Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
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W1L4_Tissue Regeneration: Mammalian Context

Hello, everyone. We will start with another session of regenerative biology. And this section is about tissue regeneration in a mammalian context. And we know that mammals have a lot of advantages when it comes to survival on the planet. But when it comes to regeneration, mammals are often found to be little debilitated compared to non-vertebrates. So mammals, in general, are excellent in a lot of their protection against pathogens, as they have supreme immunity, etc.

But when it comes to regeneration, it often lags behind in many complex tissues. Simpler tissues do regenerate like skin or nails, and if there is a bone fracture, they can heal, etc. But when it comes to complex tissues, such as the brain, spinal cord, heart, and kidney, it struggles. So let us see what the possible causes behind it are.

So, the regenerative phenomenon is widespread, but the regenerative capacity varies greatly among animals. In vertebrates, that means the ones which do have a backbone, vertebrate groups are mainly five, starting from fish, frogs, reptiles, birds, and mammals. They are the five main groups of vertebrates. So phylogenetically primitive vertebrates, such as salamanders, zebrafish, and mainly fish and amphibians, are included. They possess a higher regenerative capacity than mammals.

Even in the same individual, different tissues or organs have distinct regenerative capacities. For example, in our body, the liver can regenerate excellently, but the heart or spinal cord lags behind. In addition, the younger animal is usually easier to regenerate than the older animals. Decades of research are beginning to explain why regenerative capacity differs markedly among different cellular and molecular components and can also have some evolutionary implications. Exploring the reasons for differences in regenerative capacity has resulted in finding causes such as the properties of stem cells, the type of tissue in question, the ability to de-differentiate or trans-differentiate for a given tissue cell, the expression of specific regeneration-associated genes, and various epigenetic regulators.

And how the immune system contributes to regeneration. So the comprehensive analysis of these perspectives would allow us new insights into how to promote regeneration in mammals. So this picture you can see is a schematic of regeneration in various animals. Say planaria; you can take a planaria and cut it into pieces, as many pieces as you want. Each piece will give rise to, say if this is cut into one, two, or three pieces, give rise to three planaria.

In the same way as a hydra, it has only two germ layers. It's another primitive invertebrate. Planaria is also an invertebrate. It can cut into any number of pieces, and it regenerates. Now, coming to vertebrates, lizards' tails or limbs—you would have seen that if you cut the limb, it grows back, or even the tail.

You may have seen a wall lizard shedding its tail and growing it back. When it comes to Xenopus, normally, if you cut a tail, limb, or any other body part, it normally regenerates. And in zebrafish, it has been extensively studied for heart regeneration, retina regeneration, spinal cord regeneration, brain regeneration, etc. In this picture, heart regeneration is shown. In the case of mice, the liver is the only complex organ that can regenerate; mice are a representative of mammals.

A few tissue-specific stem cells are preferentially preserved in certain high-turnover tissues of adult mammals. What does it mean? Say your intestinal epithelial cells and skin epithelial cells are prone to damage. They are blessed to have stem cells underneath. So if an old skin is shed, the new skin comes underneath. So, for example, human skin, the blood system, you know, and bone marrow stem cells constantly keep producing your blood cells.

They have the capacity to regenerate, which is largely due to the reserve epidermal stem cells in the case of skin. Hematopoietic stem cells are also present inside your bone marrow. Unfortunately, most adult mammalian tissues have few or no resident stem cells to support them. This is likely one of the major limiting factors for regeneration. We have an explanation for why there is no regeneration, but are these explanations sufficient? It is almost like telling, "Why are you climbing the Himalayas?" The answer is yes; it is there.

That is why I am climbing. That is not the answer. In the same way, stem cells are not present. That is not the answer because, when it comes to lower vertebrates, they are regenerating in spite of not having dedicated stem cells. underneath their respective tissue.

