Regeneration Biology
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Week: 1
Lecture: 5

W1L5_Liver Regeneration: Signaling Events

Hello everyone, welcome back to another class on regeneration. We will move on to a new topic that is on liver regeneration, and we will study the signaling events in liver regeneration. As I already told you, it is the only complex organ that is capable of regeneration in mammals, whereas lower vertebrates like fish and frogs can regenerate pretty much every organ. That is a picture of Prometheus. It's a Greek legendary character who was punished by the gods for giving fire to humans because Prometheus happened to be a lover of humanity. And as a punishment, his liver will be eaten by an eagle whole day and the next day it used to regenerate.

And it's quite interesting that this character has stayed back in humans also, probably as a continuation of the blessing or benevolence we received from the god Prometheus. So compensatory regeneration is the terminology used to describe liver regeneration because it does not regenerate the lost part; rather, it compensates for the bulk. Today, the standard assay for liver regeneration is partial hepatectomy, where some lobes of the liver are removed. Liver has got its multilobular structure.

Although the removed lobe does not grow back, the remaining lobes enlarge and compensate for its loss, and they will gain in bulk. So, such compensatory regeneration is basically the division of differentiated cells to recover the structure and function of the injured organ, as has been observed in the mammalian liver and in the case of the zebrafish heart, etc. So, liver regeneration, if you look closely, occurs by two major mechanisms. So, the first mechanism is the proliferation of the existing hepatocytes. Hepatocytes are essentially the parenchyma of the liver.

The major workforce of the liver is the hepatocytes and the repopulation from the progenitor cells. These are the two approaches to liver regeneration. The human liver regenerates by the proliferation of existing cells. The liver cells do not fully de-differentiate when they enter the cell cycle, nor is there a regeneration blastema. Blastema is a group of undifferentiated cells that are formed at the site of injury or amputation in the case of salamanders or axolotls; such a blastema never forms in the liver, so mammalian liver regeneration has two lines of defense, the first one being the mature adult hepatocytes.

Normally, not dividing, they are terminally differentiated; they are in G0 arrest and are instructed to join the cell cycle, which means entering into the cell cycle and proliferating

until they have compensated for the missing part. If one gram of liver is removed, the hepatocytes will divide until they restore the lost quantity, not in the same place but somewhere else. So, there is a population of hepatic progenitor cells that are. Normally questioned but activated when the injury is severe and the adult hepatocytes cannot regenerate well due to senescence, alcohol, viral infections, other diseases, or liver cirrhosis, etc. In normal mammalian regeneration, there are five types of cells that exist in the adult liver, namely hepatocytes, ductal cells, fat-storing cells, endothelial cells, and Kupffer cells.

So these are the main five types of cells, and all begin to divide to produce more of themselves because you need all of them, although the hepatocytes are the predominant population. Each cell type retains its cellular identity, and the liver retains the ability to perform its function. Remember, if the liver is damaged, there is no blastema that is formed, and the existing cells, while performing their liver-specific duties, will continue to proliferate as well. Such functions include bile acid synthesis, albumin production, toxin degradation, etc. Even if your brain is dead, even if the brain of a person is not working, you can make that body surviving it by putting the person in ventilator.

But if the liver is down, you don't have a machine to put it in. So technically, the liver is one of the most complex organs in your body, which means it is more important. In terms of functionality, there is no replacement organ available; you can't put a heart-lung machine in place of the liver, so if the liver is down, the patient cannot move forward. Several pathways may initiate liver cell proliferation, and the end result of which downregulate and not totally suppress the genes required for the differentiated functions; some genes are induced that allow them to proliferate while retaining their liver-specific functions, and the cells will continue to do their job while activating these genes required for proliferation, and the bulk is built in this way: Proliferation of hepatocytes after hepatectomy It is the most studied model of liver regeneration in humans. Resurrection of up to 90% of the liver, averaging 70 to 90%, but a maximum of 90%.

So if your liver is around 1.5 kilos, you can remove most of the liver, retaining around 150 grams; it will bounce back and grow back. Liver can be repaired by the proliferation of the residual hepatocytes. When you donate a liver, sometimes between relatives, only a small piece will be taken from the donor and given to the recipient, and the liver will grow in that host. Hepatocyte proliferation in the regenerating liver is triggered by the combined actions of cytokines and polypeptide growth factors.

