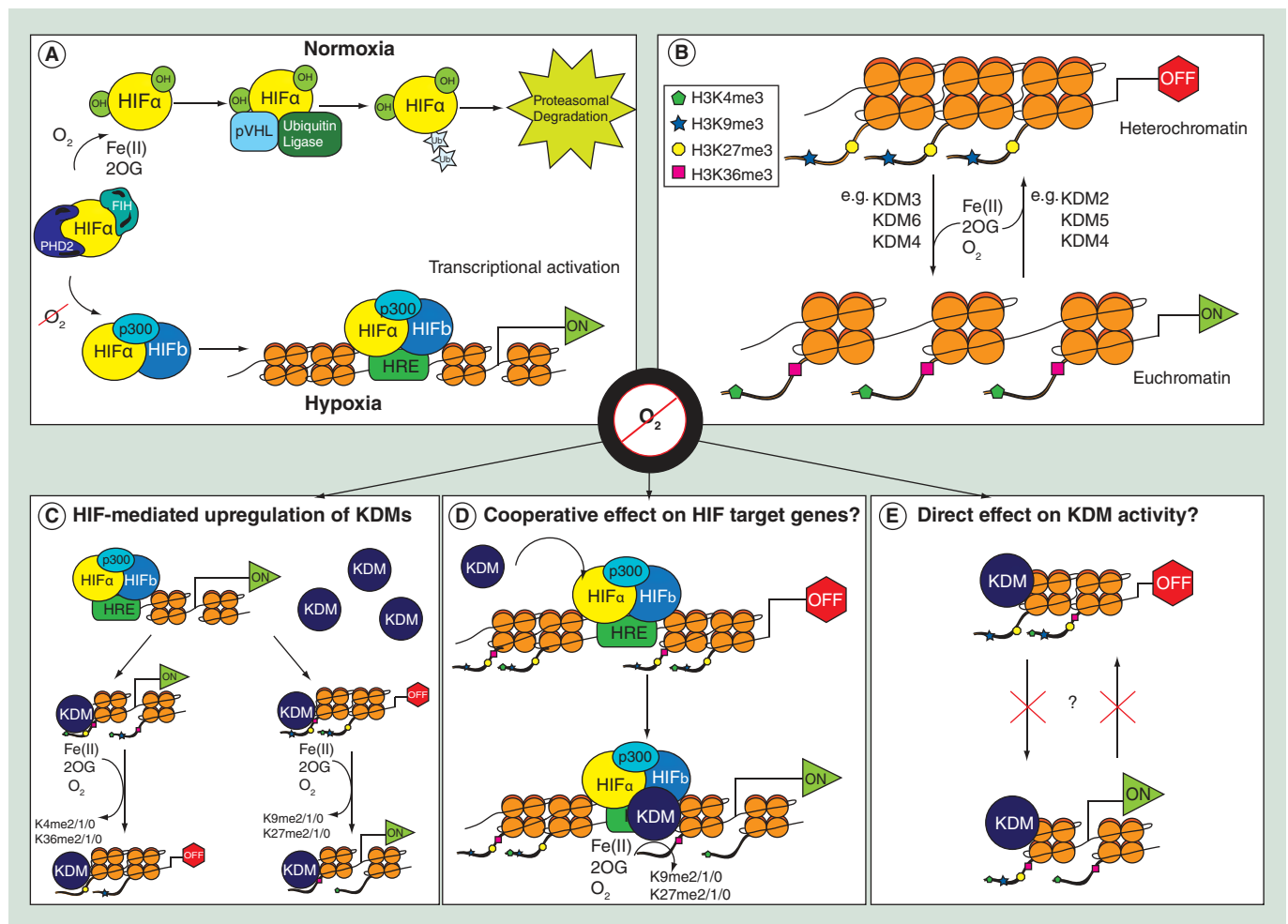

Figure 2. Transcriptional regulation by Fe(II)/2OG oxygenases. (A) The HIF-mediated hypoxic response. Under normal oxygen conditions, PHD enzymes (PHD2 is shown) and FIH hydroxylate the HIFα subunit, enabling binding of pVHL and ubiquitination by ubiquitin ligase, targeting HIFα for proteasomal degradation. In hypoxia, HIFα is not hydroxylated and translocates into the nucleus where it binds to the HIFβ subunit and the p300 coactivator. The HIF-p300 complex then binds to HREs at gene promoter regions, activating transcription [9]. **(B)** Epigenetic regulation by histone demethylases. KDMs remove methylation marks on histone tails. Changes in the methylation states of histones (and in combination with other histone modifications) can lead to either heterochromatin or euchromatin formation [12]. **(C)** Upregulation of the KDMs in hypoxia is proposed to increase demethylation of methylated lysine residues, altering gene expression [13]. **(D)** The potential for a co-operative effect between HIF and KDMs has been suggested. It is proposed that HIF recruits the KDMs to the gene locus, enabling removal of repressive marks and activation of gene expression [14,15]. **(E)** Direct oxygen-dependent changes in KDM activity and gene regulation [16]. HIF-independent induction of KDMs is also possible. In all cases **(C–E)**, changing oxygen concentrations may have the potential to affect KDM activity, thus having a direct impact on transcriptional regulation. HRE: Hypoxia response elements.

family on oxygen and their importance in human physiology suggests that other human 2OG oxygenases may be affected by oxygen availability, with implications for hypoxic disease states. In this review we discuss the evidence that JmjC-domain containing histone lysine demethylases (JmjC-KDMs), a 2OG oxygenase family of enzymes involved in epigenetic regulation, act in an oxygen-sensitive manner, and discuss these findings with respect to dynamic changes in histone methylation patterns during development leading to a regulated transcriptional program, and dysregulation of KDMs and global methylation patterns in hypoxic disease states.

