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Mini-Review

Tissue regeneration: How far away is the reality from science-fiction?

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ABSTRACT

Although the remarkable ability of organisms like the sea-stars, crayfishes, salamanders, or hydras to regrow damaged or lost body parts is very real, regeneration in humans is limited, and its potential is only fully realized in science fiction. In this review, we attempt to bring together our current knowledge of the mechanisms of regeneration in different organisms and its applications, the possible underlying causes of the low complex-regeneration capacity of mammals both from cellular and evolutionary perspectives, and exciting findings and hypotheses that may keep the hopes of transforming the fictional power of regeneration of superheroes to a reality for humans alive.

KEYWORDS: regeneration, mechanisms of regeneration, regeneration in mammals, hydra, salamanders, Acomys.

1. Introduction

Simple non chordates like the hydra posses the ability to not only regenerate part of its body lost upon amputation, but also to regenerate into whole functional organisms from dissociated cells [1]. Salamanders stand as the premier regenerator among vertebrates, having the capability to regenerate its limbs, tail, heart, and the spinal cord [2]. As we move towards more complex animals, tissue regeneration becomes limited, but some remarkable examples exist such as the ability of the lizards to regenerate its tail [3], the capacity of the deer to regenerate its antler [4], and the amazing regenerative capacity of the *Acomys* (discussed later). Humans have for a very long time coveted the ability to regenerate tissues or organs upon injury. And why not, since the ability of organisms such as the hydra to regenerate into two hydras when cut in half, of echinoderms to regrow lost limbs, of planarian worms to split down the middle to reproduce, and of salamanders to severe their limbs to escape a predator only to sprout one back later is indeed remarkable. Tissue regeneration becomes increasingly limited as organisms become more complex, with the degree of regeneration available to these organisms being markedly less dramatic (although not necessarily less remarkable) when compared to regrowing of a limb. The inabilities of humans have long inspired fiction, and the inability of humans to regenerate has inspired many classic folklore characters like the three-headed, self-regenerating serpent called the Hydra, and continues to inspire modern superhero characters. There has been considerable research in understanding the mechanisms of regeneration with the goal to develop tissue regeneration as a therapy for humans, and the field has seen significant and exciting advancements in the recent years. The central nervous system (CNS) was shown to exhibit increased axonal regeneration after spinal cord injury upon adeno-associated virus-mediated delivery of the synthetic designer cytokine hyper-interleukin-6 (hIL-6), which is the bioactive

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component of IL-6 linked to the soluble IL-6 receptor α subunit. The α subunit is poorly expressed by CNS neurons and limits the neuroprotective and regeneration-promoting effects of IL-6, and hIL-6 looks promising at circumventing this [5, 6]. Fibrosis is a major hurdle towards successful tissue regeneration, and it was recently shown that reduction of myofibroblast number through platelet-derived growth factor receptor α (PDGFRα) inhibition promotes proliferation of satellite cells, remodeling of skeletal muscle and blocks fibrosis *in situ* [7]. CRISPR/Cas-9-mediated transcriptional activation was recently successfully applied to reprogram fibroblasts into cardiac progenitor cells, which showed the ability to differentiate into cardiomyocytes, smooth muscle cells and endothelial cells *in vitro*. The loss of mature mammalian cochlear hair cells leads to permanent hearing impairment since they do not spontaneously regenerate once lost, but a recent approach that involves overexpression of the transcription factor *Atoh1* combined with *Gfi1* showed promise both *in vitro* and *in vivo* in adult mice in regeneration of the hair cells [8].

Numerous excellent reviews on the mechanisms of regeneration in various organisms, and the current application and future prospects of regenerative medicine and therapy exist, with some focusing on our inability to regenerate. Here, we try to bring together a brief overview of the marvels of regeneration in simple organisms and the knowledge they provide of the underlying mechanisms and processes, the possible reasons behind the scarce complex regeneration in mammals from both a cellular and evolutionary perspective, and discuss exciting findings and hypotheses in the field of regeneration that projects a positive outlook for inducing regeneration in mammals. We also highlight the most promising areas of research in regeneration that may help to close the gap between tissue regeneration in reality and fiction.

2. Processes of regeneration in a nutshell

Although different regenerative pathways, processes, and regulation are nuanced and differ among organisms, a broad picture of the fundamentals can still be projected.

