Regeneration Biology
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W4L19_Common cellular events during regeneration, embryonic development, and cancer

Hello, everyone. Welcome back to another class on regenerative biology. So in today's class, we will learn about what the common cellular events are during regeneration, embryonic development, and cancer. I'm sure all of you have heard about the events that occur during cancer, the events that occur during regeneration, and the events that occur during embryonic development, which we have seen on multiple occasions in the same course itself. We haven't covered embryonic development or cancer, but be assured that During regeneration, quite a lot of pathways that are turned on during embryonic development or turned on during cancerous conditions are reactivated. So we will try to understand what pathways we can think of that have in common.

So one of the transcription factors that is induced and plays a pivotal role during cancer development and regeneration is OCT4. That is one of the Yamanaka factors that is induced during development very early, and it is also hijacked by cancer, regenerating tissues, etc. So the importance of Oct4 in various pathways of embryonic development, regeneration, and cancer is being worked out in hundreds of papers. One interesting thing about Oct4 is its concentration; the level of Oct4 protein present in a tissue can decide its role.

It is just like an analogy: if your wallet is strong or thick, having lots of money will decide where you are going to have your dinner. If your wallet has only 100 rupees, you will try to have some snacks on the roadside. But if your wallet has 10,000 rupees, you will not just have a snack. You will go to a good restaurant and have a nice meal. The same logic applies to a lot of transcription factors.

Oct4 is one of them. So in a simplistic sense, when Oct4 levels build up to a certain point, it has one effect, and it goes further. It is going to have the opposite effect. So I will not discuss too much, except I will touch on how it is contributing to the regeneration of cancer and developmental biology. Oct4 is one of the pluripotency-inducing factors.

What does it mean? It can convert a fibroblast into a stem cell. That is what it means.

Somatic cells can be reprogrammed to a pluripotent state by ectopic expression of transcription factors such as Oct4, SOX2, KLF4, and MYC. They are in short form; they are called Yamanaka factors. And the scientist who won the Nobel Prize for his discovery.

So, research studies have implicated the importance of Oct4 in human zygotic genome activation. What is zygotic genome activation? During development, an egg and a sperm fertilize, and you end up with a zygote. So until a few hours of development, the first division, second division, third division, and fourth division, all this show is done by maternal deposit. That means the RNA and protein that are deposited in the oocyte are the ones that contribute to the initial stages of development until around the more or less stage. In the case of zebrafish, roughly three hours into development, it is not going to activate zygotic-specific gene expression.

Whatever is present until then in the oocyte is the one that is running the show. That is the development. So this zygotic genome activation is very important because the sperm comes with completely imprinted and completely silenced genes. So that has to be reactivated. Otherwise, paternal genes will not exist.

For example, I will tell you that human IGF and the IGF receptor are classic examples. If the maternal copy of IGF is present, a normal copy that is not going to express in the zygote will be imprinted and silenced in the same way the IGF receptor's paternal copy is not going to get expressed. So, no matter whether you have IGF and IGF receptor from both parents, only the IGF from the father will be turned on, and the IGF receptor from the mother will be turned on. the other will be the opposite will be silenced. Although the father had both copies functional, the mother had both copies functional as well, and they both contribute the genes, but the zygote will specify.

So when you say zygote genome activation, it also means the silenced one should be turned on and the turned-on one should be turned off as well, okay? So the research studies have implicated the importance of Oct4 in the activation of the human zygotic genome. Epigenetic modulation of the Oct4 gene and post-translational modifications of Oct4 protein activity are also seen in the context of different types of cancers. So what are cancers doing? They are trying to recapitulate the embryonic property or the zygotic genome activation property of Oct4, which is being hijacked by the cancerous tissues so that it can turn off any genes at will that will favor the cancerous cell. So Oct4 induction is essential for zebrafish retina regeneration as well. If you knock it down, there is no regeneration.

There is a research publication from our group. It's been available for those who are

interested in reading. So common are events during development, organ regeneration, and cancer. Let us look further. Throughout embryogenesis, cells bud off from developing tissues and move to shape and pattern the complex architecture of prospective organs.

