A finger on the pulse of regeneration: Insights into the cellular mechanisms of adult digit tip regeneration

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ABSTRACT

In mammals, multi-tissue regeneration is largely restricted to the distal portion of the digit tip and involves the formation of a blastema, a transient, proliferating cell mass that reforms the diverse tissues of the digit. Historically little was known about the mammalian blastema but with recent advances in single cell transcriptomic approaches and genetic lineage tracing, a more precise understanding of this critical structure has begun to emerge. In this review we summarise the cellular mechanisms underlying adult mammalian digit tip regeneration. We posit that understanding how some mammals naturally regenerate complex tissues will lead to strategies for enhancing regenerative abilities in humans.

1. INTRODUCTION

The number of species that are capable of some form of regeneration is extensive. Some invertebrates, such as *Hydra* and planaria, display a remarkable capacity for regeneration, being able to regenerate entire organisms from a small fragment of tissue [reviewed in 1 and 2]. Among the vertebrates, urodele amphibians (newts and salamanders) are renowned for their ability to heal in a scar-free manner and to regenerate appendages, but not an entire organism. For example, axolotl salamanders and newts can regenerate limbs, tail, lower jaw, retina, brain and many other tissues [reviewed in 2 and 3]. Studying these highly regenerative species has revealed important insights into how complex, multi-tissue appendages can be regenerated. However, as these animals are quite divergent from humans, the translation of these findings into clinical applications remains limited.

In contrast to these regenerative organisms, if one were to ask whether mammals can regenerate, the answer would typically be no. In most cases, severe damage to mammalian tissues does not induce a regenerative response but rather leads to wound healing that culminates in fibrotic scarring. There are, however, several exceptions to this rule. Examples of mammalian regenerative responses include the shedding and regrowth of deer antlers [4,5], the closing of ear hole punches in rabbits and some rodents [6,7], skin regeneration in spiny mice [8], regeneration of the heart in postnatal mice and rats [9,10], and distal digit tip regeneration that occurs in rodents, monkeys and humans [11-14].

In this review we discuss the cellular mechanisms underlying adult mouse digit tip regeneration. This system is one of the best-characterized models of tissue regeneration in a mammal and is clinically relevant, mimicking the regenerative response observed in human fingertip injuries [14,15]. Notably, digit tip regeneration is level dependent; removal of the distal potion of the digit results in recovery of the digit to its original shape after approximately 28 days. Alternatively, amputations that occur more proximally or that remove the nail bed, fail to regenerate [15,16]. The mouse digit tip, therefore, provides a powerful model for studying the transition between wound healing and regeneration in the same animal.

2. ESSENTIALS OF REGENERATION – THE BLASTEMA

Regeneration is a unique response of the distal digit tip that occurs following injury. When the terminal phalanx is amputated, regeneration proceeds in a step-wise manner that involves a transition from mature tissue, through to a relatively undifferentiated, proliferative state, ultimately culminating in re-differentiation of cells to replicate the original structure [15,17]. The transient proliferative structure that mediates the regenerative response is called the blastema and is the critical feature that distinguishes tissues that can regenerate from those that result in fibrotic scarring. What then are the cell types that contribute to the blastema and where do they come from? Is the blastema a homogenous population of multipotent progenitors or a complex mixture of cells each determined to form a specific tissue? Are the cells in the distal digit tip inherently different from those that reside more proximally? Is the blastema in the digit tip equivalent to the developing limb bud? These are some of the key questions in regenerative biology and it is clear, from this perspective, that any attempt to stimulate regeneration and repair of limbs and other tissues in mammals requires a deeper understanding of how the blastema forms and functions at the injury site.

3. THE BUILDING BLOCKS OF THE BLASTEMA

3.1 Cells of the Blastema & Regenerative Potential

The diversity and regenerative potential of mammalian blastema cells has been under continual investigation for several decades. In principle the mammalian blastema could be comprised of a single cell type that has the ability to differentiate into any of the regenerating tissues. Alternatively, tissues of the digit could each supply distinct lineage-restricted progenitors to the blastema. In recent years the prevailing view has been the latter; that the blastema is a heterogeneous population of cells that remember where they came from and only regenerate cells for their tissue of origin. Indeed, studies using mouse genetic lineage tracing demonstrated that *Sp7*- or *Sox9*-marked skeletal progenitors in the digit tip contribute exclusively to the regenerating bone and periosteum [18,19]. Similarly, endothelial cells expressing *VE-cadherin* or *Tie2* only gave rise to endothelium [19], while Schwann cells marked by *Sox2* contributed solely to the regenerated glial lineage [20]. However, while these studies clearly demonstrate that the mammalian blastema is not homogenous, a precise description of the cell types

present in the blastema is lacking and the potential for lineage flexibility was only investigated in a limited number of cell types.

