Regeneration Biology
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Week: 7
Lecture: 35

W7L35_Tgf-beta signaling during regeneration

Hello, everyone. Welcome back to another class of regenerative biology. In today's class, we will learn about TGF-beta signaling during regeneration. We have seen the involvement of TGF beta signaling in various other classes, especially in liver regeneration. And we have also learned that TGF beta signaling is generally anti-proliferative in mammals. And it is pro-proliferative in the case of zebrafish retina regeneration, especially in the early phases; the TGF beta signaling is pro-proliferative, and in the late phases, the TGF beta signaling is anti-proliferative.

We will see some other angles of TGF beta signaling in today's class. So transforming growth factor beta (TGF-beta) is a pleiotropic growth factor that is synthesized by many cells in the body, including immune cells. This growth factor is chemotactic for fibroblasts, stimulates fibroblast proliferation, and increases the synthesis of a number of extracellular matrix proteins, including collagens. TGF-beta signaling's main role is on the target cells.

The TGF-beta activator protein is a transacting factor that binds to the TGF-beta element in the distal promoter of genes such as collagen 1A1. This induces the transcription of this gene. So although transient, the TGF beta 1 activity participates in the repair and regeneration of various tissues, and its presence of persistent TGF beta function affects excessive fibrosis. Like it can be benign fibroid nodules, or there can be a patch of fibroid tissue. Everything is related to TGF beta signaling, and ultimately, scarring is the result.

We have learned about keloid formation in one of the earlier classes. These are all nothing but advanced stages of fibrosis. Scarring of both the skin and internal organs is possible because of excessive TGF-beta signaling. Scarring of internal organs such as the liver and lungs can be quite problematic. Results in loss of function and ultimately can lead to death can occur because of excessive TGF beta signaling.

If you look further, we have seen that TGF-beta signaling is a superfamily that has a lot in common with BMP signaling as well. And it also has a lot of cytokines. It can also influence the induction of ECM proteins and trigger other cytokine production. And TGF-beta, the prototype of the family, can signal through its cell surface serine threonine

kinase receptors. Besides its role in cell differentiation, migration, adhesion, etc.

, it can also induce epithelial-to-mesenchymal transition. It is acting via the SMAD pathway. The SMAD pathway followed by the MAP kinase pathway among the different types of epithelial-mesenchymal transition type 2 is described as being associated with wound healing, because epithelial-mesenchymal transition has been discussed many times, so I will not get into the details. There are two types: type 1 and type 2. You can refer to the previous classes.

But type 2 is more associated with wound healing and tissue regeneration. It can also trigger organ fibroid formation or fibrosis. And normally, the type 2 response of TGF beta signaling is induced by inflammatory stimuli. It can be triggered by the secretion of growth factors such as TGF-beta and EGF, which are other epidermal growth factors. Different endocytic routes are used to internalize the TGF-beta ligand and its receptor, and these pathways can control the activity of downstream events.

If a ligand is bound to the receptor, there is a receptor-ligand complex that will be internalized. That's a typical way of functioning. Sometimes, they get phosphorylated in the cytoplasmic domain. Sometimes the entire ligand-receptor complex gets inside the cell. Internalization can happen through different routes.

It can happen through clathrin-coated vesicles that can promote the signaling, while caveolae-mediated endocytosis, clathrin, or caveolae, either of them can happen. Plays an important role in the termination events, such as stopping cell proliferation; continuing proliferation will lead it to come out of the cell cycle. Although the steps of the termination mechanisms are not fully understood, all we know is that clathrin- or caveolae-mediated endocytosis is taking place, and the early endosome is considered. It has a great clue that indicates where this TGF beta receptor and TGF beta complex are going and promoting a proper signaling event. Recently, several research studies have been published that suggest the early endosome plays a crucial role in the termination of TGF-beta signaling occurring in a cell.

While TGF beta signaling is turned on, cells cannot continue to respond forever. Whether it is an anti-proliferative or pro-proliferative role, it must also come to a cessation. It's not that you just have an accelerator; you also need to have a brake. The early endosomes could hold the key in diverting the actual signal or the actual ligand-receptor complex to where it should go. It is important not only to maintain a spatial environment for effective signaling but also to direct the internalized cargos toward various degradative pathways so that the signaling does not continue inside the cell.

