Comparing Hedgehog-Responsive Chondrogenesis Between Lizard Tail and Mouse Digit Tip Blastemas

Zheyu Pan[12], Darian Gamble[12], Thomas Lozito[12]
[1]Department of Stem Cell Biology and Regenerative Medicine, Keck School of Medicine, University of Southern California CA,
[2]Department of Orthopaedic Surgery, Keck School of Medicine, University of Southern California CA
zheyupan@usc.edu

INTRODUCTION:
The two most well-established models of blastema-based appendage regeneration in amniotes are the lizard tail and mouse digit tip. Despite many similarities, lizard and mouse blastemas exhibit completely opposite skeletal differentiation capabilities; lizard tail blastemas differentiate into cartilage but not bone, while mouse digit blastemas form bone only. Both mouse and lizard blastema cells are derived from fibroblastic connective tissue cell (FCTC) populations. We have recently shown that lizard blastema FCTCs undergo chondrogenesis in response to Hedgehog (HH) stimulation [Vonk et al.]. Lizards treated with the HH agonist SAG express the pro-chondrogenic transcription factor Sox9, but not the pro-osteogenic factor RunX2. Here we test whether mouse blastema cells exhibit similar pro-chondrogenic programs in response to SAG treatment.

METHODS:
Histology and imaging: Lizard samples were performed as previous reported [Vonk et al.]. Mouse digit tip samples were performed in similar procedure but were decalcified for 2 weeks. Mouse cryosamples were sectioned at 12um
Drug treatment: For Hedgehog pathway signaling modulation, Mice were weighed and treated with smoothened agonist (SAG,40 μg/g) dosed per gram weight of the animal and administered via intraperitoneal (IP) injections every Monday. Wednesday and Friday. Control animals were treated with sterile water in place of drug treatments.
In situ hybridization: ISH was performed as previously reported [Vonk et al.].

RESULTS SECTION: Regenerating lizard (*Anolis carolinensis*) tails and mouse digit tips collected from animals treated with SAG or vehicle control (H_2O) 7, 14, 21, and 28 days post-amputation (DPA) were analyzed by histology/in situ hybridization (ISH) for expression of validated FCTC markers. Control tails and digits expressed Spp1 throughout all stages of regeneration, localized at proximal blastema regions. Control tails and digits also expressed blastema markers MEST and Sulf1, respectively, in distal regions. Control lizard tail, but not mouse digit tip, blastemas expressed Sox9 and Col2a1 in medial regions. SAG treatment induced cartilage formation in lizard, but not mouse, blastemas. Similarly, SAG treatment increased lizard, but not mouse, blastema expression of Sulf1, Sox9, and Col2a1. Similarly, Spp1 expression was not affected by SAG treatment in either mouse or lizard blastemas.

DISCUSSION: This work represents the first direct comparisons of lizard and mouse blastemas, and the first tests of the effects of HH stimulation on mouse bastema chondrogenesis. Mouse digit tip regeneration is distinguished from lizard tail regeneration by an unresponsiveness to HH signaling and an inability to form cartilage. Moreover, these results suggest that lizard tail and mouse digit tip represent two distinct classes of blastemas distinguished by differentiation potentials. Lizard blastema FCTCs are capable of differentiating into multiple connective tissue lineages, including cartilage, while mouse FCTCs are restricted to intramembranous ossification. Future work will characterize mouse digit tip blastemas through lineage traces and identify factors contributing to blastema fate trajectories.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): Cartilage restoration represents one of the most important areas of regenerative medicine given the prevalence of cartilage injuries, the lack of current therapies, and the minimal natural healing abilities of human cartilage. The abilities to differentiate fibroblasts into new, healthy cartilage and prevent heterotopic ossification represent profound therapeutic potentials.

REFERENCES: Vonk, A.C., Zhao, X., Pan, Z. *et al.* Single-cell analysis of lizard blastema fibroblasts reveals phagocyte-dependent activation of Hedgehog-responsive chondrogenesis. *Nat Commun* **14**, 4489 (2023)
Johnson GL, Masias EJ, Lehoczky JA. Cellular Heterogeneity and Lineage Restriction during Mouse Digit Tip Regeneration at Single-Cell Resolution. Dev Cell. 2020 Feb 24;52(4):525-540.e5.

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IMAGES AND TABLES: