Angiogenesis in Human Development

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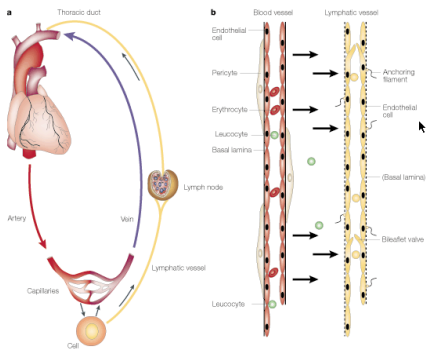
BACKGROUND READING:
Vascular Development
"Signaling Vascular Morphogenesis and Maintenance"
Douglas Hanahan. *Science* 277: 48-50. in Perspectives. (1997)

Vascular Development

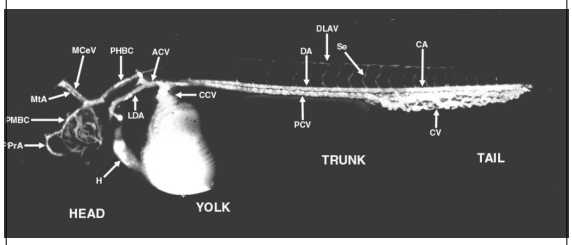
- Vasculogenesis = de novo tube formation
- Angiogenesis = sprouting of new tubes off of pre-existing tubes

- Endothelial Cell = cell type that makes up and lines blood vessels
- Mural Cells = specialized cells that surround blood vessels
  - Pericytes
  - Smooth muscle cells

- **Angiogenic Factors**
  - Vascular Endothelial Growth Factor (VEGF-A, VEGF-B, PlGF, VEGF-C, VEGF-D
  - Angiopoietins (Ang 1, Ang2, ...)
  - Notch ligands (Jagged1, Delta4)



2.5 day



Nature Biotechnology **22**, 595 - 599 (2004)

Chemical suppression of a genetic mutation in a zebrafish model of aortic coarctation

Randall T Peterson1, Stanley Y Shaw1, 2, Travis A Peterson1, David J Milan1, Tao P Zhong1, 3, Stuart L Schreiber2, Calum A MacRae1 & Mark C Fishman1, 4

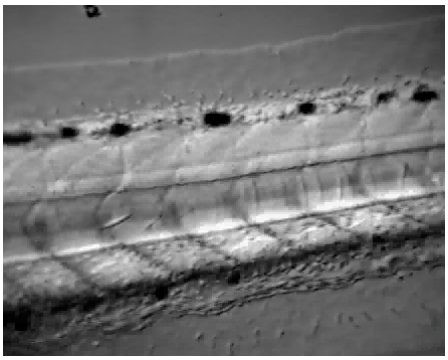
1 Developmental Biology Laboratory, Cardiovascular Research Center, Massachusetts General Hospital

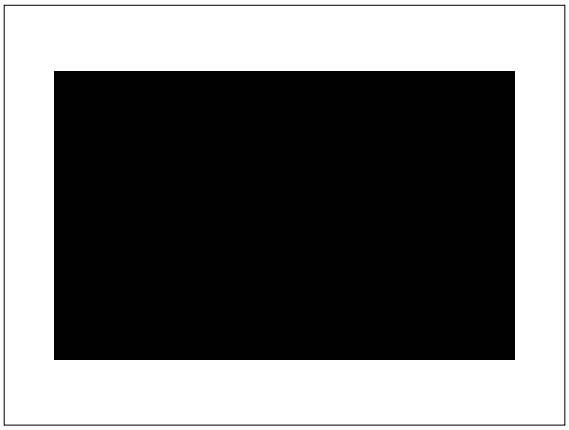
Nature Chemical Biology **1**, 263-264 (2005)

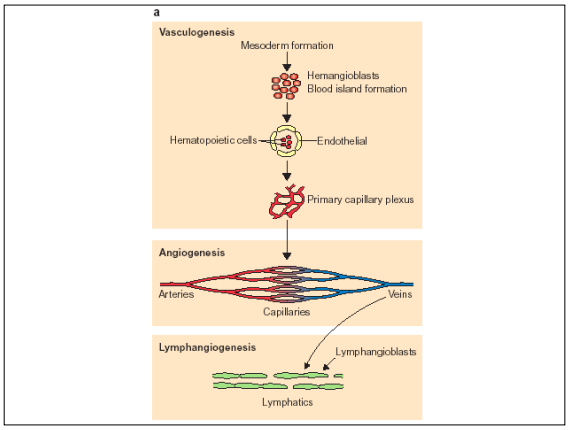
High-throughput assay for small molecules that modulate zebrafish embryonic heart rate.

