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HIF-1α stabilization requires mitochondrial respiration

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Abstract

Hypoxia inducible factors (HIFs) are oxygen sensitive transcription factors that allow adaptation to hypoxic environments by altering their metabolic programming and gene expression. HIF-1 consists of an oxygen-regulated subunit HIF-1α and a constituvely expressed subunit HIF-1β. Overexpression of HIF-1 has been found in range of human cancer cell types, and targeting HIF-1 could represent a novel approach in treating cancer. The goal of this project was to determine if mitochondrial respiratory integrity is required for HIF-1α stabilization. This was done by induction of mitochondrial respiratory defect through tetracycline-controlled expression of a dominant negative form of DNA polymerase γ followed by hypoxic exposures. NAC was added to Tet/on cultures to investigate the contribution of ROS production to HIF-1α stabilization. Tet/off and HTC116 $p53^{-/-}$ cell lines were used to further investigate if the requirement of mitochondrial respiration integrity is important for HIF-1 stabilization. HIF-1α expression was not observed in either of the cell lines with long term mitochondrial respiratory defect and addition of NAC did not have a significant different effect. Early inhibition of the mitochondrial respiratory complexes leads to a degradation of HIF-1α confirming that mitochondrial respiratory function is required for HIF-1α stabilization in hypoxic condition.

Introduction

In a solid tumor, oxygen (O_2) concentrations are known to vary substantially due to rapid cell division and aberrant tumor angiogenesis and blood flow. Tumor hypoxia has long been associated with increased malignancy, poor prognosis, and resistance to conventional therapies. In order for the cells to persist under hypoxic stress, they have to alter their transcriptional profiles to modulate glycolysis, proliferation, survival, and invasion. Studying the mechanisms the cell undergoes to pursue these alterations is essential as we seek novel therapies to target and treat cancer.

The cellular response to hypoxia is mediated in part by the hypoxia inducible factors (HIF) -1α and -2α. HIF-1 is a heterodimeric complex consisting of an O_2-labile α subunit (HIF-1α, HIF-2α, or HIF-3α) and a constitutively expressed HIF-1β subunit. This complex is primarily regulated through post-translational modification and stabilization and it signaling allows proliferating cells to sense and adapt to environment stressors by altering their metabolic programming and gene expression (Figure 1).

Hypoxic conditions within tumors can result in increased HIF-1 stability and activity. Increased expression of both HIF-1α and HIF-2α has been observed in a range of human cancer cell types either as an adaptive measure or as a result of signal transduction pathways that stabilize HIF-1α independent of oxygen. In this study we sought to investigate the mechanism by which HIF-1α is being stabilize in cancer cells. Specifically we wanted to investigate whether stabilization of HIF-1α is relevant to mitochondrial respiratory function.