So if you look into the functional domain, normally the TGF beta signaling helps in the activation of proteins called SMAD proteins. We discussed in several other classes that especially SMAD 2 and 3, with the help of SMAD 4, are contributing to the internalization of this SMAD complex into the nucleus, where they bind to different TGF beta responsive elements present in the DNA. So SMAD2, SMAD3, SMAD4, and SMAD7 are there, and they have a lot of common domains. Domains are not important, but what we should understand is that based on the presence of these domains, the SMADs can decide where and at what level they have to transactivate. The R-SMAD, which is basically SMAD2 and 3, and the Co-SMAD, which is SMAD4, have a conserved homology region called the MAD homology region, known as MH1.

And MH2 domains are connected by a linker domain, while the ISMAD, which is MAD7, lacks this MH domain. They don't have it. And the MH1 domains that contain DNA binding, except for SMAD2, because SMAD3 has the DNA binding domain and the nuclear localization signal also present in them. As you can see here, different regions are marked: MH1, MH2, and NLS, etc., are present, and NES also.

SMAD4 has a nuclear export signal, and this also has a nuclear export signal. So the different signal domain regions present in this SMAD decide where this complex has to go and act. And also the MH domain, that is, they are the functional domains that are present in these SMAD proteins, and the R-SMAD, which are SMAD2 and SMAD3, and the Co-SMAD, SMAD4, proteins have conserved MAD homology regions. which is very important for their identity and also for how they get phosphorylated because they need to undergo phosphorylation for their biological function. The ISMAD-SMAD7 lacks the distinctive MH1 domain, and the presence of these NLS and NES domains determines where the protein will go into or out of the nucleus.

So if you look further into the SMAD signal transduction pathway, they can act as an effector or the effective translator of TGF-beta signaling inside the cell. TGF-beta and its receptor are present at the cell membrane, and it goes into this SMAD2/3. SMAD2, SMAD3, and along with Co-SMAD, they can go into the nucleus and turn on a lot of genes, including the SMADs themselves, and SMAD7 is one of them. It can come inside, and it can negatively influence the whole SMAD assembly. The SMADs are a family of intracellular regulatory proteins that act downstream of the TGF-beta type 1 receptor in the cell's response to specific TGF-betas.

So the receptor-regulated type 2 and type 3 SMADs are substrates of type 1 receptor serine/threonine kinase activity. The receptor has that ability. Once these SMADs are phosphorylated, they form a complex with a common mediator SMAD known as SMAD4. It's also known as Co-SMAD. This SMAD complex translocates to the nucleus,

where it is recruited to specific DNA binding sites on various genes, and it can act as either activators of gene expression or inhibitors of gene expression, or it can also act as co-activators to regulate the transcription of specific genes.

So TGF beta in regeneration, as I already told you, we have discussed TGF beta and regeneration, but we should understand that any injury need not lead to a typical regenerative response; rather, how the body responds is decided mainly by the TGF beta signal. So the TGF beta signaling pathway in spinal cord injury is quite interesting because it's one of the well-studied injury models, even in mammals. And people have explored what the therapeutic potential is in the TGF beta signal. I would also like to tell you that sometimes this excessive TGF beta signaling can lead to fibrosis, which can act like a bottleneck or a stumbling block in the proper functioning of that organ. So these days, research also goes in those areas where you can put lots of junk DNA sequences, which have This smad binding region.

So because of this, this junk sequence that you dump in will sequester away all the excessive Smads so that it will reduce the extent of fibrosis. Instead of binding to various collagen genes and turning on a lot of extracellular matrix proteins, this junk DNA, which doesn't have any promoter, acts like a decoy to divert the activated SMAD. That treatment also is available. So pathological processes after spinal cord injury are well studied in various organisms on the basis of primary injury caused by external forces. You can do injury in multiple ways.