More recently, several studies have addressed these limitations using single-cell transcriptional profiling and genetic lineage tracing. Firstly, in 2020, both Storer and colleagues [21] and Johnson and colleagues [22] concurrently presented an unbiased description of the variety of different cell types that comprise the mammalian blastema. These cell types include vascular smooth muscle cells, pericytes, endothelial cells and lymphatic endothelium, macrophages, neutrophils, T-cells, monocytes, Schwann cells, pre-osteoclasts and numerous types of mesenchyme cells including osteoblasts [21,22]. These studies also provided support for the previous conclusion that, in spite of this heterogeneity, the majority of blastema cells were mesenchymal in nature [21,22]. Secondly, using genetic lineage tracing two independent studies provided evidence that not all blastema cells are pre-committed to generating specific tissue types. By taking advantage of the finding that many of the mesenchymal cells within the endoneurium of the nerve are neural crest-derived, Carr and colleagues [23] demonstrated that nerve mesenchymal cells contribute to bone and nerve formation during mammalian digit tip regeneration. Subsequently, Storer and colleagues [21] found that *Dmp1*-positive cells which reside in the bone and periosteum under homeostatic conditions, were able to switch cell fates during regeneration and contribute to dermis and bone in the regenerated digit tip. These results indicate that cells within the mammalian digit tip blastema are not as restricted in terms of their fate as previously thought and suggest that extrinsic cues in the local environment may, in part, specify the cell's ultimate contribution to the regenerated tissue. It will be important in the future to determine whether this lineage flexibility is limited to mesenchymal cells, as exemplified in these two studies, or if it occurs with other digit cell types as well.

3.2 Stem Cells or De-Differentiated Progenitors

In order to understand the origins of cells within the mammalian blastema, it is critical to understand whether they arise by expansion of tissue-specific progenitor cells and/or by dedifferentiation of more mature cells within local tissues. In this regard, tissue-specific progenitor cells have been reported in many adult mammalian tissues and shown to play important roles in repairing damaged muscle [24], bone [25] and skin [26]. Additionally, although less common, a growing number of studies have indicated

that when exposed to the appropriate signals, certain mammalian cell types, including Schwann cells [20,27] and epithelial cells [28], can be induced to dedifferentiate following injury. Which of these cellular mechanisms is responsible for generating the cells of the mammalian blastema? At present, the answer remains unclear. Computational analyses of transcriptomic data from two independent studies have reached somewhat opposing verdicts. In one study, cells in the uninjured and regenerating digit tips were found to be transcriptionally similar to the blastema cells, suggesting that these overlapping populations represent tissue-specific stem cells that expand to form the blastema [22]. By contrast, the second study demonstrated that blastema mesenchymal cells were distinct from the mesenchymal cells in the uninjured digit tip [21] leading to the conclusion that uninjured mesenchymal cells must somehow acquire a blastema phenotype, consistent with a dedifferentiation model. This latter conclusion was further strengthened by lineage-tracing experiments showing that uninjured bone cells that expressed *Dmp1*, a gene associated with a more mature bone cell phenotype, acquired a blastema transcriptional state after amputation and then contributed to dermis regeneration. One explanation for these apparent discrepancies in mechanisms responsible for generating the cells of the blastema may result from technical differences between the two studies. Each study used a different strain of inbred mice (FVB/NJ compared to C57 BL/6) of a different age (6 weeks of age compared to 8-12 weeks) and different methods, numbers of cells and timepoints for their analyses [21,22]. Nonetheless, it may well be that both mechanisms are important, with blastema cells being generated both by progenitors resident in the uninjured digit tip and by dedifferentiation of more biased mesenchymal lineage cells. Future studies with additional lineage tracing strategies and greater numbers of single cell transcriptomes will hopefully shed additional light on these apparent differences.

