Determination of the Roles of Keratin 18 and 19 in Early *Xenopus laevis* Development

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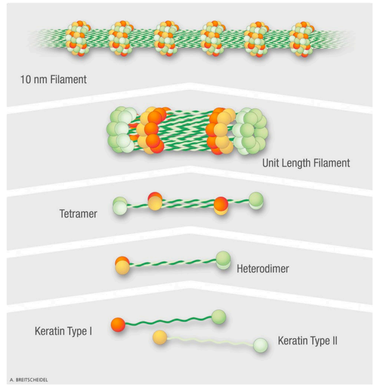
Introduction

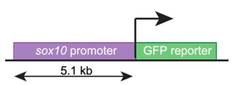
Early embryonic development is a time of rapid change. Significant cell movements are mediated by the cytoskeletal components, which also provide structure for the cells. Intermediate filaments are a class of cytoskeletal components that exhibit marked strength, one type of which is the keratin filament. Keratin filaments are made from type I and type II keratin proteins that associate by heterodimerization and further polymerize both longitudinally and laterally.



In early development of the African clawed frog, *Xenopus laevis*, keratins 18 and 19 are the primary type I keratins expressed. Prior preliminary work has shown that inhibition of keratin 19 expression by antisense morpholino (MO) results in a lethal phenotype that emerges during late gastrula to neurula stages of development. Meanwhile inhibition of keratin 18 has little to no effect on survival. Changes in production and localization of keratin in morpholino-injected embryos has not been investigated.

Methods

In vitro fertilization was performed with Sox10 transgenic *X. laevis* in order to observe potential changes to neural crest cell formation during neurulation. Male testes and female eggs from transgenic frogs were used on three separate occasions to prepare 6 distinct clutches. Fertilized eggs were separated into four groups: no injection, control MO injected, K18 MO injected, and K19 MO injected. After completing neurulation, embryos were sorted based on GFP levels, with abnormalities and viability recorded.



After reaching the desired developmental stage, some embryos were fixed in methanol for immunofluorescence and others were processed for immunobiochemical assays. Methanol fixed embryos were stained for keratins. Embryo lysates were used for Western blots and stained for keratins.

Results

Viability

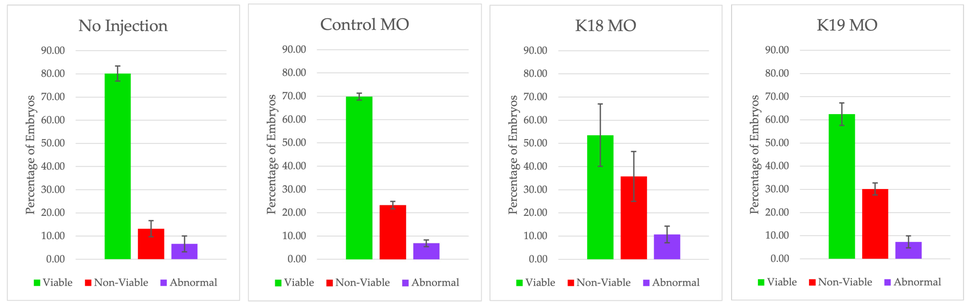


Figure 1: A comparison of embryo viability within the first 24 hours post-fertilization. Embryos that are developing atypically are labeled as "abnormal." Decreases in embryo viability can be observed in the keratin-morpholino treated groups.

GFP Expression

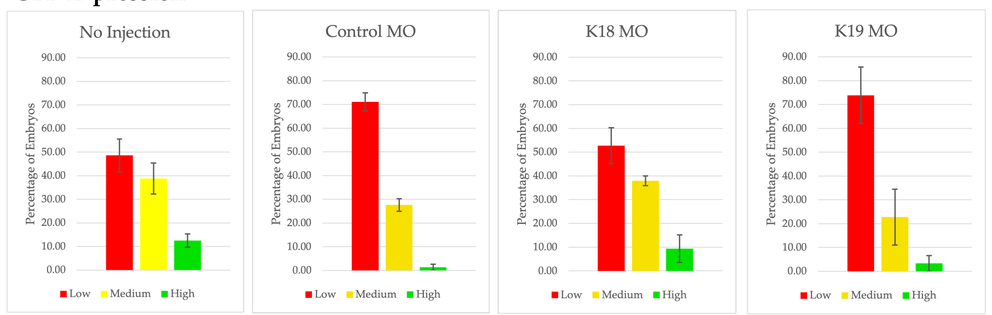


Figure 2: Observed GFP expression across the different treatment groups. Lower GFP expression is observed in the K19 MO groups when compared to other treatments.

