**Regeneration Biology**
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**Lecture: 32**

W7L32_Different cellular signaling during regeneration. Examples- Jak-STAT and Fgf signaling

Hello, everyone. Welcome back to another class on regenerative biology. So in today's class, we will try to learn how different signaling contributes to a regeneration event. And we necessarily will not stick to one particular species or one particular system simply because every system has its innate qualities; for example, liver regeneration is normal across every species, and intestinal regeneration is normal across every species, no matter whether it is regenerative or non-regenerative. Hence, we do not want to discriminate.

So, in today's class, we will try to understand what the implications of Jack, Stat, and FGF signaling are and how they contribute to a regenerative response. So, JAK-STAT signaling in the pathway is a very critical conduit in the signal transduction from the extracellular milieu to nuclear gene regulation, and it encompasses around 38 non-protein ligands and up to 36 distinct cell surface receptors that contribute. So you can imagine what the number of ligands and the number of receptors coming into the picture is. So the JAK-STAT signaling pathway incorporates about four different JAKs: JAK1, JAK2, JAK3, and TYK2, and each plays an integral role in transmitting the cytokine signals within the cell.

So we know the JAK is basically the inducer and the STAT is the one that, after phosphorylation, goes into the nucleus. The STAT family includes seven members: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. So these are all the major STAT genes, and each STAT protein contains an amino-terminal domain, a coiled-coil domain, a DNA-binding domain, and an SH2 domain; lastly, it has a carboxy-terminal transactivation domain. So once this STAT-3 binds to the DNA inside the nucleus, It will attract more activators by using its transactivation domain. So this is a simple way of functioning.

So JAK is the enzyme and STAT3 or different STATs get phosphorylated. STAT3 is one of the well-studied and predominant STAT molecules. Signal, transducers, and activators of transcription. That is the full form of STAT. So JAK-STAT, what it does before and after its activation.

Under physiological conditions, the presence and distribution of JAK1 and STAT3 indicate their integral roles in maintaining central nervous system homeostasis. So they are present, they should be there because quite a lot of normal gene expression is also driven by. So we cannot assume that JAK-STAT is meant only for regeneration every time. Many times, homeostasis running the normalcy of a tissue also needs lots of gene expression events, and that is also governed by JAK-1 and STAT-3. And after SCI, what are spinal cord incision and spinal cord injury? One of the major parts of the central nervous system is the spinal cord.

A cascade of molecular events involving the JAK-STAT pathways was initiated. So parallel to this, what happens is that the concentration of interleukin 6 (IL-6), which we know is one of the predominant pro-inflammatory cytokines in the injured tissue, rises upward from around 3 hours post-injury and peaks at around 12 hours. And then gradually diminishes. So interleukin-6 is also induced within hours of injury in the spinal cord. So the spike in IL-6 concentration is closely aligned with the phosphorylation events of JAK1 and STAT3.

So we should understand that JAK 1 and STAT 3 are important for maintaining homeostasis, and upon injury, there will be an induction of IL-6, which is also in conjunction with the phosphorylated STAT 3 levels. Following a spinal cord injury, there is a notable rise in the levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha. Both at the mRNA and the protein levels. Besides IL-6, the members of the IL-6 family of cytokines, such as IL-11, ciliary neurotrophic factor, also known as CNTF, leukemia inhibitory factor, known as LIF, cardiotropin 1, and oncostatin M, are known to activate. These molecules are known to activate the JAK-STAT signaling pathway soon after spinal cord injury.

In spinal cord injury, the phosphorylation status of STAT3 at tyrosine 705 persists for up to 160 hours. That means roughly seven days following the injury, while the interleukin 6 levels and the JAK1 phosphorylation gradually return to baseline. So up to around seven days, it contributes to a spinal cord injury response, and this is all I am talking about in an animal where regeneration is possible. Now, associated with the JAK-STAT signaling, there is another major signaling pathway that contributes, which is NF-Kappa-B, because tumor necrosis factor alpha has a strong connection with NF-Kappa-B, and NF-Kappa-B acts in the nucleus. Let us see how NF-kappa B aligns with the JAK-STAT signaling.

