Regeneration Biology
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W4L20_ (EMT) and (MET) in regeneration

Hello everyone, welcome back to another class on regenerative biology. So in this class, we will study how epithelial to mesenchymal transition and its reversal, mesenchymal to epithelial transition, are important. During regeneration and a few other biological events, such as cancer and development. So, this is a change in the identity of the cell. Epithelium to mesenchyme, mesenchyme to epithelium. Let us see how this is contributing to a regeneration context.

So, what is epithelial to mesenchymal transition? Very good questions. An epithelial to mesenchymal transition is a biological process that allows a polarized epithelial cell, which means a stagnated or fixed cell, to normally interact with the basement membrane, as we saw in the earlier class picture where the basement membrane, which is secreted, is a combined action of your ectoderm. Superficial layer of the epidermis and the dermis, so you can refer back to the previous class, but understand this epithelium has to have an attachment to the basement membrane via its basal surface to undergo multiple biochemical changes that enable it to assume a mesenchymal phenotype. Cell that exhibits certain properties, such as enhanced migratory capacity, invasiveness, resistance to apoptosis, and increased production of ECM components, extracellular matrix components.

This is needed for the mesenchyme. So, in a nutshell, you can say the epithelium is a stagnant cell, and the mesenchymal cell is migratory. So the completion of EMT, which is epithelial to mesenchymal transition, is deciphered by the degradation of the underlying basement membrane and the formation of mesenchymal cells. Naturally, if you have a dog that is tied to a pole, you have to remove the leash or unchain it so that the dog can be free. That can migrate away from the original epithelial layers.

Simple logic, but it needs to have; it's not that an epithelium can simply be detached because naturally it will undergo apoptosis. But if it is properly integrated into a mesenchyme, then it will not undergo apoptosis. What happens during an EMT? Certain events happen. Let us see what those events are. The epithelial cells lose their surface markers, one of the prominent ones being E-cadherin, which is epithelial cadherin.

Cadherin is something like your legs because you need legs to walk or even hold things with your legs and hands. Like when you travel on a crowded bus, your legs should be firm and your hands should be holding on to something so that you will stay in the same spot. If you are not holding on anywhere, the driver applies the brake, you will be out of the bus. So the epithelial cells start expressing mesenchymal markers such as vimentin and N-cadherin, another cadherin molecule that is called N-cadherin or nuclear cadherin. They started expressing.

So, the epithelial cells produce extracellular matrix. Degrading enzymes, ECM-degrading enzymes, naturally ECM is holding the epithelium tight, so you want to get rid of it; breaking the bond requires some enzymes that will break down the matrix protein so that the cell can be free. Even many matrix proteins, like matrix metalloproteases, will chop off the extracellular part of the cadherin, e-cadherin, so that it will be blunt and not have any interaction. Because the leg is broken, nobody can think of walking, or something like that. In this case, if you break the leg, that is, break the e-cadherin, it can migrate smoothly.

So the epithelial cells produce lots of matrix-degrading enzymes to facilitate these events. The epithelial cells reorganize their cytoskeletal proteins because to move, they need to have a specific shape, and the shape of a cell, like normally the spindle shape, is decided by the cytoskeleton; so it cannot have the same epithelial cell-specific cytoskeleton, thus it has to reorganize cytoskeletal proteins. The epithelial cells begin to express specific cell surface proteins, which means they are going to interact with a newer zone or niche, so it should be able to. Interact well. It has to lose its, like if you're going to pole, you'll naturally carry some heavy jackets.

If you go to the polar region without a jacket, then naturally you'll perish. So the epithelial cells change the expression of specific microRNAs. We have discussed micro RNAs multiple times. Micro RNAs, in general, are guardians of a given cell, retaining the identity of that cell because they ensure that no unwanted mRNA gets translated. which can cause losing the identity.

So the change in the microRNA expression allows certain mRNA to be translated, and the protein can be formed. So you cannot have the same microRNA load as an epithelial cell had. Once it becomes mesenchymal, it must change. So in this picture, you can see what changes happen to EMT. So you can see that this is an epithelial phenotype.

