Regeneration Biology
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Week: 5
Lecture: 25

W5L25_Spinal cord and brain regeneration-Conclusive mechanisms of regeneration

Hello everyone, welcome back to another session of regeneration biology. In this class, we will learn a brief outline about spinal cord and brain regeneration and how different mechanisms come into the picture for the regeneration of the brain and spinal cord. They are combined mainly because they are extensions of each other, basically during development from the ectoderm. Forms a neural tube and gives rise to a long neural tube, and the anterior-most portion of that neural tube expands and swells and becomes the brain. So, basically, the central nervous system ideally means the brain and spinal cord; hence, the regeneration mechanism also has a lot in common, although the medical implications of their damage vary. The brain controls the entire body, whereas the spinal cord mainly controls the motor neuron functions.

You know the rest of the body, like the arms, legs, etc., so zebrafish and various other model organisms have been used to study brain and spinal cord regeneration, and we will see how and what approaches people take to study this. So, to do brain injury studies, the first thing you need to know is How a trauma is created, how a poking is created. And normally, people use a mechanical injury method on both the brain and the spinal cord.

You can poke a needle through the nostrils, which is basically the anteriormost part of the brain that gets damaged. so you can see here there is a dot in the brain and that is a place where you damaged it and you can harvest the brain or you can section the brain and you can take the images and if you harvest the brain you can get into rna isolation protein isolation so this is the typical workflow that will be done just like what you saw in the retina regeneration study also where you do section and you take a selected block for visualization etc one such block The cross section of the brain shows one half as the left side and the other as the right side; quiescent neural stem cells contribute to brain repair and regeneration. Keep in mind that every brain undergoes some small scale of damage, but many times that will not reflect in a loss of function simply because. For a given task or function, more neurons will be allocated; for example, if 100 neurons are needed, it is not just 100, but 10,000 neurons will be allocated. Because of this, even if a few neurons are lost, I'm not talking about every function; a majority of the functions have more neurons allocated.

Then still that function will not be very badly affected. So, although this is not a rule, it is the way in which the organism survives. And in some organisms, there is persistent neurogenesis throughout the lifespan of the organism. Because, for example, in zebrafish, the brain also keeps growing. Our brains don't keep growing.

Your brain looks bigger simply because more and more neuronal connections are made. But the number of neurons is fixed in our case, in the case of humans. They don't increase in number because they do not proliferate. But newer and newer connections will be made, which increases the bulk of the brain. So in the adult mammalian brain, quiescent neural stem cells, called NSCs, possess a latent capacity to generate neurons and glia, especially in mice and rats, where there is a zone called the subventricular zone that undergoes constant proliferation in the brain.

The subventricular zone is usually mechanistic and lines the cerebral ventricles. Mechanistic insights into the origins and functional properties of quiescent anesthesia are starting to rise in rodents, Drosophila, and various other regenerative vertebrates. People explore different kinds of animals. Based on the angle from which you are looking, sometimes you may wonder why people are using different models. Why can't they stick to one model? The reason is that sometimes some models will be best suited for a given set of studies, whereas other models are suited for another set of questions.

That is why people explore different model organisms. It is becoming apparent that NSC, which are the neural stem cells, undergo different types of cohesion, such as G0, which is a G1 cell exit from the cell cycle, and G2 cohesion, which is G2 cell exiting the cell cycle. So the normal cell cycle is G1 phase. S, G2, and M. M stands for the mitotic phase.

Soon after mitosis, the cells are in the G1 phase. Then if it decides to continue in the cell cycle, it will go into the S phase. That is the DNA replication phase. Once DNA replication is perfectly completed, it will go into the G2 phase. But cells have the option of entering quiescence at the G2 stage or G1 stage.

So that is what the G1 stage quiescence is usually referred to as: the G0 stage. Environmental signals, such as exercise or feeding, might increase the activation of the quiescent NSCs. You can have NSCs activate. That means they can enter a more proliferative phase. Based on single-cell transcriptomic data, putative trajectories from quiescence to activation, from dormancy to the sleeping stage to the wakeful state, are called activation.