3.3 Cellular Sources of the Blastema

One of the fundamental questions surrounding digit tip regeneration involves the origin of the mesenchymal blastema cells. Do the blastema cells arise from tissues local to the site of the injury or perhaps from circulating cells in the bloodstream that can enter the damaged tissues? Parabiosis studies, where two genetically distinct mice are conjoined and acquire a common vascular system, showed that donor-derived cells made little to no contribution to the regenerated digit of the recipient mouse [19], indicating that the blastema cells are likely of local origin, similar to what is seen in other regenerative

species such as amphibians and zebrafish [29, 30]. Evidence also suggests that mammalian blastema cells are derived from the initial wound site. Following amputation of the distal digit tip, the remaining stump tissue undergoes histolysis, releasing cells into the local environment and, intriguingly, when this this period of bone degradation is enhanced, it results in blastemas of a larger size [31].

If the largely mesenchymal blastema is formed by local cells, then which mature tissues contribute? Following amputation of the distal digit, regeneration first involves a wound healing response that degrades the bone stump and results in a secondary amputation at a more proximal level and epidermal closure [17,32]. Although wound epidermis formation is the first observable event and is necessary for blastema formation and regeneration, the epidermis is not a source of mesenchymal blastema cells since lineage-tracing studies have shown that ectodermal and mesodermal-derived digit tip cells do not cross germ-line boundaries during regeneration [19]. It is also unlikely that the blastema cells originate from circulating or local blood cells, as indicated by both parabiosis and hematopoietic stem cell transplantation studies [19]. Finally, lineage tracing has shown that the blastema cells do not originate from the local blood vessel-associated vascular smooth muscle cells or pericytes [21]. Thus, by a process of elimination, the mesenchymal blastema must derive from local mesenchymal tissues such as the bone, bone marrow and/or dermis. Intriguingly, the blastema develops at the interface between the bone marrow cavity and the newly formed wound epidermis, suggesting that bone and bone-associated cells might be a major source of blastema cells. Indeed, genetic lineage tracing using *Dmp1,* which marks both osteoblasts and osteocytes, showed that 26% of cells in the adult murine blastema originate from bone-associated cells. These are most likely osteoblasts that have originated from the periosteum since depletion of this tissue but not the endosteum leads to deficits in digit tip regeneration [21].

Local digit nerves represent a second, more surprising source of blastema mesenchymal cells. While digit tip regeneration is known to be nerve-dependent [20,33], previous work has focused on the contribution of nerve-derived Schwann cells to successful regeneration [20]. However, a recent study by Carr and colleagues [23] showed that local injured nerves are a source of neural crest-derived mesenchymal cells that contribute to blastema formation and ultimately to bone regeneration. Thus, the

blastema cells apparently derive from local mesenchymal tissues such as the injured bone and nerves. A key remaining question then involves the potential blastema contribution made by the connective tissues of the digit tip, including the dermis, nail bed mesenchyme and bone marrow stroma, a particularly intriguing issue given the necessity of the nail bed for digit tip regeneration [34-36].

4. IS THE TIP DIFFERENT?

Many organisms experience changes in regenerative abilities throughout their lifespan. For example, studies have shown that early mouse limb buds that exhibit no overt signs of differentiation can regenerate following amputation *in vitro* [37] but that as the limb develops further regeneration becomes restricted to the distal portion of the digit tip [38]. These findings have led to the idea that the regenerative potential of the developing limb bud is linked to the cells themselves rather than to any systemic differences that may exist between embryonic and adult environments. If so, then does this mean that the distal portion of the digit tip is still able to regenerate because the cells selectively maintain their embryonic properties? Simply put, are the cells of the regenerative digit tip different from the cells that reside more proximally in the non-regenerative digit? The answer to both of these questions appears to be no. Recent studies utilising global transcriptomic approaches demonstrated that unlike amphibians [39], cells within the murine digit tip blastema do not closely resemble developing embryonic limb progenitors at the transcriptional level [21]. Instead, the blastema cells display a transcriptional profile that includes genes that are associated with tissue injury responses as well as those that are important for limb development [41]. Thus, mammalian digit tip regeneration occurs via a distinct adult mechanism rather than simply recapitulating development, suggesting that it may be the unique environment of the distal digit tip that allows for successful regeneration. Indeed, since both axolotls and mice are able to regenerate their extremities, albeit through different mechanisms, an unbiased comparison of single cell identities and/or the niche environment may provide insight into the broad conditions necessary for a successful regenerative response.

