Sonic hedgehog promotes sprouting of neurons in the pelvic ganglia and cavernous nerve during regeneration

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How does SHH pathway promote CN regeneration?

1. Normal
   - Normal erectile function
   - n=neuron, g=glial cell, v=degenerated neuron

2. Nerve crush
   - PG neurons degenerate
   - Erectile dysfunction develops
   - g=glial cell, n=neuron, v=degenerated neuron

3. Nerve crush + SHH PA
   - SHH undergoes retrograde transport
   - Neuronal, glial and target signaling is maintained
   - PG neurons do not degenerate
   - Improved erectile function
   - n=neuron, g=glial cell, v=degenerated neuron
SHH PA treatment improves CN function

(p-value=0.05, Angeloni et al., 2011)
SHH PA promotes CN regeneration

Control  SHH

4 week

6 week

(Angeloni et al., 2011)
Hypothesis

**Goal:** Better understanding of how Sonic hedgehog promotes cavernous nerve regeneration

**Hypothesis:** Sonic hedgehog promotes sprouting of pelvic ganglia and cavernous nerve neurons
Methods

Organ culture:
- Embedded in Matrigel
- Serum-free RPMI media
- 1X penicillin-streptomycin-fungizone
- Maintained at 37°C with 5% CO₂ for 3-4 days

Treatment groups

1. Normal PG/CN: SHH or PBS (control), (n=14)
2. Normal PG/CN: SHH inhibition (cyclopaamine or 5e1), (n=10)
3. Sprouting potential: CN crush at 4 and 9 day (n=5)
4. CN crush with SHH or MSA treatment in vivo 4d and in organ culture (n=12)
5. Location of delivery: CN or PG (n=6)
1. Normal PG/CN treated with SHH or PBS
SHH inhibition prevents sprouting near beads.

SHH inhibition with cyclopamine prevents sprouting near bead vehicle.
2. SHH inhibition prevents sprouting near beads

5e1 SHH inhibitor

Affi-Gel beads
3. Cavernous nerve crush 4 and 9 day

4 day crush

9 day crush

Similar sprouting potential at 4 and 9 days after crush injury.
4.4d CN crush MSA *invivo* and organ culture

Little sprouting on PG
4.4d CN crush with SHH *invivo* and organ culture

High sprouting in wide area of PG and CN. Sprouts are longer.
4.4d CN crush with MSA *invivo* and SHH organ culture

Little sprouting on CN

PA vehicle
4.4d CN crush SHH \textit{in vivo} but not organ culture

Less sprouting along CN.

No sprouting
5. SHH treatment on PG

Pronounced sprouting at CN end
SHH treatment induces nNOS

SHH upregulates nNOS in organ culture CN fibers.
Sprouts in SHH treated PG

nNOS positive sprouts in PG in response to SHH treatment.
Results/Conclusions

1. SHH treatment promotes sprouting of the PG
2. SHH promotes more sprouting in crushed CN
3. Similar sprouting potential at 4 and 9 days after crush
4. Location of SHH delivery makes a difference in sprouting potential
5. SHH upregulates nNOS
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