Actin Dynamics During Endothelial Tubulogenesis

Purpose

Endothelial tubulogenesis is the formation of functional blood vessels. The purpose of this research is to understand how actin mediates this process. Actin forms microfilaments that are dynamic structures associated with focal adhesions, cell to cell junctions, and the apical membrane. Actin acts as structural units to provide the force generation that drives morphogenesis. To observe the function of actin and actin-associated structures, transgenic gene lines were characterized. These transgenic gene lines express LifAct, Moesin, the Moesin-Actin-Binding-Domain, PH-Plc, and Alpha-Catenin fused in frame to a fluorescent protein. These gene allow visualization of actin and actin-based structures during vascular formation. This formation will be visualized using fluorescent proteins RFP and GFP. These proteins will act as markers to monitor the process of endothelial tubulogenesis in zebrafish using confocal microscopy. The zebrafish will express fluorescent using the GAL4-UAS system. This system activates the expression of our gene of interest with fluorescent. LifAct is a short motif that links the fluorescent protein to actin. This allows for the visualization of actin during blood vessel morphogenesis throughout the endothelial cells. In contrast, Moesin was specifically used to visualize the linkages of the actin cytoskeleton and the plasma membrane. Moesin-Actin-Binding Domain is similar to Moesin because it also binds to actin, but lacks a membrane binding domain. PH-Plc reflects where Moesin actually is during vessel morphogenesis. Alpha-Catenin serves as a linking protein that is associated with cellular junctions allowing co-localization of actin at adherens junctions. By observing these transgenic gene lines, we can observe how actin contributes to endothelial tubulogenesis.

Procedure and Methods

- The first step involves creating a plasmid to flank the gene of interest. The plasmid has transposon flanking arms, which allows the plasmid to integrate the desired cargo into the genome and express it in the organism.
- Each gene will be paired with either the fluorescent protein RFP or GFP. This will allow us to see the expression of our desired gene by fluorescent.
- Fluorescents will be expressed in our gene lines by using the GAL4-UAS system. GAL4-VP16 will be used with the gene Fli1b. This allows this system to be expressed in the vascular system. Then the UAS will control the expression of the target gene with the fluorescent protein.
- When the plasmid is complete, the next step involves injecting this plasmid with the gene of interest into the organism.
- After the injection, the embryos need to be screened for fluorescent. This is done under a fluorescent microscope. Then these embryos will be left to grow until endothelial tubulogenesis occurs.
- The zebrafish will then be imaged using a confocal microscope to observe the vascular formation.

Procedure and Methods

- Some alleles do not have high enough expression thresholds or broad enough expression patterns to be visualized with simple fluorescent protein knock-in. To counteract this, the GAL4-UAS system was used to optimize and amplify the signal of tagged transgenic gene lines.

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Figure 1: Embryo Injection

Figure 2: Zebrafish Lifecycle

Figure 3: GAL4/UAS System

Figure 4: Moesin-Actin-Binding Domain eGFP

Figure 5: LifeAct Tag RFP

Figure 6: Alpha-Catenin eGFP

Figure 7: PH-Plc eGFP

Figure 8: Moesin eGFP

Conclusion

The purpose of this research is to understand how actin mediates the process of endothelial tubulogenesis. Actin was observed by looking at transgenic gene lines that express LifeAct, Moesin, Moesin-Actin-Binding Domain, PH-Plc, and Alpha-Catenin. Based on the results of vasculature formation, actin is likely associated with important processes of endothelial tubulogenesis. The transgenic gene lines LifeAct, Moesin, Moesin-Actin-Binding Domain, PH-Plc, and Alpha-Catenin showed evidence that actin is very dynamic during vasculature formation. LifeAct showed that actin is present during blood vessel morphogenesis. The Moesin-Actin-Binding Domain, PH-Plc, and Moesin showed that actin linkages were used for vasculature formation. Alpha-Catenin showed the co-localization of actin at adherens junctions. By observing these transgenic gene lines, we are beginning to understand the mechanisms that drive endothelial tube formation.

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Figure 8, confocal image of Moesin eGFP in zebrafish. This image shows where Moesin actually is during vessel morphogenesis. It reflects where actin cytoskeleton linkages are based on fluorescent.

Figure 7, confocal image of PH-Plc eGFP in zebrafish. This image shows where Moesin is actually is during vessel morphogenesis. It reflects where actin cytoskeleton linkages are based on fluorescent.