2.1. Ability of cells to differentiate to replenish lost tissue is critical for regeneration

Regeneration is highly dependent on either the ability of stem or progenitor cells to differentiate into the cell-types lost upon injury, of terminally differentiated, non-dividing cells in the vicinity of the wound to dedifferentiate, which allows them to proliferate to reform the lost tissue, or of cells to transdifferentiate from one lineage to a different lineage required to reform lost tissue (Figure 1) [9, 10, 11].

2.2. Regeneration can occur with or without cell proliferation

If a hydra sustains an injury away from its mid-gastric region, it recruits and rearranges endodermal epithelial stem cells from the edges of the wound, which differentiates to form the lost tissue without any cell proliferation— a type of regeneration called morphallaxis [12]. On the other hand, a salamander exhibits epimorphosis when it loses a limb. A thin 'curtain' of cells is formed over the wound by the migration of epidermal cells, and cells from below this epidermis is released from their extracellular matrix (ECM) *via* ECM degradation, resulting in the release of fibroblasts, Schwann cells, satellite cells, and skeletal cells. These cells dedifferentiate and proliferate to form a mass of heterogeneous cells at the regenerating end called the blastema [13] (*See* BOX 1 for an overview of the regeneration mechanisms in the hydra and the salamander). Planarians exhibit a third type of regeneration, described by Agata *et al.* [14] as intercalary regeneration. In this model, the anterior and posterior blastemas act as signaling centers to direct intercalary reorganization of the planarian body. After rearrangement of the body regionality, stem cells located in the mesenchymal space become fated to differentiate into appropriate cell types according to the newly acquired positional information.

Mammals can regenerate part of lost liver tissue *via* a type of epimorphosis called compensatory regeneration, in which regeneration proceeds *via* a progenitor cell/stem cell-independent manner, and involves the direct recruitment and proliferation of differentiated cells from the surroundings of the wound [15]. Organs like the kidney and lungs can

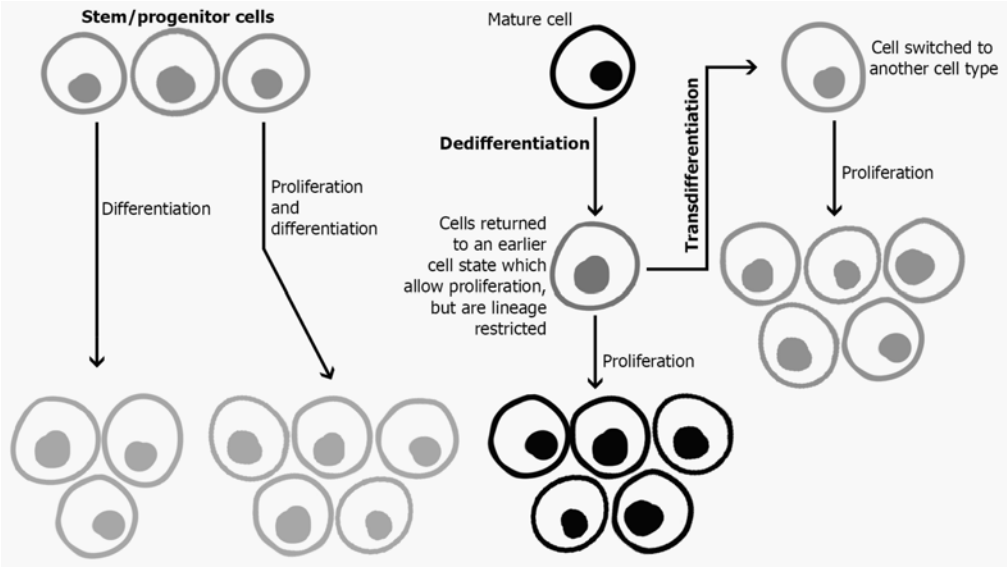

Figure 1. Modes of replacement of lost tissue during regeneration.

increase their quantity of functional tissue in response to injury or disease *via* compensatory hypertrophy which involves growth *via* increase in cell size, or *via* compensatory hyperplasia that allows growth *via* cell proliferation [16, 17].

