Regeneration Biology
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W4L16_Tissue dedifferentiation, cellular reprograming into blastema Cellular types in regeneration

Hello, everyone. Welcome back to another class on regenerative biology. Today, we will try to understand, as the title shows, tissue de-differentiation, cellular reprogramming into blastema, cellular types in regeneration, and neural crest cells in vertebrates. So there is a lot of content, but we will visit these topics as and when we study regenerative biology. But this is a prelude to understanding the whole mechanism in a nutshell. Tissue de-differentiation and cellular reprogramming are occurring.

You may have heard this terminology multiple times. So regenerative capacities vary enormously across the animal kingdom. Some animals, such as humans, have the ability to regenerate using adult stem cells, which can be found in skin, hair, nails, etc. The so-called complex organ that we can regenerate is only the liver.

In the same way, some animals like planaria, which we have seen, if you cut a planaria into four pieces, each piece will become a full-fledged animal. So, we should understand all of the animals based on their position in the evolutionary ladder, whether they are primitive invertebrates, advanced vertebrates, or protocordates, etc., based on that quality. It can have a differential regenerative capacity. In contrast to most cold-blooded vertebrates, which are basically fish and frogs, although reptiles are also cold-blooded, only mammals and birds are warm-blooded vertebrates.

Fish, frogs, and reptiles are cold-blooded. When regeneration is concerned, we are referring to fish and frogs. Mammals, including humans, do not have the ability to regenerate damaged organs; complex organs or even degeneration is happening. Although we say, "Okay, you can regenerate skin cells," we know that older people's skin shows more wrinkles, while younger people's skin looks very clean and has fewer wrinkles. Skin has many wrinkles.

Wrinkles are nothing but the accumulation of defects that have been accumulating over the years and are not getting fixed. Hence, you have this so-called old-age look. So we have to understand that despite having the regenerative ability of some tissues, it is still not perfect as you grow older. So the degeneration keeps occurring. So in this picture, you can see the regenerative capabilities of the animal kingdom.

You can see this; we have seen it, which is basically a flatworm, planaria, or any worms in that category. If you cut it, each piece develops into a normal individual. And this is a salamander, and you cut its hand or cut its tail, and all of you would have seen the wall lizard. Sometimes, if you chase behind the wall lizard, it will drop its tail; the tail will be wriggling for some time, and that is called autotomy. It will grow back after a few days.

But you can cut the tail or limb also for this, which comes under the category of frogs. Okay, this is an invertebrate, which is planaria, and this is one of the vertebrates with limbs that can regenerate. When it comes to zebrafish, it is capable of regenerating its heart, the retina, and, of course, its fin as well, because it cannot be called a limb, but it can regenerate. When it comes to chickens, they can regenerate. That is not an adult chicken.

Hens and cocks cannot, but the neonatal small chickens are able to regenerate their retina. And also, you should know that the salamander is capable of regenerating the lens of the eye, not just the retina, not just limbs. It can also regenerate the lens of the eye. When it comes to mammals, the picture of the mouse is given. It cannot regenerate any of the complex organs, but it can regenerate the liver, just like any other mammal.

So, what do you understand as you climb the ladder of complexity of the organism, like salamanders, which are less complex compared to birds and mammals. Body temperature is one example we discussed; otherwise, the physiological complexity is less in the case of fishes and frogs compared to other animals. They have a two-chambered heart, pro nephros kidneys, and do not have more advanced production of uric acid or urea; they release ammonia. Many such factors indicate their limitations, or I won't say limitations, but rather their differences. A more crude way of handling things, but for birds and mammals, it's slightly different; they do not have the ability.

It is very important to note that both birds and mammals in the neonatal stage have a good, excellent ability to regenerate, which fades as they grow older. So, as you can see here, we are trying to understand how the regulation of proliferation and de-differentiation happened in mammalian cells. We know our body cells always proliferate. Whenever there is damage, it proliferates. But much of this cell proliferation is highly regulated.

That is a cell cycle regulator. And the cell cycle also, you know, G1, S, G2, M, continues. And every time point it has to proliferate. The market, which stage it is right now, has it got the permission or the license to move to the next stage? The majority of your tissue types in your body are either in G0 or G1; G1 and G0 are technically the same, with no difference. The only difference is that you call a cell G1 simply because it has the potential to go into.

