KATHOLIEKE UNIVERSITEIT
LEUVEN

Research Group Neural Circuit Development and Regeneration

Prof. Dr. Lieve Moons

Focus 1 - How are neuronal networks formed in the developing or injured brain ?

How do neurons migrate to their position in the brain?

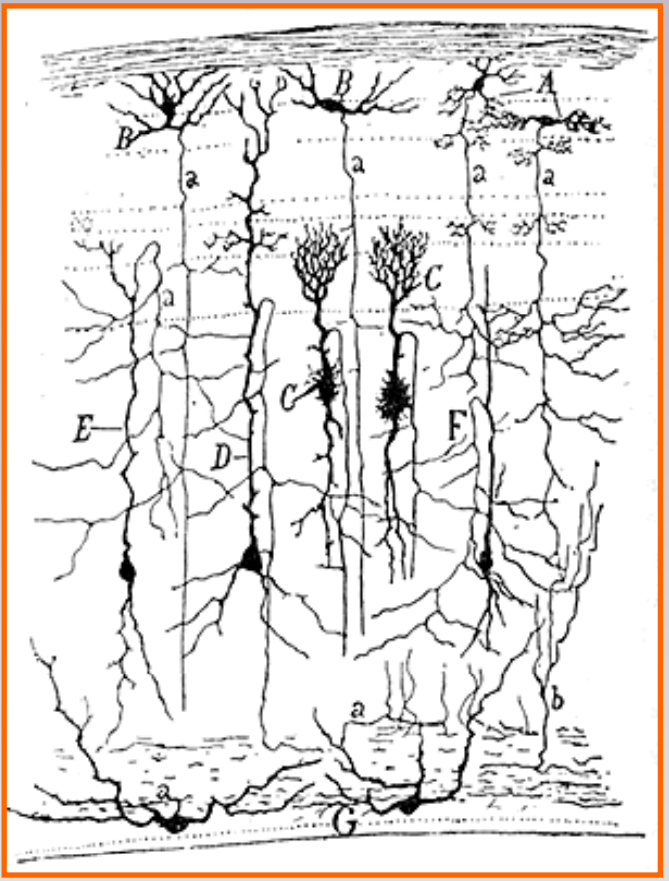
→ Neuronal patterning

How do axons find their way to their target organ?

→ Axon growth and navigation

Which molecules play a role in neuronal patterning and axon navigation?

