Identification of mir-199 Targets Involved in *Xenopus laevis* Eye Development

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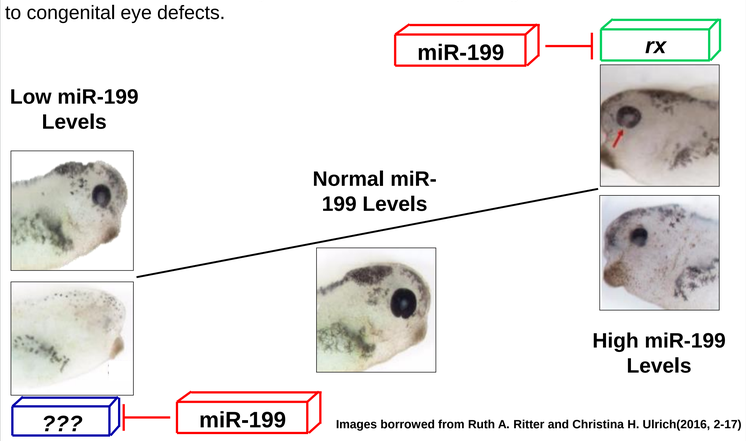
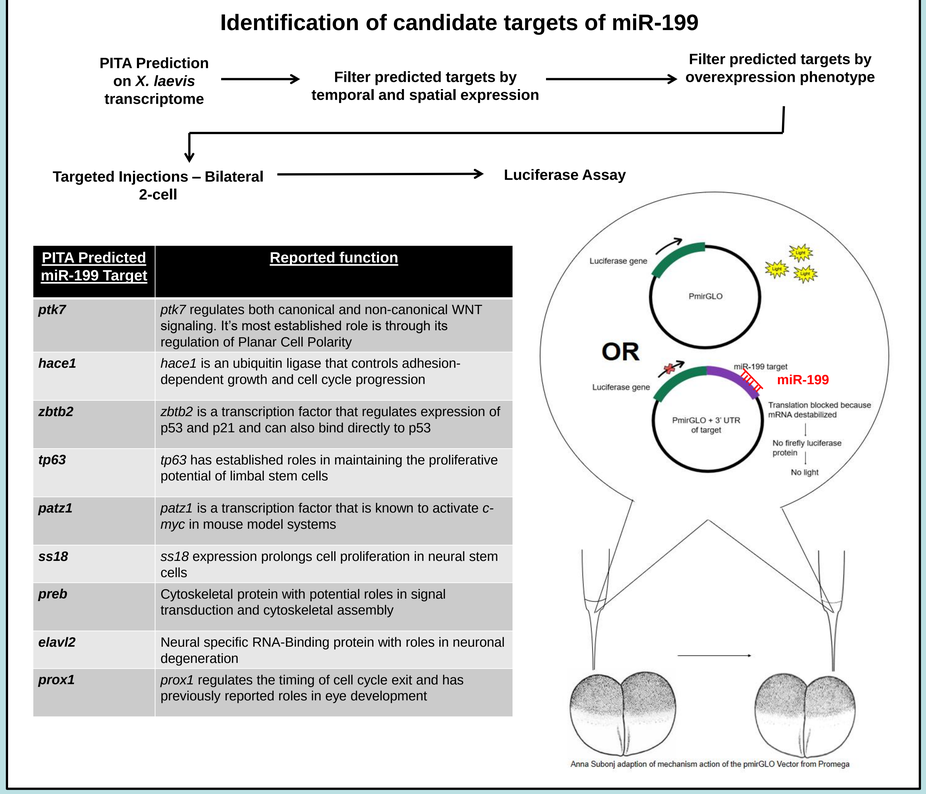
Introduction