Histone demethylation by the JmjC-KDMs

In eukaryotic cells, DNA is wound around a histone octamer, which consists of two copies of each of four subunits–H2A, H2B, H3, and H4. These DNA-histone complexes, known as nucleosomes, pack together
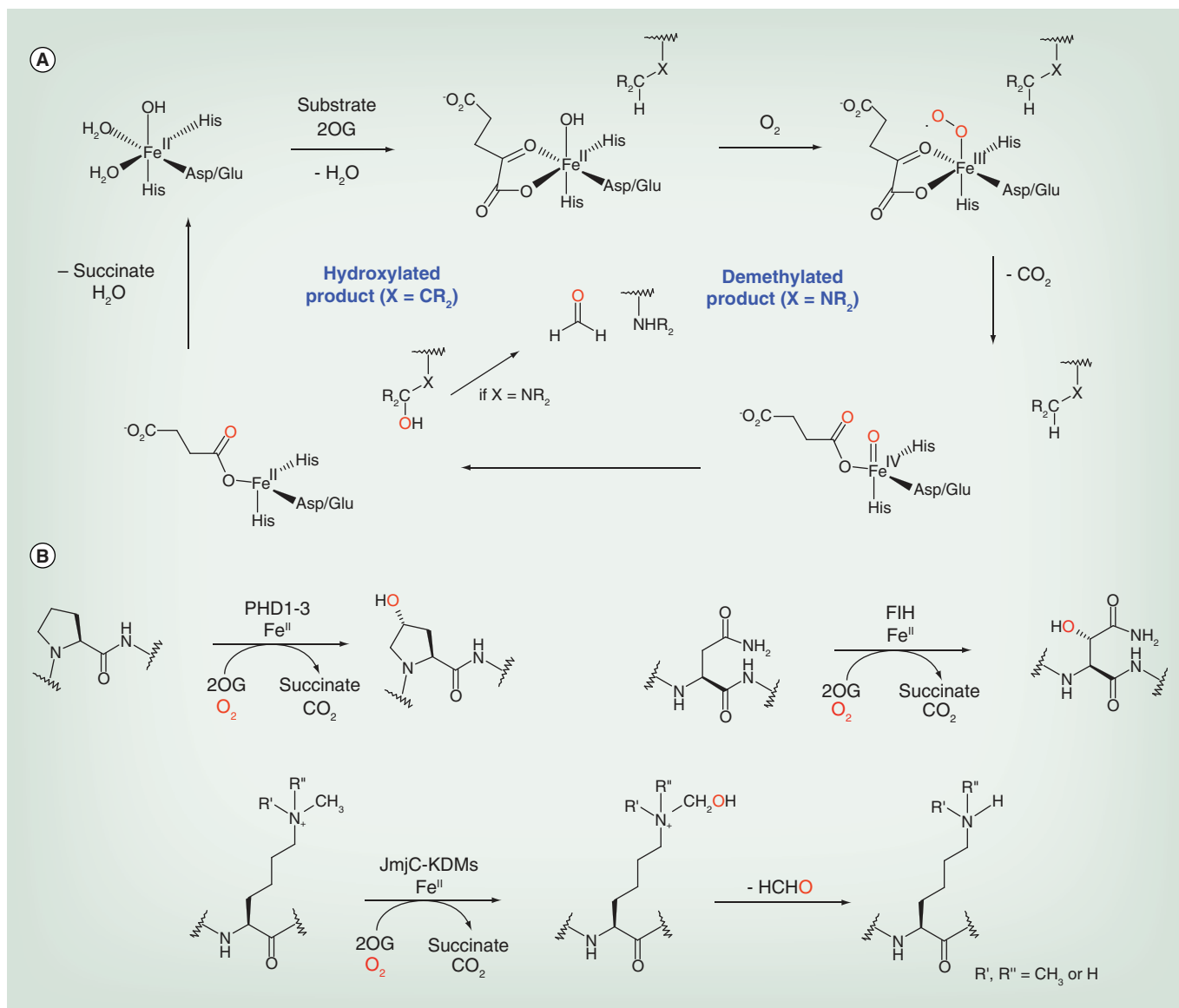

Figure 3. Conserved structural and mechanistic features of the JmjC-KDMs and HIF hydroxylases. (A) General mechanism of the Fe(II)/2OG oxygenase family of enzymes. **(B)** PHDs (PHD1–3) hydroxylate prolyl residues 402 and 564 in the *N*- and *C*-terminal Oxygen Dependent Degradation Domains of HIFα, to produce *trans*-4-hydroxyproline, while FIH catalyzes *trans*-β hydroxylation of asparagine 803 in the *C*-terminal activation domain of HIFα [9]. JmjC-KDMs hydroxylate $^\varepsilon$*N*-methylated lysine residues in the *N*-terminal tails of histone proteins, to produce an unstable hemiaminal intermediate, which generates formaldehyde and demethylated lysine [51].**(C)** Ribbon representation of KDM4A, FIH and PHD2 (PDB ID: 2OX0, 2XUM, 3HQR respectively) showing double-stranded β-helix (teal) and α-helices (white). Stick representation of active site His-X-Asp/Glu-Xn-His Fe(II) binding motif residues (magenta, pink, purple) common to this class of enzymes. *N*-oxalylglycine, an inhibitory 2OG analog is shown bound in the 2OG binding site of these enzymes (yellow). 2OG: 2-oxoglutarate.

Figure 3. Conserved structural and mechanistic features of the JmjC-KDMs and HIF hydroxylases (cont.). (A) General mechanism of the Fe(II)/2OG oxygenase family of enzymes. **(B)** PHDs (PHD1–3) hydroxylate prolyl residues 402 and 564 in the *N*- and *C*-terminal Oxygen Dependent Degradation Domains of HIFα, to produce *trans*-4-hydroxyproline, while FIH catalyzes *trans*-β hydroxylation of asparagine 803 in the *C*-terminal activation domain of HIFα [9]. JmjC-KDMs hydroxylate $^{\varepsilon}N$-methylated lysine residues in the *N*-terminal tails of histone proteins, to produce an unstable hemiaminal intermediate, which generates formaldehyde and demethylated lysine [51].**(C)** Ribbon representation of KDM4A, FIH and PHD2 (PDB ID: 2OX0, 2XUM, 3HQR respectively) showing double-stranded β-helix (teal) and α-helices (white). Stick representation of active site His-X-Asp/Glu-Xn-His Fe(II) binding motif residues (magenta, pink, purple) common to this class of enzymes. *N*-oxalylglycine, an inhibitory 2OG analog is shown bound in the 2OG binding site of these enzymes (yellow). 2OG: 2-oxoglutarate.
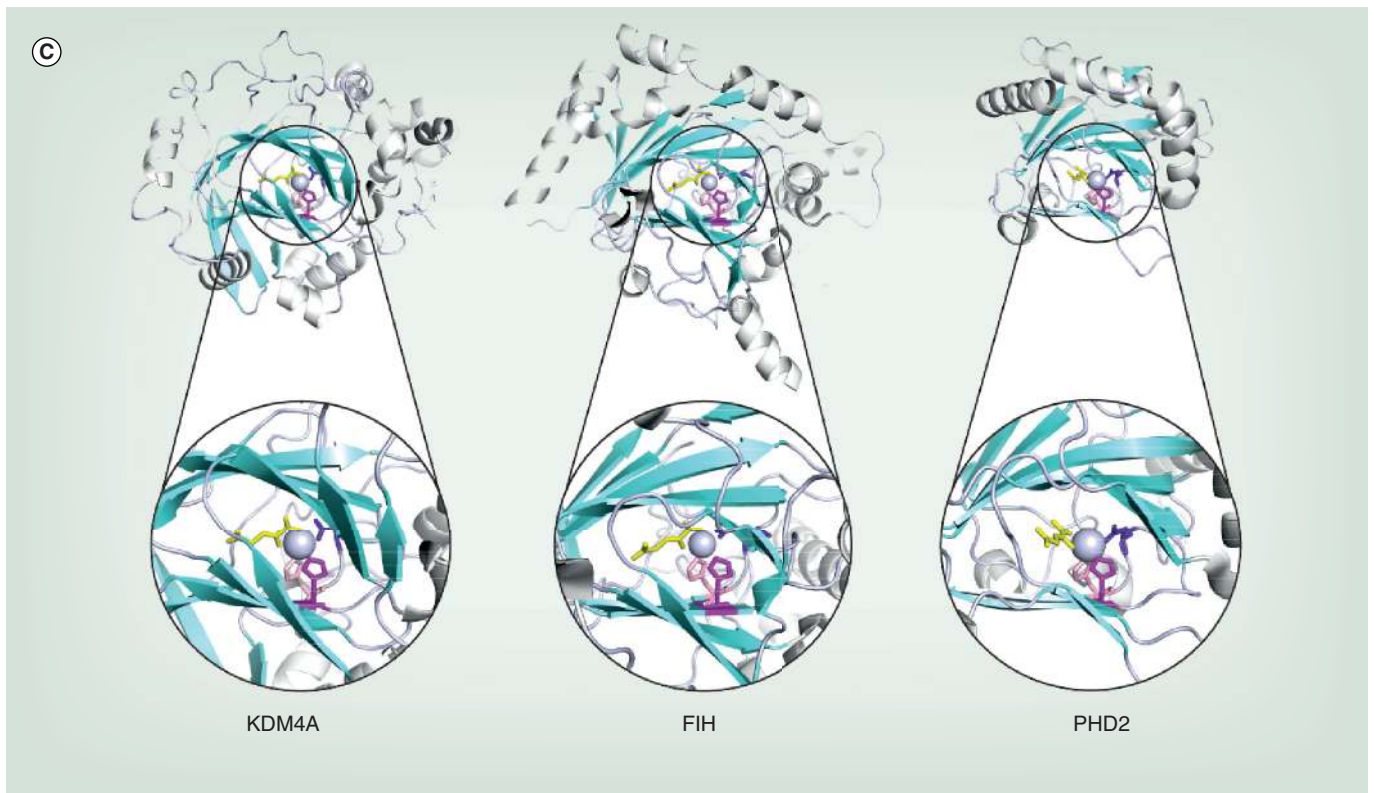
to form chromatin. Chromatin can take an open form, euchromatin, which allows transcriptional machinery to access DNA, or a closed form, heterochromatin, in which the DNA is inaccessible (Figure 2B) [12]. Dynamic covalent modifications to DNA and the unstructured *N*-terminal tails of histones alter chromatin structure thus modulating gene expression and forming the basis of epigenetic regulation [12]. Multiple post-translational modifications are found on histone tails, including acetylation, phosphorylation, ubiquitination and methylation [39].