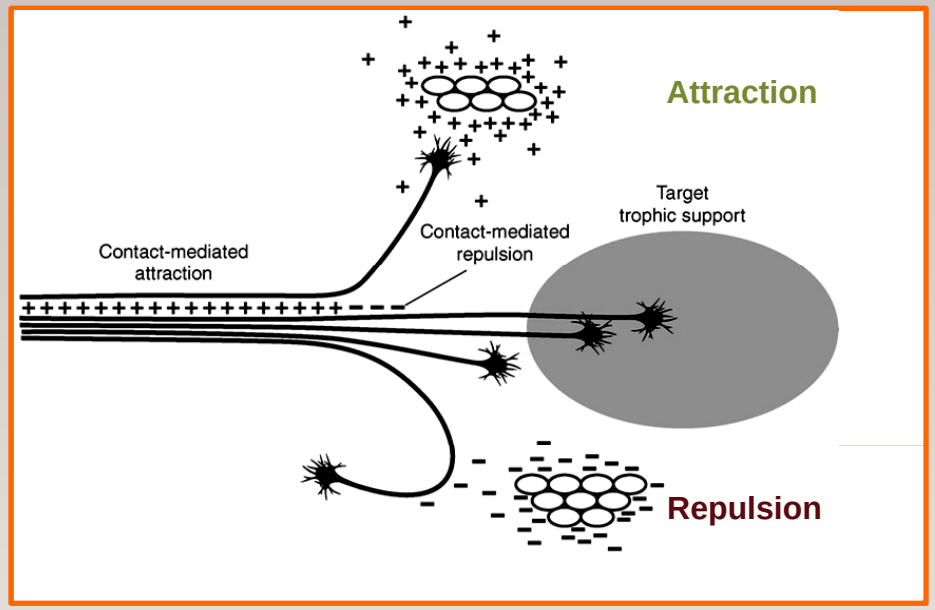
→ Functional discovery research

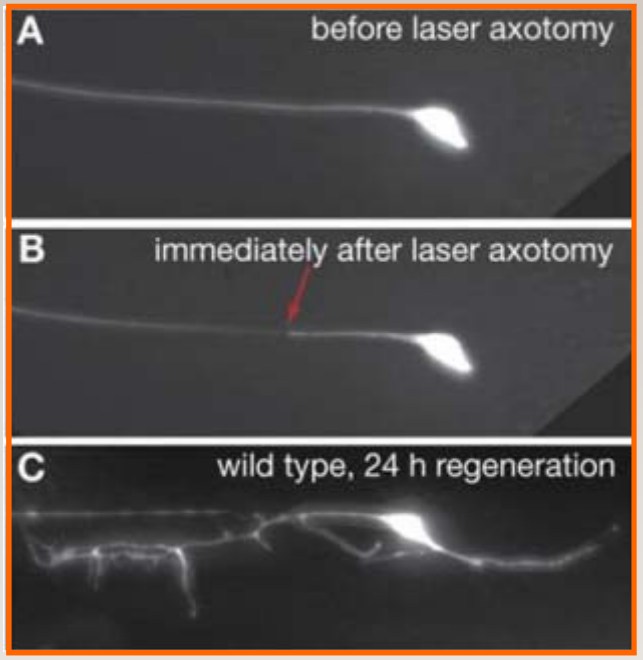


Optic Tectum (sparrow)
Santiago Ramón y Cajal

Molecules under study for neuronal patterning and axonal outgrowth

- proteinases
- neuropeptides
- (miRNA's)





Attractants ⟷ **Repellants**

Model systems

Mouse

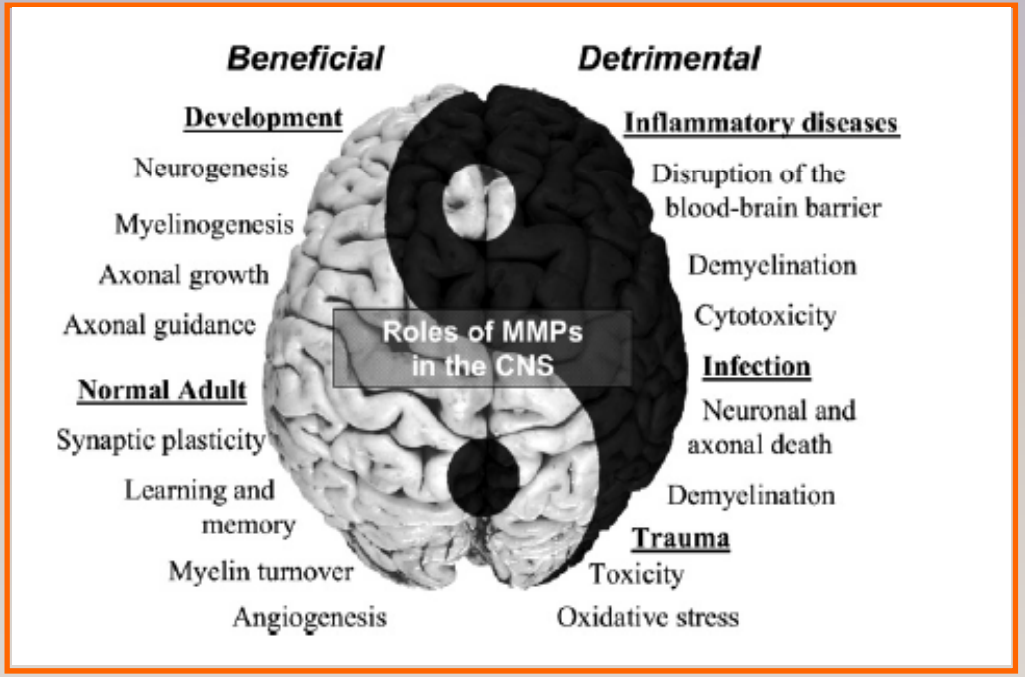
- Neuronal migration and pattern formation in the developing cerebellum
- RGC axon outgrowth using retinal explants

Zebrafish

- RGC axonal outgrowth in the retinotectal circuit of the developing zebrafish
- Regeneration of RGC axons in the retinotectal pathway of adult zebrafish

Matrix metalloproteinases (MMPs) in the central nervous system

- MMPs play a detrimental role in pathological conditions
- Recent evidence suggests a ‘good’ role for MMPs during brain development
- MMPs promote neurogenesis, remyelination and synaptic plasticity in the adult brain

