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Differences in regenerative capacity between organisms: an evaluation of the underlying mechanisms

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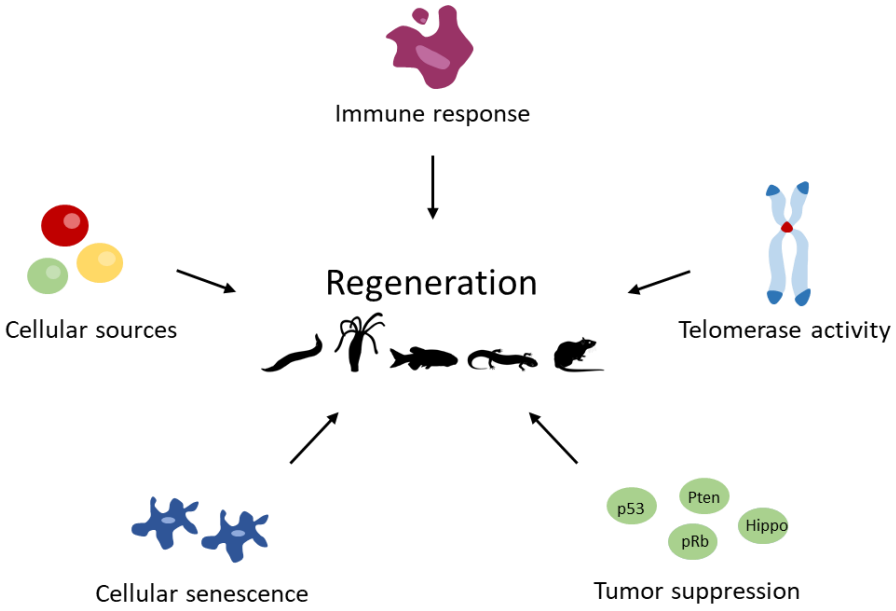
Abstract

The capacity to regenerate varies greatly within the animal kingdom. Invertebrates like planarian flatworms and Hydra are very good regenerators, while mammals are only able to regenerate a limited amount of tissue and show a lot of scar formation. How the regenerative capacity can differ so profoundly between organisms has fascinated the scientific community for many years. In this review, several mechanisms and processes that might underly the differences in regenerative capacity amongst organisms will be discussed. It focusses on the cellular basis of regeneration, immune responses, expression of tumor suppressor genes, telomerase activity and cellular senescence. A better understanding of the role of these processes in regeneration could be useful for the development of new therapies to stimulate regeneration in mammals.

Graphical abstract



Contents

Introduction

Regeneration is the regrowth of damaged or missing body parts. The capacity to regenerate varies greatly within the animal kingdom, and in particular between invertebrates and vertebrates (Tanaka & Reddien, 2011). Invertebrates such as planarian flatworms and Hydra have a very high regenerative capacity. They are capable of regenerating a new head, tail or even their entire body from only small body fragments (Tanaka & Reddien, 2011). Primitive vertebrates such as the zebrafish and salamander cannot regrow a whole organism, but have the capacity to regenerate complex structures. For example, the zebrafish is able to regrow the heart, brain, fin or even its retina (Gemberling, Bailey, Hyde, & Poss, 2013) and salamanders can also regenerate complete appendages, such as the limb, after injury (Kragl et al., 2009). Mammals have a rather limited regenerative capacity. Although mice and young children are capable of digit tip regeneration (Han, Yang, Jangwoo, Allan, & Muneoka, 2008; Illingworth, 1974), regeneration in adult humans is mainly limited to skin and liver cells and often goes together with scar formation (Iismaa et al., 2018).

Why some organisms have a better capacity to regenerate than others, especially humans, is of great interest to scientists. Although people have been doing research into regeneration for a long time, the rise of regenerative medicine caused an increasing interest in understanding the mechanisms and elements underlying regeneration, for these might be crucial for developing new therapies and promote regeneration in humans. One important underlying mechanism is the generation of new cells for the formation of new tissue. Depending on whether an organism is able to do this and to what extent, determines how quickly and how well it can regenerate. Also the immune response appears to have an effect on the regenerative capacity of organisms (Godwin & Rosenthal, 2014). With evolution, the adaptive immune response arose and the capacity to regenerate decreased. This suggests a correlation that might explain differences in regeneration ability. Tumor suppressor genes are present in both invertebrates and vertebrates and are known to be involved in cellular processes like cell proliferation (Pomerantz & Blau, 2013). Differences in expression of these genes could be one of the reasons why invertebrates regenerate but higher vertebrates don't. Another process which is involved in cell division and proliferation is telomere shortening (Shay & Wright, 2005). Telomere shortening leads to cell cycle arrest and inhibits cell proliferation, which is crucial for regeneration. The expression of telomerase prevents this from happening, and could underlie regeneration capability. A permanent cell cycle arrest leads to the formation of senescent cells. Senescence is known to be involved in ageing, but recent work also shows an important role of senescent cells during tissue repair, as their secretory phenotype induces proliferation of surrounding cells (Demaria et al., 2014).

This review of contemporary knowledge concerning biological regeneration aims to uncover the workings of the aforementioned mechanisms, and shed light on what causes the difference in regenerative capacity between organisms. First, the cellular basis of regeneration in both highly- and poorly regenerative animals will be described, making use of well-known regeneration models. Subsequently, the effect of the immune response, tumor suppressors, telomerase activity and cellular senescence on regenerative capacity will be examined.

Cellular sources for regeneration

New cells have to be generated in order to form new tissues. The generation of new cells can be achieved by activation of stem cells or progenitor cells, dedifferentiation of specialized cells to undifferentiated progenitor cells, or even the direct conversions of one differentiated cell into another, called transdifferentiation (Eguizabal, Montserrat, Veiga, & Belmonte, 2013). Which one of these mechanisms is used to yield new cells during regeneration is dependent on the organism and the tissue of origin.

Cellular sources for regeneration in invertebrates

One of the best studied regeneration models is the planarian flatworm. Planarians are found in both saltwater and freshwater ponds and have the capacity to regenerate a whole new organism out of small body fragments (Morgan, 1898). The source for this regeneration is a population of somatic dividing cells, called neoblasts, that exist throughout the whole planarian body. This population is composed of a heterogeneous pool of cells which are either lineage-restricted or pluripotent (Van Wolfswinkel, Wagner, & Reddien, 2014). The pluripotent neoblasts are called clonogenic neoblasts (cNeoblasts) and can give rise to any cell type within the body. Even after lethal irradiation, a single cNeoblast is capable of restoring regeneration (Wagner, Wang, & Reddien, 2011). After wounding, neoblasts proliferate and accumulate at the site of injury to form a blastema, out of which the new tissue will grow (Figure 1a)(Reddien, 2018).

Like planarians, Hydra also have a highly regenerative capacity and are able to form an entire organism out of small body fragments (Bosch, 2007). Hydra are freshwater hydrozoans that belong to the phylum Cnidaria. They have a polarized, primary body axis with a head and tentacles on one side and a foot on the other side. Hydra are composed of two cell layers: an ectodermal and an endodermal layer, separated by an extracellular matrix (Bosch, 2007). They possess three different cell types: ectodermal epithelial cells, endothermal epithelial cells and interstitial cells which are located in the interstitial space between the epithelial cells (Bosch, 2007). Interstitial cells are proliferative multipotent stem cells and generate neurons, gametes and secretory cells (Bosch & David, 1987). The epithelial cells in the body column continuously self-renew and therefore can be considered as stem cells which contribute to the regeneration of the ectodermal and endodermal layers (Figure 1b)(Wittlieb, Khalturin, Lohmann, Anton-Erxleben, & Bosch, 2006). Although Hydra have three types of cells, only the epithelial cells are needed for tissue regeneration (Marcum & Campbell, 1978). During regeneration, epithelial cells are activated and mobilised towards the damaged tissue (Wittlieb et al., 2006). In contrast to planarians and many other organisms, blastema formation does not take place, but the pre-existing epithelial cells are rearranged and form newly organized structures (Bosch, 2007).

Cellular sources for regeneration in vertebrates

Although vertebrates are not capable of full-body regeneration, primitive vertebrates such as the zebrafish are widely used for studying vertebrate tissue regeneration. Zebrafish live in freshwater and have the potential to regenerate complex tissues like their heart or brain (Gemberling et al., 2013). Especially their remarkable ability to perform appendage regeneration, such as the tail or fin, is a phenomenon that has been studied extensively. The zebrafish fin is build up of multiple tissues such as blood vessels, nerve fibers and bone tissue. After amputation, a blastema is formed at the tip of the fin, composed of lineage-restricted progenitor cells (Tu & Johnson, 2011). Depending on which of the tissues is formed, these progenitor cells are generated by either dedifferentiation of specialized cells

or the activation of stem cells (Figure 1c). For the regeneration of bone tissue in limbs and fins, mature osteoblasts dedifferentiate and become proliferative, but stay lineage-restricted. Subsequently, they migrate towards the blastema where they redifferentiate into new osteoblasts (Knopf et al., 2011). Regeneration of skeletal muscle in the zebrafish tail does not depend on dedifferentiation, but rather on the activation of muscle stem cells (satellite cells) (Berberoglu et al., 2017; Rodrigues, Christen, Martí, & Izpisúa Belmonte, 2012).

