Regeneration Biology
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W1L3_Regeneration: In Normal Life and Implications

Hello everyone, welcome back for another lecture. We will continue with where we left off, and this section is about regeneration in normal life and its implications. This is quite interesting, as we will compare how regeneration contributes to your life and how different animals that have the capacity to regenerate, perform the regenerative response. Mechanisms of tissue regeneration, like we saw in labile tissues, have to increase their number through rapid proliferation, and this proliferation must lead to the differentiation of various tissues. Whatever damaged area had differentiated tissue must be replaced. They will perform this task as long as the underlying basement membranes are intact.

That means every cell present in a tissue has a matrix, which is often referred to as the basement membrane, that must stay intact if you want a scarless regenerative response. The growth factors involved in this process are not very well defined, although we know a lot about them; we cannot say that only these factors are enough. It is just like if someone asks you a question, you can provide the answer. But does that mean you know the answer to it all? Does that mean you said everything you know? All we know is that if you ask a question, you have the answer to it.

Similarly, if the tissue is capable of regeneration, the adequate growth factors that will be induced will be discussed in upcoming classes, which are the growth factors. But their induction is very important; the loss of blood cells is corrected by the proliferation of hematopoietic stem cells in the bone marrow and other tissues, driven by growth factors called colony-stimulating factors, or CSF. See, many of you would have given blood donations. They take one unit of blood from your body at a time. So soon after that there is a deficiency.

Blood is the connective tissue in your body, and if there is a deficiency of cells, it will immediately trigger CSF production in the bone marrow, leading to enhanced proliferation within a few days. Your blood supply is restored, which is produced in response to the reduced number of blood cells. It can happen due to the menstrual cycle in females and due to an accident, such as a road accident, where you lost some blood or went to a blood donation camp. So tissue regeneration can occur in parenchymal organs with a stable cell population, but with the exception of the liver, this is usually a limited process. We have also seen this multiple times in the previous slides.

The pancreas, adrenal gland, thyroid, and lungs have some regenerative capacity. However, the surgical removal of a kidney elicits in the remaining kidney a compensatory response that consists of both hypertrophy and hyperplasia of the proximal ductal cells, so what does it mean that your body now lacks one kidney, and the other kidney cannot become an added mass of both the kidneys, however, causes the other kidney to automatically start performing better because there is a demand and a need for both kidneys to function, so the other kidney rises to the challenge. The mechanism underlying this response, hypertrophy and hyperplasia—hypertrophy means the existing cells become larger. Hyperplasia means the existing cells will multiply to increase in number; that is hyperplasia, and definitely, this involves the local production of growth factors and the interactions of the cells with the ECM. ECM means extracellular matrix, so every cellular response must involve an ECM-cell interaction.

The extraordinary capacity of the liver to regenerate has made it a valuable model for studying this process; we have a dedicated section that is coming up for liver regeneration, but the liver is a very classic organ when it comes to regeneration. Cells in the ECM, especially the fibroblasts, macrophages, and other cell types, mean their ECM produces growth factors and cytokines. Cytokines can be both pro-inflammatory and anti-inflammatory; pro means it is favoring inflammation, while anti means it is inhibiting inflammation. Both are needed, just like brakes and acceleration in your car. And chemokines.

What are chemokines? Chemokines allow the cells to know which direction to migrate. So chemokines provide directionality for the cells. So it acts like a route map that is critical for the regeneration and repair. So the first thing is that you need raw material. And then you need these cells, which are the raw materials, to be pasted or located in a proper place.

Although repair is a healing process, it may itself cause tissue dysfunction. For instance, in the development of atherosclerosis, many of you would have heard about atherosclerosis, which is the formation of some blockages in the blood capillaries. But why does this happen? Some internal lining, the endothelial lining, plays a role. It can get partially damaged, and now the body is trying to repair and fix it. During this process, there will be a lot of scar formation, and on top of that, a lot of lipids will come in.

Finally, your blood capillaries' diameter becomes narrower and narrower, and it can lead to blockage. So, atherosclerosis happens as a result of the repairing process; your cells or tissues were getting repaired, and that created a serious trouble. Like in a very simple example, I can tell you, say you got some injury on your face, and I am putting bandage after bandage around your nose and eyes and everywhere. What will happen is a huge structure forms on your face, and that can even affect your breathing, so your survival will be in question. So understand, sometimes the repair process can lead to an organ or tissue dysfunction unintentionally.

So if you can see here, this picture shows normal homeostasis. Homeostasis means housekeeping function; that is, the balance of proliferation and apoptosis. That means there is production and depletion. When cells are overproduced, say you need 100 cells, but somehow you ended up making 120 cells, then those 20 cells have to be killed. That is done by apoptosis.