Whether a given histone modification is activating or repressive is dependent on which residue (and originating histone subunit) is modified, the type of modification, the degree of modification (in the case of methylation) and the combination of these modifications – this is also referred to as the histone code [40]. Histone tail lysines may be mono-, di- or tri-methylated (Figure 3B). In general, methylation at H3K4, H3K36 and H3K79 is associated with active genes, whereas methylation at H3K9, H3K27 and H4K20 correlates with repressed

genes [41]. The distribution of histone methylation across the genome is an important factor in gene transcription. For example, in active genes, H3K4me3 is generally enriched in promoter and transcriptional start sites (TSS), whereas H3K79 and H3K36 methylations are abundant within the gene body itself. On the other hand, in repressed genes, H3K27me3 is generally enriched in the promoter region, and H3K9me2/3 and H4K20 marks are uniformly distributed throughout the gene [42]. Both activating (H3K4 methylation) and repressive marks (H3K27 methylation) are also found to co-exist as bivalent chromatin in embryonic stem cells (ESCs) [43].

Histone lysine methylation is dynamically regulated by histone methyltransferases (KMTs) and histone demethylases (KDMs). Methylation of lysine residues is carried out by histone KMTs, a family of enzymes that utilize *S*-adenosylmethionine (SAM) as a methyl-group donor, and include the SET domain family [44,45] and DOT1L [46]. Methylation is removed by histone demethylases (KDMs). Two mechanistically distinct

Table 1. Differential sensitivity of 2OG oxygenases to oxygen *in vitro*.			
Enzyme	$K_M(O_2)/\mu M$	**Substrate**	**Ref.**
PHD2$_{181-426}$	230-1746	HIF-1α$_{556-574}$ CODD	[24][†], [23][‡], [27][§], [28][#]
C-P4H	40	Pro-Pro-Gly	[23][‡]
mPAHX	93 ± 43	Isovaleryl CoA	[24][†]
TauD	76 ± 17	Taurine	[24][†]
FIH	90-150	HIF-1α$_{788-822}$ CAD	[24][†], [25][‡], H Tarhonskaya[§] [Unpublished Data]
FIH	110 ± 73	HIF-2α$_{832-866}$ CAD	[24][†]
FIH	100 ± 10	HIF-2α$_{832-866}$ CAD	H Tarhonskaya[§] [Unpublished Data]
FIH	10–120	Non-HIF substrates	H Tarhonskaya[§] [Unpublished Data] [32]
KDM4A$_{1-359}$	57 ± 10	H3$_{7-14}$K9me3	[26][†]
KDM4C$_{1-366}$	158 ± 13	H3$_{7-14}$K9me3	[26][†]
KDM4E$_{1-337}$	197 ± 16	H3$_{7-14}$K9me3	[26][†]
KDM4E$_{1-337}$	>93	H3$_{7-14}$K9me3	[20][§]

$K_M(O_2)$ values have been determined for a number of 2OG oxygenases using various methods:
[†]Oxygen consumption assay.
[‡]Radioactive 2OG turn over assay.
[§]Matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS) assay.
[#]Time-resolved fluorescence energy transfer (TR-FRET)-based assay.

families of histone demethylases have been identified: the LSD1 family of flavin-dependent monoamine oxidases (KDM1) and the JmjC-KDMs (Figure 2B). The JmjC-KDMs, the larger of the two KDM families, are members of the 2OG oxygenase family. Structural studies and sequence alignments reveal that all JmjC-KDMs contain a catalytic JmjC-domain with a conserved core DSBH structural motif, as exemplified by the structure of KDM4A in Figure 3C [47–49]. Evidence to date, including structural and spectroscopic observations of Enz.Fe(II).2OG.substrate complexes and kinetic investigations, indicates that the JmjC-KDMs conform to the proposed 2OG consensus mechanism (Figure 3 A & B) [20,50]. The prime substrate of the JmjC-KDMs is the unactivated C-H bond of ᵋN-methyl lysine residues on histones. The JmjC-KDMs catalyse hydroxylation of this bond, resulting in an unstable hemiaminal intermediate that fragments to produce the demethylated lysine residue and formaldehyde (Figure 3B).

The JmjC-KDMs have been categorized into 7 KDM subfamilies (KDM2–8) based on the JmjC-domain sequence homology and their demethylase activities (Table 2) [51,52]. Different JmjC-KDMs exhibit different methylation state and histone substrate specificities [52,53]. While KDM2/3/7 subfamilies are di- and monomethyl demethylases, KDM4–6/8 can also remove tri-methyl marks (Table 2). Some KDM subfamilies are highly specific for particular histone substrates whereas some have overlapping substrate selectivities. For example, the KDM5 and KDM6 members are specific for methylated H3K4 and H3K27, respec-

tively. Further, all KDM4 members can demethylate H3K9, but KDM4A-C can also demethylate H3K36 and H1.4K26 (Table 2) [51]. Most, but not all, JmjC-KDMs have domains in addition to the catalytic domain. Many contain binding domains (e.g., CXXC DNA-binding domain, Plant homeodomain [PHD] fingers, Tudor domains), which assist KDM recruitment to certain chromatin regions and/or enable substrate specificity. For instance, the PHD-finger domain of PHF8/KDM7B binds to H3K4me3 and promotes removal of the H3K9me2 mark via the catalytic domain [54]. Furthermore, JmjC-KDMs have been found in large complexes with multiple proteins including complementary KMTs and other epigenetic proteins [55]. Combinations of various marks on histone tails provide functional crosstalk, and the JmjC-KDMs and their associated complexes are key players in fine-tuning transcriptional regulation.

