Regeneration Biology
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W2L6_Liver Regeneration: Mechanistic Insights

Hello everyone, welcome back for another session of regenerative biology class. We will continue with liver regeneration, and we will try to gain more insights into the mechanistic aspects of liver regeneration. In the last class, we learned... how different cell types get regenerated, how cell cycle regulation takes place, and how various signaling molecules contribute.

And today, we will try to understand a little bit more about its mechanistic level, the different ways of injury, how the liver gets damaged, and how it responds. So the scenario can be explained in the following manner: the liver possesses a distinctive capacity for regeneration within our body, and it's one of the complex organs that is capable of remarkable regenerative capacity. This we have already seen. Under normal circumstances, the liver cells replicate themselves and maintain liver function.

We have also seen this because the hepatocytes must replicate to retain their normal function, as many hepatocytes get damaged and become defunct due to the detoxification process that takes place in the body. Usually, the replication of healthy hepatocytes is sufficient for the regeneration of acute liver injuries, depending on the extent of acuteness. When I said detoxification, we should understand it need not necessarily come from food or the atmosphere. It can also happen that the metabolic toxins your body is producing. Say you know your body produces lots of ammonia and carbon dioxide, and you know your liver is powerful in converting that into urea, which is eventually filtered by the kidneys.

The ornithine cycle, which takes place in the liver, is a detoxification process. Ammonia is dangerous, carbon dioxide is dangerous, and urea is also dangerous, but it is less dangerous; that doesn't mean it is not a toxin. In the advanced phase of chronic liver damage, a large number of hepatocytes die, and hepatocyte replication is blocked because of the extent of damage; at that time, the hepatocytes are no longer able to manage the damage, so liver regeneration has more complex mechanisms, such as transdifferentiation, which is the conversion of cell type A to cell type B. So we also saw in the previous class that when the damage is very severe, the hepatic progenitor cells come into the picture. Their distribution is in that location; we also saw them there, besides the canals of herring, but they come into the picture only when the damage is severe.

Inadequate liver regeneration causes severe chronic liver disease, and often the patient dies. Even in animal models, if the liver is failing to regenerate, the animal will die within two weeks. There is no way the animal can survive if the liver does not recover. So, this is a schematic distribution of various cells in the liver. So those who are interested can read this citation, which is a very interesting review that talks about it.

So these pictures were taken from that review. And this is the liver. As you can see here, it is a multilobular structure. And if you take a magnified portion or a portion that is under a microscope, if you take a cross section, it looks somewhat like this. You can see that the blue-colored one is the vein.

Usually, the veins are represented in blue and the hepatocytes are light brown. And these portal veins are usually distributed on average. It is arranged in a hexagonal manner, as you can see here. And there are dedicated sinusoid regions that go all the way up to the central vein. The portal vein goes and joins the central vein.

And also, there are dedicated sinusoid regions, which are the places where the signaling events take place after injury or repair. You can also see these green-colored ducts that are coming towards the central vein, and this is the bile duct, the green-colored portion, which also has a long structure called Bile canaliculus, which is the extension of the bile duct, eventually becomes a hepatic duct that releases its content into the boundary of the small intestine and stomach. Stomach and the small intestine jointly release the bile acids. And if you take a magnified portion of this further, you can see that this is the portal vein and hepatic artery, which supply the fresh blood, and you can also see the distribution of Kupffer cells, and you can also see cholangiocytes, which are dedicated cells that are embedded into the hepatocytes. The brown-colored ones are the hepatocytes.

You can also see Kupffer cells. They are basically the macrophages that are doing the phagocytic function. And you can also see the stellate cells, which are all important for various liver functions and for the signaling events. So this is the scenario. This is how the cells are distributed.