There is a basement membrane, there are epithelial cells arranged, and then there are also some villi that are projecting depending upon which tissue you are talking about. And E-cadherin is connected to different proteins: ZO1, laminin, intactin, etc. And then I also have a bunch of other identity proteins. These proteins are Desmoplakin, the miR 200 family, etc. They are not important to memorize.

However, these proteins give identity to the epithelial cells. Now, they are slowly changing their phenotypes. As you can see here, this is still epithelium. This is a little bit lost. This is a little bit lost.

This has completely lost. That is called intermediate phenotypes in the transition. And you can see here that the epithelial-specific phenotype is yellow. Mesenchymal-specific phenotype is green. And you can see that the green is slowly increasing.

The yellow color is slowly decreasing. And the mesenchymal-specific phenotype is mentioned here. That is N-cadherin and vimentin. fibronectin, beta-catenin, and OB-cadherin, etc. Name these genes, and you can see myR-10B.

Here it was myR-200, and myR-200 is lost, and myR-10B, which is a microRNA, starts expressing. So there are other microRNAs, like myR-21, and many more, as well as some genes like snail, slug, etc. also comes into the picture. So these genes retain the identity of the epithelium. These genes convert the epithelial cells into mesenchymal cells.

So this is what you should understand during this transition. Where does the EMT occur? What are the scenarios in which EMTs are needed? Epithelial-to-mesenchymal transition. During embryogenesis, it is necessary. During fibrosis, we have seen that wound healing with scar formation or prolonged inflammation can cause fibrosis. During tumor progression and metastasis, During the formation of the heart, during the formation of a secondary palate, during various phases of development, during different cancer progression metastasis, and also during embryonic development.

This EMT is necessary. EMT can be classified mainly into three categories, into three subtypes. That is type 1. What is Type 1? EMT occurs during development and includes the mesenchymal transition of primitive epithelial cells during a process called gastrulation. Gastrulation is a time when the ectoderm and endoderm are interacting together to give rise to a mesoderm. So this is a process—a very complicated process—during embryonic development and the generation of migrating neural crest cells.

Migrating neural crest cells are also formed during type 1 EMT from the neuroepithelial cells during neural tube formation and the formation of endocardial cushion tissue from the cardiac endothelial cells. So these are all the situations that come under type 1. What is Type 2 EMT? That includes the transition of secondary epithelial cells and endothelial cells. Epithelial, as well as endothelial, cells are converted to tissue fibroblasts, which can be observed during wound healing, regeneration, and fibrosis. Type 2 EMT is more prevalent during these events.

Now, what is type 3 EMT? EMT also occurs in adult tissues and involves the mesenchymal transition of epithelial carcinoma cells during cancer progression, leading to the generation of metastatic tumor cells. That means some may have a tumor originating in the liver, but the secondary may be in the brain, the secondary may be in the lungs, and then it creates a very invasive cancer, and at that time, the type 3 EMT comes into the picture. So in a nutshell, what we can say is that an epithelial stagnated or attached cell now becomes a migratory cell in a process called EMT, which is facilitated by gene expression events. You can see here that type 1, type 2, and type 3 EMTs can be seen, and type 1, which is the primitive streak, is a part of embryonic development; this is the epiblast, and you can see the primary mesenchyme, and this is the hypoblast. So these are formed during embryonic development, and type 2 is done during the wound healing process.

Epithelial cells, a basement membrane is there, and you have a mesenchymal cell that is meant for dealing with this wound or injury. Type 3, mainly during cancer, you can see that there is a tumor or a mass of tissue here, and some of it is now becoming mesenchyme. Now it is migrating away from this cancer group or cancer tissue through the bloodstream to a target organ. So these are the three events that are depicted in this picture.

Now, EMT and fibrosis. So, fibrosis, we have seen it. Prolonged inflammation can lead to fibrosis. Not that you need to have a wound for fibrosis to occur. Every wound healing with scar formation, sometimes even regeneration, is associated with having some scar in some animals. Animals have scar-free wound healing; some will have a scar that is present.