It further develops into a secondary injury. If the inflammation lasts for a longer time, then cell death will occur because of the inflammation, and it will develop into an injury ecosystem that may never heal in that place, which can present different situations, such as acute stage, subacute stage, and chronic stage. The acute stage refers to a fresh injury, while the subacute stage means it is taking time to heal, and once it is never going to heal. Heal, then it becomes a chronic stage, so you can see that a primary injury can result from a fall, a car accident, or other violence. Everything can have a spinal cord injury; it is possible, and that mainly leads to a secondary phase where inflammation, free radical edema, vascular injury, cell necrosis, excitotoxicity, etc.

, happen in that early acute phase and then in the subacute phase. What happens? Apoptosis, axonal degeneration, glial scar formation, matrix remodeling, etc. Happens in the subacute stage. And in the chronic phase, what happened? Cystic activity formation and glial scar maturation are occurring.

So a scar is already formed. Healing did not occur. It can get filled with some junk, but the organ is not functioning because the damage remains damage. They remain paralyzed

for the rest of their lives in the chronically injured spinal cord. So the TGF-beta signaling pathway in spinal cord injury, if you look further, shows that the normal TGF-beta signaling can work in multiple ways. Sometimes it is beneficial; sometimes it can be harmful, depending on which species you are talking about.

After transforming growth factor beta binds to the receptor, it phosphorylates SMAD2 and SMAD3 through the canonical pathway. Then the activated SMAD2-3 combined with SMAD4 goes into the nucleus. We have seen that very clearly. Finally, this mad complex is shuttled to the nucleus and regulates gene expression and the pathological process of spinal cord injury kick-starting. TGF-beta is also able to regulate inflammation, apoptosis, and glial scarring.

Axon regeneration can happen in those species that are capable of doing so. Mammals cannot regenerate. And after the spinal cord injury, it can also influence the spinal injury or the potential for regeneration through non-canonical pathways, which include the mitogen-activated protein kinase, MAP kinase, nuclear factor NF kappa B, and C-jun terminal kinase. Also known as JNK and RhoA-Rho associated kinase, also known as ROCK, and phosphatidylinositol 3-kinase, PI3 kinase, and protein kinase B, also known as AKT, and also the mechanistic target of rapamycin. So the PI3, AKT, and mTOR pathways can also come into the picture based on TGF-beta signaling in a non-canonical manner.

MAP kinase, NF kappa B, JNK, RO, and ROCK are activated, and PI3 kinase and AKT, along with mTOR, all lead to the regulation of inflammation, apoptosis, and scar formation. This happens during the chronic phase, whereas in the acute phase, normally this is the situation that represents the canonical signaling of the TGF-beta signaling. If you look into the mechanism of spinal cord injury, the pathological changes of spinal cord injury that are secondary injuries are quite complex and involve many mechanisms and signaling pathways related to the progression and prognosis of spinal cord injury. The TGF-beta signaling pathway is widely involved in various physiological and pathological activities of cells. It is also important during homeostasis that they are not turned on only when there is an injury.

Various research studies have been focusing on the regulatory roles of the TGF-beta signaling pathway in neuroprotection, nerve regeneration, and glial scarring after spinal cord injury. So let us see what the neuroprotective effect is. Various studies have also shown that the TGF beta signaling pathway plays an imperative part in the differentiation and maturation of neurons, astrocytes, and microglia, which have important implications in the physiology and pathology of the central nervous system. Means TGF-beta signaling is playing an important role right from the formation of the organ itself.

Astrocytes and microglia have different phenotype changes in the spinal cord injured condition, which can be divided into A1 astrocytes and M1 microglia with different neurotoxic and pro-inflammatory effects, and A2 astrocytes and M2 microglia with neuroprotective and anti-inflammatory effects, so A1 and M1.

A1 astrocytes and M1 microglia have neurotoxic and pro-inflammatory effects, whereas A2 astrocytes and M2 microglia provide neuroprotection and anti-inflammatory effects, which are just opposite to each other. This one has to be kept in mind when you study the TGF beta signaling involvement after spinal cord injury. We'll come to that in a little while. Neurotoxicity and inflammation can result in tissue damage, and neurons are vulnerable to apoptosis and demyelination soon after an injury, so adjusting the polarization of the cells can reduce inflammation and improve neuronal survival shortly after injury. Before repairing, it should also take care of the signaling events to ensure that it does not enhance the existing damage.