Histone methylation & hypoxia
Multiple studies demonstrate that exposure to hypoxia can alter the epigenetic landscape at the chromatin level in cells [14,16,64–70] (Table 3). Similar changes to epigenetic marks, including histone lysine methylation, have been observed in development and in disease states where hypoxia is known to be an important feature. The mechanisms for these alterations appear to be both direct (via altered JmjC-KDM activity or expression levels) and indirect (in a HIF-mediated manner): some JmjC-KDMs are known to be direct targets of HIF and thus their levels are upregulated in hypoxia. These include KDM3A,

Table 2. Histone substrate selectivity of JmjC-KDMs in cells.

JmjC-KDM	H3K4			H3K9			H3K27			H3K36			H3K79			H4K20			H1.4K26			O₂	HIF	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Ind	Ind	Bind
KDM2A/FBXL11/JHDM1A										✓	✓											+		
KDM2B/FBXL10/JHDM1B		✓								✓	✓											+		
KDM3A/JMJD1A				✓	✓																	+	+	+
KDM3B/JMJD1B				✓	✓																	+		
KDM3C/JMJD1C				✓	✓																	+		
HR				✓	✓																			
KDM4A/JMJD2A					✓	✓					✓	✓								✓	✓			
KDM4B/JMJD2B					✓	✓					✓	✓								✓	✓	+	+	
KDM4C/JMJD2C					✓	✓					✓	✓								✓	✓	+	+	+
KDM4D/JMJD2D					✓	✓																+		
KDM5A/JARID1A/RBP2	✓	✓																				+		
KDM5B/JARID1B/PLU1	✓	✓																				+	+	
KDM5C/JARID1C/SMCX	✓	✓																				+	+	
KDM5D/JARID1D/SMCY	✓	✓																				+		
KDM6A/UTX								✓	✓													+		
KDM6B/JMJD3								✓	✓													+	+	+
KDM7A/KIAA1718				✓	✓		✓	✓														+		
KDM7B/PHF8				✓	✓												✓					+		
PHF2				✓	✓																			
KDM8/JMJD5											✓	✓										+		
MINA53						✓																		
NO66	✓	✓									✓	✓												

The JmjC-KDMs catalyse the demethylation of mono- (1) di- (2) and/or tri- (3) methylated lysine residues on the *N*-terminal tails of histones 3 (H3), 4 (H4) and 1.4 (H1.4) and have defined substrate specificities in cells [51], although broader substrate profiles for *in vitro* catalytic domains have been shown [53]. No KDMs for H3K79 methylation marks have been identified. The substrate selectivity combines both *in vitro* and cellular data. Some JmjC-KDMs are induced under low O₂ concentrations (O₂ Ind, +) [14,56] and several have been identified as being regulated by HIF (HIF Ind, +) [14,56–61] and/or interacting with HIF (HIF Bind, +, [15,62–63]).
Bind: Binding; Ind: Induced.

KDM4B, KDM4C, KDM5B and KDM5C [14,57–61], and more recently KDM6B (Table 2) [56]. However, other JmjC-KDMs have been found to be upregulated in hypoxia (at the mRNA level) but thus far have not been identified as HIF target genes [14,62,71]. Intriguingly, Beyer *et al.* demonstrated differential sensitivity to hypoxia of overexpressed KDM3A and KDM4B in HeLa cells incubated at 0.2% oxygen. Demethylation of H3K9me3 by KDM4B was attenuated, while the activity of KDM3A was maintained under the same conditions [57]. It has been proposed that the upregulation of KDMs acts as a compensatory mechanism whereby increased levels of these enzymes compensate for their reduced activity in depleted oxygen conditions [14,57]. However, the disparity in apparent oxygen sensitivity of two HIF-target JmjC-KDMs revealed in this study suggests that the relationship between HIF and the JmjC-KDMs may be more complex. Of note, both KDM4C and KDM6B have been shown to bind to HIF in a potential coactivating role (Table 2; [15,72]), and the HIF-mediated upregulation of several JmjC-KDMs in cancer has been well documented (see below).

Given their absolute requirement for oxygen and relationship to the HIF hydroxylases, the question has been raised as to whether the JmjC-KDMs could be similarly sensitive to oxygen availability, in other words, whether their activity diminishes in hypoxic conditions. Biochemical data to validate the sensitivity of JmjC-KDM activity with respect to oxygen are to date limited, partly due to the relatively recent development of robust assays for their activity (including with respect to oxygen). K_M values for oxygen have been reported for KDM4A, KDM4C and KDM4E at 57, 157 and 197 µM, respectively (Table 1), measured using an oxygen-consumption assay with an 8-mer peptide substrate [26]. KDM4E activity measured over a range of oxygen concentrations, 0–20%, detected by mass spectrometry analysis of demethylation of an 8-mer peptide

Table 3. Changes to histone methylation status observed in hypoxia.					
Cell line	% O$_2$	Changes to global methylation	Gene locus and change in expression levels	Altered methylation status at promoter	Ref.
A549	NS	H3K9me2 ↑	-	-	[64]
		H3K9me3 ↑			
Fetal lung type II	2.0	H3K9me2 ↑	*SP-A* ↑	H3K9me2 ↑	[65]
A549, HOS, HEK293	0.5	H3K9me2 ↑	*MLH1* ↓, *DHFR* ↓	H3K9me2 ↑	[66]
Hepa1-6	0.2	H3K4me2 ↑	*AFP* ↓, *ALB* ↓	H3K9me2 ↑	[67]
		H3K4me3 ↑	*EGR1* ↑	H3K27me2 ↑	
		H3K79me2 ↑		H3K9me2 ↓	
		H3K27me3 ↓		H3K27me2 ↓	
		H3K9me2 ↓			
		H3K4me1 ↑			
HepG2	0.1-5	H3K4me2 ↑	-	-	[14]
		H3K4me3 ↑			
		H3K9me2 ↑			
		H3K36me3 ↑			
BEAS-2B, A549	1.0	H3K4me3 ↑	-	-	[68]
RAW264.7		H3K9me2 ↑	*CCL2* ↓, *CCR1* ↓, *CCR5* ↓	H3K9me2 ↑	[16]
		H3K9me3 ↑		H3K9me3 ↑	
		H3K36me3 ↑			
McA-RH7777	1 or 5	H3K9me3 ↑	-	-	[69]

Cellular studies have demonstrated both global increases (↑) or reduction (↓) in methylation at several histone lysine marks in hypoxia. Further analyses of altered gene expression accompanied by locus-specific lysine methylation changes are detailed. Adapted and updated from [70].

substrate confirmed a graded response to oxygen over this range [20]. Pre-steady state kinetic analyses of KDM4E activity with oxygen revealed a slow rate of oxygen activation similar to that observed for PHD2 [20]. These published data (Table 1) indicate differential sensitivity of some JmjC-KDMs toward oxygen suggesting the potential for some, though likely not all, of the JmjC-KDMs to act in an oxygen-sensitive manner [20,26].