Functional NF kappa B complexes are present in all nervous system cell types; every cell type, whether it is a Schwann cell, oligodendrocyte, radial glia, or neurons, is expressed, including neurons, astrocytes, microglia, and oligodendrocytes. So NF kappa B activation in glial cells is generally associated with inflammation. So, like I told you, inflammatory markers are normally IL-1, IL-6, and TNF alpha. And TNF alpha has a significant role to play in the activation of NF kappa B. Studies have shown that NF kappa B is activated by amyloid beta peptide toxicity and excitotoxic or oxidative stress.

Many times, if you have too much signal, even from free radicals or maybe any other external chemicals, too much excitation happens to the neuron, which will lead to excitotoxicity. And this oxidative stress also plays a major role in the induction of NF-kappa B because all of these will cause an inflammatory response. NF-kappa B can exert a positive or negative influence depending on the cell type and the nature of damage, because although we are mentioning cellular damage, it can happen in different ways. For example, apoptosis is a form of cellular damage, necrosis is also cellular damage, and even cutting with a knife or scissors is also a form of cellular damage. So, depending on what cellular damage occurs, NF-kappa B can play a differential role.

Traumatic brain injury means you are creating a real wound or injury, and excited toxicity and ischemia—ischemia means cutting the blood supply to that area, which is a lack of oxygen—are characterized by NF-kappa B's activation; no matter which part of the central nervous system you are injuring, whether it's the brain or spinal cord, NF-kappa B gets activated. Simultaneously, the basal level of NF-kappa B activity in neurons is necessary for protecting these cells from traumatic damage, with the effectiveness of protective effects depending upon the timing post-injury. So soon after the injury, there will be some damaged cells, so they have to be cleared. There is no point in protecting those damaged cells. However, this traumatic response can trigger damage to the existing cells, so you don't want them to die.

Say, for example, you have a wall and you created a hole in the wall, so 100 bricks are lost from that wall. There is no point in protecting those lost bricks, but surrounding that hole there are so many bricks you don't want to fall down. If they also start falling down, the entire wall will collapse. So, adjacent to the injury spot, they need to be protected. This is what we are referring to by Protection is based on the timing of the injury.

Soon after an injury, they are vulnerable to being damaged. Otherwise, this injury was in the X area, and now, because of the cell death or lack of protection, that X will now become a 100X area. So you don't want that to happen. So endogenous NF-kappa B activation might protect neurons under stress conditions caused by damage by suppressing p53-mediated apoptosis. P53, as we all know, is basically induced based on DNA damage.

Soon after DNA damage, P53 is expressed and it turns on P21, which prevents the cell from continuing the cell cycle. That is the worst step. Then it will turn on the DNA repair gene. And again, if the damage persists, it will turn on the apoptotic genes. So P53, basically a tumor suppressor gene, has one of its roles in apoptosis; provided the damage is not fixable, in that situation it undergoes apoptosis.

We should not think, "Oh, if P53 is induced, the cell will die," but P53-mediated apoptosis can also be prevented; it can protect the neurons from undergoing apoptosis. Additionally, the protective role of NF-kappa B in neurons includes anti-apoptotic effects mediated by the induction of caspase inhibitors. Caspase means it is a protease. So wherever there is an aspartic acid residue, there is an amino acid; it will cleave aspartic acid, which is casp, and caspase is a marker of cellular apoptosis. Thus, this NF kappa will induce caspase inhibitors so that this protease will not act on the cell.

It will also help in the expression of several antioxidant genes that can scavenge free radicals. So these are all meant to protect the cells. In contrast, inhibiting NF kappa B in microglia and astrocytes may contribute to a favorable outcome following a central nervous system injury; this is also being observed in the interaction between the NF kappa B and STAT3 pathways. Now let us see how NF kappa B is contributing to or aligned with STAT3. You can see the interaction between the NF kappa B and STAT3 pathways, particularly in response to TNF alpha stimulation.

