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REVIEW

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Spiny mice (*Acomys*) have evolved cellular features to support regenerative healing

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Abstract

Spiny mice (*Acomys spp.*) are warm-blooded (homeothermic) vertebrates whose ability to restore missing tissue through regenerative healing has coincided with the evolution of unique cellular and physiological adaptations across different tissue types. This review seeks to explore how these bizarre rodents deploy unique or altered injury response mechanisms to either enhance tissue repair or fully regenerate excised tissue compared to closely related, scar-forming mammals. First, we examine overall trends in healing *Acomys* tissues, including the cellular stress response, the ability to activate and maintain cell cycle progression, and the expression of certain features in reproductive adults that are normally associated with embryos. Second, we focus on specific cell types that exhibit precisely regulated proliferation to restore missing tissue. While *Acomys* utilize many of the same cell types involved in scar formation, these cells exhibit divergent activation profiles during regenerative healing. Considered together, current lines of evidence support sustained deployment of proregenerative pathways in conjunction with transient activation of fibrotic pathways to facilitate regeneration and improve tissue repair in *Acomys*.

KEYWORDS
cell cycle, fibrosis, injury response, regeneration, spiny mice, wound healing

INTRODUCTION

Studying otherwise routine physiological processes in certain animals that appear extraordinary (compared to humans) is one path toward uncovering new solutions to frustrating biomedical problems.[1,2] Our inability to regenerate complex tissue assemblages in response to injury is one such problem where basic research using an array of highly regenerative organisms may one day provide a breakthrough that could unlock regenerative mechanisms in humans. Indeed, leveraging carefully chosen adult animal models of enhanced regenerative ability alongside a strong conceptual appreciation for the complexities of tissue repair is a good place to begin. Although the impact of regeneration studies in fishes, amphibians, and lizards awaits translational applications, the emergence of mammalian models of complex tissue regeneration affords opportunities to discover key features that can explain how these animals sidestep scar production to generate new functional tissues.[3–7]

In this vein, spiny mice (*Acomys*) have emerged as promising models to study complex tissue regeneration across multiple organ systems. Those *Acomys* species examined thus far all exhibit the capacity to fully regenerate skin[7–9] and musculoskeletal tissues,[10–12] while also resisting injury-induced fibrosis to regain function of the heart, kidneys, and spinal cord after injury[13–16] (Figure 1). In contrast, species such as the laboratory mouse (*Mus*) and other closely related murid rodents undergo fibrosis following identical injuries.[10] Thus, unlike most studies using nonmammalian vertebrate regenerators, research with these animals can leverage cross-species comparisons to discover genomic, cellular, and physiologic characteristics that facilitate regeneration.

Quite simply, studies directly comparing closely related regenerative and nonregenerative animals hold the most promise for

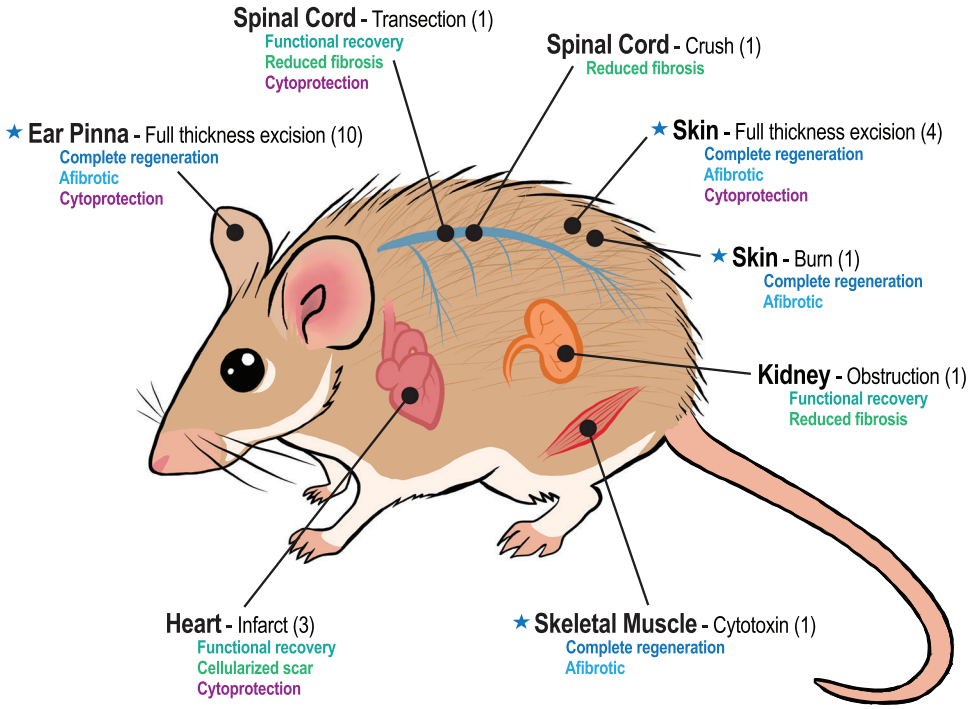

FIGURE 1 Multiple *Acomys* tissues display regeneration or functional recovery following injury. *Acomys* exhibit the ability to regenerate or functionally recover following injuries to the ear pinna, heart, kidney, skeletal muscle, skin, and spinal cord. For each surveyed tissue, the wounding model and supporting citations are indicated. *Acomys* skin and musculoskeletal tissues (blue stars) undergo complete regeneration and as a result are histologically afibrotic (blue; ear pinna—full-thickness punch,[7,10–12,41,65,90,105,116] skin—full-thickness excision,[7,106,107,120,164] skin—burn,[165] muscle—cytotoxin[63]). Heart, spinal cord, and kidney all display enhanced healing and functional recovery following injury (green; spinal cord—transection,[15] spinal cord—crush injury,[166] heart—cardiac infarction,[13,14,167] kidney—ureteral obstruction and ischemia/reperfusion[16]). Histologically, these tissues display reduced fibrosis and/or increased scar cellularity compared to *Mus* that received identical injuries. Molecularly, cytoprotective processes have been identified in cases of regeneration and enhanced healing in *Acomys* (purple).

uncovering constraints operating to inhibit or restrict regeneration. However, multispecies studies require additional levels of validation and reproducibility compared to studies focused on highly inbred model species. First, it is essential to establish anatomical and physiological baselines so studies at the cell, tissue, and organ levels are interpretable across species. Along these lines, researchers using *Acomys* have taken important steps to establish cross-species protocols for the ear pinna, heart, and spinal cord injury models.[10,13,15] Second, studying a new species and verifying new discoveries requires reproducibility and repeatability. To this point, results using the above-mentioned injury models have been replicated within and across multiple labs, supporting the robustness of the recovered phenotypes given the existing genetic diversity among populations (Figure 1). The preponderance of data strongly supports that *Acomys* regenerate multiple tissues. Third, comparative studies utilizing multiple regenerative and nonregenerative models allow for the distinction of species-specific factors from phenotype-specific factors. For example, regenerative studies in the ear pinna have used multiple *Acomys* species alongside rabbits (*Oryctolagus*) to increase sampling of the regeneration phenotype in comparison to *Mus*, rats (*Rattus*), and other nonregenerative rodents.[10,17] Similarly, studies investigating tissue repair in response to myocardial infarct have leveraged inbred and outbred *Mus* lines to help substantiate superior healing and survival in *Acomys*.[13,14] In addition to *Mus* and *Rattus*, laboratory gerbils (*Meriones*) are valuable murid scarring models and phylogenetically the closest research species to *Acomys*. Unfortunately, the expense of doing experiments with mammals often makes it impractical to include the number of comparisons that might be desired. Though not ideal *sensu stricto*, one-way comparisons with *Mus* or *Meriones* have and will continue to provide important data for future studies.

With these points in mind, this review explores cell states and behaviors that exhibit consistencies across *Acomys* injury models that result in regeneration or enhanced healing. We explore comparisons across multiple tissue types to pinpoint overarching proregenerative features in *Acomys*. These consensus features can guide future multispecies studies designed to determine which characteristics are incidental or specific to mammalian regeneration. That said, we contextualize our focus on two generalizable hypotheses for how regenerative healing is activated in lieu of scar production, noting that they are not mutually exclusive: (1) activation of unique regeneration-specific responses to a common injury signal, and (2) differential activation of common pathways between fibrosis and regeneration that vary in magnitude and duration. Below, we present general cell- and tissue-level characteristics observed in *Acomys*, then discuss tissue-resident cell types that are likely responsible for these characteristics. Evaluating recent studies across *Acomys* tissues and injury models, we find

support for both hypotheses. Specifically, *Acomys* responds to injury with the activation of both unique proregenerative pathways and pathways associated with scar formation. Importantly, while proregenerative activity is maintained, fibrosis-associated pathways only appear to be activated transiently.

ACOMYS AS A REGNERATIVE MODEL

There are several features of *Acomys* that make it an excellent model to study tissue repair, the most notable being that they are a bona fide mammalian model of complex tissue regeneration that extends beyond those cellular compartments exhibiting physiological regeneration using dedicated adult stem cell populations (e.g., hair follicles, endometrium, intestinal epithelium, etc.) (Figure 1). This is underscored by the fact that the evolution of homeothermy generated additional changes that do not necessarily reflect cell states observed in poikilothermic vertebrates; specifically, those related to basal metabolic rate, the excess generation of metabolic heat, and relatively high baseline levels of reactive oxygen species.[18] Other mammalian models of complex tissue regeneration are thus far limited to digit tip regeneration as observed in *Mus*, *Rattus*, and primates (where there is no interspecific variation in ability),[19–21] deer and reindeer antlers, including velvet,[5,22,23] and ear pinna regeneration in *Oryctolagus* (New Zealand rabbits).[10,24,25] Compared to these models, *Acomys* exhibits a greater array of regeneration across tissues and is an easier lab animal to work with than rabbits, primates, and cervids. From a technical standpoint, several annotated, chromosome-level genomes are publicly available for transcriptomic analysis (Wellcome Trust Sanger Institute, spiny mouse genome project; mAcoPer2_REL_1905, mAcoDim1_REL_1905, mAcoKem2_REL_1905, ASM2989020v1, and mAcoRus1.1),[26,27] and a wide array of commercially available antibodies exhibit robust reactivity to specific proteins and cell types. As a result, *Acomys* is readily available for cellular and genetic studies.