S phase, that is, it will synthesize the DNA and go to G2 phase and then M phase; but if that G1 cell does not have any plan of going forward, then we essentially call it G0, which means it has quit the cell cycle. Now this G0 cell can be coaxed to get into G0 and G1. Once it got into the G1 phase, it may be forced or directed to enter proliferation. So let us see what this quiescent and differentiated cell is. So every differentiated cell technically is in the G0 phase, or we can call them quiescent cells.

What is the pathway that works? Quiescent and differentiated cells are affected when the activity of the cyclin D CDK4-6 complex is inhibited by cyclin-dependent kinase inhibitors. Say, for example, P27, P21, P15, and P16. These are some of the naturally occurring proteins. And another molecule is PRB. RB stands for retinoblastoma.

It's found in its inactive, hypophosphorylated state. When? In a quiescent state. It has no plans of proliferating. Active PRB blocks proliferation by sequestering members of the E2F family. They are a group of transcription factors, the E2F family of transcription factors.

And it can also recruit histone deacetylases, HDACs. Their main role is to deacetylate proteins. So many proteins are functional when they are acetylated and non-functional when they are deacetylated, and vice versa. So HDACs also come into the picture with E2F and PRB to further repress the expression of cell cycle genes. When HDACs are active on the histones, they remove the acetyl groups, which affects the transcribability of those genes.

So that is the involvement of HDACs. Additionally, this PRB retinoblastoma protein can enhance the recruitment of lineage-specifying transcription factors, for example, myoD. We have seen MyoD in planaria. That's the marker of longitudinal muscles, etc. And this is an indicator of differentiation.

That is, you're converting the stem cell into a differentiated state. That is MyoD in skeletal muscle cells. That normal muscle cells, called skeletal muscle cells, promote the expression of differentiation genes or the genes that keep a given cell in a differentiated state and the stable maintenance of specific somatic cell identity, so these molecules contribute to the retention of quiescence, as you can see here how p27, p15, p21, etc., contribute. To inhibit the cycling once the cyclings are active, they will push the cell into the cell cycle; it will get into S phase, G2/M, like that.

So this is mainly done by retinoblastoma and by interacting with the E2F proteins, a group of proteins, and also HDAC further contributes to it by repressing the whole complex that represses the genes. Deregulation happens in this pathway, making the cell vulnerable to proliferation, which we can see in the next slide. In a proliferating cell, cdk inhibitors are inactive; we saw many inhibitors, such as p21, p16, and p15, that are inactive, which allows the cyclin D cdk4-6 complex to phosphorylate and inactivate the pRB. The pRB is no longer interacting with e2F. PRB inactivation, or retinoblastoma protein inactivation, causes an exit from quiescence.

That means it's decided. I don't want to stay in quiescence anymore. I don't want to be in the G0 phase anymore. As E2F transcription factors are now available to drive the expression of genes involved in DNA replication, That is nothing but the S phase and entry into the S phase. of the cell cycle. That means the G0 cell transitioned into G1 and then entered the S phase.

Moreover, independently of the induction of proliferation, PRB inactivation and retinoblastoma protein inactivation can decrease the expression of differentiation genes because it has entered the cell cycle now. Say if a muscle is entering proliferation, it no longer wants muscle-specific genes. A kidney cell has now entered proliferation; it no longer wants kidney-specific genes to be expressed. It will perform the task of proliferation only, thereby favoring de-differentiation. So this process, in a nutshell, we call de-differentiation.

Even in the retina, when regeneration is happening, it is the glial cells that forget their glial cell identity and enter the proliferating phase. And we call this phase de-differentiation. In other words, cellular dedifferentiation and proliferation can both be favored by PRB inactivation. Retinoblastoma protein, if you inactivate it, can force any cell to enter a proliferative phase. So retinoblastoma being active is the main cause of cells remaining in a quiescent state.

