Regeneration Biology
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W6L29_Importance of regeneration associated gene induction events

Hello, everyone. Welcome back to another session of Regenerative Biology. So in today's class, we will try to learn about the importance of regeneration associated with various factors, that is, the gene, which includes gene induction events or sometimes the early immune response, etc. The broad categorization will be the activators and repressors of the regeneration response. So in this lecture, we will be trying to cover what the major events are that are occurring and how and why these events contribute significantly to a successful regeneration. So if you look at an overview, we know from a retina regeneration point of view that we will restrict this observation with regard to retina regeneration.

The reason is that it is one of the complex tissues, and it is unclear why and how a similar tissue in mammals, such as Müller glia, does not respond effectively. So we will get to know them a little bit more in detail. So hence we restrict this with retina itself. So Muller glia is the major component of the retina, consisting of around 40% of the total number of cells.

They are one of the last retinal cell types that are born during development, and they function to maintain retinal homeostasis and integrity because the neurons need a lot of support from the glial cells; hence, the neurons are able to perform their higher-order tasks. So in mammals, the Müller glia respond to injury in various ways that can be protective, or they can be detrimental to retinal function, but they are often limited in their ability to create an effective regenerative response. They indeed make some cell types, but they are not effective in bringing about a proper regenerative response. By contrast, in teleost fish such as zebrafish, we have all seen how zebrafish respond to injury. The response of the Müller glia to retinal injury involves reprogramming of the Müller glial cells.

and even imparts the retinal stem cell with unique characteristics of a pluripotent cell, allowing it to produce a proliferating population of progenitors that are capable of fixing all the damaged retinal cell types. And many recent studies have revealed several important mechanisms underlying Müller glia reprogramming and retina regeneration. And that will help researchers design strategies. How can we implement these discoveries

from the zebrafish into a mammalian system? So if you look at Müller glia, it has a transformation phase. You can see the adult Muller glia after injury; it becomes reprogrammed Muller glia.

That means you have a color change now here in this picture. And it also has interkinetic migration and asymmetric cell division. So this reprogrammed Muller glia is now getting into a proliferative phase. And often the hallmark is becoming asymmetrical. It does not have symmetrical cell division.

And these divided progenitors now expand in number, and they will form some columns of cells in the retinal section. You can see it. They will form an expanded number of progenitor amplifications, and these amplified progenitors will now give rise to all retinal cell types. So this is the overall theme of normal regeneration. So adult Muller glia and zebrafish respond to the retinal injury by reprogramming their genome because all six pluripotency-inducing factors are naturally induced in the Muller glia, which allows them to enter a pluripotent stem cell status.

To acquire stem cell properties. This reprogramming results in interkinetic nuclear migration to the outer nuclear layer because the damage has occurred in all layers, and the Müller glia cell body resides in the inner nuclear layer. So unless these progenitors are migrating to various layers, you cannot repair the damaged cell types in different cell layers. So, cell division has to continue along with interkinetic migration. And this asymmetric cell division, which, like I told you, this reprogrammed Müller glia undergoes asymmetric cell division.

Asymmetric cell division generates multipotent progenitors that transiently proliferate and restore the original Müller glia. So it's not that there will be a short supply of the Muller glia because they are all reprogramming. First of all, all of them reprogram. Even if the Muller glia reprogram, only a subset of cells that are derived from this reprogrammed Muller glia are undergoing proliferation. And the multipotent progenitors migrate to all cell layers, exit the cell cycle when the purpose of regeneration is completed, and regenerate all major retinal cell types.

Now comes an important table. As you can see here, this was taken from a review published in Nature Reviews Neuroscience. And it's a huge table that depicts the factors affecting Müller glia reprogramming and proliferation. A lot of factors are listed here. Its function is listed here, animal testing is listed here, and its expression following an injury is listed here.

And what is its effect, the role in the regenerative response? For example, delta notch is

one of the first two factors and its function is basically a transmembrane ligand and receptor. Both of them are present in the membrane. It has been tested in animals such as birds, mice, and rodents, as well as fish; all these species have been tried for retinal regeneration, which is induced soon after injury, and the effect can be quite unique in the sense that it stimulates in birds and mice but inhibits in fish, showing one signaling pathway that is induced in both. And you see that in one case it is activated, in another case it is repressed. So we should understand the same signaling based on the species you are studying that is able to perform a different task.