So, proliferation is the process of the production of cells. In a normal homeostasis situation, if there is an injury, the body can respond in multiple ways; one way is regeneration. Regeneration occurs in renewing tissues, where complete regeneration happens, such as in the epidermis, respiratory epithelium, hematopoietic system, etc., and in stable tissues such as the liver. A part is amputated, as you can see here; this amputated plane and a huge chunk are removed.

You have a leftover liver, as you can see here, but it does not replace this lost part; rather, it undergoes compensatory growth. Like I gave an example in the previous class, if you have five fingers in your hand and three fingers are chopped off, they are never coming back. The existing two fingers occupy the space of those three fingers, so this is called compensatory growth. When it comes to repair, if the tissue is incapable of regeneration, then will indulge in repair, and say you have a wound; then it will lead to wound healing by scar formation. Like you can see here, there was a wound, it got filled with some junk, and it doesn't have the proper color of this tissue, or the color is basically for our understanding.

It is replaced by some other tissue, and then comes another scenario where there is chronic inflammation. What does it mean? There is no proper injury, but rather it has a scenario that mimics the injury; that means soon after the injury, there is an inflammation that is helpful for the regenerative or repairing response. However, in this case, you have chronic inflammation; that means, for example, if there are hungry people or hungry mouths, there are food complements. But there are zero hungry people; there are people who have stomachs full of food taken, but now you have a food supply. Then what will happen? It will give an unwanted response because there is no damage, but there is a response to damages already present that is chronic inflammation due to various environmental or pathological conditions.

That situation can lead to fibrosis because there is too much production of collagen or ECM proteins, and it leads to fibrosis. Coming back to repair and regeneration, repair begins. by an early inflammatory response called inflammation. Regeneration refers to the growth of cells and tissues to replace the lost structure. As liver and kidney growth occur after partial hepatectomy, renalectomy, or unilateral nephrectomy, there will be compensatory growth.

This response occurs through two processes: one is a regenerative response, and the other is fibrosis. Remember, liver damage, kidney damage, or amputation can often lead to a partial regenerative response and can also lead to fibrosis, depending on the condition of the patient. If it is vulnerable to inflammation, then fibrosis is an assured or most probable

outcome, so the repair normally involves fixing the damage; the regeneration of injured cells by cells of the same type is what we often refer to as regeneration, as with the regeneration of skin and oral mucosa, which requires a proper basement membrane. We have already discussed that the basement membrane is formed by secreted ECM proteins and the replacement. By the fibrous tissue that is fibroplasia or scar formation first, once the regeneration has occurred, if the regeneration is not complete or the regeneration is inadequate, in that situation, you will have the fibrous tissue that will be formed, and both these processes, both regeneration and replacement, require cell growth, differentiation, and cell matrix interaction.

So this is the picture of the basement membrane. All of you would have seen this picture in your school days, where you have an epithelium that is the outermost surface of an organ. We often refer to it as epithelium; it has multiple areas like the lateral surface, apical surface, etc. It also has a nucleus and supplies neuronal connections and blood vessels, etc. Just below that epithelial layer, you have the basal lamina.

Below that is the reticular lamina; you can see they are slightly different. These cells contribute to the basal lamina, and the bottom cells-connective tissue contributes to the reticular lamina. Both the basal lamina and reticular lamina together are called the basement membrane. As long as this basement membrane is intact, that means in the case of a superficial wound, say a scratch wound, a mosquito bite, etc.

, or an ant bite, etc. Then the connective tissues are not disturbed, the basal membrane is fine, and the stem cells can contribute to the growth properly. So the basal lamina from the epithelial cell, which is basically composed of collagen fibers, and the reticular lamina are secreted by the connective tissue underneath, or reticular fibers. And their functions basically guide cell migration during development, which is the formation of that organ, and may become thickened due to the increased collagen and lamin production. As you grow old or the tissue ages, there is a thickening of the basal lamina. For example, in the case of diabetes mellitus, the basement membrane of small blood vessels, especially those in the retina and the kidney, becomes thickened.

Normally, it is a condition-dependent phenomenon, and the basement membrane can have different morphologies. So during tissue regeneration, which is basically controlled by the biochemical factors released in response to cell injury, cell death, or mechanical trauma. So there has to be a proper response if tissue regeneration is to happen; the affected area must respond to that injury; only then can it consider either repair or regeneration. The most important control is by inducing the resting cells to enter the cell cycle, that is, G0 cells into G1. This is the first direction or way of moving forward.

A balance of stimulatory or inhibitory factors is needed, and it has to have a shortened cell cycle; it cannot take a whole day or a whole year to proliferate; it has to be fast and decrease the rate of cell loss. This means the produced cells should not disappear; they should be

present. All these things work together, and you end up getting a regenerative response. Healing is usually a response of tissue. You would have heard healing many times.