After passing this checkpoint, a particular cell is committed to a single round of division. Whether it will persuade further depends on other supporting factors that are available. Now let us see how the cell cycle comes to a halt, that is, cell cycle arrest. And we have seen that whenever there is damage that normally happens in an S/G2 checkpoint, if there is any damage in the DNA replication, it has to be taken care of. So, cell cycle arrest—how does it happen? However, if DNA damage occurs, ATR and ATM protein kinases are two proteins.

They get activated because they detect that there is damage in the DNA. They phosphorylate checkpoint kinases 1 and 2, CHK1 and CHK2, which in turn phosphorylate and activate a protein called p53. P53 is a well-studied tumor suppressor protein because when mutated, it makes a given cell vulnerable to cancer due to uncontrolled proliferation. Active p53 drives the expression of p21, the downstream molecule, because p53 indicates that there is some damage to the DNA when it is turned on. That is why P53 is now capable of turning on P21.

What will P21 do? It will immediately go and prevent the cyclin-CDK complex. P21 makes sure that the cell cycle cannot proceed. It is just like a dog running around. You immediately put a chain on it so that you are making sure that it does not bite anyone anymore.

You are just harnessing it. And that is what P21 will do. P53 also activates. And by doing this, what is it doing? It is buying extra time to fix this DNA damage. P53 also activates the DNA repair machinery. The same P53 that turned on the P21 now turns on the machinery for DNA repair.

And if the DNA damage is too extensive, it will wait for quality control to say yes. And if the p53 is still active, that indicates that the CHK1 and CHK2 are still active. If p53 is still active, what will it do? It will eventually induce the cells to undergo apoptosis. So p53 will turn on p21 to stop the cell cycle, halting the cell cycle. Then it will turn on the cell cycle DNA repair proteins once the cell cycle is arrested.

DNA repair proteins. If p53 is still active, it will activate the apoptosis genes, which will kill the cell. And this is killed by apoptosis or by senescence. PRB and p53 operate as part of a larger network wired for functional redundancy. So this means that the differentiation and re-entry of a given cell cycle cannot be induced simply by inactivation of PRB. That means, like I told you earlier, if you are in the middle of the ocean, you need to know which side is land.

That doesn't save you. At least if you don't know which side is land, you don't know where to sail your boat. So this is what PRB kick-starts. But the involvement of P53 is also very important. Indeed, in the absence of PRB, oncogenic stimulation can activate another protein, ARF, which leads to P53-mediated growth arrest.

This can also happen. In fact, active ARF can inactivate mouse double minute homolog MDM2, an E3 ubiquitin protein ligase that normally stimulates p53 degradation. So, p53 stability is a factor. Its activation is another factor. If both are in conjunction with the retinoblastoma protein, pRB, then there is a chance that it can continue the proliferative phase. That is what we described as being shown in cartoon form on this site, where the different checkpoints are working together purely based on the DNA damage.

Now let us get into how the epigenetic landscape and the fate of a cell are changing. This comes in the picture of within the line of development. Development happens with a zygote, and it follows certain gradients or morphogenetic gradients that give rise to all the differentiated cell types we are now talking about. Can they go back in time? In this picture, you can see it's a three-dimensional image that looks like hills and valleys. If you have a rounded ball and you're rolling it around in a valley, that ball will go and stop in a place where.

.. The deepest point it will go and settle down is the most stable point; the ball is rolling because gravity is acting on it, and it will go and settle down at a point where it finds its most stable position. Your cells are also like that; sometimes, if there is a value here and here, this cell will decide whether to go this way or that way, and the same applies here as well: should I go this way? Or should I jump and come this way? So everything is just like a rolling ball. So we can see de-differentiation; just now we saw the term associated with lineage reprogramming, meaning that fully differentiated cells revert back to a less differentiated and proliferative stage. Within their own lineage, without reaching pluripotency, the differentiated cells are not reaching the pluripotency stage, but that is the right track; they have the ability to go back into the pluripotency stage. Also, reprogramming is a terminology we use that refers to a full restoration of pluripotency to a stage where the cells can switch lineages and differentiate into any cell type.

