August 2, 2022

Facundo Batista, Ph.D.

Chief Editor

*The EMBO Journal*

Dear Dr. Batista:

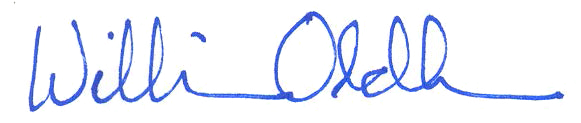
We are delighted to submit for your consideration at *The EMBO Journal* our manuscript entitled, “MYC overrides HIF to regulate proliferating primary cell metabolism in hypoxia.” As you know, the study of hypoxic cancer cell metabolism is an extremely active area of investigation, while comparatively less is known about how human primary cells adapt to oxygen limitation. In this study, we sought to develop metabolic flux models of human primary cells exposed to hypoxia. Classically, hypoxia is thought to increase glycolysis through the activation of hypoxia-inducible factor (HIF) and increased glycolytic enzyme expression. Surprisingly, we found that hypoxia suppressed glycolytic flux in proliferating primary cells despite HIF accumulation and glycolytic gene expression. Interestingly, hypoxia also suppressed the increase in glycolysis following pharmacologic HIF stabilization in normoxia. These observations led us to perform metabolomic and transcriptomic profiling of our primary cells which suggested that hypoxia-induced MYC activation may antagonize the effects of HIF stabilization in hypoxia. Indeed, using MYC knockdown and overexpression approaches, we show that MYC inhibits HIF-dependent glycolytic flux.

We expect these findings to be of interest to your readers. In particular, they highlight the critical importance of HIF-independent regulation of the cellular metabolic response to hypoxia. They demonstrate the limitations of inferring changes in metabolic flux from changes in gene expression. They highlight fundamental differences in how primary cells respond to hypoxia as compared to cancer cells. Finally, they identify a new paradigm of HIF-MYC cross-talk whereby MYC antagonizes the metabolic consequences of HIF activation in hypoxia. We anticipate these findings raise important questions for future research, including what role other HIF-independent events play in the hypoxia response, how MYC activity is regulated by hypoxia, and whether these regulatory pathways contribute to hypoxia responses *in vivo*.

We respectfully suggest the following reviewers: Ralph DeBerardinis ([Ralph.Deberardinis@UTSouthwestern.edu](mailto:Ralph.Deberardinis@UTSouthwestern.edu)) based on his familiarity with the metabolic flux analyses described in the manuscript; Kurt Stenmark ([kurt.stenmark@cuanschutz.edu](mailto:kurt.stenmark@cuanschutz.edu)) based on his work in hypoxic primary cell metabolism; Mark Gladwin ([gladwinmt@upmc.edu](mailto:gladwinmt@upmc.edu)) based on his work related to cellular responses to redox stress; Brian Graham ([Brian.Graham@ucsf.edu](mailto:Brian.Graham@ucsf.edu)) who has previously done some stable isotope labeling in related systems; and Stephen Chan ([chansy@pitt.edu](mailto:chansy@pitt.edu)), who is an expert in pulmonary vascular cell metabolism.

Thank you for your consideration of our manuscript.

Sincerely,

  
William M. Oldham, M.D., Ph.D.­­­