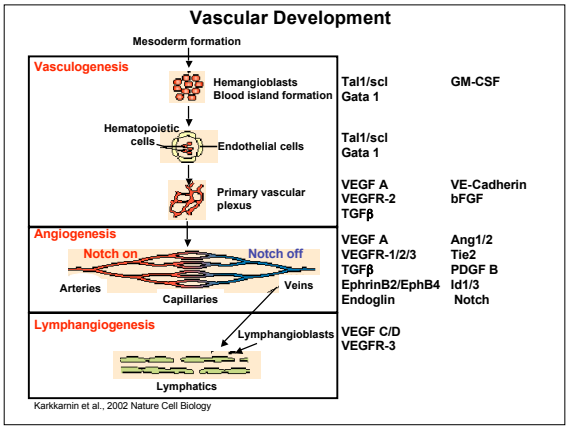
Burns CG, David J Milan, Grande DJ, Rottbauer W, Calum A MacRae & Mark C Fishman

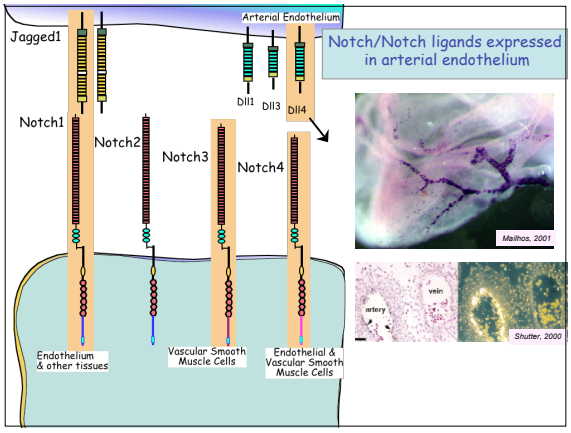
Developmental Biology Laboratory, Cardiovascular Research Center, Massachusetts General Hospital

