Tail regeneration in the Tokay Gecko (Gekko gecko)

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Chapter 4. Single Cell Sequence Analysis of Tokay Gecko Tail Regeneration

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Abstract

Regeneration is the replacement of missing or lost tissue with a functional copy. The capacity for regeneration in different species is influenced by various factors including the characteristics of the stem cells involved. In axolotl limb and zebrafish caudal fin regeneration, there is activation of developmental patterning genes in the regeneration blastema (cell mass). The properties of the blastema are in some respects similar to an embryonic limb bud or tailbud. In this study, we examined whether the tail regeneration blastema in the adult tokay gecko involves similar cell populations and programming as in embryonic tail development. We used single-cell mRNA sequencing of the regeneration blastemata of the adult tokay gecko tail (16, 20, 24 and 28 days post-autotomy) and compared it with the embryonic tailbud (3 and 7 days post-oviposition). Our analysis reveals differences in the cell populations of the regeneration blastema and the embryonic tailbud/tail. The regeneration blastema is dominated by fibroblasts from 16 days post-amputation onwards, whereas the tailbud shows a substantial population of pluripotent stem cells. In the regeneration blastema, RNA velocity analysis estimated that stromal cells (resident stem cells) and fibroblasts were the initial state, while the terminal states predominantly developed within their own lineages. Notably, keratinocytes only developed from their own cluster. By contrast, in the embryonic tailbud/tail, we estimated mesenchymal cells to be the initial state, with high probabilities of becoming chondrocytes and myocytes, medium-to-low probabilities of becoming endothelial cells, and very low probabilities of becoming nerve cells. In summary, our findings suggest that gecko tail regeneration predominantly relies on the activation of resident stem cells in the uninjured tissue, rather than on dedifferentiation. This contrasts with the blastema-based regeneration in the zebrafish and many urodeles, and is more similar to regeneration in mammals, both of which primarily involve activation of resident stem cells.

Introduction

Regeneration is the replacement of missing or lost tissue with a functional copy. It is important as a one of the survival strategies used by animals to deal with injuries (Bely and Nyberg, 2010; Gurtner et al., 2008). It is seen at various levels of biological organization, ranging from the cell to the whole body, and can be induced at different stages of the lifecycle (Bely and Nyberg, 2010). It involves a program of cell-proliferation, pattern formation and differentiation after injury (Ricci and Srivastava, 2018; Tanaka and Reddien, 2011).

It is important to study the similarities and differences in regeneration in different species because, for example, it may help us understand why mammals, including humans, have such a limited regeneration capacity (Alibardi, 2010; Daponte et al., 2021; Zhao et al., 2016). Indeed, one goal of regeneration research is to translate this knowledge to regenerative medicine (Ahmed et al., 2014; Oviedo and Beane, 2009; Stocum, 2006).

Regeneration is dependent on stem cells (Poss, 2010; Ricci and Srivastava, 2018; Tanaka and Reddien, 2011). Species differences in the availability or source of stem cells might partly explain the differing regenerative capacity of different species (Tanaka and Reddien, 2011; Zhao et al., 2016). Other factors possible influencing regeneration capacity include the characteristics of the stem cells involved, the potential for dedifferentiation and transdifferentiation, the expression patterns of genes associated with regeneration, the presence of epigenetic regulators, and immune system responses (Zhao et al., 2016).

Regeneration in the vertebrates is more limited compared to that of invertebrates such as *Hydra* and planarians. In humans, for example, the capacity for true regeneration is limited to the fingertips, and then only takes place when the nail bed is preserved (Gang and Lenghi, 1982; Narayanan, 2015; Tang et al., 2014). The liver in humans and other mammals can restore lost parts, but some researchers regard this as physiological size regulation rather than true regeneration (Michalopoulos, 2007; Wang et al., 2019b). Some vertebrates, however, do show an impressive capacity for regenerations. These include teleosts such as the zebrafish (Pfefferli and Jazwinska, 2015), Lissamphibia such as salamanders (Joven et al., 2019) and the axolotl, (Polikarpova et al., 2022) and Squamata such as lizards (Alibardi, 2014; Alibardi, 2018; Li et al., 2015)

Interestingly, in the axolotl limb (Gardiner et al., 1995; Gerber et al., 2018; Kragl et al., 2009; Muneoka and Bryant, 1982; Muneoka and Bryant, 1984) and the zebrafish caudal fin (Pfefferli and Jazwinska, 2015), regeneration bears a number of similarities to the embryonic development of those same structures. For example, an entire suite of

developmental patterning genes is activated in the regeneration blastema (Blum and Begemann, 2012; Gerber et al., 2018; Pfefferli and Jazwinska, 2015; Sinclair et al., 2021). Furthermore, the tissue organization of the blastema is similar to an embryonic limb bud or tailbud. Thus, the early blastema is small, and is composed of a mass of undifferentiated cells capped by a wound epithelium. This is similar to the embryonic limb bud in which there is a 'progress zone' of undifferentiated mesenchymal cells capped by an apical ectodermal ridge according to some models (Tickle, 2015; Vargesson, 2020). The embryonic tailbud has a simlar structure (Goldman et al., 2000). In the green anole, some developmental pathways are re-activated during tail regeneration, but are not expressed in a growth zone. Rather, they are distributed throughout the blastema (Hutchins et al., 2014). Furthermore, the regeneration blastema in lizards is relatively large compared to that in the axolotl and zebrafish.