So to study regeneration, you need to have an injury model. Any regeneration study requires a proper injury model, and the injury model is selected in such a way that it mimics the natural damage an organ can sustain. So one of the easiest and well-studied models is the amputation model, also known as the PHx model, in which a surgical procedure is performed to remove a specific section of the liver without causing direct harm to the liver cells or the organ's functioning, as a portion is being removed, and there is an advantage to doing this because you can. damage consistently say if you are doing by some other method say you put a toxin and it depends the effect of that toxin depends on the ability of this animal to deal with the toxin and it won't be same for all whereas amputation is concerned it is going to be fixed if you amputate a given quantity of tissue or remove a given quantity

of tissue across different animal the damage is going to be More or less the same; however, there are other methods like this. Chemically induced liver injury models also exist because we know the persistent damage to the liver that takes place due to various chemicals.

Chemically induced liver injury models include the administration of diethyl nitrosamine, carbon tetrachloride, or ethanol. We know that ethanol is one of the major causes of liver cirrhosis all over the world. Because the ethanol creates an intermediate called acetaldehyde, which is very dangerous and very toxic, it can damage the liver in no time. Even in this chemically induced injury model, there are two types or two subcategories. Acute liver injury models.

In acute liver injury, it often occurs with a high dosage of toxin. let it be any of them let it be diethyl nitrosamine or carbon tetrachloride or alcohol you give a high dose in minimum time less amount of time that is one way acute liver injury model and there is chronic injury model of the liver where the chronic injury is induced with sustained, mild, or very moderate levels of the toxin at frequent intervals. It is something like that of a person who gets addicted to alcohol. No one starts drinking large quantities of alcohol. People start slowly and gradually increase the dosage.

In very small quantities, your body may be able to deal with it, although a toxin is a toxin. But you keep increasing the dose and doing something similar. It has been done in a chronic liver injury model. However, in a laboratory setting, the chronic injury method takes a lot of time to standardize because if you are using a rodent model, we don't have the luxury of running the study for six months or one year since the mice will grow old. Many a times, the PHx method, which is amputation or the acute liver injury model, is used.

However, the chronic liver injury model has also been done because it is the most prevalent in humans. For instance, long-term excess alcohol consumption and chronic viral hepatitis. Sometimes it happens, like, for example, the hepatitis C virus. Once it enters your body, it never leaves.

It is always there. All you can do is support your liver as long as you want to live. There is no way you can get rid of the hepatitis C virus. You may have heard in the news that some celebrities have gotten infected, so if it normally spreads through blood transfusion, once it enters your body, your body cannot clear it, which is the same with many other viral diseases. However, in some chronic viral diseases, what happens is that viral hepatitis always keeps on damaging your liver. To study such scenarios, you need to have chronic liver injury models as well.

So this is a picture that shows it was taken from the same review. You can see that chronic liver injury can also happen due to type 2 diabetes. So obesity is one of the major causes; when you eat a lot, your body deposits those carbohydrates or fats with a consequence that damages your liver because the liver has fat-storing cells, and your body does not want to

lose a single nutrient that is put into it, even though it is damaging to the body. So you end up, to start with, getting fatty liver disease, and fatty liver means your body has a disproportionate amount of fat that is deposited. It can manifest in the form of some liver pain, but the body tries to repair itself; for example, if you reduce your food intake, this fat will be metabolized, and if you do exercise, it will also be metabolized, allowing the liver to reduce the fat load in the body.

However, if it progresses to a cirrhosis stage, then the liver has to engage its regenerative mechanism, and hence the problem can arise from various situations. Normally, fatty liver or obesity-related liver damage occurs because of insulin resistance, which is essentially type-2 diabetes. Excessive alcohol consumption and hepatitis-causing viruses, such as hepatitis A, B, and C, can affect the liver; another example can be a tumor that develops in the liver and starts consuming a lot of the nutrients that are coming into the liver. Many times, doctors will take a surgical approach to remove the tumor if there is no secondary spreading, etc. That is also something similar to traumatic liver damage, and of course, in a natural environment, if two animals are fighting and the horn of one animal enters the belly of another, its liver can get damaged.