During embryonic development, there is too much movement of the cells, which is essential because an organism is being formed from the zygote. So, if the cells are not moving, there is no movement. Different germ layers like ectoderm, endoderm, and mesoderm, etc. Will not be formed properly unless the cells are moving. So a similar process occurs in adult life during wound healing and tissue repair when lingering cells migrate to injury sites to recreate the pre-existing structures.

During regeneration, it is very similar to development, where the cells have to move. So in the case of injury, it has to move to the spot where there is an injury so that it can colonize and restore normalcy. So acquiring cell motility is necessary but not sufficient. Motility is needed, but not sufficient for this event. It's like you got admitted to a good college.

You have to go to that college, but that doesn't mean you will get the degree. You need to move. The cell has to move and then behave in a certain way. Credit goes to its gene expression events in that cell after reaching that particular location. So cells that detach from their neighbors must elude a process called anoikis, which is a form of apoptotic cell death that occurs when cells lose their adhesion to the extracellular matrix.

Whenever a cell moves from its comfort zone, say a given tissue had 100 cells, one cell decides to move as part of being proliferating or migrating, etc. But the moment it loses contact—normally, every cell will have around six to seven contact points, of which losing two or three points is good enough—that cell is now vulnerable to apoptosis, and that process is called anoikis. However, it has to resist; it's just like you are not ready to move away from your home. And you always need support from the parents. If you move out, you will perish.

But during that process, you should develop. Although I am dependent on my parents as of now, I should be self-reliant. I should be standing on my own two feet. Only then can I run the show.

Cell also have to do this. It has to resist this matrix detachment related to anoikis, which is a form of apoptotic cell death. Moreover, the migratory cells undergo extensive mitotic divisions to produce founder populations, which settle in newly forming organs during development. So these migrating cells during development are also occupying a particular area. Say that your liver is formed in a particular place. Your lung is formed in

a particular location.

Your kidneys are formed in a particular location. Your heart is formed in a particular place. So this all happens because of the migrating cells, and then they differentiate, not randomly, according to the demand of the morphogenetic gradient that is present in that location. The same logic applies when you are talking about cancer metastasis or when you are talking about wound healing. So during development, an organ is being formed; during regeneration, a new tissue is being formed in that lost area, and they must colonize.

After moving, the newly formed cells colonize worn tissues during the repair. So the typical phases of embryogenesis and organ regeneration strongly resemble the pathological processes of tumor invasiveness. You may have heard about tumor invasiveness many times; if anyone in your non-circle gets cancer, doctors will say, "Oh, there is a secondary tumor" or "there is a metastasis." They use this word. This is because you have a tumor cell in a particular location, but it has spread to a secondary site in a different tissue.

At that time, the cancer becomes complex, and this is called tumor invasiveness, or we refer to it as an invasive tumor or invasive cancer. Similarly to cells at the wound edge, So the tumor invasion happens at the cells in the wound edge. Think about a lake. You want to close that leak. How will you close the lake? You will start putting sand or mud in the surrounding periphery, and you will keep walking into the middle of the lake.

That's how you don't randomly throw mud or sand into the middle of the lake. Usually, you don't do it. In the same way, at the periphery of the wound site, the cells have to migrate, and the cells have to proliferate, walking into the center of the wound. This is what usually happens. The wounded cells at the tumor's leading front disrupt intercellular contacts and infiltrate the adjacent surroundings, where they resist anoikis, which is a form of apoptosis, and grow before lodging in the blood vessels for systemic dissemination.

So when a tumor is growing, and normally it happens when doctors take a biopsy, it also brings tumor cells into the bloodstream. Once they get into the bloodstream, they are now looking for a new colony to establish, and often the cancer cells resurrect. The latent schemes of cellular reorganization that are usually confined to embryonic development. Resurrect means they reinvent and rediscover the properties that embryonic cells have because embryonic cells are very sturdy, have lots of good gene expression, and facilitate proliferation, migration, and differentiation. This is what the cancer cells also make use of.