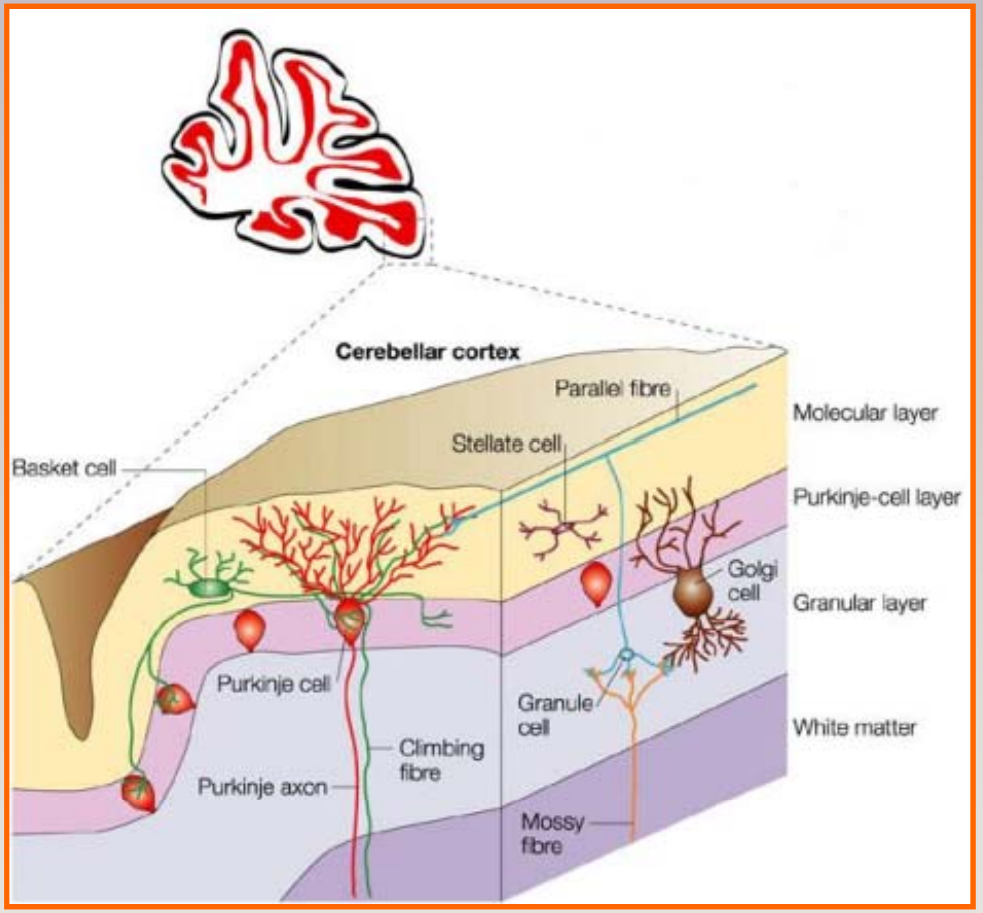


✓ Does MMP-2 play a role in neuronal proliferation, migration, differentiation or synaptogenesis?

Role of matrix metalloproteinase 2 (MMP-2) in neuronal migration and pattern formation in the developing cerebellum

Background

- The cerebellum is an ideal model system to study processes of proliferation, apoptosis, migration and synaptogenesis
- Only 10% of the total brain volume, > 50% of all CNS neurons
- Important for motor coordination, motor learning and cognition



Role of matrix metalloproteinase 2 (MMP-2) in neuronal migration and pattern formation in the developing cerebellum

Experimental model

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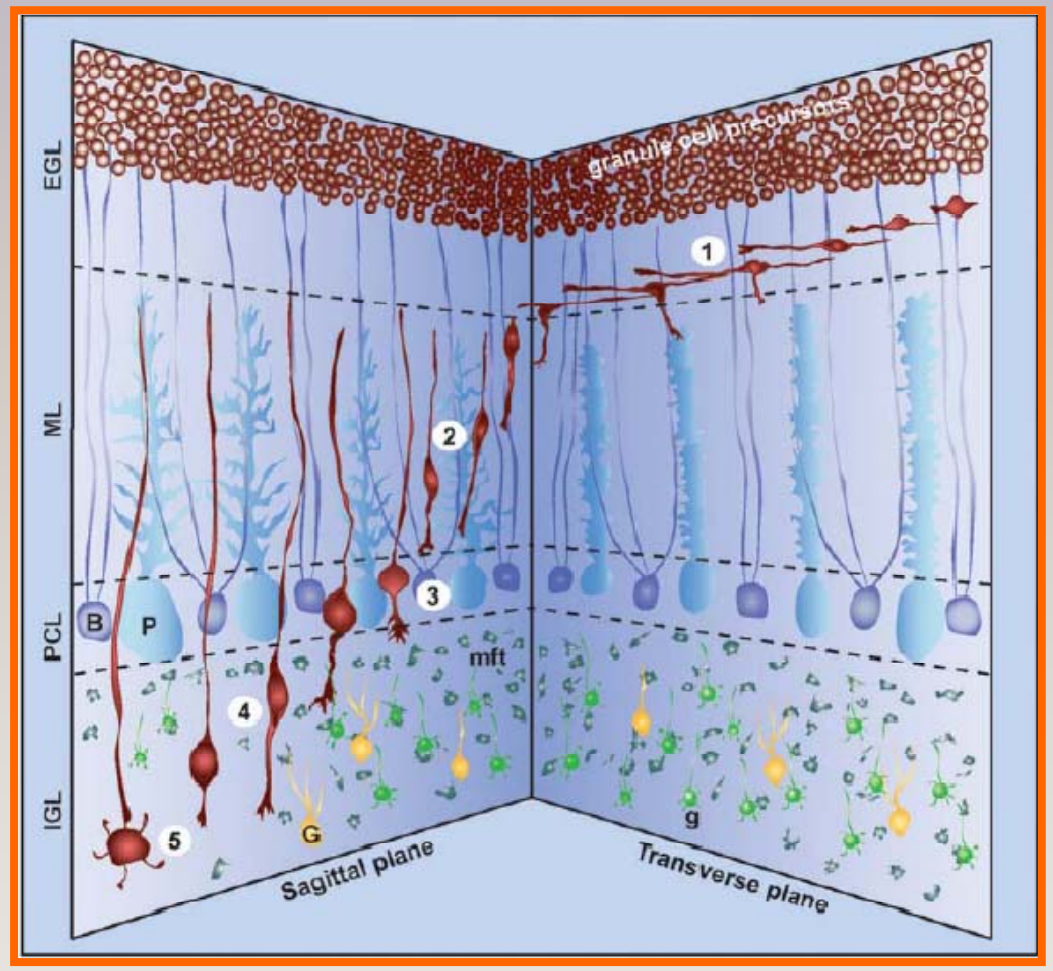
→ Is there a role for MMP-2 during cerebellar development?



