Migration Pattern Dynamics during Choroid Fissure Closure in Zebrafish

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Migration Pattern Dynamics during Choroid Fissure Closure in Zebrafish

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Abstract
Successful closure of the choroid fissure (CF) is essential for proper development of the vertebrate eye. The impermanent structure forms as the optic cup surrounds the invaginating lens allowing hyaloid vasculature to enter the developing eye. If the choroid fissure closure (CCF) fails to close, a coloboma develops associated with approximately 3-11% of childhood blindness worldwide. The CF cells are distinct prior to fusion as they remain undifferentiated until fusion of the opposing sides and have unique H-cadherin expression. During CFC, cells must breakdown basement membrane to allow fusion between both sides producing a seamless ventral retina. Both breakdown and fusion of the opposing sides of the CF initiate from the central region and proceeds bi-directionally. It is unknown however where or if the CF cells migrate away from an aligned fusion point. In vivo confocal microscopy of transgenic Hsp70.1:Gal4;UAS:Kaede zebrafish embryos during CFC allows distinctive contrast of photoconverted cells to give insight to their spatial temporal movement. We partitioned our analysis into three distinct regions along the proximal/distal axis of the CF to determine if cellular movement at the choroid fissure edges maintained distinct cellular migration patterns prior to differentiation. Preliminary analysis from 44 to 48 hpf in the proximal and distal regions demonstrate movement distinct from those within the central region. In both proximal and distal CF, upper (dorsal) CF cells move towards the central CF changing their proximal/distal axis in the opposite direction of fusion. Lower (ventral) CF cells move further ventrally towards the apposed side in order to fuse the CF. This contrasts with the central CF upper and lower cells that move directly towards the apposed sides. These results further support the hypothesis that cells at the CF edge are regulated differentially from the remaining differentiating retina.

Zebrafish Choroid Fissure Closure

The eye develops from neural tissue and bulges out to create the eye shape. The lens then develop from epithelial tissue.

Choroid Fissure Closure
The choroid fissure (CF) is a transient opening at the last major morphogenesis change in eye. The mechanism of the CF closure has three major stages. The first stage involves proliferation and elongation of the cells at the CF. The second stage is the breakdown of the laminin basement membrane. The last is the fusion of the two apposed sides into one seamless structure. The closure begins in the central region along the proximal/distal axis and closes bidirectionally in a zipper-like fashion.

Stage 1
Stage 2
Stage 3

Open
Closed

Improper Choroid Fissure Closure
If the CF does not properly close it will result in a coloboma. A coloboma is visible as a tear through the iris however there is no retinal tissue present within the opening. Colobomas are associated with 3-11% of childhood blindness and tend to present as a phenotype of multiple syndromes.

Successful Photoconversion of Transgenic Embryo

UV Activation of CF Cells and Cell Tracking

UV laser is focused on CF cells to track movement. The UV laser excites the protein Kaede, activating and photoconverting the protein.

Movement of Choroid Fissure Cells

Figure 1. Hsp70.1:Gal4 and UAS:Kaede heatshock embryos are UV activated in the CF cells. Before exposure to a 405 nm UV laser, Kaede emits at 518 nm wavelength (green). The UV laser allows for conformational change of the Kaede protein shifting the emission to 582 nm (red). Precise photoconversion was done on the confocal to activate the cells at the CF. Z-stack images of CF were taken every 15 minutes after the one exposure and sectioned into 2 µm slices. Analysis was done using Imager.

Figure 2. Successful heatshock activation and photoconversion of Kaede protein. A, B, and C demonstrates conversion of somites. D, E, and F visualize the photoconversion of the skull. Kaede is ubiquitously expressed with no phenotypic abnormalities.

Future Directions
• What are the fate of these cells at their final position after fusion?
• What factors are involved that initiate these unique dynamics?

Acknowledgements
• University of Northern Colorado College of Natural and Health Sciences Student Research Fund and TriBeta
• Jeffery Gross and the Gross lab for the transgenic fish donation to the James Lab
• Chad Wangeline for access to the imaging suite for confocal imaging
• McNair Scholars Program and Krista Kaufman
• Knights Templar Career Starters Grant

Successful Conversion of CF Cells

What are some key findings from the study on choroid fissure closure in zebrafish, and how do these findings contribute to our understanding of eye development?

The study provides evidence as to why the CF closes at the central region first. When exposed to 405 nm UV laser, the Kaede protein converts to a red fluorescence, allowing for temporal and spatial study of the cells. This provides insight into the movement and differentiation of the cells during choroid fissure closure. The results suggest that cells at the edge of the CF are regulated differently from those within the central region. The study also highlights the role of the Hsp70.1:Gal4 transgene in activating the Kaede protein, which is crucial for understanding the dynamics of cell migration and differentiation during choroid fissure closure.

What are the implications of these findings for understanding eye development and potential treatments for disorders like colobomas?

The findings of this study have significant implications for understanding eye development and potential treatments for disorders like colobomas. By elucidating the distinct movement patterns of cells at the CF edge versus those within the central region, the study provides new insights into the mechanisms governing cell behavior and differentiation. The use of transgenic zebrafish models allows for precise visualisation and tracking of cell movement, which can inform future research on congenital disorders affecting eye development. Understanding these complex cellular interactions can guide the development of targeted therapies aimed at preventing or treating conditions associated with abnormal CF closure, such as colobomas, and may contribute to the advancement of regenerative medicine approaches for eye repair and restoration.