Have been reconstructed bioinformatically. People have explored those angles. In

general, quiescent NLCs are restricted to producing specific neuronal subtypes after activation in vivo. It is possible to modify these outputs. Experimentally, in some instances, you can tweak the output.

What are the possible cell types that are going to come from a particular activation event? The ability to control the outputs of quiescent NSEs will be an essential step in harnessing them for brain repair. Brain repair means brain regeneration. Those who are interested can read this article in Trends in Neuroscience. So one factor that is significantly contributing to brain regeneration is called BDNF. Zebrafish is a very well-studied model organism to understand brain regeneration.

BDNF, brain regeneration, and insights from zebrafish. Zebrafish are teleost fish that are widely accepted as model organisms for neurophysiology. Regeneration or neuroscientific studies. Adults show common vertebrate brain structures. Whatever the typical vertebrate, if you take any vertebrate of your choice, including humans, the typical structures are maintained.

Although the zebrafish brain is small and the human brain is big, the typical structures are restored as they are in zebrafish, similar to those in humans. And it has similar key neuroanatomical and neurochemical pathways relevant to various human diseases. That is why we use zebrafish to study regenerative biology. However, the brain of adult zebrafish possesses different characteristics. There are a lot of differences compared to those of mammals.

Intense neurogenic activity, which can be correlated with high regenerative activity. Properties. This is something we should understand: the neurogenic activity is quite intense. That means the zebrafish brain is waiting for an opportunity to make more neurons that are neurogenic and pro-neurogenic. One such factor that is contributing to or favoring is called brain-derived neurotrophic factor.

In short, it is called BDNF. It is also important for synaptic plasticity and the maturation of neurons, etc. It is playing a vital role in various aspects of brain function. It is a member of the neurotropin family and has multiple roles in the brain due to several biologically active isoforms interacting with different types of receptors. So BDNF is a well-studied molecule.

Tropic factors mean something acting on the surface that is called a tropic factor. If you are applying it like you may have seen in ointment, for tropical application only, that means on the surface. So BDNF is a trophic molecule that is called a tropic factor. BDNF is a well-conserved vertebrate protein across millions of years of evolution, with the

primary amino acid sequence between zebrafish and human BDNF being 91% identical, not similar. Identical means its similarity will go much higher.

Similarity basically means that basic amino acids or acidic amino acids are called homologous, but if they are identical, it means the amino acid is not changing at all. So, 91% of BDNF is identical, which tells you that no testing is done in evolution on that protein because it plays a vital role. You can see the same thing about histone proteins as well. Histone proteins are conserved from yeast to humans. You can imagine yeast is the most primitive eukaryote which just started having a nucleus.

Bacteria don't have histones. So that is also a highly conserved protein. So BDNF is also a highly conserved protein. The zebrafish brain is a valuable model for adding new insights into the future of BDNF in regeneration studies. Brain-derived neurotrophic factor and its mRNA distribution. So how are regeneration studies done? If you say, "Oh, gene A or gene B is important in regeneration.

" The first thing is that you should show proof. After the injury, what is its status? Does it come with extra? Does it have a differential location or a differential quantity of expression? And is the level going down? So, mRNA distribution is very vital in understanding that mRNA means protein will also be there. That is the assumption. And all of these are done in the cross section. Different regions are the telencephalon and mesencephalon.

And this is the broad categorization: diencephalon, rhombencephalon, and different regions of the brain. You don't need to worry about that anatomy section. All you understand is that there are different extremes. Extreme is telencephalon, then mesencephalon, and then towards the spinal cord region, which is rhombencephalon, you can see. And a normal injury will occur in the telencephalon.

So the cells containing BDNF RNA, which are seen as red dots because this is a cartoon, not a real picture. But you can see different areas here. There are red dots you can see here and also in this part. And then there is something called a PCNA, proliferating cell nuclear antigen, which is indicative of the cell proliferating.