There is an involvement of immune system, which we studied in the previous class; there has to be a proper immune response. How is the injury to the liver sensed to trigger the regeneration? Someone has to tell them that there is no CCTV camera to watch. So when there is an injury, someone has to detect it. What is that molecule? It is very interesting to follow. The removal or injury to the liver is sensed through the bloodstream, and there are some liver-specific components.

That will be leaked into the bloodstream; that means it can be bile acid, or it can be some of the liver-specific factors. Otherwise, if the liver is damaged, there is no point in the kidney regenerating or some other organ regenerating, so it has to be liver-specific factors that are lost, while others, such as bile acids and gut lipopolysaccharides, will increase. Gut lipopolysaccharides are supposed to be controlled and kept in check by the liver, and if this is not happening, you may have seen it in jaundice. The person's eyes will become yellow in color, skin will become, nails will become, all will become yellow in color because the liver is not doing the duty. And these are indications for the liver to start proliferating and growing back in bulk.

These polysaccharides induce some non-hepatocytes to secrete paracrine factors. Paracrine factors are the factors that allow the neighborhood to respond in a given way. They are some of the signaling events that induce the hepatocytes to enter the cell cycle. The Kupfer cells secrete interleukin-6, one of the pro-inflammatory cytokines, because they are similar to those of macrophages. They can secrete IL-6.

TNF alpha, tumor necrosis factor alpha, while the stellate cells can secrete hepatocyte growth factor, also known as HGF, and another factor called TGF alpha and TGF beta, transforming growth factor alpha and transforming growth factor beta. The specialized blood vessels of the liver can also produce HGF and Wnt2. Wnt2 is a ligand of wind signaling, which is an essential molecule for cellular proliferation. As you can see in this picture, it is basically a sine wave. That basically means a cavity of the liver sinusoid, which is lined by endothelial cells.

And there are Kupfer cells, stellate cells, and neighboring hepatocytes. So, one hepatocyte will be primed by the Kupffer cells through the secretion of interleukin 6 and TNF alpha, and this Kupffer cell, in turn, will prime the neighboring Kupffer cells and neighboring hepatocytes to enter the cell cycle, and so the neighboring hepatocytes are primed. And the stellate cell is also releasing HGF and TGF-alpha to the same hepatocytes, and now it is primed; so it was in a G0 arrest stage, now it will enter into the G1 phase. Also, the extracellular matrix is capable of releasing some of the growth factors, such as TGF-alpha and Hgf. And all these together will make hepatocytes enter the cell cycle; however, you don't want unwanted proliferation, so the hepatocyte that entered the G1 phase is now acted upon by TGF-beta, which is one of the anti-proliferative molecules, similar to the brake in a car.

You have the accelerator; the moment you lift the accelerator, you need to use the brake if you want to reduce the speed of the car, so TGF-beta tells. This hepatocyte is not to go. This doesn't mean that the proliferation is inhibited. The proliferation is happening at a reduced rate. So the same stellate cell is telling, on one hand, the hepatocytes to enter the G1 phase.

The same stellate cell is releasing TGF beta and telling the other cell not to proliferate. So this allows for controlled proliferation, and it will progress from G1 to S, while the

metabolic pathways also have to continue, and it will enter the mitotic phase. In this way, you will generate the bulk of the hepatocytes as part of liver regeneration. In the first priming phase, the cytokines such as interleukin 6 are produced mainly by the Kupffer cells, and they act on the hepatocytes to make the parenchymal cells competent enough to receive and respond to the other growth factor signals. In the second phase, the growth factor phase, the first phase is the priming phase, and the second is the growth factor phase.

The growth factors, such as HGF and TGF alpha, produced by many different cell types do that. And they act on the primed hepatocytes, not the normal hepatocytes. And they stimulate the metabolism and entry of the cells into the cell cycle. And in the last phase, that is where the hepatocytes are quiescent, and it takes them several hours to enter the cell cycle as they progress from G0 to G1. Basically, both are in the same phase.