So these are all things that occur naturally, and no animal can afford to lose its liver. because it has to survive its body so these are all traumatic brain injury and then lot of chemicals which you take like even simple paracetamol what you are eating for fever the maximum your body can tolerate paracetamol in a day is 8 gram thankfully every paracetamol is only 500 milligrams if you take 10 paracetamol in a day it is only 5 gram but more than 8 gram In a day, it can cause liver damage because paracetamol also needs to be detoxified like many other tablets and chemicals that you are consuming daily. Even if it is not studied for liver toxicity, some drugs can cause damage to your liver, so it is always good to stay away from drugs unless they are essential for living or survival. Animal drugs should be life-saving drugs rather than, like, you know, some people take a painkiller and then keep playing the game or keep damaging their body further. So basically, if pain is tolerable, it's better not to get into a painkiller or any other drug.

Now let us see what the growth factors are that can influence the regeneration of the liver after a liver resection. As I told you, it can be any damage, hepatocyte growth factor and epidermal growth factor; these are both predominant growth factors. Hepatocyte growth factor, also known as HGF in short form, is one of them. They are secreted by hepatic macrophages such as Kupffer cells. They secrete HGF and mediate hepatocyte proliferation through its receptor cMET.

cMET is a receptor that is essential for hepatocytes to respond to HGF. It activates downstream effectors such as extracellular signaling-regulated kinase, also known as ERK1 and ERK2. Another downstream gene that is activated because of HGF binding to the cMET is protein kinase B, also known as AKT. Protein kinase B is another name for AKT. And then comes the signal transducer and activator of transcription known as STAT3.

So ERK1 and ERK2. AKT and STAT3. Remember, this pathway can be turned on by other mechanisms also. But HGF binding to the cMET receptor indeed can trigger these mechanisms. And these are all pro-mitotic. That means it will trigger the cell to enter a proliferative phase. And driving the proliferation and survival of hepatocytes.

Just the proliferation is not good enough. Unbounded proliferation; your body will... make it die by apoptosis so this is also you should keep in mind that whenever there is a proliferation not that it will stay forever your body is waiting for killing all type of cells that is formed inadvertently, but here, these mechanisms also ensure that they don't die by preventing apoptosis Epidermal growth factor (EGF).

EGF is another factor, like HGF. EGF binds to a receptor called EGFR, which means epidermal growth factor receptor. It initiates the hepatocyte proliferation signaling pathway and promotes hepatocyte proliferation and survival. So both HGF and EGF contribute to hepatocyte proliferation and its survival. In the PHx model, which is the amputation model we discussed, after blood flow was restored, soon after cutting, there will be a blood clot and the blood flow will restore. HGF and EGF are rapidly activated to promote hepatocyte division and proliferation.

So one of the prime responses is through HGF and EGF induction. And the other signaling that is induced is wind proteins. In the previous class, we also discussed one of the winds and how it contributes, playing a major role in the proliferative event along with wind proteins and signals. So regeneration after hepatic injury relies heavily on Wnt beta-catenin signaling. So normally, I can quickly explain that beta-catenin is produced in every cell; every cell needs beta-catenin for its survival.

It's part of cell communication. Cytoskeletal elements like tubulin and actin filaments are connected with alpha-catenin, which is linked with beta-catenin. Beta-catenin is linked with integrins, so this is needed for every living cell to have. This beta-catenin, however, the excess beta-catenin is degraded by a process called the destruction complex. We will not go into the details of the destruction complex, but understand that destruction happens inevitably to the beta-catenin so that it never gets a chance to go to the nucleus; it will be degraded in the cytoplasm. However, when the wind binds to its receptor called a frizzled, what happens? This destruction complex is no longer able to function because wind binding to the frizzled receptor will take away one of the pivotal players of this destruction complex.

Then it's like you have a four-legged table. I broke one table. Then that table will not stand. A three-footed table or a chair cannot stand. So, all four legs are important. So one leg will be snatched by a wind-frizzled complex.