So demyelination, etc. Happens, then the neighboring part will also get affected. So TGF-beta signaling promoted the transformation of A1 to A2. That is pro-inflammatory to anti-inflammatory astrocytes in a dose-dependent manner, increasing neuronal survival and improving motor function recovery after spinal cord injury. So, this is the role played by TGF beta signaling. Interestingly, if you look further, many studies have shown that inhibiting the TGF beta signaling pathway is beneficial in alleviating spinal cord injury.

It may look like a conundrum. On one side, you are saying it is helpful in making the region A1 to A2 switch, and it's helping in apoptosis survival. And another study suggests that inhibiting TGF beta signaling is beneficial. So we can get a little confused. Is it a helper or a destroyer? Studies demonstrated in rats that the spinal cord injury model, once it is made, shows that apoptosis in spinal cord-injured conditions is mitigated by inhibiting the TGF-beta/Smad signaling pathway. Remember, rats are not zebrafish; rats are mammals.

Other studies have shown that inhibiting the TGF beta signaling pathway can reduce the cellular inflammatory response after spinal cord injury. In addition to this, it has a similar effect in other diseases as well, not just in spinal cord injury. In mild traumatic brain injury, TGF-beta 1 induced inflammation is not alleviated; that is why removing TGF-beta signaling is going to be beneficial. Apoptosis was reduced, and inflammation was reduced by inhibiting TGF-beta R1 or reducing TGF-beta 1 itself. So here you are delineating that TGF-beta signaling, when activated, induces inflammation.

And it also induced apoptosis, but this allows less and less TGF beta signaling to happen because the TGF beta receptor is downregulated and the ligand is downregulated. So

what you learn from here is that although the initial TGF beta signaling is problematic, it prevents further problems from existing in that spot by getting rid of it. It's like if your ear has some water in it; whenever you try to get it out while taking a bath, it will not come out, but if you pour some more water into the ear, it may help. And you fill your ear with water, then you bend it; even the trapped water will come out. Something similar logic works: TGF beta signaling initially looks like it is causing trouble, but it will prevent its own production and its own receptor so that no further TGF beta signaling is happening.

These studies indicated that the TGF beta signaling pathway plays an important regulatory role in the pathophysiology of spinal cord injury by regulating inflammation and apoptosis after TGF beta signaling. Spinal cord injuries improve, and neuroprotection after spinal cord injury can be promoted by properly manipulating, which is the correct term, the TGF beta signaling based on which species you are discussing. Now it's a regulation in nerve regeneration. Spinal cord-injured rats treated with progesterone showed an enhanced oligodendrocyte differentiation and promoted myelin regeneration via TGF beta 1-mediated effect. Research has reported that TGF beta upregulated SMAD6 expression, one of the SMADs Promoting the differentiation of neuronal stem cells.

SMAD6 also plays a role in the BMP signaling. And neural stem cells differentiate into neurons in a spinal cord-injured rat. Neural stem cells are now capable of fixing the damaged spinal cord by converting themselves into neurons; NSCs getting converted into neurons, and TGF beta 1 could improve the synthesis of extracellular matrix components following the spinal cord injury, promoting neuronal survival as well. However, some other studies have found that TGF-beta signaling could inhibit neurite outgrowth. Neurite outgrowth means the growth of the axon. Whenever there is damage, on one hand you are saying neurons are formed, and on the other hand you are saying neurons do not extend their axons.

So how do they make contact? So the promotion or inhibition of TGF beta signaling in axonal regeneration may depend on which cell type affects which organ, the local environment, and the microenvironment. Or at what stage of development this animal is: is it a neonatal animal, or is it an adolescent, or is it an old animal? So that is the question of which stage of development and the physiological state of the cell itself can decide. It's just like you are a great singer, but you are singing in front of a wall; the wall will not clap after your performance. So this logic you should have, or the listener should know what song or how tough that song is in order to appreciate it. And also the regulation of glial scar formation can influence TGF beta signaling; we have seen it as a rescue measure, but it can come with its own baggage.