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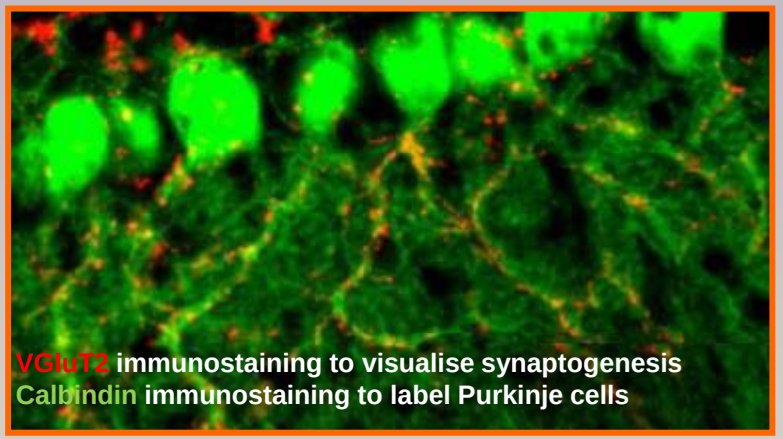
Role of matrix metalloproteinase 2 (MMP-2) in neuronal migration and pattern formation in the developing cerebellum

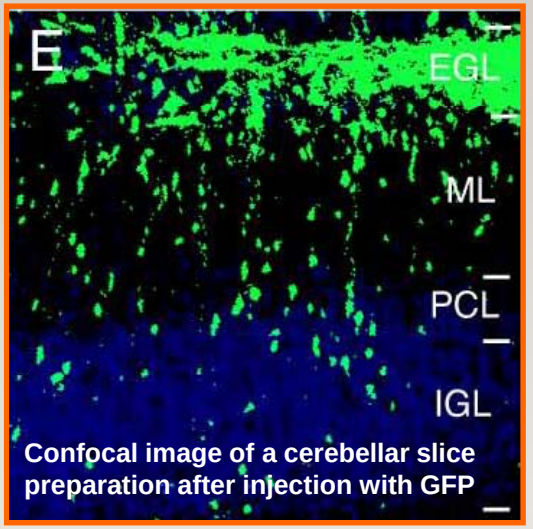
Objectives

- To unravel the exact role of MMP-2 in the histogenesis of the cerebellum
  - → Comparing cerebellar development in MMP-2 deficient and wild-type mice



Methods

- *In vivo* stainings on cryo sections
- *Ex vivo* cerebellar slices (GFP plasmid electroporation)
- *In vitro* cell cultures:
  - cell culture to study proliferation/apoptosis
  - glia culture to study radial migration
- Fluorescent and confocal microscopy
- morphometric analysis



Supervisor

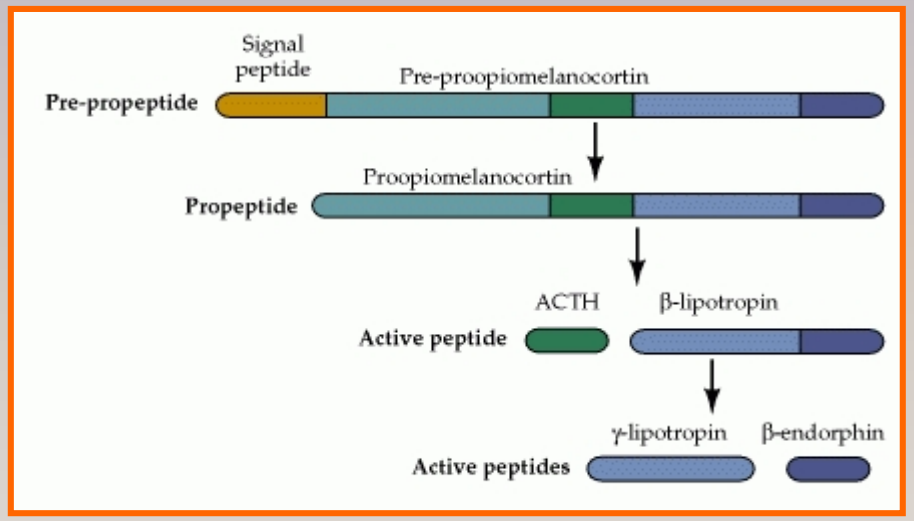
Mieke Verslegers (mieke.verslegers@bio.kuleuven.be)

