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CK1δ restrains lipin-1 induction, lipid droplet formation and cell proliferation under hypoxia by reducing HIF-1α/ARNT complex formation

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ABSTRACT

Proliferation of cells under hypoxia is facilitated by metabolic adaptation, mediated by the transcriptional activator Hypoxia Inducible Factor-1 (HIF-1). HIF-1α, the inducible subunit of HIF-1 is regulated by oxygen as well as by oxygen-independent mechanisms involving phosphorylation. We have previously shown that CK1δ phosphorylates HIF-1α in its N-terminus and reduces its affinity for its heterodimerization partner ARNT. To investigate the importance of this mechanism for cell proliferation under hypoxia, we visually monitored HIF-1α interactions within the cell nucleus using the *in situ* proximity ligation assay (PLA) and fluorescence recovery after photobleaching (FRAP). Both methods show that CK1δ-dependent modification of HIF-1α impairs the formation of a chromatin binding HIF-1 complex. This is confirmed by analyzing expression of lipin-1, a direct target of HIF-1 that mediates hypoxic neutral lipid accumulation. Inhibition of CK1δ increases lipid droplet formation and proliferation of both cancer and normal cells specifically under hypoxia and in an HIF-1α- and lipin-1-dependent manner. These data reveal a novel role for CK1δ in regulating lipid metabolism and, through it, cell adaptation to low oxygen conditions.

1. Introduction

Oxygen deprivation of cells and tissues (hypoxia) causes a dramatic alteration in gene expression and characterizes major pathological processes like ischemia and cancer. The response to hypoxia is mainly mediated by the hypoxia-inducible factors (HIFs) that control the expression of genes involved in metabolic reprogramming as well as angiogenesis, cellular proliferation and survival under low oxygen conditions. HIFs are therefore essential for adaptation to hypoxia that allows cancer cell proliferation in the hypoxic tumor microenvironment or survival of normal cells in ischemic tissue [1,2]. A major part of this adaptation involves HIF-1-mediated stimulation of anaerobic carbohydrate metabolism and repression of oxidative phosphorylation [3]. In addition, HIF-1 is implicated in lipid metabolism by supporting fatty acid synthesis via glutamine metabolism [4], decreasing β-oxidation of fatty acids [5], and increasing lipin-1-dependent neutral lipid synthesis and lipid droplet formation [6].

HIFs act as heterodimers and consist of the regulatory HIFα subunits and the constitutively expressed HIFβ (or aryl hydrocarbon receptor nuclear translocator; ARNT) subunit. Under normal oxygen levels, HIF-1α is modified by prolyl hydroxylases (PHDs), polyubiquitinated and degraded by the proteasome [7]. Under low oxygen tension, hydroxylation is inhibited, and HIF-1α is stabilized and translocated into the nucleus where it interacts with ARNT to form an active DNA-binding heterodimer that associates with hypoxia-response elements (HREs) and activates the transcription of target genes.

HIF-1α expression and transcriptional activity are additionally regulated by oxygen independent mechanisms involving the NF-κB, PI3-K, MAPK and STAT3 pathways [8,9] or interactions with many other proteins including HSP90, RACK1 and MgcRacGAP [10–13]. A key aspect of HIF-1α regulation involves its post-translational modification by protein kinases. Phosphorylation can regulate HIF-1α protein stability, as reported for modifications introduced by kinases GSK3, PLK3 and CDK1 [14–16], or it can affect HIF-1 transcriptional activity. In the latter

Abbreviations: HIF, hypoxia-inducible factor; ARNT, aryl hydrocarbon receptor nuclear translocator; CK1, casein kinase 1; hBSMC, human bronchial smooth muscle cells; PLA, proximity ligation assay; FRAP, fluorescence recovery after photobleaching

