Abstract: P560

Arterial Flt1 regulates de novo arteriologenesis and arteriolar caliber involving novel endothelial remodeling events.

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Introduction:
Ischemic cardiovascular diseases are amendable for therapeutic revascularization. Stimulation of de novo arteriologenesis and formation of large caliber arterioles that can conduct flow to the hypoperfused regions are considered medically relevant. Here we addressed the cellular and molecular events regulating arteriologenesis and evaluated the therapeutic potential of arterial endothelial Flt1. We demonstrate that arterial Flt1, by acting as a rheostat safe-guarding local arterial Vegfa availability, regulating both spatial distribution and Vegf dosage, can control VE-Cadherin - actin mediated stretching of endothelial cells promoting diameter growth and flow during arteriologenesis with obvious impact for designing revascularization strategies.

Methods & Results:
Arterial endothelial cells expressed soluble flt1 (sflt1) and membrane bound flt1 (mflt1) as evidenced by Tg(flt1BAC:YFP) and whole mount in situ hybridization. To determine the distribution of Flt1 protein we generated Flt1-HA (TgTm(flt1_E3_2HA)) and sFlt1 knock-in transgenics (Tg(flt1enh:sflt1_?7-2HA)). Anti HA-tag IHC revealed high mFlt1 and sFlt1 protein levels at the arterial endothelial cell membrane. Constitutive and inducible vegfa gain of function induced instable, vascular networks in line with high levels of Vegfa being detrimental. Vegfa competes with Plgf and Vegfb for binding to Flt1, and we hypothesized that competing Vegfa away from Flt1 by plgf or vegfb gain of function should release a physiologically relevant and safe Vegfa dosage promoting arteriolar growth. Accordingly, in tissue specific plgf or vegfb gain of function transgenics we observed significantly increased arteriogenesis, and two fold increase in structural arteriolar diameters. In line with requirement for arterial Flt1, flt1ka601 or mflt1ka605 loss of function mutants showed significantly less diameter growth compared to plgf in wt. Mechanistically, Vegfa-Kdrl converging onto P-Akt and nitric oxide accounted for the plgf induced effects, independent of macrophages. Imaging in Tg(fli1a:lifeact-GFP);Tg(musc:plgf) and Tg(fli1a:nEGFP) transgenics revealed that the remodeling around a larger arteriolar lumen required endothelial stretching events. Endothelial actin bundles condensed at VE-Cadherin positive junctions, prior to stretching and enlargement of the endothelial cell, followed by alignment around a larger lumen. Conversely, loss of VE-cadherin and inhibition of actin prevented lumen remodelling.

Conclusion:
Arterial Flt1 by titrating arterial Vegfa regulates the caliber of arterioles during de novo arteriologenesis involving a unique sequence of collective endothelial cell behaviors and actin remodeling events. Plgf and Vegfb, by shifting the Flt1-Vegfa binding equilibrium toward Vegfa release, promote arteriologenesis. Our model suggests
that arterial Flt1 may be used as a local cargo to deliver safe Vegfa dosages in revascularization strategies.