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Review

Chromatin as an oxygen sensor and active player in the hypoxia response

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ABSTRACT

Changes in the availability or demand for oxygen induce dramatic changes at the cellular level. Primarily, activation of a family of oxygen labile transcription factors, Hypoxia Inducible Factor (HIF), initiates a variety of cellular processes required to re-instate oxygen homeostasis. Oxygen is sensed by molecular dioxygenases in cells, such as the prolyl-hydroxylases (PHDs), enzymes which are responsible for the oxygen-dependent regulation of HIF. As HIF is a transcription factor it must bind DNA sequences of its target genes possibly in the context of a complex chromatin structure. How chromatin structure changes in response to hypoxia is currently unknown. However, the identification of a novel class of histone demethylases as true dioxygenases suggests that chromatin can act as an oxygen sensor and plays an active role in the coordination of the cellular response to hypoxia. This review will discuss the current knowledge on how hypoxia engages with different proteins involved in chromatin organisation and dynamics.

Contents

1. Introduction

Oxygen is essential for the majority of multicellular organisms. As such, variations in oxygen supply and demand within a given time frame activate a variety of pathways, the ultimate aim of which is to re-instate oxygen homeostasis. This is true at the organism level but also true at the cellular level. Oxygen is required for efficient ATP production via oxidative phosphorylation in the mitochrondria, whilst ATP production via glycolysis does not require oxygen, it is much less efficient.

Hypoxia is an important stimulus for physiological processes such as development and adaptation to high altitude living, but it is also an

Abbreviations: HIF, Hypoxia inducible factor; ARNT, Aryl hydrocarbon nuclear translocator; VHL, von Hippel Lindau; PHD, Prolyl-hydroxylase; FIH, Factor inhibiting HIF; ChIP, Chromatin immunoprecipitation; SWI/SNF, Switch/Sucrose NonFermentable; ISWI, Imitation switch; CHD, chromodomain helicase DNA-binding; NURF, nucleosome remodelling factor; CHRAC, Chromatin remodelling and assembly complex; ACF, ATP-utilising chromatin remodelling and assembly factor; NoRC, nucleolar remodelling complex; RSF, Remodelling and spacing factor; WICH, WSTF–ISWI chromatin remodelling complex; NuRD, nucleosome remodelling and histone deacetylase; SRCAP, SNF2-related CBP activator protein; TRRAP, transformation/transcription domain-associated protein/Tip60; HAT, Histone acetyl transferase; HDAC, Histone deacetylase; LSD1, lysine-specific demethylase-1; JmjC, Jumonji C domain.

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important factor in the pathology of many human diseases [1,2]. These include cancer, diabetes, ageing, and stroke/ischaemia [1,2]. Furthermore, it plays a role in the resistance to therapeutic approaches such as radiotherapy [1,2].

Whilst the understanding of how whole organisms respond to variations in oxygen availability has been greatly enhanced over the last century, with physiology studies [3], the molecular understanding of how oxygen is sensed at the cellular level is much more recent, with the findings made thus far likely being the tip of the iceberg.

The research into oxygen sensing at the cellular level, was greatly enhanced with the discovery of a family of transcription factors that respond to hypoxia, called Hypoxia Inducible Factors (HIF) [1]. HIF is a heterodimer of an oxygen labile subunit, HIF-α, and an oxygen-insensitive HIF-1β, also known as aryl hydrocarbon nuclear translocator (ARNT).

The tumour suppressor von Hippel Lindau (VHL), as part of the E3 ubiquitin ligase complex, targets HIF-α in the presence of oxygen to be degraded by the proteasome. VHL recognises HIF-α mostly in normoxia, through interaction with hydroxylated proline residues within the oxygen-dependent degradation domain of HIF-α (Fig. 1). Biochemical studies demonstrated that VHL has a 1000 fold increased affinity for hydroxylated HIF, compared to non-hydroxylated [4]. This specific modification of prolines, is mediated by a class of dioxygenases, called Prolyl-Hydroxylases (PHDs). There are 3 PHDs that have demonstrated effects on HIF, PHD1-3 and these enzymes require molecular oxygen for their activity. Another dioxygenase with known effects on HIF is the Factor Inhibiting HIF (FIH). FIH mediates the hydroxylation of asparagine residues within the C-terminus transactivation domains of HIF-α, preventing binding to co-activators such as p300 or CBP [5], and thus limiting HIF transcriptional activity (Fig. 1).

HIF can activate many genes involved in many important cellular processes such as cell cycle and cell growth, metabolism, oxygen homeostasis, apoptosis and autophagy (Fig. 2). In fact, recent studies using genomic chromatin immunoprecipitation (ChIP) techniques, ChIP-on-ChIP and ChIP-Sequencing, have demonstrated hundreds of genomic loci, where HIF binds [6,7], suggesting that many genes are under the direct control of these transcription factors [6,7].