And then, hue. Hue is a freshly formed neuron that will have a marker called hue. And this BDNF mRNA is present normally in the mature neurons. In several areas, they are close to the radial glia, which are the black dots. What you are seeing as black dots are the radial glial cells that have the marker aromatase. And proliferating cell green dots, which have the PCNA marker, and the Q positive azure dot, they are normally young neurons.

You can see them in certain regions: the red dots, which are BDNF positive, and also the green, which is proliferating cell; they all converge and merge together, and the hue cell, because normally if they are proliferating, that is also giving rise to new neurons. That is why the blue marker. And also, you can see there is a supporting cell that is the radial glial cell, which is seen as aromatase B enzyme positive, and the abbreviations are given for different regions, that is, cerebellar body, dorsal tail, and cephalon, so you can read it. It is not important just for understanding, but this picture is put in just to show that the expression of BDNF has a strong correlation with. Proliferation and also formation of new neurons.

That is the whole message given by this image. And if you look further, the BDNF is involved in brain regeneration. You can see here that there is a telencephalic injury caused by a needle. We already saw this picture. And you can see as the days pass: 1 DPI, 4 DPI, 7 DPI, 15 DPI.

And it continues. It takes for complete regeneration up to six months. But the studies are normally done for around two weeks because, whether it is regenerating or not, the story will be complete by around two weeks of studies. An interesting thing is that BDNF mRNA distribution in cross sections can be done at various times post-injury. So you can see this width of BDNF is highest here, and by around 15 days, it has become narrow. So this means the expression: the spread of expression.

It starts with a lot, as you can see here, plenty, because this is an injured area. There is plenty of BDNF expression, and it becomes less, less, less, less, and by day 15, it becomes very much restricted to very close to the injury spot. So, BDNF mRNA distribution in cross sections of zebrafish telencephalon after injury shows cells containing BDNF mRNA, which are shown as red color dots. Are and also in young hue positive and mature neurons have acetylated tubulin; that marker is an indicator of a mature neuron, whereas hue marker expression is a neuron only, but it is a young neuron, and they are shown in blue dots. So, acetylated tubulin is an old neuron or mature neuron that has blue dots and is hue positive.

They are being given a blue color that is freshly formed neurons, and in several areas, they resulted in close proximity to the proliferating cells. The message is that the newly formed neurons are seen very close to the BDNF-positive cells, indicating that the expression of BDNF is widespread. This is indicative of PCNA, which is the indicator of proliferation. BDNF and hue co-expressing cells are given a circle label, which you can see here, because the hue is blue inside and the red is outside; it's a cartoon, and that's how they have depicted so many hue-expressing cells that also have BDNF. So the take-

home message from this picture is that the expression of BDNF is very high soon after injury.

And as the regeneration is completed, the BDNF level goes down. But the expression of BDNF is associated with the proliferation of newly formed neurons. So these things tell you that BDNF is a pro-regenerative or pro-proliferative marker. And if you get rid of BDNF, you do not achieve normal regeneration. And you can see a schematic representation of adult neurogenesis: a comparison of the neural stem cell niche to the telencephalic ventricular zone of zebrafish and mice.

So now in this picture, it's a cartoon that compares how a zebrafish brain behaves versus how a mouse brain behaves after an insult. So A is basically the transfer section that is the zebrafish transfer section through the encephalon of adult zebrafish, illustrating different cell types present within this niche. A dash is the panel that depicts the differentiation of radial glial cells. In short form, it is written as RGC in neurons and some signaling pathways controlling the activity of RGC, which occurs in zebrafish. In zebrafish, quiescent RGCs that are type 1 are activated to become the proliferative RGCs that are type 2, which can regenerate proliferative neuroblasts that are type 3.

So type 1 becomes type 2 and later type 3, eventually differentiating into mature neurons. Type 1 is basically quiescent, and type 2 becomes proliferative. Type 3 becomes a neuroblast that is capable of giving rise to other neurons. Transverse sections, that is, the B panel in the mice, through the telencephalic region of the adult mouse.