Hypoxia & development

The gestation environment is hypoxic, and oxygen concentration varies during development (Figure 4) [73,74]. One single cell, the zygote, gives rise to all cells of an ordered multicellular organism. This is achieved through differentiation, where spatiotemporal regulation of gene expression under epigenetic control causes pluripotent cells to differentiate through increasingly differentiation-restricted cell types to yield a wide range of specialized cell lineages. Differentiation can be regulated by intracellular genetic mechanisms and by the extracellular microenvironment. It is possible that external stimuli, such as oxygen tension, and genetic modulators, such as histone modifications, may interact in a regulatory manner to drive development.

Hypoxia has an essential regulatory role in normal development. Insults to the normal gestational oxygen conditions, both hypoxic and hyperoxic, lead to morphological defects [5,82–83], and oxygen tension has been shown to regulate differentiation potential in various embryonic and induced stem cell models [84]. Incubation of embryos at low oxygen during *in vitro* fertilization has been shown to improve both embryo quality and clinical outcome, possibly because this mimics the conditions *in vivo* [85].

TrX, polycomb & bivalency, & their roles in development

In undifferentiated cells pluripotency genes are activated, while lineage-associated genes are repressed. Upon differentiation pluripotency genes become repressed, while lineage-associated genes are activated, yielding a specialized cell phenotype [86]. Bivalency, the co-localization of one activating and one repressing histone modification, is one potential mechanism by which histone methylation states regulate early development. Bivalent histone states (H3K4me3 and
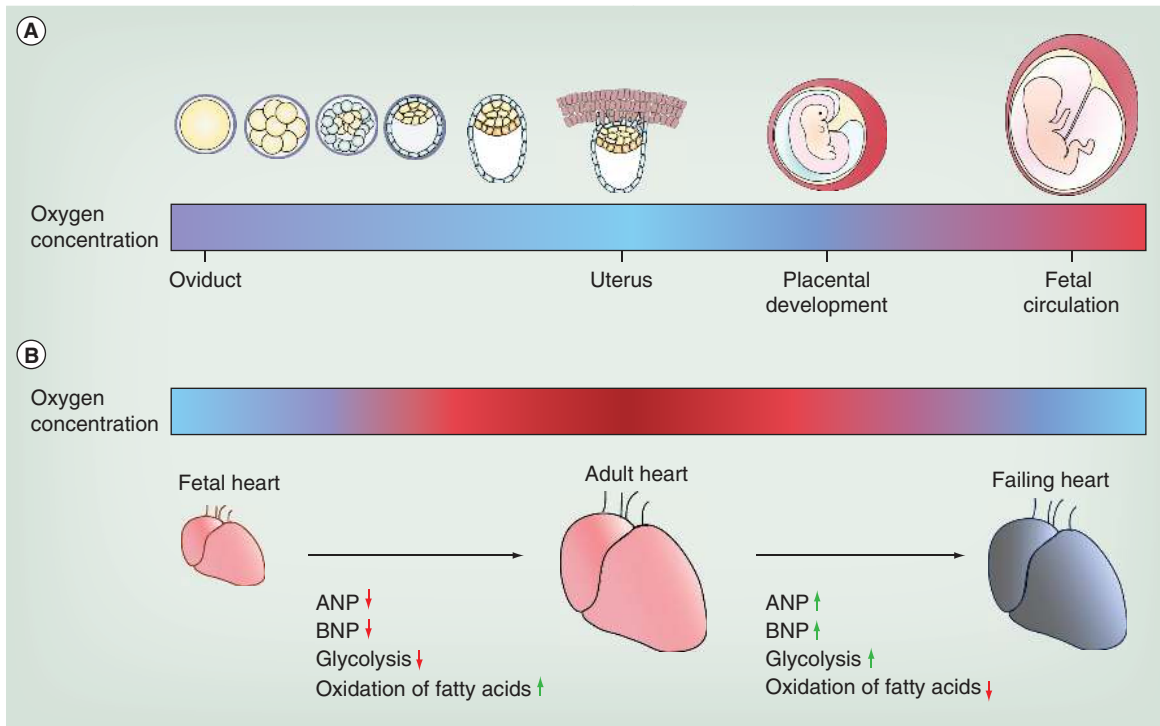
Figure 4. Oxygen environments in development and disease. (A) Oxygen tension in the oviduct and uterus varies between 2–8%. Oxygen available to the early embryo is limited by diffusion from these tissues. After fertilization in the oviduct the embryo migrates, experiencing a decreasing oxygen gradient, to the uterus [3]. Even after placental development, oxygen conditions are still low as the placenta acts as a barrier to oxygen [75]. It is not until fetal circulation is established that oxygen available to the fetus increases [76]. **(B)** The different oxygen environments experienced by the fetal, adult and diseased heart. The fetal heart exists in a hypoxic environment [77], with a postnatal transition into a more oxygenated state [78] accompanied by remodeling and changes in gene expression. Many cardiovascular disease states are characterized by hypoxia, and the failing heart possesses a gene expression profile similar in many ways to that of the fetal heart [79–81].

H3K27me3) have been identified in a number of studies in human ESC, murine and zebrafish models of development [43,87–92].

Bivalent states are maintained at developmental target genes. The H3K27me3 mark represses expression of the lineage gene, protecting pluripotency, while the H3K4me3 mark 'poises' target genes for activation enabling rapid expression upon differentiation. Upon differentiation, the bivalent histone state is resolved yielding either a stable activate or stable repressive histone state [86]. Bivalent histone states are established and resolved by antagonistic Polycomb group (PcG) and Trithorax group (trxG) complexes, which contain specific methyltransferases for depositing H3K27me3 and H3K4me3, respectively. The PcG and trxG complexes also possess other components involved in recruitment to genomic loci, chromatin condensation and other histone modifications. PcG and trxG mediated deposition of both marks results in a bivalent state, but this equilibrium is disturbed by activating stimuli. KDMs are then recruited to remove either H3K27me3 or H3K4me3 resulting in stable gene activation or repression [93].

Bivalency has been indicated in control of *Hox* genes, [94] cardiac differentiation potential and tooth development [92,95]. Indeed, KDM6s have been associated with decreased H3K27me3 levels and activation of *Hox* genes during development [96], while KDM5A regulates *Hox* gene expression and is recruited by PcG to developmental genes in murine ESCs [97].

JmjC-KDMs & development

The JmjC-KDMs are thought to regulate development by maintaining or removing histone lysine methylation states which repress or activate gene expression, such as H3K27me3 or H3K4me3, respectively. Histone lysine methylation is a relatively stable histone modification and thus is ideally suited to maintain gene expression patterns and cell identity during development [98].

A considerable number of studies have linked KDMs to embryogenesis and post-implantation development including organogenesis [98–100]. Spatiotemporal expression profiles of JmjC-KDMs have been characterized in murine and bovine embryos [101,102]. Deletion of specific JmjC-KDMs in numerous developmental models results in embryonic lethality and

morphological defects [98–100]. Additionally, JmjC-KDM mutations have been linked to several developmental syndromes [103–105]. Various studies have linked each of the JmjC-KDM subfamilies to developmental roles (see Pedersen and Helin review [99]).