According to the CDC, between the years 2004-2006 congenital eye defects were present in 1 of every 5,349 births. Our research proposes to uncover the underlying regulation of eye development through identifying proteins that, at high levels, block normal eye development. The network of proteins responsible for eye development (the EFTF) is well understood and conserved across vertebrates. Previous research from our lab has identified post transcriptional regulation of this network through microRNA regulation, in particular through miR199. We found
that in addition to its regulation of the EFTFs, miR199 also regulates proteins blocking correct eye development. This allowed us to conclude that balanced expression of miR199 is necessary for normal eye development. Our lab identified several potential targets that could block normal eye development and are predicted to be regulated by miR199. My role in this project has been to validate miR199 regulation of the targets hace1, ptk7.L, and prox1. To do this, I have cloned fragments of their 3' untranslated regions (3' UTR) and performed luciferase assays on them. Greater understanding of the underlying systems regulating eye development will pave the way for future research regarding how these proteins lead to congenital eye defects.



Images borrowed from Ruth A. Ritter and Christina H. Ulrich(2016, 2-17)

Methods



PITA Predicted miR-199 Target	Reported function
ptk7	*ptk7* regulates both canonical and non-canonical WNT signaling. It's most established role is through its regulation of Planar Cell Polarity
hace1	*hace1* is an ubiquitin ligase that controls adhesion-dependent growth and cell cycle progression
zbtb2	*zbtb2* is a transcription factor that regulates expression of p53 and p21 and can also bind directly to p53
tp63	*tp63* has established roles in maintaining the proliferative potential of limbal stem cells
patz1	*patz1* is a transcription factor that is known to activate *c-myc* in mouse model systems
ss18	*ss18* expression prolongs cell proliferation in neural stem cells
preb	Cytoskeletal protein with potential roles in signal transduction and cytoskeletal assembly
elavl2	Neural specific RNA-Binding protein with roles in neuronal degeneration
prox1	*prox1* regulates the timing of cell cycle exit and has previously reported roles in eye development

Anna Subonj adaption of mechanism action of the pmirGLO Vector from Promega

Results

Which target(s) of miR-199 might act as negative regulators of eye development?

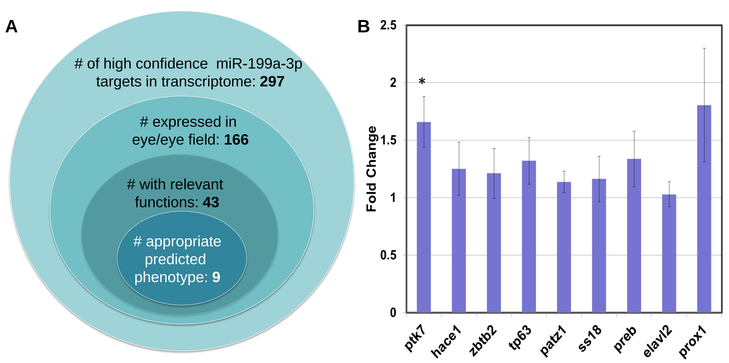


FIGURE 1 – Targets of miR-199 independent of the EFTF network. A) Identification of potential targets of miR-199 that could act as negative regulators of eye development. Target predictions for miR-199 were carried out against the *X. laevis* transcriptome using PITA (Kertesz et al., 2007); subsequent analyses identified nine potential targets. B) qRT- PCR of miR-199 knockdown embryos. Ct values were normalized to the geometric mean of *HisH4* and *odc* and presented as -fold difference from Ct values of control LNA-injected embryos. Eight of the nine candidate genes showed increased expression in miR-199 knockdown embryos. Error bars show S. E. M. N ≤4, *p ≥ 0.05.

Is hace1 regulated by miRs?