G0 means it will not enter the cell cycle. G1 means it can proceed to the S phase, then to the G2 phase, and finally to the M phase. Almost all hepatocytes of the parenchymal type replicate during liver regeneration. After partial hepatotomy, not just that area alone, because that area is not the one that is going to be replaced; the entire liver has to respond, and it will become bigger. The wave of hepatocyte replication is followed by the replication of non-parenchymal cells such as Kupffer cells, endothelial cells, and stellate cells, so they continue to contribute; they continue to proliferate.

However, that is a second wave because the major bulk player is the... Hepatocytes. The second number represented is the Kupffer cells, endothelial cells, stellate cells, etc.

So during the phase of hepatocyte replication, more than 70 genes are activated that contribute to this priming and proliferation. These include genes encoding transcription factors, cell cycle regulators, regulators of energy metabolism, and many others. They contribute to this. And now comes the final phase, which is called the termination phase, where the hepatocytes return to quiescence. Quiescence means we are returning to the G0 phase.

This is what happens. Hepatocytes that are still connected to each other in the epithelium cannot respond to HGF. Then if so happens, how is regeneration triggered? So what we should understand is that in a given scenario, every hepatocyte is capable of responding to hepatocyte growth factor, and it will work only when there is damage; then this response becomes interesting. Let us see how this is regulated. The hepatocytes activate a gene called cMet. cMet stands for the receptor for HGF; HGF is a ligand, a secreted molecule.

But everyone cannot say that a satellite is releasing a signal from space onto your house; you cannot watch the TV; you need to have a dish antenna. And a TV, dish antenna, and wire connected to your TV, then you can watch. Otherwise, you cannot watch. So the cMet acts like a receptor or a dish antenna for receiving HGF. Within an hour of partial hepatectomy, the cement is induced.

So those cells that have got cement can now respond to HGF. The trauma of partial hepatotomy may activate several metalloproteases; metalloproteases are enzymes that do a scavenging job or a cleaning job. For example, if you are entering a room or your own house, and your house is full of furniture, you should first clear the furniture so that you can open the door and put your foot inside. Your living room, if there are 2000 chairs in the way, how are you going to enter? This damaged area, the clearing job is done by matrix metalloproteases that digest the ECM. What is ECM? Extracellular matrix permits the hepatocytes to separate and proliferate.

Otherwise, every cell is anchored to the extracellular matrix, and that has to be cleared. So that they can be free, they can be free birds in order to proliferate. Metalloproteinases also cleave HGF into its active form. HGF is usually secreted in an inactive form as a precaution and protection, and the HGF is now acted upon by these proteases, which will convert it into active HGF that can bind to the cMet Together, the factors produced by Kupffer cells, endothelial cells, and stellate cells allow the hepatocytes to divide by preventing apoptosis. If apoptosis is called cell death and it is occurring, the number will decrease.

If your mother is making dosa, and every dosa is made while you are constantly eating, then by the end of the day, the plate will be empty. There won't be any dosas. So at least Mother should stop you from eating for at least 10 or 15 minutes. So that there are at least one or two doses on the plate.

The same logic applies. So apoptosis will be prevented because you need to increase in bulk. And this is done by activating cyclins D and E, which will help increase proliferation and repress cyclin inhibitors such as p27. So, activators are turned on, cell cycle activators are turned on, and the inhibitors are turned off. Both are needed. So the proliferation of non-hepatocytes in the liver typically lags behind that of the hepatocytes by a couple of days.

Non-hepatocytes refer to the non-parenchymatous tissue. That means the cells that are doing a supportive role, like stellate cells, Kupffer cells, and endothelial cells—those kinds of cells. They lag behind by a couple of days, with the result that the small clusters of newly produced hepatocytes take form before they interact with other elements of the liver to reconstitute the normal internal hepatic structure. Because if all cells are dividing in equal phases, every cell is dividing, then at the end of the day, each one will have one-to-one ratio.

You don't want that. Because the majority, around 30 to 40% of the total cell types, belongs to hepatocytes. So little is known about what brings about the cellular regenerative ability in the liver. Hurt, but one major molecule, TGF-beta, produced by the stellate cells, is known to inhibit the re-entry of the hepatocytes into the mitotic cycle, so the same stellate cell is allowing the primary cell to enter from G0 to G1, and the same cell is releasing transforming growth factor beta to prevent it from continuing to be in that phase. Because this is a system

of checks and balances in cell proliferation. In some situations of chronic toxic damage to the liver, such as alcoholic cirrhosis or repeated exposure to carbon tetrachloride, hepatocyte regeneration is not sufficient.