Another well-studied model for complex tissue regeneration is limb regeneration in salamanders. As in zebrafish, a heterogeneous blastema is formed upon limb amputation, consisting of lineage-restricted progenitor cells (Kragl et al., 2009). Also in these animal, both dedifferentiation of mature cells and activation of stem cells take place (Figure 1c). Where in zebrafish no dedifferentiation is observed during skeletal muscle regeneration, it is observed in some salamanders. Sandoval-Guzmán et al. (2014) showed that in newts, dedifferentiation of myofibers is essential for the regeneration of muscle tissue. While in axolotls, satellite cells are activated upon amputation and no dedifferentiation occurs (Sandoval-Guzmán et al., 2014). In addition, salamanders possess some tissues that have the potential to transdifferentiate. They are one of the few animals that are capable of complete lens regeneration. After removal of the lens, dorsal pigmented epithelial cells transdifferentiate into lens cells and a full new lens is regrown (Stone, 1955).

Regeneration in higher vertebrates like mammals is very limited. Some tissues do show regeneration, but this often goes together with the formation of scar tissue (Iismaa et al., 2018). An example of a tissue that shows regeneration is the liver. The liver regenerates by the proliferation of existing liver cells; cells divide but maintain their differentiated function. So instead of forming a blastema and regrowth of the excised parts, the remaining liver tissue expands (Miyaoka et al., 2012). Other tissues that are capable of regeneration are the intestine and the skin. These tissues possess multipotent stem cells which are capable of differentiating into various cell types needed for restoration of the wounded tissue (Figure 1d)(Iismaa et al., 2018). However, skin repair often results in scar formation. Although the protective barrier is restored, it lacks structures such as hair follicles and sweat glands, which are required for proper skin function. Also dedifferentiation is very rare in mammals, only showing dedifferentiation and proliferation of Schwann cells during peripheral regeneration (Chen, Yu, & Strickland, 2007). Despite higher vertebrates being poor regenerators, there are a few examples of mammalian regeneration that resemble regeneration of lower vertebrates and in which blastema formation does take place. One example is the digit tip regeneration in mice. It is shown that regeneration happens by the formation of blastema-like cluster consisting of lineage-restricted proliferating cells (M. Han et al., 2008; Rinkevich, Lindau, Ueno, Longaker, & Weissman, 2011). Still, regenerated digits were shorter than unamputated digits and the degree of regenerative success was very much dependent on the site of amputation, which makes it less efficient than limb regeneration in salamanders. It has also been documented that young children can regenerate their fingertips after amputation (Illingworth, 1974), but how this is done remains largely unknown.

Comparing these various regeneration models, teaches us that there is a difference in the cellular basis underlying regeneration in highly- and poorly regenerative organisms. Highly regenerative invertebrates like the planarian flatworm and Hydra make use of a large amount of highly proliferative stem cells during regeneration, while regeneration in vertebrates is mainly due to blastema formation of a small number of lineage-restricted progenitors. This is one of the most prominent reasons why invertebrates are capable of regenerating an entire body out of small body fragments and vertebrates are not. The pluripotent stem cells of planarians can differentiate in all types of somatic cells, including

germline cells (Newmark, Wang, & Chong, 2008). In vertebrates, in contrast, there is a distinction between germline and somatic stem cells and in addition they are in need of tissue-specific stem cells for each of their tissues to regenerate. Also the body complexity of invertebrates is lower than in vertebrates. Hydra have two main tissues, each containing continuously proliferating stem cells. Vertebrates and especially mammals have a lot more different tissues and not all of these tissues possess abundant resident stem cells. Though, primitive vertebrates such as zebrafish and salamanders, are able to generate new cells by dedifferentiation of specialized cell, but the capacity of mammals to do this is very rare. Therefore, more knowledge about how dedifferentiation is induced might be crucial for increasing the regenerative potential in mammals.