Despite a number of studies, the role of the KDM2 subfamily in development is poorly understood [98]. Homozygous knockout of KDM2B in mice causes partial peri- and post-natal death, with pups displaying exencephaly and an increased number of mitotic neural progenitor cells [106]. KDM2A knockout in mice results in embryonic lethality, downregulation of PcG, decreased cell proliferation and increased apoptosis [107]. In cattle, a KDM2B missense mutation causes congenital multiorgan developmental dysplasia [108,109]. KDM3A regulates male sex determination during development in mice; KDM3A-mediated demethylation of H3K9 leads to transcription of Sry, a 'master switch' of sex determination [110]. KDM3A also has a role in maintaining pluripotency in ESCs by demethylation of H3K9 leading to expression of pluripotency factors. The same study found that KDM4C also regulates maintenance of pluripotency via H3K9 demethylation [111]. KDM4A and KDM4B are essential to *Drosophila* development and mediate ecdysteriod hormone signaling leading to transcriptional activation of ecdysone response genes [112].

Key roles in neural development have been identified for KDM5B and KDM5C. KDM5B is essential to neural differentiation of ESCs [113] and deletion caused defects in eye and cranial nerve [114]. Mutations in KDM5C have been found in patients with X-linked mental retardation and epilepsy [115], and studies in zebrafish have identified potential roles in brain patterning and neuronal survival [116].

Homozygous deletion of KDM6A causes embryonic lethality in mice, *C. elegans* and *Drosophila* in a sex-specific manner [117], while in humans, constitutional KDM6A mutations have been identified as a cause of Kabuki syndrome [103]. KDM6A and KDM6B have key roles in neural and cardiac development. KDM6B is upregulated during differentiation of ESC to neuronal lineages [118], and overexpression of KDM6B in neural progenitor cells (NPCs) lead to expression of neuronal genes [119]. *In vivo* experiments in chick neural tubes found that KDM6B acts with SMAD to co-activate the neural gene program [120]. KDM6A is essential for retinoic acid induced neural differentiation in an ESC model [121]. KDM6A and KDM6B null ESCs have reduced capacity for cardiac lineage commitment [122,123]. Severe cardiac defects and embryonic lethality is seen in KDM6A deficient mice, accompanied by reduced expression of key cardiac genes, while KDM6B deficient embryos die at the blastocyst stage [122,123].

Within the KDM7 cluster, PHF8 (KDM7B) has been found to be important in neural development [124]. Mutations in PHF8, including some which are catalytically inactivating, have been identified in patients with X-linked mental retardation and cleft lip/palate [125]. PHF8 deletion in zebrafish leads to animals with fewer neurons and craniofacial alterations [124,126] and loss of the PHF8 PHD finger in *C. elegans* causes impaired locomotion [127]. In chickens, KDM7A/KIAA1718 promotes neural induction [128].

Understanding the role of the JmjC-KDMs in the hypoxic environment of development is likely to be useful in the context of hypoxic disease states. Uncovering the mechanisms by which these enzymes may be regulating pluripotency and differentiation may be useful in the production of therapeutic cell populations. Both oxygen tension and the JmjC-KDMs have regulatory roles in development, and there is a need to characterize the relationship between these two influences. Further, as stated above, HIF regulates expression of a number of JmjC-KDMs and thus it is likely that HIF-mediated expression of JmjC-KDMs is significant during development (for a more detailed discussion of HIF and development see Dunwoodie, 2009 [73]).

Cancer & hypoxia

Hypoxia is a common feature of solid tumors. As tumors grow, they surpass the diffusion limits of the local blood supply, resulting in regions where the concentration of oxygen is severely reduced relative to normal tissue. This leads to stabilization of HIF to activate the hypoxic transcriptional response (including angiogenesis), and a shift in energy metabolism from oxidative phosphorylation to glycolysis (known as the 'Warburg effect' [129], a recognized hallmark of cancer). The HIF response can partially restore oxygen supply to tumors, but nevertheless, oxygen supply can fluctuate and cells adapt to an ever-changing microenvironment. While the HIF system is the major factor affecting gene expression changes associated with oxygen concentrations, it is feasible that epigenetic changes, both dependent and independent of HIF pathways, also have significant implications in transcriptional regulation and cellular adaptation at different oxygen concentrations in disease (reviewed in Kaelin and Ratcliffe 2008 [6]).

Cancer & JmjC-KDMs.

Many epigenetic proteins are dysregulated or mutated in cancers [130]. Aberrant gene expression and epigenetic patterns are associated with cancer occurrence and progression, often associated with poor prognosis [131].

Emerging evidence suggests that many KDMs are implicated in tumor growth, with a number of KDMs identified to be oncogenes (see Højfeldt et al. for a recent review on KDMs and cancer [52]).

Of the HIF-regulated JmjC-KDMs, KDM3A has been implicated in numerous cancers including bladder cancer [132], renal cell carcinoma [13], hepatocellular carcinoma [133,134] and colorectal cancer [13,135]. The KDM4s play key roles in the cell cycle and proliferation [136,137] and have been strongly linked to cancer. KDM4A and B are overexpressed in breast, prostate, colorectal, bladder and gastric cancers, and KDM4B, which associates with the androgen receptor (AR) and the estrogen receptor (ER), is being actively pursued as a prostate and breast cancer target [138]. KDM4C is amplified in squamous cell carcinoma [136] and required for growth in many cancer cells including esophageal [136], prostate [139] and breast [140] cancers. KDM4A and C have been shown to form a complex with the AR in prostate cancer cells and co-operatively demethylate the repressive histone marks (H3K9me3/me2) to activate transcription of androgen receptor target genes responsible for tumor proliferation [139,141–142]. KDM5B is required for growth of breast cancer cells [143] and of slow-cycling melanoma cells [144], and is linked to prostate cancer malignancy [145]. KDM5A/B are found to be overexpressed in multiple cancer cells, including cervical, gastric, lung, bladder and breast cancer [146]. KDM6B is highly expressed in T-cell acute lymphoblastic leukemia (T-ALL) cells and is essential for the initiation and maintenance of T-ALL [147]. It is also upregulated in renal cell carcinoma [148].

It is possible that, for at least some JmjC-KDMs, their association with cancer may be related to hypoxia. Recently, KDM3A, KDM4B/C and KDM5B have been identified as HIF target genes and are thus upregulated in hypoxic conditions (Figure 2C) [14,57–59,61]. This hypoxic JmjC-KDM induction is found to further amplify HIF target gene expression, either by direct interaction with HIF or by separate promoter binding and demethylation (Figure 2E). In hypoxic conditions, KDM3A removes H3K9 methylation from the promoters of HIF target genes, such that KDM3A activity is required for their full hypoxic induction [13]. Consistent with this, significant differences in gene expression profiles are observed in KDM3A knockout mouse ESCs under hypoxia [149]. Hypoxic induction of KDM3A in several cancer cell lines enhanced cell proliferation and tumor growth via transcriptional activation of several tumorigenic and proliferative genes [134]. Hypoxia has been shown to increase expression of prostate-specific antigen (PSA) in LNCaP cells, via direct interaction of KDM3A, HIF-1α and the AR resulting in recruitment of KDM3A to the PSA enhancer region [63].