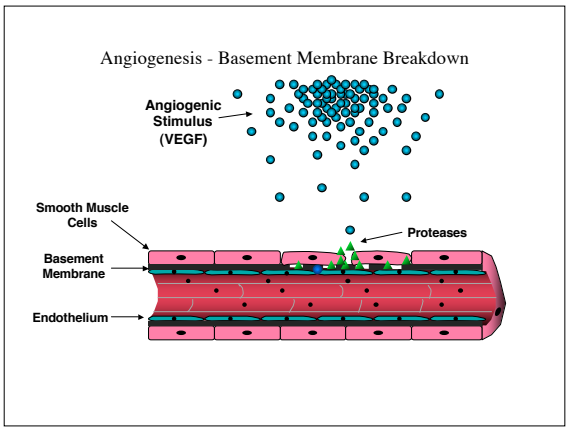


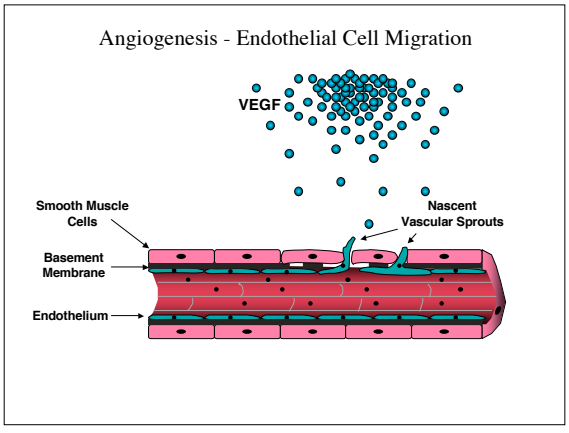


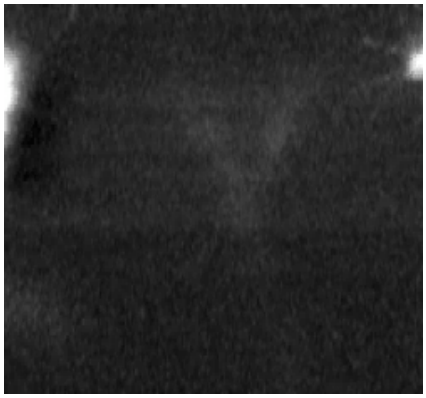

a
Vasculogenesis
Mesoderm formation
Hemangioblasts
Blood island formation
Hematopoietic cells
Endothelial
Primary capillary plexus
Angiogenesis
Arteries
Veins
Capillaries
Lymphangiogenesis
Lymphangioblasts
Lymphatics


Vascular Development
Mesoderm formation
Vasculogenesis
Hemangioblasts
Blood island formation
Tal1/scl
Gata 1
GM-CSF
Hematopoietic cells
Endothelial cells
Tal1/scl
Gata 1
Primary vascular plexus
VEGF A
VEGFR-2
TGFβ
VE-Cadherin
bFGF
Angiogenesis
Notch on
Notch off
Arteries
Veins
Capillaries
VEGF A
VEGFR-1/2/3
TGFβ
EphrinB2/EphB4
Endoglin
Ang1/2
Tie2
PDGF B
Id1/3
Notch
Lymphangiogenesis
Lymphangioblasts
VEGF C/D
VEGFR-3
Lymphatics
Karkkamin et al., 2002 Nature Cell Biology

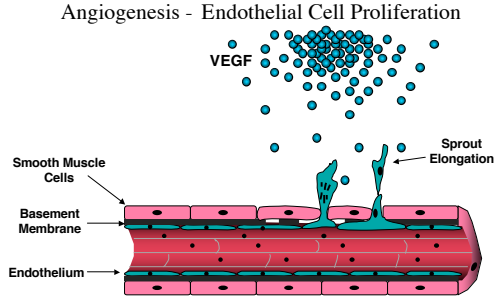

Arterial Endothelium
Jagged1
Dll1
Dll3
Dll4
Notch/Notch ligands expressed in arterial endothelium
Notch1
Notch2
Notch3
Notch4
Mailhos, 2001
vein
artery
Shutter, 2000
Endothelium & other tissues
Vascular Smooth Muscle Cells
Endothelial & Vascular Smooth Muscle Cells


Angiogenesis - Basement Membrane Breakdown
Angiogenic Stimulus (VEGF)
Smooth Muscle Cells
Proteases
Basement Membrane
Endothelium

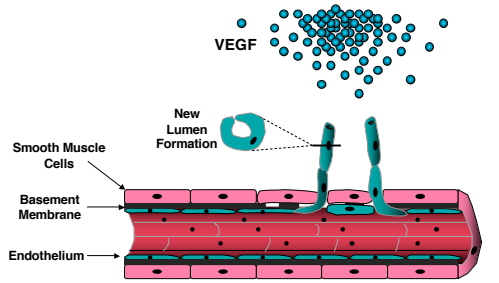

Angiogenesis - Endothelial Cell Migration
VEGF
Smooth Muscle Cells
Nascent Vascular Sprouts
Basement Membrane
Endothelium

