THE ROLE OF SEMA6D AND ITS RECEPTORS IN CARDIOVASCULAR DEVELOPMENT
By: Alison Phua Referees: Mauro Giacca and Guido Tarone

ABSTRACT

Heart valves malformation is a serious problem that affects 1% of live births in the world. Hence, it is crucial to understand better the molecular mechanisms responsible for heart valve development. Recently, semaphorins and their receptors have emerged as a new class of molecules involved in the regulation of cardiovascular development. In particular, it has been previously shown that soluble semaphorin 6D (Sema6D), that binds to a holoreceptor complex formed by plexinA1 and of vascular endothelial growth factor receptor 2 (VEGFR2) or protein tyrosine kinase 7 (PTK7), could regulate heart valve formation in developing chick embryos through unknown mechanisms. Therefore, we decided to investigate the role of Sema6D in chick heart development. We found that overexpression of soluble dimeric Sema6D inhibited the development of the right (tricuspid) but not the left (mitral) valves. This indicated that Sema6D acted differently on the right and left atrio-ventricular (AV) valves. While trying to understand the molecular mechanisms underpinning the Sema6D activities on AV valves, we uncovered that a segregation of VEGFR2 and PTK7 expression, starting as early as E7, occurred on the mitral, but not on tricuspid valves of chick embryos. Indeed, VEGFR2 and PTK7 were respectively expressed at significantly higher levels on the ventricular (non-flow) and atrial (flow) side of the mitral valves. Notably, a similar segregation of PTK7 and VEGFR2 expression was also found on the opposite sides of the aortic valves. Instead, these two receptors were distributed homogeneously on the tricuspid valves, suggesting that higher blood shear stress in the left part of the heart could have resulted in the inverse relationship between PTK7 and VEGFR2 expression on mitral valves. Hence, we hypothesized that the differential distribution of Sema6D holoreceptor components PTK7 and VEGFR2 expression on mitral and tricuspid valves could play a role in the activity of soluble dimeric Sema6D. To further understand the signaling of dimeric Sema6D via its receptors, we decided to conduct in vitro shear stress experiments and results showed that steady laminar shear stress upregulated both PTK7 and VEGFR2 expression. On the other hand, since the coupling of PTK7 and PlexinA1 has been shown to have a forward inhibitory signaling upon Sema6D stimulation, we decided to investigate the activity of dimeric Sema6D in vitro and interestingly, we discovered that dimeric Sema6D caused a collapse in cells co-transfected with PlexinA1 and PTK7. Further investigation on the activity of dimeric Sema6D via adhesion and haptotaxis assays showed that dimeric Sema6D had a pro-adhesive/migratory activity on Human Umbilical Vein Endothelial Cells (HUVECs). In addition, our observations also showed that dimeric Sema6D had a pro-adhesive/migratory activity on HUVECs compared to inhibitory dimeric Sema6A, another member of the semaphorin family. Further work is to be done to better characterize the role Sema6D and its holoreceptors both in vitro, as well as in vivo.