KDM4C has been shown to facilitate the hypoxic response by directly interacting with HIF and thereby enhancing its transcriptional activity. KDM4C binds directly to HIF-1α, which directs it to HIF target genes, promoting H3K9 demethylation of the HRE and gene induction. The histone demethylation activity of KDM4C is required for HIF transactivation and knockdown of KDM4C inhibits breast tumor growth and lung metastasis in mice [15]. There may also be other factors required to promote the HIF-mediated upregulation of KDMs. siRNA knockout of the paired-like homeodomain pituitary gland transcription factor (PITX1) results in increased transcriptional activity of HIF and changes in JmjC-KDM levels. Mudie et al. demonstrated that PITX1 and HIF-1α modulate JmjC-KDM/KDM4B expression, and hence H3K9me3 levels, in a co-operative manner, via interaction of PITX1 with HIF-1β at the KDM4B promoter in MDA-MB-231 and MCF-7 breast cancer cells [150].

Therapeutic targeting of KDMs in cancer

The significance of JmjC-KDM activity in cancer progression means that KDM families are now emerging as potential new targets for cancer [52] and there has been much progress in recent years toward development of small molecule inhibitors against JmjC-KDMs [151–153]. The majority of the small molecules reported to date contain a metal-chelating motif and inhibit demethylase activity via chelation of the active site Fe(II). (For a recent review see [154]).

N-oxalylglycine (NOG), an inactive 2OG analog, inhibits all subfamilies of JmjC-KDMs with differential activity (IC_{50} range of 0.3–250 μM) [155]. DMOG, a cell permeable form of NOG which is often used to inhibit HIF hydroxylases to upregulate HIF and mimic hypoxia, also targets JmjC-KDMs in cells resulting in increased global histone methylation levels [155]. 5-carboxy-8-hydroxyquinoline (IOX1), a more potent inhibitor, has been developed as an alternative generic inhibitor for 2OG oxygenases [155,156] and was established as a chemical tool [157]. There have been some recent developments of promising KDM selective inhibitors. Both methylstat and JIB-04 (pyridine-hydrazone) inhibit JmjC-KDM activity in vitro, with approximately ten-fold selectivity for JmjC-KDMs over PHD2 [158,159]. Methylstat inhibits prostate cancer cell growth, while JIB-04 inhibited both lung and prostate cell growth in vivo and in tumors [158,159]. Interestingly, a hybrid compound consisting of KDM1 and JmjC-KDM targeting motifs

(4-carboxy-4′-carbomethoxy-2′-2-bipyridine) inhibited both KDM families (KDM1 and JmjC-KDMs) with high selectivity over HIF hydroxylases *in vitro*, and demonstrated growth arrest and apoptosis on prostate and lung cancer cells [160].

A major challenge in KDM inhibitor development is achieving selective inhibition within the JmjC-KDM subfamily. One of the first promising leads developed against a JmjC-KDM subfamily is GSK-J1/J4 [161], a cell-active compound targeting the KDM6 subfamily. While it also weakly inhibits KDM5 [162], GSK-J4 (the prodrug form) has proved to be a useful tool in epigenetic research [147,161]. Most recently, Epitherapeutics reported EPT103182 as a KDM5-targeting small molecule inhibitor with potent KDM5 inhibition *in vitro* and in cells [163]. EPT103182 shows an antiproliferative effect in many cancer cell lines, particularly in hematological and solid cancer cell lines, and demonstrates dose-dependent tumor growth inhibition in xenograft models [163].

Cardiovascular disease & hypoxia

Heart failure is the common end-point of various cardiovascular conditions, including cardiac hypertrophy, myocardial infarction and myocardial ischemia [164,165]. As in cancer, a shift in metabolism from oxidation of fatty acids to glycolysis is seen in heart failure, which is accompanied by cardiovascular remodeling and the reactivation of fetal genes such as atrial natriuretic peptide and brain natriuretic peptide (*ANP* and *BNP*, respectively) [79,80] (Figure 4). This reprogramming of fetal genes has been proposed as a cardioprotective response, aimed at increasing cardiac efficiency under conditions of stress [81]. The mechanisms underlying the convergence of these diseases on a common gene expression profile are yet to be understood [166]. Cardiac hypoxia

may be the result of an ischemic event, or due to systemic hypoxia or anemia [77] and is often a feature of cardiac hypertrophy [167], as well as a key contributor in the progression of hypertrophy to heart failure [168]. The contribution of epigenetic changes to cardiovascular disease is less well studied than in cancer. However, the possibility exists that focal or chronic hypoxic conditions in cardiovascular diseases may affect JmjC-KDM levels and activity and thus histone methylation status. Here we discuss evidence available to date that demonstrates associations of JmjC-KDMs with cardiovascular disease progression.

JmjC-KDMs & cardiovascular disease

Altered histone lysine methylation patterns resulting in changes to gene expression profiles have been observed in a number of cardiovascular disease states, particularly cardiac hypertrophy and cardiomyopathy (Table 4) [169–174]. While studies to date have analyzed the downstream effect of changes to methylation status at specific gene promoter regions, genome-wide ChIP in hypertrophic cardiomyocytes revealed redistribution of methylated histone marks to both promoter and enhancer regions of specific genes associated with the disease state [175]. This suggests that changes to histone modifications are not only a result of disease, but can, in fact, drive maladaptive phenotypic changes.

Recent studies have implicated several JmjC-KDMs in a number of cardiovascular disease states. KDM4A protein levels were reported to be upregulated in hypertrophic cardiomyopathy patients [171]. Heart-specific overexpression of KDM4A in mice exacerbated cardiac hypertrophy following TAC-induced cardiac pressure overload, while heart-specific KDM4A knock-out blunted the hypertrophic response [171]. Increased levels of four and a half LIM domains 1 (FHL1, a biomechani-

Table 4. Epigenetic modulations associated with cardiovascular disease states.				
Disease state	**Changes to lysine methylation status**	**Gene locus and change in expression level**	**KDM implicated?**	**Ref.**
Heart failure	H3K4me3	Global study	-	[169]
	H3K9me3			
PTIP knock out mouse (reduced global H3K4me3 model)	H3K4me3 ↓ (globally induced)	*KCNIP2* ↓	-	[170]
Cardiac hypertrophy	H3K9me3 ↓	*FHL1* ↑	KDM4A ↑	[171]
Cardiomyopathy	H3K36me3 ↓	*DUX4* ↓	-	[172]
Cardiomyopathy	H3K9me2/3 ↓	*ANP* ↑	KDM3A ↑	[173]
		BNP ↑	KDM4A ↑	
			KDM4B ↑	
Several studies have demonstrated changes in gene expression levels and associated changes to histone lysine methylation in cardiovascular disease states. Altered KDM expression levels were also reported in some cases.				

cal stress sensor implicated in the development of cardiac hypertrophy [176]) were found to be due to binding of KDM4A to the *FHL1* promoter, decreasing H3K9 trimethylation and activating transcription [171]. Cardiac hypertrophy is stimulated by pressure overload, which increases myocardial oxygen demand and can lead to myocardial hypoxia [166], hence elucidating the effect of low oxygen concentrations on the JmjC-KDMs could prove to be therapeutically beneficial in this context. Importantly, increased KDM4A, KDM4B and KDM3A expression was found in heart failure patients [173]. Double knockdown experiments in neonatal rat cardiomyocytes correlated KDM3A and KDM4A expression levels with changes in expression of ANP and BNP, apparently mediated by changes to the methylation status of H3K9 in the promoter region of these genes, and it was suggested that this evidence supported a regulatory role for KDM3A and KDM4A in the reactivation of fetal genes in a disease-specific context [173].