The importance of the HIF pathway has been extensively demonstrated by genetic studies: HIF-1β null mice are embryonic lethal with severe defects in many organs [8,9]. Furthermore, conditional HIF-1β knockouts have been made in T-cells [10], β-cells [11] and skin [12], and all of these tissues and cells have several defects. HIF-1α null mice are also embryonic lethal, with defects in heart, brain, vasculature and bone [13–15]. In addition, conditional deletion of HIF-1α has been achieved in macrophages and neutrophils [16], neural cells [17,18], keratinocytes [19], the colon [20] and the liver [21], to name a few, and has been shown to be required for proper function of these tissues. HIF-2α deleted mice present phenotypes that are strain specific [22–24]. However, they all have severe defects in development.

All the PHDs have been deleted in mice, but only PHD2 is embryonic lethal with placental defects [25]. PHD2 also regulates the vascular system in adult mice [26,27]. PHD1 null mice are apparently normal, but demonstrate increased muscle fatigue [28], protection against ischemic/reperfusion injury to the liver [29], and against colitis [30]. PHD3 null mice are born but have defective sympathoadrenal development and are systemically hypotensive [31]. More recently, FIH was deleted in mice, and these were viable, with no apparent developmental defects. Interestingly, FIH null mice have alterations in their metabolism, presenting lower body weight, increased response to insulin and importantly, protection against diet induced weight gain [32]. These results suggest that FIH does not play a role in the control of HIF in developmental hypoxia but perhaps only in the case of pathological hypoxia.

2. Chromatin structure

As mentioned before, HIF is an important transcription factor, and as such it requires binding to DNA target sequences in the context of chromatin. Chromatin is a dynamic and complex structure composed of DNA and many proteins. The basic unit of chromatin is the nucleosome. The nucleosome consists of 147 bp of DNA wrapped around an octomer of histones (2 copies of each of the core histones: H2A, H2B, H3 and H4) [33,34]. Nucleosomes are linked with stretches of linker DNA, which incorporate linker histones such as H1 [34]. Nucleosome arrays are further compacted into higher order of chromatin, however detection and analysis methods for these higher order chromatin structures are still not routinely available. In addition, there is no detailed information on how chromatin structure changes in hypoxia.

There are two types of chromatin recognised according to its compaction: heterochromatin and euchromatin. Heterochromatin, is

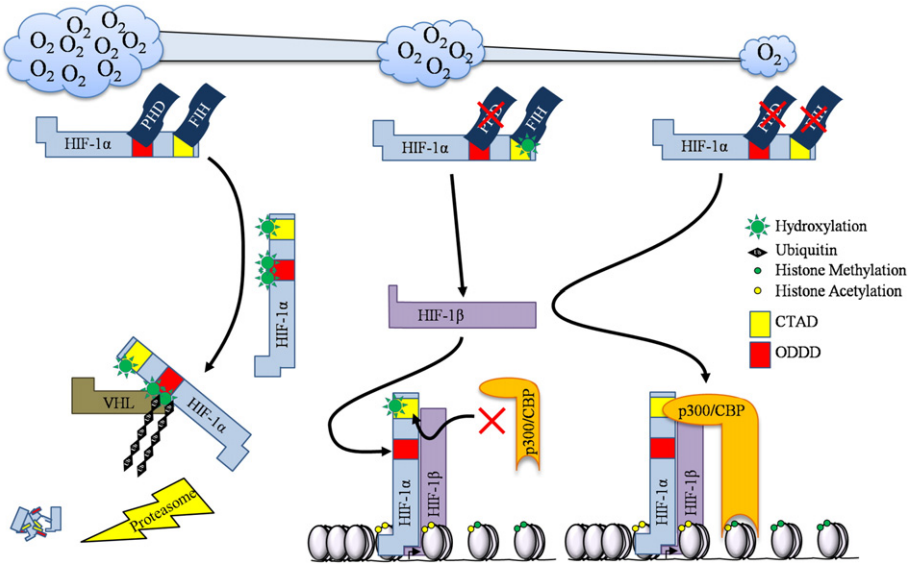

Fig. 1. The HIF degradation pathway. In normoxia the hydroxylases (PHDs and FIH) use O_2 to hydroxylate HIF-1α in the Oxygen Dependent Degradation Domain (ODDD) and the C-Terminal Activation Domain (CTAD). Hydroxylation in the ODDD targets HIF-1α for ubiquitination by the VHL containing E3 ligase complex and HIF-1α is then degraded by the proteasome. In moderate hypoxia the PHDs are inhibited causing HIF-1α accumulation and its dimerisation with HIF-1β. Further decreases in oxygen cause FIH inhibition and subsequent interaction of the HIF-1α-CTAD with co-activators such as p300/CBP.