2.3. The formation of a blastema is a hallmark of complex regeneration

The formation of the blastema is critical for regeneration in invertebrates like the planarians and hydras to primitive vertebrates like salamanders and zebrafishes. Blastema originates mostly from dedifferentiation of the surrounding cells, but the activation of local stem cells is also shown to be important [18]. Although the presence of a blastema is common, the mechanisms leading to the formation of a blastema is diverse, as is evident by the differences in limb regeneration in newts and axolotls—newt limb regeneration is mostly dependent on dedifferentiation to produce progenitor cells, while axolotls regenerate limb muscles from muscle satellite cells (a kind of stem cell) produced from the fragmentation of mature myofibers [18].

2.4. The blastema is not an absolute necessity for epimorphic regeneration

Regeneration of skin to close wounds, heart regeneration in the zebrafish, lens regeneration in newts do not involve the formation of a blastema [19, 20, 21]. It seems as long as proliferation can occur properly to produce the target cells, regeneration can occur.

2.5. The ECM is an important player in regeneration

In an interesting 2016 study [22], preparations of cell-free ECM from zebrafish healing hearts were able to recover cardiac function and promote regeneration in adult mouse heart tissues after acute myocardial infarction. When similar mammalian ECM preparations are used, fibrosis instead of regeneration occurs. The same study showed that the zebrafish ECM preparation also promoted proliferation of human cardiac precursor cell populations *in vitro*.

It is known that perineuronal nets, a specialized ECM structure that forms mesh-like structures around points of synaptic contacts, can be removed *via* degradation to restore plasticity and allow regeneration of function in damaged neurons [23]. It seems that somewhere down the evolutionary pathways, the ECM in mammals must have evolved away from its regenerative capabilities.

2.6. The precise regulation of the immune system is essential to avoid fibrosis and drive regeneration

While inflammatory pathways are essential in wound healing and fighting against invading pathogens, prolonged inflammation can severely

hinder regeneration, as shown in studies on salamanders, where enhancing the inflammatory pathways after limb amputation slowed or completely halted limb regeneration [24]. Regulatory T-cell (Treg), a type of T-cell that suppresses inflammatory responses, is essential in checking inflammation to promote healing in wounds, and is known to be essential in regeneration of the zebrafish heart [25]. Interestingly, animals with weakened adaptive immune responses, like the nude mice, and the *Acomys*, show increased regenerative abilities [24, 25, 26].

BOX 1

The incredible hydra

If a hydra is blended into individual cells, and those cells are then centrifuged to stick them together, whole new hydras can form from those lumps of cells! [1].

The epithelial cells of the body column of a hydra are continuously dividing, moving into the extremities, and getting either sloughed off or developing into buds to form new hydra [27]. They are made of three distinct cell populations (Figure 2). The ectodermal and endodermal epithelial cells are terminally differentiated at the head and the foot region, but are unipotent stem cells in the body column. The third lineage, the interstitial stem cells, found in the spaces among the epithelial cells of both layers is multipotent, and provide nerve cells, nematocytes, gland cells as well as germinal cells [12]. The axis patterning of the hydra body is governed by the head organizer (HO), a cluster of cells at the hypostome. The HO transmits two signals to the body column. One sets up a 'head activation' (HA) gradient in the body column, and the other is a graded distribution of the 'head inhibition' (HI) signal which prevents body column tissue from undergoing head formation [28, 29]. Upon amputation away from the mid-gastric region, foot regeneration undergoes a comparatively simple morphallactic process in which the epithelia from around the wounded area stretch to cover the wound, and the endodermal and ectodermal epithelial cells rearrange and differentiate without any proliferation or the involvement of the interstitial stem cells [12, 30]. In contrast, head regeneration is a complex process that relies on the activity of the HO, which forms within 8 hours of injury. Upon head amputation, activation of the MAPK pathway leads to the apoptosis of the interstitial cells, which activates the Wnt3 pathway and causes nearby interstitial stem cells to proliferate. The Wnt pathway also plays a critical role in establishing the HO from the epithelial stem cells of the body column, with its component genes such as the *HyWnt*, *Hyβ-cat*, *HyTcf* and *HyBra* playing important roles in patterning and reorganizing the HO. Although the proliferation of the interstitial stem cells may allow the head regeneration process to be categorized as epimorphosis, interestingly, it is shown that head regeneration can proceed, albeit at a slower pace, when the cell cycle is transiently halted or the interstitial stem cells eliminated [31, 32].

