CDB SEMINAR

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15:00~16:30 C1F CDB Auditorium

Spatio-temporal activation of ERK through Vegfrs regulates identity of segmental artery in zebrafish

Summary
Several signaling pathways downstream of vascular endothelial growth factor receptors-2 and -3 (Vegfr2 and Vegfr3; *Kdrl* and *Flt4* in zebrafish, respectively), including ERK1/2, JNK, p38 and PI3K/AKT, play crucial roles in a variety of developmental and physiological processes upon activation in response to Vegf ligands in vascular endothelial cells (VECs). However, when and where these signal effectors downstream of Vegfrs are activated *in vivo* during vascular development is less clear. By immunostaining zebrafish embryos with phospho-specific antibodies, we found that ERK1/2 is preferentially phosphorylated in segmental artery (SeA) tip cells (TCs) as they sprout from the dorsal aorta (DA). Furthermore, ERK phosphorylation in the SeA cells is dependent on Kdrl and Flt4 through phospholipase C gamma 1 (Plcg1). By contrast, Notch activation restricts phospho-ERK1/2 to sprouting SeA cells, suggesting that this may be a central regulatory point at which Notch and Vegf regulate the behavior of sprouting VECs. To determine the role of phospho-ERK1/2 in this context, the phosphorylation was blocked by a MEK inhibitor (SL327) or by an ERK-specific phosphatase (Dusp6). In SL327-treated embryos, SeA TCs sprout normally, but then stall, similar to defects caused by loss of *Flt4*. Interestingly, SL327 treatment leads to downregulation of *Flt4* and *Dll4*, and downregulation of Notch activity specifically in SeA cells caused by the downregulation of *Dll4*. Finally, endothelial cells expressing exogenous Dusp6 prior to SeA formation lose ERK1/2 activity and do not contribute to growing SeAs. Thus, we propose critical roles of phospho-ERK1/2 in SeA cells through Vegfrs, 1: for selecting SeA cells from DA early on, 2: for reinforcing the TCs identity, mainly by regulating the spatial expression of *Flt4* and *Dll4* later on.

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