While *Acomys* may not display quite the same regenerative capacity as some other vertebrate models of regeneration such as salamanders and zebrafish, their status as an intermediate regenerator has advantages in our view. For example, *Acomys* may represent an important intermediate between humans and other vertebrate regenerators, informing both the evolution of regenerative abilities and facilitating the development of new clinical approaches to stimulate regeneration, or to inhibit fibrosis. Similarly, because they exhibit alternative forms of repair in the heart, spinal cord, and kidney that may not amount to complete regeneration, they have the potential to transform our idea of beneficial clinical interventions in these injury paradigms. Lastly, the translational potential of studying vertebrate regenerators such as axolotl and zebrafish is somewhat limited by their indeterminant growth mode, poikilothermic physiology, and aquatic lifestyle. *Acomys* are one of the few vertebrate regeneration models that do not display indeterminant growth; a feature that is highly correlated with regenerative ability.[28] By studying tissue repair in *Acomys*, we can tease apart proregenerative mechanisms that function in the absence of consistent cell proliferation. In addition, we can study their ability to regenerate under the biomechanical load created by a terrestrial environment.

We also note three unique features of the *Acomys* model that can further expand our understanding of wound healing more broadly. First, *Acomys* are a model for naturally occurring type II diabetes.[29,30] Anecdotally, older and heavier *Acomys* exhibit reduced healing rates, although they still appear to regenerate (unpublished observations from our breeding colony). This feature has obvious potential for studying diabetic wound healing and treatment. Second, other than primates, bats, and the elephant shrew, *Acomys* are one of the few mammals known to menstruate.[31,32] This offers an unprecedented opportunity to better understand how hormone cycles and wound healing mechanisms interact in females. Considering the observation that blood draws induced inflammation and accelerated early wound healing in wild female *Acomys kempi*,[10] further study of menstruation status, inflammation, and wound healing could open unique insights into regeneration. These aspects of *Acomys* biology provide excellent opportunities to expand the study of regenerative mechanisms against the backdrop of human-like physiological traits. Lastly, recent studies have found that *Acomys* tails have osteoderms along their entire length.[33] While the tail itself cannot regenerate following amputation, this presents an interesting opportunity to investigate the regenerative ability of tail skin, especially as it relates to its unique osteogenic requirements.

CELL AND PHYSIOLOGICAL TRENDS IN *ACOMYS* TISSUES

Vertebrate regeneration is an epimorphic process whereby cell proliferation is required to produce new cells that undergo morphogenesis to replace the missing structure. In nonmammalian vertebrates, this process has been well-characterized and can be divided into several conserved, albeit general, phases: (1) injury response and inflammation to initiate and then limit tissue damage; (2) cell activation/cell cycle re-entry and proliferation of resident cells to provide new tissue; and (3) patterning of new tissue to restore structure and function.[34,35] Whether mammalian regeneration also proceeds through these processes in the same way is less certain, although studies to date suggest that it does.[36,37] Below, we discuss general cellular properties observed during regeneration and tissue repair across *Acomys* tissues and how these properties are similar to, or different from, those properties observed in nonregenerative mammals. Collectively, these properties act as signals and environmental states that allow the accumulation of regeneration-competent cell phenotypes rather than profibrotic cell phenotypes following injury (Figure 2). Importantly, several features of resident cells within various *Acomys* tissues contribute to regeneration that we will expand upon in the proceeding section.

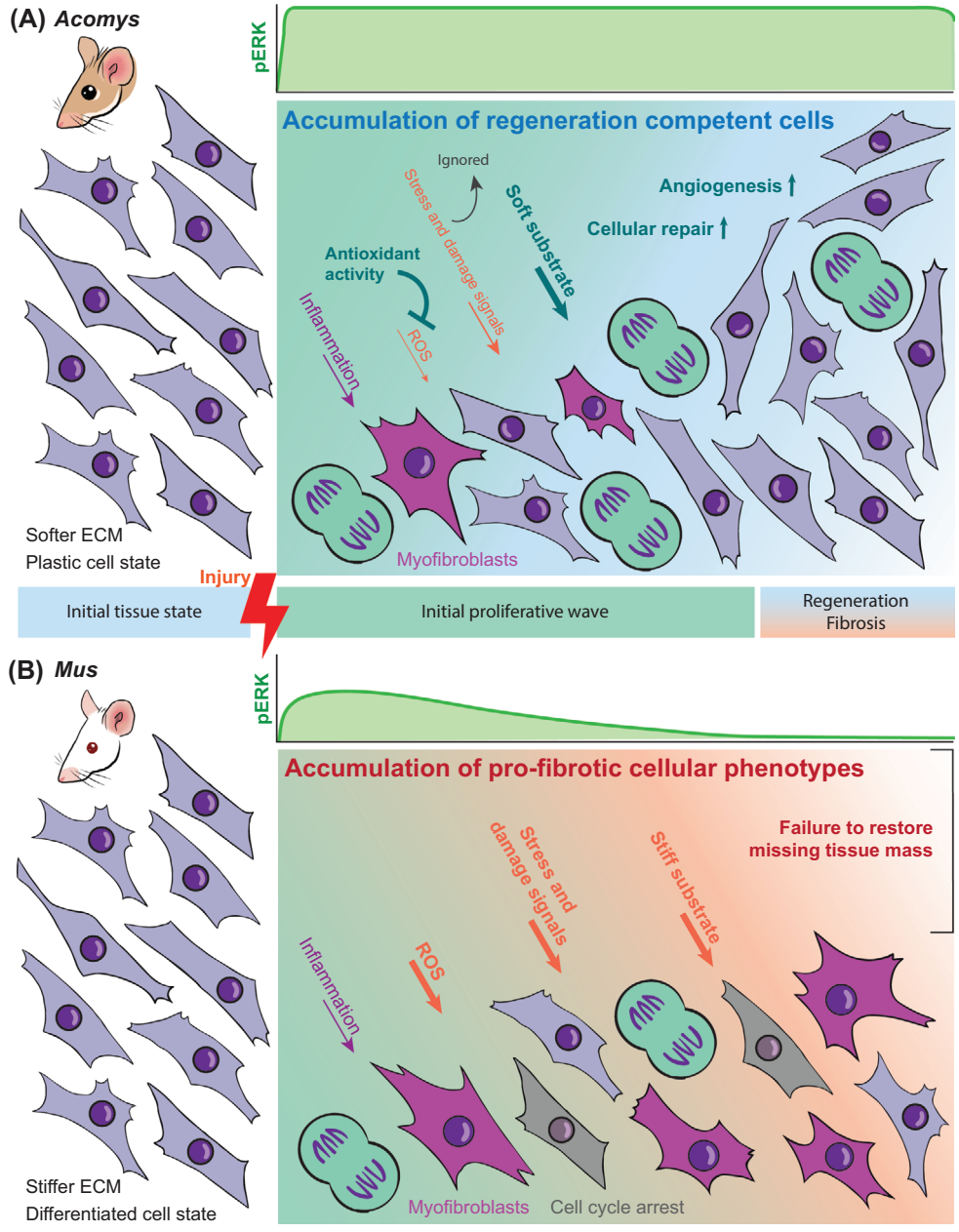

FIGURE 2 Initial tissue state and early injury response drive regeneration or fibrosis through the maintenance or loss of regeneration-competent cell phenotypes. (A) *Acomys* accumulate regeneration-competent cells following injury, while (B) *Mus* accumulate profibrotic cellular phenotypes. Histologically, *Acomys* and *Mus* have similar tissue architecture, but *Acomys* tissues are softer and their cell phenotypes more plastic. Following injury, resident cells of both species (purple) experience a wave of Erk signaling that corresponds with cell cycle re-entry. (A) In *Acomys*, this Erk signal persists after inflammatory resolution. *Acomys* mitigate ROS and other stress and damage signals with enhanced mitigation and altered damage sensing. Soft substrates, enhanced repair, and robust angiogenesis likely further support a proregenerative response. Subsequently, regeneration-competent cells maintain proliferation to restore missing tissue mass. (B) In *Mus*, Erk signaling is not maintained after inflammatory resolution. Inflammation, ROS, and other stress and damage signals correlate with cell cycle arrest and differentiation of profibrotic cell types, such as myofibroblasts. Accumulation of these profibrotic phenotypes prevents the restoration of missing tissue mass. Abbreviations: ECM, extracellular matrix; pERK, phosphorylated Erk, ROS, reactive oxygen species.

Acute damage sensing and stress perception

Tissue damage provides acute signals that are collectively experienced as cellular stress. These signals arise from the loss of tissue integrity and perfusion, generation of excess reactive oxygen species (ROS), release of nucleic acids, damage-associated molecular pattern molecules (DAMPs), and pathogen-associated molecular pattern molecules (PAMPs) from cells, and infiltration of exogenous agents into the damaged tissue. In most mammals, cells respond to injury-induced stress along a spectrum that includes repair, senescence, or

cell death. Indeed, excessive senescence and cell death are associated with poor wound healing outcomes.[38–40] For regeneration to proceed, the stress response must be regulated to limit senescence and cell death, and instead harnessed to stimulate repair, cell cycle progression, proliferation, and tissue growth. There are two primary strategies for avoiding the negative effects of stress signals that have been observed in *Acomys*: an increased capacity to mitigate intracellular damage signals and an apparent insensitivity to signals associated with scar formation (Figure 2).[17,41–43]

In vitro studies have shown that *Acomys* primary fibroblasts resist senescence in response to high levels of hydrogen peroxide and gamma irradiation; a result which also extends to primary *Oryctolagus* cells.[17] In contrast, gamma irradiation and hydrogen peroxide at far lower doses induce senescence in *Mus* and *Rattus* cells. The high threshold for resisting oxidative damage appears to stem from the activation of rapid and robust antioxidant activity, particularly glutathione peroxidase (Figure 2).[17] In nonregenerative models, antioxidant therapy improves skin wound healing and reduces apoptosis in ischemia-reperfusion injury, supporting a connection between high endogenous antioxidant activity and improved healing outcomes.[44,45] Interestingly, *Acomys* primary fibroblasts seem to also exhibit increased resistance to genomic instability, as evidenced by their apparent resistance to induced pluripotent stem cell (iPSC) reprograming.[46] While this may simply be a byproduct of inadequate markers for identifying *Acomys* iPSCs, a concurrent activation of the DNA damage response suggests enhanced activation of repair processes.[47] Extending these collective findings, recent work in vivo found that *Acomys* have a high tolerance to lead acetate toxicity, supporting increased resistance to genotoxicity and oxidative damage.[43] Thus, cellular resistance to stress appears conserved in two mammalian genera (*Acomys* and *Oryctolagus*) and coincides with enhanced regenerative ability in both cases.

