RGC and optic nerve
Literature review 2018

Nitza Goldenberg-Cohen, MD
Bnai Zion Medical Center, Haifa
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Optic nerve models and RGC regeneration

- RGC are terminally differentiated CNS neurons
- Limited endogenous regenerative capacity
- RGCs cannot regenerate axons after optic nerve damage
- RGCs undergo cell death and lead to permanent visual loss
Regenerated or spared axons?

• Can mature RGC axons regenerate after optic nerve injury following genetic manipulations?
• How to differentiated regenerating axons from spared axons?
• Careful examination of axonal projection patterns and morphology may facilitate distinguishing spared from regenerating RGC axons

Fischer et al. ON regeneration in mammals: Regenerated or spared axons? Exp Neurol. 2017
Microphotograph shows a section of this nerve with spared axons on the edge that project through and beyond the optic chiasm.

A. Spared axons project as a narrow bundle near the lesion A (arrows in A’ and A’’)

bundle of spared axons with variable width along the optic nerve. Depending on the orientation and angle at which the optic nerve is sectioned (i.e. cryosectioned), spared axons in some tissue sections appear narrow near the lesion area, but are often wider and more obvious in the distal optic nerve regions (red rectangle). Asterisk indicates the lesion site.
3D neurite tracing of single fiber showing different growth patterns. Some axons loop back towards the eye as shown in B, and others generate branches as they extend within the optic nerve as shown in C.
Profiling transcription factors

1. Activation of the Ascl1 gene turn Muller into RGCs
2. SoxC transcription factors promote contralateral RGC differentiation and axon guidance
3. Transcription factor SOX11 kills α-type RGCs
4. Zinc: A surprise target in regenerating optic nerve
5. Elevated transcription factor Stat3 by Wnt3a protects RGC and enables axonal regeneration
6. Mitochondrial protein Armcx1, RGC survival and axonal regeneration
7. Enzyme DINE and axonal regeneration
8. GSK3 activity and axonal regeneration
1. Genetic activation of the Ascl1 gene turn Muller glia into RGCs

- Müller glia are the cells from which all other types of retinal cells are regenerated in the fish.
- Muller cells in injured eye can turn into regenerating neurons and appear to integrate themselves into the eye's circuitry.
- In newborn mice, Müller glia can be directed to become retinal neurons by activating transcription factor Ascl1, which in turn activates a suite of genes involved in regeneration.

Jorstad et al. *Nature*, 2017
Regenerating Müller glia in the mouse retina

- Müller glia in the adult mouse can give rise to new neurons
- The regenerated cells were making synapses and integrating into both presynaptic and post-synaptic sides

2. SoxC Transcription Factors Promote Contralateral RGC Differentiation and Axon Guidance

• TFs control cell identity by regulating diverse developmental steps such as differentiation and axon guidance

• The transcriptional code for contralateral RGCs differentiation is now identified

• SoxC TFs -Sox4, 11, and 12- are highly expressed in contralateral RGCs

• SoxC TFs are important in the differentiation and guidance of RGCs that project contra-laterally

Kuwajima et al. Neuron 2017
Functions of SoxC TFs in Contralateral Retina
3. Transcription factor SOX11 kills α-type RGCS

- RGCs' functional degeneration in optic nerve injury is subtype dependent
- At least 30 types of RGCs exist
- Different subtypes of neurons may respond differently to the same factors
- **SOX11** TF regulates the early differentiation of RGCs, when axon growth is initiated
- **SOX11** is critical in a mouse model for RGCs regeneration of their axons but simultaneously kills α-RGCS

4. Zinc: A surprise target in regenerating the optic nerve after injury

- **Mobile zinc** increases rapidly in the retina after optic nerve injury
- Mobile zinc regulates RGCs survival and optic nerve regeneration
  - $\text{Zn}^{2+}$ increases rapidly in the processes of amacrine cells and then transfers via vesicular release to RGCs
- **Chelating zinc**
  - Elongates RGCs survival
  - Led to axonal regeneration in a mouse model
  - $\text{Zn}^{2+}$ chelators already exist and could potentially be given either systemically or through IVT

Yiqing Li, PNAS 2017
5. Wnt/B-catenin Signaling Pathway

- Activation of the canonical Wnt/β-catenin signaling pathway led to axonal regeneration in a mouse optic nerve injury model
- Wnt3a ligand activator of the Wnt/-catenin pathway
- ONC model in a transgenic Wnt reporter mouse, followed by IVT injections to deliver Wnt3a or saline
- **Wnt3a**-induced axonal regeneration through TF **Stat3** contributing to RGC survival
- **Novel role for retinal Wnt signaling in axonal regrowth**

Patel et al, Neuroscience, 2016
Increased numbers of microglia in Wnt3a-injected retinas

Patel et al, Neuroscience, 2016

Wnt3a injection induced prominent Wnt signaling in the INL and GCL
Axonal regeneration after injury following a single intravitreal injection of Wnt3a