As I already told you, TNF alpha is a pro-inflammatory cytokine that involves a complex cascade of events, as you can see in this picture. Complex leads to the transcription of certain genes, including interleukin 6 (IL-6), which we know is a very strong pro-inflammatory cytokine, and leukemia inhibitory factor. The IL-6 produced by this process can act in an autocrine or paracrine manner. Autocrine means the cell that produces a ligand utilizes it. Paracrine means the cell that released the adjacent one, just like a mosquito bite on your hand while you are scratching yourself or scratching your neighbor's back.

So, for example, scratching your own hand is called autocrine, while scratching your neighbor's hand is called paracrine. So, that is the main difference. So IL-6 can act in either way leading to further signaling events. So pro-inflammatory cytokines can have serious roles in triggering the early signaling events. So this activation involves IL-6, which has its own dedicated receptor, the IL-6 receptor glycoprotein complex GP-130, and another molecule, JAK-1,2 Janus kinase.

Jack 1/2 and kinase, which then phosphorylates, are capable of phosphorylating statically. So what you understand is that interleukin 6, when it binds to its own receptor, can trigger the activation of JAK1 and JAK2. That will end up getting activated as a phosphorylated Stat3. Phosphorylated stat3 enters the nucleus to initiate the transcription of several target genes, including the suppressor of cytokine signaling known as SOCS3. The cellular inhibitor of apoptosis known as CLAP2 illustrates what you can see in this picture: the intricate details that are interconnected between these two pathways during cellular signaling.

As you can see here, TNF alpha is present and the NF kappa B complex is present. and it will turn on a bunch of NF Kappa B complex genes. The names of genes are not important, but we understand that at the end of the day, you will end up getting more IL-6 and LIF, which in turn can bind to GP-130, a receptor for IL-6, and it can trigger the transcription or activation of STAT3 and the formation of SOX3. That is the link between NF-kappa B and statins; this is their intercommunication upon damage. The astrocytes are characterized by the activation of inflammatory pathways, including NF-kappa B.

So let us see inflammatory mediators such as interleukin-1 beta. We also remember that interleukin-1 beta is involved in fin regeneration as well. And tumor necrosis factor alpha and IL-6 are induced no matter which tissue you are talking about. These astrocytes exhibit hyperphosphorylation and activation of the STAT pathway, typically in response to injury, so we can now confidently say that if there is inflammation, if there is TNF alpha, if there is NF kappa B, and if there is IL-6 activation, phosphorylated STAT3 will be produced, which can turn on several genes, including IL-6 itself, typically in response to injury, additionally, what you see is the inhibition of anti-inflammatory PI3 kinase. Phosphoinositide 3-kinase, which is the PI3 kinase AKT pathway in these cells, results in the decreased expression of neurotrophic factors, which contributes to synaptic loss and apoptosis; this is the flip side of it.

Therefore, when these anti-inflammatory pathways are kick-started in the very beginning, you are not allowing this inflammation to initiate. Then you end up having some synaptic loss. So the take-home message is that early inflammation is beneficial for the cell from a regenerative point of view or a cell protection point of view. So, that is what you are seeing here. There are different types; this is a picture of a neuron.

You can see normal glycolysis, etc. Takes place, and the STAT3 pathway is turned on, the NF-kappa pathway is turned on, and the PI3K-AKT pathway is turned down. And TGF beta levels are turned down. So all this work together so that the neuron can be protected, provided proper inflammatory pathways are kicked in. So cellular responses to the injury after a spinal cord injury are being shown here. What you are seeing here is an overall view of a neuron, and two kinds of classification are present here: whether it's a neuron, microglia, or macrophage; it doesn't matter.

There are two classifications. One is a protective pathway on the left-hand side, which is shown in green. And on right hand side, you are seeing a harmful pathway. So we should understand that harmful pathways are there not to cause any harm, but rather they are countering the protective pathway because sometimes every cell does not necessarily need to be protected, and there is a TGF-beta receptor. Additionally, there are many genes induced, such as GAP-43 and GFAP, and most importantly, you can see the JAK-STAT signaling that is turning on, which is a protective pathway. of the NF Kappa B complex, which in turn triggers the formation of several factors such as CCL2 and VEGF.

