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Commentary

Single-cell revolution unveils the mysteries of the regenerative mammalian digit tip

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ABSTRACT

The digit tip is an exciting model for studying regeneration in mammals, but the precise mechanisms and the populations of cells involved in the formation and remodeling of the blastema remain unknown. In an exciting new work, Storer et al. take advantage of single-cell RNAseq combined with Pdgfra+ lineage-tracing to open the way into the enigmatic world of mammalian tissue regeneration.

Regeneration remains one of nature's most exciting mysteries. Several organisms can regenerate diverse parts of their bodies with different efficiencies. Although important progress in the understanding of this phenomenon has been made in the last decades, the precise spatio-temporal cellular dynamics of the regenerative response remains an unresolved puzzle. Interestingly, mammals present a particular regenerative paradigm, as they are able to robustly regenerate tissues such as blood and intestinal epithelium, and digits to a limited extent, but are unable to regenerate a host of other tissues. Digit tip regeneration is complex and involves a series of steps such as a wound response, formation of a blastema and reestablishment of the missing tissue, resembling the epimorphic regeneration previously observed in non-mammal organisms.

Historically, we have learned much of the requirements for appendage regeneration from axolotls. Both axolotls and mammals depend on the formation and maturation of a blastema for successful multi-tissue regeneration following damage. The blastema is a transitional structure that contains all the signaling cues, in addition to progenitor and descendant cells required for successful regeneration. Among the several cell types and tissues that participate in appendage regeneration, the connective tissue (CT) plays a critical role by providing many blastema-resident cells and by harboring the positional identity of limbs (Reviewed in Tanaka, 2016). CTs are defined not only by their location and arrangement of collagenous and elastic fibers but also by their distinct functions. Stromal mesenchymal progenitors (MPs) synthesize the extracellular matrix of connective tissues and play an essential role in modeling and remodeling the structural integrity of tissues in health and disease (Lemos and Duffield, 2018; Uezumi et al., 2011). MPs display functional specialization according to their tissue of origin, body site, and precise spatio-temporal location. Researchers have long suspected that one type of MP may be converted into or replaced by another, because of their closely related functions and derivation from a common type of ancestral cell. Hence, both the cellular plasticity and heterogeneity exhibited by MPs are of particular interest given their requirements for wound healing and regeneration.

A key marker of MPs is platelet-derived growth factor receptor α (PDGFRα) (Contreras et al., 2019a; Farahani and Xaymardan, 2015; Uezumi et al., 2010), whose activation triggers several cellular responses such as migration, differentiation, and proliferation of CT cells (Contreras et al., 2019a,b; Hamilton et al., 2003). Thus, it is not surprising that connective tissue-derived PDGF signaling is essential for the formation of the blastema during axolotl limb regeneration (Currie et al., 2016).

Are mesenchymal progenitors required for mammalian digit tip regeneration? Previous work by Johnston et al., 2016 and Carr et al.,

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2019 showed the relevance of Pdgfra+ MPs to mammalian tissue repair and regeneration. Following digit tip amputation, nerve-derived mesenchymal progenitors directly promote regeneration and repair of injured bone and skin tissues. However, there are still open questions about the origin, behavior, plasticity, and heterogeneity of those CT stem/progenitor cells during regeneration.

In this context, Storer et al., 2020 took full advantage of scRNA-seq and lineage tracing to establish the transcriptional identity and lineage trajectories of tissue-resident MPs during the regenerative response, shedding lights on the role of MPs at unprecedented resolution. First, the authors showed that Pdgfra+ progenitors, rather than Pdgfra- cells, form the blastema, and therefore, represent most of the cellular portion at 14 days post-amputation (DPA). Second, their progeny locates to the regenerating dermis, bone marrow stroma, and bone matrix at 28 DPA, suggesting that the blastema originates from MPs. Third, by ablating Pdgfra+ cells, the authors observed an impaired digit tip regeneration, confirming the importance of these cells for regeneration (Fig. 1).

Using scRNA-seq, the authors profiled and characterized all the populations of cells found in both uninjured and regenerating digit tip. Among the different groups identified, the authors found that Pdgfra+ MPs were segregated from other mesenchymal populations, and therefore, represented different sub-populations. Remarkably, the gene signature of regenerating Pdgfra+ MPs is heterogeneous and progresses over time after amputation. The team established that MPs acquire a unique plastic transcriptome that changes as the blastema forms, mature, and resolves. A key question in regenerative and developmental biology is whether regeneration recapitulates development, as it was recently reported for axolotl limb (Gerber et al., 2018). To further characterized the developmental-related gene signatures of the mesenchymal compartment and to address this question, the authors sampled the developing E11 hindlimbs, E14 digits, and P3 digit tips, and compared them with blastema scRNA-seq data sets. These analyses indicated that MPs express mesenchymal-related developmental genes during regeneration, but this transcriptional profile is not a complete recapitulation of a developmental state, providing evidence that MPs do not acquire an embryonic state upon digit amputation.