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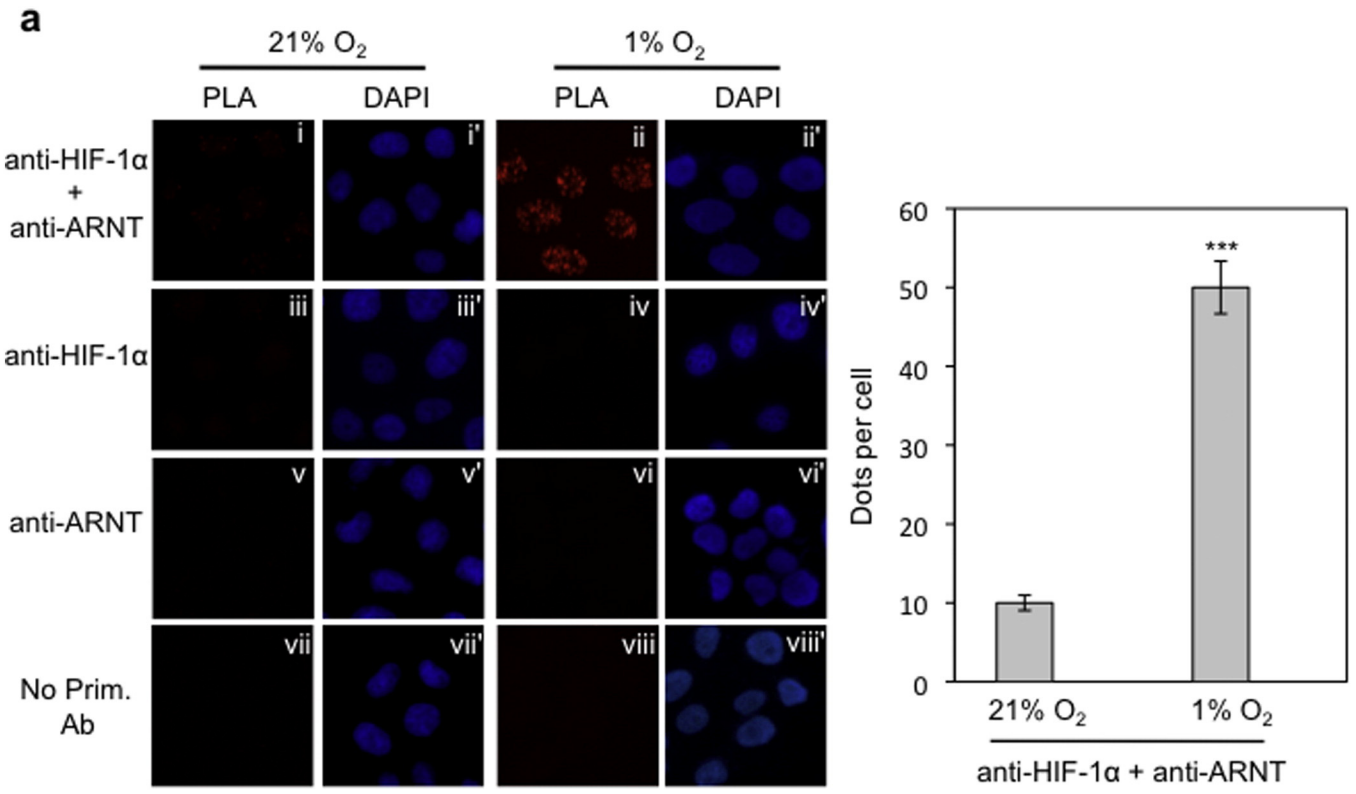

