Abstract Title:

Protection Against Hypoxic Injury Is Mediated By Regulators Of Glucose and Lipid Metabolism

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Introduction: Injury to developing organs at birth can lead to cerebral palsy, neurocognitive impairment, and other learning and behavior disabilities. Interestingly, a remarkable phenomenon known as hypoxic preconditioning arises when a brief hypoxia exposure leads to substantial protection against subsequent, severe hypoxia. Although the protective phenomenon of hypoxic preconditioning (hPC) has been demonstrated in several model organisms, its molecular underpinnings remain poorly understood and are likely distinct from the acute hypoxia response. Accordingly, our aim is to discover novel hypoxia-protective genes using a functional genomic approach in a zebrafish developmental stress model.

Methods: Previously, we established conditions for reproducible acute hypoxic injury at various stages of development, including optimization of a robust hypoxic preconditioning protocol that was able to protect against an overwhelmingly lethal hypoxia exposure (Fig. 1). To identify differentially expressed transcripts induced by hypoxic stress, we utilized genome-wide transcriptional profiling at gastrula and

segmentation stages. We subsequently validated the hypoxia-inducibility of highly-induced genes individually and then tested individual gene function via knockdown in the hPC assay.

Results: Analysis of these data prominently identified the prolyl hydroxylase egln3 (Fig. 2A, arrow; 2B), a key regulatory enzyme of the Hif1a response pathway, as the single most highly-upregulated and statistically significant gene detected, among multiple additional novel or uncharacterized hypoxia-regulated genes. Hypoxia-regualted expression was validated for individual candidates with qPCR or in situ hybridization, and further functional characterization with antisense oligonucleotide injection and mRNA rescue (n=11). Of six morphants without global defects in normoxia, five showed significant impairment of hPC in comparison to control-MO-injected embryos, including genes functioning in the insulin, cAMP/creb, and hematopoiesis pathways (Fig. 2C-E; Manchenkov et al., 2015, G3:Genes|Genomes|Genetics).

Conclusion: These results demonstrate that the embryonic stress model in zebrafish can be used for discovery of novel hypoxia response genes essential for hypoxic preconditioning, and that "tuning" of energy metabolism contributes to hypoxia protection.