B dash shows the panel that represents various steps. Of neural regeneration, starting from slowly dividing neural cells, that are NSCs. Their rate of division is milder compared to that of zebrafish, which are referred to as B cells. The B cells give rise to C cells corresponding to highly proliferative progenitors, also called transiently amplifying progenitors or TAPs. The C cells differentiate into neuroblasts, which are A cells in mice, and subsequently mature into neurons.

So this is the pathway that is seen in mice. Note that there is a difference. You may wonder, oh both clays, there is a proliferation, there is neuron formation, so why can't mice also regenerate? So there is a catch; let us see that. Note that the ventricular zone of the zebrafish telencephalon, which corresponds to the RGCs and also the neurogenic niches highlighted in red, is similar to the ventricular subventricular zone (VSVZ) in mammals, while in zebrafish it is referred to as VZ, not to mention that in mice the NSCs. Astrocytes in zebrafish are NSCs, while RGCs are the radial glial cells. Although these two glial cell populations have different anatomies, they share very similar functions, and genetic markers are also similar in the ventricular zone of the telencephalon.

So you can see here that the quiescent type 1 becomes type 2 and type 2 becomes type 3. Type 3 gives rise to neurons. Whereas in B, the B cells, because of the notch signaling, can give rise to a transiently amplifying population called TAMPs. And they give rise to A cells, which are similar to those of these proliferating cells, as seen in the neuroblast, similar to what is observed in the zebrafish.

And the neuroblasts give rise to neurons. So this is a normal scenario that happens in both. But the regeneration context is slightly different. Activation of cellular and molecular processes upon injury in the telencephalon; when you compare it, you will see the difference. In zebrafish, you are seeing the same zebrafish and mouse; the top panel is zebrafish, and the bottom panel is mouse. Both zebrafish and mouse injury areas are labeled with capital A, small a, capital B, small a, aa, and ba, which lead to apoptosis and necrosis.

In zebrafish. As a consequence, inflammatory signals are activated in zebrafish, recruiting immune cells such as neutrophils, microglia, and macrophages to the injury site, which you can see in AC and BC. In this picture, you can see very clearly how injuries cause the recruitment of various immune cells. In zebrafish, the activation of immune cells and inflammation is transient. Note that point very clearly.

You do not want that inflammation to persist. You want it. You can't skip it. You want inflammation, but it is transient. And plays a positive role in activating RGCs, that is, the radial glial cells. Leading to neurogenesis without scar formation. What is a scar? Scar is nothing but ECM, extracellular matrix, especially collagen, which is produced by fibroblasts and various other immune cells.

These cells, when they are produced, will create havoc. They are protective in role, but the protection becomes so much that the cells don't have space to occupy in the place where they should be. Say, for example, you are a furniture fan. Even a simple example exists. You like furniture. One chair you bought, two chairs you bought, three chairs you bought, and you want to sit differently.

And now your whole house is full of furniture. Now, how will people come and move around in the house? Your house has only furniture, one on top of the other, because you like it. Furniture is needed; otherwise, you can't sit on the floor. In the same way, cells are attached to the ECM. They want to hold onto the ECM. But the production of ECM becomes so high that it will automatically lead to a condition called fibrosis.

And there will be a scar that will be formed. The beauty of regeneration in zebrafish is

forming without scar formation. However, in mouse, let us see what happens. Inflammation leads to the proliferation of astrocytes. It is not like RGC in the case of zebrafish astrocyte proliferation, and in most cases, astrocytes are involved in glial scar formation; additionally, extracellular matrix deposition does not impede neurogenesis and neurite plasticity because there is a lot of Blockade is just like traveling, even taking your bicycle through a very crowded road; a traffic jam road.

Whether you are riding a bicycle or walking, it will be very difficult. There is too much junk formed that will prevent neurogenesis, the formation of new neurons, and neuroplasticity, making new connections. hindering successful regeneration. So the formation of too much ECM hinders successful regeneration. Unlike in zebrafish, not that point either. Unlike in zebrafish, In both models, there is an observed increase in oligodendrocyte recruitment and their proliferation, which are central nervous system-specific glial cells.