A related fundamental question involves the transcriptional state and nature of cells found in the regeneration incompetent regions of the digit tip. Why do cells form a blastema and regenerate following distal amputations, but simply repair the injured

digit stump with more proximal amputations? Insights into this question come from experiments transplanting cells from regeneration incompetent regions of the body into the amputated distal third of the digit. Remarkably, these cells were able to engraft, participate in blastema formation and ultimately contribute to the newly regenerated tissues of the digit [22, 41]. Additionally, recent studies have shown that even following non-regenerative amputations digit tip mesenchymal cells acquired some facets of a blastema transcriptional state [22, 42], suggesting that a regenerative response was initiated but was then aborted or circumvented. These findings suggest that a regenerative response is the default state, but that successful regeneration also requires an environment with the appropriate regeneration-specific cues [22, 42]. Together, this new research supports the concept that the blastema state is at least in part, determined by external environmental cues and raise the possibility that if we can identify the relevant cues we may be able to exogenously promote regeneration.

5. FUTURE PERSPECTIVES

If the ability to regenerate a human limb and/or even to promote regenerative repair rather than fibrotic scarring is to one day become a reality, we must first understand the difference between the remarkably regenerative distal digit tip and the majority of non-regenerative mammalian tissues. While this may seem like a daunting task, the last few years have yielded significant breakthroughs in our understanding of the cell populations and interactions necessary for mammalian blastema formation, the critical structure underlying digit tip regeneration. Moreover, these studies have led to the conclusion that, at least within the mesenchymal lineage, adult cells can initiate a regenerative response following injury, and that the difference between regeneration and fibrotic scarring may simply be the injury environment. These findings pave the way for future studies defining the regenerative environment. They also provide hope that we will ultimately be able to exogenously promote human regeneration and repair by altering the local environment, particularly in light of previous studies reporting fingertip regeneration in humans from childhood through to old age [13,14,43]. In the best-case scenario, this would involve adding a single factor that would galvanize a regenerative response, but such a factor has not yet been discovered and may well not exist. Instead, establishment and maintenance of a pro-regenerative response is likely to involve multiple parameters including, but not limited to, paracrine ligands, extracellular matrix composition and immune status. Ultimately, we may also need to

combine strategies manipulating the injury environment with transplanted cells to ensure a robust regenerative response. Nonetheless, we now understand that mammalian cells and tissues can regenerate under the right circumstances, and future research holds the promise of moving this conceptual framework into a therapeutic reality.

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REFERENCES & RECOMMENDED READING

1. Reddien PW: **The cellular and molecular basis for planarian regeneration.** *Cell* 2018, **175:** 327-345 https://doi.org/10.1016/j.cell.2018.09.021

2. Tanaka EM, Reddien PW: **The cellular basis for animal regeneration.** *Dev. Cell* 2011, **21:** 172-175 https://doi.org/10.1016/j.devcel.2011.06.016

3. Tanaka EM: **The Molecular and Cellular Choreography of Appendage Regeneration.** *Cell* 2016, **165:** 1598-1608 https://doi.org/10.1016/j.cell.2016.05.038

4. Goss RJ: **Experimental investigations of morphogenesis in the growing antler.** *J. Embryol. Exp. Morphol.* 1961, **9:** 342-354.