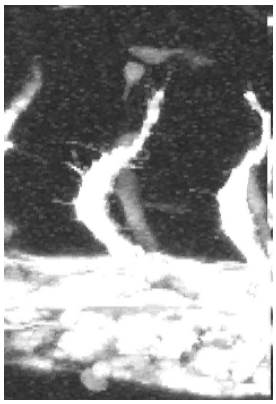


Angiogenesis - Endothelial Cell Proliferation

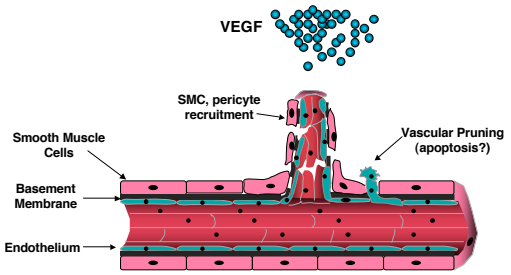


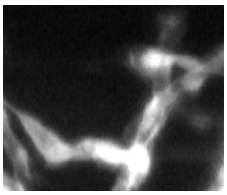
Angiogenesis - Capillary Morphogenesis



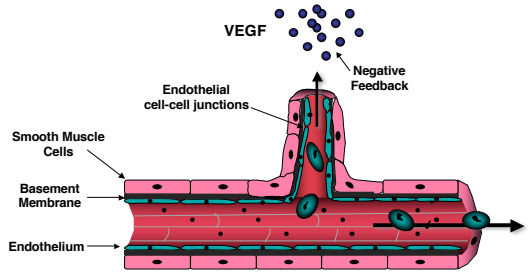


Angiogenesis - Vascular Maturation

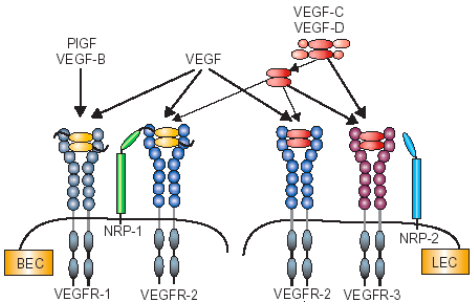




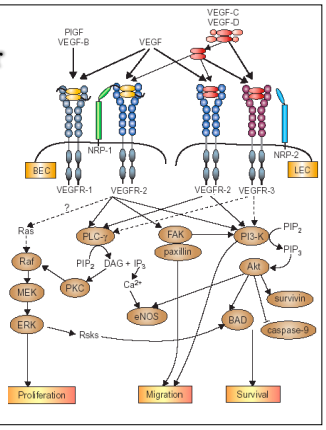
Angiogenesis - Vascular Maturation

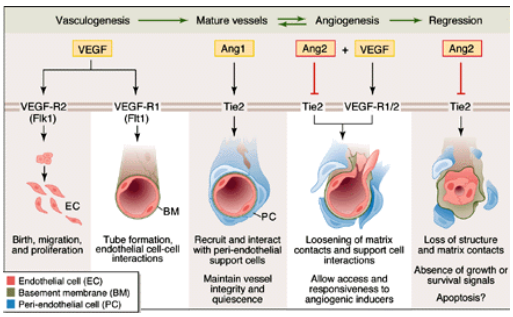


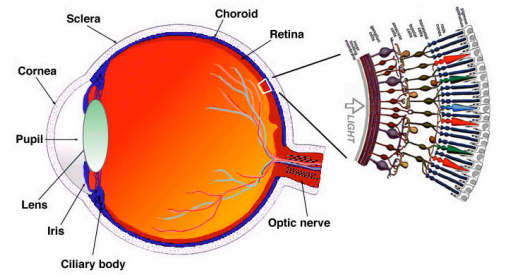
VEGF and VEGF Receptors

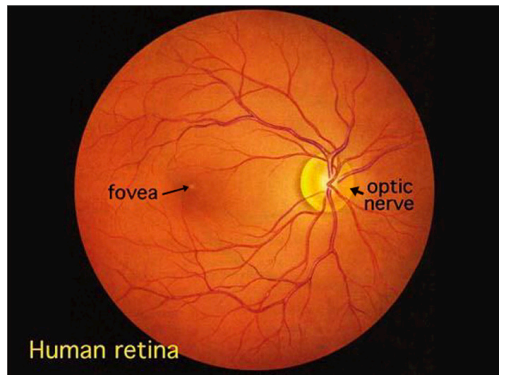


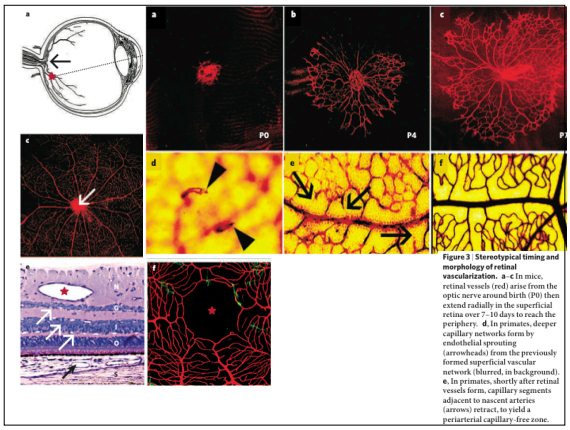
VEGF-receptor signaling

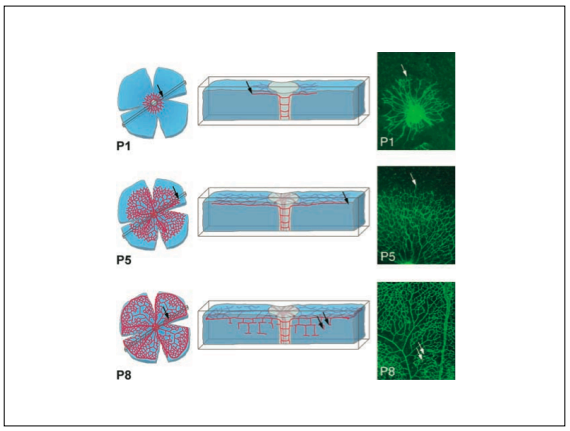



Vasculogenesis
Mature vessels
Angiogenesis
Regression
VEGF
Ang1
Ang2
+
VEGF
Ang2
VEGF-R2 (Flk1)
VEGF-R1 (Flt1)
Tie2
Tie2
VEGF-R1/2
Tie2
EC
BM
PC
Birth, migration, and proliferation