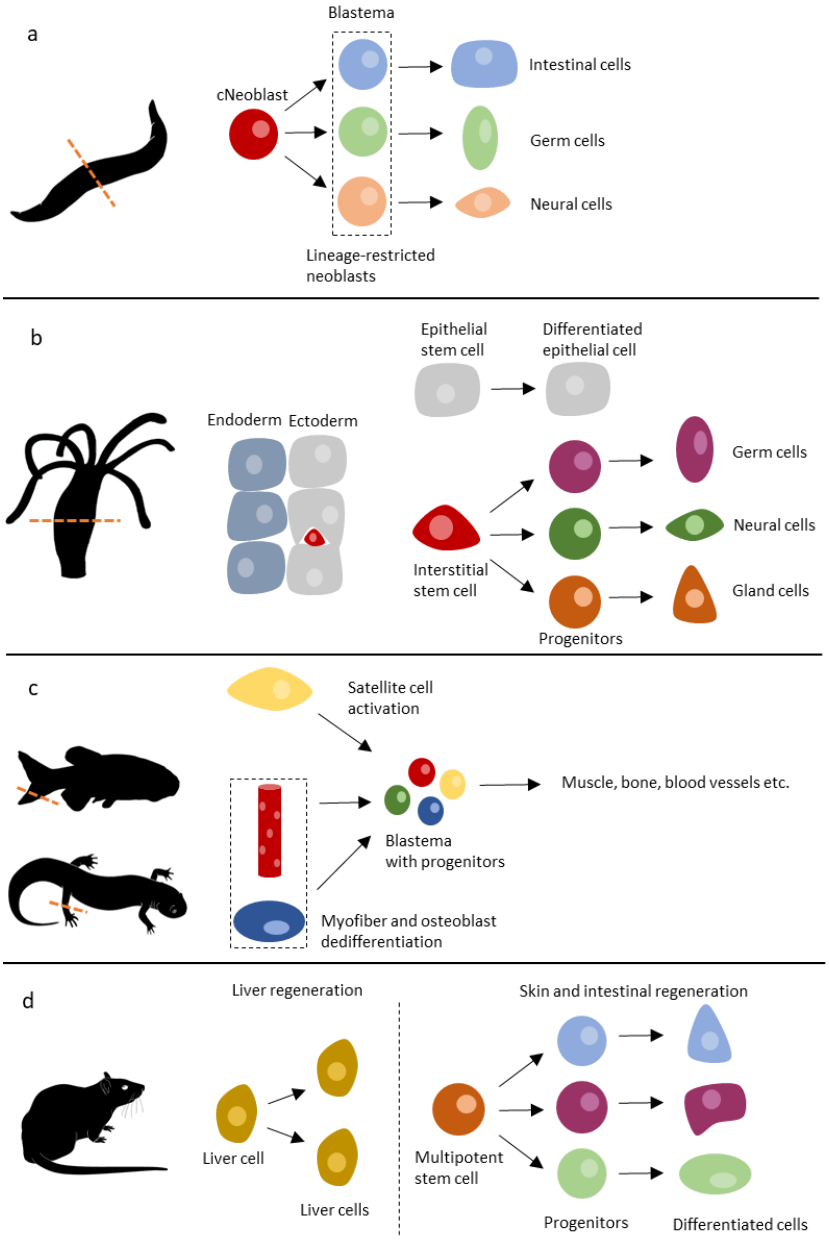


Figure 1. Schematic representation of the cellular sources for regeneration in different regeneration models. (a) Planarian regeneration after bisection. Pluripotent and lineage-restricted cells are activated and differentiate. (b) Hydra regeneration after bisection. Epithelial cells are rearranged and form the endodermal and ectodermal layers. Interstitial stem cells are activated and form the other cells. (c) Zebrafish and salamander appendage regeneration. Specialized cells dedifferentiate or stem cells are activated and form a pool of lineage-restricted progenitor cells after which they differentiate into a specific tissue. (d) Regeneration in mammals. Differentiated liver cells divide and expansion of the remaining liver tissue takes place. During skin and intestinal regeneration, multipotent stem cells differentiate into various cell types.

More advanced immune response causes a decrease in regenerative capacity

Looking at these regeneration models, we can say that the regenerative capacity of organisms generally declined during evolution (Bely & Nyber, 2010). Together with evolution, the complexity of the immune response against microorganisms increased (Matsunaga & Rahman, 1998). This suggests that there could be a correlation between these two and that a more developed immune response might deplete regenerative capacity.

Protection against pathogenic microorganisms during injury is crucial for wound repair and survival in all individuals. Even in the most simple organisms, coexistence of the immune system and regeneration has been observed. Research showed that in planarian flatworms, phagocytic cells surround the area of injury after making a small incision (Morita, 1991). This suggests that planaria possess an innate immune system, which is activated after wounding. Also other important molecules associated with the innate immune response, such as pathogen recognition receptors, complement factors and cell adhesion molecules, have shown to be present in planarians (Peiris, Hoyer, & Oviedo, 2014). The activation of these molecules during regeneration implies that they are involved with the protection of damaged tissue.

In addition to the innate immune response, vertebrates developed the adaptive immune system. In these animals the immune response consist of two components: the non-specific innate immune system provides the first response against invasive cells, after which the more slower and specific adaptive immune system is activated. The development of adaptive immunity is closely linked to a decrease in regenerative capacity, not only in evolutionary aspect but also in an age-dependent manner. An animal not yet discussed, but which also has a high regeneration competence, is the Xenopus frog. It is shown that the ability of the Xenopus to regenerate its tail decreases with age and goes together with the excessive production of collagen and connective tissue (Dent, 1962; Wolfe, Nye, & Cameron, 2000). The immune system of larval frogs is still not well developed, while the adult Xenopus immune system is comparable to that of mammals, showing both a cellular and humoral immune response (Robert & Cohen, 1998). In contrast to Xenopus frogs, the regenerative capacity of salamanders is maintained throughout their whole life (Eguchi et al., 2011). Salamanders possess a strong innate immune system, but their humoral immune response is very slow and lacks memory cells (Tournefier et al., 1998). Another indication for a weaker adaptive immune response in salamanders is their ineffective clearance of certain viral infections, while Xenopus frogs are able to do so (Cotter, Storfer, Page, Beachy, & Voss, 2008).