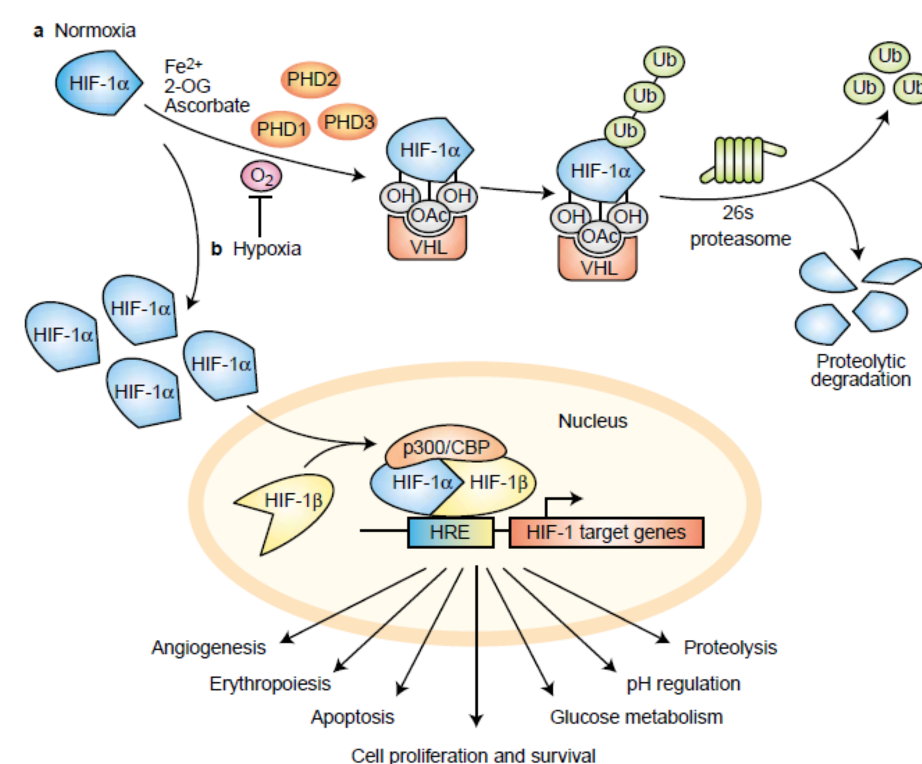

Figure 1. HIF-1α regulation by proline hydroxylation. (a) In normoxia, HIF-1α is hydroxylated by proline hydroxylases and recognized by pVHL, which, together with a multisubunit ubiquitin ligase complex, tags HIF-1α with polyubiquitin allowing recognition by the proteasome and subsequent degradation. (b) In response to hypoxia, proline hydroxylation is inhibited. VHL is no longer able to bind and target HIF-1α for proteasomal degradation, leading to HIF-1α accumulation and translocation to the nucleus. There, HIF-1α dimerises with HIF-1β, binds to hypoxia-response elements (HREs) within the promoters of target genes and recruits transcriptional co-activators for full transcriptional activity.

Hypothesis

We hypothesize that stabilization of HIF-1α is dependent on mitochondrial respiration integrity.

Methods

Cell Culture

Tetracycline (Tet)-off cells were maintained in high glucose (4.5 mg/ml) DMEM with 10% Tet-free fetal bovine serum (FBS). Tet-on cells were maintained in high glucose (4.5 mg/ml) DMEM with 10% FBS, 0.05 mg/mL uridine, and 2 mM sodium pyruvate. 1 ug/ml doxycycline was added to the T-on cell cultures to induce POLGdn expression. HTC116 $p53^{-/-}$ cells were maintained in McCoy's 5A medium supplemented with 10% FBS. HL60 cells were maintained in RPMI 1640 medium supplemented with 10% FBS and C6F cells were maintained in RPMI 1640 medium with 10% FBS, 4.5mg/ml glucose, 0.05 mg/mL uridine, and 2 mM sodium pyruvate. Cultures were maintained at 37° C in a 5% CO_2/95% air incubator.

Treatment and hypoxic exposures

0.5 mM NAC was added to Tet-on cultures overnight before exposure to the hypoxic condition. Normoxic cells (21% O_2) were kept at 37 ° C in a 5% CO_2/95% air incubator while hypoxic cells (5% O_2) were placed in a control atmosphere chamber at 37 ° C for 4hrs followed by protein extraction. 100 nM rotenone, 500 µM sodium azide or 1 mM potassium cyanide was added to Tet-off and HTCp53116 $p53^{-/-}$ cultures for 2 hours or overnight before hypoxic exposures.

Western Blot Analysis

Protein extracted from cells were resolved by electrophoresis using a 10% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were probed with anti-HIF1α (Becton Dickinson), overnight followed by incubation with appropriate secondary antibodies and detection with a SuperSignal Enhanced Chemiluminescence kit (Pierce, Rockford, IL). For sequential blotting, the membranes were stripped with Stripping Buffer (Pierce) and reprobed with proper antibodies.

References

Dang CV, Hamaker M, Sun P, Le A (2011) Therapeutic targeting of cancer cell metabolism. J Mol Med 89: 205-212.

Ke Q and Costa M (2006) Hypoxia Inducible Factor-1 (HIF-1). Mol Pharmacol 70: 1469-1480.

Keith B, Johnson RS, Simon MC (2012) HIF1α and HIF1β: sibling rivalry in hypoxic tumor growth and progression. Nature Reviews Cancer 12: 9-21.

Lu W, Hu Y, Chen G, Chen Z, Zhang H, et al. (2012) Novel Role of NOX in Supporting Aerobic Glycolysis in Cancer Cells with Mitochondrial Dysfunction and as a Potential Target for Cancer Therapy. PLoS Biol 10 (5): e1001326. doi:10.1371/journal.pbio.1001326.

Shay JES and Simon MC (2012) Hypoxia-inducible factors: Crosstalk between inflammation and metabolism. Semin Cell Dev Biol 23: 389-394.

Results

Mitochondrial respiratory function is required for HIF-1α stabilization in hypoxic condition.