(Neuro)peptides in the central nervous system

Neuropeptides

- are small protein-like molecules
- are signaling molecules between neurons, hormones and growth factors
- can promote axon regeneration *in vitro*

A number of previously unidentified bioactive peptides were isolated from several mouse tissues with the help of peptidome technology

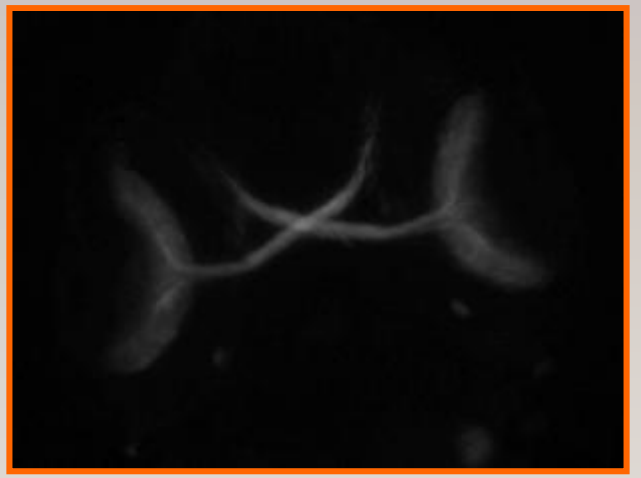


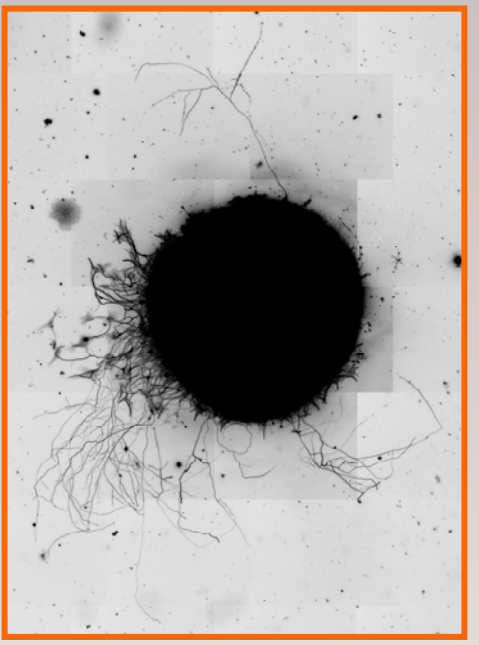
✓ Are these bioactive peptides involved in axonal outgrowth and/or regeneration of axons ?

Screening and *in vivo* validation of bioactive peptides on axonal outgrowth and regeneration

Experimental models

- *Mouse Retinal explants*
  - Culture retina in presence of peptides
  - Analyse neurite outgrowth





- *Zebrafish*
  - Raise larvae in water containing peptides
  - *Analyse in vivo effect on d*evelopment of the retinotectal system

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Screening and *in vivo* validation of bioactive peptides on axonal outgrowth and regeneration

Objective

Screening and characterization of bioactive peptides for their role in axonal outgrowth on mouse retinal explants and in developing zebrafish larvae

Methods

- Isolation of retina from neonatal mice
- Screening for neurite outgrowth in retinal explants
- Immunohistochemistry on retinal explants and sections
- Morphological analysis via fluorescent and confocal microscopy
- *In vivo* study in zebrafish during development and regeneration

Supervisor
Tom Buyens (tom.buyens@bio.kuleuven.be)