Acomys also show enhanced resistance to ischemia as it occurs in different injury paradigms. Following myocardial infarction, *Acomys* resist extensive functional tissue loss compared to inbred and outbred *Mus* strains.[13,14] Similarly, *Acomys* resist extensive loss of kidney architecture following ischemia-reperfusion, suggesting organism-wide cytoprotective mechanisms to hypoxia.[16] While *Acomys* exhibit enhanced angiogenesis following myocardial infarction and ear pinna injury,[13,14] resulting in reduced temporal hypoxic effects, it is unlikely angiogenesis is rapid enough to fully account for the observed retention of functional tissue. Additional differences in metabolism and cellular repair mechanisms likely contribute to this phenomenon.

Upstream of damage mitigation, stress perception further modifies the cellular response to injury signals. The ability to ignore signals that hamper wound healing has been noted in planaria, which display temporary resistance to radiation-induced apoptosis when injured.[48] These results suggest regenerative healing may require a relative reduction in the amount of intracellular damage cells perceive to maintain a sufficient proliferative population. In vitro studies have revealed that *Acomys* primary fibroblasts are insensitive to TGFβ-induced differentiation.[41] In the skin, TGFβ acts as a stress signal to initiate the transformation of fibroblasts to myofibroblasts, which are biomarkers for the early stages of fibrosis and popular targets for antifibrotic therapies.[49,50] In vitro, exogenous TGFβ causes *Mus* cells to upregulate myofibroblast markers and stress fiber formation. In contrast, while *Acomys* primary fibroblasts increase pSmad3 in response to TGFβ, they do not display any significant increases in myofibroblast markers or stress fiber formation.[41]

Insensitivity to environmental signals is also evident in the ability of primary *Acomys* fibroblasts to ignore environmental stiffness.[42] In *Mus*, generating a soft environment by inhibiting lysyl oxidase–driven extracellular matrix (ECM) crosslinking or inducing the perception of a soft environment through YAP1 inhibition reduces fibrosis following skin injury.[6,51,52] Because *Acomys* skin is softer and weaker compared to *Mus* skin,[7,9] it was presumed that a softer wound healing environment allowed *Acomys* to avoid fibrosis. However, while *Mus* cells upregulate *Acta2* expression to stiff substrates in vitro, *Acomys* cells appear to have a muted response to variable stiffness in vitro.[42] These results suggest that *Acomys* can reduce or sidestep fibrosis in part by ignoring the biomechanics of their substrates. This insensitivity may result from differences in the propagation of biomechanical sensing pathways such as Hippo/YAP1.[6,51] The soft homeostatic environment of *Acomys* tissues may also prime resident cells to resist changes in their substrate biomechanics through mechanical memory.[53,54] Together, these results suggest an overall insensitivity of *Acomys* cells to stress responses induced in mammals that normally exhibit fibrotic repair and support the importance of intrinsic mechanisms controlling the perception of exogenous damage signals (Figure 2).

Senescence resistance

Senescence is an irreversible cell state of replicative arrest. Most tissues exhibit a homeostatic population of senescent cells that increases with aging and are associated with diminished wound healing ability.[55] In addition, injury and DNA damage can induce cellular senescence acutely and chronically.[56] Importantly, senescent cells cannot re-enter the cell cycle to generate new tissue. As a result, excessive senescence is associated with poor wound healing outcomes.[38,39]

As described in the previous section, primary dermal fibroblasts from *Acomys* resist senescence in response to a variety of damaging conditions in vitro.[17] In response to an ear punch injury, *Mus* cells upregulate the consensus senescence markers p21 and p27 at 10, 15, and 20 days post-injury, whereas few p21 and p27 positive cells are detected in *Acomys* (Figure 2).[10] It is unclear if there is a significant increase in baseline senescent cells in *Acomys* with aging, though senescent cells can be detected in uninjured skin.[10] Mining recently published gene expression datasets of additional injury models reveals other trends. Following ureteral obstruction, *Acomys* and *Mus* kidneys showed similar activation of *Cdkn1A*, the gene that codes for p21, suggesting that senescence is activated to a similar degree in this tissue.[16] In contrast, *Cdkn1* was not detected in either *Acomys* or *Mus* following spinal cord transection.[15] However, gene expression is a poor proxy for the activation of senescence-associated proteins. The dynamics of senescence in tissues outside the skin compartment obviously requires

further study to determine if the observed differences between *Acomys* and *Mus* are indeed key features that help facilitate regeneration.

Interestingly, senescence induction can have beneficial roles in wound healing and regeneration.[57] Injury-induced cell senescence can help prevent damage propagation and the secretome of these cells is important for directing elements of the early wound healing process, including re-epithelialization, angiogenesis, and cell proliferation.[39,56,58–60] *Acomys* may have evolved the ability to initiate these processes in the absence of injury-induced senescence. Alternatively, it remains entirely possible that *Acomys* may have cells that enter a senescent-like state that awaits identification.[61] While senescence is considered an irreversible state, there is mounting evidence that in some contexts it can be reversed or escaped,[62] leaving the possibility that *Acomys* could activate transient or intermediate senescent-related states. Although the study of senescent cells in *Acomys* is currently limited to a comprehensive marker set that awaits the development of lineage tracing tools, the topic is one that deserves further investigation.

Proliferation

Complementary to senescence resistance, *Acomys* tissues exhibit a strong proliferative inclination. During regeneration, sustained cell proliferation of appropriate cell types is required to restore missing tissue. In *Acomys* and *Mus*, epidermal stem cells and satellite cells in muscle fibers show similar rates of proliferation,[63] which is unsurprising given these tissues undergo physiological regeneration in mammals. However, *Acomys* maintain the proliferation of connective tissue cells and exhibit enhanced healing in the skin, ear pinna, and heart, while *Mus* cells are not maintained in a proliferative state (Figure 2).[8,10,13,14,64]

The ability of *Acomys* to sustain cell proliferation following injury has been best characterized by using the ear pinna model. In vitro, *Acomys* and *Oryctolagus* primary ear pinna fibroblasts will maintain cell proliferation in concentrations of hydrogen peroxide and gamma irradiation that induce senescence in poorly regenerative *Mus* and *Rattus* primary fibroblasts.[17] Following a full-thickness punch through the ear pinna, *Acomys* and *Mus* appear to broadly initiate cell proliferation in the epidermis and dermis.[8,10] At 10 days post-injury, however, there are significantly more cycling cells within the mesenchymal compartment in *Acomys* based on the cell cycle markers phosphorylated retinoblastoma, phospho-histone H3, and EdU.[10] By 15 days post-injury when *Acomys* proliferative rates remain high, *Mus* proliferative rates precipitously drop. The divergence in proliferation correlates with two features of tissue healing: resolution of inflammation and ERK signaling dynamics. Although ERK phosphorylation occurs immediately after injury and is independent of the healing phenotype, the propensity for continued cell cycling and proliferation 10 days later in *Acomys* is dependent on the maintenance of high ERK activation during regeneration (Figure 2).[65]

Both *Acomys* and *Mus* undergo an initial wave of proliferation in response to myocardial infarction, but *Acomys* sustains proliferation past this initial burst to contribute to angiogenesis.[13,14] This proliferation results in the restoration of coronary circulation that supports the maintenance of heart function. Within the heart tissue itself, RNAseq analysis has demonstrated that *Acomys* cardiomyocytes (CMs) express more proliferative markers than *Mus* following infarction.[14] In addition, *Acomys* efficiently restores the epicardium through the expansion of existing cells. While *Acomys* do not fully restore heart form and function in response to an infarct, the increased proliferation of different cardiac tissue types contributes to significantly improved survival compared to inbred and outbred mouse strains. Thus far, there is evidence of limited CM proliferation, indicating they may have some capacity to regenerate the myocardium itself.[13] This echoes data from some amphibians that exhibit enhanced CM proliferation without complete ventricular regeneration.[66–68]

While sustained proliferation is clearly an essential component of tissue repair and regeneration in multiple *Acomys* tissues, further studies are necessary to identify molecular regulation of cell cycle re-entry and progression. Because regeneration and tumorigenesis utilize many of the same molecular pathways, especially during increased cell proliferation and generation of a promigratory environment, some speculate that attempting to induce regeneration in the clinic may put patients at higher risk of tumorigenesis.[69] Furthermore, the genomic stress induced by high levels of proliferation may further contribute to an increased risk of cancer development.[70,71] Despite these theoretical risks, there is some evidence that highly regenerative animals are resistant to tumorigenesis.[72,73] This is further supported by the strong connection between tissue stiffening, fibrosis, and tumor formation in aged, poorly regenerative organisms.[74,75]

Anecdotally, regenerative poikilotherms like axolotls and zebrafish do not form tumors at higher rates than other species, and in fact may possess some tumor resistance. However, since these species are indeterminant growers, mechanisms for maintaining proliferative compartments and organ size likely diverge from species that exhibit determinate growth. Attempts to reprogram *Acomys* fibroblasts into iPSCs have shown that *Acomys* fibroblasts upregulate tumor repressors to resist transformation,[46] a fact that may drive molecular resistance to tumorigenesis. Yet, *Acomys* fibroblasts immortalize in culture over a similar time course to *Mus*,[76] suggesting that this species does not have a particular susceptibility or resistance to the development of cancer. Interestingly, our lab has observed masses in both the skin and gastrointestinal compartments of *Acomys* (Figure 3A). Notably, the skin masses that form on the ears of older animals appear to contain differentiated tissues including cartilage, glands, and hair follicles (Figure 3B). Indeed, this may represent reactive tissue growth in response to injury that occurs in conjunction with reduced tissue size control in older animals. However, these masses also have some characteristics of papillomas, including hyperkeratosis and fingerlike projections that might suggest other origins like infection or tumor formation.[77,78] Further studies into the activation of tumor repressors by *Acomys* cells will be important to understand this process and further explore a potential connection between tumorigenesis, cancer, and enhanced regenerative ability.