In the same way, you can say BMP/smad. And it's a secreted factor tested in rodents; it is induced and stimulates. You can see here another factor, dkk, which is a negative regulator of Wnt signaling. It's a secreted factor tested in fish, and it is suppressed. The dkk is suppressed soon after injury, and it is an inhibitor of regeneration, so the suppressor is.

.. blocked, then the regeneration is favored in zebra fit. So you can see I will not go into each of these pathways. Those who are interested can read it. But what we should understand is that there can be genes that are induced for a better cause in terms of regeneration, or there are some genes that are inhibited for a better cause of regeneration, and vice versa. So, you can see some genes, such as TGF-beta, which is a secreted factor tested in rodents and fish, and it is suppressed.

It has been shown to inhibit, so in zebrafish, TGF-beta 1. Some studies have shown to activate also, but in general, it is an inhibitor, which means it is controlling; it acts like a brake in a vehicle. So, this kind of regulation, on one hand, you are pampering; on the other hand, you are punishing. So this is the way various factors act. The same table is continuing; you can see TNF alpha, and you have got HSPD1.

These are all different groups of proteins, families of proteins that have been tried, and some of them are transcription factors, some of them are signal-inducing factors, and some of them are transmembrane factors, and different species have been tried. A lot of them are tested in zebrafish simply because it is a well-regenerating model. That is why many of these pathways and factors are discovered in zebrafish. And you can see that not all of them are induced; some of them are suppressed. Let-7 microRNA, which we have discussed multiple times, is a suppressed molecule, and if present, it inhibits.

So the inhibitors of regeneration are often suppressed, whereas the activators of regeneration are often induced in a well-regenerating model. So the important question based on these factors is, how do the Muller glia sense the injury? Someone has to... tell the Müller glia that there is an injury; of course, many factors contribute to it.

Sometimes it may be reactive oxygen species, sometimes it may be the blood clot, and sometimes it may be the PDGF, etc., that is induced because of an injury, a bloody wound. So these things contribute to how the Müller glia is able to respond, however. What are the factors a Muller glia will be looking for? So secreted factors such as Heparin-Binding Epidermal Growth Factor, Tumor Necrosis Factor, Wnts, and Ciliary Neurotrophic Factor have been reported to contribute to injury-induced Müller glia reprogramming and progenitor formation. But there are some interesting factors.

CNTF is not reported in zebrafish. It is normally present in mammals. The CNTF gene is not reported in zebrafish. However, if you provide CNTF from mammals to zebrafish, it favors regeneration. So, that is something interesting we should keep in mind.

Other pro-inflammatory cytokines, such as interleukin-6, commonly known as IL-6 family cytokines, can contribute to and supplement or complement the absence of CNTF in the case of zebrafish. This doesn't mean that CNTF is nonfunctional in zebrafish. The only thing is that the gene for CNTF is not present in zebrafish. Cytokines, probably IL-6, are taking that job. Contribute to retinal regeneration in this species, which is zebrafish.

Two factors that regulate Müller glia proliferation, that is, ADP. You know, ATP, when it is degraded, becomes ADP. It can further become AMP, adenosine diphosphate, or adenosine monophosphate. Adenosine triphosphate is the energy source. And ADP can also act as a signaling molecule for Müller glia proliferation.

And TNF-alpha is not only produced in the Muller glia, but also appears to be released by the dying retinal neurons. So whenever there is an acute injury, these dying retinal neurons need not be glia because the retina has many neurons as well. So when they are dead, it will release this ADP and TNF-alpha, which will stimulate the Müller glia. TNF-alpha contributes to the injury-dependent induction of ASCL1A and STAT3, which are two transcription factors. ASCL1A is a BHLH, basic helix-loop-helix transcription factor.

STAT3 is a component of the JAK-STAT signaling pathway. STAT3 is phosphorylated, enters the nucleus, and activates many genes. and whose expression is necessary for the generation of the Müller glia-derived progenitors. So this picture kind of depicts various signaling cascades that contribute to immunoglobulin reprogramming and progenitor proliferation in zebrafish. One point is that retina regeneration requires the activation of a variety of signaling cascades, which I already told you; not every induced gene or induced pathway is pro-regeneration.