Inflammation & JmjC-KDMs

Atherosclerosis is a non-resolving inflammatory disease, and presents an interesting link between inflammation, hypoxia and cardiovascular disease. Atherosclerotic plaques are inherently hypoxic [177], demonstrating HIF-1α stabilization [178], while atherosclerosis is itself an underlying cause of ischemic heart disease, angina, myocardial infarction and stroke.

KDM3A, KDM4B and KDM4D are upregulated in macrophages in response to hypoxia, concomitant with a global increase in levels of H3K9me2, H3K9me3 and H3K36me3, likely due to attenuation of JmjC-KDM activity [16]. Downstream reduction in expression of chemokine ligands and receptors was also observed. Interestingly, one such affected ligand, CCL2, is similarly downregulated in a murine model of atherosclerosis upon treatment with the iron chelator desferrioxamine [179], which is a generic inhibitor of the 2OG oxygenase family, including the HIF hydroxylases and the JmjC-KDMs [Unpublished data, author's laboratory].

KDM6B has been linked to inflammation in several studies. Macrophages stimulated by both lipopolysaccharides [180,181] and Serum Amyloid A (SAA) [182], an acute phase protein associated with chronic disorders including atherosclerosis [183], demonstrated upregulation of KDM6B [180–182] and KDM6B-mediated modulation of expression of inflammatory genes [181,182]. Furthermore, shRNA knock-down of KDM6B reduced uptake of oxidized low density lipoproteins by macrophages, thereby reducing the SAA-induced formation of foam cells [182], which contribute to the pathogenesis of atherosclerosis [184].

Published work has demonstrated that changes to histone lysine methylation at specific gene loci have direct consequences for the development and progression of several cardiovascular and inflammatory diseases. These changes are likely mediated, at least in part, by JmjC-KDMs, although further investigation of the specific role of JmjC-KDM activity in these disease states is required. Given the links between hypoxia and heart disease, and the hypoxic regulation of a number of these enzymes, the relationship between reduced oxygen concentration and epigenetic regulation in the context of cardiovascular disease presents an interesting avenue for further research.

Conclusion & future perspective

Evidence is amassing that phenotypic changes in hypoxic diseases are not solely a result of the HIF-driven hypoxic response. Altered histone lysine methylation in hypoxia, resulting in changes at the transcriptional level, has been demonstrated in a variety of contexts, as described above. As with the related 2OG oxygenases, PHD and FIH, JmjC-KDMs have been shown to have different oxygen sensitivities, at least *in vitro* (Table 1 [26]), and their activity has the potential to be affected by changing oxygen concentration [20]. JmjC-KDMs may therefore regulate gene expression at specific gene loci in an oxygen concentration-dependent manner, although further evidence to support this hypothesis is needed.

Challenges remain in elucidating the relationship between oxygen availability and epigenetic regulation by the JmjC-KDMs. Potential redundancy [107,117,185] and differential HIF regulation within JmjC-KDM subfamilies [14,57–58] are factors that may prove complex. It is unclear how HIF-mediated gene upregulation in hypoxia affects overall KDM activity levels since both HIF-dependent (KDM upregulation/association) and HIF-independent (effects on catalytic activity) mechanisms (Figure 2C–E) may be at play. Several JmjC-KDM sub-families are known to have demethylase-independent roles in gene regulation [186–188]. In the context of epigenetic changes and oxygen availability, it is also important to consider DNA methylation patterns which are altered in hypoxia [189,190]. While it is beyond the scope of this review, DNA demethylation mediated by Ten-eleven translocation methylcytosine dioxygenases (TETs, including TET1–3), another family of 2OG oxygenases, may also be key players in epigenetic regulation under hypoxic conditions.

In order to address these questions, development of specific and potent chemical probes for these enzymes will be essential. Indeed, some promising chemical probes for this enzyme family have already been identified [153,154]. There is also a need to develop more precise and reliable methods for measurement of oxygen

concentration in cells and tissues, such as fiber-optic, small molecule or cell-permeable nanoparticle probes.

A large body of evidence indicates that the JmjC-KDMs have key roles in gene regulation and that mis-regulation of the JmjC-KDMs is a pathological feature in hypoxic disease states. Reduced activity of at least some of the JmjC-KDMs in hypoxia may be an influencing factor. Further biochemical, kinetic and physiological characterization is necessary to validate this proposal, and will confirm the importance of the JmjC-KDMs as therapeutic targets for the treatment of diseases of the maladaptive hypoxic response.

Executive summary

Hypoxia, HIF & the HIF hydroxylases
- HIF mediates the hypoxic response and HIF levels are regulated by the HIF hydroxylases, oxygen-dependent enzymes which are members of the 2OG-dependent oxygenase family.

Histone demethylation by the JmjC-KDMs
- JmjC-KDMs are 2OG oxygenases, which play key roles in epigenetic regulation and act by modulating dynamic histone lysine methylation states.
- Levels of some JmjC-KDMs are upregulated in hypoxia, and kinetic studies suggest their activity could be sensitive to decreased oxygen availability, with potentially important consequences for gene regulation in hypoxia.

Hypoxia, development & the JmjC-KDMs
- Hypoxia is an essential regulator of normal development, while various JmjC-KDMs are critical to different types of development.
- Understanding how the JmjC-KDMs contribute to gene regulation in development may prove useful in understanding how aberrant function, especially in hypoxia, may contribute to disease states.

Hypoxia, cancer & the JmjC-KDMs
- Hypoxic conditions commonly occur within solid tumors and cause a HIF-mediated hypoxic transcriptional response.
- Hypoxia-induced epigenetic changes may contribute to altered gene expression in cancer.
- Many JmjC-KDMs have been implicated in cancer, and some have been identified as oncogenes.
- Inhibitors targeting JmjC-KDMs are being developed as cancer therapeutics.

Hypoxia, cardiovascular disease & the JmjC-KDMs
- Altered gene expression and histone methylation patterns have been identified in several cardiovascular disease states.
- Data are emerging that indicate JmjC-KDMs may contribute to these conditions in a pathological manner.

Conclusion
- Hypoxia may be a contributing factor to the aberrant function of various JmjC-KDMs that have been implicated in hypoxic disease states.
- There is a need to further characterize the effect of hypoxia on the activity and expression of JmjC-KDMs.
- To this end, further biochemical and physiological study, and the development of potent and selective inhibitors will be essential.

Unanswered questions
- What are the oxygen sensitivities of JmjC-KDMs? Do they vary between different JmjC-KDMs families and isoforms? Can the JmjC-KDMs act as oxygen sensors?
- Is gene expression regulated by JmjC-KDMs in an oxygen dependent manner?
- Are the JmjC-KDMs overexpressed to compensate for loss of enzyme activity at low oxygen concentrations?
- Does both HIF-dependent and HIF-independent transcriptional regulation by JmjC-KDMs occur under different oxygen stress?
- What is the actual, absolute concentration of oxygen experienced by cells exposed to a hypoxic environment, or by cells within a physiologically hypoxic microenvironment?

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