The damaged adult organs leverage themselves to become competent for metastasization, which is the act of metastasis, so these activities, which are basically the mortality survival, mean resisting apoptosis and proliferation that occur during development and also during injury response; this can be either regeneration or wound healing, it doesn't matter. Neoplastic tissue is the one that migratory cancer cells embody, defined as invasive growth, which means invasive growth occurs during development, regeneration, wound healing, and also during neoplasia or cancer metastasis; thus, this property is essential. This is triggered by extracellular stimuli that regulate the activity of several transcription factors, which in turn modulate the expression of many other proteins ranging from cytoskeletal and cell-cell junctional components to cell cycle regulators and anti-apoptotic effectors. That means the cell should have the ability to proliferate, migrate, and not undergo apoptosis.

Then it is a fit show or a fit cell. One major environmental inducer of invasive growth is hepatocyte growth factor (HGF). We have seen this during liver regeneration, which is also known as a scatter factor. The ligand for the MET is a tyrosine kinase receptor. MET stands for mesenchymal-to-epithelial transition. So, the MET tyrosine kinase receptor is also known as the HGF receptor.

So HGF can bind to MET, which is a receptor tyrosine kinase. It can also do kinase activity, but it can also bind to the ligand HGF. The MET function is required for various morphogenetic events in both embryonic and adult life, and it drives the malignant progression of several types of tumors. It is a necessary thing, but during cancer, it goes haywire. For this purpose, MET propagates an intricate system of signaling cascades that results in the comprehensive rewiring of gene expression.

Because the MET binds to the HGF, many changes in gene expression occur inside the cell. HGF is secreted as a single-chain inert precursor and is converted into a two-chain functional heterodimer by extracellular proteases. Proteases are induced due to inflammation or injury, among other factors. and they contribute to the proper maturation of HGF. Pro-inflammatory cytokines such as interleukin-1, interleukin-6, tumor necrosis factor alpha, also known as TNF alpha, and transforming growth factor beta (TGF beta) induce transcriptional upregulation of both HGF.

HGF is produced because of this inflammation in fibroblasts and resident macrophages. Macrophages are phagocytic cells. They are part of the immune system. And also, fibroblasts are the glue cells, which are present pretty much everywhere. and MET that will not only produce this HGF, but it is also produced by MET in epithelial cells so that the ligand is there and the receptor is there.

Now they have to act, and the gene expression even starts. Following HGF binding, the kinase activity of MET is switched on by receptor dimerization and transphosphorylation of two catalytic tyrosine residues, namely tyrosine 1,2,3,4 and tyrosine 1,2,3,5. Within the kinase activation loop. That is the first event that occurs. The subsequent step is the phosphorylation of two additional docking tyrosines in the carboxy-terminal tail.

That is tyrosine 1349 and 1356. So we have got a total of four tyrosines in the picture. When phosphorylated, these tyrosines act as a degenerate motif. For the recruitment of many signal relay molecules, this means they can act as mediators for passing on the signal, somewhat like intermediates, or as if they are facilitators. Metis also serves as a substrate for several protein tyrosine phosphatases known as PTPs, including the receptor PTP and Density Enhanced Phosphatase 1, which is also known as DEP1, and has another name, PTPRJ, because in different articles, when you read, you will also see this name, as well as a leukocyte common antigen related. This is another protein that is contributed by the signaling event HGF2-MET, which triggers these sets of events so that the cell now turns on a bunch of genes.

So now let us see what the signal cooperation is between MET pathway components. So, as you can see here, this is the plasma membrane where the receptors are located, and the HGF ligand is seen here, and the MET receptor is seen here. And this will trigger a bunch of internal action. or internal signaling events. As you can see here, the names of these proteins are not necessary for you to remember or understand.

But this picture shows how complex these signaling events are that are occurring. So this is inside the cell, and this is outside the cell. Once the ligand binds to the receptor, you can see many related pathways. In one scenario, you see this alpha 6 beta 4 integrin coming into the picture, and they are coming together because of this interaction.

In another scenario, the same HGF binds to the MET. These proteins are not there, but you have a different set of proteins, which is part of the CD44V6 protein that came into the picture. In another scenario, what happens? You also have this MET bound by another protein called SEMA, and then the class B Plexin comes into dimerization. So you have different types of scenarios that are occurring with the same kind of ligand-receptor. It's almost like a Swiss army knife, and based on which tissue you are talking about, this can have a differential behavior. This is what you should keep in mind: it's just like if you say you are in India, you will read a Hindi newspaper, or if you are in the US, you may not get a Hindi newspaper.