So these ones beyond certain limit they can trigger a harmful effect. So you can see in this box pro-inflammatory, prolonged pro-inflammatory. Can be harmful, and similarly, you can see factors here: IL-6, TNF-alpha, IL-1, and IL-12 are all mentioned here. The same factors are present in the protection as well: IL-6, LIF, CNTF, etc. However, the protective pathway acts from a protection point of view only if it is short term, as I mentioned first—maybe around seven days only.

You don't want that to continue for long. And now we will look into the FGF family and FGF signaling. Because of this class, we will address both of them. FGF signaling is of two types. Canonical FGF signaling, hormone-like FGF signaling, and intracellular FGF signaling.

There are different molecules. We will not read their name, FGF 1, 2, 3, like that is there. And there are also plenty of FGFs here. So they can act in a canonical way, a hormone-like way, and an intracellular FGF way. So in mammals, there are 22 members of the FGF family, and these are relatively small proteins, with a molecular weight of around 20 kilodaltons. Most FGFs, which are the canonical FGFs, are secreted and function as paracrine or autocrine growth factors.

Like I told you, autocrine and paracrine already. So they do not act very far away. They don't have any far-reaching role. For a typical canonical signaling pathway molecule, there is a subfamily of FGF that includes four intracellular proteins: FGF 11, FGF 12, FGF 13, and FGF 14. They are involved in regulating ion channels, and they can have a completely different role than the conventional canonical molecule.

Another group includes FGF 15, 19, 21, and 23. They consist of secreted, hormone-like proteins. That means they can travel distances through the bloodstream. Hormone-like proteins regulate various aspects of organism metabolism. So they are important for housekeeping.

They are important for providing cellular protection. and they are also important in communicating over longer distances. So FGF can act as a stimulant for regeneration and repair. And this is a complex picture; don't worry, we will dissect it. Unlike higher vertebrates, such as birds and mammals, the lower vertebrates, such as fishes and amphibians, have a pronounced capacity to regenerate amputated organs. Zebrafish and the amphibian Exolotl, Ambystoma mexicanum, represent two good models to study organ regeneration.

And particularly, the role of FGFs is well studied in this species. As you can see here, there is induction of FGF, and it binds to the FGF receptor. The FGF receptor, which has an activation role, involves many cellular proteins; the activated receptor can now cause the phosphorylation of Stat 1, Stat 3, and Stat 5, and it can enter the nucleus. Another pathway is that it can influence the Ras sos 2 and Grb2 pathway, which can create. A PI3 kinase, phosphatidylinositol 3-kinase activity, which in turn causes the phosphorylation of AKT, can also enter the cell and induce cellular proliferation.

Another pathway it can take is through the RAF, MEK, and MAF kinase pathway, and it can also create a pro-regenerative environment. Another factor is the presence of PIP2, which occurs more frequently due to the switch between PIP2 and PIP3; this can lead to the creation of a PLC gamma and DAG, diacylglycerol, and protein kinase C pathway. Another connection is through the second messenger IP3 and calcium signaling. So one signaling, FGF signaling, once it is activated, depending on the cell type and depending on what cellular or intracellular molecules are utilized, it can trigger many pathways including STAT3, AKT, MAP kinase, and protein kinase, etc. And you can see that the specific inhibition of the FGFR receptor suppressed caudal fin regeneration in zebrafish.

This effect was mediated by the suppression of blastema formation. It does not allow the blastema to form, and the mass, whatever cell mass that should have been formed and is meant for active proliferation, is not occurring; if you block it, the effect is basically the blocking of the FGFR receptor. That means FGF can act only on the FGFR receptor; if the receptor is blocked, then the fin amputation will not cause a regenerative response. And overexpression of the dominant and negative mutant of FGFR1. Dominant negative basically means it acts exactly like the receptor, but it will not do the job of a receptor.