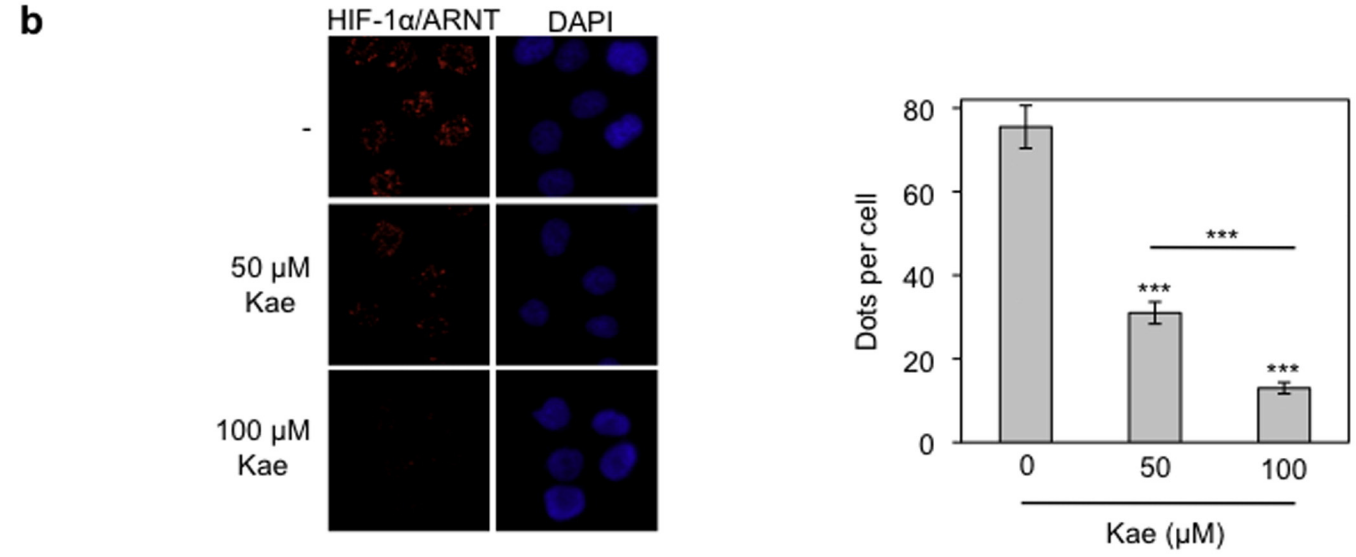

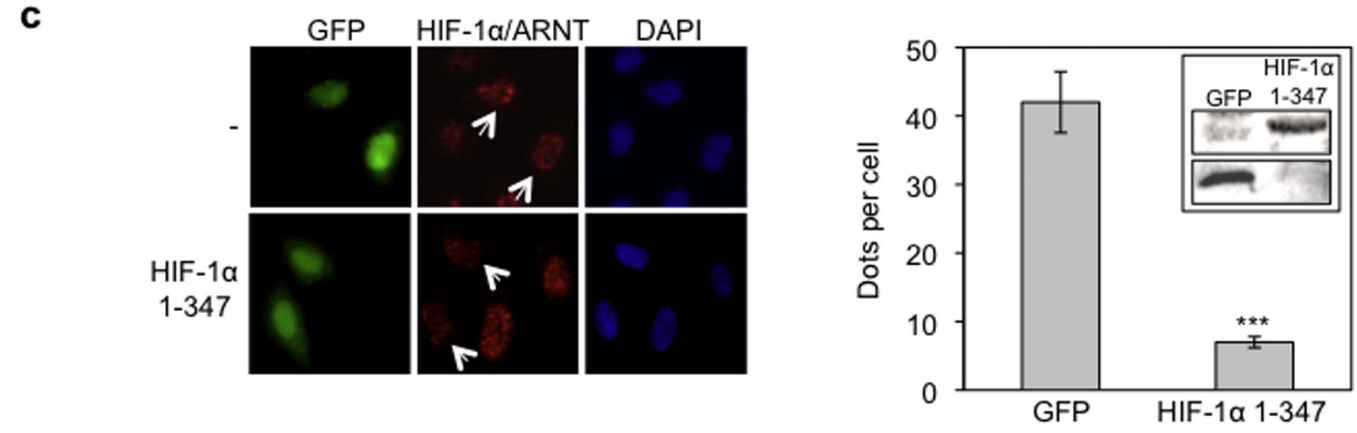

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