The king of vertebrate regeneration: the salamanders

Salamanders stand as the champion of regeneration among vertebrates, having the ability to regenerate parts of the eye such as the retina and the lens, regions of the brain and spinal cord, the heart, the jaws, the tail, and its limbs [2]. Upon amputation of the limb, a wound epidermis is formed from migrating epidermal cells that close the wound (Figure 3) [33]. The death of neutrophils at the cut end *via* apoptosis attracts macrophages, which plays an important role in limb regeneration by shifting the cytokine balance in favor of the anti-inflammatory subset, reducing inflammatory responses, and promoting ECM degradation that enable tissue remodeling [34]. Rapid dedifferentiation occurs that produces undifferentiated, lineage-restricted cells that arise from fibroblasts, Schwann, skeletal and myogenic cells. These cells re-enter the cell cycle and proliferate to produce the blastema [35]. As the blastema grows, the wound epidermis thickens to produce the apical epidermal cap (AEC). The AEC becomes innervated from an underlying nerve at the wounded end, inducing the AEC to become a signaling hub that produces a plethora of morphogens and growth factors, mediating the growth of the blastema [36]. Newt anterior gradient (nAG) protein secreted by the nerves act as ligands for the blastema cell-surface protein called Prod1, which shows a graded expression along the proximal-distal axis, hence implicating this protein as an important component for the determination of positional identity in regenerating tissues [36]. It has also been shown that stable FGF signaling is important for continual blastemal cell proliferation, and the expression of *FGF8* is supported by Hedgehog signaling from the posterior side of the limb [37, 38].

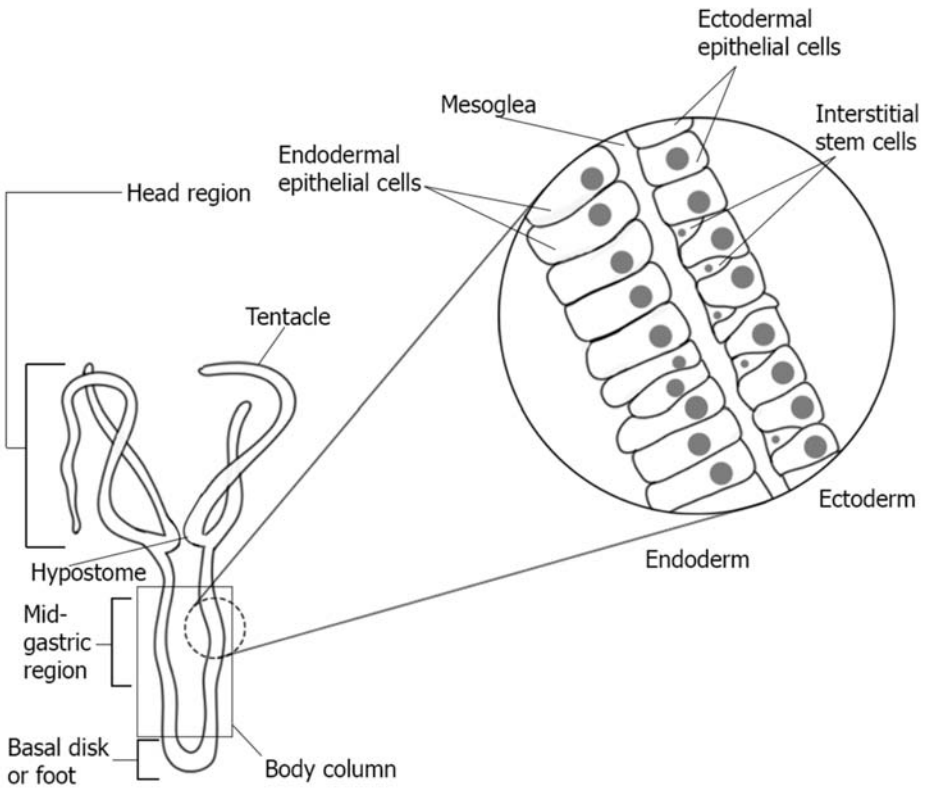

Figure 2. The hydra.

