Genomics Data 6 (2015) 83–88

Contents lists available at ScienceDirect

Genomics Data

journal homepage: http://www.journals.elsevier.com/genomics-data/

Data in Brief

Transcriptome analysis of severe hypoxic stress during development in zebrafish

I.G. Woods [a], F.B. Imam [b,c,*]

[a] Department of Biology, Ithaca College, Ithaca, NY, USA
[b] Department of Pediatrics, Division of Neonatology, University of California San Diego School of Medicine, La Jolla, CA, USA
[c] Rady Children's Hospital-San Diego, USA

ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form 22 July 2015
Accepted 26 July 2015
Available online 4 August 2015

Keywords:
Hormesis
Hypoxia–ischemia
Metabolism
Preconditioning
Stress tolerance

ABSTRACT

Hypoxia causes critical cellular injury both in early human development and in adulthood, leading to cerebral palsy, stroke, and myocardial infarction. Interestingly, a remarkable phenomenon known as hypoxic preconditioning arises when a brief hypoxia exposure protects target organs against subsequent, severe hypoxia. Although hypoxic preconditioning has been demonstrated in several model organisms and tissues including the heart and brain, its molecular mechanisms remain poorly understood. Accordingly, we used embryonic and larval zebrafish to develop a novel vertebrate model for hypoxic preconditioning, and used this model to identify conserved hypoxia-regulated transcripts for further functional study as published in Manchenkov et al. (2015) in G3: Genes|Genomes|Genetics. In this Brief article, we provide extensive annotation for the most strongly hypoxia-regulated genes in zebrafish, including their human orthologs, and describe in detail the methods used to identify, filter, and annotate hypoxia-regulated transcripts for downstream functional and bioinformatic assays using the source data provided in Gene Expression Omnibus Accession GSE68473.

Specifications	
Organism/cell line/tissue	Zebrafish (*Danio rerio*)
Sex	Mixed sex
Sequencer or array type	NimbleGen Gene Expression Danio Rerio 385k Array [071105_Zv7_EXPR]
Data format	RMA Normalized (R/Bioconductor, Limma)
Experimental factors	Hypoxia vs. normoxia; shield (gastrula) and 8-somite (segmentation) stages
Experimental features	Study of hypoxia-induced differential expression during zebrafish development at gastrulation (shield) and segmentation (8-somite) stages
Consent	N/A
Sample source location	Boston, Massachusetts, USA

1. Experimental design and data

To identify novel hypoxia protective factors, we developed a novel *in vivo* hypoxic stress assay in zebrafish. First, we optimized hypoxic stress parameters at two different timepoints during zebrafish development and collected control and hypoxia-exposed embryos at gastrula (shield, 6 h postfertilization or hpf) and segmentation (8 somite, 12 hpf) stages to identify shared hypoxia responses at two timepoints (Fig. 1A). We reasoned that hypoxia response genes activated at distinct developmental timepoints were likely to be enriched for evolutionarily conserved hypoxia-protective components relevant for human biology and disease. We performed RNA extraction, labeling, and hybridization to NimbleGen zebrafish microarrays and show both the raw and normalized intensity plots and histograms for these data (Fig. 1B, C). We combined data from normoxic control samples at both gastrula and segmentation in comparison with matched hypoxia-exposed samples and identified 3768 of the 37,157 transcripts measured to be differentially expressed greater than 2-fold under hypoxia (Fig. 2). To further increase the utility of this dataset for the scientific community, we provide additional annotations in this article that includes human homologs and functional properties/domains for all transcripts measured, including the 300 most hypoxia-induced and hypoxia-repressed genes passing statistical significance criteria (Supplementary Tables 1–3). From these lists of genes, we validated the expression of a subset of individual hypoxia-induced genes using qPCR and/or in situ hybridization, with *irs2* shown as a representative hypoxia-induced gene that exhibited increased and ectopic expression at both gastrula and segmentation (Fig. 3). We further tested individual expression-validated genes for hypoxia-protective function in both acute hypoxic stress buffering and hypoxic preconditioning assays in zebrafish (Fig. 4) and identified several novel hypoxia-induced genes

* Corresponding author at: 9500 Gilman Drive #0734, La Jolla, CA 92093-0734, USA.
E-mail address: f2imam@ucsd.edu (F.B. Imam).