It can render the tissue non-functional, so the glial scar formed in the acute stage after spinal cord injury is beneficial for limiting the inflammatory response and preventing the spread or expansion of the injury. However, the glial scar inhibits axonal regeneration. Once the scar is formed, no stem cells can move. It acts like a wall, and nerve recovery will be affected after entering the chronic stage.

So you don't want a scar to be formed. Spinal cord injury scars can be fibrous or glial scars, including a lesion core composed of fibroblasts and inflammatory immune cells, and the lesion boundary is formed by hyperchronic astrocytes. When the scar is formed, it can also attract many inflammatory cells because the scar is essentially a disturbance, which will keep the injury a chronic injury. Moreover, the TGF-beta secreted by M2 macrophages, which are anti-inflammatory, induced glial cell scarring through the activation of astrocytes. Although M2 macrophages are supposed to be anti-inflammatory, they also secrete TGF-beta, which can create glial scarring by activating astrocytes. Blocking the activation of the TGF-beta signaling pathway can inhibit glial scar formation and promote axonal regeneration.

That is another benefit. Reactive astrogliosis, which means astroglia that accumulate become astrogliosis, negatively affects recovery from spinal cord injury. Research has found that TGF beta-dependent SMAD2,3 signaling induced astrocyte proliferation. Once there are more astrocytes, naturally it will create astrogliosis. which is triggered by the TGF beta signaling. Various other studies have confirmed that the TGF beta can promote fibrosis and pathological processes of a disease, including the formation of fibrotic scars in spinal cord injury.

So if you look further into how the regulation of TGF beta signaling is possible in an actual spinal cord injury. Conservative and surgical procedures are the currently available mechanisms for the treatment of spinal cord injury. Conservative means how you deal with the fact that restoring the physical damage can be fixed to some extent. It will not make a fully functional spinal cord. However, these methods are ineffective; they are useless in alleviating the pathological progression caused by the secondary injury that one has to deal with.

If the secondary injury has kick-started, it is a long-standing trouble which can eventually become a chronic injury. Finding an effective target is the key to treating the secondary injury. Which pathway should be blocked? That is what the research is focusing on in the current scenario. The TGF-beta signaling pathway, as a classical signaling pathway, plays an essential role in spinal cord injury; especially its involvement in secondary injury could be used as a target for intervention. If TG-beta signaling is

maneuvered properly, you can prevent getting a secondary infection.

secondary acute injury and/or developing a chronic injury. So numerous studies have shown that activating or inhibiting the TGF-beta signaling pathway can be used as an attractive candidate for managing spinal cord injury pathology. Here, what we have discussed so far is the role of these factors in treating spinal cord injury by regulating the TGF-beta signaling and SMAD pathway. So there is one more approach called the cell transplantation approach. When there is a spinal cord injury, you can inject some cells. The kind of cells we will see are mesenchymal stem cells, known as MSCs, which are a promising treatment for spinal cord injury.

They offer a favorable environment for axon regeneration so that they can bridge the gap. If there is a gap, it can bridge the gap, and functional recovery is possible. In spinal cord injured rats, TGF-beta secreted by the bone marrow mesenchymal stem cells, also known as BMSCs, upregulated the expression of Smad6, and in the late phases of spinal cord injury, it promotes the differentiation of NSCs, that is, the neural stem cells, into neurons; thus, late phase TGF-beta is a favorable molecule. Research has demonstrated that intravenous infusion of MSCs and extracellular vesicles in spinal cord injured rats could target macrophages to upregulate TGF-beta. Activate the TGF beta signaling pathway so that it can make more and more neurons.

This results in increased expression of blood-spinal cord barrier protein, also known as SCBSCB-related protein, because the spinal cord needs to have a blood-brain barrier established, ultimately promoting functional recovery. If blood and neurons are mixed, it won't be able to restore normalcy. Also, adipose-derived stromal cells (ADSCs), when transplanted, can activate the TGF-beta, SMAD, and PLOD2 signaling pathways to reduce SCI neuronal apoptosis and glial scar formation, thereby improving neuronal functional recovery. So we have seen different angles played by the TGF-beta signaling in restoring normalcy after a spinal cord injury. But it is also playing a role in various other tissues, including heart regeneration.

We'll study more in detail about other signaling pathways in the next class. Thank you.