Like the development of the adaptive immune system in primitive vertebrates, the inflammatory response became more developed in higher vertebrates. Vertebrates with less well-developed adaptive immunity, such as the zebrafish and salamander, show minimal inflammatory responses to injury. In mammals, a high inflammatory response often goes together with tissue atrophy or fibrosis. With higher inflammatory response, more macrophages are recruited to the site of wounding. Macrophages stimulate the proliferation of fibroblasts and myofibroblasts that produce collagen, which ultimately leads to the formation of scar tissue (Ploeger et al., 2017;Wynn & Barron, 2010). However, macrophages do not only have a negative effect on regeneration. Aurora et al., (2014) showed that neonatal mice with a depletion of macrophages are unable to regenerate their hearts and form fibrotic tissue. Also during appendage regeneration in zebrafish and salamanders, macrophages are needed for proper healing (Godwin, Pinto, & Rosenthal, 2013; Petrie et al., 2014). An explanation for this difference in macrophage function might be that there are two distinct types of macrophages: M1 and M2. M1 macrophages are pro-inflammatory and stimulate fibrosis, while M2 macrophages are

anti-inflammatory and stimulate growth and regeneration (Ploeger et al., 2017). It is shown that after spinal cord injury in mice, there is an upregulation of M1 macrophages, while there is a loss of M2 macrophages, which goes together with an impaired recovery (Kigerl et al., 2009). This indicates that the evoked macrophage response might differ between highly- and poorly regenerative vertebrates and that in higher vertebrates it are mainly M1 macrophages that are stimulated.

These findings all show that the regenerative capacity is indeed strongly associated with immunological responses. In both highly- and poorly regenerative animals, the innate immune response is crucial for stimulation of tissue growth and restoration after injury. In higher vertebrates, the adaptive immune response causes an inflammatory response which can lead to production of collagen and ultimately scare formation. An explanation for the development of the adaptive immune system in vertebrates could be the need for better protection against injuries and infections. Adaptive immunity arose together with jaw structures in primitive fish, which indicated a more predatory life style. Ingested food might be harmful to their intestines and lead to infections (Matsunaga & Rahman, 1998). To combat invading pathogens, a quicker and more specific immune response might have been needed and evolutionary more favourable. The transition of aquatic to terrestrial animals might be a reason for the development of a more severe inflammatory response. On land, there is a higher diversity of microorganisms, which leads to a higher chance of getting an infection (Whitman, Coleman, & Wiebe, 1998). Therefore, the need for quicker wound healing to prevent wounds from getting infected got bigger. It is likely that the rapid growth of tissue to form a protective barrier at the site of injury was advantageous. In addition, in the case of mammals, this response probably proves beneficial as a lower rate of reproduction causes a higher need of a quick and effective immune response. Thus, rapid repair of injuries to protect against infection could have been more favoured with regard to survival than the restoration of lost structures to maintain complete function.

Alterations in tumor suppressor pathways during evolution

Tumor suppressor genes are so called because of their inactivation in mammalian tumors. They also have important functions in fundamental cellular processes like the response to DNA damage, apoptosis, cell proliferation and growth (Pomerantz & Blau, 2013). Regeneration of complex tissues goes together with cellular proliferation, which is a process that should be strictly regulated. Therefore, tumor suppressors might be crucial for proper regeneration because they provide negative control of cell proliferation by inducing cell cycle arrest. On the other hand, the impediment of proliferation could also inhibit regenerative processes because activation and proliferation of stem cells is needed to generate new tissues. Differences in tumor suppressor pathways between invertebrates and vertebrates, could be related to their ability to regenerate.

There are multiple tumor suppressor pathways, each containing one or more tumor suppressor genes. Four important tumor suppressor pathways are p53, retinoblastoma, Pten and Hippo signalling. These pathways all appear to be evolutionarily ancient and are present in both highly- and poorly regenerative organisms (Pomerantz & Blau, 2013). The p53 family exists of three members: p53, p63 and p73. The origin of the p53 gene is a p63/p73 ancestor gene which was first observed in Hydra. Here, the gene has mainly a germline protective function, as it responds to DNA damage after ultraviolet irradiation by causing apoptosis in germ cells but not in somatic cells (Pankow & Bamberger, 2007). In vertebrates, expansion of the p53 family takes place by duplication of the p63/p73 ancestor. In addition to the germ line protection of the p63/p73 hybrid, the p53 gene focusses on surveillance of DNA damage in somatic cells (Belyi et al., 2009). It is likely that this extra tumor suppressor gene

was needed because besides germline stem cells, vertebrates also possess distinct somatic stem- and progenitor cells that need protection against duplication errors to prevent them from developing in potential cancer cells.

