

Regeneration Biology
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W6L30-Roles of different cellular signaling in regeneration: Examples- Wnt signaling

Hello, everyone. Welcome back to another class on regenerative biology. In today's class, we will learn more in detail about Wnt signaling. Different extracellular or cellular signaling events need to be studied and addressed, which we will do in the subsequent classes. And in today's class, we will try to understand how Wnt signaling influences different tissue types, how and why Wnt signaling becomes pivotal in a regeneration event. And we also see some examples.

Wnt signaling is not very welcome for the regeneration of some tissues. And here, this is not exclusively meant for zebrafish because we have seen that Wnt signaling is an important signaling event for retina regeneration; as we studied in the previous classes, Wnt signaling can allow a regenerative response even without any injury at all. We will study how other organisms respond to Wnt signals. The importance of Wnt signaling during tissue regeneration has been well characterized, and regenerative medicine is a translational field that combines different aspects of cell biology, molecular biology, and tissue engineering.

It constructs spare organs that help injured or defective tissues or generate or restore a damaged portion and restore normal function; for example, a person who has a myocardial infarction and whose blood supply to the heart muscle is stopped. And this often leads to some scar formation after healing, and this scar is not welcome because it will not contract. For the heart to function, the wall must contract, so this scar will be a hindrance in the contraction. It is just like, you know, you are chewing something, some nice food, and I put a stone in between, so you cannot break it; you cannot chew the stone, and it will stop the process. Smooth grinding of your existing food.

So contraction has to happen perfectly for the heart muscle to function properly. So if you can get rid of this scar by some means, that is a welcome sign in itself. You need not restore a heart muscle, but at least you can get rid of this scar. That is one of the right approaches to perform or restore normal heart function so that the heart can function properly. So this is particularly important with specific organs such as the heart, central nervous system, retina, or limbs, which possess very limited regenerative capacity, especially in mammals, which is what we are talking about.

Wnt is normally a wingless type MMTV, integrating integration site family. So it is a larger protein than the name. Wnt signaling is an evolutionarily conserved system that is highly active during embryogenesis. Some researchers point out that there are Wnt-like molecules present even in plants. Animals are there, but plants also have molecules similar to those of Wnt signaling.

Some researchers say that they can cross-react, so I will not dwell in detail, just to tell you that it is an evolutionarily conserved pathway. As such, regenerative medicine has received peculiar attention in the last few years, and in this regard, the Wnt/beta-catenin signaling pathway has been the subject of intensive research. It plays many essential roles in regulating progenitor cell fate, developmental decisions, and proliferation during embryonic development and adult tissue homeostasis. So the roles of Wnt signaling in various aspects have been well characterized and well studied. So regeneration is nothing but an extension or a recapitulation of regenerative biology.

So the Wnt beta-catenin signaling pathway contributes to both stem cells and cancer stem cell maintenance. So this is also another factor that you should keep in mind. During regeneration, many progenitors are induced, but you don't want any cancerous tissue to form because of an injury response. Wnt dysregulation and excessive activation during pathological conditions led to morphological and functional changes in the heart, resulting in impaired myocardial regeneration under pathological conditions such as myocardial infarction, heart failure, and coronary artery disease. So Wnt signaling, if it is overactive, is also a problem; if it is not acting, it is also a problem when regeneration is concerned, so the adequate Wnt signaling response is expected for perfect regeneration.

Several groups of Wnt inhibitors have demonstrated the ability to modulate the Wnt pathway, thereby significantly reducing fibrosis and improving cardiac function after myocardial ischemia. Like I told you, if you can get rid of the fibrosis itself, it is a Right approach. Their inhibitory effect can be realized at multiple levels, which include the inhibition of Wnt ligands, the inhibition of Frizzled receptors, the stabilization of the beta-catenin destruction complex, and the disruption of the nuclear beta-catenin interaction at different levels. Say, for example, I gave you a plate of food, and you have to eat, and I can stop you in multiple ways. Either I can put a tape on your face, tie both your hands or cover the plate or remove the food from your plate.