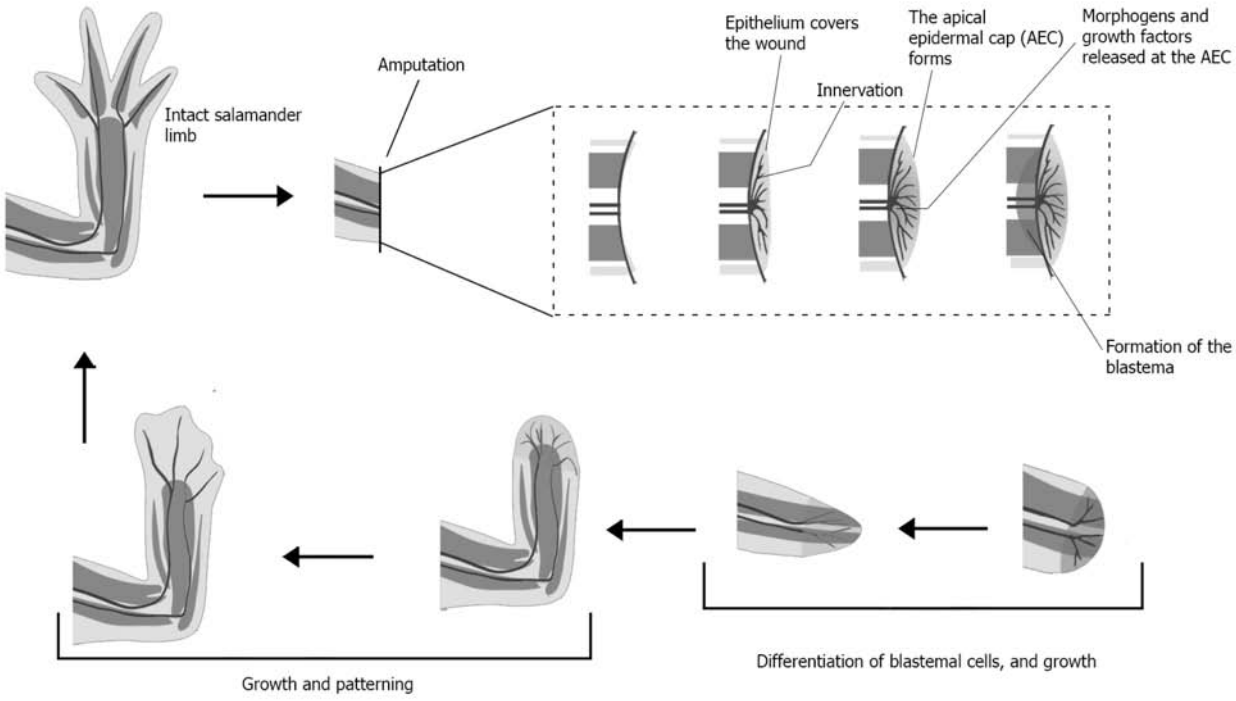

Figure 3. Overview of salamander limb regeneration.

3. Why can they do it, but not us?

3.1. Our cellular shortcomings

The ability of cells to proliferate into cell types of the lost tissue is indispensable in the process of regeneration. Invertebrates like the planarians and the hydra, and invertebrates like *Xenopus laevis* possess a large number of stem cells, and they all have immense regenerative abilities. Stem cells are rarely found in tissues of adult mammals, and tissues that do contain them, like the skin with its epidermal stem cells, and the blood cells with their hematopoietic stem cells, do exhibit regenerative capacity. But even without the presence of a plenty of stem cells, zebrafish and salamanders show remarkable regenerative abilities. They achieve this *via* dedifferentiation of cells to allow proliferation to regenerate lost tissue [39]. Dedifferentiation in mammals is rare [40, 41], and may be an important reason why regeneration in mammals is rare as well. Some tissues still possess the ability of dedifferentiation, like the renal epithelial cells of the kidney that allow restoration of lost cells upon kidney injury, and Schwann cells which enable peripheral nerve regeneration [42, 43]. Proteins that mediate the cell-cycle, like the retinoblastoma protein (pRB) and *p53*, could be, in part, responsible for the reduced abilities of dedifferentiation in mammals. After injury, terminally differentiated newt muscle cells can dedifferentiate after inactivation of pRB *via* phosphorylation, but mammals do not phosphorylate pRB upon injury, blocking the pathways to proliferation [44]. Similarly, downregulation of *p53* is essential during limb regeneration in salamanders, which does not occur in mammals [45]. Proteins like the pRB and *p53* are essential in maintenance of the cell cycle and suppression of tumor formation, but are implicated in mediating cellular senescence as well, which may contribute to age-related disorders [46]. Could it be that evolution drove the selection of strict tumor-suppressing mechanisms that imparted higher organisms with a more immediate defense against tumors, but at the cost of possible regenerative processes?