If, however, the small number of resident stem cells in adult mammalian tissues could be stimulated and recruited, it would be possible to promote regeneration. So this is the conjecture that one has to test. For instance, the mouse can achieve digitative regeneration by stimulating a few distinct lineage-restricted progenitor cells, and it can form a blastoma-like structure, so that even if your finger is cut, if the nail bed is preserved, then there is a good possibility that the fingertip can grow, but it may not be as long as your digit; however, if the nail bed is not restored, then the chances of the fingertip appearing or growing back are limited. So let us understand what the differentiation potential is, a new terminology you are learning in a simplistic sense. I'm sure all of you who are taking this class have completed your plus two, and maybe you have completed your bachelor's or even master's.

But let us take a bracketed group. All of you who are taking this class would have completed your plus two, which means you are all 12th pass. Now, if you are asked to go and sit in your kindergarten, say UKG, KG, Class 1, or Class 2, what do you do? Understanding means you are erased of all your knowledge or whatever learnings have happened over these many years, and you have been asked to learn right from kindergarten, such as the alphabet, numbers, counting, writing, etc. Something like that is in a simplistic form: the differentiation that is going backward in time. Mammals fail to regenerate tissue after the amputation of their bones.

Although internal bone defects can be healed if they are below a critical size. So if the bone has severe damage, it cannot heal. But if there is a fracture, there is a crack, and when you put these bone parts together, they grow. In mammals, what happened? The de novo osteoblasts derived from the mesenchymal stem cells contribute to the bone healing process without osteoblast de-differentiation. This is what we observed in the case of mammals.

The scarcity of differentiation in mammalian osteoblasts may be the underlying reason why the bone cannot regenerate. If you say you removed a bone, new bone cannot form, or the damage to the bone is very severe. It cannot be fixed. So there can be a limited amount of damage to the bones. It can heal; rather, it can fuse.

So, retina regeneration in zebrafish is a good example, and even chick neonates soon after birth can regenerate their retina; however, in mammals, this often fails and depends on the de-differentiation of the molecular pathways. The differentiation of malaria occurs, although the mammalian retina has a much lower regenerative capacity than that of zebrafish and chick. What does it mean? It simply means that they do not know the exact mechanism or the exact processes through which it can reprogram or differentiate. This is probably ascribed to the lower differentiation potentials in malaria in mammals. than in zebrafish and chickens.

Having said that, you should also understand less capability means less instructions are gone on to that Mullerglia. Mullerglia is a cell, and it is ready to express any gene, but it is not being given the proper instructions. You should read in that way. Not that the muller blade cannot be reprogrammed, it does not reprogram. So that is what we should understand.

Although most mammal tissue cells lose de-differentiation potential, several cells retain this ability; for instance, Schwann cells undergo de-differentiation for peripheral nerve regeneration. Say you got a burn wound and new skin is being formed; for a few days, you will not have sensation, but after a few months, you will regain your ability to feel touch if someone touches you. In the same way, people who get leprosy lose their sensation in the skin because the Mycobacterium leprae damages the peripheral nervous system, but if it is healed, it can bounce back thanks to Schwann cells, which are the glial cell population of the peripheral nervous system. Although mammalian cells cannot undergo natural de-differentiation after injury, de-differentiation can be induced in vitro. Mouse myotubes are induced to de-differentiate and proliferate after treatment with extracts from the regenerating limbs of newts following ectopic expression of one transcription factor.

It's called the MSH homeobox, also known as MSX1. So it can be induced. This indicates, like I told you, it's not that it cannot, but it does not. De-differentiate naturally. This indicates that mammalian cells like myotubes have the potential to de-differentiate, although this potential needs to be stimulated or induced.

Why do the cells of primitive vertebrates such as fish and frogs undergo de-differentiation more easily than what you see in the case of mammals? That is the question. Although the specific mechanisms are not clearly understood, cell cycle regulators play a major role. They are found to play an essential role in controlling the differentiation process. Terminally differentiated means from stem cell to the final differentiated cell. Finally differentiated cells are called terminally differentiated.