Rather, you will get a newspaper that is written in English. Or if you are in France, you

may find a newspaper that is made in French. So, what is the difference? Everywhere you are reading newspapers, provided you know the language, but you cannot expect this particular thing to be there in this place. The same logic you should have is based on who you are.

But the... The event is the newspaper being delivered to your residence. In the same way, HGF binds onto the mat. What action will happen depends on which tissue or scenario you are talking about. So one good signaling event that I mean is a well-studied signaling event is head jog signaling, which is important during prostate development, regeneration, and cancer. So you may have seen that one of the very deadly cancers is prostate cancer, which affects many males, especially in their post-middle age years.

So let us try to understand what the contribution of hedgehog signaling is. Hedgehog signaling in the developing prostate involves understanding what the signaling will do during development, during maintenance homeostasis, and during cancer. Are they doing the same throughout all three phases? Hedgehog signaling in the developing prostate involves both autocrine and paracrine signaling. Autocrine means a cell is releasing hedgehog signaling and acting on itself.

That means you are cooking food for yourself. Paracrine signaling means that, like when your mother is cooking food. She doesn't make just food for you. Mother makes food for herself and also for the family members.

This is paracrine signaling. And both hedgehogs can do. And remember, a hedgehog is a molecule that has got... A migratory ability of up to 3 mm.

One of the longest distances it can pass. Because of its credit, it goes to its lipid moiety added onto it, etc. We will not go into the details of it. Auto crane signaling drives. The androgen is independent. Androgen means it is a male sex hormone, and autocrine signaling drives the progenitor cell proliferation at the bud tip.

And remember, this phenomenon is androgen-independent. That means it can happen right from the early stage of development. You don't have adequate levels of androgens. In them, much later after puberty, only you will have that level of paracrine signaling that is autocrine signaling, what we saw, which is androgen-independent, that is progenitor cell proliferation at the bud tip of the prostate gland, and paracrine signaling elicits a complex transcriptional response determined by the development stage, which stage of development you are in, that exerts various effects. On the epithelial proliferation and differentiation. So this is paracrine signaling because paracrine signaling can act a little bit away.

Autocrine signaling occurs right in the same cell where it is produced. In the prenatal prostate, which is very early in development, hedgehog pathway activity drives epithelial proliferation and ductal growth. This is very early in the development. In postnatal prostate, which means post-delivery, what happens? Hedgehog pathway activity inhibits epithelial proliferation and ductal growth. So what do you understand from this? The same signaling event occurs during early development. It allows the organ formation normally present, but soon after birth, it does not want any more proliferation.

Because it is done and dusted. The same signaling is doing the same job. You may have seen in some movies where the villain pays money to the bodyguards of the rich man. And then the rich man thinks, "Oh, these people are my bodyguards." But the villain already paid them, and they are gone, so the rich man is now alone. So, you would have seen such kinds of stories.

In the same way, the hedgehog signaling is now done. Earlier, before birth, it was pro-proliferative. After birth, it does not favor proliferation. It is stopping it. So androgen-independent progenitor cells generated by autocrine Hedgehog signaling during development can survive castration. That means if you remove the organ and enable prostate regeneration in response to testosterone supplements.

So now you are talking about a third scenario. Prenatal and postnatal, we saw it. Now something happens due to androgen supplementation. That is why the levels of androgen, if they are very high, can act as a condition, but not everyone who has a high level of androgen will end up getting prostate cancer.

Not like that. But it will favor a situation because it is androgen-dependent. So, so far what you saw is androgen independent. Sometimes, the hedgehog signaling can be facilitated by androgens. That is why even if you castrate, there is a possibility of the proliferation of this gland because of the androgen supplementation. So this is what you can see here in this picture: how Sonic Hedgehog signaling happens, the GLE as the actual transcription factors, epithelial progenitor cells, and mesenchymal cells, and how they are contributing to the proliferation. Red means stop, yellow means caution, and green means go in terms of proliferation.