The mouse digit provides an exciting model to understand the cues involved in a pro-regenerative response since in the same structure, different planes of amputation result in successful regeneration versus just wound healing, respectively. The authors compared the transcriptional changes of Pdgfra+ cells during regeneration with those during a non-regenerative amputation, achieved by the complete removal of the nail bed. Interestingly, they found that Pdgfra+ cells from a non-regenerative environment partially expressed blastema-associated genes, suggesting that they might be primed to take part in regeneration but lack the decisive cues that would reveal their broader lineage potential (Fig. 1). The authors hypothesized that the environment plays a role in the induction of regeneration and by transplanting Pdgfra+ neonatal dermal fibroblasts into a regenerative or a non-regenerative amputated digit, they observed that those cells from the regenerative environment contributed to blastema formation, while those from the non-regenerative environment contributed only to formation of a stump. Therefore, the environment in which these cells are activated is critical for the regenerative response. Another possibility to test would be whether MPs from a non-regenerative context become intrinsically suppressed even when regenerative cues are present. In this context, an interesting experiment would be to isolate MPs from a proximal digit tip location (e.g. second phalanx) where amputations fail to regenerate, and transplant them into a regenerative blastema. Would they be able to contribute to the blastema as efficiently as regeneration-competent MPs from the terminal phalanx? This would corroborate the existence of distinct innate positional characteristics of connective tissue MPs that command their regenerative capabilities (Wu et al., 2013). Further studies should also focus on the mechanisms by which mesenchymal progenitor heterogeneity arises, including lineage restriction by transcriptional regulatory networks and epigenetic factors in combination with extrinsic effects of the spatial context within a tissue.

The study of Storer et al., 2020 unravels exciting new details on the requirements and plasticity of Pdgfra+ MPs for proper tissue regeneration in mammals. The importance of these cells has gained increasing attention as growing evidence of their active participation in the regeneration and repair of most tissues is revealed (Carr et al., 2019; Contreras et al., 2019b; Harvey et al., 2019; Malecova et al., 2018; Wosczyna et al., 2019; Lemos and Duffield, 2018; Uezumi et al., 2010). However, there are still open questions that need to be addressed. What are the cues that the environment provides, and which cells are producing them? The fact that MPs that participate in regeneration don't resemble their counterparts in development, might be one reason for the reduced regenerative capabilities from mammals compared to axolotls. The potential of connective tissue MPs to participate in regeneration remains underexplored,

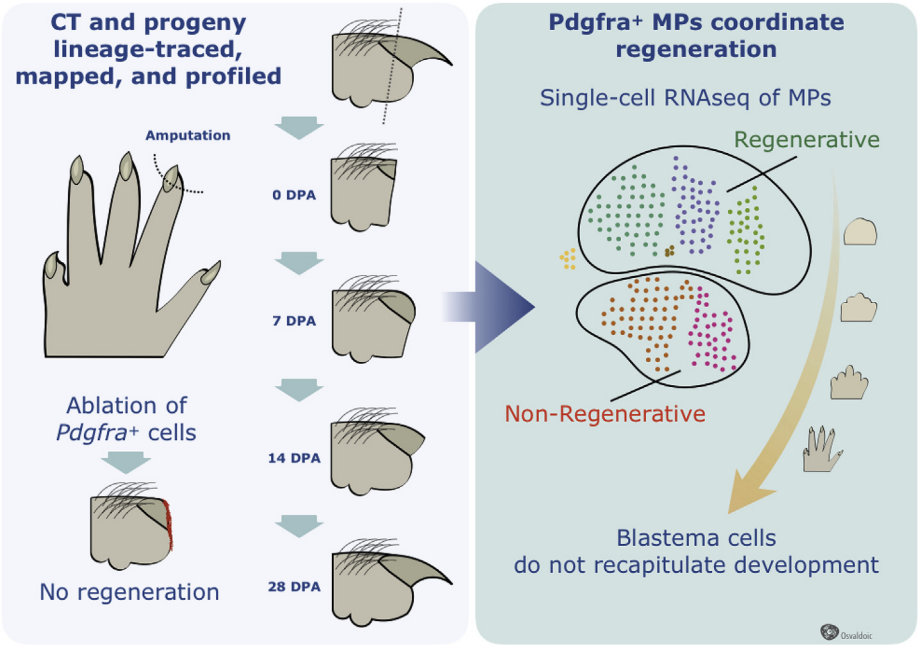

Fig. 1. Mapping MPs Cell Lineage, Identity, and Plasticity at Single-Cell Resolution in the Regenerating Digit Tip.
Left: Digit tip regenerates post-amputation. Pdgfra+ CT cells are labeled using the inducible Cre-loxP system and lineage traced via TdTomato expression. Pdgfra+ progenitors and their derivatives were isolated and profiled via scRNA-seq at different DPA. These experiments demonstrated that, independently of their origin, Pdgfra+ cells repopulated the blastema and are required for appendage regeneration. Right: scRNA-seq approach to map the cell trajectories based on the gene signatures of single MPs following damage and during digit development. These experiments demonstrated that a permissive regenerative blastema, but not non-regenerative fibroblasts, is what primed these cells to regenerate. Comparisons between digit development and regeneration in mammals suggest that blastema progenitor cells do not recapitulate development.

and thus more research about the extrinsic and intrinsic mechanisms regulating their plasticity, heterogeneity, and behavior is needed. The Storer et al., 2020 study points to a future where we will be able to translate that knowledge into efficient medical applications.

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