Patel et al, Neuroscience, 2016
6. Armcx1 Regulates Mitochondrial Transport during Axon Regeneration

- The mitochondrial protein Armcx1 is upregulated during RGCs axonal regeneration
- Armcx1 overexpression mobilizes mitochondria
- In vivo, Armcx1 overexpression enhances neuronal survival and axonal regeneration
- Armcx1 upregulation is necessary for mouse model with high regenerative capacity

Cartoni et al., 2016, Neuron
Armcx1 Regulates Mitochondrial Transport

• In all species, axonal mitochondria move bi-directionally along microtubule tracks
• Mitochondrial transport is crucial for neuronal and axonal physiology
• Armcx1 overexpression enhances mitochondrial transport in adult RGCs
• Armcx1 also promotes both neuronal survival and axon regeneration after injury
• Armcx1 controls mitochondrial transport during neuronal repair

Cartoni et al. Neuron 2016
Mice injected with AAV-PLAP, AAV-Armcx1, or AAVArmcx1DTM 15 days after optic nerve crush. Axons were labeled with CTB injection.
Armcx1: a new molecular player in neuronal injury

Mammalian-specific mitochondrial protein
Armcx1 mobilizes mitochondria and promotes neuronal survival and axonal regeneration

Cartoni et al. Neuron 2016
7. Enzyme DINE and regeneration

- Damage-Induced Neuronal Endopeptidase
- A new enzyme, DINE was identified as a nerve regeneration-associated molecule
- Common injury-inducible transcription factors: ATF3, STAT3 and cJun
- The transcriptional response of DINE to nerve injury is regulated by ATF3, which is a core transcriptional factor to initiate nerve regeneration
- DINE and ATF3 were upregulated in injured RGCs

Kaneko, Cell Death and Disease, 2017
DINE is a Regeneration-associated Molecule

- Overexpression of ATF3 promotes neurite elongation and neuronal survival
- DINE is involved in the gene network system for regeneration downstream of ATF3 after optic nerve injury
- DINE had impact on αRGCs with axon-growth potential as well as on other types of RGCs

Kaneko et al. Cell Death and Disease, 2017
DINE is a Regeneration-associated Molecule

Injured RGCs activate and orchestrate multiple signaling pathways, such as mTOR-, STAT3- and Rho-mediated pathways, to enhance robust nerve regeneration.

DINE-ablated RGCs fail to regenerate even after treatment with the regeneration promoting reagent zymosan.

Kaneko, Cell Death and Disease, 2017
8. GSK3 activity and axonal regeneration

• The role of GSK3 in axon regeneration is controversial
• Increased GSK3 activity accelerates peripheral nerve regeneration but not the CNS
• KO/knockdown of GSK3β in growth-stimulated RGCs was disinhibitory and potentiated optic nerve regeneration
• CRMP2 compromised RGCs’ ability for axon growth
• Both GSK3 inhibition or neuronal expression of constitutively active CRMP2 (CRMP2T/A) potentiated optic nerve regeneration

Leibinger et al. PNAS 2017
Inflammation Vs Regeneration

1. Oroxylin A, an anti-inflammatory agent, promotes RGC survival in a rat ONC model

2. MSC exosomes promote survival of RGCs through miRNA-Dependent Mechanisms

3. Microglia are irrelevant for neuronal degeneration and axon regeneration

4. Inflammatory caspases and Pyroptosis, inflammatory programmed cell death
   - a novel non-apoptotic role for caspases in RGC death and axon regeneration

5. Intranasal delivery of an anti-inflammatory agent preserved the optic nerve
1. Oroxylin A Promotes RGCs Survival in A Rat ONC Model

- **Single SC injection** of Oroxylin A immediately post ONC in rats Preserved RGC density in the retinas
  - 15mg/Kg in 0.2ml PBS
- Improved VEP
- Reduced apoptosis
- Reduced microglial infiltration

Lin et al, PloS ONE 2017
2. MSC Derived Exosomes Promote RGC Survival Through miRNA-Dependent Mechanisms

- Mesenchymal stem cells (MSC) have demonstrated significant neuroprotective and axogenic effects on RGC
- MSC derived exosomes promote survival of RGCs through miRNA-Dependent Mechanisms
- 21d after ONC and weekly intravitreal exosome injections prevented RGC and axonal loss
- Possible to use the BMSC-derived exosomes as a cell-free therapy for traumatic and degenerative ocular disease
3. Microglia are irrelevant for neuronal degeneration and axon regeneration

• The role of microglia and infiltrating macrophages in regenerative processes after damage of the CNS is not clear
• Treatment with CSF1 receptor inhibitor PLX5622 eliminated virtually all microglia in mice retina and optic nerve but spared the macrophages
• Microglia depletion impaired the removal of dead cells but had no influence on degeneration of RGCs after ONC