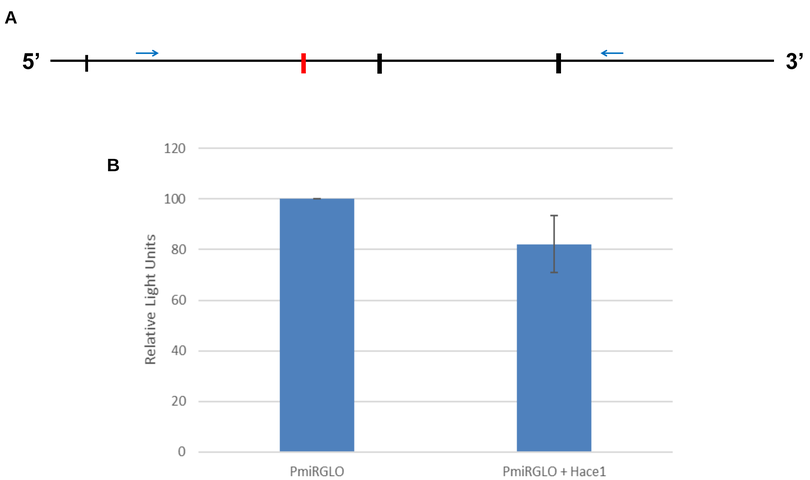


FIGURE 2 – Post-transcriptional regulation of *hace1* in *Xenopus laevis* embryos. A) Representation of the PITA predicted miR-199 binding sites on *hace1* 3'UTR Red mark indicates strongest predicted target sequence. Blue arrows indicate the portion of 3'UTR cloned into the pmiRglo (PMG) plasmid for the following luciferase assays (Forward and reverse primers). B) Reduction of relative light units when comparing PmiRGLO + hace1 3' UTR to PmiRGLO only was observed by St. 12.5-13.This indicated that the mRNA transcript for *hace1* is under post-transcriptional regulation. n≥4

Is ptk7 regulated by miRs? Does ptk7 play a role in eye development?

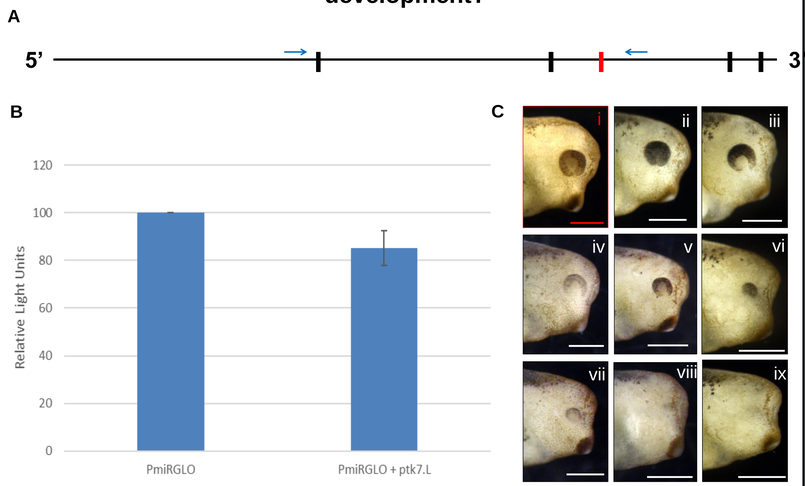


FIGURE 3 - *ptk7* post-transcriptional regulation and role in eye development. A) Representation of the PITA predicted miR-199 binding sites on *ptk7* 3'UTR Red mark indicates strongest predicted target sequence. Blue arrows indicate the portion of 3'UTR cloned into the pmiRGLO plasmid for the following luciferase assays (Forward and reverse primers). B) A reduction of relative light units was observed when comparing PmiRGLO + *ptk7* 3' UTR to PmiRGLO by St. 12.5-13.This indicated that the mRNA transcript for *ptk7* is under post-transcriptional regulation. n≥6. C) *ptk7* overexpression effects on eyes as seen at St 35. Surplus *ptk7* during eye development causes a range of phenotypes from colobomas to small eyes to no eyes.

Conclusions

- The microRNA miR-199 is required for eye development and outgrowth of the optic cup. This finding suggests that a target of miR-199 is a negative regulator of eye development and optic cup morphogenesis.
- Late tailbud embryos overexpressing *ptk7* exhibit eye defects, indicating that *ptk7* negatively regulates eye development and optic cup outgrowth.
- *hace1* and *ptk7* both have multiple putative recognition sites for miR-199 in their 3'UTR's. Luciferase assays demonstrate that the *hace1* and ptk7 exhibit post-transcriptional regulation. Whether this is due to miR-199 regulation specifically remains to be determined.

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