http://dx.doi.org/10.1016/j.gdata.2015.07.025

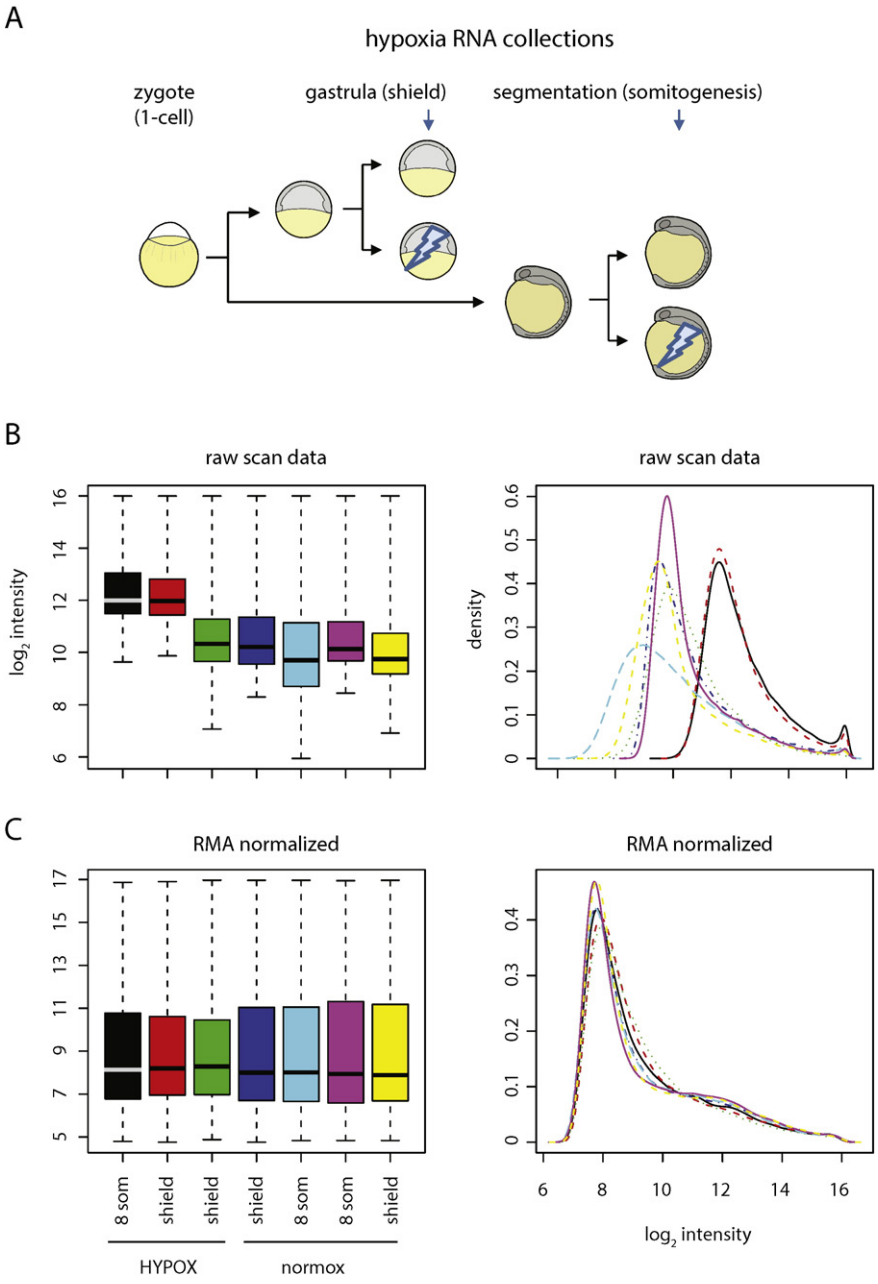

Fig. 1. Hypoxia sample collection and quality control. (A) RNA collection scheme. Stage-synchronized embryos were collected in duplicate at gastrula (shield, 6 h) and segmentation (8-somite, 12 h) stages for RNA extraction with or without 2 h of severe hypoxia exposure at 0.3% oxygen (blue arrows). (B) Raw data from individual samples is shown both as a log-intensity boxplot and histogram. (C) Data after Robust Multi-array Average (RMA) normalization showing alignment.

that function to protect against acute hypoxic stress and/or provide hypoxic preconditioning protection [1].

2. Materials and methods

2.1. Zebrafish husbandry and strains

Zebrafish from the TL/AB strain were maintained using standard procedures and developmental stages were determined as previously described [2]. Embryos were raised at 28.5 °C in embryo water containing 0.1% Methylene Blue hydrate (Sigma, St. Louis, MO, USA) and 0.03% Instant Ocean sea salt (United Pet Group, Cincinnati, OH, USA). Experiments were performed under UCSD IACUC protocol S13006.

2.2. Hypoxia exposure and RNA isolation for microarrays

For hypoxia treatment of embryos used for RNA isolation and microarray analysis, a closed hypoxia chamber was used with a flow meter attached to a gas source (Billups-Rothenberg, Inc.). Hypoxia chambers were flushed for 4 min at 20 L/min, and repeated 30 min later with nitrogen gas. 50 mm Petri dishes containing 4 mL of embryo water were pre-equilibrated in the hypoxia chamber prior for at least 4 h prior to embryo transfer, as this treatment was determined to be the minimum time necessary to reach <1% oxygen as measured by a dissolved oxygen meter (DO-5509; Alfa Electronics), and gave similar results when compared with longer pre-equilibration times up to 24 h. A colorimetric resazurin indicator was used to monitor the hypoxic environment during pre-treatment of media and the duration of each experiment (<1% oxygen if colorless; Bio-Bag, Becton, Dickinson and Company).