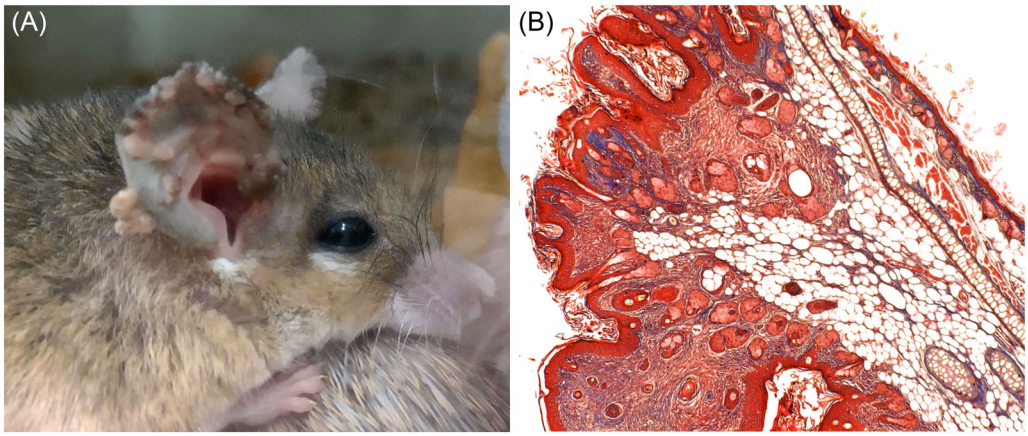

FIGURE 3 *Acomys* can develop skin masses. (A) Multiple masses visible on the ear pinna of a 2.5-year-old male *Acomys*. (B) Masson's trichrome stained tissue section of *Acomys* ear pinna mass from a 2-year-old female *Acomys*. In trichrome sections, nuclei appear dark brown/black, cell cytoplasm is pink, connective tissue and extracellular matrix (collagen) stain blue, and epidermis (keratin) and muscle stain red. Tissue section is oriented with dorsal side to the right, ventral left, proximal up, distal down.

Cell plasticity and potency

As previously discussed, current dogma based on a handful of highly regenerative vertebrates purports that epimorphic regeneration requires cells to reacquire the capacity for proliferation, which in turn generates new progenitor cells that can undergo morphogenesis.[79–81] This process may involve dedifferentiation of cells to a less specialized state. While the definition of dedifferentiation with respect to cell identity remains under debate,[34] the process of dedifferentiation as defined in salamanders appears linked to an increase in cell potency. Some evidence suggests that *Acomys* cells exist in or readily enter a state of increased potency, whether or not these cells make more of themselves or can contribute to a range of new tissues awaits validation.

In vertebrates at least, the accumulation of proliferating progenitors is referred to as a blastema and one need not invoke dedifferentiation to observe one.[37] Indeed, robust blastema formation has been observed in *Acomys* ear pinna regeneration.[7,10] Preliminary work by our lab has found that *Acomys* primary dermal fibroblasts may have more efficient differentiation toward specific lineages. *Acomys* dermal fibroblasts show nominally improved adipogenesis compared to *Mus* (Figure 4A,B). While *Acomys* and *Mus* efficiently generate lipid vacuoles as shown with Oil Red O staining, *Mus* cells retain a more fibroblastic shape, while *Acomys* cells take on a more rounded morphology resembling adipocytes (Figure 4A,B). Differentiation of scar-forming myofibroblasts into adipocytes during wound healing has been shown to reduce fibrosis.[82] Thus, increased differentiation capacity may help *Acomys* cells resist fibrosis. In addition, *Acomys* cells are more efficient in vitro when induced toward chondrogenesis (compared to *Mus*) (Figure 4A,C). They readily proliferate in a 3D cell culture, generate a robust glycosaminoglycan-rich matrix as evidenced by Alcian blue staining, and even appear to efficiently remodel the matrix, generating large lacunae resembling mature cartilage (Figure 4A,C). *Mus* ear pinna fibroblasts, in contrast, generate a matrix but appear to proliferate and remodel inefficiently in a 3D cell culture (Figure 4A,C). These data may indicate an increased potency of *Acomys* cells, and merit further exploration.

Fetal features

For some researchers, the early vertebrate embryo is the paradigmatic regenerative model.[83–85] Early vertebrate embryos can repair certain tissues, like skin, scar free, and late-stage embryos retain enhanced wound healing capacity. Loss of regenerative ability in the embryo also correlates with the termination of developmental patterning programs and maturation of the immune system.[83] Some recent work has revived the hypothesis that regeneration requires cellular reprogramming to an embryonic state and these studies equate this process with cellular dedifferentiation.[86–88] However, a more objective viewpoint argues that resident cells need only access to certain developmental pathways or cell states when regenerating new tissue. This latter hypothesis is supported by the observation that many *Acomys* tissues retain adult cells that express certain features observed in embryonic cells.[10,13,14,16,89,90]

Several cellular-level observations have supported the expression of fetal cell features in adult *Acomys*. Differentiated connective tissue cells in the *Acomys* ear pinna are generally euchromatic compared to *Mus* cells.[10] In *Acomys* bone marrow, most neutrophils are maintained as immature band cells rather than more mature segmented cells found in *Mus*.[89,90] There is a greater abundance of mononucleated CMs in the *Acomys* heart,[13,14] which is associated with greater proliferative potential in newborn *Mus* and humans,[91] and some *Acomys* CMs exhibit a T-type calcium channel that is strictly associated with fetal *Mus* CMs.[13]

At the molecular level, several well-characterized developmental signaling cascades and cell states have been observed to occur during tissue regeneration in *Acomys*. In skin and ear pinna regeneration, hair follicle neogenesis follows a developmental program. As new tissue is

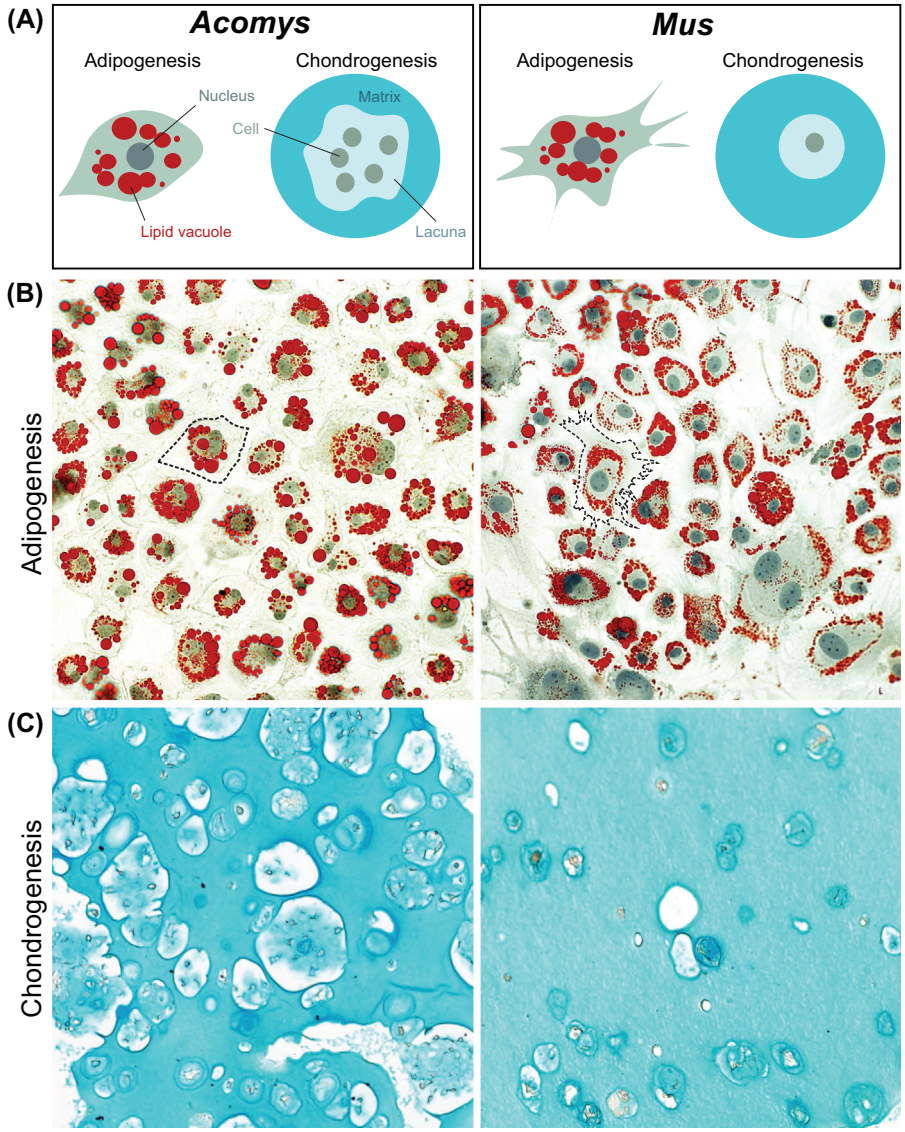

FIGURE 4 Differentiation capacity of *Acomys* and *Mus* primary ear pinna fibroblasts. (A) Schematic representation of *Acomys* (left) and *Mus* (right) primary ear fibroblasts differentiated into an adipogenic or chondrogenic fate. (B) Oil red O staining of *Acomys* and *Mus* primary ear pinna fibroblasts induced to undergo adipogenesis. Nuclei counterstained with hematoxylin. Representative cell membrane outlined in each species. (C) Alcian blue staining of *Acomys* and *Mus* primary ear pinna fibroblasts induced to undergo chondrogenesis in 3D alginate culture. Nuclei counterstained with Brazilliant. $n = 2$ for each panel.

produced, Wnt signaling is upregulated in *Acomys* epidermis and dermis where the Wnt signaling transcription factor LEF1 is upregulated in epithelial placodes and dermal papilla cells. Coincidentally, epidermal hair placodes express keratin 17, which subsequently becomes restricted to new hair follicles as regeneration proceeds.[7] Importantly, these events are not observed after wounding in *Mus* ear pinnae and back skin, and stimulating Wnt signaling via LEF1 induction can induce hair follicle neogenesis.[92] Interestingly, observations of fibroblasts in neonatal, newborn, and adult *Mus* skin suggest that it is the loss of one or more embryonic fibroblast lineages that can explain the propensity for *Mus* skin scarring or regeneration.[93–95] Whether or not a similar embryonic fibroblast lineage is maintained in adult *Acomys* and contributes to their enhanced regenerative ability awaits further investigation.