Still, this recruitment or proliferation is much more pronounced in mice than the required response seen in zebrafish. Not that the direct experimental evidence about the recruitment of peripheral immune cells to the injury site of the adult zebrafish tail encephalon is lacking; that means there is not too much aggressive recruitment. Of immune cells in zebrafish, because of which the immune response is minimal, and hence the ECM or scar formation is minimal, the newly formed neurons get a chance to migrate effectively and form new connections. So the details are available for those who are interested, and they are well described in the image. And if you look at the other part, this topic is also about spinal cord regeneration.

Spinal cord injury is one of the most prevalent examples of roadside accidents. People get spinal cord injuries in road accidents and they become paralyzed below their waist. Typically results in axonal damage and death of the neurons and glial cells, which results in paralysis in the case of humans and any other animal. The spinal cord is a very important part of the central nervous system. The secondary injury phase is primarily caused by uncontrolled inflammation; remember, inflammation came once again as excitotoxicity.

Edema, ischemia, and chronic demyelination. Myelin basically means the covering of your neurons. Demyelination means something like a multiple sclerosis condition where signals will get leaked out. So the brain is sending a signal, but it is not reaching the target.

In between, it got leaked out. So demyelination causes that at the site of injury. Glial scars generated at the wound site can inhibit axonal regeneration. The same issue that we

discussed a while ago, too much scar formation, prevents the prospect of regeneration. A comprehensive illustration of six major challenges in spinal cord injury treatment. Challenge one: unclear cell types. What are the cell types? Unclear nerve regeneration cell types limit stem cell therapy because some junk gets filled in that space.

Challenge 2. Abnormal microenvironment. Injury site conditions impair the survival and function of transplanted cells. So even if you put in stem cells, it doesn't help much. Inhibitory immune response. A prolonged dysregulated immune response hinders nerve regeneration. and challenge four glial scarring reactive astrocytes from the barriers inhibiting axonal regeneration that is another trouble as you can see here glial scars are being formed here and challenge five bridging large gaps that is after spinal cord injury the spinal cord is separated this much gap is there creates gaps challenging the axonal regeneration in an unknown territory like if you have one building and two building if it is one meter distance you will jump if one building and second building have got 10 meter how will you travel you can't no scope of jumping from that same logic applies here Axonal regeneration and neuronal connections challenge limited functional recovery, even though mechanically they are linked; functionally, they are not the same.

Functional recovery is limited due to axonal integration challenges, so these are all the main challenges faced. Comprehensive spinal cord injury response. A comprehensive schematic of the zebrafish experimental model utilized for the system of regeneration. How do people do? Zebrafish do this very effectively. So starting from the zygote, if you see, zebrafish are vulnerable to transgenesis, genome editing, etc.

You can use either juvenile zebrafish or even use the embryos themselves. Seventy-two hours post-fertilization, embryos are used for spinal cord injury. SI approaches can involve mechanical damage, such as a stab wound or a scissor cut wound. And you can also isolate the number of regenerative cells that are responding from the young embryos much more easily because the tissues are not very harsh. And people also do cell transplantation therapy. You can take the cells, whichever cells, and you can put them in that place and see what happens to the fate of those cells.

Do they transdifferentiate or do they differentiate into neurons? So that transplantation therapy is possible. The introduction of regenerative cells into the lesion can stimulate regeneration and recovery. So these are all the approaches that are normally done using zebrafish as an excellent model. Coming back to this point, we should understand that every situation that each challenge presents, six challenges we saw, each challenge comes with its own baggage. Inflammation creates lots of free radicals, and although inflammation is needed for the initial immune response, these free radicals can also cause cell death.

An injured spinal cord thinks of regeneration, but the ROS formed can inhibit normal survival, and there are several axonal growth inhibitors. The central nervous system does not want any new communication because any new communication will alter the stored information. Therefore, these inhibitors have to be removed so that they can be told, "Oh, now regeneration has to happen, so let us keep away from these troubles." We will study regeneration biology in the next class. Thank you.