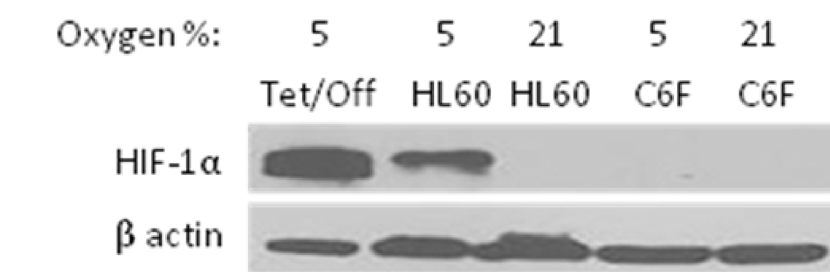

Figure 1. HIF-1α is stabilized only in cells with mitochondrial respiratory integrity (Tet/off and HL60 cells).

Addition of NAC does not change HIF-1α stabilization in hypoxic condition.

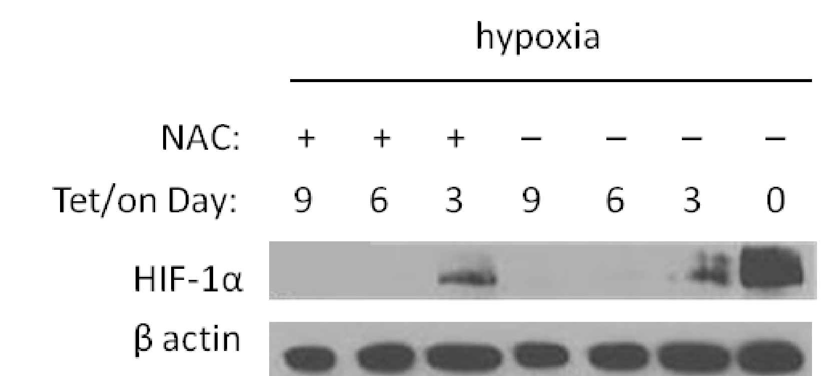

Figure 2. Dramatic decrease in HIF-1α expression followed tetracycline induced expression of POLGdn. When NAC is co-administrated, we do not see a significant difference in HIF-1α expression.

Inhibition of mitochondrial respiratory complexes led to degradation of HIF-1a.

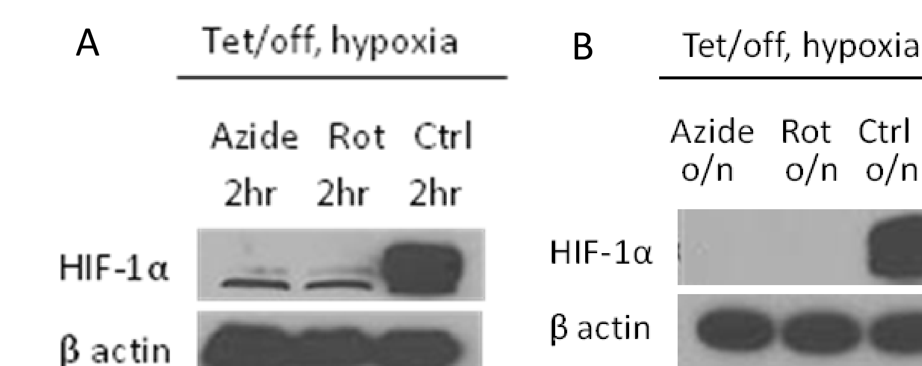

Figure 3. 2 hour (A) or overnight (B) inhibition of mitochondrial complex I or complex IV does not induce HIF-1α expression.

Dramatic decrease of HIF-1α expression followed inhibition of mitochondrial respiratory complexes.

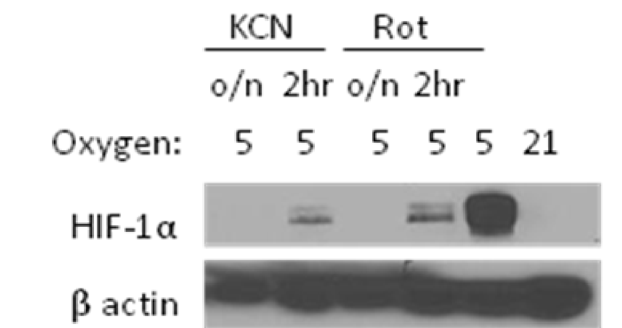

Figure 4. Inhibition of mitochondrial complex I decreased HIF-1α expression at 2 hours, while inhibition of mitochondrial complex IV lead to degradation of HIF-1α at both 2 hours and overnight.

Conclusions

- Under hypoxic conditions, HIF-1a expression is not detectable in cells with compromise mitochondrial respiration.
- HIF-1α stability did not alter following NAC treatment in the hypoxic condition.
- Expression of HIF-1α was significantly decreased in Tet/off and HCT116$p53^{-/-}$ cells after short-term inhibition of either mitochondrial complex I or complex IV.
- Mitochondrial respiration integrity is essential for HIF-1α stabilization.

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