Together, these observations provide tantalizing clues that support access to developmental programs and cell states in *Acomys* tissues following injury. Future studies will delineate which embryonic cell features are maintained into adulthood and which are specifically activated in response to injury. Exploring the control mechanisms that regulate these cell states should provide new avenues for biomedical scientists to leverage in the context of regenerative medicine.

Tissue biomechanics

During the original investigation of complex tissue regeneration in *Acomys*, we observed much weaker skin compared to *Mus*.[7] Subsequent work used atomic force microscopy to show that the wound

bed of healing *Acomys* skin is much softer than *Mus*.[9] Anecdotal evidence further supports that several other *Acomys* tissues are softer and perhaps weaker than identical *Mus* tissues. While the evolutionary origins of weak tissue in mammals remains a mystery, links between tissue stiffness and wound healing outcomes suggest that the soft tissue environment observed in *Acomys* may help facilitate regeneration (Figure 2). Softer substrates help tissues resist fibrosis and tumorigenesis,[74,96,97] and age-related tissue stiffening negatively impacts wound healing while increasing the risk of developing tumors in older organisms.[98–100] Conversely, during regeneration, a soft tissue environment likely enables cell migration, cell proliferation, revascularization, axon sprouting, and possibly, epithelial-to-mesenchymal transitions.[101] For example, in the transected *Acomys* spinal cord, evidence of axons spanning the wound space support that a soft substrate can facilitate reinnervation.[15]

The biomechanics of *Acomys* tissues may also contribute to the differentiation and patterning of *Acomys* cells. In vitro, soft substrates promote adipogenesis, while hard substrates promote chondrogenesis and osteogenesis.[9,51,102,103] In the stiff environment of the ear pinna scar, *Mus* generate some disorganized new cartilage but cannot regenerate hair follicles.[7,10] In contrast, *Acomys* efficiently regenerate the elastic cartilage, hair follicles, hypodermis, and other components of the ear pinna. The ability to restore elastic cartilage may reflect the reduced role substrate biomechanics play in influencing cell behavior and suggest a robust patterning signal in the ear. Conversely, the soft tissue environment of *Acomys* may facilitate the differentiation of myofibroblasts into adipocytes to prevent fibrosis.[82,104] An interesting line of future research will be to investigate the resistance of *Acomys* tissues to misspecification of cell fate, such as heterotopic ossification following burn and blast injuries.

The softer nature of *Acomys* tissues is at least partly established by specific ECM components in tandem with their modification and arrangement.[9,105] For example, uninjured *Acomys* skin has less type I collagen (COL1) than uninjured *Mus* skin.[8,105,106] *Acomys* skin and heart both generate a more disorganized basketweave matrix following injury, unlike *Mus* which generates a highly parallel matrix typical of scarring.[9,13,14,107] ECM composition during *Acomys* ear pinna regeneration superficially resembles the ECMs from other regeneration models including late-stage mammalian embryos and salamanders.[8,108,109] Despite similarities across regenerative models, a more comprehensive comparison reveals large differences in the respective matrisomes, indicating that multiple combinations of proteins can generate an ECM that supports regenerative healing. The bigger question, of course, is the degree to which specific matrisomal combinations promote, rather than simply permit, regeneration.

Considered together, the properties reviewed above paint a picture of an animal whose cells are poised to respond to various insults with some degree of plasticity instead of the stereotypical vigor that rapidly repairs damage with scar tissue. Increased stress resistance allows *Acomys* to escape processes such as senescence that protect against damage propagation and intense fibrosis. This allows damage signals to promote cell cycle progression and sustained cell proliferation of resident cells to restore tissue mass (Figure 2). Combined with a soft tissue environment and the ability to access developmental signaling pathways, these features ultimately synergize to replace tissue architecture and restore tissue function.

PROLIFERATING CELL POPULATIONS

To further investigate cell-intrinsic (i.e., cell-autonomous) behaviors that permit proliferation during mammalian regeneration, it is valuable to identify those cell types that can generate new tissue. In this vein, the proliferative population during regeneration could be generated by the expansion of adult progenitors, partial reprogramming (dedifferentiation) of resident cells, or via differentiated cells that retain the capacity to proliferate; all remain possibilities. Evidence from other regenerative models and skin wound healing studies implicate tissue progenitor cells and connective tissue fibroblasts as orchestrators of fibrosis or regeneration. As mentioned above, cell subtypes within larger populations of fibroblasts or monocytes are suggested to underlie differences in repair outcomes in *Mus*, supporting the need for careful evaluation. Although many of the cell type–specific markers used in *Mus*, *Rattus*, and human cells appear to mark similar cells in *Acomys*, careful work is required to determine if these markers faithfully identify the same cell populations across all these species. Despite the absence of transgenics for robust lineage tracing, scRNA-sequencing and immunohistochemistry have provided a starting place for cross-species comparison.

Stem and progenitor cells

Stem cells reside within specific niches where they undergo self-renewal and generate progenitors through asymmetric cell division. How stem cells participate in regeneration has long been a topic of intense focus as many highly regenerative invertebrate species mobilize stem cells to facilitate epimorphic regeneration, and normal physiological regeneration in mammals occurs via activation of dedicated stem cell populations. Many invertebrate groups exhibiting whole-body regeneration (e.g., Platyhelminthes, Cnidarians, etc.) use pluripotent or totipotent somatic stem cells to regenerate missing or damaged tissues. Basal epidermal, satellite, and hematopoietic stem cells are all well-characterized stem cell populations in vertebrates that maintain the epidermis, skeletal muscle, and circulating blood cells, respectively. Aside from the germline, available data in vertebrates support that adult stem cells are tissue-specific, and to date, no study has identified a connective tissue stem cell that directs complex tissue regeneration in vertebrates. However, because cellular reprogramming is achievable in the laboratory, there is intense interest in learning how local cell populations (stem and progenitor) behave during natural regenerative phenomena. Recently, in vivo reprograming has shown promising potential for rejuvenation and regeneration in tissues that normally scar.[88,110] Understanding how stem cells and differentiated cells contribute to new tissue in a highly regenerative mammal can help us refine therapies for more effective use.

Multiple stem cell types reside in the skin compartment, including interfollicular epidermal stem cells, hair follicle stem cells, sebaceous gland stem cells, melanocyte stem cells, and neural progenitor cells.[111] Following skin and ear pinna injury, *Mus* and *Acomys* both efficiently re-epithelialize. However, only *Acomys* regenerates epidermal appendages (hair and glands),[7,8] and in the case of the ear pinna, continuous cartilage, peripheral axons, and muscle.[7,10,11] These data suggest that skin stem cells in *Acomys* possess a greater capacity for expansion, differentiation, and replacement in new tissue compared to similar cell types in other murid rodents. In the kidney, the presence of stem or progenitor cells is supported by expression data. Following ureteral obstruction, *Acomys* kidneys were found to upregulate markers of embryonic nephrogenic progenitors (e.g., *IgF2* and *H19*).[16,112] Normally in *Mus*, nephrogenic progenitors become depleted shortly after kidney organogenesis. These data suggest either retention, activation, and/or expansion of an existing stem cell pool or partial reprogramming of resident cells into a more stem-like fate to restore function.

The resistance of *Acomys* to functional failure following myocardial infarction and the presence of higher numbers of mononuclear CMs suggests a tissue state reminiscent of the fetal heart.[91] In vivo CM reprograming using the Yamanaka factors in *Mus* hearts affords increased protection to myocardial infarction.[110] Interestingly, these reprogrammed hearts resemble *Acomys* following MI in that they still develop some scar tissue, but do not undergo the same degree of ventricular dilation and functional loss as is normally observed in untreated *Mus* hearts. The increased proliferative drive in *Acomys* heart tissue previously discussed may contribute to cytoprotection, but further study will be required to determine if engagement of heart progenitor cells is a prerequisite for heart regeneration.

The identification of bona fide stem cells and their niches in regenerating *Acomys* tissues will be an important next step in understanding how tissue mass is restored. scRNA seq studies have identified some potential stem cell markers and further validation has the potential to generate new tools for studying regeneration. Comparative frameworks for determining cell abundance will need to be rigorously established to account for the size difference in *Acomys* and *Mus*. Still, there is strong circumstantial evidence for the presence of cells in progenitor-like states in different *Acomys* tissues. Studying stem cell dynamics in *Acomys* provides a new model to better understand how resident stem and progenitor cells may be maintained and activated to enhance healing.

Fibroblasts

Connective tissue fibroblasts have long been identified as a critical population for mediating injury signals, initiating repair, and determining wound healing outcomes. In most tissues, this heterogenous population of cells is activated following injury to proliferate, migrate, secrete factors, and/or differentiate.[113] Fibroblasts make up a large fraction of the mesenchymal tissue in the heart, kidney, and muscle, where they have been shown to drive fibrosis in *Mus* and humans.[94,114] In particular, the ECM generated by fibroblasts responding to injury appears to have significant effects on wound healing outcomes. Given the ability of *Acomys* to resist fibrosis or initiate regeneration in these tissues, *Acomys* fibroblasts likely have unique cell-specific qualities, phenotype trajectories, and tissue proportions that differ from poorly regenerative mammals (Figure 5).