Hepatocyte regeneration will be sufficient when the damage is minimal. If the damage is severe, it is not sufficient to prevent the fibroblast cells in the liver from producing the connective tissue scarring. This will lead to scar formation, and the liver is not going to regenerate properly. There is a junk region that will be created in the liver, and the regeneration will be compromised. As you can see in this picture, this is a cross-section of a normal hepatic globule, and this is a larger area.

You can see there is a portal triad where you have the hepatic artery, portal vein, and bile duct acting together. You can see that whenever an injury occurs, there is injury to the cells, and here on the right-hand side, you have injured cells and matrix. You can see the matrix; it looks intact, but the cells are kind of damaged and not looking healthy, as you can see here. They are damaged, however. The proliferation can continue, and then this normalcy, as you can see here in the top right-hand side, is restored because the matrix was intact.

What happened was an injury to the cells; as you can see here, the cells are rearranged, but the matrix is not there. Like you can see here, the matrix is properly here; there is no matrix. Then what happened is that the matrix is non-existent, so the regeneration is not proper. See how many hepatocytes are formed here; hardly any hepatocytes regenerated. But meanwhile, the matrix has given rise to scarring that is called repair by scarring.

Scarring is nothing but the abundant production of the extracellular matrix, which is mainly done by fibroblasts, which are not tissue-specific cells, and are basically glue-like cells. Liver regeneration occurs from the progenitor cells in situations where the proliferative capacity of hepatocytes is impaired or inadequate. Such as after chronic liver injury or inflammation, the progenitor cells in the liver contribute to the repopulation, so moderate damage to hepatocytes will take care; but if there is severe damage, the liver stem cells or liver-specific progenitor cells—progenitor cells and stem cells are more or less the same—contribute. In rodents, these progenitor cells have been called oval cells because of the shape of their nuclei; they are oval in shape. And some of these progenitor cells reside in a specialized niche called the canals of Herring.

They are located where the bile canal connects with the larger bile ducts. We have a picture here. You can see it here. This is basically what you are talking about.

This is the hepatic artery. And you have plenty of hepatocytes, which are brown in color. And these canals of herring, which are labeled here, you can see these yellow-colored cells. They are basically hepatic progenitor cells. They will come into the picture if the damage to the liver is severe.

If there is extensive damage, then they come into the picture. For example, if the liver size became 150 grams, as I mentioned earlier, or if you received a new liver from a donor, in that situation, the stem cells in that liver fragment have to proliferate. Hepatocyte proliferation alone will not be sufficient, so this is what you should understand about the stem cells. It's kind of a second line of defense when it comes to liver regeneration. To summarize the second line of regenerative ability in the mammalian liver, you can see that hepatocytes are unable to regenerate. More than being unable to regenerate, inadequate is the correct word.

Inadequate to regenerate the liver sufficiently within a certain amount of time. That means there is a waiting period. A certain amount of time after the second line is activated, which involves the progenitor cells called oval cells, in the case of rodents, a small population of oval cells, a small progenitor cell population that can produce hepatocytes and biliary cells, starts dividing, and oval cells appear to be kept in reserve and are used only after hepatocytes have attempted to heal the liver. You have the ability to regenerate simply because any toxic food or any toxic substances going into your body, including alcohol, have to be detoxified by your liver; in other words, every day your liver is dying, regardless of whether you are consuming alcohol or, as long as you are eating some natural products, some toxins present in those will be damaging your liver; hence the hepatocytes have to fix it, and the regeneration by hepatocytes is a daily process. And if that one doesn't happen because of, you know, type 2 diabetes or maybe fatty liver disease, non-alcoholic fatty liver, or non-alcoholic liver cirrhosis, all the situations, hepatocytes fall behind, and intercellular signaling contributes a major role when it comes to regeneration of the liver.

It includes mainly three pathways: pathway one is called autocrine. In autocrine signaling, cells have receptors for the secreted factor on their own. For example, let us assume I am a cook and I am not giving food to anyone. I am cooking and making food only for myself, and I won't give any to anyone.

I will cook and eat. So this can be easily compared to that of autocrine signaling. It do not allow anyone else. Let us assume paracrine signaling. What does it mean? Cells respond to the secretions of nearby cells.