Tube formation, endothelial cell-cell interactions
Recruit and interact with peri-endothelial support cells
Maintain vessel integrity and quiescence
Loosening of matrix contacts and support cell interactions
Allow access and responsiveness to angiogenic inducers
Loss of structure and matrix contacts
Absence of growth or survival signals
Apoptosis?
Endothelial cell (EC)
Basement membrane (BM)
Peri-endothelial cell (PC)


Sclera
Choroid
Retina
Cornea
Pupil
Lens
Iris
Ciliary body
Optic nerve
LIGHT

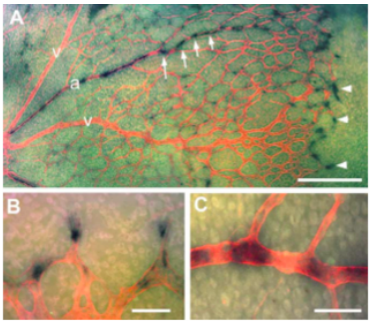

fovea
optic nerve
Human retina





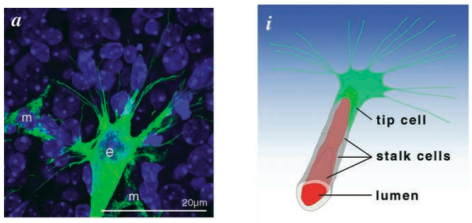
Delta4 expressed in retinal vasculature

arterial expression *tip cell expression*



in situ hybridization

Fruttinger, 2004



Normal intraretinal vasculature

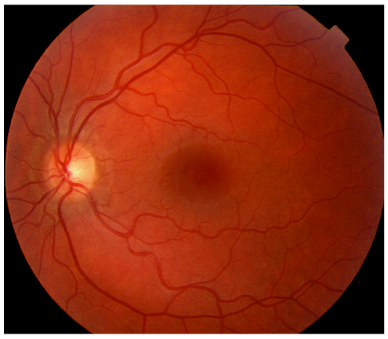
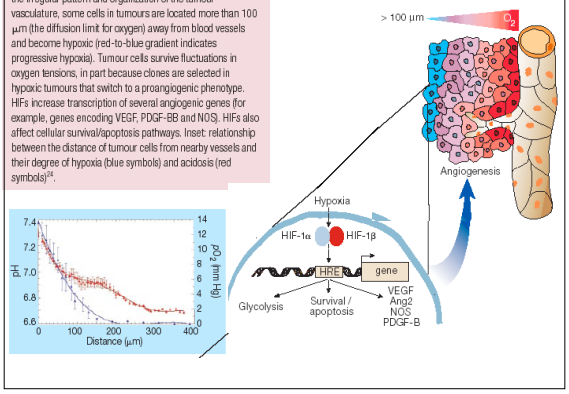


