Effects of Osteoclast Depletion on Lizard Tail Regeneration

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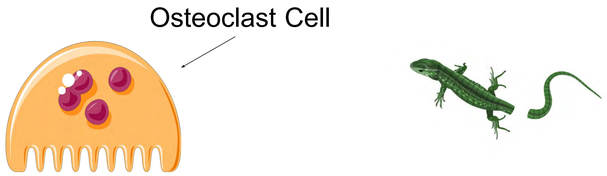
Abstract

Lizards are the closest relatives of mammals capable of tail regeneration. Lizard tail regeneration involves the growth of a specialized structure called a blastema. Blastemas are made up of tail cells that are released from tissues by enzymes secreted by immune cells, such as macrophages and osteoclasts. Here we test the effects of targeted osteoclast depletion on lizard tail regeneration. Zoledronic Acid (ZA) is a drug that inhibits osteoclast activity. Lizards were treated with ZA following tail amputation, and the effects on tail regeneration were studied. ZA treatment reduced osteoclast levels and inhibited normal blastema formation.



Methods

Cryostat

A cryostat is a machine that operates in -25 Celsius and cuts thin slices (16 µm) of tissue. For this project, I used a cryostat to prepare sections of lizard tail samples. I made around 40 slides which had 3 samples each.

Pipette

A pipette is a handhold device that works similar to a syringe. With a pipette you can move liquids that are in miniature quantities with ease and precision. To prepare ZA drug treatments, I added 2 µl of ZA drug to 1 ml of PBS. The lizards were treated with 200µg/kg ZA three days a week.

Crested Gecko

Green Anole

What I have been doing in my Lab

Cleaning out the lizard cages happened every 2 weeks

Collected our first tail with mutated blastema due to no Osteoclasts inside the Lizard.

Last collection of green anole tails with modified blastema

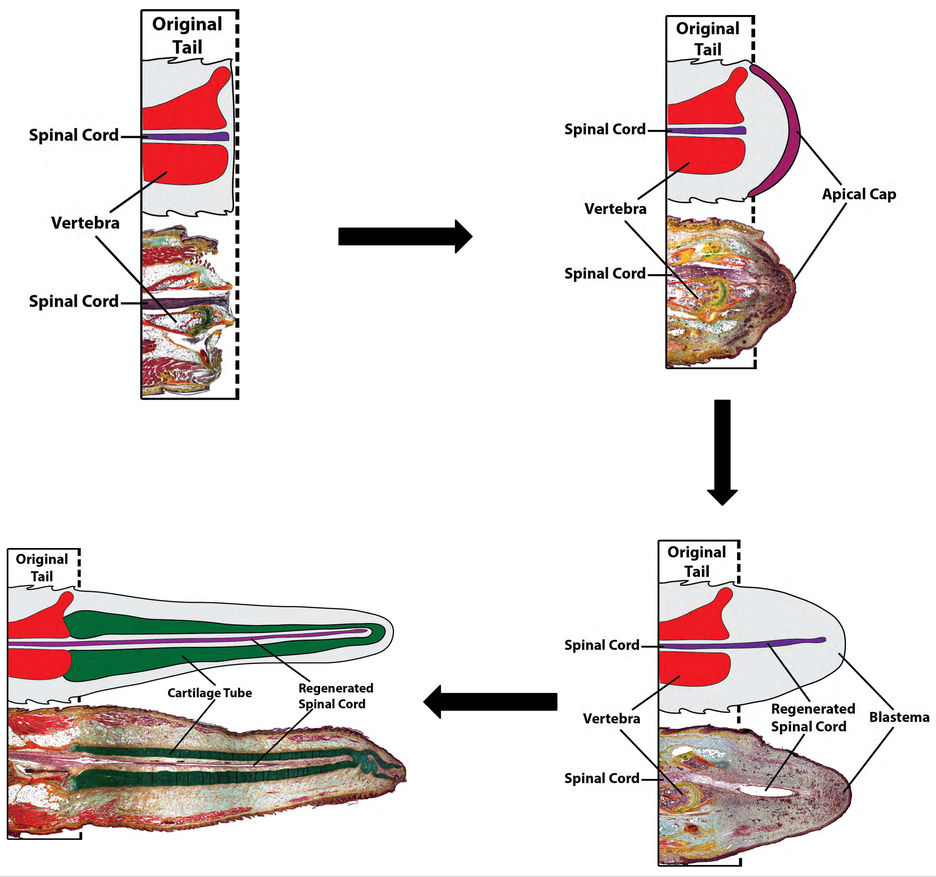
6/23/23 First injection into green anole's using Zoledronic Acid (ZA)

6/26/23 First Amputation on green anole's. We collected the tail with room to form a blastema

7/10/23 Feeding the lizards accured every Tuesday and thursday of every week

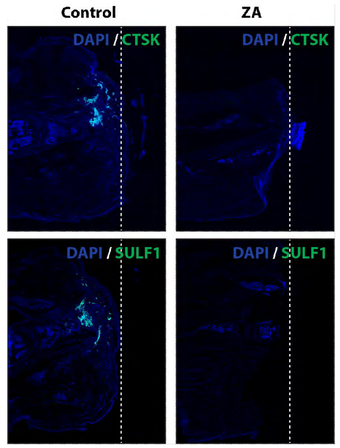
7/21/23

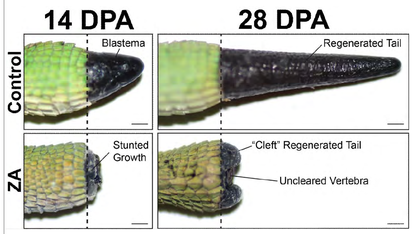
The Lizard Tail Regeneration Process



Results

Tail samples collected from control and ZA-treated lizards were analyzed by in situ hybridization for CTSK and SULF1 expression. CTSK is an osteoclast marker and SULF1 is a blastema-specific marker. The picture on the right demonstrates how ZA treatment/osteoclast depletion lizard tail regeneration. The control lizard tail expresses both CTSK and SULF1, while ZA-treated tails do not.





The picture on the left compares control and ZA-treated tails collected at 14 and 28 days post-amputation (DPA). At 14 DPA, ZA treatment stunted blastema growth. By 28 DPA, ZA treatment interfered with vertebra clearing and caused deformed, W-shaped regenerated tails.

Summary

I learned by treating lizards with zoledronic acid that osteoclasts are important for lizard tail regeneration. If osteoclasts are depleted, then tail vertebrae will not deteriorate, leaving the blastema damaged. The blastema is crucial for the tail to regrow and, when it was damaged, I learned that the lizard tail will not regenerate. Throughout my summer research experience, I have learned many different techniques and tools for studying lizard tails. I have worked on many different machines and microscopes that have helped me with my project. Considering that I was the first person ever to study the effects of zoledronic acid on lizard tail regeneration, I am proud of what I have accomplished and look forward to learning more about tissue regeneration.

CONTACT US

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