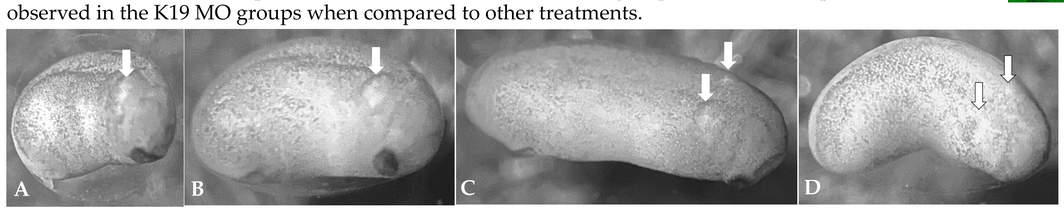


Figure 3: Images of GFP expression in embryos for uninjected (A), control morpholino (B), K18 morpholino (C), and K19 morpholino (D) treatment groups. Slight decreases in GFP expression are visible in the injected groups.

Conclusions

Decreases in embryo viability are observed in keratin-morpholino injected groups, along with decreases in Sox-10 related GFP expression.

A variety of changes in localization and production are observed in morpholino-treated embryos, though variable by embryo. K18-inhibited embryos exhibit less cortical keratin, and intracellular collapse of keratin networks in mucociliary cells. K19-inhibited embryos show very little cortical keratin, and some exhibit a lack of keratin network in the mucociliary cells, while others show intracellular collapse of the network.

Additional trials should be done to further characterize the disruption.

Acknowledgements

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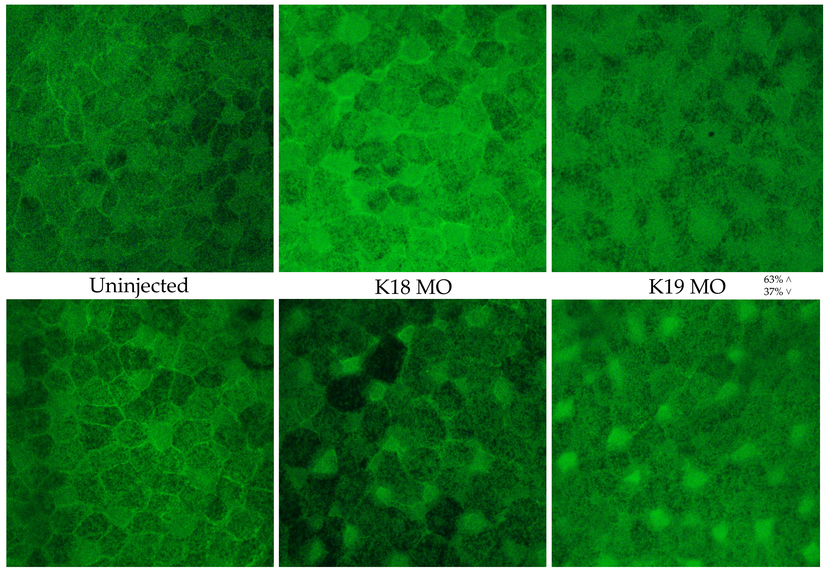
Literature Cited

1) Yoon, S.; Leube, R. E. Keratin Intermediate Filaments: Intermediaries of Epithelial Cell Migration. *Essays in Biochemistry* **2019**, *63* (5), 521–533. https://doi.org/10.1042/EBC20190017.
2) Alkobtawi, M.; Ray, H.; Barriga, E. H.; Moreno, M.; Kerney, R.; Monsoro-Burq, A.-H.; Saint-Jeannet, J.-P.; Mayor, R. Characterization of Pax3 and Sox10 Transgenic Xenopus Laevis Embryos as Tools to Study Neural Crest Development. *Developmental Biology* **2018**, *444*, S202–S208. https://doi.org/10.1016/j.ydbio.2018.02.020.

Keratin Expression and Localization



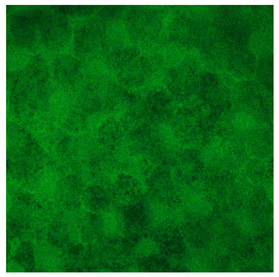


Figure 4: Fluorescence microscopy images for each treatment group. Uninjected controls exhibit robust cortical staining and keratin networks particularly in the apical surface of mucociliary cells. This is somewhat maintained in the control MO group but decreases in cortical staining and disruption of the intracellular keratin network within mucociliary cells can be visualized in K18 and K19 MO groups.

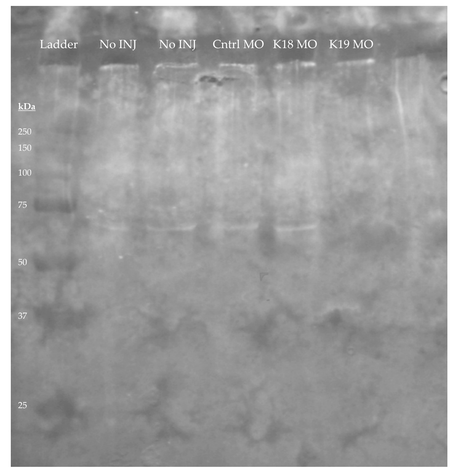


Figure 5: A Western blot for keratin. Left to right: protein standard ladder, uninjected, uninjected, control MO, K18 MO, K19 MO. The keratin band can be seen in lanes 2-5 but is missing in lane 6 with K19 inhibited embryos.