That means you can compare yourself to your mother. Mother does not cook only for her. If a four-member family, the mother will cook for her and her family members, and this can be compared to paracrine signaling, which means it works in the vicinity. Then comes endocrine signaling. What is endocrine signaling? Endocrine signaling is essential for a lot of distantly acting processes. Let us assume a restaurant is making food and, through Swiggy, Zomato, or any other online distribution system, is sending it to various customers, and this is the endocrine system.

That means cells respond to factors and hormones. Now I'll show the previous picture once again so that you will know it much better. Here you see there are a lot of hormones coming

into the picture, such as the thyroid hormone T3 and the epidermal growth factor (EGF) from the duodenum; these are endocrine signals, along with the pancreas, which secretes insulin, and the adrenal gland, producing norepinephrine. All these factors are endocrine factors that contribute to the regeneration of the liver, and you can see here this, uh. An individual cell that released IL-6, TNF alpha is basically paracrine signaling.

And this cell's hepatocytes are priming the neighboring cell. This is paracrine signaling. And if some of the factors released by the hepatocytes act on themselves, that is called autocrine signaling. This cell itself is releasing TGF beta and negatively influencing the hepatocyte.

That is a paracrine signaling. It is not released into the bloodstream. What is released into the bloodstream is the endocrine signaling. So all three kinds of signaling come into picture when it comes to the regeneration of the liver. You can see here in this picture, in this cartoon, that the cell releases its own ligand and that the receptors are needed for that as well. It is releasing, acting on the receptors, and responding accordingly.

It can also be compared to that of, you know, stimulating yourself. You may have sometimes seen people who are playing sports; after performing well, they will say, "Okay, well done, man, well done." You did a good job; you are talking to yourself, and it's self-boosting. So this is basically done in the case of autocrine signaling, whereas paracrine signaling is basically a molecule that does not act in the same cell, but it acts by diffusion into the neighboring cell. Paracrine signaling is very predominant.

Autocrine signaling and endocrine signaling happen in a routine manner. But paracrine signaling is normally done very effectively in the case of liver regeneration. Endocrine signaling, such as growth hormone and insulin, etc. They are all released into the bloodstream. As you can see here, there are endocrine cells present.

They are released into the bloodstream and can reach distant target cells. But remember, distant target cells can respond only if they have the receptor for it. If it does not have the receptor, it cannot act. So now, some questions come up. How does the liver stop growing when it reaches its appropriate size? Very good questions.

Like I told you, 1.5 kilos. Who is telling? Is there a weighing machine situated inside? How can I stop it? And the answer is not known. Having said that, we have some clues. Let us see. One clue comes from the parabiosis experiment. What is the parabiosis experiment? Partial hepatotomy in one of the parabias rats was performed.

will cause the other liver to emerge. What does it mean? You take the bloodstream of one rat and fuse it with the bloodstream of the other rat. You keep two rats together. And connect the blood vasculature of one mouse in one rat to the other rat; this means their circulatory systems are now united. Of course, they don't have this blood mismatch issue like what humans have. Even if humans have B positive with B positive, nothing should

happen, but no experiments will be done on humans in this case.

In one rat, the liver is damaged, but why does the other liver have to become bigger? This does happen. Some factors in the blood released from the amputated liver of a rat enter a normal rat's bloodstream. To establish the liver's size. So Huang and colleagues proposed in 2006 that these factors are bile acids. So partial hepatotomy stimulates the release of bile acids in that rat, but since its bloodstream is connected to a normal rat, this activates the transcription factor FXR.

FXR in hepatocytes promotes their proliferation. As a result, it becomes bigger, and we also understand that liver hepatocytes have the ability to enhance their size; they will become hypertrophied. Now we have a few questions remaining: what are the molecular mechanisms by which these regeneration-promoting factors interact? How are they interacting? Is there any master regulator? How is the liver first told to begin regenerating? Or proliferating, or we also said that stem cells come into progenitor cells; they will wait. Who is telling them how it is being measured? How is the liver told to stop regenerating when it reaches an appropriate size? Say the moment it reaches from 150 grams to 1.5 kilos, it will not grow to 2 kilos. Who is telling them how it is being stopped? So these are some of the interesting factors to explore. We will continue more about liver regeneration in the next class. Thank you.