In this thesis, we have studied the tokay gecko (*Gekko gecko*). This is one of many lizard species which shows caudal autotomy. This process is the spontaneous shedding of the tail when the animal is attacked by a predator. The tail fragment may continue to twitch after it is shed, distracting the predator and allowing the gecko to escape (Rumping and Jayne, 1996). After the tail has been shed by autotomy, the tail undergoes regeneration.

The normal (unregenerated) tail of the tokay gecko consists of structures including the spinal cord, caudal vertebrae, blood vessels, skeletal muscles, connective tissue and adipose tissue, all covered by the epidermis. By contrast, the regenerated lizard tail (RLT) is a non-identical, but functional, replica of the original (Alibardi, 2014; McLean and Vickaryous, 2011). The differences found in the RLT compared with the original tail are: (1) the axial skeleton is replaced by a continuous cartilage tube; (2) there is a lack of true tendons, presumably due to the lack of vertebrae for them to attach to; (3) the spinal cord is replaced by the ependymal cells and their covering of meninges; (4) the pattern of scales (scalation) is slightly different and the scales are smaller (Higham et al., 2013; Nurhidayat et al., 2020a; Nurhidayat et al., 2020b).

In this chapter, we have examined the question of whether the tail regeneration blastema in the adult tokay gecko contains a similar cell population as the embryonic tailbud. We examine this question using single cell RNA sequencing (scRNA-seq). We apply RNA velocity analysis to the data in order to estimate the cell fate probabilities.

Material and Methods

Ethics statement

The tokay gecko (*Gekko gecko*) is listed as 'least concern' in the International Union for Conservation of Nature *Red List* (IUCN, https://www.iucnredlist.org/species/195309/2378260) and also listed in Appendix II in

the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; https://cites.org/eng/taxonomy/term/3919). In Indonesia, the tokay gecko is not a protected species. All animals were collected in Yogyakarta, Indonesia under a license issued by the Ministry of Environment and Forestry, the Republic of Indonesia (permit number SK.83/KSDAE/SET/KSA.2/5/2021 signed on 7 May 2021) and a recommendation letter issued by The Indonesian Institute of Sciences (Lembaga Ilmu Pengetahuan Indonesia/LIPI; Recommendation Number: B- 2158 /IV/KS.01.04/3/2021 signed on 19 March 2021). The transport of samples from Indonesia to the Netherlands was conducted under CITES permit numbers 05717/IV/SATS-LN/2021 and 09160/IV/SATS-LN/2022 issued by the Ministry of Environment and Forestry, the Republic of Indonesia.

All experimental and surgical procedures needed for samples collection were carried out at Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia with the approval from The Ethical Committee of the Integrated Laboratory for Research and Testing (Laboratorium Penelitian dan Pengujian Terpadu/LPPT) Universitas Gadjah Mada (Ethical Clearance number: 00014/04/LPPT/IV/2021 signed on 30 April 2021).

Experimentation and sample collection

Regenerated tail samples

Twenty-one adult tokay geckos were anaesthetized with intramuscular ketamine injection (50–75 mg/Kg body weight) administered in the thigh prior to the autotomy procedure. Autotomy was performed by gently restraining the gecko manually, while leaving the tail accessible. Using the thumb and index finger, a firm pinch was applied to the required autotomous region (every segment after the 5th caudal vertebra) of the tail causing the gecko to spontaneously shed its tail. The wound was painted with Betadine® iodine antiseptic solution but no dressing was applied. After the autotomy procedure was performed, the geckos were allowed to regenerate a new tail. The geckos were distributed into experimental groups based on the number of days post-autotomy, each consisting of three geckos. After ketamine anesthesia, the tails of geckos in each group were re-autotomized 2 cm cranially from the previous autotomy site at 16, 20, 24, and 28 days post-autotomy (dpa). The regenerated tail samples were washed and further handled in phosphate-buffered saline (PBS, 4 °C) and subsequently processed with tissue dissociation protocols.

Embryo samples

Eighteen adult tokay geckos were grouped and bred as described above. The terraria were checked daily for the presence of gecko eggs. The eggs were left in the terrarium for the required number of days of incubation at ambient temperature (which in Yogyakarta ranged from 24–26 °C). The embryos were then harvested at 3 days post-oviposition (dpo) for the tailbud, and 7 dpo for the embryonic tail (we refer to the later

sample as embryonic tail because it was more extensive than the tailbud alone). The tailbuds and embryonic tail were then cut off and transferred to cold PBS and subsequently processed for tissue dissociation (see below).