To date, most in vitro studies using *Acomys* cells have been carried out on a heterogeneous population of plastic-adherent primary dermal fibroblasts from the skin or ear pinna that exhibited vimentin and heterogenous smooth muscle actin (αSMA) positivity along with expression of *Col1a1*.[17,41,42,46,115,116] Although primary isolates used to acquire fibroblasts might also contain perichondrocytes, fibro-adipogenic progenitors, and epidermal lineages, the fact that these cell types require specialized cell culture conditions supports that they are lost during passaging. Therefore, we accept that in vitro observations published so far (i.e., high resistance to stress, low incidence of cellular senescence, increased resistance to iPSC reprograming, and increased differentiation efficiency) are attributable to ear pinna and skin fibroblasts.[17,41,42,46]

While transcriptional studies suggest that all dermal fibroblasts have some regenerative potential given the right environment,[117] several subtypes preferentially contribute to fibrosis or scar-free wound healing in *Mus* skin. Broadly, the dermis is divided into a superficial papillary layer ($Dpp4^{+}/Dlk1^{-}/Ly6a^{-}$) and a deeper reticular layer ($Dpp4^{-}/Dlk1^{+}/Ly6a^{\pm}$) overlying the hypodermis ($Dpp4^{-}/Dlk1^{-}/Ly6a^{+}$).[92,93,118,119] Cells in the papillary layer, and in particular $LEF1^{+}$ cells, are important for *Mus* skin regeneration.[92] LEF1 is highly expressed during hair placode formation in *Acomys* skin and ear pinna regeneration,[64,120] supporting that $LEF1^{+}$ cells are important for the regeneration of skin adnexa. Conversely, cells of the reticular layer have been associated with fibrosis.[51,121] In *Mus*, the papillary layer thins with age, resulting in a proportionally thicker reticular layer and concomitant reduction in healing capacity over time.[122] *Acomys* have overall thicker skin than *Mus* and appear to retain this overall thickness with age, though the actual proportions of papillary and reticular dermis have yet to be determined. Complicating these comparisons are the dynamic expression patterns of fibroblast markers. DPP4 in particular can be found diffusely throughout the skin, and $DPP4^{+}$ cells from the deep dermis give rise to Engrailed-1 positive fibroblasts (EPFs), which have been hypothesized to play a major role in driving fibrosis.[51,121] Blocking EPF mechanosensing with the YAP inhibitor, verteporfin, promotes scar-free healing in *Mus* skin.[51,121] Determining the dynamics of these different fibroblast subtypes in *Acomys* skin will provide key information as to whether their ability to facilitate scar-free healing is similar to, or diverges from, enhanced healing in *Mus*.

Mining of published gene expression datasets collected for *Mus* and *Acomys* skin and ear pinna has revealed differential changes in dermal subtype markers during fibrotic repair and regenerative healing, respectively.[10,120] *Mus* skin has increased *Dpp4* expression at 14 days post-injury suggesting papillary fibroblasts or EPFs are the dominant subtype during fibrotic repair. In contrast, *Acomys* exhibit increased *Dlk1* expression at 7 and 14 days post-injury, with a moderate increase

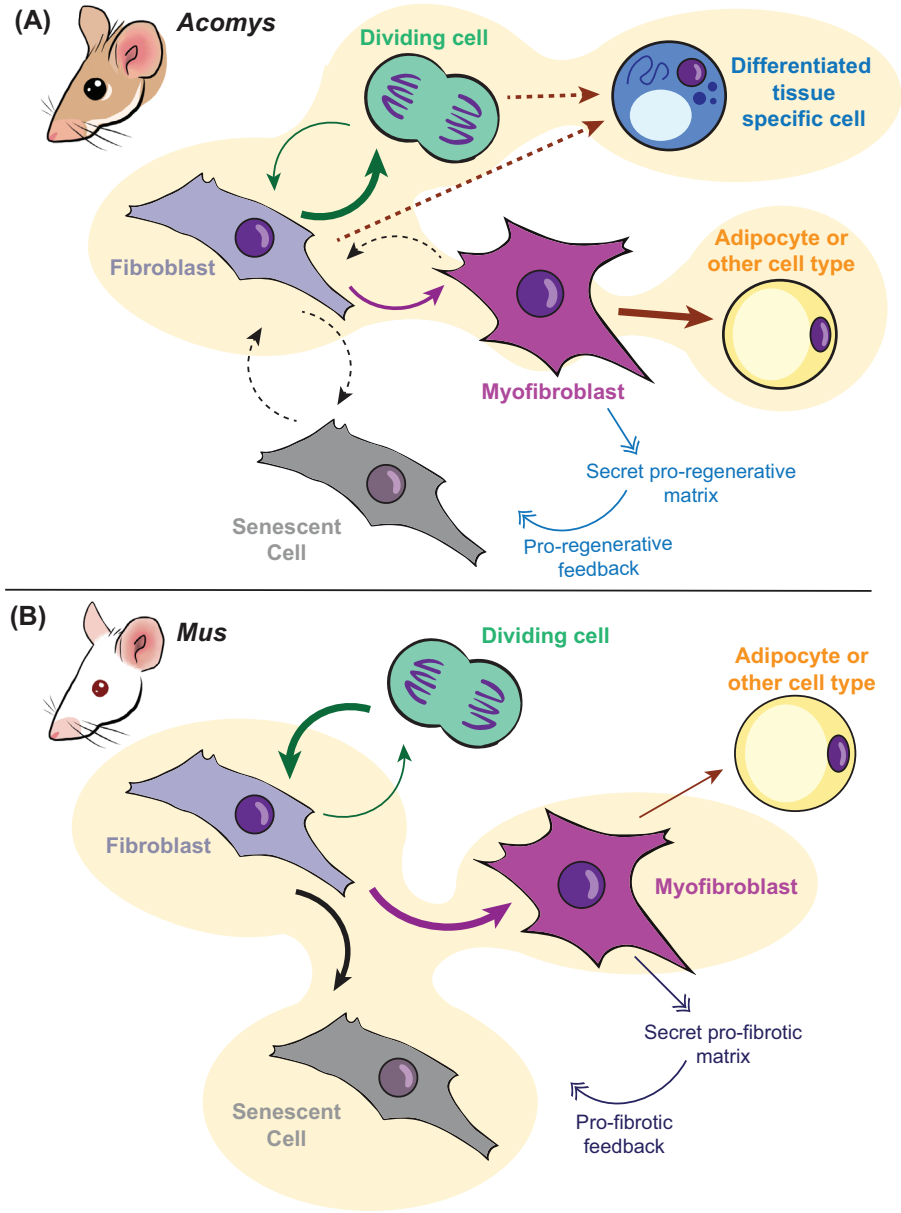

FIGURE 5 Cell phenotype trajectories during regeneration and fibrosis. (A) *Acomys* and (B) *Mus* show evidence of differential tissue-resident fibroblast trajectories that influence wound healing. Arrow thickness indicates relative likelihood a cell proceeds down a given trajectory (thick—more likely, thin—less likely). Dotted arrows indicate hypothesized trajectories that await experimental confirmation. (A) *Acomys* cells display robust proliferative maintenance and increased cell plasticity that allows for the generation of new, appropriately patterned tissue. Little senescence appears to be activated and may be reversible. While myofibroblasts accumulate in damaged *Acomys* tissue, they are fewer in number compared to *Mus* and are only transiently maintained. Differentiation of myofibroblasts to adipocytes or other cell types may aid in their clearance. This collectively leads to the accumulation of proliferating or homeostatic differentiated cells. (B) Conversely, *Mus* fibroblasts fail to maintain proliferation and accumulate high numbers of profibrotic identities such as myofibroblasts and senescent cells.

in *Ly6a* at day 7 indicating enrichment for reticular fibroblasts and hypodermis. These data support that different subtypes within the dermis are activated and expanded between regeneration and fibrosis. In addition, the paradigm of papillary cells contributing to regeneration, and reticular cells contributing to fibrosis may not hold in *Acomys*. Engrailed-1 mRNA expression is increased only in *Mus* skin 7 and 14 days after injury,[120] suggesting that this cell subtype is not active in *Acomys* skin regeneration. Additionally, *Crabp1*, a marker of dermal papilla cells,[93,123] is highly upregulated in *Mus* and *Acomys* 5–15 days following ear pinna injury.[10] While *Crabp1* expression precipitously drops by 20 days post-injury in *Mus*, it is maintained at high levels in *Acomys*. Importantly, this differential expression timing correlates with a divergence point when proliferation is maintained in *Acomys* or terminated in *Mus*. Future studies will be required to determine if these dermal markers identify the same populations of cells in *Acomys* as *Mus*, or if the *Acomys* dermis has a unique composition.

Fibroblasts play an important role in the nervous system as well. Fibrosis induced by acute or chronic injury in the central nervous system (CNS) is characterized by the formation of a unique scar with an inner fibroblast-rich core and an outer glial scar.[124,125] Both these scar layers inhibit axon growth through a physical barrier and secreted factors, resulting in failure to recover function. Inhibiting fibroblast aggregation to combat the fibrotic scar results in improved functional recovery following spinal cord injury.[126] Following spinal cord transection in *Acomys*, newly generated tissue at the injury site is confined to the area of the original tissue.[15] In contrast, *Mus* spinal cords form an extensive scar that protrudes from the original transection area. In addition, *Acomys* deposits ECM that facilitates axon migration and results in functional recovery. Together, these data suggest that *Acomys* CNS fibroblasts have reduced fibroblast proliferation and ECM deposition after injury.

Since fibroblasts have proregenerative and protective properties during wound healing in the right context,[5,93,127,128] it is not a stretch to assume that the *Acomys* tissue environment facilitates activation of a proregenerative subpopulation that in turn contributes to regeneration. Based on in vitro studies, *Acomys* fibroblasts themselves appear to have many features that correlate with a progenitor-like phenotype. Given these cells are major contributors of mesenchymal tissue throughout the body, it is unsurprising that *Acomys* fibroblasts contribute significantly to their wound healing ability.

Myofibroblasts

Myofibroblasts are a population of transiently activated fibroblasts that express αSMA in response to injury.[129,130] These cells are contractile, facilitate wound closure, and deposit large amounts of COL1 and other ECM components. Myofibroblasts can have diverse developmental origins, arising from local fibroblasts, epithelium, endothelium, and infiltrating bone marrow-derived cells.[82,115,129] The persistence of myofibroblasts in tissues is linked with fibrosis and exuberant ECM production,[129–131] making them a popular target for antifibrotic therapies. As previously noted, *Acomys* primary fibroblasts appear to resist TGFβ-induced myofibroblast differentiation in vitro.[41] In addition, the soft tissue environment in *Acomys* may limit biomechanical myofibroblast activation and accumulation.[132,133] Supporting the link between tissue mechanics and regeneration, experiments in *Mus* blocking YAP-mediated mechanosensation in the skin promote scar-free wound healing.[6,51] Therefore, while *Acomys* cells may have the capacity to become myofibroblasts, they may do so with reduced vigor due to intrinsic and extrinsic control mechanisms. Surveying the various injury models carried out in *Acomys* supports that the enhanced wound healing ability is facilitated by reduced activation or efficient clearance of myofibroblasts.