Regenerative animals also differ from animals with lower or no regenerative capacity at the genetic level. Blastema associated genes, such as the glycosylphosphatidylinositol-anchored protein coding gene *Prod1*, has no ortholog in mammals [47], and some genes like the *Fgf20a*, a fibroblast growth factor found in both zebrafish and mammals, promotes regeneration in zebrafish, but not in mammals [48].

Differences in DNA methylation levels and histone modifications may have important effects on regenerative capacity of an organism. Low DNA-methylation levels generally promote gene expression and are associated with regenerative capacity of tissues. Planarians show low levels of DNA methylation, which may allow many regeneration/pluripotency supporting genes to be hypomethylated and active [49]. The Murphy Roths Large (MRL/MpJ) mice show high regenerative capacity after injury of the ears and other organs, and exhibit some DNA methylation patterns similar to that of its embryonic stage [50]. DNA methylation also seems to regulate dedifferentiation capacity of tissues, as seen in Zebrafish [51]. Post-translational modifications of the histones are found to be important in the maintenance of pluripotency of cells in planarians and regeneration in zebrafish [52, 53]. Increased histone acetylation is also implicated in the high regeneration capacity of the peripheral nervous system compared to that of the CNS [54].

As discussed previously, the enhanced cellular and adaptive immune system of higher organisms impedes regeneration. In fact, the gradual loss of regenerative capacity in *Xenopus* is attributed to the maturation of the adaptive immunity [55].

3.2. The evolutionary perspective

Did regeneration evolve independently in different organisms? If so, maybe mammals were never under strong enough selection pressures to evolve regenerative capabilities, or in simple terms: they did not lose a limb frequently enough, and if they did, that did not hurt their survivability or reproducibility. On the other hand, it is well documented that organisms like the lizard very frequently loses its tail [56], which is an important appendage for the lizards partially because of its expendable nature that helps in escaping predators or distracting them with the wriggling of the detached tail [57], and may hence have evolved to regenerate it back. But the hermit crab, which

only exposes its first two legs, can regenerate the rest of the three legs protected inside the shell just as well [58]. It could be that processes of regeneration that evolved for the exposed legs are not bound to those legs exclusively, and can help regenerate the other three as well. However, it is also well known that lower vertebrates can regenerate internal organs like the heart. It is difficult to imagine persistent selection pressures that would challenge the ability of the heart to regenerate. Was regeneration then common in all organisms, and higher organisms lost the ability during evolution due to it being a selective disadvantage? The ECM might have forgone its ability to regenerate to allow it to support more complex structures and processes where mechanisms of regeneration could not co-exist. The immune system of more complex organisms may have evolved to provide better immediate protection against invading pathogens at the cost of regenerative options. This view is supported by the fact that the fundamentals of the mechanisms of regeneration are similar among organisms, especially among vertebrates [59].

4. Will superhero-like regenerative abilities always remain a fantasy?

Dendrocoelum lacteum, a planarian that is unable to regenerate its head, can successfully do so after inhibition of just the β-catenin signaling pathway [60]. The effect of simple inhibition of just one gene on the Wnt pathway among the thousands of genes required for regeneration in planarians shows that it may not be so outrageous to imagine regenerative abilities in mammals, and provides great stimulus towards the search for ways of inducing regeneration in mammals. At the molecular level, humans, and other 'non-regenerative' mammals show extensive regeneration in the form of replacement of membranes, proteins, blood cells, surface epithelia lining the gut and the airways, healing of wounds, and turnover of stem cells, which shows that the ways of regeneration is definitely not completely lost in higher organisms. Examples of more complex regeneration in mammals exist, which includes the remarkable complete regeneration of deer antlers annually. Antler regeneration does not involve cell dedifferentiation and a blastema. Instead, antler regeneration is a stem-cell-based process that initiates from the proliferation and differentiation of the pedicle periosteal cells, although the cells at the tip of the new budding antler resemble blastemal cells and are highly proliferative [61, 62]. Digit tips of monkeys and humans can regenerate upon amputation through the formation of a blastema which consists mostly of osteoblasts formed from bone fragmentation, which then proliferates and redifferentiates into new bone. The cells are lineage restricted, and their proliferation is mediated by Wnt signaling from the nail bed and factors secreted by Schwann cells [63, 64]. Probably the most impressive among the regeneration abilities of the *Acomys* is its remarkable ability of heart regeneration as a mammal, which is lost in the *Mus* after about a week of birth. After myocardial infarction, the *Acomys* can almost completely regenerate its heart back to normal [65]. And including the compensatory regeneration of the liver, the compensatory hypertrophy of the kidney and lungs upon injury and loss of tissue in mammals [16, 17], and the regeneration of the mouse pancreas after surgical removal of most of the tissue [66], the number of organs in mammals that can exhibit significant regeneration is not as few as one might think.