Tissue dissociation and ACME Fixation

Samples were cut into pieces and were transferred into 15 mL tubes containing cold 1% trypsin (Sigma Aldrich®; T7409) 1mM EDTA (380 mg/L) in Hanks' Balanced Salt solution (Sigma Aldrich®; H9394), which was without magnesium and calcium, with phenol red. Samples were then incubated for 90–120 min on a shaker at 200 rpm with trituration every 30 minutes. The samples were then centrifuged at 440 RCF (relative centrifugal force) which equates to 16 radian/minute, at 4 °C for 5 minutes and the supernatant was then carefully discarded. Acetic-methanol (ACME) solution was made according to (Garcia-Castro et al., 2021). ACME solution without methanol (8.5 mL) was added to the tube. After 20 min incubation, 1.5 mL of methanol was then added to each tube and incubated for another 20 min. The dissociated tissue of all samples was then strained through a 70 µm Corning® cell strainer (cat. 431751). The cell suspensions were then centrifuged at 440 RCF at 4 °C for 5 min and the supernatant was then discarded. Bovine serum albumin (BSA; 1% in PBS) was added to resuspend and wash the cells and they were subsequently recentrifuged at 440 RCF at 4 °C for 5 min. One and a half mL of 1% BSA in PBS was then added to resuspend the cells. About 100 µL of dimethyl sulfoxide (DMSO) was added to each tube containing the cell suspension, and the tubes then snap-frozen with liquid nitrogen. They were then stored at −80 °C until being shipped on dry ice.

Single-cell RNA sequencing Analysis

The single-cell library preparation was done by the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany using 10X genomics v3 (28bp). The sequencing was done using Illumina NextSeq 2000 with a read length of 2×50 bp.

Gene expression data were obtained using Cell Ranger v. 6.1.2 pipelines (https://www.10xgenomics.com/support/software/cell-ranger/latest) carried out within the computing resources of the Academic Leiden Interdisciplinary Cluster Environment (ALICE) of Leiden University. The genome of Townsend's least gecko *Sphaerodactylus townsendi* (GCF_021028975.2) was used as a custom genome reference by utilizing 'cellranger mkref' pipeline. The transcript quantification and gene expression data matrix were produced by using 'cellranger count' pipeline.

Seurat5 v. 5.0.0 (https://satijalab.org/seurat/articles/install.html) was used to analyze the scRNA-seq sample datasets, including standard quality control and pre-processing, data integration applying the reciprocal principle component analysis (RPCA) approach, data normalization, linear dimensional reduction, cells clustering, non-linear

dimensional reduction, differential gene expression (marker genes determination), and cell type assignment (Hao et al., 2024). Cell type assignment was done manually by curating the cell type prediction results with the clustermole package (https://igordot.github.io/clustermole/) and SCtype pipeline (Ianevski et al., 2022). RNA velocity was determined using Velocyto and analyzed with Cellrank (Lange et al., 2022).

Results

Different cell populations in the regeneration blastema and embryonic the tailbud

To test the hypothesis that adult regeneration is based on the reactivation of embryonic developmental pathways, we compared cell populations in the regenerating and embryonic tails. We used single-cell RNA sequencing (scRNA-seq) on the following tissue samples: embryonic tailbud/tail (3 dpo and 7 dpo), and regenerating tail blastemata (16, 20, 24 and 28 dpa; (Figure 4-1A; all samples *N*=1). Note that we sampled the whole regeneration blastema (Figure 4-1A). After data preprocessing, data integration, cell clustering and semi-automatic cell type assignment, we identified 13 cell clusters (Figure 4-1B) based on the differential expression of the panel of genes shown in Figure 4-2.

Interestingly, the embryonic tailbud/tail did not, for the most part, cluster with the regenerating tail blastema, even though there are some common cell clusters (Figure 4-1C). These common clusters shared by both the tailbud/tail and the blastema were chondrocytes, endothelial cells, erythroid cells and myocytes. Cell clusters unique to the embryonic tailbud/tail samples were tailbud mesenchymal cells, neuroblast, neurons and mesenchymal stem cells. Clusters unique to the regeneration blastema, on the other hand, were fibroblasts, keratinocytes, stromal cells, macrophages and myoblasts. Note that we have named the pluripotent stem cell cluster in the embryonic tailbud/tail as ‘tailbud mesenchymal cells’ at 3 dpo and as ‘mesenchymal stem cells’ at 7 dpo.

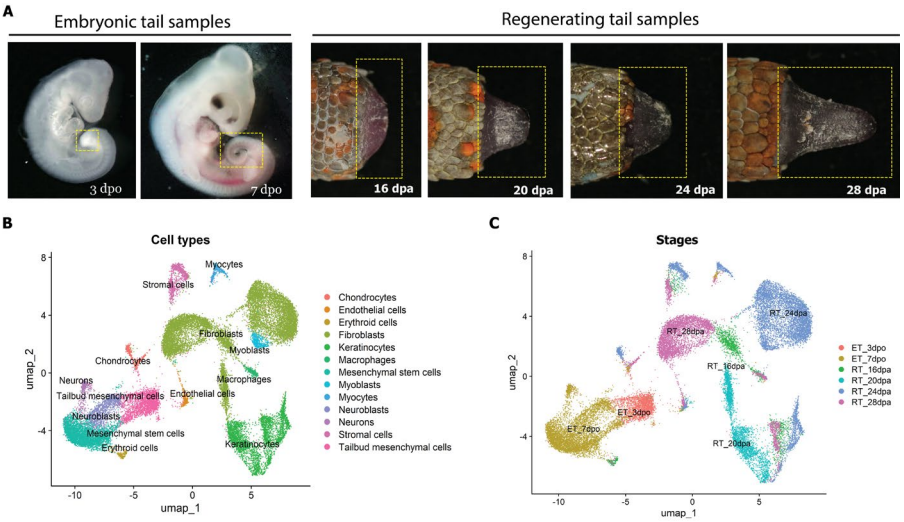

Figure 4-1. **Different cell populations in the regeneration blastema and embryonic the tailbud.** (A) Samples used for single-cell RNA sequencing (area boxed with yellow dashes). (B) UMAP plot showing cells in the regeneration blastema and embryonic the tailbud clustered by cell type. (C) UMAP plot of cells in the regeneration blastema and embryonic tailbud clustered by stages. For the panel of genes used for determining the cell types, see Figure 4-2.