Newt myotubes, that is, newt means basically frog. Amphibian species, myotubes can de-differentiate after injury because of the tumor suppressor retinoblastoma, RB. RB proteins are phosphorylated, and they are very important for regulating your cell cycle, thereby allowing cells to re-enter the cell cycle. Means cells that are arrested in the cell cycle, which is normally called G0 arrest, can re-enter and will then become G1. However, mammalian myotubes do not phosphorylate RB proteins after an injury and fail to re-enter the cell cycle.

And one of the causes of why it is not able to de-differentiate. This suggests that RB phosphorylation may be a crucial barrier to mammalian muscle de-differentiation. In support of this, do we have any evidence? Transient inactivation of RB and the alternative reading frame ARF tumor suppressor, another protein, forces the mammalian myotube to reenter the cell cycle and lose its differentiation properties, which means myotubes can be pushed into the differentiated phase if you inactivate the RB protein. If it is not happening spontaneously, then you can forcefully inactivate it and push them into the cell cycle. RB and another RB family member, P130, can block cell cycle genes and maintain the post-mitotic state of the mammalian adult cardiomyocytes.

Knockdown of RB and P30 together leads to cell cycle re-entry in adult cardiomyocytes of mammals. As a cell cycle inhibitor, p53, one of the well-studied and famous tumor suppressor proteins, also hinders differentiation, which means that during salamander limb regeneration, early downregulation of p53 is a prerequisite, or essential. For mesenchymal cell de-differentiation and blastema formation. In our own lab's research, we have seen another tumor suppressor called P10, phosphatase and tensin homolog, spontaneously get downregulated during retina regeneration. If you prevent that, then you won't get retinal regeneration.

So what does it mean that tumor suppressor cells also contribute to making the cell de-differentiate? Because cell cycle inhibitors block de-differentiation in mammalian cells, targeted modification of these inhibitors will likely promote de-differentiation and regeneration. In addition to these cell cycle regulators, several epigenetic factors also contribute. They control the differentiation and maintain the differentiated state. They are also important. If any tweaking happens to these epigenetic factors, it can push them into de-differentiation.

So sometimes a tissue needs to be maintained in a differentiated status. So, epigenetic factors do contribute. It can be histone modifications such as histone methylation, histone acetylation, etc. Targeting the epigenetic regulators has been applied to facilitate differentiation. For example, the forced expression of transcription factors or treatment with small molecules that can change epigenetic regulators such as DNA methylation or histone modification.

Result in the complete dedifferentiation of the somatic cells into pluripotent cells. So, these are some of the interesting observations that are being shown by artificially tweaking the scenario for a mammalian cell. Accordingly, the dedifferentiation potential may be enhanced artificially by targeting cell cycle regulators or epigenetic regulators. So then comes the transdifferentiation potential. What is transdifferentiation? The differentiation we have seen is going back in time.

That means an intermediate student is going to kindergarten. Now you're not going all the way to kindergarten. You are, say you are a Bachelor's student who has studied biology for your BSc; now you are going up to plus two, and instead of studying BSc biology, you are now going into medicine, or you are going into LLB, or you are going into engineering. That means it appears like a person who was studying BSc biology has now become a lawyer or an engineer. That is called a transdifferentiation; you have to go back in time, but there is no need to go all the way back in time.

So, for example, nudes and frogs can completely regenerate their lenses via cellular transdifferentiation. In nudes, once the lens is removed, pigmented epithelial cells from the dorsal iris—dorsal means top, ventral means down—are observed. So in your back and front, if you look at that angle, you can see the dorsal-ventral concept in the book. Basically, if I want to express your dorsal and ventral, your back is your dorsal and your chest is your ventral in simplistic terms. The dorsal iris transdifferentiates into lens cells and regrows the entire lens if you remove the lens from your eye.

That is the part that is removed during cataract surgery; if your lens becomes opaque, you can't see, and in cataract surgery, you remove the opaque lens. Similarly, the frog lens can regenerate through the transdifferentiation of the corneal epithelium into lens cells during the larval stage. In contrast, the mammalian lens only has the ability to achieve incomplete regeneration from lenses of epithelial cells without the transdifferentiation of other cells. Mammals lag behind here too. Therefore, the loss of natural transdifferentiation in mammals impedes complete lens regeneration.

