MYC overrides HIF-1α to regulate proliferating primary cell metabolism in hypoxia

Courtney A. Copeland1,2, Benjamin A. Olenchock1,2, David R. Ziehr1,3,2, Sarah McGarrity1,2,4, Kevin Leahy1,2, Jamey D. Young5, Joseph Loscalzo1,2, and William M. Oldham1,2,‡

1 Department of Medicine, Brigham and Women’s Hospital, Boston, MA 02115, U.S.A.  
2 Department of Medicine, Harvard Medical School, Boston, MA 02115, U.S.A.  
3 Department of Medicine, Massachusetts General Hospital, Boston, MA, 02114 U.S.A.  
4 Center for Systems Biology, School of Health Sciences, University of Iceland, Reykjavik, Iceland  
5 Departments of Chemical & Biomolecular Engineering and Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN 37240, U.S.A.

‡ Correspondence: [William M. Oldham <woldham@bwh.harvard.edu>](mailto:woldham@bwh.harvard.edu)

# Abstract

Hypoxia requires metabolic adaptations to sustain energetically demanding cellular activities. While the metabolic consequences of hypoxia have been studied extensively in cancer cell models, comparatively little is known about the metabolic response of primary cells to hypoxia. We performed metabolic flux analyses of human lung fibroblasts and pulmonary artery smooth muscle cells proliferating in hypoxia. Unexpectedly, hypoxia decreased glycolysis despite activation of hypoxia-inducible factor 1α (HIF-1α) and increased glycolytic enzyme expression. While HIF-1α activation by prolyl hydroxylase (PHD) inhibition did increase glycolysis in normoxia, this effect was abrogated by hypoxia. Multi-omic profiling revealed distinct molecular responses to hypoxia and PHD inhibition, suggesting a critical role for MYC in modulating HIF-1α responses to hypoxia. Consistent with this hypothesis, MYC knockdown in hypoxia increased glycolysis and MYC overexpression in normoxia decreased glycolysis stimulated by PHD inhibition. These data suggest that MYC signaling in hypoxia uncouples an increase in HIF-dependent glycolytic gene transcription from glycolytic flux.

# Keywords

hypoxia / metabolic flux analysis / hypoxia-inducible factor / prolyl hydroxylase / MYC

# Introduction

Cellular responses to hypoxia propel many physiologic and pathologic processes from wound healing and angiogenesis to vascular remodeling and fibrosis (J. W. Lee et al., 2019; Semenza, 2012). These activities require cells to continue energetically demanding tasks, such as macromolecular biosynthesis and proliferation, despite limited oxygen availability. Since respiration is the most efficient way for cells to produce energy, cell metabolism must adapt to meet energy demands when oxygen supply is limiting. Understanding how these metabolic adaptations sustain critical cellular processes in hypoxia is fundamentally important to our understanding of human health and disease.

Cells typically respond to hypoxia by shifting energy production away from respiration and toward glycolysis. This response is mediated primarily by stabilization of the hypoxia-inducible transcription factor 1α (HIF-1α). HIF-1α activates the transcription of glucose transporters, glycolytic enzymes, lactate dehydrogenase, and pyruvate dehydrogenase kinase, while decreasing the expression of tricarboxylic acid (TCA) cycle and electron transport chain enzymes (Lee et al., 2020; Semenza, 2012). Although HIF-1α is constitutively expressed, it is hydroxylated by prolyl hydroxylase enzymes (PHDs) in normoxia and targeted for proteasomal degradation. PHDs are the principal oxygen sensors in metazoan cells (Kaelin and Ratcliffe, 2008). PHDs are α-ketoglutarate-dependent dioxygenase enzymes that require molecular oxygen for their enzymatic activity. When oxygen tension falls, PHD activity decreases, leading to HIF-1α stabilization and activation of its downstream transcriptional program. Overall, this transcriptional program should increase glycolytic capacity and divert glucose-derived pyruvate from oxidative phosphorylation toward lactate fermentation to maintain glycolytic ATP production.

