ROLE OF N-MYC DOWNSTREAM REGULATED GENE 1A (NDRG1A) IN MEDIATING ANOXIA-INDUCED CELL CYCLE ARREST

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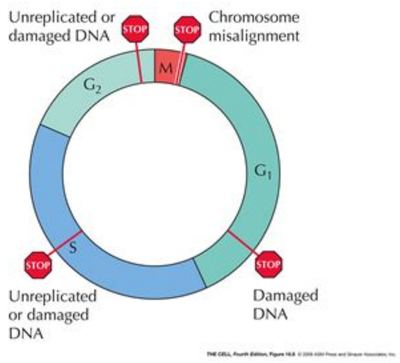
Abstract

Zebrafish embryos can survive for up to 50 hours in absence of oxygen (anoxia). N-Myc Downstream Regulated Genes (NDRGs) are transcriptionally upregulated under low oxygen and have been linked to adaptive responses of hypoxic cancer cells. The Brewster lab has shown that *NDRG1a* is implicated in physiological adaptation of zebrafish kidney cells to prolonged anoxia, by downregulating the ATP-demanding sodium-potassium ATPase pump. My research project aims to determine whether members of the NDRG family also play a role in mediating anoxia-induced cell cycle arrest, which is expected to be energy-conserving and pro-survival. I hypothesize that *NDRG1a* is activated in response to anoxia and blocks mitosis. To test this, we are comparing the mitotic index in dome-stage *NDRG1a*-depleted embryos raised under anoxic conditions (2h and 4h) to control groups. The mitotic index (number of M phase cells/total cell number) is assessed following imaging and quantification of embryos labeled with P-Histone 3 (M phase marker) and DAPI (nuclear marker). Preliminary data indicate that the mitotic index is higher in anoxia-treated *NDRG1a*-depleted embryos than in controls, supporting my hypothesis. Future directions of this project will include analyzing the role of other members of the NDRG family, specifically *NDRG3a*, in cell cycle arrest.

Background

- The cell cycle is the repeated progression by which DNA is replicated (S phase), the cell divides (M phase), and in between these two steps the cell checks to make sure this process of proliferation runs smoothly without error (G1 and G2)[1]



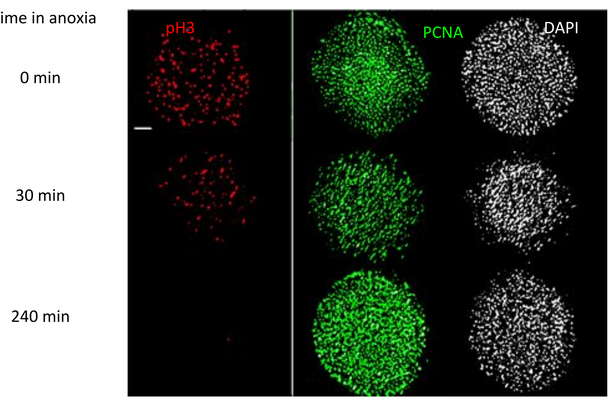


Figure 2A: Time for anoxia-induced cell cycle arrest in zebrafish. Animal poles of dome-stage (4 hpf) embryos exposed to increasing durations of physical anoxia 0, 30 and 240 minutes shown here. Embryos were then labeled via immunofluorescence with anti-pH3 (mitotic marker)(A-C), anti-PCNA (replication marker), and stained with DAPI (nuclear marker). Scale bar is 100µm.