It could be seen as a case of extreme de-differentiation. A little bit going back in time. Is the differentiation going further back in time; we call it reprogramming, involving dramatic changes at the levels of both gene expression and epigenetic signatures. That means if a person is getting Alzheimer's, they will forget their latest memories first and their oldest memories will stay intact. So the oldest memory of a given cell is about its childhood or infancy.

That means it is a zygote. So it will go back in a stepwise manner and that is reprogramming. Now, what is transdifferentiation? Transdifferentiation describes the conversion of a fully differentiated cell of a given tissue lineage directly to one of a distant lineage without passing through the pluripotent intermediate. But it has to go back in time a little. But it doesn't have to go into a pluripotency stage.

And there is another term that is called transdetermination. What does it mean? Transdetermination describes the conversion of adult multipotent stem cells from their typical lineage to a closely related one. Say I have skin stem cells, and now I am using them to make nails. So nail also a different issue. Skin is also a different issue.

But now skin stem cells are giving rise to nails. Both are adult stem cells. So this is trans determination. So this is a picture, as you can see here. It is totipotent and pluripotent. And when in differentiation, it is standing here and reprogramming is going back.

And transdifferentiation is moving from one line to the other. Jumping across this hill. And here is something called a unipotent cell, and this is called a multipotent cell. Unipotent cells cannot have the luxury of making different types of cells. Multipotent cells can give rise to multiple cell types. And de-differentiation is a differentiated cell going back to an undifferentiated state.

to not reach the multipotent or go beyond the multipotent stage. It doesn't reach the pluripotency stage, but it is powerful enough. It came up to here, powerful enough to jump into another lineage. So that is the differentiation. So now we will learn about another type of cell; they are called neural crest cells, which are left out during development.

They are cells involved in tissue regeneration. Neural crest stem cells, called NCSCs, are a transient multipotent cell population that originates along the border of the neural plate during early vertebrate development. During vertebrate development, the first organ system that is formed is your nervous system. And the neural tube has to form. And this tube is the one toward the anterior-most portion.

It swells, folds, expands, and becomes the brain. And the rest of the neural tube remains as your spinal cord. But during the folding of this tissue, some cells will escape at the folding crest, and those cells are called the neural crest cells. which are capable of replicating and maintaining their number. And neural stem cells are very important because, during embryonic development, not every organ—hands, legs, etc.

—is formed. Can be formed, but some organs are formed much later. Say, for example, eyelids. Or, say for example, muscles on your nostrils, or say for example you have got, you know, some small twitching muscles; how you move your eyeball, the eye itself is not formed, whereas eyeball muscles like that, the formation of your jaw, these kinds of intricate skeletal muscles are all derived from neural crest cells much later in development. At that time, you don't have stem cells available for you to do the job, so neural crest cells become very handy; they are able to survive on their own and can give rise to different cell types, like skin color, which is determined, head skeleton, teeth formation, and heart formation. A newborn baby hardly has any skin color; later, it acquires the skin color.

And the intricate connections of the nervous system, jaw formation, etc., are all decided by the neural crystal. So this is a cartoon that shows the schematic of the neural crystal. This is the neural tube formation; as you can see, here is the epidermis, and this is the place where the neural tube is being formed. You can see here the general model of neural crystal development in a given vertebrate embryo.

Invertebrates are also shown. The left is a cross-section of the open neural plate, which is not closed like yours; this is a neural tube. The blue color is trying to form a neural tube in an embryo with a neural plate, and the neural plate is NP and CNS primordium.

CNS stands for central nervous system primordium, and in the middle... It is flanked bilaterally by the neural plate borders, which elevate the neural folds enough, and ventrally by the notochord, which will be formed. What you can see here is the notochord; the epidermal ectoderm is here, and shown on the right is a cross-section of the neural tube. So, during this formation, there are two types of neural. Crest cells are present. One is a premigratory neural crest cell that has yet to start migrating, and another is migratory neural crest cells.

Both are present here, but they originate, as you can see, on the crest of a neural tube. Hence, they are called neural crest cells, and they will be wandering around the body. Different groups of animals have the peripheral nervous system (PNS) and central nervous system (CNS). As you can see in the pink and blue colors, vertebrates, tunicates, nematodes, and arthropods. Arthropods, nematodes, and tunicates are all invertebrate groups, and the bilateralian ancestor is much older.