Some of them will be anti, too. But that doesn't mean that the anti-regenerative response or pathway is unwanted. It is needed. It's almost like telling me I need a car without a brake. So this is what you should keep in mind. Signaling pathways shown to regulate retinal regeneration are indicated in this picture by solid arrows, while those that are indirectly implicated and hypothesized are shown by dotted and dashed arrows.

One factor that is secreted is normally a group of factors, such as Wnt and SFRP; many are involved, including Sonic Hedgehog. And this is indicated to be expressed outside the cell in this picture, which can impact the Müller glia response or reprogramming, and arrows are pointing to the top half of the nucleus that is present, which they stimulate and maintain positively, while the blunt or blocked arrow indicates that it is a negative regulator. So wnts are secreted in a lipid-modified fashion, glycoproteins, and they can bind to the frizzled family of receptors that allow the stabilization of beta-catenin. That is one of the pivotal pathways. We will see more about the Wnt signaling in the coming class as well.

And this allows for the sequestering of dishevelled from this degradation omplex , where normally the excess produced beta-catenin is degraded and the dislodging of the dishevelled occurs through cytoplasmic phosphorylation acting downstream of these Wnt signaling events. Stabilize; because of this, the degradation of beta-catenin will be prevented. This will stabilize the beta-catenin, which will go to the nucleus and act on various target genes. Another factor, insulin and IGF (insulin-like growth factor 1), are secreted proteins that bind to the tyrosine kinase receptor that signals via IRS, which is the insulin receptor signaling protein called IRS, and an adapter protein. couples the insulin and IGF receptor to PI3 kinase, which is phosphatidylinositol 3-kinase, and AKT.

So this is the pathway through which insulin and IGF act together. Another factor is HBEGF, which is heparin-binding epidermal growth factor, a transmembrane protein that undergoes ectodomain shedding because it needs to be processed; the enzymatic action causes ectodomain shedding, and it is a member of the EGF family of growth factor ligands, acting via epidermal growth factor receptors. Heparin binding epidermal growth factor acts via the EGF receptor, as you can see here. This is also taken from this review. You can see there are so many factors; there are membrane-bound receptors that act on HBGF given here.

Insulin and IGF are given here, and the DKK, which is a negative regulator, so Wnt signaling is given here because these are all secreted factors outside the cell. Inside the cell, this is the nucleus, and this is the cytoplasm. Outside the nucleus is the cytoplasm, and this is the membrane. This is the nuclear region of the Muller glia itself, so beta-catenin usually will be degraded in a steady-state cell, but When signaling is on, beta-

catenin will be stabilized and will go to the nucleus and create a lot of pro-mitotic genes that will be turned on. You can also see Lin28 negatively regulating let-7, which indeed influences the translatability of various regeneration-associated genes.

So you can see this picture in a nutshell: there are both pro- and anti-regenerative responding molecules combined together. In general, the reprogramming of the Müller glia depends on how effectively these pro- and anti-regenerative events are triggered. Another factor is FGF, or fibroblast growth factors. They are secreted molecules that bind to the fibroblast growth factor receptor. EGF, epidermal growth factor, and FGF receptors are tyrosine kinase receptors that can signal via MAP kinase, which is mitogen-activated protein kinase, and ERK kinase, which is extracellular signal-regulated kinase.

This is the role in which the EGF and FGF are acting. Cytokines, which are mainly secreted by your immune cells, are secreted proteins that often signal through receptors that lack their intrinsic tyrosine kinase activity. They don't do their jobs unless they bind to a ligand. Cytokine receptors are often coupled to JAK, which is also known as Janus kinase.

which is meant for the STAT3 activation. JAK proteins are non-receptor tyrosine kinases that transduce cytokine-mediated signals by phosphorylating another protein called STAT, which stands for signal transducers and activators of transcription. So the phosphorylated STAT can go to the nucleus and act on the target genes. Then comes the TGF-beta, which is transforming growth factor beta, a secreted protein that has differential roles in mammals. It is normally anti-proliferative, whereas in zebrafish it can be pro-proliferative depending on which TGF-beta is present; TGF-beta 1 is pro-proliferative, whereas TGF-beta 3 is not. Another microRNA molecule is Let7 microRNA, which we have discussed sufficiently; hence, I will not go into the details, as it is a negative regulator of reprogramming.

