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Extra View

Genetic Instability

The Dark Side of the Hypoxic Response

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ABSTRACT

Under low oxygen tension, the activated transcription factor HIF-1α upregulates an array of hypoxia-inducible genes via heterodimerization with ARNT and binding to the hypoxia-responsive element in the promoter. Alternatively, HIF-1α regulates hypoxia-responsive genes by functionally antagonizing the oncoprotein Myc via protein-protein interactions. This so-called HIF-1α-Myc mechanism apparently not only accounts for the gene upregulation, but also for the gene downregulation during hypoxia, depending upon the activating and repressive nature of Myc in gene expression. Indeed, our recent study demonstrated that both mismatch repair genes, *MSH2* and *MSH6*, are inhibited by this mechanism in a p53-dependent manner. In particular, the constitutively bound transcription factor Sp1 serves as a molecular switch by recruiting HIF-1α in hypoxia to displace the transcription activator Myc from the promoter. Therefore, our findings shed light on the mechanisms underlying hypoxia-induced genetic instability, an "adverse" effect of the hypoxic response, and yet a germane process to tumor survival and progression.

The cellular response to oxygen deprivation—hypoxic response—is essential for cell proliferation and survival. The hypoxia-inducible transcription factor, HIF-1α, apparently acts as a master regulator of oxygen homeostasis by virtue of its critical role in regulating an array of hypoxia-responsive genes.[1-9] HIF-1α is known to upregulate hypoxia-inducible genes such as *EPO*, *VEGF* and *PGK1* through dimerization with ARNT (also known as HIF-1β),[10] recruitment of the transcription coactivator p300/CBP,[11-13] and binding to the hypoxia-responsive element in the promoter.[14] Furthermore, our previous study demonstrated that HIF-1α also utilizes a novel HIF-1α-Myc mechanism to activate *CDKN1A* (also known as $p21^{cip1}$) expression, involving functional antagonism of the transcription repressor Myc via protein-protein interactions.[15] Remarkably, this alternative mechanism is independent of HIF-1α DNA binding and transcriptional activity; instead, HIF-1α displaces Myc from binding to the *CDKN1A* promoter, resulting in gene activation.[15]

It is noteworthy that cells not only upregulate genes in adaptation to oxygen deprivation, but downregulate hypoxia-responsive genes to complement the cellular event, both of which merit equal amount of attention for a comprehensive understanding of the hypoxic response.[16] We have shown recently that the HIF-1α-Myc mechanism also accounts for hypoxic downregulation of *MSH2* and *MSH6*,[17] whose protein products constitute MutSα, a MSH2-MSH6 heterodimer that is crucial for DNA mismatch repair.[18-20] The fate of the hypoxia-responsive genes in this category seems to be determined by the intrinsic function of Myc that controls transcription. In stark contrast to its repression of *CDKN1A*, Myc activates *MSH2* expression.[21] Hence, the HIF-1α antagonism of Myc results in *MSH2* downregulation.[17]

Intriguingly, neither HIF-1α nor Myc binds directly to the MSH2 promoter. Rather, both HIF-1α and Myc discretely interacts with the constitutively bound transcription factor Sp1 on the *MSH2* promoter, whereas HIF-1α dominates Sp1 binding in hypoxia by competing against Myc. As a result, Sp1 acts as a molecular switch by recruiting HIF-1α for the hypoxic repression of *MSH2*. The identification of Sp1 in the HIF-1α-Myc pathway may provide a framework for the further understanding of how other hypoxia-responsive genes are downregulated by hypoxia.

A salient point of this study is the requirement of the tumor suppressor p53 for the hypoxic downregulation of *MSH2* and *MSH6*. Either deletion or knockdown of *TP53* reduced basal expression of *MSH2* and *MSH6*, resulting in a loss of further inhibition by hypoxia. Conversely, introduction of *TP53* into *TP53*-null cells recapitulated the *MSH2* and

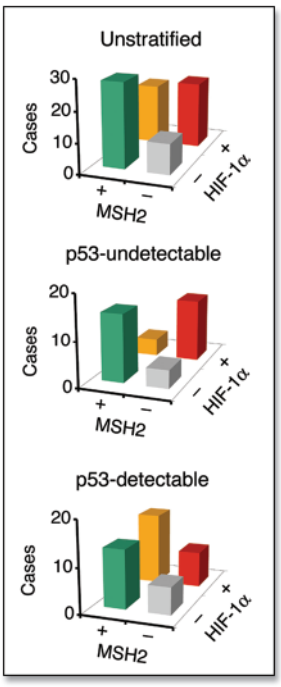

Figure 1. An inverse correlation of HIF-1α and MSH2 expression in p53 stratified nonhereditary colon cancers. The immunohistochemistry results from a total of 80 specimens (*Unstratified*) and from p53 stratified groups (*p53-undetectable* and *p53-detectable*) were plotted in axes, labeled with the number of cases, MSH2, and HIF-1α respectively. The plus sign indicates positive staining, and the minus sign negative staining. The green column depicts specimens stained positive for MSH2 but negative for HIF-1α, whereas the red denotes the reverse. Likewise, the orange column represents both MSH2 and HIF-1α positive, and the grey means both negative.

MSH6 inhibition by hypoxia. However, no such regulation was seen in p53-inactivated cells. Although the p53 core domain has been reported recently to bind the HIF-1α ODD domain,[22] the latter is not required for HIF-1α repression of *MSH2* and *MSH6* expression. Thus, the mechanism by which p53 engages in repressing hypoxia-responsive genes remains obscure.

The human DNA mismatch repair system plays a crucial role in safeguarding the genomic integrity by correcting DNA replication errors and by blocking recombination events between divergent DNA sequences.[18,23] Mutations in the mismatch repair genes are associated with the development of both hereditary and sporadic cancers, and germline mutations in *MSH2* or *MLH1* are the most prevalent cause of hereditary nonpolyposis colorectal cancers.[20] To demonstrate the intricate relationships among p53, HIF-1α, and MSH2 in vivo, we examined the gene expression patterns with immunohistochemistry in nonhereditary human colon cancers. Stratified results based on the p53 status manifest a striking inverse correlation between HIF-1α and MSH2 in the p53-undetectable group harboring wild-type p53 (Fig. 1). However, no such correlation exists in the p53-detectable group harboring p53 mutants. Therefore, these results substantiate the role of HIF-1α in suppressing MSH2 expression in the presence of wild-type p53.

A highly relevant question about these findings is how the reduction of MutSα levels by HIF-1α relates to the cellular adaptation to hypoxia. To that end, we have proposed that balancing the supply and demand of theintracellular ATP is key to this adaptive response. In essence, not only does HIF-1α maintain intracellular ATP production by stimulating angiogenesis and glycolysis, but also reduces ATP consumption by inhibiting cell proliferation and DNA repair. This theory apparently explains why cell populations under hypoxic stress experience multifaceted consequences, including gene mutation and cell death, in addition to cell survival. These adverse events are seemingly an inevitable price associated with the survival of cell populations under hypoxic stress. Accordingly, during tumor development hypoxic microenvironment is created in the name of cell survival, at the expense of gene mutation, and even of cell death.

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