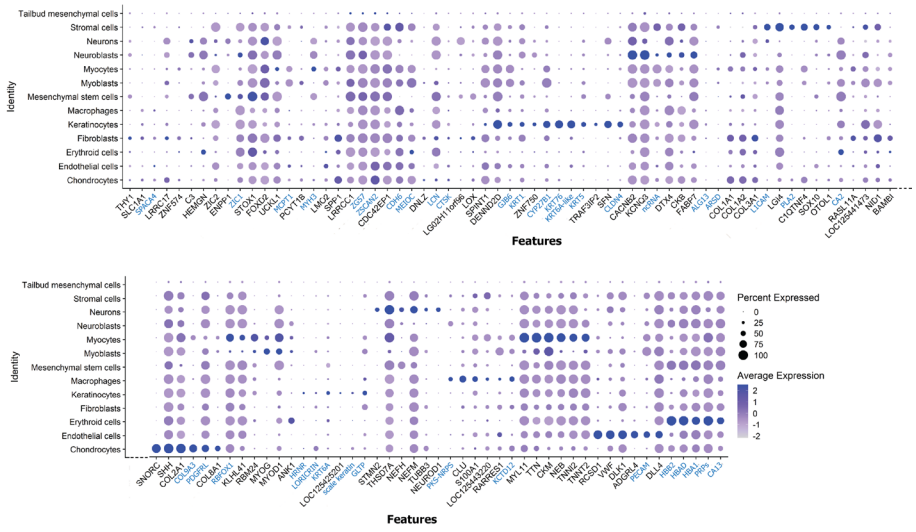

Figure 4-2. **Genes making up the expression profiles of the different cell types in Figure 4-1B and C.** The gene symbols highlighted in blue represent human gene orthologs identified based on gene descriptions from the *Sphaerodactylus townsendi* genome. The mapping of gene symbols and corresponding gene identifiers in *Sphaerodactylus townsendi* genome is provided in the Table S 4-1.

Blastema is lineage-restricted

We estimated the terminal state, and cell fate probability, of cell clusters using Velocyto and Cellrank. In the embryonic tailbud/tail, mesenchymal cells were identified as the initial state (Figure 4-3A). They have a high probability of becoming (as terminal states) chondrocytes and myocytes (Figure 4-3B and C), a medium-to-low probability of becoming endothelial cells (Figure 4-3D), and a very low probability of becoming nerve cells (Figure 4-3E). In the regeneration blastema, we estimated stromal cells (resident stem cells in mesenchymal lineages) and fibroblasts to be the initial state (Figure 4-4A). However, the terminal states in the tail regeneration blastema predominantly develop within their own lineage (Figure 4-4B–F). Notably, keratinocytes develop only from the keratinocyte cell cluster (Figure 4-4E).