Myofibroblasts drive fibrosis in acute and chronic heart pathologies in laboratory mice.[134–136] As in other tissues, damage-induced TGFβ signaling activates differentiation and migration of myofibroblasts, which exert contractile forces and deposit an ECM rich in COL1 and type III collagen (COL3).[134–136] This leads to structural changes in the heart including loss of contractibility. Interestingly, two myocardial infarction studies in *Acomys* have shown they retain structural components of the damaged ventricle and restore some function, whereas *Mus* develop and ultimately succumb to dilated cardiomyopathy.[13,14] Much of the retained heart function in *Acomys* is related to the deposition of an ECM containing thick fibers arranged in a basketweave formation. In contrast, *Mus* repair heart tissue with thinner fibers are in a densely parallel arrangement. Interestingly, *Acomys* present a greater density of PDGFRα$^+$ fibroblasts at the wound site compared to *Mus*, although the extent of myofibroblast activation has not been assessed. The deposition of collagen fibers in *Acomys* after infarction does suggest the activation of matrix-synthesizing cells, such as myofibroblasts. If myofibroblasts are indeed driving collagen production, then their function must be altered to deposit ECM in an arrangement that resembles uninjured tissue.

In the kidney, myofibroblasts originate from proliferating resident fibroblasts and infiltrating bone marrow-derived cells.[114,137] In fact, blocking fibrosis in the kidney is complicated by the multiple sources and mechanisms of expansion (proliferation and migration) employed to expand myofibroblasts. Following ureteral obstruction, *Acomys* exhibit significantly fewer myofibroblasts and have reduced ECM production compared to identical injuries in *Mus*.[16] Whether these myofibroblasts are eventually cleared from the kidney tissue has not been determined.

Multiple studies have identified the presence of αSMA$^+$ cells in *Acomys* skin and the ear pinna where they accumulate but do not persist in the wound like they do in *Mus*.[41,64] While some have proposed that skin scarring is driven by contractile forces resulting in the deposition of aligned collagen matrixes,[138] robust contraction is still observed in *Acomys* following full-thickness skin wounds.[7] The gene transcript for ANKRD1, a protein that facilitates wound contraction via fibroblast matrix interaction, is highly expressed in *Acomys* following ear pinna punch,[10,139] supporting that contraction does not counteract regeneration. Interestingly, blocking YAP signaling, and presumably myofibroblast activation, delayed ear pinna regeneration in *Acomys*,[41] supporting that myofibroblasts are important for the early stages of regeneration. Indeed, αSMA$^+$ cells are required for skin healing in *Mus*.[140] These results suggest myofibroblasts and YAP pathway activation do not exclusively promote fibrosis during wound healing. YAP activity itself is important for organ size control and has been shown to induce proliferation and even promote regeneration in the heart.[66,141–144] The transience of myofibroblast activation in *Acomys* skin following injury may drive later regenerative processes through relief of ECM deposition, contraction, and other profibrotic signaling processes during the regeneration/scaring decision point. At present, the developmental origin of *Acomys* myofibroblasts remains unknown and lineage tracing experiments will be required to better understand how they contribute to regeneration specifically.

The mechanism by which myofibroblasts are cleared from regenerating *Acomys* tissue remains unresolved. To date, no increase in apoptosis has been observed during skin or ear pinna healing.[17] This suggests that myofibroblasts are cleared through differentiation to other identities such as mature dermal fibroblasts or some other

mesenchymal cell (Figure 5). Multiple studies have shown that myofibroblasts can differentiate into adipocytes,[82,104,115] and recent work in *Acomys* showed that inhibiting YAP decreased adipose formation in the regenerated *Acomys* ear.[41] However, it is unclear if the decrease in adipose is due to YAP inhibition and or delayed differentiation following delayed closure. Abundant adipose tissue in *Acomys* skin and ear pinna, coupled with the efficiency by which their fibroblasts can differentiate into adipose-like cells in vitro (Figure 3), implicates this differentiation pathway as an important factor in regeneration. Overall, it appears that modest activation and efficient clearance of myofibroblasts is an important contributor to regeneration (Figure 5).

The extracellular matrix

As one of the primary matrix synthesizers, myofibroblast dynamics have a profound effect on the structure and composition of new tissue. Following wound healing, a provisional matrix is established to support new tissue growth, and this matrix is subsequently remodeled to create the final regenerated tissue. The *Acomys* matrisome has been investigated in several tissues and found to consist of features that facilitate proliferation and migration. Most recently, a comprehensive proteomic analysis of the regenerating *Acomys* ear pinna following 2 mm ear punching added to previous gene expression analysis of the healing matrisome.[105] Several general features stand out and are shared by other regeneration models: an increase in proproliferative/proregenerative provisional matrix proteins such as fibronectin (FN1) and tenascin (TNC), a decrease in fibrillar collagens, an increase in matrix metalloproteinases, and a more natural alignment of collagen fibers compared to the dense, parallel arrangement observed in fibrosis.[7,83,109,145]

Abundant deposition of aligned COL1 fibers contributes to the general structure of a scar in *Mus*, whereas *Acomys* produce a matrix with less total collagen and a more disordered arrangement after tissue injury.[13,15,90,105,145] A recent study investigating COL3-mediated COL1 assembly during skin healing showed that *Acomys* have enriched COL3 compared to *Mus* early in wound healing.[107] This study further demonstrated that loss of COL3 leads to increased COL1 alignment in *Mus* skin scar tissue. As a result, the accumulation of COL3 likely helps to prevent the assembly of a highly aligned COL1 matrix in *Acomys*. Interestingly, while collagen-producing myofibroblasts are required for *Mus* skin healing, COL1 itself appears to be dispensable, indicating that other collagens and ECM components are sufficient to support the new tissue.[140] Activation of myofibroblasts in *Acomys* wound healing without concomitant deposition of a collagen-rich matrix may be part of the key processes that determines the regenerative or fibrotic trajectory of a wound.

The provisional matrix components, TNC, FN1, and periostin (POSTN), are upregulated during *Acomys* skin and ear pinna regeneration.[10,105,106] Because of high expression in other regeneration models such as fetal wound healing and axolotl limb regeneration, these ECM proteins have been dubbed proregenerative. During ear pinna regeneration in *Acomys*, the proximal side of the injury where the blastema forms quickly generates an ECM rich in FN1, while the distal side displays delayed FN1 production; and no indication of blastema formation.[12,65] Paradoxically, persistent expression of TNC, FN1, and POSTN have also been shown to drive fibrosis.[146–148] These data indicate that like myofibroblasts, transient expression of these proteins is required for regeneration to proceed. TNC, FN1, and POSTN are all directly or indirectly responsive to tissue stiffness.[149] As a result, the *Acomys* soft tissue environment along with feedback loops with ECM-depositing myofibroblasts may aid in the persistence or clearance of these proteins.[148,150–152]

Several matrix-modifying enzymes are upregulated during *Acomys* regeneration, including the matrix metalloproteinases MMP9 and MMP13 in skin and musculoskeletal injury, and keratin-modifying enzymes in response to CNS injury.[10,15,105] The gelatinase MMP9 is highly upregulated in other regenerative models and plays an important role in preventing dense deposition of collagen.[153–158] In the injury environment, keratins and their modifications have important roles during axon regrowth,[159] as specific modifications on keratin proteins can stimulate or inhibit axonal growth.[15] Collectively, these modifying enzymes likely facilitate migration and infiltration of cells, vasculature, and nerves to restore tissue.

Considered *in toto*, many general wound healing features are common to both regeneration and fibrosis. Known adult stem cell niches, mesenchymal fibroblasts, and injury-responsive myofibroblasts all contribute to regenerated tissue or a scar. *Acomys*, however, appears to have access to additional progenitor populations in some tissues that are not maintained in adult *Mus*, such as mononuclear CMs with fetal calcium channels and kidney cells with progenitor-like gene expression profiles. Fibroblast subtypes and their specific functions show several important differences between the two healing modalities. *Acomys* appears to mobilize proregenerative fibroblast subtypes and generate different myofibroblast proportions. Additionally, differential fibroblast and myofibroblast activity ultimately alters local ECM composition. All indications point to subtle, but specific phenotypic shifts at the cellular level that appear to drive regenerative or fibrotic healing. Further defining active cell states and their secreted products that favor regeneration and suppress fibrosis should provide an entry point for biomedical scientists to begin designing therapies to restore missing tissue.

CONCLUDING THOUGHTS

Although regeneration research using spiny mice remains in its infancy, important clues are emerging that paint a picture for how regenerative healing occurs in these rodents. First, many of the same cell types and processes are utilized during fibrosis and regeneration, with differences emerging among cell subtypes and processes in the post-inflammatory phases of wound healing (Figure 6). Second, there seems to be a critical point around inflammatory resolution when the trajectory toward fibrotic or regenerative healing rapidly diverges. The consequences of this decision point result in the resolution or persistence of certain generally shared cell types (myofibroblasts