In addition to metabolic changes designed to maintain energy supply, hypoxic cells also reduce energy demand through down-regulation of Na+/K+-ATPase, slowing protein translation, and decreasing cell proliferation (Hubbi and Semenza, 2015; Wheaton and Chandel, 2011). In particular, HIF-1α decreases cell proliferation by activating cyclin-dependent kinase inhibitor expression, inhibiting cell-cycle checkpoint progression (Gardner et al., 2001), and antagonizing pro-proliferative MYC signaling (Koshiji et al., 2004). Despite these canonical effects of HIF-1α activation, there are many examples where cells continue to proliferate despite hypoxic stress, including cancer cells, stem cells, and lung vascular cells (Hubbi and Semenza, 2015). How these cells meet the metabolic needs of sustained proliferation in hypoxia and how these adaptations are regulated are active areas of investigation (Jain et al., 2020; Lee et al., 2020; Oldham et al., 2015). Since hypoxia is a prominent feature of cancer biology as tumor growth outstrips blood supply, most detailed metabolic studies of hypoxic cell metabolism have used tumor cell models, yielding important insights into the metabolic pathobiology of cancer (Garcia-Bermudez et al., 2018; Jiang et al., 2016; W. D. Lee et al., 2019; Melendez-Rodriguez et al., 2019; Metallo et al., 2011; Wise et al., 2011). For example, stable isotope tracing and metabolic flux analyses identified a critical role for the reductive carboxylation of glutamine-derived α-ketoglutarate for lipid biosynthesis in supporting tumor growth (Gameiro et al., 2013; Metallo et al., 2011; Scott et al., 2011; Wise et al., 2011), and metabolomic studies identified aspartate as a limiting metabolite for cancer cell proliferation under hypoxia (Garcia-Bermudez et al., 2018). By contrast, comparatively little is known about metabolic adaptations of primary cells to hypoxia. Indeed, the importance of reductive carboxylation or aspartate biosynthesis remains to be elucidated in primary cells. A more complete understanding of primary cell metabolic adaptations to hypoxia would provide an important context for understanding how metabolic reprogramming supports normal cellular responses to hypoxia, how these responses may be (mal)adaptive in a variety of disease contexts, and how the hypoxia metabolic program in primary cells differs from that observed in cancer cells.

To address these questions, we have developed models of bioenergetic carbon flux in human lung fibroblasts (LFs) and pulmonary artery smooth muscle cells (PASMCs) cultured in 21% or 0.5% oxygen. These cells may be exposed to a wide range of oxygen concentrations *in vivo*, continue to proliferate despite hypoxic culture conditions *in vitro*, and play important roles in the pathology of non-cancerous diseases in which tissue hypoxia is a prominent feature, including pulmonary hypertension and pulmonary fibrosis. We found that hypoxia fails to increase glycolysis in these primary cells despite robust up-regulation of the HIF-1α transcriptional program. In normoxia, HIF-1α stabilization by the PHD inhibitor molidustat (BAY-85-3934, “BAY”) (Flamme et al., 2014) did increase glycolysis and lactate efflux; however, hypoxia blocked this response. These findings suggested that important hypoxia-dependent regulatory mechanisms override the metabolic consequences of HIF-1α-dependent glycolytic gene expression. Transcriptomic profiling identified a critical role for the transcription factor MYC in the adaptive response to hypoxia. Using knockdown and overexpression approaches, we demonstrated that MYC attenuates HIF-driven glycolysis in hypoxia and following HIF stabilization in normoxia.

# Results

## Hypoxia uncouples HIF-dependent glycolytic gene expression from glycolytic metabolic flux

The goal of this study was to characterize hypoxia-induced metabolic changes in proliferating primary LFs and PASMCs. To accomplish this goal, we adopted a metabolic flux analysis technique that enabled us to link intracellular metabolic fluxes to cell proliferation rates. Metabolic flux analysis fits cell proliferation rate, extracellular flux measurements, and 13C intracellular isotope labeling patterns to a computational model of cell metabolism (Antoniewicz, 2018). This analysis reconstructs comprehensive flux maps that depict the flow of carbon from extracellular substrates, through intracellular metabolic pathways, and into cell biomass and metabolic by-products (Young, 2014). These models assume that cells are at a metabolic pseudo-steady state over the experimental time course (Buescher et al., 2015). Exponential growth phase is thought to reflect metabolic pseudo-steady state as cells in culture steadily divide at their maximal condition-specific rate, provided nutrient supply does not become limiting (Ahn and Antoniewicz, 2011; Buescher et al., 2015). Thus, we first set out to define experimental conditions to capture exponential growth phase in normoxic and hypoxic cultures.

Cells were seeded and placed into hypoxia for 24 h prior to sample collection to provide adequate time for activation of the hypoxia-dependent transcriptional program (**Figure 1A**). We selected 0.5% oxygen for hypoxia as this level yielded the most reproducible phenotypic differences compared to 21% oxygen culture, while being physiologically relevant and above the KM of cytochrome *c* oxidase (electron transport chain complex IV) for oxygen (Lee et al., 2020; Wenger et al., 2015). From this starting point, we identified the optimal cell seeding density and time course to capture exponential cell growth (**Figure 1B**). LFs cultured in 0.5% oxygen grew more slowly than LFs cultured in 21% oxygen (**Figure 1C**), but slower growth was not associated with decreased cell viability (**Fig S1A**).

