Identification and Validation of Therapeutic Targets for Vascular Disease

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Endothelial Signaling Regulating Arterial Venous Specification

Embryonic development:
the dorsal aorta (DA) and cardinal vein (CV)

Disease pathogenesis:
arteriovenous malformation (AVM)

On blood vessels

Ancient Greek physician Galen proposed blood does not circulate but is locally generated

1628 William Harvey discovered the heart: arteries carry blood away and veins return blood back to the heart

1661 Marcello Malphighi identified capillaries as the smallest vessels that close the circulation
Chick Vasculature

Drawing by Malphighi (1661)

Arterial venous hierarchy is essential for vascular function

Notch Pathway Genes are Expressed in Arteries but not Veins

Ligand Dll4-lacZ Adult Brain

Bendito et al., Gene Expression Patterns 2005

Notch, a Universal Arbiter of Cell Fate Decision

Harvey and Rosenthal, Heart Development, 1999
Notch Signaling Promotes Arterial Differentiation

Signaling Cell

Constitutively Active Notch4* (int3)
Notch1* (N1ICD)

Notch1, Notch2, Notch3, Notch4

Jagged1, Jagged2, Dll1, Dll3

Nucleus

Hes & Hey

HDAc

MAML

MAD

GSR

Receiving Cell

CV DA

CV DA

CV DA

CV DA
Morphogenesis of the Dorsal Aorta and Cardinal Vein - the first artery-vein pair in the body

Constitutively Active Notch4 Leads to Enlarged Aortae and Diminished Cardinal Veins

Notch4\(^*\) Increases the Size of the DA At the Expense of the CV

Notch4\(^*\) Does Not Affect Proliferation or Total Number of Endothelial Cells
Dorsal Aorta is Reduced while the Cardinal Vein Primordium is Enlarged in Notch$^{-/-}$ Mice


Notch Determines Arterial Differentiation Thereby Balancing the Allocation of Endothelial Cells between the DA and CV

Wild-type Tie2-tTA;TRE-Notch4$^*$

Notch1$^{-/-}$

Temporal and Spatial Effect of Notch4$^*$

Notch$^+$

Phenotype: embryonic AVM in Brain, AV coordinationSkin but not Brain

Publication: Development 08 PNAS 08 PNAS 2005
Brain Arterial Venous Malformation (BAVMs)

Six million people (young) harbor BAVM

Significant contributions to stroke
  • half of the hemorrhagic stroke in children
  • 2% of all strokes

Current surgical treatment has limited benefit
  • ongoing trial to test surgery against natural course

Little is known about the pathogenesis
  • no animal model

Hallmarks of BAVMs

- Operational definition of BAVM
- Arteriovenous shunting
- Enlarged blood vessels
- Hemorrhage
- Symptoms of stroke
- Ataxia

Enlarged and Tangled Vessels in the Mutant

Tie2-tTA

Tie2-tTA;TRE-Notch4*

Brian Arterio-venous Malformations (BAVMs)
Contains Enlarged and Tangled Vessels

Friedlander, NEJM, 2007
Hemorrhage and Necrosis in the Mutant

\[ \text{Tie2-tTA} \quad \text{Tie2-tTA;TRE-Notch4*} \]

Notch4* Leads to Brain AV Shunting

\[ \text{V} \quad \text{V} \quad \text{A} \quad \text{A} \]

Arteriovenous Connections are Larger than Capillaries

\[ \text{Tie2-tTA} \quad \text{Tie2-tTA;TRE-Notch4*} \]

Illness and Ataxia Are Reversed by Repression of Notch4*

\[ \text{P20} \quad \text{P23 (3 days Notch4* repression)} \]

Carotid Injection of 15µm FITC-beads
Summary

• The luminal sizes of the growing artery and vein are reciprocally balanced
  • Notch dictates this balance.
• Increase in Notch signaling promotes and sustains arterial venous malformation
• Notch activation is up-regulated in human Brain AVM patients
• A robust and faithful animal model for BAVM progression and regression