Figure 2 Role of hypoxia in tumour angiogenesis. Because of the irregular pattern and organization of the tumour vasculature, some cells in tumours are located more than 100 μm (the diffusion limit for oxygen) away from blood vessels and become hypoxic (red-to-blue gradient indicates progressive hypoxia). Tumour cells survive fluctuations in oxygen tensions, in part because clones are selected in hypoxic tumours that switch to a proangiogenic phenotype. HIFs increase transcription of several angiogenic genes (for example, genes encoding VEGF, PDGF-BB and NOS). HIFs also affect cellular survival/apoptosis pathways. Inset: relationship between the distance of tumour cells from nearby vessels and their degree of hypoxia (blue symbols) and acidosis (red symbols)[24].



Retinopathy of Prematurity

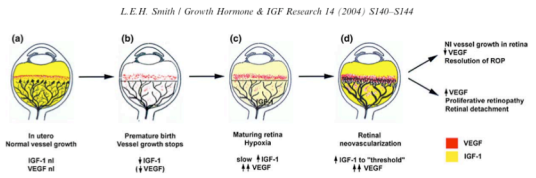
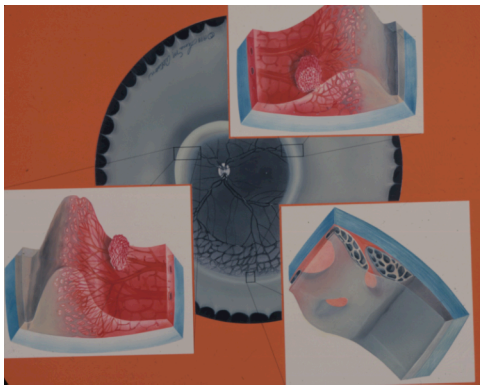


Fig. 1. Mechanism for retinopathy of prematurity (ROP) (Reprinted, with permission, from [37]).



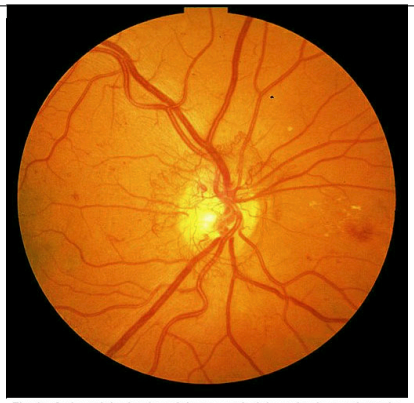
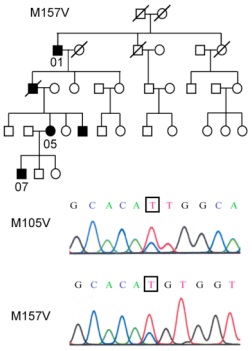


Fig. 25. A view of the fundus of the eye and of the retina in a patient who has advanced diabetic retinopathy.

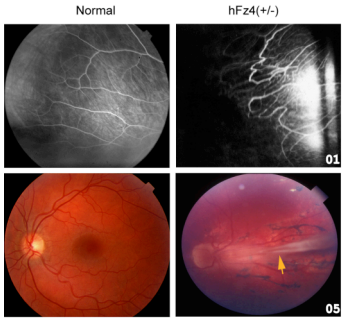
Familial Exudative Vitreoretinopathy

- First described by Criswick and Schepens [Am. J. Ophthalmol. 68: 578-594 (1969)]
- Autosomal dominant, recessive, and X-linked forms; variable phenotype
- Clinical characteristics
  - mild to severe vision loss
  - retina: avascular peripheral retina, exudates, neovascularization, fibrovascular masses, traction or rhegmatogenous retinal detachment
  - vitreous: posterior vitreous detachment, fibrovascular membranes, hemorrhage
  - other: cataract, neovascular glaucoma