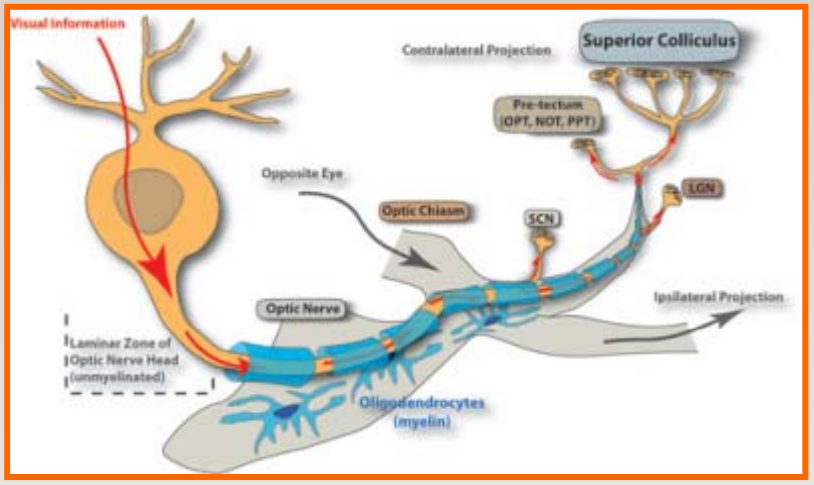
Focus 2 - How can we increase neuronal survival in neurodegenerative diseases?

Neurodegenerative diseases lead to neuronal death and axonal degeneration

Which molecules play a role in neuronal and axonal survival and regeneration?

→ Functional discovery research

→ Research on glaucoma



Glaucoma as a neurodegenerative disease

Background

Glaucoma is a multifactorial neurodegenerative disease

- ultimately leads to irreversible blindness
- the second most important cause of blindness, estimated to affect more than 60 million people worldwide
- characterized by progressive apoptosis of RGCs and degeneration of the optic nerve
- most important risk factor is elevated intraocular pressure (IOP)
- many questions about the pathological events that translate elevated IOP into progressive RGC death remain to be elucidated

Investigating the role of MMP-3 in glaucoma disease progression in the mouse

Experimental model

Induction of increased IOP and glaucoma

- injection of plastic beads in the forechamber of the eye
- eye pressure measurements
- analysis of RGC apoptosis
- analysis of optic nerve degeneration

Eye pressure measurement

✓ Does MMP-3 influence the survival of RGCs and their axons in glaucoma?

✓ By which molecular mechanisms ?

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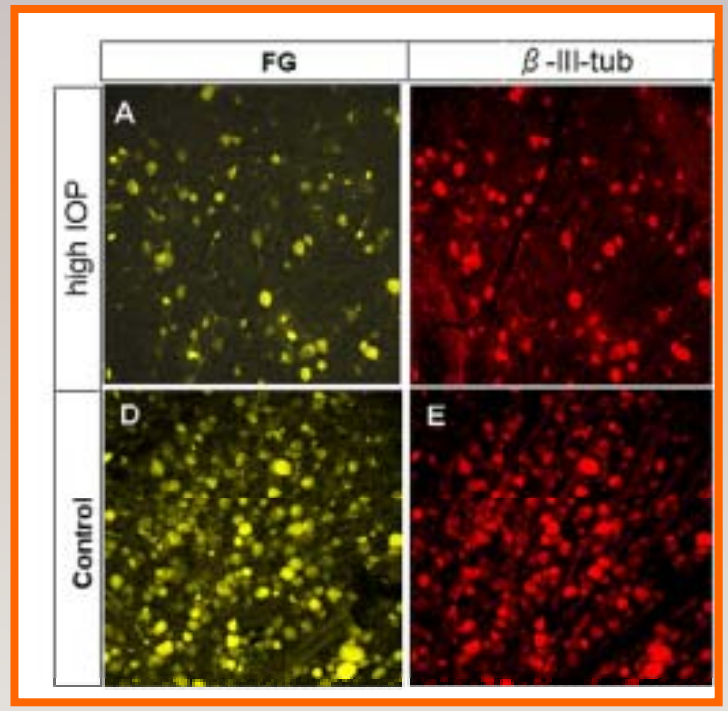
Investigating the role of MMP-3 in glaucoma disease progression in the mouse

Objectives

To unravel the role of MMP-3 in RGC death and optic nerve degeneration induced by glaucoma

Methods

- Breeding and handling of mice
- Genotyping (PCR & gel ectrophoresis)
- Injection of plastic beads in the forechamber of the eye
- Eye pressure measurements
- Dissection to isolate the retina and optic nerve
- Immunological stainings
- Fluorescence microscopy
- Cryosectioning
- Performing various microscopic analyses

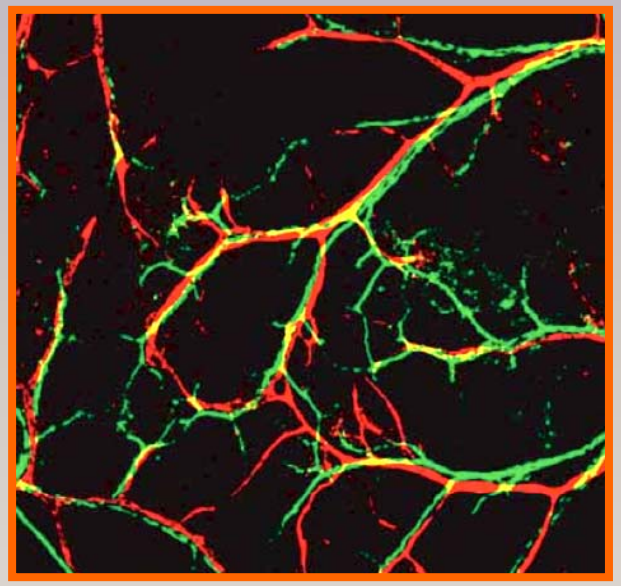


Degeneration of RGCs by increased eye pressure

Supervisor

Djoere Gaublomme (djoere.gaublomme@bio.kuleuven.be)

