Cartilage Formation in Re-regenerated Lizards

Hannah Chuabanlue, Thomas Lozito

Lozito Lab, Dept of Stem Cell Biology and Regenerative Medicine, Bridge Institute, University of Southern California, Los Angeles, CA , USA

Bridge UnderGrad Science (BUGS) ***Summer Research Program***

USC University of Southern California

Abstract

Lizards are amniotes with the ability to regenerate their tail. However, regenerated tails are imperfect: they lack the tissue organization seen in original tails, and consist only of a single unsegmented cartilage tube surrounding the spinal cord rather than proper vertebrae. But different regions of the cartilage form differently. The most proximal region (CC) of this cartilage tube, closest to the amputation point, reflects a fracture healing system. Periosteal stem cells from the original tail vertebra respond to BMP and Ihh signaling to form a cartilage callus. These chondrocytes undergo hypertrophy and endochondral ossification that replaces the cartilage with bone continuous to the original spine. Meanwhile, more distal areas (CT), referring to all area beyond the proximal cartilage callus (CC) exhibit blastema based regeneration. This is because CT forms from blastemal cells in response to Shh signals from the ependymal tube, the center of the regenerating spinal cord.

In this study, lizards were amputated within the CT of already regenerated tails. A previous experiment showed that the new cartilage tube formed did not develop hypertrophic cartilage like in the CC of an original amputation. This was repeated with SAG treatment to see if hedgehog stimulation would induce cartilage in a re-regenerating tail like it does in original re-generating tails. Comparing SULF1 expression affirmed that it did, albeit more delayed than in an original regenerated tail.

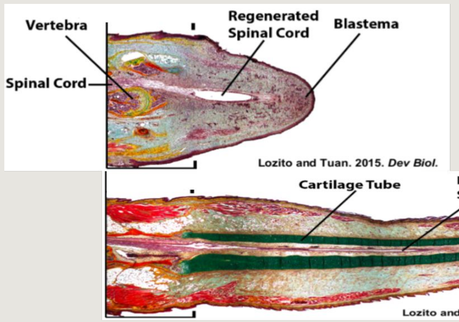


Fig. 1: Lizard tail regeneration, 2 week (top), 1 month (bottom)

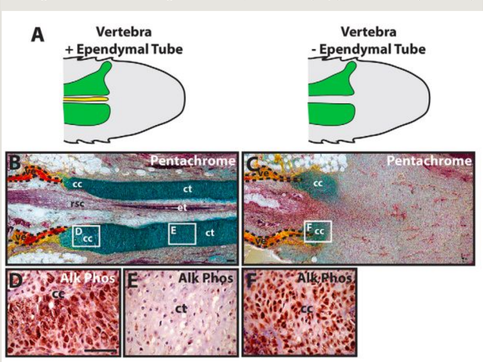


Fig. 2: Proximal and distal regenerated cartilage form independently

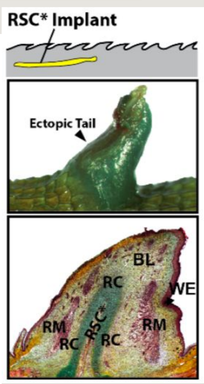


Fig. 3: RSC induces regeneration

OBJECTIVE

In this study, lizards were amputated within the CT of already regenerated tails. A previous experiment showed that the new cartilage tube formed did not develop hypertrophic cartilage like in the CC of an original amputation. This was repeated with SAG treatment to see if lizards could be encouraged to produce even more cartilage when re-regenerating their tails.

Materials

Smoothened agonist (SAG): The Smoothened (SMO) protein plays an important role in the hedgehog signal pathway, and SAG amplifies SMO expression, therefore also increasing Shh signals from ependymal tube. The histological effect is that more cartilage is formed.

For this study, we injected lizards with 300 µl of 800µg/ml concentration SAG solution every other day, starting 3 days post amputation.

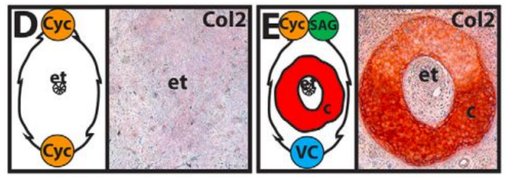
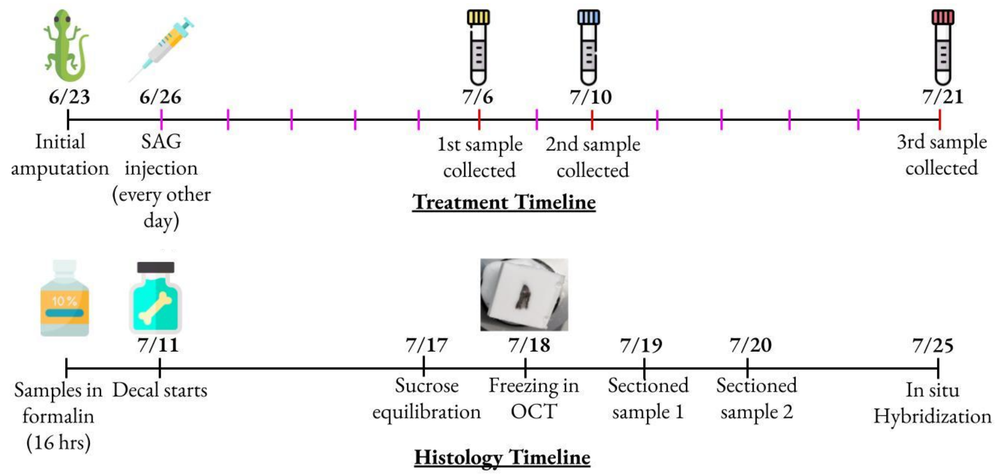