So a lot of molecular and cellular developmental hallmarks are associated with neural crest cells. And long predating vertebrate and chordate origins are depicted here. And the most important thing is we have to understand that there is a BMP and Wnt signaling that contribute to the expression of a bunch of genes such as fox, twist, snail, etc. That contributes to the EMT, which is the epithelial to mesenchymal transition and cell migration.

If a cell has an epithelial identity, that means it won't move. But if it acquires a mesenchymal identity, it is capable of moving. So stem cells should naturally have this mesenchymal identity. There is no point in having a stem cell and sitting anchored to a spot. It should be able to migrate.

So, this EMT transition happens mainly because of these markers. And it is true for neural cluster cells as well. And there are many proteins. The names of these genes or proteins are not important.

And many are there. NKX transcription factors, forkhead box transcription factors, etc. Contributes to the formation or maintenance of this neural crest population. And as you can see in this picture, evolutionarily speaking, although all of them have got their relative distribution is slightly different. You know, vertebrates, it is much smoother.

PNS and CNS are located. Whereas in others, they are more like distributed. However, they need to have the involvement of neural crest cells for their later development or fine-tuning during the developmental stage. So this is a model of the stepwise evolution of the neural crest from the ancestral precursors mapped into chordate phylogeny. The previous one included the invertebrates. Now we are looking into the chordates, which are cephalocordates, tunicates, jawless vertebrates, and jawed vertebrates.

Jawed vertebrates are the ones that include fishes and frogs. All five groups of vertebrates are in this category, and these are jawless vertebrates and tunicates. They are more primitive; however, they are chordates as well. And they do have, as you can see in this picture, multipotent NCCs with long range. As you can see here, how much is involved, whereas in this boundary, that is tunicates to the jawless and jawed boundary, you can see they have almost 50-50.

Here, you have got three types of black, like NPB. Which is based on their marker expression, and also both migratory and non-migratory forms are present because of the presence of their marker. So, you can say that in jawed vertebrates, the class of neural crest is a little more diverse compared to that of tunicates, as well as jawed and jawless forms. This boundary, when you see the caudate ancestor, indicates they have only NPP, which has a packs mask. and ZIC, which is mainly dealing with the PNS peripheral nervous system neurons.

They have very minimal complexity to their body. Hence, they are not depending too much on the neural crystal because they don't have that fine-tuning or the complex body structure. So, neural crystal stem cells are for tissue regeneration. Neural crystal cells are a transient population of cells that arise early during vertebrate development. They harbor stem cell properties such as self-renewal.

These cells form at the interface of the non-neuronal ectoderm and the neural tube. Ectoderm and the neuron, that boundary, you have the neural crest. Undergo extensive migration. They contribute to various cell and tissue derivatives, ranging from craniofacial tissues to peripheral nervous system cells. They can give rise to a variety of cell types. Neural stem cells can be derived from pluripotent stem cells, placental tissues, adult tissues, somatic cell reprogramming, and have differentiation capabilities similar to those of NCSCs, and they possess great potential for regenerative medicine.

You may wonder why there is so much excitement about neural crust cells. They are somewhat similar to those of neoblasts present in planaria. What we lack is an adequate supply of neural crust cells. If you have, you may not have so much difficulty in regenerating a complex organ. Now in this picture, you can see the sources and potential applications of neural crest cells; they are summarized. It may look like a complex slide, but it is a schematic representation of the potential applications of neural crest cells in regenerative biology or regenerative medicine.

You can see that from the fetus, an entire organism is forming, and the neural crest cells, as you can see here, are purple in color; they are migrating and are capable of giving rise to a bunch of. Cell types can be isolated, and you can also happily isolate them; they can also be converted. Neural crest cells can be made from pluripotent cells or even from fibroblasts. Once you have neural crest cells, you can utilize a lot of tissue-specific regenerative capabilities.

They can be used in tissue engineering, which we will see in the future. Upcoming classes and various cell derivatives make this very much possible, and those who are interested can read this article. We will continue discussing stem cells and regeneration in the next class. Thank you.