Peppi Karppinen: Cardiac Deletion of Hypoxia-Inducible Factor Prolyl 4-Hydroxylase-2 Improgves Survival and Cardiac Function Following Ischemia-Reperfusion Injury

Prolyl 4-Hydroxylase

Oxygen sensor, regulates HIF-a (under hypoxia no hydroxylation and thus not target for degradation via ubiquitin and can move to the nucleus to form active transcription factor with HIF-b)
3 isoforms: isoform 2 is most important (knock-out is lethal unlike with isoforms 1 and 3)

Mouse hypomorph of Prolyl 4-Hydroxylase-2 (8% of normal mRNA levels in heart, in other tissues up to 85%)

stabilization of HIF
mouse are fine
very little or no changes in Epo values
hearts are roughly equal to wt mice
on microarray significantly upregulated genes: some glycolytic genes, Ang2 (not VEGF-A, -B!)
increased capillary area/eNOS/NO, (p)Tie-2

Ex-vivo & in-vivo challenging by ligation-induced ischemia followed by reperfusion

no baseline differences between wt and hypomorph
hypomorphs recover better (measured by coronary flow rate & LDH washout)
better survival (30% of wt die, all hypomorphs survive) of 30 min ischemia
Evans blue staining? What did they show? Vascular leakage or cardiac cell damage?

Inhibition of Tie-2 signaling in mice by Tie-2ECD/AAV

increased angiogenesis in hypomorphs only → pulmonary edema

Didier Stainier: Endothelial cell behaviour during blood vessel formation in zebrafish

Cell specification (tip vs stalk) and sprouting, model system: ISV (intersomitic vessels) that develop by sprouting from the DA (dorsal aorta) to form the DLAV (dorsal longitudinal anastomotic vessel)

- SPIM movies
- what transcription factors define/determine cell specification and sprouting?

- Cardinal vein formation by selective (ventral) sprouting from dorsal aorta, not by vasculogenesis as previously thought (Herbert 2009, Science).
- Subsequent dorsal sprouting of ISV, merge to form DLAV (on the video the stalk cells had as many filopodia as the tip cells?)

"Standard model":
tip cell:VEGF-VEGFR-2 → Dll4↑
stalk cell: Dll4-Notch → VEGFR-2↓
JAG-1-Notch → SMC

Blocking by PY inhibition (SU5416)/β-secretase inhibition (DAPT):

DAPT: more sprout cells
SU5416: deletion of the sprout
Titration of inhibition: at intermediate concentration premature lumen formation
lumen formation at tipping point between vegf/notch signaling

Differential expression profiling
identified Hlx1. Expression start when sprouting of ISV starts, gets downregulated in tip, remains strong in stalk (Hlx1 is also expressed in other branching tissues)

Morpholinos
blunt-ended sprouts, no sprouts, gaps in DLAV

Mosaic of wt and morpholino-knocked-out cells
Hlx1 MO cells mostly located in tip
Hlx1 overexpression → no significant difference in cell distribution