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Abstract: 173P

Cardiac forces regulate zebrafish heart valve delamination by modulating Nfat signaling

Renee Wei-Yan Chow[1], Hajime Fukui[1,2], Wei Xuan Chan[3], Kok Soon Justin Tan[3], Stéphane Roth[1], Anne-Laure Duchemin[1], Nadia Messaddeq[1], Hiroyuki Nakajima[2], Fei Liu[4], Nathalie Faggianelli-Conrozier[1], Andrey S. Klymchenko[4], Yap Choon Hwai[5], Naoki Mochizuki[2], Julien Vermot[1]

Background: In the clinic, most cases of congenital heart valve defects are thought to arise through errors that occur after the endothelial–mesenchymal transition (EndoMT) stage of valve development. Although mechanical forces caused by heartbeat are essential modulators of cardiovascular development, their role in these later developmental events is poorly understood. This project aims to use the zebrafish superior atrioventricular valve (AV) as a model to determine the role of mechanical forces in these later developmental events.

Methods: To characterize valve development, we imaged the live beating heart over developmental time, and performed photoconversion fate mapping, immunostaining, and electron microscopy experiments. To determine the role of mechanical forces during valve development, we examined the *gata1* mutant, which has defects in blood cell formation and thus lower wall shear stresses (WSS) acting on luminal valve cells. Finally, to determine the mechanotransduction pathway underlying later stages of heart valve development, we imaged the valve using several transgenic reporter lines and performed drug treatments and RNAscope assays.

Results/Conclusions: We showed that cellularized cushions of the superior atrioventricular canal (AVC) morph into valve leaflets via mesenchymal–endothelial transition (MEndoT) and tissue sheet delamination. *Gata1* mutants showed defects in delamination that result in thickened, hyperplastic valves. Computer modelling showed that the WSS acting on luminal valve cells decreased by a factor of 4 in *gata1* mutants compared to controls. The *gata1* phenotype could be partially rescued by injecting a viscous medium into the bloodstream, suggesting that mechanical stimuli are important regulators of valve delamination. Mechanistically, we show that forces modulate Nfatc activity to control delamination. Together, our results establish the cellular and molecular signature of cardiac valve delamination *in vivo* and demonstrate the continuous regulatory role of mechanical forces and blood flow during valve formation. (Please see the figure below for a summary of the results.)

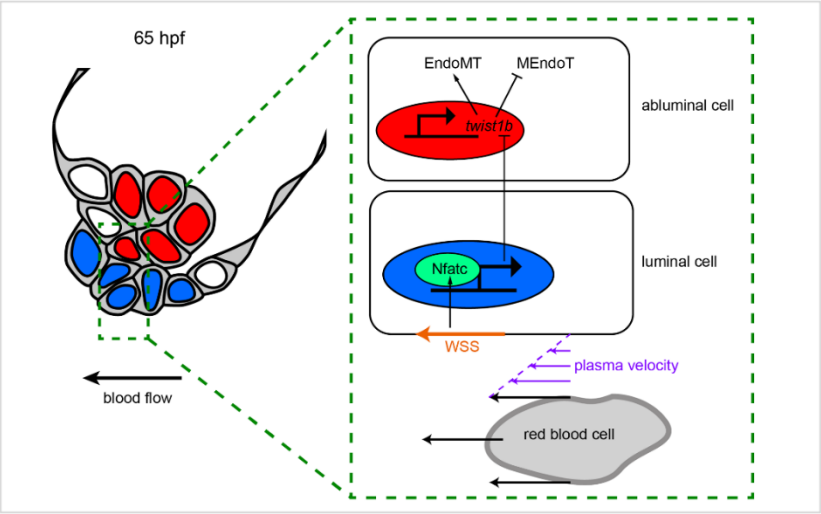

Figure 1: At 65 hours post fertilization (hpf), just prior to delamination, wall shear stress (WSS) activates Nfatc signalling in luminal cells. This leads to the inhibition of *twist1b* expression in abluminal cells, allowing MEndoT and tissue sheet delamination to occur normally.