So Let7 must go off if the reprogramming of the mullerglia has to happen. Tumor necrosis factor alpha, which is a secreted cytokine that acts via the TNF receptors that regulate cell signaling and gene expression. And delta notch signaling is another signaling event that has both of them as single-pass transmembrane proteins, and they have to be present in the adjacent cells, which we often refer to as juxtacrine signaling. And then comes the last part, which is the ECM. Extracellular matrix can also signal the adjacent cells via the transmembrane integrin receptor to regulate cell function.

So from this, we understand that the signaling events are not just initiated by one point. They are acted upon by a plethora of angles and directions on a given cell. So let us quickly look into what the major activators of a regenerative response in zebrafish are.

Retinal injury results in Wnt gene expression and beta-catenin stabilization in the Müller glia-derived progenitors.

Inhibition of Wnt signaling. It can be done in multiple ways. You can use overexpressed DKK, or you can knock down some of this gene or create a knockdown of beta-catenin itself. There are different ways to block Wnt signaling suppressors, and if you block the Wnt signaling, it can suppress progenitor formation in the injured retina. So the pharmacological activation of the beta-catenin signaling pathway means you are stabilizing the beta-catenin, which is independent of the availability of the Wnt ligand. So the Wnt ligand acts on the Frizzled cell receptor, which is a cell membrane-associated job.

Now you bypass the whole thing. You, no matter what is happening, can stabilize the beta-catenin by blocking one of the phosphorylation events that are necessary for beta-catenin degradation, so one of the phosphorylating molecules of the destruction complex is Gsk3-beta. Glycogen synthase kinase 3 beta. So, if you block it using an inhibitor, it can stimulate the Müller glia reprogramming and progenitor formation even without an injury. This is what you should keep in mind. If you can stabilize the beta-catenin without any injury to the retina, it will start a regenerative response.

These treatments can bypass an inhibitory retinal environment that results in part from a pan-retinal expression of the Wnt antagonist DKK, which is present throughout the retina. You don't want any unwanted proliferation to happen in the retina. So soon after injury, in a normal regeneration event, the DKK will be downregulated. But when you are stabilizing beta-catenin, you do not have to bother about DKK at all.

Let the DKK block the Frizzled co-receptor; it doesn't matter. You are dealing directly with the inside of the cell, and that too at the level of beta-catenin. So if you look into a few more factors, CNTF, ciliary neurotrophic factor, can stimulate Müller glia to generate progenitors in the uninjured zebrafish retina, which suggests that the JAK-STAT signaling may also be involved in Müller glia reprogramming and retina regeneration. So they also contribute, which is done exclusively by CNTF. Induced retinal injury can stimulate STAT3 expression in both quiescent Müller glia and in the Müller glia-derived progenitors, and the STAT3 knockdown can inhibit progenitor formation if you block STAT3, although other signaling events are still occurring. You cannot get a normal regenerative response if you get rid of one component; it's almost like assuming that if there are 10 components needed to make a dish, and you got rid of one component, say salt, you have not made the dish because you put everything else in but did not add the salt.

So you cannot say I put everything, but I just skipped the salt; it will not taste correct, and it will not be a servable dish. The same logic applies to individual signaling events: a Müller cell acquires the properties of the retinal stem cell usually by the reprogramming of its genome to express genes that allow it to generate multipotent progenitors for the purpose of regeneration of the retina, so gene expression has to. underlie before a Mueller glia is reprogrammed. And there are also many negative regulators in the regenerative response.

There are plenty of negative regulators. Retinal regeneration is driven by the activation of signaling pathways that stimulate Müller-glial reprogramming and progenitor formation because of these activators, as well as suppression pathways that drive Müller glial differentiation in quiescence. Sometimes, some pathways do not want the Müller glia to continue to proliferate. They want the Müller glia to differentiate. So if the differentiation is happening in a premature manner, then it is not happening. It's almost like, you know, someone who is a five-year-old person; you are expecting them to go for a job.