Healing is a response that occurs on the outermost surface of the injury. That is what is called healing. When you say healing, it means that the open nature of the wound is gone. But it's actually not fixed. Underneath, the trouble is still present.

So, a wound that is commonly on the skin also leads to healing and an inflammatory process in the internal organs, and it can occur in a necrotic condition, like when someone slaps you or you fall down somewhere; there is no bleeding, but there is internal damage, so some cells will die by necrosis. And it's in all these scenarios when the healing is not properly organized, they are incapable of proper regeneration, so the healing often results in a fixing of the trouble rather than a proper regeneration; thus, this scar formation is basically the repair by the connective tissue deposition. It's a way of wound healing, but there is too much disorganization after the wound healing or after the repair, which makes it easy to recognize where the damage was. That is called wound healing by scar formation. Repair begins within 24 hours of the injury.

The components involved are the formation of new blood vessels, also referred to as angiogenesis, and the granulation tissue that takes around three to five days, which includes the migration and proliferation of fibroblasts and the deposition of connective tissue. Abundant blood vessels, new thin-walled delicate capillaries, and interspersed leukocytes and macrophages indicate that granulation is occurring in a damaged area around two to three days after the injury, and then comes the maturation and reorganization of the fibrous tissue, which we often refer to as remodeling, to produce a stable fibrous scar. Remember, although the scar is non-functional or can be a burden, but it restores the structure and allows the organ to function, although the damaged or repaired area is not contributing to the function; it prevents it, just like a tank that is leaking and you are filling that hole with hay, cardboard, a stone, or something else. Contributing actively, however, it is preventing the damage. Like you can see here, this is normal tissue and a site of injury.

You can see the tissue injury has led to some disorientation, and then there is inflammation, credited to the pro-inflammatory cytokines that are produced by your immune cells. That migrates to the place, and remember, whenever there is tissue damage, the extracellular matrix is damaged, which automatically attracts immune cells because they have to protect your body from infection; hence, they allow a lot of these inflammatory cytokines, which enable the WBCs to come out of the blood vessels into the site of injury to tackle the injury response. And then there is a formation of granulation tissue, as you can see here. That means a lot of granulocytes will be occupying that area for 3 to 5 days post-damage. And then, once it is fixed, it leads to scar formation.

As you can see here, there is an abundant deposit of fibroblasts as well as the ECM, extracellular matrix. So this is a picture of the granulation tissue. As you can see here, there

is too much deposition of collagen and few cells that are deposited, so this is how granulation tissue looks. Now, if you look into the regenerative response of different animals, in a normal scenario, the liver, blood, and skin of the majority of the animals can regenerate, and to some extent, the skeletal muscles, gut epithelium, pancreas, etc., can regenerate, but when it comes to the central nervous system, heart, and limb, and kidney, the regenerative capacity of the is minimal, as you can see in this arrow, and the regenerative potential is minimal for mammals when it comes to the central nervous system.

On the other hand, if you observe animals such as urodeles and teleosts, which are amphibians and fishes, they have the maximum ability to regenerate the central nervous system, unlike what is seen in the case of mammals. So our lab focuses mainly on the regeneration of the zebrafish retina. It's a very interesting animal. You can see here; this is a zebrafish. And if you look into the eye, it is very similar to that of any other vertebrate.

Vertebrates, such as humans, have a cornea, a lens, and a retina. This yellow color is the retina, and if you look at the ultrastructure of that retina, it has rods, cones, and a photoreceptor layer. You have an extensive inner nuclear layer with bipolar cells, horizontal cells, amacrine cells, and also one population of glial cells called Müller glial cells. Then you have a ganglion cell layer that gives rise to the optic nerve. So whenever there is an injury, it is the Müller glia that respond because neurons cannot divide; they don't have centrioles, so they are permanently unable to divide.

It has to be the glia that are entering the cell cycle from G0 to G1, and they increase in number. We focus on a needle poke; you take a 30-gauge needle and make a hole in the back of the eye, which is very easy to perform. You anesthetize the fish, and also give a focal injury, while the rest of the retina remains unaffected. Then we study the injury spot, but what happens after the injury is the interesting thing. Soon after the injury, you can see that there are multi-layered cells present, and what happens in the injured area, especially with the Müller glial cells, is that they undergo a process called de-differentiation.

That means Müller glia is differentiated tissue. It undergoes something called reprogramming, and who does that? It is because of the injury response, and Müller glia also have the capacity for phagocytosis; they take up this damaged tissue and start expressing some of these factors that allow them to reprogram, and we call them activated Müller glia, as you can see here. And this activated Muller glia now start proliferating, and while proliferating, they are not Muller glia; they have all pluripotency factors like Yamanaka factors and Thomson factors expressed. Probably you would have heard about how you can convert a fibroblast into a stem cell, so these factors are induced. During the retina regeneration, it occurs spontaneously. So, de-differentiated and proliferating Müller glia are present, and this peak of proliferation in the case of needle poke is around four days post-injury.