Homeostasis and neoplasia allow us to see what epithelial cells will do: they will release the hedgehog ligand, leading to the production of Gli, which is the transcription factor based on the hedgehog signaling in the stromal cells. Stromal cells will release many paracrine factors, allowing the epithelial cells to proliferate during carcinogenesis. What happens is homeostasis in a normal scenario. During carcinogenesis, the same event

occurs; however, these paracrine factors allow for the disorganization of the stromal cells and epithelial cells.

Here, it is well organized; here, it is not organized. This is what happens: the target genes are activated by paracrine hedgehog signaling in the adult prostate, and the phenotype of this stroma determines the effect on epithelial tumor cell proliferation. And in normal adult prostate and prostate cancers with a normal fibroplastic stroma, the target gene profile resembles that of the postnatal prostate. Postnatal prostate proliferation, you know, is minimal.

Same signaling event. And the effect of paracrine hedgehog signaling is homeostatic. It is involved in maintaining homeostasis. In prostate cancer, let us see what happens. The so-called reactive homeostasis is. Myofibroblastic stroma; the target gene profile resembles that of the prenatal prostate.

That means proliferative. Prenatal means proliferative prostate tissue. And the effect of paracrine signaling is to promote the proliferation of epithelial tumor cells. So the autocrine signaling and the tumor cell have a strong connection both during homeostasis and in neoplasia. So this is a schematic review of another tissue called the urothelial cells. Urothelium is the lining of your urinary bladder, which is one of the simple organs as well as one of the tough organs in your body, regulatory pathways, and is important for urothelial proliferation, differentiation, and regeneration upon injury. So you can see in this picture, urothelial cells and markers; this is a typical urothelium.

You have got superficial cells here, intermediate cells here, basal cells here, and stromal cells. It's a very simple structure; dedicated cells are there because it contains urine, which is a toxic, hypertonic liquid that has to resist the damage that can occur. On to so you can see here in A and B, this is urothelium and urothelial markers. As you can see, different markers are present in different tissue types.

What you are seeing is color-coded here. You can see stem cells, basal cells, intermediate cells, and superficial cells; they are color-coded. BMP4 is expressed in the mesenchymal compartment and signals to the urothelium through its receptor BMPR1A to regulate proliferation and terminal differentiation status. Estrogen can regulate urothelial regeneration by regulating the BMP pathway directly or indirectly by downregulating the cytokine interleukin-6, which is a pro-inflammatory cytokine. Retinoic acid and Wnt signaling govern urothelial regeneration by regulating genes such as BMP-4.

Because BMP4 starts with it, it will facilitate its own production. During a UTI, or

urinary tract infection, what happens? Uropathogenic E. coli invades and rapidly replicates inside the superficial cells. What you are seeing here, the blue-colored one or the violet-colored one? Then the host tissue responds by exfoliating the damaged superficial cell layer and recruiting neutrophils and monocyte macrophages to clear the infection. The urothelium mounts a rapid, proliferative, regenerative response to reestablish the barrier.

So now, if you look at this picture, you'll know the stem cell is doing this much action. It has to respond to the Wnt signaling and BMP signaling. As you can see here, it produces these cells; stromal cells produce BMP4, and it responds to the Wnt signaling, which then triggers a sonic adjuvant signaling in the stem cell, resulting in the production of BMP R1a. That is its receptor, BMP's receptor, and it relays this information to the basal cells, which will lead to terminal differentiation and restore the superficial cells. This is the normal course. But when there is damage, as you can see here in this picture, it must super activate this pathway so that it can restore normalcy.

So this is how your urinary bladder epithelium gets damaged and restored. So, in the regulation of urothelial development, regeneration, and cancer, the urothelium plays a critical role as a permeability barrier to cytotoxic factors and waste products present in the urine. The essential barrier is maintained by the specialized superficial P1 urothelial cells that we saw in the picture. A dysfunctional response to urothelial injury and dysregulated repair and restoration of the superficial barrier can lead to chronic bladder disease. This process of underlying urothelial repair is complex, involving structural elements, signaling pathways, and tropic factors. So we'll study more about it in future classes. Thank you.