Hence, the destruction of beta-catenine will not take place effectively. This stabilized beta-catenin will now go to the nucleus, with the help of another collaborator called TCF. They

will bind to the DNA targets and turn on transcription, so this is what happens following PHx: the Wnt/beta-catenin axis is activated to facilitate quick liver regeneration by enabling hepatocytes to enter the G1 phase. All hepatocytes are in the G0 phase; they will now. Enter the G1 phase, promoting DNA synthesis in a cell.

Additionally, by controlling the downstream effector genes such as cyclin D1 and cMYC, these two genes are expressed downstream of Wnt/beta-catenin signaling. And this cyclin D1 and cMYC will favor the proliferation of a cell. The wind signaling interacts with other signaling events, such as Notch and HGF, because wind signaling can influence the production of ligands of Delta-Notch signaling, like Jagged and DLL; those kinds of genes are turned on. By wind signaling. So they can influence delta node signaling and also HGF (hepatocyte growth factor) production, which we discussed some time ago, forming a complex regulatory network.

Then comes the presence of cytokines. Cytokines are secreted molecules that can influence a target cell. They are normally of two types: broad categories, pro-inflammatory cytokines and anti-inflammatory cytokines. So they do either of these functions. In general, cytokines are chemicals, mainly proteins. Protein chemicals or peptides that can drive a cell to perform a function.

And this is the simple meaning of cytokines. Interleukins are classic examples, such as IL-6 and IL-33. They exert important roles in liver regeneration after hepatic resection, which means damage to the liver and acute liver injury. Through a single dose of a toxic chemical, IL-6 contributes significantly to liver regeneration because IL-6 is a predominant pro-inflammatory cytokine. During COVID, many of the people who died had exceptional high levels of IL-6 in their lungs and bloodstream, which affects their bodies, as it is produced by the immune system. But beyond a certain limit of IL-6, it will be too difficult for your body to handle.

But here in liver regeneration, IL-6 has to be induced to make the environment pro-inflammatory. After hepatic resection, acute liver injury and IL-6 contribute significantly to liver regeneration. And IL-6, mainly secreted by the cases or Kupffer cells, activates the Janus kinase, JAK, and signal transducer and activator of transcription. Janus kinase and STAT3.

They are called the JAK-STAT pathway. So JAK binds to the STAT, and the STAT normally stays in the cytoplasm, but because of this binding, it gets phosphorylated and goes to the nucleus, acting like a powerful transcription factor, STAT3. Signaling pathway: JAK-STAT signaling pathway after binding to its receptor, IL-6R. Interleukin-6 has its own receptor, so it will activate only those cells that have the IL-6 receptor. So IL-6 is secreted, IL-6 binds to those cells that have the IL-6 receptor, and it will activate the JAK and STAT3 pathways. And IL-6 JAK-STAT3 signaling promotes the G1 to S transition.

If this transition from G0 to G1 is done by HGF and EGF, it now has to move from G1 to S. Only then will the cell cycle proceed. So this JAK-STAT pathway has the power to convert the cell from G1 to the S phase, which is the DNA synthesis phase. This is mainly done by upregulating the expression of cyclin D1. If there is no cyclin D1 expression, then it will not go into the G1 to S phase.

So STAT3 plays a major role. STAT3 activation induces the upregulation of several anti-apoptotic genes, such as MCL1 and BCL2. So, these are very powerful anti-apoptotic genes. Even if you have some potential for many cancer cells, use this strategy because cancer cells don't want to die, so a healthy cell should not have these Mcl-1 and Bcl-2 levels. If these genes are high, even a mad cell or a cancer cell will not die, so you don't want that to happen. But in this case, it is okay because STAT3 makes sure that BCL2 levels are high and MCL levels are high, thereby enhancing the anti-apoptotic ability of hepatocytes in the injury environment because any new cell is welcome since the liver badly wants to gain bulk.