The appearance of a new p53 gene shows that there have been alterations in the tumor suppressor pathways during evolution. It is possible that these modifications underlie the differences in regenerative capacity between invertebrates and vertebrates. Another evolutionary alteration is the emergence of the tumor suppressor Arf in non-regenerative vertebrates (Hesse, Kouklis, Ahituv, & Pomerantz, 2015). Arf stabilizes p53 by inhibiting Mdm2. Mdm2 normally binds to p53 and inhibits its transcriptional activity. Therefore, Arf is very important for cell cycle control and loss of Arf might lead to unlimited cell proliferation. Arf orthologs have not been found in highly regenerative organisms. Notable is that human Arf expression in zebrafish, supresses their capacity to fulfil fin regeneration after amputation (Hesse et al., 2015). Another tumor suppressor gene that does not exist in planaria, but does in mammals, is p21 (Pearson & Alvarado, 2010). p21 is a target of p53 and is an inhibitor of cyclin dependent kinases (CDKs). These kinases are important for cells to complete full cell cycles and inhibition of CDKs by p21 leads to cell cycle arrest (El-Deiry et al., 1993). Bedelbaeva et al. (2010) showed that mice that are capable of regeneration after ear injury have a downregulation of p21. Moreover, deletion of p21 in nonregenerative mice improved their healing potential. p21 is a strong regulator of the G1 cell cycle checkpoint. In the mice lacking p21, they observed an accumulation of cells in het G2/M phase. Hydra have also been shown to have a high number of cells in the G2/M phase (Buzgariu, Crescenzi, & Galliot, 2014), which could suggest that they also lack the p21 gene. These findings all indicate that the appearance of some tumor suppressor genes in higher vertebrates has a negative impact on their regenerative capacity.

In addition, the function of several tumor suppressors were shown to be different in invertebrates and vertebrates. In planaria, the p53 homolog Smed-p53 is necessary for proper stem cell function and regeneration as it controls proliferation and promotes differentiation (Pearson & Alvarado, 2010). To the contrary, for limb regeneration in salamanders a down-regulation of p53 is required (Yun, Gates, & Brockes, 2013). A similar contrast is noticed for the tumor suppressor gene Pten. The Pten homolog Smed-Pten is upregulated during regeneration in planaria, while in mice the regenerative response is enhanced by deletion of Pten (Liu et al., 2010; Oviedo, Pearson, Levin, & Sánchez, 2008). When Smed-Pten was supressed in planaria, this resulted in inappropriate proliferation of neoblasts, abnormal tissue growth and failure of regeneration.

Although the core tumor suppression pathways are present in both invertebrates and vertebrates, there are crucial distinctions that might underly the differences in their regenerative capacity. Striking is that some tumor suppressor genes are needed for regeneration in invertebrates, while they hinder regeneration in vertebrates. As discussed in the first paragraph, planarians form a regeneration blastema of hyperproliferating neoblasts. After proliferation these cells lower their activity and differentiate to form new tissue. Considering the necessity of Smed-p53 during this process, the initial function of tumor suppressors might be to support regeneration by monitoring appropriate cell proliferation and differentiation. With the evolution of long-lived and larger animals, like vertebrates, the need for more strict regulation of cell divisions became higher. Larger animals have more somatic stem cells per tissue that have the potential to accumulate mutations and also the available time for cells to undergo mutations is higher. Therefore, they have a high risk of developing cancer. Extension of the p53 tumor suppressor pathway with Arf and p21 has shown to be only present in poorly-regenerative organisms. This suggests that evolutionary selection has enhanced tumor

suppression at the expense of regeneration. How the evolved mechanisms to fight tumors impede regeneration in mammals is something that should be examined in more detail in future research.

Telomere length is maintained in highly regenerative organisms

Telomeres are short repetitive sequences of DNA, which are located at the end of chromosomes (Blackburn & Gall, 1978). Their main function is to prevent chromosomes from deterioration or end-to-end fusion with other chromosomes. However, DNA polymerase is unable to fully replicate the ends of DNA strands during cell division, which leads to loss of DNA and telomere shortening. When telomere lengths become too short, the cell will go in cell cycle arrest (Shay & Wright, 2005). Telomerase is a reverse transcriptase which is able to add telomere sequences to the chromosomes and in this way prevents telomere attrition (Greider & Blackburn, 1985). Activation of telomerase is strictly regulated in most multicellular organisms, because it allows for indefinite proliferation. Because regeneration involves a lot of cell divisions and proliferation, loss of telomere length might be a reasons for a lower regeneration potential.