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# Author contributions

W.M.O. conceived and designed the analysis. C.A.C., B.A.O., D.R.Z., S.M., K.L., and W.M.O. collected the data. J.D.Y. and W.M.O. contributed data or analysis tools. W.M.O. performed the analysis. W.M.O. drafted the manuscript. All authors participated in interpreting the results and revising the manuscript. All authors approve the final submission.

# Conflict of interest

The authors declare that they have no conflicts of interest.

# References

Ahn WS, Antoniewicz MR. 2011. Metabolic flux analysis of CHO cells at growth and non-growth phases using isotopic tracers and mass spectrometry. *Metab Eng* **13**:598–609. doi:[10.1016/j.ymben.2011.07.002](https://doi.org/10.1016/j.ymben.2011.07.002)

Antoniewicz MR. 2018. A guide to 13C metabolic flux analysis for the cancer biologist. *Exp Mol Med* **50**:1–13. doi:[10.1038/s12276-018-0060-y](https://doi.org/10.1038/s12276-018-0060-y)

Buescher JM, Antoniewicz MR, Boros LG, Burgess SC, Brunengraber H, Clish CB, DeBerardinis RJ, Feron O, Frezza C, Ghesquiere B, Gottlieb E, Hiller K, Jones RG, Kamphorst JJ, Kibbey RG, Kimmelman AC, Locasale JW, Lunt SY, Maddocks OD, Malloy C, Metallo CM, Meuillet EJ, Munger J, Noh K, Rabinowitz JD, Ralser M, Sauer U, Stephanopoulos G, St-Pierre J, Tennant DA, Wittmann C, Vander Heiden MG, Vazquez A, Vousden K, Young JD, Zamboni N, Fendt SM. 2015. A roadmap for interpreting (13)C metabolite labeling patterns from cells. *Curr Opin Biotechnol* **34**:189–201. doi:[10.1016/j.copbio.2015.02.003](https://doi.org/10.1016/j.copbio.2015.02.003)

Flamme I, Oehme F, Ellinghaus P, Jeske M, Keldenich J, Thuss U. 2014. Mimicking hypoxia to treat anemia: HIF-stabilizer BAY 85-3934 (Molidustat) stimulates erythropoietin production without hypertensive effects. *PLoS One* **9**:e111838. doi:[10.1371/journal.pone.0111838](https://doi.org/10.1371/journal.pone.0111838)

Gameiro PA, Yang J, Metelo AM, Perez-Carro R, Baker R, Wang Z, Arreola A, Rathmell WK, Olumi A, Lopez-Larrubia P, Stephanopoulos G, Iliopoulos O. 2013. In vivo HIF-mediated reductive carboxylation is regulated by citrate levels and sensitizes VHL-deficient cells to glutamine deprivation. *Cell Metab* **17**:372–85. doi:[10.1016/j.cmet.2013.02.002](https://doi.org/10.1016/j.cmet.2013.02.002)

Garcia-Bermudez J, Baudrier L, La K, Zhu XG, Fidelin J, Sviderskiy VO, Papagiannakopoulos T, Molina H, Snuderl M, Lewis CA, Possemato RL, Birsoy K. 2018. Aspartate is a limiting metabolite for cancer cell proliferation under hypoxia and in tumours. *Nat Cell Biol* **20**:775–781. doi:[10.1038/s41556-018-0118-z](https://doi.org/10.1038/s41556-018-0118-z)

Gardner LB, Li Q, Park MS, Flanagan WM, Semenza GL, Dang CV. 2001. Hypoxia inhibits G1/S transition through regulation of p27 expression. *J Biol Chem* **276**:7919–7926. doi:[10.1074/jbc.M010189200](https://doi.org/10.1074/jbc.M010189200)

Hubbi ME, Semenza GL. 2015. Regulation of cell proliferation by hypoxia-inducible factors. *Am J Physiol Cell Physiol* **309**:C775–82. doi:[10.1152/ajpcell.00279.2015](https://doi.org/10.1152/ajpcell.00279.2015)

Jain IH, Calvo SE, Markhard AL, Skinner OS, To TL, Ast T, Mootha VK. 2020. Genetic Screen for Cell Fitness in High or Low Oxygen Highlights Mitochondrial and Lipid Metabolism. *Cell* **181**:716–727 e11. doi:[10.1016/j.cell.2020.03.029](https://doi.org/10.1016/j.cell.2020.03.029)

Jiang L, Shestov AA, Swain P, Yang C, Parker SJ, Wang QA, Terada LS, Adams ND, McCabe MT, Pietrak B, Schmidt S, Metallo CM, Dranka BP, Schwartz B, DeBerardinis RJ. 2016. Reductive carboxylation supports redox homeostasis during anchorage-independent growth. *Nature* **532**:255–8. doi:[10.1038/nature17393](https://doi.org/10.1038/nature17393)