There are multiple ways in which I can prevent you from eating food. In the same way, you can also influence the signaling pathway at different phases. The role of the beta-catenin pathway in tissue development and regenerative medicine. So this is a cartoon that explains how Wnt signaling is important during development and also during tissue

healing. So, blastocyst, this is the inner cell mass from which a developmental stage can be identified where embryonic stem cells can be collected, and the maintenance of pluripotency is necessary if you want to passage onto the cell.

And you can see that frizzled receptors are expressed in these cells, and they have to be constantly active if they are to remain in a stem cell state. So for which people supplement the Wnt3a, that is, Wnt3 ligand externally into the cells and the establishment of stem cells, their nature must be maintained. The GSK3 beta usually inhibits because GSK3 beta causes the degradation of beta-catenin, and the Wnt signal will be turned off, so Wnt has to be expressed by these stem cells if you want to properly maintain it; different Wnts include Wnt 3, Wnt 5, and Wnt 5b. Different winter ligands are expressed. Some tissue has got more of a given ligand than the others.

Since these cells are embryonic stem cells, they have a variety of winter ligands that are being produced. And when it comes to bone marrow or umbilical cord, both have some kind of stem cells: bone marrow stem cells or umbilical cord stem cells. And they are often referred to as mesenchymal stem cells. Cell proliferation occurs at a rapid rate in these cells. And they also have frizzled receptors that are meant for activating the Wnt signaling, as you found with the embryonic stem cell.

Their visceral receptors are expressed in these cells, and Wnt5a, instead of Gsk3b, is acting as a negative regulator, so it plays an inhibitory role. However, just Wnt5a and the maintenance of pluripotency in these cells, whether umbilical cord or bone marrow stem cells, require adequate and constant expression of a lot of factors. Wnt ligands, as mentioned here, Wnt 2, Wnt 5A, and Wnt 4, and some of them, even if they are negative regulators, make sure that they are produced at a constant rate and a balanced rate, not that the balance is skewed towards either the activation side or the inhibition side, so this is also important to note. So coming back to this terminology, regenerative medicine, many of you would have heard about it, and it was coined around 1992. And since then, this field has gained a lot of popularity, and it has increased because of the potential that regenerative medicine has had.

It created a lot of excitement and interest in the field of regenerative biology. And regenerative medicine is one of the major causes driving the regeneration biology field. One of the most important reasons is the design of novel biological substitutes with the ability to restore and maintain normal function, which is the main scope of tissue engineering as the major component of regenerative medicine. If I tell you about a person who has insulin deficiency due to type 1 diabetes and does not have enough insulin-producing cells in their body, which are supposed to be produced by the beta cells of the pancreas. Now they may have an autoimmune condition in which the beta cells are

destroyed.

So think about a situation where you can take cells from the same patient, culture them in a petri dish, force them to become beta cells, and supply them back into the pancreas. Then that person will have a normal insulin production. A simple solution to control his type 1 diabetes is needed. So these are all the potentials that regenerative medicine is offering. Tissue engineers combine cell transplantation, biomaterial science, life science principles, and mechanical engineering principles to construct new tissues for implantation.

So tissue is necessary for implantation. Like people have used it, sometimes the pig urinary bladder is used in implants. A human urinary bladder troubles, but the ideal scenario would be to create a more humanized or human-derived urinary bladder rather than using a donor animal's urinary bladder. The urinary bladder is not a complex tissue, but it's an important organ. If there is a hole or damage, then individuals have to roam around with a urine bag because they cannot hold their urine.

Thus, one of the simpler structures has seen many successful transplantations of the urinary bladder. In various developed countries. As inferred from its name, a cellular matrix consists of a matrix with virus vectors or plasmids to secrete specific growth factors or hormones in the extracellular matrix proteins, inducing collagen, hyaluronic acid, and fibronectin to ensure biocompatibility and to prevent any cells from binding to the host and the matrix cell interaction. One of the other approaches that has been done is to take some people and use some, you know, cotton candy. You may have seen that kind of matrix.

or otherwise you take a donor organ say heart or a kidney or something and get rid of all the cells decellularize it and you got rid of all the cells all you have now is a skeleton of extracellular matrix ecm now you colonize the stem cells uh from the host of or the Host or the patient-derived stem cell, you colonize it into that matrix so that eventually the matrix will be dissolved by these host cells and the new matrix will be secreted by these colonized cells. And so that this organ will become completely humanized or a host-derived organ you made outside the cell, this is what you should keep in mind: the potential of regenerative medicine, how the Wnt/beta-catenin pathways come into stem cell control. That is something we should look into. Embryonic stem cells, as we all know, respond to the activation of the Wnt and beta-catenin signaling pathways. It is necessary, and if you put more of Wnt3a protein, then it will stimulate proliferation more and more.

