REPLY TO MOHLIN ET AL.:

High levels of *EPAS1* are closely associated with key features of low-risk neuroblastoma

Isabelle Westerlund[a], Yao Shi[a], Konstantinos Toskas[a], Stuart M. Fell[a], Shuijie Li[a,b], Erik Södersten[a], Susanne Schlisio[a,b], and Johan Holmberg[a,1]

Our principal aim was not to explore the role of *EPAS1*/HIF2α in neuroblastoma (NB). Neither do we claim to present conclusive data showing that HIF2α is a bona fide NB tumor suppressor. We simply followed our analysis of the transcriptional response induced by 5-aza-deoxycytidine (AZA)+retinoic acid (RA) treatment to the analysis of large (n = 498 + 649) sequenced/microarrayed NB cohorts. We show that *EPAS1*/HIF2α expression strongly correlates with better outcome and low-risk NB, while being inversely correlated with key features of high-risk NB. Thus, our analysis indicates a possible tumor suppressor role and question whether HIF2α is a NB oncogene (1).

The claim that we have not measured HIF2α protein levels in NB (2) is simply not correct (figure 7*H*). We did not measure HIF2α at the cellular level. However, a previous study shows HIF2α enrichment in *MYCN*-nonamplified NB, whereas HIF1α immunoreactivity is abundant in *MYCN*-amplified NB (3), supporting our analysis. Furthermore, conflicting results between studies utilizing the same technique (3–5) makes it desirable to use methods that are more robust.

Mohlin et al. (2) claim that, in the NB datasets we use, *EPAS1* mRNA is derived from tumor-associated macrophages (TAMs). As pointed out in a study coauthored by Påhlman (6), aggressive NB typically contains high numbers of TAMs. Since high levels of TAMs are a feature of high-risk NB (6, 7) and high levels of *EPAS1*/HIF2α are correlated with low-risk NB, we are somewhat perplexed by their argument. In another NB study, Påhlman and coworkers (8) conclude "that the majority of HIF-2α$^+$ cells are indeed tumour-derived". Why then would *EPAS1*/HIF2α expression not be tumor-derived in the NB datasets we have analyzed? Notably, Påhlman and coworkers have themselves used a smaller (n = 88) NB dataset to visualize *EPAS1/IGF2* expression in *MYCN*-amplified vs. nonamplified tumors (4), without accounting for TAM interference.

We never claim that AZA+RA treatment induces differentiation to bona fide sympathetic nervous system (SNS) neurons. We show that it impedes tumor growth and at day 14 induces a transcriptional response characterized by up-regulation of hypoxia-associated genes and down-regulation of genes typical for cycling tumor cells. At the end point of treatment, the transcriptional response contains a strong "neuron differentiation" component. In contrast to what Mohlin et al. claim, we do not merely base this on expression of two markers. Our conclusion is based on RNA sequencing of tumors at different time points. *SCG5*, *STMN2*, and the other three genes (S4O-Q) are just examples of neuronal genes. Despite their criticism of our including *STMN2* (*SCG10*), the authors themselves utilize it as a sympathetic neuronal marker (4, 9). Whether AZA+RA treatment induces SNS neurons is, in our opinion, less important than that it impedes tumor growth.

Mohlin et al. claim that no previous observations support our findings. As we state in our *Discussion*, Simon and coworkers (3) have shown that HIF1α, but not HIF2α, is enriched in high-risk *MYCN*-amplified NB. Mohlin et al. claim that HIF2α has solely oncogenic functions in other tumors. This is a misrepresentation of the literature; several studies show the opposite (e.g., refs. 10 and 11).

Thus, both our analysis and the literature imply that a deeper understanding of HIF2α in NB biology is warranted before NB patients receive HIF2α inhibitors.

[a]Department of Cell and Molecular Biology, Ludwig Institute for Cancer Research, Karolinska Institutet, 171 77 Stockholm, Sweden; and [b]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 171 65 Solna, Sweden

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The authors declare no conflict of interest.

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[1]To whom correspondence should be addressed. Email: johan.holmberg@ki.se.

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