Kaelin WG, Ratcliffe PJ. 2008. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* **30**:393–402. doi:[10.1016/j.molcel.2008.04.009](https://doi.org/10.1016/j.molcel.2008.04.009)

Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. 2004. HIF-1alpha induces cell cycle arrest by functionally counteracting Myc. *EMBO J* **23**:1949–1956. doi:[10.1038/sj.emboj.7600196](https://doi.org/10.1038/sj.emboj.7600196)

Lee JW, Ko J, Ju C, Eltzschig HK. 2019. Hypoxia signaling in human diseases and therapeutic targets. *Exp Mol Med* **51**:1–13. doi:[10.1038/s12276-019-0235-1](https://doi.org/10.1038/s12276-019-0235-1)

Lee P, Chandel NS, Simon MC. 2020. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nat Rev Mol Cell Biol* **21**:268–283. doi:[10.1038/s41580-020-0227-y](https://doi.org/10.1038/s41580-020-0227-y)

Lee WD, Mukha D, Aizenshtein E, Shlomi T. 2019. Spatial-fluxomics provides a subcellular-compartmentalized view of reductive glutamine metabolism in cancer cells. *Nat Commun* **10**:1351. doi:[10.1038/s41467-019-09352-1](https://doi.org/10.1038/s41467-019-09352-1)

Melendez-Rodriguez F, Urrutia AA, Lorendeau D, Rinaldi G, Roche O, Bogurcu-Seidel N, Ortega Muelas M, Mesa-Ciller C, Turiel G, Bouthelier A, Hernansanz-Agustin P, Elorza A, Escasany E, Li QOY, Torres-Capelli M, Tello D, Fuertes E, Fraga E, Martinez-Ruiz A, Perez B, Gimenez-Bachs JM, Salinas-Sanchez AS, Acker T, Sanchez Prieto R, Fendt SM, De Bock K, Aragones J. 2019. HIF1alpha Suppresses Tumor Cell Proliferation through Inhibition of Aspartate Biosynthesis. *Cell Rep* **26**:2257–2265 e4. doi:[10.1016/j.celrep.2019.01.106](https://doi.org/10.1016/j.celrep.2019.01.106)

Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Johnson ZR, Irvine DJ, Guarente L, Kelleher JK, Vander Heiden MG, Iliopoulos O, Stephanopoulos G. 2011. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* **481**:380–4. doi:[10.1038/nature10602](https://doi.org/10.1038/nature10602)

Oldham WM, Clish CB, Yang Y, Loscalzo J. 2015. Hypoxia-Mediated Increases in L-2-hydroxyglutarate Coordinate the Metabolic Response to Reductive Stress. *Cell Metabolism* **22**:291–303. doi:[10.1016/j.cmet.2015.06.021](https://doi.org/10.1016/j.cmet.2015.06.021)

Scott DA, Richardson AD, Filipp FV, Knutzen CA, Chiang GG, Ronai ZA, Osterman AL, Smith JW. 2011. Comparative metabolic flux profiling of melanoma cell lines: beyond the Warburg effect. *J Biol Chem* **286**:42626–34. doi:[10.1074/jbc.M111.282046](https://doi.org/10.1074/jbc.M111.282046)

Semenza GL. 2012. Hypoxia-inducible factors in physiology and medicine. *Cell* **148**:399–408. doi:[10.1016/j.cell.2012.01.021](https://doi.org/10.1016/j.cell.2012.01.021)

Wenger RH, Kurtcuoglu V, Scholz CC, Marti HH, Hoogewijs D. 2015. Frequently asked questions in hypoxia research. *Hypoxia (Auckl)* **3**:35–43. doi:[10.2147/HP.S92198](https://doi.org/10.2147/HP.S92198)

Wheaton WW, Chandel NS. 2011. Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am J Physiol Cell Physiol* **300**:C385–93. doi:[10.1152/ajpcell.00485.2010](https://doi.org/10.1152/ajpcell.00485.2010)

Wise DR, Ward PS, Shay JE, Cross JR, Gruber JJ, Sachdeva UM, Platt JM, DeMatteo RG, Simon MC, Thompson CB. 2011. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of alpha-ketoglutarate to citrate to support cell growth and viability. *Proc Natl Acad Sci U S A* **108**:19611–6. doi:[10.1073/pnas.1117773108](https://doi.org/10.1073/pnas.1117773108)

Young JD. 2014. INCA: a computational platform for isotopically non-stationary metabolic flux analysis. *Bioinformatics* **30**:1333–5. doi:[10.1093/bioinformatics/btu015](https://doi.org/10.1093/bioinformatics/btu015)

# Figures

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