Another exciting facet of this topic, even if not as fantastical as the idea of spontaneous regeneration in humans, is regenerative medicine and therapy, which focuses on application of stem cells and/or progenitor to stimulate or promote repair and restore function in damaged body tissues or organs. This field has gained a lot of attention in recent years, and has made significant advancements. Multipotent adult stem cells derived from adipose tissue hold a lot of promise in cell-based therapy and tissue engineering, and multiple phase I or II clinical trials are underway testing the effects of injection of adipose-derived stem cells in different diseases including osteoarthritis, ischemic heart disease, limb Ischemia, amyotrophic lateral sclerosis, multiple system atrophy, and spinal cord injury (for a full review, see E. Ntege *et al*., 2020) [67].

5. Conclusions and Future Perspectives

The ability to spontaneously regenerate, or at least the ability to reliably and stably induce regeneration using stem-cell-based therapies would be

revolutionary for human healthcare, and although significant advancements have been made in the recent years in elucidating the mysteries of different regeneration processes and their underlying cellular and molecular mechanisms in various animals, much remains unknown. But with the hopes of one day being able to induce regeneration in humans, we suggest studies to be directed in a few key areas. In general, animals with higher number of stem cells possess higher regenerative capacity. It is, hence, important to understand the mechanisms by which stem/progenitor cells are sustained in such high numbers in regenerative organisms, and how they are activated to promote regeneration. Dedifferentiation, the process that confers vertebrates like the axolotls high regenerative capacity despite their lack of numerous stem cells, is scarce in mammals. However, mouse myotubes were shown to be inducible to allow dedifferentiation after they were treated with preparations of regenerating newt limbs [68]. If mammalian cells retain the potential to dedifferentiate, attempting to find ways to locally stimulate dedifferentiation to allow regeneration could be exciting. Mammals also seem to have lost the ability of transdifferentiation, but it is well known that mammalian cells still possess the ability to transdifferentiate *via* numerous tissue-reprogramming experiments [69], and hence exogenous stimulation of this ability holds promise in regeneration therapies. Many genes responsible for regeneration are either lost or silenced in the course of evolution or development, and hence the reactivation or reintroduction of these genes or the application of the proteins these genes code for may give rise to regenerative abilities in mammals. Also, being able to create or induce a microenvironment that supports regeneration after injury is critical for the success of any attempts at implementing regeneration in therapy, as exemplified by the low success of the stem-cell-based therapies that exist today. The inverse relationship of a more developed and complex immune system with the ability to regenerate in organisms implicates that the immune system plays a crucial part at blocking regeneration, and hence attuning the immune response to injury at the right moment by just the right amount should be looked at when developing strategies for regeneration.

Overall, as discussed before, there exist a significant number of tissues and organs that can regenerate in mammals within the small number of mammals studied. Numerous experiments and studies have been conducted on the regenerative abilities of simple invertebrates like the planarians, and vertebrates like the salamanders, *Xenopus,* and zebrafish. However, it is much more difficult to work with, and conduct experiments on, mammals largely due to the costs involved, and the ethical issues. For example, the antler regeneration in the deer is a remarkable feat of regeneration in mammals, but conducting experiments on them is difficult, especially when many of the species are endangered. Nevertheless, it is not unreasonable to argue that among the approximately five and a half thousand species of mammals, there could be more mammals with regenerative abilities. Search for more mammals with regenerative abilities, and large scale comparative phylogenetic analysis of regenerative and non-regenerative mammals is essential to understand when and how in development and evolution particular animals lost or gained the capacity to regenerate. And since there exists many shared features of regeneration among different animals [59], with the basic underlying requirement being proliferation or dedifferentiation to replace lost tissue, both of which either exist or is inducible in models of mammalian regeneration, one can hope that someday induction of regeneration in humans would be possible, and superheroes would need to find some other kind of superpower to keep us interested.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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