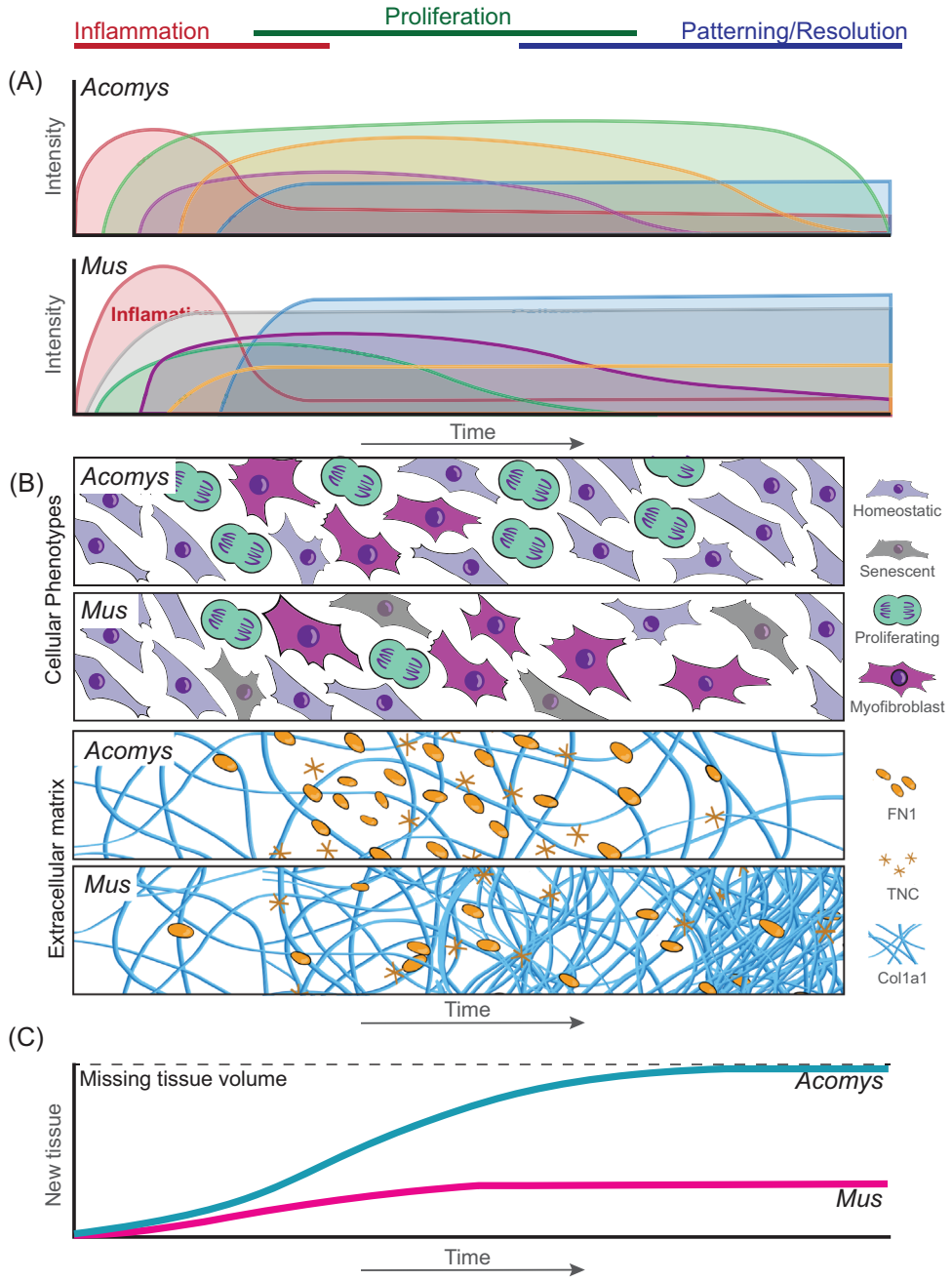

FIGURE 6 Temporal dynamics of regeneration and scaring. (A) Activation intensity of different processes during tissue repair relative to each other (in *Acomys* and *Mus*). *Acomys* has a muted inflammatory response and reduced type I collagen deposition compared to *Mus* and only transiently upregulates myofibroblasts and provisional matrix components. In addition, *Acomys* activates sustained proliferation and little cellular senescence. (B) Proportions of cell phenotypes and extracellular matrix components over time during injury response in *Acomys* and *Mus*. *Acomys* exhibit transient activation of myofibroblasts and sustained proliferation, while *Mus* only transiently activate cell cycle re-entry and accumulate senescent cells and myofibroblasts. *Acomys* generates a basket-weave collagen matrix that resembles uninjured tissue and transiently activates provisional matrix components. *Mus* generate a dense, highly aligned collagen matrix and may not clear provisional matrix components. (C) Ability to restore missing tissue volume over time in *Acomys* and *Mus*. *Acomys* can restore missing tissue mass in skin and musculoskeletal tissues through regeneration facilitated by the trends highlighted in A and B. *Mus* fail to restore missing tissue volume due to fibrosis.

and senescent cells) and differential maintenance, termination, and activation of pertinent signaling pathways (ERK signaling, cell cycle progression, matrix deposition, etc.). The evidence for myofibroblast transience and provisional ECM proteins supports that these components of the wound response are not inherently fibrotic, but rather that the timing of their accumulation (and clearance) is key to determining healing outcomes. In addition, while many of the general cell identities at play may be the same between highly and poorly regenerative species, there are likely specific subpopulations that dominate in each. Lastly, once fibrotic or regenerative healing is underway, what follows

are more canalized processes that ultimately form a persistent scar or direct tissue morphogenesis (differentiation and matrix modification). An open question is the degree to which interventions in these later phases might alter healing outcomes.

Conservation of the early wound response

Regardless of wound healing outcomes, the initial injury response across almost all animals studied includes hemostasis, restoration of a tissue barrier (re-epithelialization), removal of foreign agents, and activation of cell cycle progression through ERK signaling.[34,65] All these processes can be observed during *Acomys* and *Mus* tissue repair. While not discussed in this review, several studies have demonstrated the importance of immune cell types during *Acomys* regeneration, specifically macrophages.[36,116] Though certain proinflammatory molecules appear dampened during *Acomys* inflammation (compared to other nonregenerative mammals),[8,65,90] the timing of inflammatory induction and then resolution seems quite conserved between species and healing types[90,116] (Figure 6). During the inflammatory phase, expansion and activation of fibroblasts and myofibroblasts occurs in both species. Like inflammation, there appears to be differences in the magnitude of individual cell type activation, but these cell types clearly play an important role in both processes.[41]

We would like to note that because of these common injury response processes, the healing of small wounds becomes difficult to distinguish between regenerators and nonregenerators.[10] As discussed at the beginning of the review, the first divergence between *Mus* and *Acomys* injury response occurs during perception and response to stress and damage. In the case of a very small wound (e.g., 2-mm ear punch, thin line skin cut, etc.), a stress threshold may not be activated to elicit a differential response. Further investigation into a potential stress threshold that results in differential responses can aid in the establishment of therapies to prevent fibrosis.

Experimental design and induction of the wound response

Given the similarities we and others observe in the early wound response between robust and poor regenerators, wound models need to be rigorously designed to properly differentiate between regenerative and fibrotic repair. Designing models requires that relative tissue architecture and organ sizes are accommodated such that identical injuries are induced across species. For example, to properly compare cardiac infarction between *Mus* strains and *Acomys*, anatomical studies needed to establish that the coronary vasculature was similar across species.[13] This ensured that despite size differences between *Mus* and *Acomys* hearts, a myocardial infarction would induce comparable amounts of damage. Furthermore, histological, anatomical, and physiological methods were required to rigorously define the initial damage, healing, and recovery. Similarly, in assessing restoration of kidney function, ureteral ligations were used to ensure a similar induction of injury between species and to demonstrate a significant difference in the propagation of injury.[16]

Wound size is a particularly important factor for induction of the wound response.[160] In the ear pinna, a 4-mm excisional wound appears to be the critical size for differentiating ear hole closure resulting from a true regenerative response rather than exuberant scar tissue formation via fibrosis. Mouse strains, such as Murphy's Roth Large, LgJ, or p21 null *Mus*, close 2-mm holes but not 4-mm punches.[10] Interestingly, 4 mm is also the skin incision size at which lamb embryos usually form scars.[161] Thus, *Acomys* ear pinna studies normally use 4 mm punch injuries to provide a basis for cross-study comparison.[7,10–12,36,65,90,116] However, recent studies have employed 2-mm ear punch injury, a decision that likely altered the wound response and has made it difficult to compare to most other studies.[41,105] For example, insufficient activation of myofibroblasts and other wound response pathways in response to small wounds make it difficult to fully differentiate a fibrotic from a regenerative response. In addition, a recent study showed that the proximodistal position of an ear punch can affect the rate of ear hole closure, where distal wounds take weeks longer than proximal wounds to close.[65] As a result, proximodistal position must be carefully controlled to allow accurate comparisons. Lastly, studies that employ multiple wounds at different time points have to account for the possibility of wound priming: the activation of wound response pathways by an initial wound that allows organisms to respond and heal subsequent wounds faster.[162,163] Establishing rigorous wound models for cross-study comparisons is essential to improve reproducibility and will necessarily lead to a more robust understanding of regeneration in mammals.

Divergence of regeneration and fibrosis

As noted in this review, there are numerous places where processes that occur during regeneration and scarring differ in magnitude or where the cellular participants vary. As discussed above, these differences appear to accumulate over the progression of wound healing with the greatest differences occurring at the final patterning stage (Figure 6). Inherent differences between uninjured tissue from regenerative and nonregenerative species likely contribute to healing outcomes. In *Mus* and *Acomys*, baseline features suggest the expression of certain fetal-like characteristics in adult *Acomys*. *Acomys* have softer and weaker tissues, they maintain cells in a more adaptable or plastic state, and they appear to resist age-related tissue changes such as thinning and stiffening. These features set the stage for different outcomes following the activation of common injury response pathways.

While many general cell types and signaling pathways commonly appear between regeneration and fibrosis, we have identified several important points of divergence between healing trajectories (Figure 6). In the initial inflammatory response, *Acomys* exhibit reduced responses to stress signals and increased damage mitigation. In addition, inflammatory cells exhibit an anti-inflammatory phenotype while also expressing proinflammatory molecules.[90,116] As proliferation proceeds, *Mus* and *Acomys* likely activate different subpopulations of

resident cells to generate tissue mass. Ultimately, the final patterning phase shows differences in the resolution of intermediate healing states, cell differentiation to restore missing tissue types, and ECM remodeling to restore tissue architecture.

In response to tissue injury, data to date supports that activating a variety of pathways is common across mammals regardless of the ultimate healing outcome. This degree of conservation provides hope that a therapeutic path to regeneration is not as remote as we may think. At present, our understanding of mammalian regeneration suggests that at least two key inputs are necessary to stimulate regeneration: modulating the magnitude of the initial injury response to activate and recruit cells away from rapid senescence and fibrosis, and post-inflammatory maintenance of signaling inputs that maintain cell states necessary for cell cycle progression and tissue morphogenesis. The spiny mouse stands as one of our most promising models with which to make these discoveries.

AUTHOR CONTRIBUTIONS

Robyn S. Allen: Conceptualization; writing—original draft; writing—review and editing. **Ashley W. Seifert**: Conceptualization; writing—review and editing; supervision; project administration; funding acquisition.

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COMPETING INTERESTS

The authors declare they have no competing interests.

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