However, mammalian cells retain the transdifferentiation potential that needs to be improved. Followed up further and see whether we can make it through an exogenous stimulus. Recent reprogramming strategies have been extensively done to confirm that latent trunk differentiation in mammals can happen. Somatic cells, such as fibroblasts, can be induced into another lineage; for example, neurons, cardiomyocytes, hepatocytes—anything you want can be converted by several reprogramming approaches. And this includes the production of a lineage factor-based reprogramming, and we also call them iPSCs, induced pluripotent stem cells.

This is basically directing a fibroblast or a normal cell into a stem cell. These reprogramming approaches, especially small molecule-mediated reprogramming, will offer very meaningful results. Opportunities that allow for the deliberate transdifferentiation of one cell type into another either in vitro or in vivo. So this is a picture that shows how transdifferentiation is done or how reprogramming is done. You take a somatic cell and can convert it back due to various signaling events using either small molecules or transcription factors.

Ideally, you can make iPSC-based reprogramming, where you convert the somatic cell into iPSCs, which can give rise to any cell through differentiation, or lineage factor-based reprogramming, where you directly convert the somatic cell into cardiomyocytes by expressing cardiomyocyte-specific factors in it, or neurons. Cardiomyocytes can regenerate, so this is very much possible in vitro, but in vivo the research is still ongoing. The regenerative species depend on the ability of adult stem cells in their bodies or have a huge potential to undergo differentiation and transdifferentiation in adult cells; this is the hallmark of regeneration, and mammals often lag behind. However, a few mechanisms are lacking in most adult mammals, limiting their regenerative capacity, such as the activation of stem or progenitor cells. We know progenitor cells are needed, but are they able to make it? The answer is no.

The reversion of differentiated cells into their progenitor, called de-differentiation, does not happen effectively. The conversion of one tissue into another is called strand differentiation. So these are the limiting factors. When it comes to mammalian regeneration. The selected expression or silencing of regeneration-associated genes also affects regeneration, and the genes might be possessed or expressed exclusively in a regenerative species, but not in a non-regenerative species such as the mammoth.

In recent years, accumulating evidence has strongly suggested that epigenetic regulators exert enormous influence on the so-called regeneration-associated gene expression. They can modulate the epigenetic landscape, and they can influence regeneration. And let us see what the regeneration-aiding genes are. Almost all animals can heal wounds, but only some can regenerate.

We know that. One hypothesis is that certain regeneration-specific genes are expressed exclusively in regenerating species. Let us see whether it is true or not. One salamander-specific gene, say PROD1, which encodes the glycophosphatidylinositol-anchored protein. It has been found to support this hypothesis. What does it say? Prod1 is expressed in the blastoma and is essential for patterning and growth during salamander limb regeneration.

More importantly, no ortholog of Prod1 has been found in Xenopus, zebrafish, or mammals. So what does it tell you? This Prod1 is a unique feature found in salamanders, which can regenerate a damaged organ. Therefore, the regeneration gene is specifically possessed by salamanders. One could argue that it is not always the case, but this is one example of a gene. Another possibility is that non-regenerative and regenerative species both carry certain regeneration genes, but these genes promote regeneration only in a regenerative species; the same gene does not promote regeneration in a non-regenerative species.

So this is another possibility. Let us see whether there is any evidence that this possibility is verified by specific expression of. Growth factor FGF20A in zebrafish. FGF20A is expressed early after fin amputation and initiates fin regeneration. By contrast, the ortholog of the same gene, FGF20A, in mammals is not associated with regeneration.

It is not turned on. It is not contributing to regeneration. It is worth mentioning that selective expression of regeneration genes affects the regenerative capacity of different tissues and even in the same animal. So we cannot generalize that this gene must help in regeneration or that this gene is essential for regeneration. Newt lens, which we already discussed, can regenerate from pigment epithelial cells of the dorsal iris, but not from the ventral iris. Both are irises; one is dorsal and one is ventral. What is the difference between them? One study has shown that this situation is due to a deficiency of one lens-specific regeneration gene, that is, sign oculus-related homeobox, also known as 6-3.