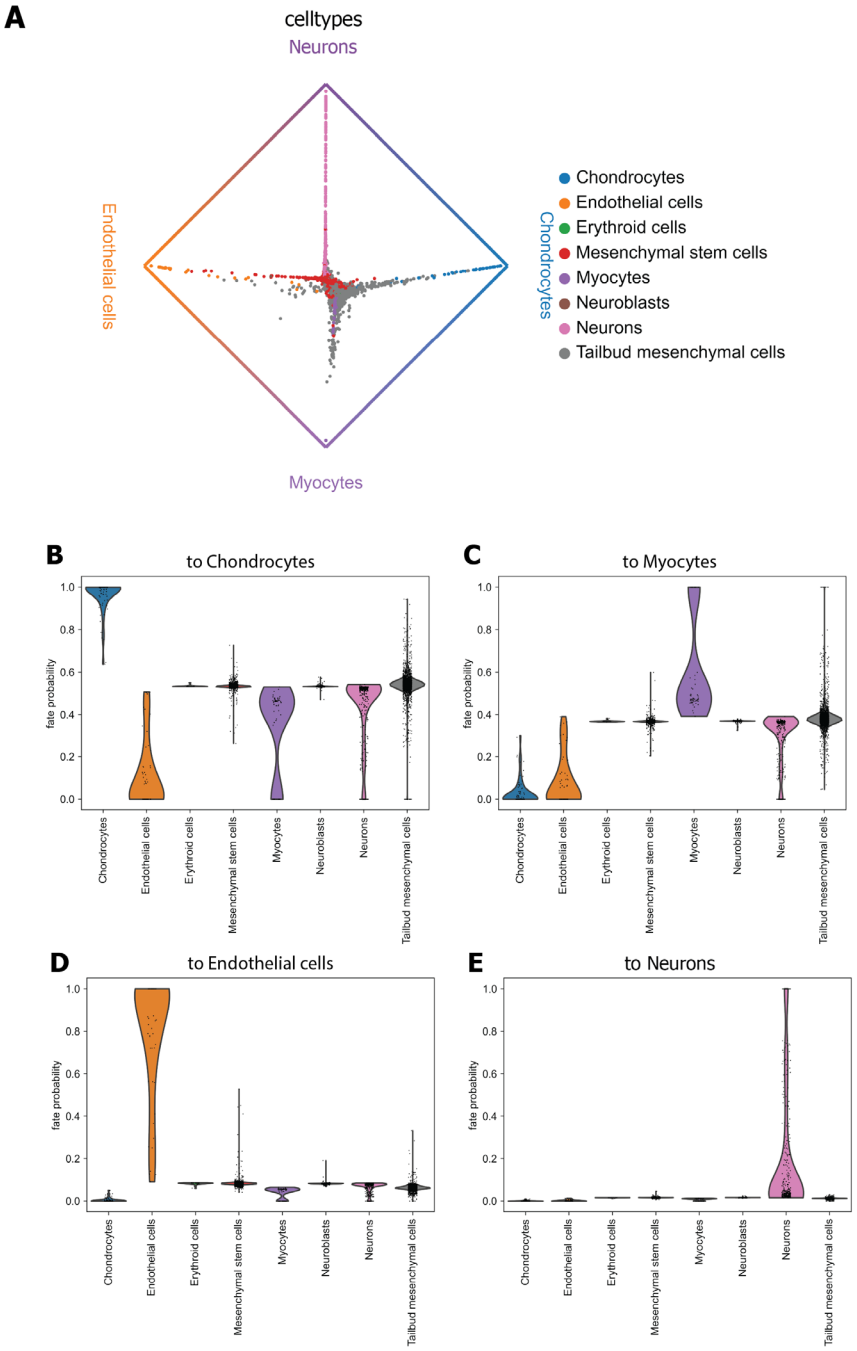

Figure 4-3. **Cell fate probability estimation for the embryonic tailbud and tail.** (A) fate probabilities of merged [embryonic tailbud + embryonic tail] cell clusters towards estimated terminal states. (B–E) fate commitment of each cell cluster in the merged [embryonic tailbud + embryonic tail] data towards terminal states.

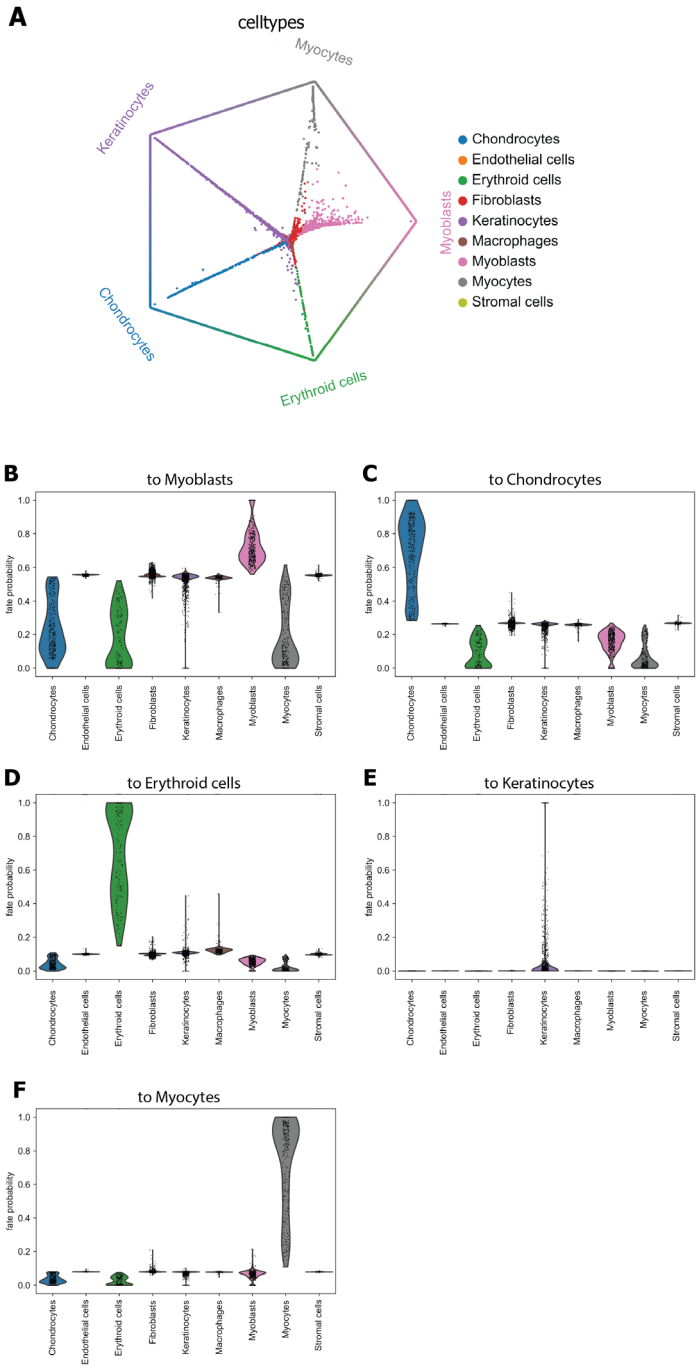

Figure 4-4. **Cell fate probability estimation of tail regeneration blastema.** (A) fate probabilities of tail regeneration blastema cell clusters towards estimated terminal states. (B–F) fate commitment of each cell cluster in the tail regeneration blastema towards terminal states.