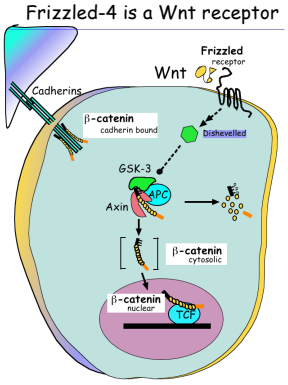
Autosomal dominant FEVR mutations In the cysteine-rich domain (CRD) of Fz4



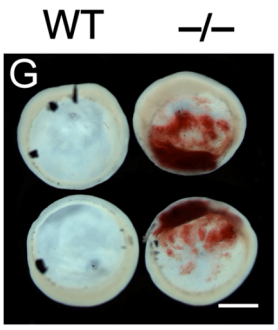
Retinal defects in FEVR patients heterozygous for Fz4 M157V



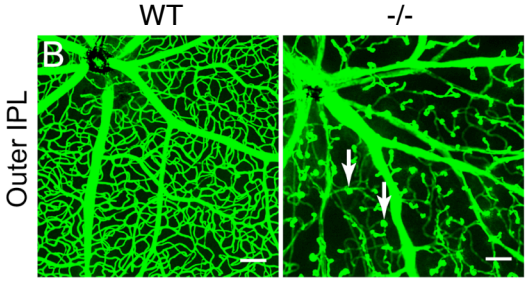
Frizzled-4 is a Wnt receptor



Intraocular hemorrhage in Fz4(-/-) mice



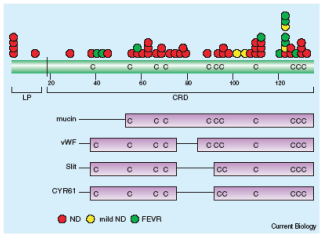
Absence of intra-retinal capillaries in Fz4(-/-) mice



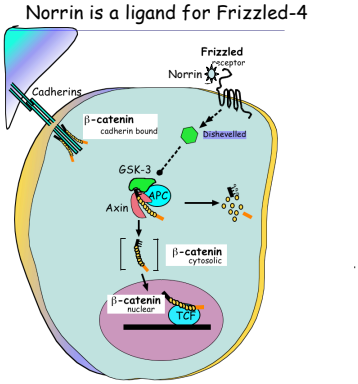
Norrie Disease

- First described by Norrie (1927) and analyzed systematically by Mette Warburg [Acta Ophthalmologica 39: 757-772 (1961); 41: 134-146 (1963); 89: 1-147 (1966)]
- X-linked recessive with variable phenotype
- Clinical characteristics
  - moderate vision loss to congenital blindness
  - retina: retinal folding and detachment, retinal degeneration, fibrovascular masses, vitreoretinal hemorrhage
  - vitreous: persistent primary vitreous
  - other: progressive sensorineural deafness

Figure 1. The Norrin protein.
Missense and nonsense mutations in Norrin leading to classic or mild Norrie disease (ND) and to FEVR. Amino acid numbering is given on the scale below the protein. LP, leader peptide; CRD, cysteine-rich domain. The CRD is homologous to domains in the proteins indicated below (adapted from [1]).



Norrin is a ligand for Frizzled-4



Molecular genetics of Norrie Disease and FEVR

- FEVR

  One autosomal dominant FEVR gene identified by Robaitaille et al [Nature Genetics 32: 326-330 (2002)] encodes Frizzled4, a putative Wnt receptor. A second autosomal dominant FEVR locus encodes the Wnt co-receptor Lrp5 [Toomes et al [IOVS 45: 2083-2090 (2004)]; Jiao et al [Am J Hum Genet 75: 878-884 (2004)].

- Norrie disease

  Gene identified by Berger et al and Chen et al [Nature Genetics 1: 199-203 and 204-208 (1992)]

  The encoded protein is small (133 amino acids in length), has the same pattern of cysteines as seen in transforming growth factor beta, and begins with a signal sequence (i.e. it looks like a secreted protein). No known biochemical function.

Vessel component to human disease

- Tumor angiogenesis
- Diabetic vascular complication
  - Diabetic retinopathy
  - Stroke
  - Ischemia
  - Wound repair
- Heart disease
- Obesity
- Blindness
  - Wet Macular Degeneration
  - Retinopathy of Prematurity