5. Wang D, Berg D, Ba H, Sun H, Wang Z, Li C: **Deer antler stem cells are a novel type of cells that sustain full regeneration of a mammalian organ – deer antler.** *Cell Death Dis.* 2019, **10***:* 443 https://doi.org/10.1038/s41419-019-1686-y

6. Williams-Boyce PK, Daniel JC Jr: **Regeneration of rabbit ear tissue.** *J. Exp Zool.* 1980, **212:** 243-253 https://doi.org/10.1002/jez.1402120211

7. Gawriluk TR, Simkin J, Thompson KL, Biswas SK, Clare-Salzler Z, Kimani JM, Kiama SG, Smith JJ, Ezenwa VO, Seifert AW: **Comparative analysis of ear-hole**

closure identifies epimorphic regeneration as a discrete trait in mammals. *Nature Commun.* 2016, **7:** 11164 https://doi.org/10.1038/ncomms11164

8. Seifert AW, Kiama SG, Seifert MG, Goheen JR, Palmer T M, Maden M: **Skin shedding and tissue regeneration in African spiny mice (Acomys).** *Nature* 2012, **489:** 561-565 https://doi.org/10.1038/nature11499

9. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA: **Transient regenerative potential of the neonatal mouse heart.** *Science* 2011, **331:** 1078-1080 https://doi.org/10.1126/science.1200708

10. Wang H, Paulsen JM, Hironaka EC, Shin HS, Farry JM, Thakore AD, Jung J, Lucian HJ, Eskandari A, Anilkumar S *et al*.: **Natural heart regeneration in a neonatal rat myocardial infarction model.** *Cells* 2020, **9:** 229 https://doi.org/10.3390/cells9010229

11. Borgens, RB: **Mice regrow the tips of their foretoes**. *Science* 1982, **217:** 747-750. https://doi.org/10.1126/science.7100922

12. Singer M, Weckesser EC, Geraudie J, Maier CE, Singer, J: **Open finger tip healing and replacement after distal amputation in rhesus monkey with comparison to limb regeneration in lower vertebrates.** *Anat. Embryol.* 1987,**177:** 29-36 https://doi.org/10.1007/BF00325287

13. Illingworth, CM: 1974 **Trapped fingers and amputated finger tips in children.** *J. Pediatr. Surg.* 1974, **9:** 853-858 https://doi.org/10.1016/S0022-3468(74)80220-4

14. Miller TJ, Deptula PL, Buncke GM, Maan ZN: **Digit Tip Injuries: Current Treatment and Future Regenerative Paradigms.** *Stem Cells Int.* 2019, **9619080** http://doi.org/10.1155/2019/9619080

15. Simkin J, Han M, Yu L, Yan M, Muneoka K: **The mouse digit tip: from wound healing to regeneration.** *Methods Mol. Biol.* 2013, **1037:** 419-435 https://doi.org/10.1007/978-1-62703-505-7_24

16. Chamberlain CS, Jeffery JJ, Leiferman EM, Yildirim T, Sun X, Baer GS, Murphy WL, Vanderby R: **Level-specific amputations and resulting regenerative outcomes in the mouse distal phalanx.** *Wound Repair Regen.* 2017, **25:** 443-453 https://doi.org/10.1111/wrr.12544

17. Fernando WA, Leininger E, Simkin J, Li N, Malcom CA, Sathyamoorthi S, Han M, Muneoka K: **Wound healing and blastema formation in regenerating digit tips of adult mice.** *Dev. Biol.* 2011, **350:** 301–310 https://doi.org/10.1016/j.ydbio.2010.11.035

18. Lehoczky JA, Robert B, Tabin CJ: **Mouse digit tip regeneration is mediated by fate-restricted progenitor cells.** *Proc. Natl. Acad. Sci. USA*. 2011, **108:** 20609–20614 https://doi.org/10.1073/pnas.1118017108

19. Rinkevich Y, Lindau P, Ueno H, Longaker MT, Weissman IL: **Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip**. *Nature* 2011, **476:** 409–413 https://doi.org/10.1038/nature10346

20. Johnston AP, Yuzwa SA, Carr MJ, Mahmud N, Storer MA, Krause MP, Jones K, Paul S, Kaplan DR, Miller FD: **Dedifferentiated Schwann cell precursors secreting paracrine factors are required for regeneration of the mammalian digit tip.** *Cell Stem Cell* 2016, **19:** 433–448 https://doi.org/10.1016/j.stem.2016.06.002

21. Storer MA, Mahmud N, Karamboulas K, Borrett MJ, Yuzwa SA, Gont A, Androschuk A, Sefton MV, Kaplan DR, Miller FD: **Acquisition of a Unique Mesenchymal Precursor-like Blastema State Underlies Successful Adult Mammalian Digit Tip Regeneration.** *Dev. Cell* 2020, **52:** 1-16 https://doi.org/10.1016/j.devcel.2019.12.004
** This is one of the first molecular characterisations of the adult mammalian digit tip blastema at single cell resolution and this paper demonstrates that cells of the bone are also able to contribute to dermal tissue in the newly regenerated digit tip.