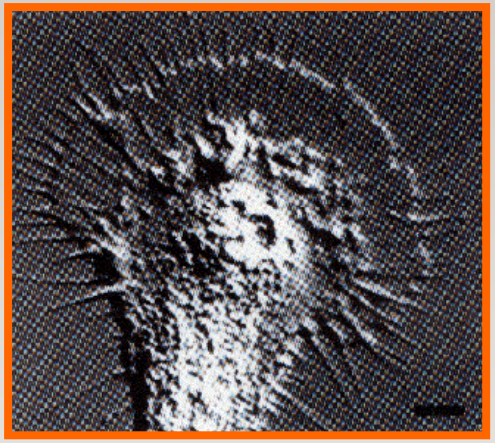
Focus 3 - How do blood vessels grow in development and disease?



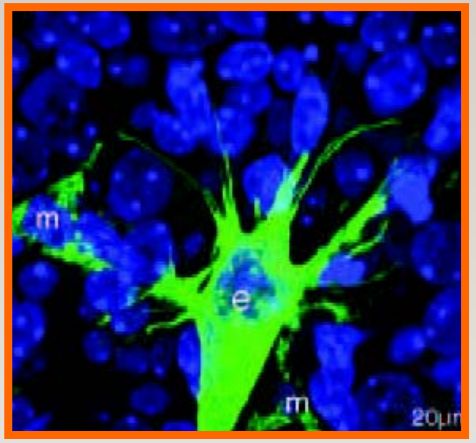
Nerves
Blood vessels

Blood vessels and nerves:
- similar patterning
- similar morphology while growing

➡ **common molecular players ?**



axon growth cone



endothelial tip cell

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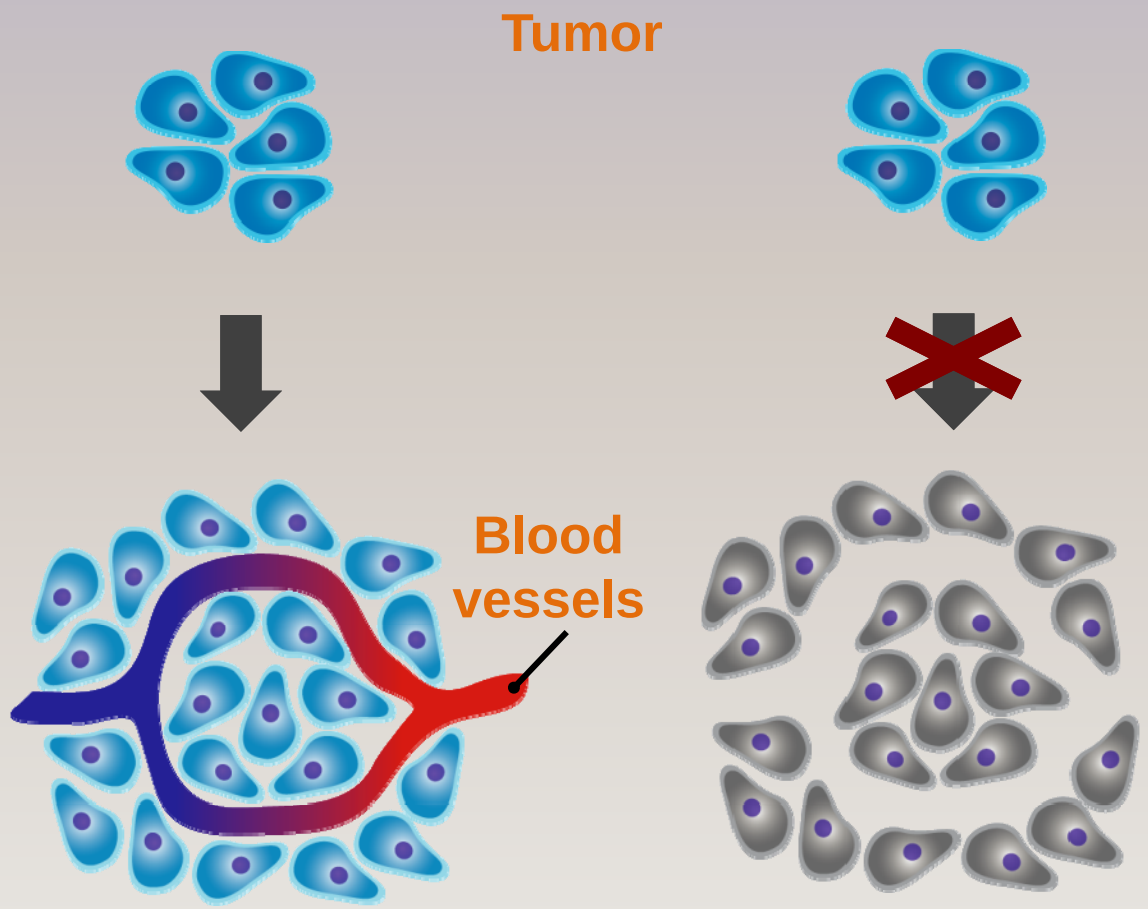
Studies on the role of microRNAs in the formation of blood vessels in developing zebrafish via confocal/fluorescence microscopy and histological phenotyping