Hilla, Diekmann and Fischer, Jneurosci 2017
Microglia are irrelevant for neuronal degeneration and axon regeneration

- Optic nerve regeneration remained unaffected upon microglia depletion
- Gliosis of optic nerve lesion site was delayed
- Concurrent double depletion of microglia and infiltrated macrophages significantly compromised optic nerve regeneration
- Microglia neither promote nor inhibit neuronal degeneration or axonal regrowth

Hilla, Diekmann and Fischer, J. Neurosci ; 10.1523/JNEUROSCI.0584-17.2017
Microglia are irrelevant for neuronal degeneration and axon regeneration
Microglia are irrelevant for neuronal degeneration and axon regeneration

Hilla, Diekmann and Fischer; J. Neurosci; 10.1523/JNEUROSCI.0584-17.2017
4. Caspases in RGC death and axon regeneration

- RGC die by caspase-dependent mechanisms
  - during development
  - after ocular injury
  - progressive degenerative diseases

- Pyroptosis, mediated by inflammatory caspases, can also occur in RGCs
  - a specialized form of inflammatory apoptosis

- Initiator caspases activate executioner caspases through catalytic cleavage of their activation domain
  - Initiators: caspase-2, -8, -9 and -10
  - executioner: caspase-2,-3, -6 and -7

- caspase-2 can act as both an initiator and an executioner caspase, and does not fit into either intrinsic or extrinsic apoptotic pathways

Thomas et al, Cell Death Discovery 2017
Apoptotic caspases in the canonical intrinsic and extrinsic pathways. Death receptor activation mediates the extrinsic pathway. The intrinsic pathway is mitochondria-dependent. Caspase-8 can also activate the intrinsic pathway.

*Thomas et al. Cell Death Discovery 2017*
Caspases inducing RGC death through apoptosis and pyroptosis after trauma and disease. Inhibition of caspases with pharmacological or genetic inhibitors promotes RGC survival.

*Thomas et al. Cell Death Discovery 2017*
Inflammatory caspase-1 is activated within the inflammasome protein complex

The NLRP3 inflammasome activates caspase-1, which cleaves precursor cytokines IL-1β and IL-18 into their active forms and gasdermin-D into its N-terminal fragment. The N-terminal fragment of gasdermin-D forms a plasma membrane pore facilitating pro-inflammatory cytokines release and inducing pyroptosis.

Thomas et al. Cell Death Discovery 2017
Caspases and axon regeneration
caspase-2, -6 and -8

• Caspases promote RGC axon regeneration
• Pharmacological inhibition of caspase-6 and -8 provide RGC neuroprotection with limited RGC axon regeneration
• Inhibition of caspase-6 provide few RGC axons regeneration
• Combined suppression of caspase-2 and -6 promoted significant axonal regeneration
• The neuroprotective and pro-regenerative effects of caspase-6 inhibition are mediated indirectly by CNTF upregulation in retinal glia

*Thomas et al. Cell Death Discovery 2017*
Caspases and axon regeneration
caspase-2,-3,-6 and -8

• Caspase-3 is implicated during RGC development, whereas most apoptotic and inflammatory caspases are implicated in trauma and disease
• siRNA knockdown of caspase-2 provide the greatest neuroprotection after axotomy, optic neuritis and AION
• Non-apoptotic roles of caspases, such as inflammatory pyroptotic death or facilitating formation of necroptotic complexes are also critical in RGC death

Thomas et al. Cell Death Discovery 2017
5. Intranasal delivery of an anti-inflammatory agent preserved the ON

• Intranasally delivered amnion cell derived biological secretome in the EAE animal model of MS to
  – suppress inflammation
  – prevent neuronal damage
  – preserve neurologic function
• ST266, the biological secretome of amnion-derived multipotent progenitor cells, contains multiple anti-inflammatory cytokines and growth factors
• Intranasal delivery of neuroprotective ST266 is a potential novel, noninvasive therapeutic modality for the eyes, optic nerves and brain

Khan et al. Sci Rep 2017
Visual threats are translated rapidly into defensive behavioral responses

• Animals promote their survival by avoiding rapidly approaching objects that indicate threats

• In mice, looming-evoked defensive responses are triggered by the superior colliculus (SC) which receives direct retinal inputs

• The specific neural circuits that begin in the retina and mediate this important behavior remain unclear
Visual threats are translated rapidly into defensive behavioral responses

- Subset of RGCs controls mouse looming-evoked defensive responses through axonal collaterals to the dorsal raphe nucleus (DRN) and SC
- Activating DRN GABAergic neurons that in turn inhibit serotoninergic neurons
- Dedicated population of RGCs signals rapidly approaching visual threats and their input to the DRN controls a serotonergic self-gating mechanism that regulates innate defensive responses

Huang et al. NATURE COMMUNICATIONS | DOI: 10.1038/ncomms14908