And it is important for maintaining pluripotency. Wnt signaling is a must. Mouse

embryonic stem cells are maintained with LIF. LIF stands for Leukemia Inhibitory Factor and STAT3 signaling. Leukemia Inhibitory Factor and STAT3.

Because normally these stem cells are vulnerable to transformation. It can become cancerous. You don't want that. So LIF comes into the picture. Normally, you have a feeder layer that secretes the LIF.

Or sometimes you have a feeder layer, and you supplement the lift from outside. Either way, leukemia inhibitory factor is necessary to maintain the stem cells and lift Stat3 signaling. Stat3, we have already discussed in the previous class, signals through the transcriptional activation of the Myc gene; Myc is a proto-oncogene. And MYC is a common transcriptional target of LIF and STAT3. And canonical Wnt signaling cascades turn on these MYC genes.

And MYC is also a very important gene for regenerative biology. Although MYC is normally associated with metastasis, cancer, etc. It is one of the pluripotency-inducing factors as well. Because the MYC protein is further stabilized by the canonical Wnt signaling cascade, this pathway is necessary for the maintenance of undifferentiated mouse embryonic stem cells. Without this pathway, it will not hold up.

Stem cells and Wnt signaling in regenerative medicine. How is it contributing? Stem cells, including embryonic stem cells, bone marrow mesenchymal stem cells, which are also known as BMMSC, and umbilical cord-derived mesenchymal cells (UCMSC), induced pluripotent cells, where you can take the fibroblasts and convert them into stem cells that are iPSC, and other cells derived from fetal tissue or adult sources such as bone marrow, fat, skin, etc., can act as sources with the future potential to be used therapeutically because these cells can be maneuvered on a petri dish and made suitable for transplantation, at least as a cell type. Mesenchymal stem cells, such as BMMSCs derived from the bone marrow and umbilical cord, are among the most practical somatic stem cell sources that can be used for regenerative medicine. These cells are found in large numbers in an organism.

Including the marrow, umbilical cord, blood, and adipose tissues. It is available, so there is no shortage or short supply of these cells, allowing you to access them easily and culture them. The MSCs, that is, mesenchymal stem cells, differentiate into a wide range of cells such as osteocytes, fibroblasts, mesoderm-derived chondrocytes, myocytes, adipocytes, non-mesodermal-derived hepatocytes, and even neurons. So maneuvering these cells has a huge potential when it comes to regenerative medicine. These cells can be isolated as a fraction of adherent bone marrow colony-forming units, fibroblasts, also known as CFU-F.

The umbilical cord mesenchymal cells are also similar to the bone marrow mesenchymal cells in terms of gene expression profile; although they are from two different sources, they have a lot in common when it comes to gene expression profile. They will be able to differentiate into adipocytes, osteoblasts, and hepatocytes; of course, you have to provide ample environment or stimulation for them to do so. It's not that you take an umbilical cord cell and it will only give adipocytes or only neurons; it's not like that; you have to give it the proper conditions. stimulation. Similar to embryonic stem cells, the regulation of proliferation and differentiation of these mesenchymal stem cells is also affected by Wnt beta-catenin signaling.

No matter what the source of the cells is, they depend a lot on this Wnt beta-catenin signaling. And they are important for controlling stem cell proliferation. Various studies have reported the involvement of Wnt beta-catenin signaling pathways. They are important in the maintenance of murine and human embryonic stem cells; one of the most interesting factors about the impact of the Wnt/beta-catenin signaling on the embryonic stem cells has been achieved from the observation that the Wnt proteins contribute to the maintenance of pluripotency, as they are required for maintaining the pluripotency of the human and murine embryonic stem cells during the treatment period. The experimental regime is an important factor that affects the outcome you get because of the Wnt proteins acting on the cell proteins in the embryonic stem cell pluripotency; some studies have shown that long-term treatment of embryonic stem cells with Wnt3a, which is the ligand of the Wnt signaling pathway, causes differentiation towards a mesodermal lineage.