Background

- abnormal blood vessel growth plays a role in numerous diseases, including cancer
- there is a therapeutic need to identify molecules that regulate blood vessel growth!
- microRNAs are small endogenous RNA molecules that regulate gene expression post-transcriptionally
- we identified several novel microRNAs with unknown function that are expressed in blood vessels
- the zebrafish is an ideal model to identify novel targets involved in blood vessel growth



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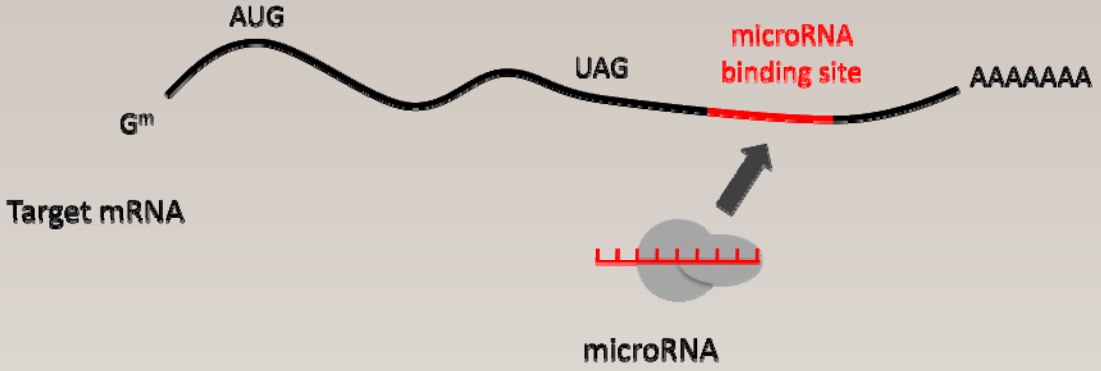

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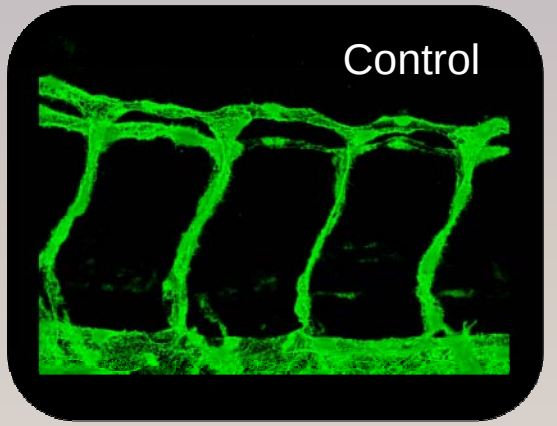
Studies on the role of microRNAs in the formation of blood vessels in developing zebrafish via confocal/fluorescence microscopy and histological phenotyping

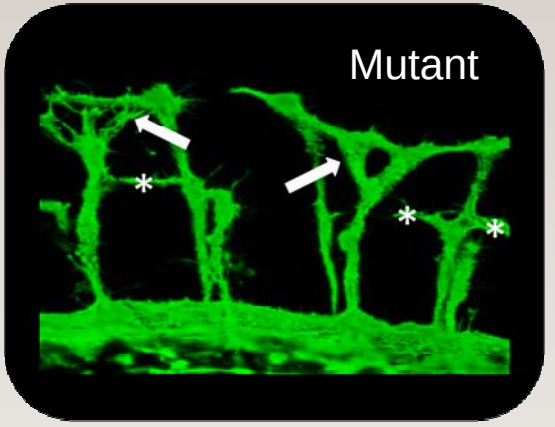
Objective

To identify novel microRNAs that regulate blood vessel growth

Methods

- Knockdown of selected microRNAs via morpholino injections in transgenic zebrafish lines (expressing eGFP in their ECs)
- Live screening of knockdown embryos
- Normal fluorescent and confocal microscopy
- Morphometric analysis of stainings via fluorescent microscopy
- ISH or IHC stainings on cryo- or paraffin sections or whole mount





Supervisor
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