Deubiquitinating enzyme mutagenesis screens identify a USP43 driven HIF-1 transcriptional response.

Tekle Pauzaite, Niek Wit, Rachel Seear, and James A Nathan
Cambridge Institute for Therapeutic Immunology and Infectious Disease. University of Cambridge.

Transcriptional activation of the hypoxia response involves stabilisation of Hypoxia Inducible Factor α (HIF-α) and binding of the HIF-α/HIF1β heterodimer to HIF responsive elements (HRE). How HIFs interact with specific HREs is dictated by a complex interplay of recruitment of co-activators, chromatin accessibility, and histone modifications. The central role of VHL-mediated ubiquitination in governing the HIF response to oxygen gradients is evident, but the involvement of deubiquitination in oxygen-sensing remains unsolved. This is surprising when considering analogous signalling pathways, such as the NF-κB response, that are regulated by multiple ubiquitin enzymes and several deubiquitinating enzymes (DUBs). To globally understand the involvement of DUBs we used CRISPR/Cas9 mutagenesis screens in HIF reporter human cell lines that are sensitive to endogenous HIF levels and activity. We performed reciprocal HIF activator and suppressor screens using a pooled DUB sgRNA library to address the role of deubiquitination in HIF regulation, and how HIF isoform responses are determined. Our work identified a previously unappreciated role for an orphan DUB, USP43, in the selective regulation of a HIF-1 response. USP43 loss prevented activation of the HIF-1 response across multiple cell types, without altering HIF-2 signalling. Depletion of USP43 did not alter stability of the HIF complex. Instead, USP43 loss prevented the accumulation of HIF-1 within the nucleus and decreased HIF-1 binding to chromatin within minutes of hypoxic exposure. USP43 recruited HIF-1 to chromatin through the hypoxia-sensitive engagement of 14-3-3 proteins, and while we showed that USP43 is a catalytically active DUB, its enzymatic activity was not essential for the activation of HIF-1 target genes. The association of USP43 to 14-3-3 proteins and HIF-1 was dependent on hypoxia-induced serine phosphorylation, highlighting the ability of DUBs to have wider roles aside from their catalytic activity. In summary, our studies identify USP43 as a mediator of HIF activation and demonstrate that the non-catalytic recruitment of 14-3-3 proteins can facilitate HIF signalling.