Mesoderm means muscle, etc., so they are mesoderm-derived embryonic origin. We will not go into the details of those tissues right now. In addition, there are also other studies; the previous research documents have indicated that the key components of the Wnt beta-catenin signaling include Wnt3, Wnt5b, Wnt7a, and Wnt7b. That list continues with Frizzled receptors, which are over two, and they are expressed in embryonic stem cells, meaning they are waiting for a chance to. Interact with the respective ligand when available, either externally supplied or from the cell itself that is secreting it.

Embryonic stem cells respond to the activation of the Wnt/beta-catenin signaling pathway in a very positive manner, especially in maintaining pluripotency, which is the ability to differentiate into a given cell type; that is what we are referring to by pluripotency. Activation of the canonical Wnt signaling with the beta-catenin signaling cascade is necessary for the maintenance of undifferentiated mice, such as bone marrow-derived mesenchymal stem cells and umbilical cord-derived mesenchymal stem cells, which are somatic cells and the most practical cell sources of regenerative medicine,

because their abundance is very high, making it not too troublesome for the host or the donor organism. The mesenchymal stem cells can differentiate into a wide range of cells, such as osteocytes, fibroblasts, mesoderm-derived cells, myocytes, adipocytes, hepatocytes, and even neurons. Similar to embryonic stem cells, the regulation, proliferation, and differentiation of these mesenchymal stem cells are also affected by Wnt beta-catenin signaling. So what we should understand is that a huge number of studies have shown that the Wnt ligands play pivotal roles in triggering the Wnt signaling pathways such as Wnt 2, Wnt 4, Wnt 5, etc.

, and also their receptors known as Frizzled 2, 3, 4, 5, and 6, etc. Additionally, there are other related LRP coreceptors that also come into the picture, and needless to say, the involvement of Wnt negative regulators, such as Wif, DKK, and SFRP, also comes into play. Furthermore, the canonical Wnt beta-catenin signaling pathways play a substantial role in the maintenance and self-renewal of these cells until they receive a signal for differentiation. Until then, the Wnt signaling has to be turned on and active in a prudent state. Due to an increase in self-renewal potential and a decrease in apoptosis.

Cell culture treatment with Wnt3a. Normally, in all these studies, people use Wnt3a because it is more active on the cells. So it doesn't mean that Wnt4 is not good or that Wnt2 is not good. People usually use Wnt3a. Enhance the multi-potential population of MSCs as well as human adipose-derived stem cells; additionally, the overexpression of LRP5, which is a core receptor, has been shown to increase the proliferation rate of mesenchymal stem cells in contrast to Wnt3a and Wnt5a, which inhibit the proliferation of mesenchymal stem cells.

by activating a non-canonical pathway. It still binds to the receptor, but compared to Wnt3, Wnt5a triggers a non-canonical pathway. The dual effect that you see in Wnt beta-catenin signaling is that Wnts increase the mesenchymal stem cell proliferation rate at a low dose. at very low dose, it is increasing proliferate rate while suppressing it. The proliferation rate at a high dose reveals that the intensity of Wnt signals can result in various or even opposite biological functions.

So you need food to live properly. But if you are overdosed on food, you will end up in the hospital. But despite having an idea that food is a necessary component. So this is the logic you should have in your mind. Wnt signaling in cardiac regeneration. Let us see how cardiac regeneration is influenced by Wnt signaling.

Reactivation of Wnt beta-catenin signaling following cardiac injury has been shown to have pivotal impacts on cardiac progenitors in several ways. Many research authors have reported that beta-catenin depletion induced recovery in a resident cardiac progenitor cell

population exhibiting alpha-MHC, MHC promoter activity, and expression of cardiac transcription factors such as GATA4, which is a cardiac-specific transcription factor, and TBX5 after infarction.