We explored these results further (in the blastema only) using pseudotime estimation with Cellrank (Figure 4-5A). In this analysis, keratinocytes were the earliest of all cell clusters followed by endothelial cells and fibroblasts (Figure 4-5A). We then subset the cell clusters of mesenchymal lineages since our previous cell fate probability analysis showed that the keratinocytes only develop from their own cell cluster. We found that the stromal cells were the earliest among the mesenchymal lineages, followed by fibroblasts and chondrocytes (Figure 4-5B).

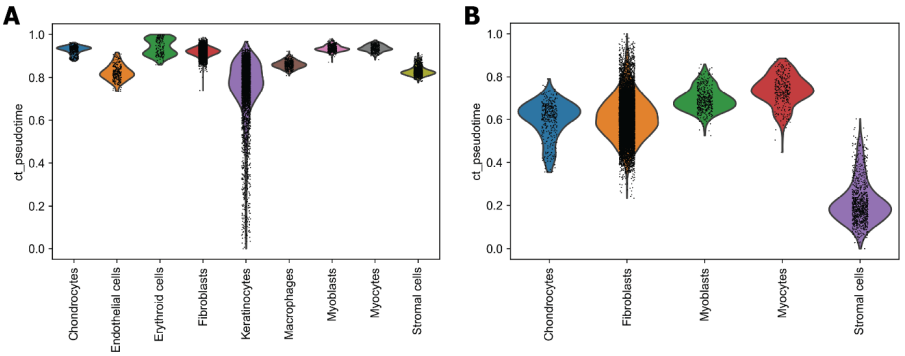

Figure 4-5. **Pseudotime analysis of tail regeneration blastema.** (A) distribution of pseudotime values of all cell clusters of the tail regeneration blastema. (B) distribution of pseudotime values of mesenchymal lineage cell clusters of the tail regeneration blastema.

Discussion

In this chapter, we compared the adult tail regeneration blastema with embryonic tailbud and embryonic tail samples in the tokay gecko using single cell RNA sequencing (scRNA-seq). We wanted to examine whether regeneration in the blastema on the one hand, and normal development in the embryonic tailbud/tail on the other, involve similar cell populations and their lineage restriction. Our analysis does reveal some differences in the cell populations in tail regeneration vs. tail development. Thus, the regeneration blastema is dominated from 16 dpa onwards by fibroblasts. By contrast, the embryonic tailbud/tail shows a substantial population of pluripotent stem cells, which we classify these cells as 'tailbud mesenchymal cells' at 3 dpo and as 'mesenchymal stem cells' at 7 dpo.

Blastema formation is a key feature of epimorphic regeneration, which is the type of regeneration seen in salamanders and zebrafish (Joven et al., 2019; Lee et al., 2009; McCusker et al., 2015). The regeneration blastema consists of proliferative, undifferentiated cells that give rise to the regenerated structure (Seifert and Muneoka, 2018). In axolotl limb regeneration the blastema, at least in some respects, recapitulates embryonic limb bud development at certain stages. For example, in the axolotl limb, the

mid-late regeneration blastema shows similar cell clusters to the embryonic tailbud (Gerber et al., 2018). In zebrafish tail regeneration, the blastema cells also share developmental signatures with the embryonic tailbud (Blum and Begemann, 2012; Pfefferli and Jazwinska, 2015; Sinclair et al., 2021) .

Interestingly, our single cell data analysis in this chapter shows that the majority of clusters are not shared between the tokay gecko regeneration blastema on the one hand, and its embryonic tailbud/tail bud on the other. The regeneration blastema in the tokay gecko is predominantly composed of fibroblasts and does not exhibit a distinct pluripotent stem cell population. A transcriptomic study of tail regeneration in the green anole (a lizard) also found that the blastema formed is not similar to the typical blastema of the axolotl and zebrafish. For example, the green anole blastema is highly vascular, unlike the axolotl and zebrafish blastema. Our histological findings confirm this for the tokay gecko (See Chapter 2). They further suggest that the green anole tail regeneration is more likely to involve tissue-specific regeneration through stem/progenitor populations (Hutchins et al., 2014). Our data in Chapter 2 found that the no apical growth zone in the tokay gecko regeneration blastema. This suggests that the regenerative process in the lizard may involve distinct mechanisms compared to the blastema-based regeneration seen in zebrafish and salamanders (Hutchins et al., 2014).

Cellular dedifferentiation and transdifferentiation are hypothesized to be the key mechanisms underlying regeneration in vertebrates and other animals (Jopling et al., 2011). Dedifferentiation involves a change in cell state of a terminally differentiated, quiescent cell into a pluripotent, undifferentiated cell capable of proliferation (Yao and Wang, 2020). Transdifferentiation by contrast is when a terminally differentiated cell transforms into a terminally differentiated cell from a different lineage; this sometimes involves an intermediate stage where the differentiated cell first reverts to a stem cell like state (regression; (Debuysschere et al., 2024; Jopling et al., 2011). Transdifferentiation and dedifferentiation are both thought to occur in the formation of the regeneration blastema in teleost (Knopf et al., 2011; Sousa et al., 2011) and urodele models (Echeverri et al., 2001; Stocum, 2017; Tanaka and Reddien, 2011; Tsonis et al., 1995). Transdifferentiation between muscle and cartilage may occur in tail regeneration in the Mourning Gecko (Londono et al., 2017). One exception among the urodeles to the patterns outlined above is limb regeneration in the Eastern Newt *Notophthalmus viridescens* which requires the dedifferentiation of myotubes, whereas myogenesis during limb regeneration in axolotls relies on the activation of resident stem cells (Sandoval-Guzman et al., 2014).