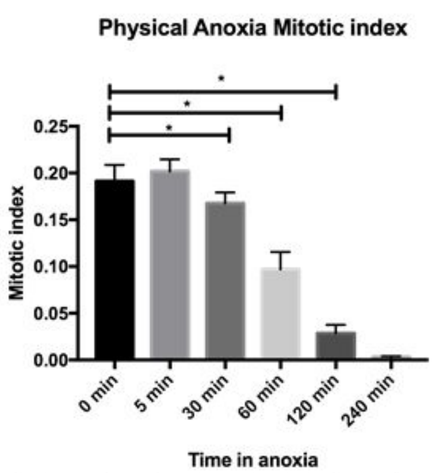
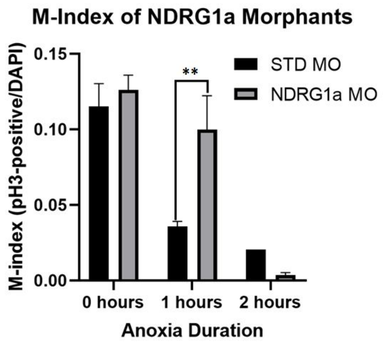


Figure 2B: Preliminary data showing mitotic indices of physical anoxia. These were calculated by dividing the quantified number of pH3-positive cells by the total number of DAPI stained nuclei.



** p<0.005 Determined with Two-way ANOVA and Sidak's Multiple Comparisons Test

- *NDRG1a* morpholino injected embryos exhibit a greater proportion of cells in mitosis following 1 hour of anoxia compared to embryos which were injected with a Standard (STD) control morpholino.

- Overexpression of NDRGs results in decreased cell numbers and DNA replication, knockdown leads to increased proliferation.[2]

NDRGs
↓ Cancer
NDRG1
↓
Regulates spindle organization

- NDRG1 is important in maintaining correct microtubule function, whose disturbance may lead to genomic instability and a block in the progression through the M-phase

Questions

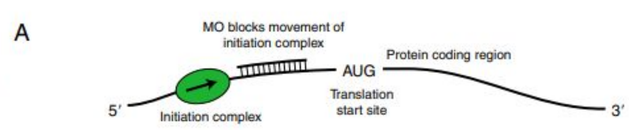
Is *NDRG1a* involved in mediating anoxia induced cell cycle arrest?

Methods

Morpholino Knockdown Approach

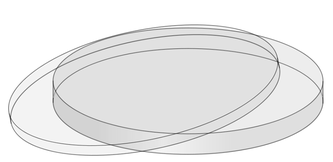
Inject groups of fertilized embryos with a standard morpholino and an *NDRG1a* morpholino

[3]



- Morpholinos are synthetic oligonucleotides composed of chains of about 25 subunits that are similar to DNA and RNA oligonucleotides, except that they have a morpholine ring rather than a ribose ring
- Morpholinos designed to bind to the START codon (ATG) result in a translation blockage

Raise the embryos to dome stage



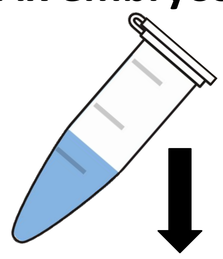
[4]

↓

Anoxia treatment
0, 1, 2, and 4h

↓

Fix embryos in PFA



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Immunolabel
Using mitotic markers
→ PH3 & DAPI

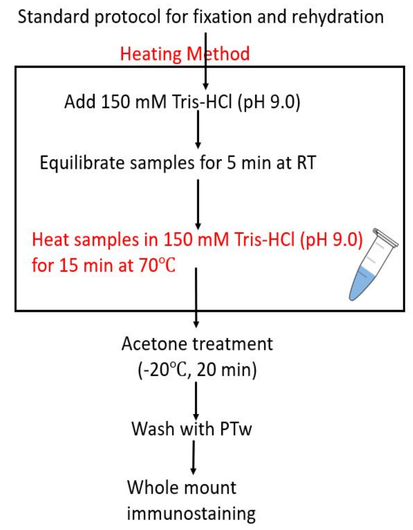

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Image & Quantify

Future Directions

- Continue initial experiments, running more trials with a greater a sample size.
- Analyze the role of other members of the NDRG family in cell cycle arrest

References

1. Ortmann et al., 2014
2. Kitowska and Pawelczyk, 2010.
3. Eisen and Smith, 2008
4. Kimmel et. al, 2013

Acknowledgements

UMBC Department of Biological Sciences. This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers TL4GM118989, UL1GM118988, and RL5GM118987. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. I would like to thank Dr. Rachel Brewster and my graduate mentor, Timothy Hufford.