Mechanisms of CNS Axon regeneration

Homaira NAWABI,
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PNS vs CNS: PNS is able to regenerate

Zion Harvey, 8 years old
Double hands transplant
Children’s Hospital of Philadelphia,
July 2016
CHRISTOPHER REEVE The man of steel

Traumatic injury
Spinal cord
Brain (TBI, Stroke)

PNS vs CNS: CNS cannot regenerate

Acute CNS injuries

Chronic diseases

Stephen Hawking

Neurodegenerative diseases
Alzheimer
Parkinson
ALS
Glaucoma

https://www.christopherreeve.org/
http://www.unadev.com/
http://chnfoundation.org/
https://wwwfrm.org/urgence/maladies-neurodegeneratives.html
Central Nervous System Regeneration

Extrinsic and intrinsic barriers of axon regeneration

Inhibitory role of the environment

Translation increase ($mTOR$)
Transcription modulation ($KLF$, $SOCS3$, $DLK$)

Myelin debris (Nogo, MAG, Omgp)

Glia scar (reactive astrocytes, CSPG)

He & Jin, Neuron 2016
Optic nerve: part of the SNC

The eye is a window to the brain

Relative easy surgery
Fast model
Less invasive
Results easily transposable to spinal cord/brain
Modulation of RGC and injury model

Retina Ganglion cells

AAV injection

Day 1
AAV2-GFP

Day 2
AAV2-RFP

Week 2
Termination

AAV2-RFP
AAV2-GFP
AAV2-RFP/AAV2-GFP
AAV2-RFP/Tuj1
Promoting Axon Regeneration in the Adult CNS by Modulation of the PTEN/mTOR Pathway

Kevin Kyungsuk Park,* Kai Liu,* Yang Hu,* Patrice D. Smith,* Chen Wang, Bin Cai, Bengang Xu, Lauren Connolly, Ioannis Kramvis, Mustafa Sahin, Zhigang He†
Neuronal response to injury: optic nerve model

Belin et al., Neuron 2015
Characterization of RGCs response to injury

Bioinformatic approach

Target approach

Log2 ratio of expression

Proteins

Belin et al., Neuron 2015

Nawabi et al., Neuron 2015
DCLK2 a structural protein to regulate axon regeneration

DCLK2 belongs to the DCX superfamily

DCX (doublecortin) is a microtubule associated protein (des Portes et al 1998; Gleeson et al 1998; Gleeson et al 1999)

DCX is expressed in post mitotic neurons (Gleeson et al 1999; Francis et al 1999)

DCX has a critical role in neuronal migration (Gleeson et al 1999; Francis et al 1999)
DCLK2 promotes survival and its kinase domain is dispensable

MT binding domain

Actin regulating domain

**MT binding domain**

**Actin regulating domain**

**WT intact**

**WT/PLAP**

**WT/DCLK2**

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**Wildtype intact**

**Wildtype/PLAP**

**Wildtype/DCLK2**

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**MT binding domain**

**Actin regulating domain**

**DCX**

**DCX-domain 1**

**DCX-domain 2**

**Ser/Pro Rich**

**DCLK1/2**

**DCX-domain 1**

**DCX-domain 2**

**Ser/Pro Rich**

**KINASE**

**DCX-270**

**DCX-domain 1**

**DCX-domain 2**

**DCX-SP**

**Ser/Pro Rich**

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**% of Tuj1+ RGCs**

**% of Tuj1+ RGCs**

---

**intact**

**PLAP**

**DCLK2**

---

**% of Tuj1+ RGCs**

**% of Tuj1+ RGCs**

---

**intact**

**PLAP**

**DCLK2**

**DCX-270**

**DCX-SP**

---

**% of Tuj1+ RGCs**

**% of Tuj1+ RGCs**

---

**intact**

**PLAP**

**DCLK2**

**DCX-270**

**DCX-SP**
DCLK2 promotes axon regeneration in WT

Estimated number of axons vs. distance from lesion site (mm)

- WT/AAV2-DCLK2
- WT/AAV2-PLAP

Significance levels: *p < 0.05, **p < 0.001
DCLK2 enhances PTEN$^{-/-}$ regenerative phenotype

**Estimated number of axons**

- **Distance from lesion site (mm)**

  - **PTEN$^{-/-}$/DCLK2**
  - **PTEN$^{-/-}$**

  ![Graph showing the estimated number of axons at different distances from the lesion site for PTEN$^{-/-}$/DCLK2 and PTEN$^{-/-}$ genotypes.](image)
DCLK2 enhances growth cone formation

- P21 PTEN−/− mice
- AAV2-Cre+PLAP
- AAV2-Cre+DCLK2
- PTEN−/−
- PTEN−/−/DCLK2
- Anti-Tuj1
- Number of fibers growing from the explant
- Distance from explant (um)

**Legend:**
- *: Significant difference
- **: Very significant difference
DCLK2 enhances growth cone formation

- P21 PTEN^-/^- mice
- AAV2-Cre+PLAP
- AAV2-Cre+DCLK2

Week 2
- Explant culture

Week 4
- Laser cut/
  Live imaging
DCLK2 enhances growth cone formation by stabilizing actin

Riedl et al. Nat Protoc. 2008
DCLK2: Summary

Regulates injury signals transport
Neuron survival

Actin stabilization

Growth cone formation
Axon elongation
Long distance regeneration: several signaling pathways

Belin et al., Neuron 2015
Tissue clarification for in-depth analysis

sections

- treatment | + treatment

Benzyl benzoate/Benzyl alcohol
iDisco/3Disco

clariﬁcation

- treatment | + treatment

Optic nerve

Adult Brain
Long distance regeneration: guidance defects

mTOR+JAK/STAT+c-myc

Belin et al., Neuron 2015
The journey of CNS regeneration

80’s  90’s  2008

PNS Grafting (David & Agayo 1981)
NoGo (Chen et al 2000)

2010’s

Intrinsic factors
(Park et al 2008)

Extrinsic factors

Specificity of action

Function

Guidance/circuit

Stem cells transplantation (Lu et al 2014)

Electrical/chemical stimulation (van den Brand R et al 2012)
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