Regeneration of the tokay gecko tail may rely on the activation of resident stem cells, rather than the dedifferentiation seen in some urodeles and in zebrafish. This has also been suggested for regeneration in other lizard species (Hutchins et al., 2014; Londono et al., 2018). We found no common pool of mesenchymal precursors in the

tokay gecko regeneration blastema. Instead, the terminal states develop mostly from their own resident stem cells. Resident stem cells (stromal cells) are a population of stem cells in adult mesenchymal tissues that sometimes contribute to cancer progression (Zhao et al., 2023). The activation of resident stem cells has been proposed as a key mechanism underlying the regeneration of complex body structures in amniotes (Hutchins et al., 2014; Johnson et al., 2020; Kierdorf et al., 2009; Lehoczky et al., 2011; Wang et al., 2019a) and myogenesis during limb regeneration in the axolotl (Sandoval-Guzman et al., 2014).

Furthermore, we find that the development of keratinocytes is different from that of mesenchymal cell clusters. This difference makes it unlikely, we believe, that the epidermis contributes in any way to the blastema (for example by epithelial-mesenchymal transition). We found no evidence of transdifferentiation and dedifferentiation in the tokay gecko regeneration blastema. Instead, our data support a model in which the tail regeneration blastema in the tokay gecko is composed of multiple stem cells that were already committed to a particular lineage at the start of regeneration. A prime candidate for these stem cells are resident stem cells from the uninjured stump tissue. Regeneration in mammals, such regeneration of the rodent digit tip (Johnson et al., 2020; Lehoczky et al., 2011) and regeneration of deer antlers (Qin et al., 2023; Wang et al., 2019a), also relies on resident stem cells that were already committed to a particular lineage.

Axolotl limb development involves a major component of dedifferentiation supplemented by the activation of resident stem cells. Thus, in the axolotl limb a major source of to muscle and cartilage in the blastema is dedifferentiation of fibroconnective tissue of the stump (Gerber et al., 2018). However, (Sandoval-Guzman et al., 2014) have suggested that muscles in the regenerating axolotl limb may originate from resident stem cells. Our pseudotime analysis suggests that the cluster ‘stromal cells’ has a significant role in initiating blastema formation (followed later in this role by fibroblasts and other cell types). And, although axolotls and geckos both need resident stem cells from existing mature tissue to form a blastema, we did not find evidence of dedifferentiation in the tokay gecko, although it has been reported during tail regeneration in the Mourning Gecko *Lepidodactylus lugubris* (Londono et al., 2017).

In conclusion, our findings indicate that tokay gecko tail regeneration predominantly relies on the activation of tissue-resident stem/progenitor cells, rather than the formation of a blastema formed by dedifferentiation. This contrasts with the blastema-based regeneration observed in amphibians such as urodele and teleost model species. Instead, the regenerative process in lizards is more akin to the mechanisms found in mammals, which primarily involve the utilization of resident stem cell populations.