IL-33, interleukin 33, induces intestinal mucosal cells because it enters the bloodstream. Mucosal cells release more serotonin because 95% of the serotonin in your body is produced by your intestine, not by the brain. Only 1 to 5% of serotonin is in your brain. The rest are produced by your intestine.

And it will release more serotonin into the portal bloodstream. And it activates an HTR2A receptor. P30S6K in hepatocytes, which is also helpful in proliferation. So now the bile acids come. In the previous class, we also discussed that in the parabiosis experiment, the bile acids are one of the components influencing the liver. So bile acids are created by the hepatocytes, which are then discharged into the biliary duct.

And the intestine eventually gets into the intestine before being absorbed again, so it goes in a circle because it is released, and immediately it will be released once its job is done. The bile acids are returned to the liver via. Enterohepatic circulation. There is a unique circulation called enterohepatic circulation that is involved in releasing and absorbing.

Releasing and absorbing. Because bile acids mainly act like a detergent. What is the use of detergent for cleaning your laundry? Same job. It acts like an emulsifier. Lipids and water will form an emulsion with the help of bile acid. Bile acid levels dramatically increased immediately after PHx, which means partial hepatotomy.

It is called PHx in both rodents and humans. And bile acids accumulate, you know, in the bile. The coloration of the pigment becomes yellow in the case of jaundice, and if your liver is not functioning, you appear yellow in color. Bile acids accumulate something called the Farnesoid X receptor, also known as FXR, and then activate another G protein-coupled receptor known as GPBAR1. And it's also referred to as TGR5; these are some names of the genes and receptors, and they play essential functions in regulating bile acid balance and

preventing liver damage.

Once damage occurs, you don't want further damage to happen. FXR activation stimulates gene expression in bile acid synthesis, detoxification, and transport, so this helps. To reduce liver damage caused by bile acids and support liver regeneration. Furthermore, we should know that bile acids modulate the activity of various growth and regeneration-related pathways. For instance, the wind beta-catenin signaling pathway.

Moreover, the FXR and TGR5 signaling we just discussed. have been demonstrated not only to promote hepatocyte proliferation but also to protect hepatocytes from apoptosis. Protection is one thing; protecting is another. So do they. So it produces and protects, thereby facilitating liver regeneration following the injury by the partial hepatotomy method. You can see here in this picture the signaling pathways that we have observed through different methods; whatever we have seen is mentioned here: the EGFR receptor and MET receptor bind, and the PI3K AKT mTOR pathways are turned down, leading to the kickstart of proliferation.

And you also have the CCR5 receptor, CCL5 binds, and you have the CCR1 receptor. It all turns on the FOXO3A signaling pathway, which in turn activates the macrophage and then eventually produces a lot of hepatocyte growth factor. And you also have the IELTS 33, which I discussed just now about activating the intestinal mucosa to secrete serotonin, which eventually reaches the liver and turns on the HTR2A and p70S6K pathways, which also help in liver regeneration. There is something very interesting: the hypoxia-inducible factor, which is normally formed because of the blood supply; when the blood supply to the liver is cut, the HIF-1α and NRF-2 are affected. These will stimulate the production of some unique substances and also the level of cholesterol because cholesterol is something that is produced in your liver.

And if the cholesterol level is fluctuating, that means the liver is functioning poorly. That is also an indication of your liver malfunctioning. They can also stimulate starting from the cholesterol level, and this will trigger the HIF and NRF. To trigger the activation of liver regeneration. So in general, we can see liver regeneration and chronic liver damage, which happens through the normal proliferation of the hepatocytes or can normally occur through de-differentiation.

Additionally, dedicated stem cells can come into the picture and contribute to the Wnt signaling and Jagged production, and the Notch signaling gets activated. The NICD goes to the nucleus and can turn on a bunch of genes like DNMT, YAP, TED, SOX9, and also hepatic nuclear factor. Make the liver cell able to regenerate; we will study a new subject in the next class. Thank you.