Telomerase activity has shown to be upregulated in organisms with strong regenerative abilities. A recently introduced regeneration model is the freshwater annelid *Aeolosoma viride.* This ringworm also exhibits a great regenerative capacity and can form a whole new body out of three or more body segments. During regeneration, they show an upregulation of telomerase expression and maintenance of telomere length. Although telomere attrition is often observed during ageing, *A. viride* does not show any telomere shortening during their lifespan (Chen, Sung, Chen, & Chen, 2018). Also lower vertebrates like zebrafish have a constitutively high telomerase activity throughout their whole lives (Kishi et al., 2003). Their efficiency of tissue regeneration seems to be related to the expression of telomerase. Anchelin et al., 2011 showed that fish with higher telomerase expression have a higher ability to regenerate their fin upon amputation. Also their telomere lengths increase after amputation, even when this is repeated multiple times. Another study showed that telomerase is activated after wounding in lizards. This indicates that telomerase expression is needed to trigger cell proliferation and blastema formation (Alibardi, 2016).

In animals that have a low regenerative capacity, like mammals, telomerase activity is much lower. In mice, telomerase only exists in limited tissues like the liver and testis (Prowse & Greidert, 1995). When focussing on regeneration, it was shown that telomerase is very important for liver regeneration in mice. Depletion of telomerase in liver cells leads to an increase in collagen deposition and liver fibrosis and hinders regeneration (Lin et al., 2018). New-born mice are able to regenerate their heart to a limited extend until the age of 7 days (Porrello et al., 2011). A recent study showed that after birth, the telomerase activity rapidly declines together with regenerative ability and that telomerase-negative new-born mice are not able to properly regenerate but instead produce fibrotic tissue (Aix, Gutiérrez-Gutiérrez, Sánchez-Ferrer, Aguado, & Flores, 2016). In humans, there is an upregulation of telomerase during embryonic development. However, after birth, the expression is limited to only high proliferating germline cells and adult stem cells, while somatic cells lack telomerase activity (Kim et al., 1994).

Why telomerase activity is primarily a feature of highly-regenerative organisms remains unanswered. The explanation could be closely related to that of the evolution of tumor suppressor genes. Telomerase upregulation is present in more than 90% of human cancers (Kim et al., 1994). As was already discussed in the previous paragraph, cancer cells arise by accumulation of mutations. However, before enough mutations have accumulated to obtain malignant characteristics, a lot of cell

divisions are needed. With every cell divisions the telomere ends become shorter. To prevent the formation of cancer cells, short telomeres might have become a DNA-damage signal which induces cell cycle arrest. So in long-lived species like humans, repression of telomerase in somatic tissues may have been evolved to reduce the probability of cancer. It is proposed that the mechanism by which short telomeres inhibit regeneration, is the induction of cell-cycle arrest via tumor suppressor genes. The aforementioned telomerase-negative mice that were poorly able to regenerate heart tissue, had higher levels of the cell-cycle inhibitor and tumor suppressor gene p21 (Aix et al., 2016). It is shown that this p21-induced cell-cycle arrest eventually leads to cellular senescence. There has been additional evidence that telomere shortening is responsible for cellular senescence, as the expression of the catalytic component of telomerase in telomerase-negative somatic cells allows them to divide indefinitely (Bodnar et al., 1998). So telomeric shortening might inhibit regeneration in mammals via tumor suppressor genes and cellular senescence, a phenomenon that will be discussed further in the following section.

Effective clearance of senescent cells is crucial during tissue repair

Cellular senescence is a permanent state of cell cycle arrest in which cells lose their ability to proliferate. It is induced by various types of stress stimuli like tumor suppressors and telomere shortening, but also genotoxic stress or inflammatory cytokines. Cellular senescence therefore acts as an effective anti-tumorous mechanism (d'Adda di Fagagna, 2008). One of the characteristics of senescent cells is that they secrete cytokines and growth factors which gives them their senescence-associated secretory phenotype (SASP). It appears that senescent cells and SASP have positive effects on regeneration. Work of Demaria et al., 2014 revealed that in response to cutaneous wounds in mice, senescent fibroblasts and endothelial cells induce myofibroblast differentiation by secretion of certain growth factors. Also the elimination of senescent cells in wounds delays tissue repair, whereas the consecutive treatment with specific growth factors again initiates myofibroblast differentiation and tissue repair. Similar findings were obtained in structural remodelling of pericardial interstitial cells derived from patients with pericarditis, and during heart regeneration in mice (Bednarek et al., 2015; Han et al., 2017). It has also been shown that senescence occurs during embryonic development in mammals and is involved in several developmental processes (Muñ oz-Espín et al., 2013). Moreover, the loss of senescent cells resulted in morphological abnormalities. There also appears to be an important role for macrophages in this process, as they infiltrate the developing tissue and clear the senescent cells. This induction of cellular senescence and the subsequent clearance of senescent cells by macrophages has also been shown to be present during limb regeneration in salamanders (Yun, Davaapil, & Brockes, 2015).