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Supplementary Information

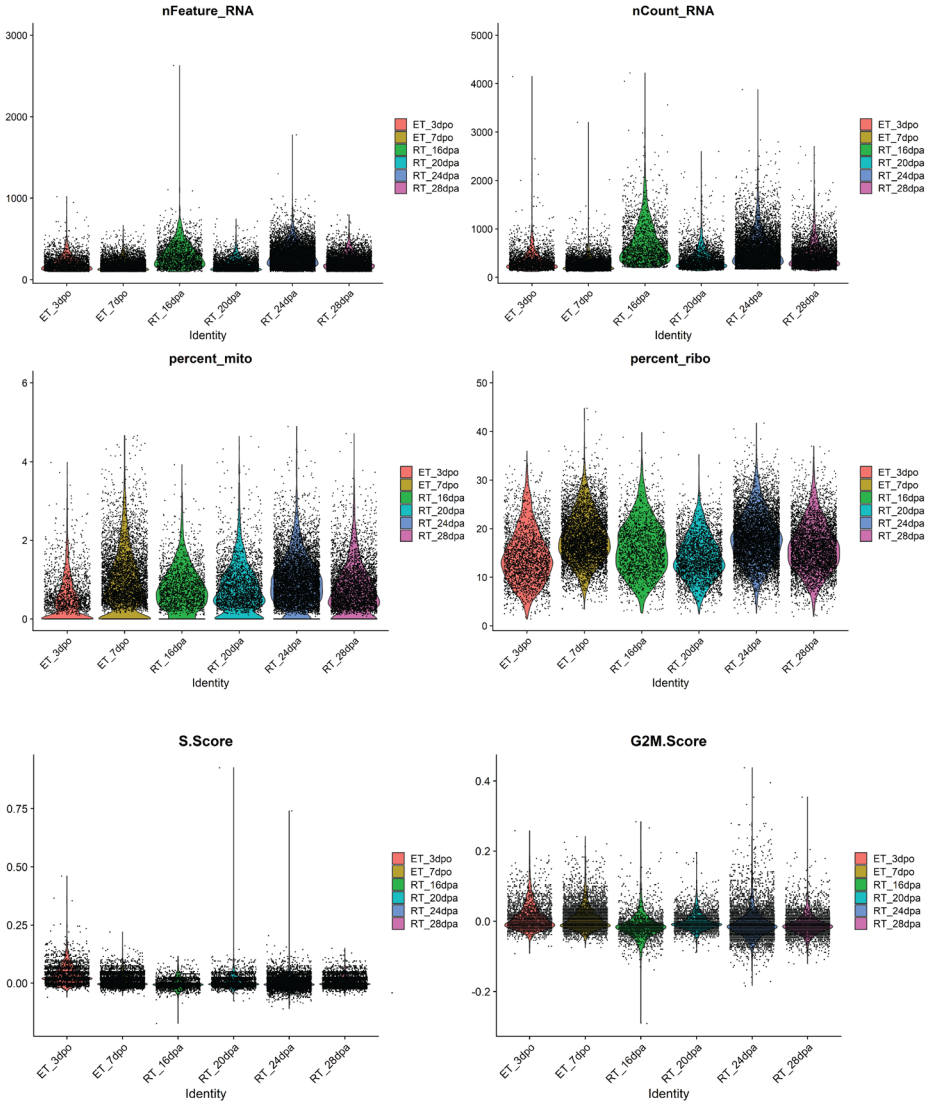

Figure S 4-1. **Quality Control assessment of single cell data used in this chapter.**

Table S 4-1. **The mapping of gene symbols and corresponding gene identifiers in *Sphaerodactylus townsendi* genome**.

No.	Gene symbol	Gene Identifier	Gene description
1.	SPACA4	LOC125430222	sperm acrosome membrane-associated protein 4-like
2.	CDH6	LOC125439157	cadherin-6
3.	LCN	LOC125442120	lipocalin-like
4.	ZIC1	LOC125437433	zinc finger protein ZIC 1 (ZIC 1)
5.	CA13	LOC125438970	carbonic anhydrase 13-like
6.	CA2	LOC125438766	carbonic anhydrase 2-like
7.	HBA1	LOC125431165	hemoglobin subunit alpha-A (HBA1)
8.	ZG57	LOC125428732	gastrula zinc finger protein XlCGF57.1-like
9.	PRPs	LOC125435388	PRPs-basic proline-rich protein (PRPs)
10.	ZSCAN2	LOC125428616	ZSCAN2-zinc finger and SCAN domain-containing protein 2-like
11.	MCPT1	LOC125433220	MCPT1-mast cell protease 1A
12.	MYH3	LOC125429055	MYH3-myosin-3
13.	RBFOX1	LOC125445098	RBFOX1 RNA binding fox-1 homolog 1
14.	KCTD12	LOC125442777	BTB/POZ domain-containing protein KCTD12
15.	MEIOC	LOC125440652	MEIOC-meiosis-specific coiled-coil domain-containing protein MEIOC
16.	PDGFRL	LOC125428064	PDGFRL-platelet-derived growth factor receptor-like protein
17.	CTSK	LOC125444042	cathepsin K-like
18.	GJB6	LOC125432195	GJB6-gap junction beta-6 protein-like
19.	KRT76	LOC125427915	KRT76-keratin, type II cytoskeletal 60 kDa, component III
20.	KRT1	LOC125427914	KRT1-keratin, type II cytoskeletal 1
21.	KRT6A	LOC125429328	KRT6A-keratin, type II cytoskeletal 6A
22.	GLTP	LOC125442796	GLTP glycolipid transfer protein
23.	CYP27B1	LOC125427926	Cyp27b1-25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial
24.	KRT6A-like	LOC125429093	keratin, type II cytoskeletal 6A-like
25.	KRT5	LOC125429094	KRT5-keratin, type II cytoskeletal 5
26.	LOC125441473	LOC125441473	uncharacterized LOC125441473
27.	ncRNA	LOC125444722	ncRNA
28.	ALG13	LOC125442804	ALG13-UDP-N-acetylglucosamine transferase subunit ALG13 homolog
29.	ARSD	LOC125431876	arylsulfatase D-like
30.	L1CAM	LOC125428002	L1CAM-neural cell adhesion molecule L1
31.	PLA2	LOC125438409	phospholipase A2 inhibitor alpha-like protein
32.	COL9A3	LOC125433411	COL9A3-collagen alpha-3(IX) chain gene
33.	LORICRIN	LOC125439757	loricrin-like
34.	HRNR	LOC125432313	hornerin-like
35.	LOC125425201	LOC125425201	uncharacterized LOC125425201
36.	scale keratin	LOC125439306	scale keratin-like
37.	PKS-NRPS	LOC125440523	hybrid PKS-NRPS synthetase fsa1-like
38.	PECAM	LOC125430216	PECAM-platelet endothelial cell adhesion molecule-like
39.	HBB2	LOC125430938	HBB2-hemoglobin subunit beta-2-like
40.	HBAD	LOC125431166	HBAD-hemoglobin subunit alpha-D-like
41.	CLDN4	LOC125444890	CLDN4-claudin-4
42.	LOC125443220	LOC125443220	5-hydroxyisourate hydrolase-like (Transthyretin)