22. Johnson GL, Masias EJ, Lehoczky JA: **Cellular Heterogeneity and Lineage Restriction during Mouse Digit Tip Regeneration at Single-Cell Resolution.** *Dev. Cell* 2020, **52:** 525-540 https://doi.org/10.1016/j.devcel.2020.01.026
** This is one of the first molecular characterisations of the adult mammalian digit tip blastema at single cell resolution.

23. Carr MJ, Toma JS, Johnston APW, Steadman PE, Yuzwa SA, Mahmud N, Frankland PW, Kaplan DR, Miller FD: **Mesenchymal Precursor Cells in Adult Nerves Contribute to Mammalian Tissue Repair and Regeneration.** *Cell Stem Cell* 2019, **24:** 240-256 https://doi.org/10.1016/j.stem.2018.10.024
** This is the first paper to demonstrate that neural crest derived mesenchymal cells that reside in nerves are an important source of cells for the mammalian digit tip blastema.

24. Lepper C, Partridge TA, Fan C-M: **An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration.** *Development* 2011, **138:** 3639–3646 https://doi.org/10.1242/dev.067595

25. Dawson LA, Simkin J, Sauque M, Pela M, Palkowski T, Muneoka K: **Analogous cellular contribution and healing mechanisms following digit amputation and phalangeal fracture in mice.** *Regeneration* 2016, **3:** 39-51 https://doi.org/10.1002/reg2.51

26. Aragona M, Dekoninck S, Rulands S, Lenglez S, Mascre G, Simons BD, Blanpain C: **Defining stem cell dynamics and migration during wound healing in mouse skin epidermis.** *Nat Commun.* 2017, **8:** 14684 https://doi.org/10.1038/ncomms14684

27. Clements MP, Byrne E, Camarillo Guerrero LF, Cattin AL, Zakka L, Ashraf A, Burden JJ, Khadayate S, Lloyd AC, Marguerat S, Parrinello S: **The Wound Microenvironment Reprograms Schwann Cells to Invasive Mesenchymal-like Cells to Drive Peripheral Nerve Regeneration.** *Neuron* 2017, **96:** 98-114.e7 https://doi.org/10.1016/j.neuron.2017.09.008

28. Fu XB, Sun XQ, Li XK, Sheng ZY: **Dedifferentiation of epidermal cells to stem cells *in vivo.*** *The Lancet* 2001, **358:** 1067-1068 https://doi.org/10.1016/S0140-6736(01)06202-X

29. Currie JD, Kawaguchi A, Traspas RM, Schuez M, Chara O, Tanaka EM: **Live Imaging of Axolotl Digit Regeneration Reveals Spatiotemporal Choreography of Diverse Connective Tissue Progenitor Pools**. *Dev. Cell* 2016 **39:** 411-423 https://doi:10.1016/j.devcel.2016.10.013

30. Tu S, Johnson SL: **Fate restriction in the growing and regenerating zebrafish fin.** *Dev. Cell* 2011, **20:** 725-732 https://doi:10.1016/j.devcel.2011.04.013

31. Sammarco MC, Simkin J, Cammack AJ, Fassler D, Gossmann A, Marrero L, Lacey M, Van Meter K, Muneoka K: **Hyperbaric oxygen promotes proximal bone regeneration and organized collagen composition during digit regeneration**. *PLoS ONE* 2015, **10:** e0140156 https://doi.org/10.1371/journal.pone.0140156

32. Simkin J, Sammarco MC, Dawson LA, Tucker C, Taylor LJ, Van Meter K, Muneoka K: **Epidermal closure regulates histolysis during mammalian (*Mus*) digit regeneration.** *Regeneration* 2015, **2:** 106 – 119 https://doi.org/10.1002/reg2.34