Fig. 4: SAG induces cartilage regeneration and can reverse the inhibitory effect of Cyc (cyclopamine)

Methods

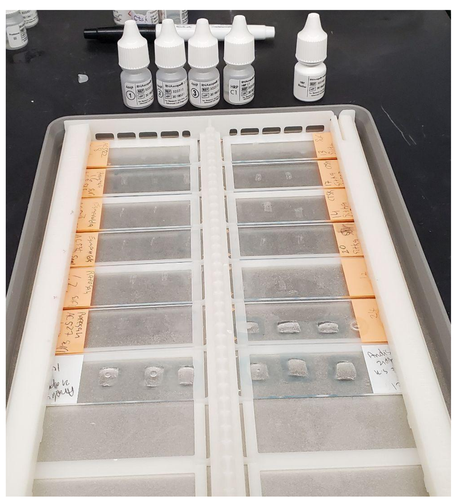


In SItu Hybridization

This is a process for preparing slides for fluorescent microscopy. LIving cells transcribe DNA into mRNA products. In this study, we searched for the Sulf 1 gene which is a sign of cartilage regeneration. To do this, we have to attach fluorescent markers to cells of interest by In Situ Hybridization.

Day 1: pre treatments
1. wash with PBS to remove OCT
2. fix in 10% NBF (neutral buffered formalin)
3. ethanol gradient: permeabilize membrane
4. add hydrogen peroxide
5. mark hydrophobic barrier around samples

Day 2:
1. add protease
2. add (up to 3) probes, marks cells of interest
3. heat at 60 deg for 2 hours
4. amplification 1
5. amp 2
6. amp 3
7. add fluorescent dye
8. hrp block: reduce non specific binding
9. add DAPI dye
10. seal slide



Results

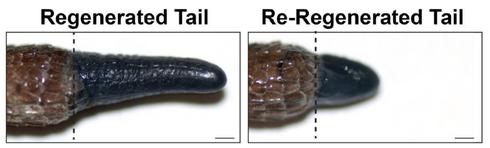


Fig. 5: regenerated vs re-regenerated tail 21 days post amp. on SAG treatment; dash line represents amputation point

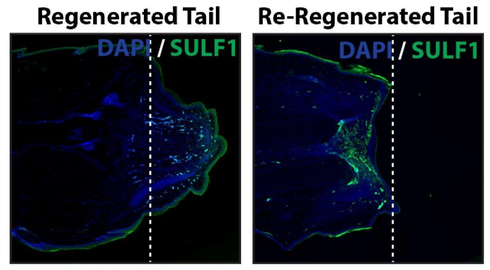


Fig. 6: DAPI/SULF1 expression in regenerated vs re-regenerated tail

Comparing pictures of a regenerated tail and a re-regenerated tail both 21 days post amp, the growth of the latter is visibly less than the previous. In addition, when comparing expression of DAPI/SULF1, while the regenerated tail shows SULF1 expression in the blastema, the re-regenerated tail displayed SULF1 around the amputation point, but barely any within the blastema. This might imply that something important to regeneration was in decreased supply in the re-regenerated tail. This could include blood vessels, blood, vertebrae, etc. However, the presence of SULF1 stains in the re-regenerated tail affirms that hedgehog stimulation does induce cartilage, although slower or more delayed in a re-regenerated tail.

Summary

Sulf1 is a gene uniquely expressed in tail regeneration, not even found in regenerating lizard digit tips. It is responsible for hedgehog signaling which is vital to regeneration and is a marker for cartilage regeneration. Expression of Sulf1 in regenerated tails versus re-regenerated tails, observed through in situ hybridization and fluorescence microscopy, revealed that re-regenerated tails are delayed in regeneration. However, SAG treatments, which were shown to induce cartilage formation by being a hedgehog agonist, held the same effects in the re-regenerated tail. This means that hedgehog signaling, like in an original regeneration, also plays a vital role in cartilage formation for re-regeneration.

Acknowledgements

Thank you to Dr. Thomas Lozito and all other members of the Lozito Lab for graciously allowing me the opportunity to work and learn in the lab and for guiding me through this project.

REFERENCES

[1] Lozito, Tuan, et al. Lizard Tail Regeneration As An Instructive Model of Enhanced Healing Capabilities In An Adult Amniote 2017. PMID 27459585
[2] [3] [4] Lozito, Tuan, et al. Lizard tail skeletal regeneration combines aspects of fracture healing and blastema-based regeneration 2016. PMID 27387871

CONTACT US

bridge.usc.edu/bugs