It will bind to the ligand and sequester it. Just act like a sponge. How a sponge will suck up the water. Same way, dominant negatives act like a mutant. The mutant of FGFR1 can have a similar effect. Scientist Keating and colleagues found that FGF20 is essential for zebrafish fin regeneration, which we discussed in our previous classes.

Those who want to revisit it can look back at other class lectures. Zebrafish FGF signaling is also known to be required to generate the spinal cord, liver, heart, lateral line neuromast hair cells, and rod photoreceptor cells, as well as extraocular muscles, meaning any tissue, no matter what tissue you are talking about. The FGF signaling is pivotal. An enhanced expression of FGF8 was detected in the regenerating larval limbs of the African frog Xenopus, and the FGF inhibitor also suppressed the regeneration of the Xenopus tadpole tail, which means that, similar to zebrafish, FGF is important for Xenopus as well. The cooperative application of FGF2, FGF8, and BMP7 to the skin wounds of axolotl normal skin wounds, not amputation wounds, involved just putting FGF2, FGF8, and BMP7 not only on axolotls but also on newt, which resulted in ectopic limb formation instead of simply wound healing.

That means when these molecules come together, FGF2, FGF8, and BMP7 can create a limb in an unwanted place. So, limb regeneration in axolotls is known to be nerve-dependent. So, it has been shown that FGF8 in the spinal ganglia of the axolotl is derived from the long axons of the regenerating limbs, wherever there is a nerve in that limb that secretes these FGF8 molecules. So the studies on lower vertebrate models demonstrate the importance of FGF in organ regeneration. So if you look cellular responses, they are processes that is regulated by FGF.

If you look here, you can see that FGF is important; it can trigger lots of proteases, it can trigger a proliferative response, it can trigger stemness, it can trigger senescence, cellular senescence, which means age response, and it can inhibit cellular senescence. It's a role activation, and it can also trigger mortality and angiogenesis. It can trigger differentiation. It can inhibit apoptosis. So this symbol shows I inhibit apoptosis, and it can also trigger inflammation.

during a regenerative response. So, to summarize further, what you can see here is that the members of the FGF family function as potent stimulators of tissue repair. The pro-reparative, that is, regenerative effects of FGF are based on their ability to stimulate cell proliferation and migration in one way and enhance angiogenesis, which is the formation of blood vessels, and regulate inflammation. That means you need inflammation, but you don't want inflammation for a prolonged period. Just like whenever you talk about inflammation, you remember how much salt you have put in your dish.

It is needed, but more is not good. So regulate inflammation, maintain cell stemness, and promote differentiation. The expression of various proteases, which are necessary for ligand maturation, is important. Many ligands have to be matured, including TGF-beta and tumor necrosis factor-alpha. Various other ligands need to mature through protease action. Many proteases are also important for clearing the extracellular matrix, the junk that is formed after injury.

So the bioavailability of FGF in damaged tissues is ensured by stress-promoted gene expression and, at least in the case of the FGF1 molecule, can act as a pro-regenerative gene. Many questions remain to be answered in FGF-regulated tissue repair. Although we know a lot, it also brings in many other leading questions, such as which branches of the FGF signaling pathway are responsible for specific pro-reparative cellular effects of FGF. Which action is inhibiting apoptosis or triggering inflammation to help regeneration? Or all of it.

So, such questions are always there. What are the relative contributions of individual cellular effects of FGF to tissue repair? That is a very interesting question to explore. How does the interplay of FGF with other growth factors and cytokines regulate repair? Is FGF good enough? Or, along with FGF, some other factor is also necessary. So these are all the angles or areas of research that are going on: what is the role of negative regulation of FGF signaling in the repair process? Can we know about FGF 20? If you remove it, you don't get fin regeneration; we know that, but why only FGF 20? What about other FGFs? Are they not doing their job? What is the mechanism? When there is trauma like ischemia.

That is trauma. So how is it contributing? There is no cellular damage. But the blood supply has been cut. How will you bring back normalcy? Especially the connections in the neurons are important. The expanding arsenal of modern methods.

And genetic information. Genetic analysis. It has a huge potential to help in answering these questions. Okay. We will study more about regenerative biology in the next class. Thank you.