The HIF-Dependent Transcriptional Response

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The Hypoxia Inducible Factor 1 (HIF-1) plays a major role in the cellular response to hypoxia by regulating the expression of many genes involved in adaptive processes that allow cell survival under low oxygen conditions. Adaptation to the hypoxic tumor micro-environment is also critical for cancer cell proliferation and therefore HIF-1 is also considered a valid therapeutical target. Despite the huge progress in understanding regulation of HIF-1 expression and activity by oxygen levels or oncogenic pathways, the way HIF-1 interacts with chromatin and the transcriptional machinery in order to activate its target genes is still a matter of intense investigation.

Keywords: hypoxia ; HIF-1 ; transcriptional regulation

1. Introduction: The Cellular Response to Hypoxia and the Role of HIFs

Hypoxia or lack of sufficient oxygen can occur under either physiological or pathological conditions such as intense muscular exercise or ischemic diseases, respectively. Hypoxia also characterizes the micro-environment of solid tumors and potentiates the aggressiveness and resistance of cancer cells to therapy. A key element in the cellular response to hypoxia is the stabilization of the alpha subunits of the hypoxia inducible factors (HIFα) and the subsequent activation of the HIF heterodimers, that upregulate the transcription of many genes required for adaptation at low oxygen conditions. The HIF family of heterodimeric transcription factors comprises three HIFα members (HIF-1α, HIF-2α, and HIF-3α) and one HIFβ member (HIF-1β, also known as aryl hydrocarbon receptor nuclear translocator, ARNT).

The breakthrough work by G. Semenza, Sir P. Ratcliffe and W. Kaelin (2019 Nobel prize in Physiology or Medicine) led to the characterization of the cellular oxygen sensing mechanism that controls the expression levels of HIFα [1][2][3][4][5]. Briefly, under atmospheric oxygen concentrations (normoxia), oxygen sensitive enzymes hydroxylate HIFα and cause its degradation and/or block its binding to transcriptional co-activators. The inactivation of these enzymes under hypoxia leads to stabilization of HIFα, its translocation into the nucleus, the formation of functional HIF heterodimer with ARNT, through their Per-Arnt-Sim (PAS) homology domains, and binding to specific DNA sequences called hypoxia response elements (HRE), through their basic helix-loop-helix (bHLH) domains. Thus, the transactivation domains (TAD) of HIFα can then interact with transcriptional coactivator proteins such as CREB-binding protein (CBP) and stimulate expression of genes containing HREs in the promoter or enhancer regions.

2. The HIF-Dependent Transcriptional Response

Early analysis of several different individual validated hypoxia-responsive and HIF-dependent target genes, revealed that the HRE comprises the short core consensus sequence 5′-RCGTG-3′, as originally determined in the erythropoietin enhancer, which led to the first purification and identification of HIF-1 [6][7]. In addition, early transcriptomic analyses using microarrays in different cell lines identified 500–4000 genes that changed their expression after exposure to hypoxia, while studies using chromatin immunoprecipitation (ChIP) coupled with analysis on microarrays (ChIP-chip) identified a much smaller number (approx. 300–500) of HIF-1 binding sites [8][9][10][11][12][13]. Several important conclusions were drawn from these studies.

First, a surprisingly small overlap between genes deregulated by hypoxia was detected among different cell types, suggesting that the transcriptional response to hypoxia depends a lot on cellular context [9][11][13]. Second, the majority of hypoxia responsive genes did not contain a detectable HIF-binding site in their proximal promoter, although the majority of HIF-1-binding sites were localized in close proximity to genes [9][10][12]. This indicates that a significant part of the transcriptional response to hypoxia is only indirectly regulated by HIF-1 through induction of other transcriptional regulators, in agreement with the observed large difference between the number of deregulated genes and the number of true HIF-1 binding sites. Furthermore, HIF-1-binding sites were mostly absent from genes down-regulated by hypoxia, suggesting that HIF-1 functions predominantly or even solely as a transcriptional activator [10][12]. Therefore, any

transcriptional repression observed under hypoxia must be a result of HIF-1-dependent induction of repressor proteins and/or non-coding RNAs. Third, less than 1% of the DNA promoter sequences containing the core RCGTG motif bound HIF-1 or HIF-2 [10] and extended sequence preferences beyond the core motif could not explain the lower than-predicted number of observed HIF-1-bound sites [12], raising the issue of how productive HREs are selected. In relation to this, although many loci containing the core motif bound both HIF-isoforms, substantially more bound HIF-1 than HIF-2 [10]. This was in agreement with the considerably smaller contribution of HIF-2 to the transcriptional responses to acute hypoxia [8], at least under the conditions and cell lines studied, further underlining the question of selectivity.

Subsequent and more detailed studies utilizing RNA-seq and/or Chip-Seq [14] in combination with analysis of the non-coding transcriptome [15] and the role of HIF-α hydroxylases [16] or HIF-α isoforms [17] in many different cell lines [18][19] largely corroborated and extended the previous conclusions. These studies confirmed that only a relatively small set of genes (less than 50) are upregulated consistently and substantially by hypoxia or hydroxylase inhibitors in different human cell types, which may form the core of a hypoxia responsive gene signature [16][18][19]. It was also shown that, at genome-wide level, HIF-binding sites were enriched in the vicinity of gene promoters and their majority overlapped with DNAse1-hypersensitive peaks, i.e., open chromatin, although only approx. 1% of hypersensitive RCGTG motifs were bound by HIFs, indicating again that functional HREs may be defined by epigenetic mechanisms [14]. Interestingly, despite the fact that HIF-1 and HIF-2 share a common consensus DNA-binding motif, they were shown to bind different but overlapping sets of sites in chromatin and transactivate only partially overlapping sets of genes, in accordance with their distinct physiological functions and roles in disease [17]. HIF-1 binding sites were more often close to transcription start sites than those of HIF-2 and the binding site distribution was suggested to be caused by inherent properties of each isoform rather than by the severity or the duration of the hypoxic stimulus itself [17]. Concomitant analysis of RNA Pol II binding and histone H3 modification suggested that both HIFs may act predominantly through release of pre-bound promoter-paused RNA Pol II [15]. However, HIF-1 associated more strongly with histone H3 modifications (H3K4me3 and H3K9ac) that mark primarily promoters and proximal regulatory elements while HIF-2 interacted more strongly with H3 modifications (H3K4me1 and H3K27ac) often found in enhancers and other distal regulatory elements [17]. These studies suggested that functional HREs may be largely defined by preformed chromatin structures (i.e., present also under normoxia) which are not affected by HIF binding.

Overall, the genome-wide transcriptomic studies support the idea that HIFs do not alter the chromatin accessibility by their binding but rather associate with already defined and partially active promoters or enhancers, as also suggested by the fact that most HIF-target genes display normoxic expression which is further enhanced by hypoxia [20][21]. However, this is not an absolute rule as recent studies utilizing other than ChIP-seq methodology such as Micrococcal Nuclease (MNase) protection assays [22] and Assay for Transposase-Accessible Chromatin (ATAC)-seq [21] suggest that HIF binding at certain genes can also have a significant effect on nucleosome organization and chromatin accessibility. In either case, isoform specificity, gene selection and cell-type differences cannot be explained by a simple HIF-HRE association and must be conferred through interactions between HIFs and distinct transcriptional and chromatin-associated cofactors. Indeed, recent single-gene studies have identified a significant number of HIF-1α physical partners, several of which are involved in transactivation and act as HIF-co-regulators [23].

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