And now, after four days, the proliferation decreases; it doesn't continue to proliferate. These proliferated cells, which we call Müller glia-derived progenitor cells, start migrating to different layers, and while migrating, they also differentiate back. This is the outer nuclear layer, where rods and cones are present, and in the inner nuclear layer, you have bipolar cells, amacrine cells, and horizontal cells. So, the Müller glia itself gives rise to the ganglion cell layer in the GCL, restoring homeostasis. Remember, this happens in an organism called zebrafish, which is capable of regenerating pretty much every organ, and we are talking only about the retina.

As a result of this repair or regenerative response, the normalcy of the retina is restored and the fish can continue to do so as many times as it needs. But if you look into retina regeneration at a glance, there are so many factors that have been worked out. So you can see here a lot of cytokines and molecules like leptin contribute to JAK-STAT signaling, and they can give rise to the induction of factors like Heparin-binding epidermal growth factor (HB-EGF), which can trigger MAP kinase signaling and then the induction of pro-neural genes like ASCL1A, which in turn can induce. Factors like Lin28, which negatively regulates a Let-7 microRNA, are required for the maintenance of the differentiated status of a given tissue. If Let7 is downregulated, it makes that tissue or cell vulnerable to proliferation.

As long as Let7 is present in that tissue, many pro-proliferative genes, even though they are expressed, will never get translated. Hence, Let7 can be called a guardian microRNA. And this will lead to the retention of the tissue in a differentiated status when lead 7 is present, and other examples include ascl1a, which can negatively affect a repressor called insm1a, and positively affect a repressor called insm1a, which is a negative regulator of many inhibitors of Wnt signaling. For example, DKK is basically a negative regulator of Wnt signaling, which prevents. The proliferative response occurs in a normal scenario, but when INSM1A is induced because of ASCL1A, what will happen is that the inhibitor DKK is removed; as a result, the environment will become pro-proliferative.

This means that Wnt signaling can kickstart, and someone who was a blocker of Wnt signaling is gone. Wnt signaling normally acts by stabilizing beta-catenin, which is normally produced when the cell... In the cell, it is degraded because it is needed for cell-cell communication and cell junctions, also by combination with alpha-catenin and integrins.

Beta-catenin is important for cell-cell communication. But when beta-catenin is not degraded, it gets stabilized, goes to the nucleus, and leads to cellular proliferation. Another regulator of the regenerative response of the retina is various epigenetic regulators, such as HDAC and other gene or genome-regulating factors. And another molecule that is contributed is delta-notch signaling. Delta-notch signaling is very important during embryonic development and also in the formation of hematopoietic stem cells or maintaining a fixed number of stem cells, and a group of stem cells now gets differentiated. For example, if you have one liter of milk in a glass and you took 100 ml from that, you now have 900 ml, so what you can do is pour.

100 more mL of milk. So that milk will remain milk. If you pour 100 ml of water, it will become diluted milk. The same logic applies when it comes to stem cells. If 100 cells are removed, 100 cells should be replaced. That is done through proliferation.

So delta-node signaling is very important for this. Delta-notch signaling produces another transcriptional repressor, HER4. And this is the way in which cellular signaling is regulated in an orchestrated manner. There are many more factors that contribute to it; I am mentioning a few of them due to the complex nature of this regulation, and tissue regeneration, like we saw, is a blessing for a A given set of organisms or some organisms from a given set of cells exists in our body; every cell cannot regenerate as strongly, no matter what the age or species of that organism is. The regeneration of injured cells and tissues often involves cell proliferation, which is driven by growth factors that are produced at that site.

For example, if you get a hand cut and there is... Blood that will come out has platelets; platelets will rupture when they come in contact with air, releasing a factor called platelet-derived growth factor (PDGF). Every cell has a PDGF receptor, so the moment PDGF is made available, the PDGF receptor will get activated, and that cell will start proliferating. This is a routine process that occurs throughout your body. With or without your knowledge, this is the importance of growth factors and is critically dependent on the integrity of the extracellular matrix for the development of mature cells from stem cells, and we have seen three categories of tissues: labile cells, stable cells, and permanent tissues. So the cell proliferation and the signals that control the mechanisms include several cell types that proliferate during tissue repair.

We know that. The remnants of the injured tissue, which attempt to restore the normal structure, are significant players in this regeneration; vascular endothelial cells create new blood vessels, and fibroblasts are the source of the fibrous tissue that forms the scar to fill the gap. Needed for the regeneration, these functions work together to restore the normalcy of damaged tissue. We will see more about this topic in the next class. Thank you.