It doesn't help because there is a maturation process that has to build up. There has to be a sufficient buildup of progenitors. So gene expression has to continue for a fixed amount of time. And if it continues forever, then there will be a cancerous kind of response because you have only progenitor after progenitor after progenitor.

You don't want that. So the progenitor should come and then it should differentiate. So let-7, as we discussed many times, that another negative regulator, let-7 microRNA, is another negative regulator of the regenerative response, and DKK which is another negative regulator, which is let-7, an RNA molecule, whereas DKK is a protein molecule. Let-7 RNA, when present, acts as a microRNA; it ensures that no translation occurs for those mRNAs that have the let-7 binding site and many of the regeneration-associated genes. have let-7 binding sites. Hence, you can expect that even though those genes are induced, they will never get translated.

Same goes for the DKK. Even if the Wnt ligand is present, unless the DKK goes off, the Wnt ligand simply will not be able to do any job on the Frizzled receptor. And they are inhibitory pathways that help to maintain zebrafish Müller glia in a quiescent state. It means an inactive state, which is necessary for a normal retina. TGF-beta signaling inhibitors can enhance progenitor proliferation, especially in the mammalian retina.

And it can also be done in the zebrafish retina to some extent. And TGF-beta induced homeobox protein, called TGIF1. and Sine Oculus Homeobox 3B (Six3B). These proteins are involved in implementing the TGF-beta signaling and how effectively it is

regulated during TGF-beta signaling. These inhibitory molecules, or inhibitors, are transcriptional co-repressors with multiple targets, and their effect on the TGF-beta signaling in the injured zebrafish retina remains under exploration because, as I told you, TGF beta-1 acts in a pro-proliferative manner in the early phases, whereas the same TGF beta-1 acts as an anti-proliferative in the late phases, while TGF beta-3 acts as an anti-proliferative molecule throughout retina regeneration, so these are all some nuances one has to test. Notch signaling also appears to play an inhibitory role during zebrafish retina regeneration, so this must be understood very clearly: it does not inhibit the proliferation in total; rather, it inhibits the expansion of the zone.

Say, for example, there is an injury and you want to... Injury response should be in a one square centimeter area; I'm giving a very random figure: one square centimeter area. You don't want it in a 10 square centimeter area, but if the notch signaling is inhibited, instead of one square centimeter area, it becomes a 10 square centimeter area. So, if you want a responsive zone to be restricted in the required spot, then the notch signaling should be active; if you block it, It will become almost like tying a dog to a leash. If the leash is one foot long, it will only run around a one square foot area.

But if it is 10 feet long, it will act on a 10 square foot area. In the same way, delta notch signaling restricts the zone of regenerative response. So that is why not signaling is also considered a negative regulator; however, unlike most inhibitory pathways that are suppressed following retinal injury, not signaling components such as delta A, B, C, notch one, and notch target genes such as her four are induced by injury. So this is what you should, like I told you earlier, not every gene that is induced is. Favoring regeneration, sometimes some active signaling does not favor regeneration; in the case of delta not signaling these ligands, the receptor delta is the ligand and notch is the receptor. They are induced mainly to restrict the zone of regeneration in the case of retina regeneration.

So in this picture, you can see how different gene expression events congregate together and where the central focus is given to ASCL1A, which is a basic helix-loop-helix protein. It impacts the Mülle glia reprogramming. And proliferation by regulating the Wnt signaling pathway, but it can inhibit DKK. Being a transcriptional activator, it can inhibit DKK, and it is indeed necessary for the action of the Wnt protein on its target. But how ASCL1A is doing this, because ASCL1A is a transcriptional activator, is through the activation of a repressor called INSM1A.

So, ASCL1A regulates the expression of insulinoma-associated 1A, short form known as INSM1A, which is a transcriptional repressor that affects. Both Muller glia reprogramming and progenitor cell cycle exit. So it acts in two ways. INSM1A can block

its own expression.

INSM1A can block DKK expression, and INSM1A can block various other factors. So those are listed here. ASCL1A acts on various signaling and growth factor responses to it, and how ASCL1A influences different factors is listed in this graph. We will study the regeneration response in more detail in the next class. Thank you.