33. Rinkevich Y, Montoro DT, Muhonen E, Walmsley GG, Lo D, Hasegawa M, Januszyk M, Connolly AJ, Weissman IL, Longaker MT: **Clonal analysis reveals nerve-dependent and independent roles on mammalian hind limb tissue maintenance and regeneration.** *Proc Natl Acad Sci U S A* 2014, **111:** 9846-51 https://doi.org/10.1073/pnas.1410097111

34. Takeo M, Chou WC, Sun Q, Lee W, Rabbani P, Loomis C, Taketo MM, Ito M: **Wnt activation in nail epithelium couples nail growth to digit regeneration.** *Nature* 2013, **499:** 228–232 https://doi.org/10.1038/nature12214

35. Lehoczky JA, Tabin CJ: **Lgr6 marks nail stem cells and is required for digit tip regeneration.** *Proc Natl Acad Sci U S A* 2015, **112:** 13249-54 https://doi.org/10.1073/pnas.1518874112

36. Yu Y, Cui H, Zhang C, Zhang D, Yin J, Wen G, Chai Y: **Human nail bed extracellular matrix facilitates bone regeneration via macrophage polarization mediated by the JAK2/STAT3 pathway.** *J Mater Chem B.* 2020, **8:** 4067-4079 https://doi.org/10.1039/c9tb02910a

37. Lee KK, Chan WY: **A study on the regenerative potential of partially excised mouse embryonic fore-limb bud.** *Anat Embryol (Berl)* 1991, **184:**153-7 https://doi.org/10.1007/BF00942746

38. Reginelli AD, Wang YQ, Sassoon D, Muneoka K: Digit tip regeneration correlates with regions of Msx1 (Hox7) expression in fetal and newborn mice. *Development* 1995, **121:** 1056-1076

39. Gerber T, Murawala P, Knapp D, Masselink W, Schuez M, Hermann S, Gac-Santel M, Nowoshilow S, Kageyama J, Khattak S, Currie JD, Camp JG, Tanaka EM, Treutlein B: **Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration.** *Science* 2018, **362:**eaaq0681 https://doi.org/10.1126/science.aaq0681
** This paper uses single-cell transcriptomics together with Brainbow-based lineage tracing to demonstrate that the blastema is formed from fibroblasts that de-differentiate to form multi-potent skeletal progenitors during axolotl limb regeneration.

40. Qu F, Palte IC, Gontarz PM, Zhang B, Guilak F: **Transcriptomic analysis of bone and fibrous tissue morphogenesis during digit tip regeneration in the adult mouse.** *FASEB J.* 2020, **34:**9740-9754 https://www.doi.org/10.1096/fj.202000330R

41. Wu Y, Wang K, Karapetyan A, Fernando WA, Simkin J, Han M, Rugg EL, Muneoka K: **Connective tissue fibroblast properties are position-dependent during mouse digit tip regeneration.** *PloS One* 2013, **8:**e54764. https://doi.org/10.1371/journal.pone.0054764

42. Dawson LA, Schanes PP, Marrero L, Jordan K, Brunauer R, Zimmel KN, Qureshi O, Imholt FM, Falck AR, Yan M, Dolan CP, Yu L, Muneoka K: **Proximal digit tip amputation initiates simultaneous blastema and transient fibrosis formation and results in partial regeneration.** *Wound Repair Regen.* 2021, **29:**196-205 https://doi.org/10.1111/wrr.12856
* This paper demonstrates that amputations proximal to the nail bed result in partial regeneration that is characterised by an absence of periosteal cell contribution.

43. Lee LP, Lau PY, Chan CW: **A simple and efficient treatment for fingertip injuries.** *J. Hand Surg. Br.* 1995, **20:**63-71 https://doi.org/10.1016/S0266-7681(05)80019-1

FIGURE LEGEND

Figure 1. ***Summary of the cellular mechanisms that underlie mammalian digit tip blastema formation.*** Top Panel: The nerve and bone have been shown to contribute cells that form the mammalian digit tip blastema following injury. Middle Panel: At early stages, regeneration of the adult mammalian digit tip occurs through mechanisms that are distinct from embryonic limb development which include wound healing and blastema formation. The latter stages of regeneration however utilise many molecular pathways important for limb development. Bottom Panel: Potential mechanisms for producing new cells that contribute to the blastema include the activation of stem cells and de-differentiation of mature cells to produce dividing cells that display progenitor like properties.