Senescent cells are also known to be involved in ageing, as they accumulate with time and cause several age-related diseases (Demaria et al., 2014). Mammals have shown to lose their regenerative capacity with ageing, while invertebrates and salamanders can regenerate throughout their whole life (Eguchi et al., 2011; Porrello et al., 2011). In contrast to the formation of senescent cells during ageing in mammals, Hydra do not show any sign of senescence during lifespan (Martínez, 1998). This implies that Hydra possess mechanisms that prevent accumulation of DNA damage and therefore the formation of senescent cells. Highly regenerative organisms like the zebrafish show a very gradual senescence during ageing, but this is almost negligible when compared the senescence in mammals (Kishi et al., 2003). These findings suggest that during ageing, senescent cells have a negative

effect on regeneration, as they limit regeneration in older animals and are not present during ageing in highly regenerative organisms.

From these studies, it can be stated that senescence is a double-edged sword. On one hand senescent cells seem to be beneficial during embryonic development and tissue repair, but become detrimental during ageing. So at some point, there is a shift between this good and bad phenotype. What underlies this shift seems to be the ineffective clearance of senescent cells. The initial, positive, role of senescent cells is the secretion of various SASP factors These SASP factors activate the proliferation of surrounding cells which are needed for tissue repair. SASP is also important for the extensive recruitment of immune cells, like macrophages, which have shown to be crucial for the removal of senescent cells. Demaria et al., 2014 showed that during tissue repair, senescent cells are only transiently present, while in aged and chronically damages tissue there is a persistent presence of senescent cell. This indicates that in aged and severely damaged tissues, senescent cells are not properly cleaned up, after which they accumulate and impair tissue functioning. It could be that lower vertebrates, like salamanders, have a more effective way of senescent cell clearance during regeneration than mammals and humans. This is inextricable linked to ageing, as we do see clearance during embryonic development in mice. During pericardial remodelling in humans, the main function of senescent cells is to induce an inflammatory response. There is also a recruitment of monocytes, but here they predominantly induce fibronectin and collagen production (Han et al., 2017). How it can be that Hydra do not show any senescence during their life, is not yet clear. As discussed, Hydra have stem cells that constantly proliferate and replace existing cells. However, since they have a constant proliferation of stem cells, one would expect that the potential for mutations is also larger. Therefore, it is assumable that they possess mechanisms that prevent damage accumulating over time or that they can remove these cells very efficiently.

Conclusion

The evaluation of various mechanisms and processes that underly regeneration in both highly- and poorly regenerative animals, has helped to come to a better understanding of why regenerative capacity differs between organisms. During regeneration, invertebrates use pluripotent or multipotent stem cells, which can differentiate in both germline cells and somatic cells. Vertebrates only possess lineage-restricted progenitor cells that have limited differentiation potential, which restrains their regenerative capacity. In order to create enough new cells, lower vertebrates are able to dedifferentiate specialized cells, a trait that does not occur in mammals. Furthermore, vertebrates developed the adaptive immune system in addition to the innate immune system that was already present in invertebrates. Especially the humoral immune response seems to be involved in lowering the regenerative capacity in higher vertebrates, as e.g. this response is very slow in salamanders. Phagocytic macrophages have shown to be critical during tissue repair, but might have a more pro-inflammatory phenotype in mammals which leads to fibrosis instead of tissue growth. Also the processes that lead to cell cycle arrest, like tumor suppression and telomeric shortening, are present in a higher extend in vertebrates. The tumor suppressors Arf and p21 are only present in mammals and suppress regeneration. Also the function of several tumor suppressors is different in invertebrates and vertebrates. A mechanisms to prevent telomeres from shortening is telomerase activity. The expression of telomerase is primarily a feature of highly-regenerative organisms and only exists in germline cells in humans. When cells reach a permanent state of cell cycle arrest, they become senescent. Senescent cells are needed for regeneration because they secrete SASP factors that active

surrounding cells. However, they are only beneficial if they are properly cleared afterwards by macrophages and appear to have a negative effect on regeneration during ageing.

Taken together, it seems that the development of more advanced protection mechanisms in higher vertebrates limits their capacity to regenerate. There is an higher control of cell proliferation due to the presence of more extensive tumor suppression mechanisms and the absence of highly proliferating stem cells. Although each of the processes mentioned in this review show an effect on regenerative capacity when looking at them individually, the findings suggest that these processes might also have an underlying relationship and that one process reinforces the other. Telomeric shortening leads to DNA damage, which is a trigger for the activation of tumor suppressor genes. Tumor suppressor genes cause cell cycle arrest, which ultimately leads to the formation of senescent cells. If these cells are not properly disposed of by macrophages, they will accumulate and eventually impair tissue functioning and regeneration. The alteration of one of these processes, could therefore also influence the other processes in a positive manner, which might eventually enhance regeneration in mammals.

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