In the ventral iris, the dorsal iris has 6-3, but the ventral iris doesn't have it. If you remove the lens, 6-3 is required for lens development during embryogenesis but is only expressed in the dorsal iris after the removal of the newt lens. Then what did people do? If you express 6-3 in the ventral iris through transfection, it generates the lens. You don't need a dorsal iris. If you get rid of the dorsal iris, the ventral iris can give rise to all it wanted, which was the 6-3 gene.

Altogether, certain species and tissues, like you said about the dorsal and ventral, are both iris, but there is a difference between dorsal iris and ventral iris. Have high regeneration and have a special regeneration-specific gene in a given species or tissue after injury. So now let us see the immune responses. The more phylogenetically primitive Urodial amphibians, such as salamanders, appear to have weaker cellular and humoral immune responses regarding specificity, speed of onset, and memory compared to that of an anurine amphibian. That is normally frogs that live on land compared to their siblings or related members who live in water.

In response to the larval stage, it is relatively ancestral and much less well-developed than that of the post-metamorphic stage, which is highly evolved. As the organism evolves, it has a better-matured immune response than the larval stage. In mammals, the transition from fetal scarless wound healing to adult typical scarring is accompanied by a gradual increase in inflammation, which is contributed to by immune cells and various pro-inflammatory cytokines. These studies indicate that animal regeneration or an animal's ability to regenerate develops. A more advanced immune system is not allowing you to have a proper regenerative response.

The immune response does not always hinder regeneration. One should not think that if the immune system is powerful, you can't get regeneration. But there is a catch to it. Successful regeneration, in effect, demands a proper immune response. You should know when to give acceleration and when to give the brake; this is what is understood. For example, immune responses are indispensable to salamander limb regeneration and neonatal mouse heart regeneration.

If you interfere with the immune system, both cannot regenerate at all; that means the immune system is needed. Common functions of the immune responses in regeneration include scavenging the cellular debris whenever there is an injury, activating the progenitor cells, and promoting angiogenesis, which means the formation of blood vasculature. Many immune cells, such as cytokines and components, are engaged in these processes. Among them, the macrophage response is very classical; they play an important role in the regeneration after amputation of the axolotl limbs. Macrophages are recruited early into the regenerating blastema; in contrast, the systemic depletion of macrophages leads to failure of full limb regeneration.

And it leads to extensive fibrosis, as you can see in the case of damage to a human limb or a mouse limb. So tremendous strides have been made in delineating the regeneration process and the cellular and molecular mechanisms of regeneration in animal models. After comparing many aspects of animal regeneration, it can be suggested that several possible reasons exist to explain why regenerative capacity differs among animals. Those reasons may account for the low regenerative capacity observed in the case of mammals, and they can provide a novel avenue for promoting regeneration in mammals.

Because adult mammals have insufficient stem cells. One can always tell why it is not able to regenerate effectively. The induction of differentiation and transdifferentiation is crucial for obtaining cellular sources for regeneration. You want to make a building; you need to have bricks. And for regeneration, the bricks are nothing more than cells. In high stability of adult mammalian cells, they can prevent changes in their cell states, which considerably restrains their de-differentiation and trans-differentiation potentials.

So many research studies have shown that in a neonatal pup, a postnatal chicken, the regenerative capacity is tremendous, whereas the same animal, the same species, once it differentiates, the immune system advances; all the immune system is necessary, and the advanced immune system makes the environment rather more pro-inflammatory, and this can interfere with the ability to. regenerate effectively. So what we should understand from here is that as organisms grow older, the macrophages we already discussed consist of two types: M1 and M2. M1 is always pro-inflammatory, whereas M2 is always anti-inflammatory.

The levels of M2 macrophages deplete as the organism grows older and older. M2 macrophages are very low in the case of diabetes patients, which could explain why they have too many ulcers and difficulty in healing even a simple wound. It is not regeneration. So we'll study more on regeneration in the next class. Thank you.