They are turned on. These effects... was mediated by enhancing the differentiation of cardiomyocytes from alpha MHC major histocompatibility complex plus SCA1 plus cardiac progenitors. So there is a congregation of these three. And also, the stabilization of beta-catenin by pharmacological methods using the same promoter decreased the cardiomyocyte differentiation of this cardiac progenitor. So we can understand how Wnt signaling is influencing cardiac repair or cardiac regeneration. Knockdown of another protein called KLF15, which is a Kruppel-like family protein transcription factor and a negative regulator of beta-catenin signaling, TCF transcriptional activity, resulted in cardiac beta-catenin transcriptional activation along with functional cardiac deterioration in normal homeostasis.

No need for an injury, even in a normal situation, if you knock down the KLF15, as this can often lead to hypertrophy. So homeostasis is also conditioned by tweaking the Wnt signaling; you will often end up getting some very dramatic effects. KLF15 deletion induced endothelial lineage differentiation in cardiac progenitor cells. They concluded, the researchers who discovered it, that KLF15 controls the cardiac progenitor cell homeostasis in the adult heart, similar to embryonic cardiogenesis when the heart is being formed, via the inhibition of beta-catenin transcription. So KLF15 has a significant role in controlling or maintaining the level of beta-catenin at a threshold level.

The treatment of SCA1, the promoter of Wnt inhibitor, and the secreted frizzled-related protein, SFRP, which is a negative regulator of Wnt signaling. It is secreted by the cells. It will go and sequester the Wnt ligands. So, before the Wnt finds the actual frizzled receptor, this SFRP will fool the Wnt inhibitor. And act like a sponge; it will get rid of how sponge is removing water.

The Wnts will be removed by SFRP; they promote differentiation of the cells after ischemia-reperfusion injury. In other words, what we understand is the treatment of the progenitor cells with SFRP, which is a negative regulator of Wnt signaling. Suppressed their proliferation and primed them for cardiac differentiation. That means you will end up getting more cardiac-type cells if you tweak the SFRP and deliver more of it, which is a negative regulator.

of Wnt signaling. Beta-catenin overexpression and consequently the activation of the Wnt beta-catenin pathway decreased myocardial neovascularization through the differentiation of fibroblasts into myofibroblasts and resulted in a slight improvement in cardiac function

and a decrease in the infarct. In fact, what it basically means is that fibroid tissue is formed after myocardial infarction. So, Wnt signaling leads to the differentiation of epicardial cells into fibroblasts via the process of epithelial to mesenchymal transition. So, what is its role in Wnt signaling on lung regeneration? Increased Wnt beta-catenin signaling may contribute to epithelial cell injury and hyperplasia, as well as impaired epithelial to mesenchymal crosstalk in idiopathic pulmonary fibrosis, which is a condition that typically results in fibrotic lungs, reducing lung function. Canonical beta-catenin signaling is activated during lung epithelial regeneration and regulates bronchovascular system cell proliferation after lung injury.

So the regeneration of the injured lung seems insufficient to repel the fibrotic disease. So Wnt signaling can play very interesting roles in kidney regeneration. So this is slightly different from and opposite to what you have seen so far. When macrophages are depleted from the injured kidney, the canonical Wnt pathway responses in the kidney epithelial cells are decreased. The administration of the Wnt pathway negative regulator Dkk2 increased the repair process, which means the kidney has received more Wnt signaling by default, so you want to decrease the activation of beta-catenin by Wnt1 or stabilize beta-catenin.

Protected tubular epithelial cells from apoptosis. Hence, the endogenous beta-catenin is substantial for renal tubular protection via acute kidney injury by provoking cell survival through multiple mechanisms. So now, if you look into bone regeneration, the gain of function of the beta-catenin signaling pathway resulted in high bone mass in mouse models. And therefore, not surprisingly, in mouse models with an activated form of beta-catenin in osteoblasts, which are the bone cells that give rise to bone, the knockout of axin-2 has a considerable increase in bone healing. So the administration of recombinant Wnt 3A and lithium chloride can increase the healing potential following the start of treatment after a bone fracture.

This has been demonstrated in mice. Wnt-responsive cells that are undergoing a transient step of cell differentiation induced by local Wnt stimuli can increase or restore the regenerative capacity of the bone. So we have seen how important Wnt signaling contributes to different tissue regeneration and repair processes. We'll study more about the other signaling events in the next class. Thank you.