This is also due to fibrosis or extensive deposition of ECM. Here, you can see a normal tissue, and the basement membrane is present, along with E-cadherin expression. What happens next? Two categories occur: resident fibroblasts and inflammatory cells, which are influenced by inflammation. The TGF-beta, MMP2, MCP1, and also hypoxia come into play, allowing this pro-inflammatory milieu to stimulate these cells to become mesenchymal.

There are no injuries. Mind it. There are no injuries. It is favoring a normal cell to become more mesenchymal-specific. As a result, you end up with a mesenchymal cell formed by EMT. So the EMTs related to fibrosis are associated with inflammation. Remember, mesenchymal cells are capable of producing too much ECM, which is an extracellular matrix and a feature of fibrosis.

Fibrosis is associated with inflammation and the generation of numerous types of molecules by inflammatory cells and resident activated fibroblasts, which are myofibroblasts. These molecules cause disruption of epithelial layers through degradation of the basement membrane. Remember, pro-inflammation also creates lots of matrix metalloproteases, which will disrupt the basement membrane. The epithelial cells lose polarity either by undergoing apoptosis, which is normal in a typical scenario, or by EMT. Any damage that happens to cells will cause them to undergo apoptosis, but not all will.

Some of them will survive, and they will undergo an EMT transition. The minority of the cells doesn't matter; they can contribute to too much ECM deposition, and MCP-1, which stands for monocyte chemoattractant protein 1, contributes to this minority of groups that stay in that locus and produce too much ECM. And it will lead to the formation of fibroid tissue, fibroid tumors, or benign fibroid tumors because of this EMT. The origin of fibroblasts during fibrosis and its reversal by BMP7.

Now, something interesting. We know our class titles are EMT and MET. The moment EMT happens, you know that it should have a reversal as well, just like when you started studying for a course. You cannot say that; you know, I will continue to study for the rest of my life. It doesn't happen because you are studying for a purpose. You have to finish the study and utilize it in your life.

So, that is what happens. Epithelial cells became mesenchymal with a purpose. Its purpose is not to cause fibrosis. Its purpose is to migrate, to locate in a place, to do something, and to do a job. Either it can be organ formation or wound repair. And then this migrated EMT has to return to the epithelial phenotype.

That is called MET, or mesenchymal-to-epithelial transition. This is very important. If this doesn't happen, then there is no, uh, what you call it, there is no purpose in doing this EMT at all, so a reversal can happen because of BMP7. Different sources of fibroblasts are possible in the formation of organ fibrosis. Four possible mechanisms are listed here in this picture.

One study suggests that about 12 percent of fibroblasts are from bone marrow. Fibroblasts mean we know they are basically the glue tissue; they are the main cells which do not have any. Unique or a specific function, but they provide structural stability to a given tissue, and they are 12 percent formed from the bone marrow. About 30 can arise via local EMT, which means the epithelial-mesenchymal transition creates the fibroblasts because fibroblasts are the migratory cells. Involving tubular epithelial cells under inflammatory stress, some people can experience this because of food habits, or maybe because of the abusive use of chemicals or drugs, or possibly due to environmental pollution, which can create an inflammatory milieu; additionally, in old age, there can normally be an inflammatory milieu in the body, and about 35 percent are from the end.

Empty, that is what you can see here, is formed by some unique stress of mesenchymal transition, which is mainly caused by stress-related inflammation. The remaining percentage likely emerges via the proliferation of the resident fibroblasts and other still unidentified sources. So you can see there are bone marrow-derived and endometrial mesenchymal transitions that can contribute, and you can see this is the activated fibroblast that has been mentioned here, and this is the resident fibroblast, and these are the epithelial cells that undergo EMT, and as a result, you end up getting a pool of activated fibroblasts that have a mesenchymal phenotype, and in B. The panel shows that systemic treatment of mice with renal fibrosis using recombinant human BMP7 reverses the renal disease due to severe attenuation of EMT and end-EMT-derived fibroblast formation. So BMP7 can prevent this transition of EMT or the production of too many fibroblasts, which can eventually cause fibrosis; remember, it can be reversed by BMP7 treatment.

So what you understand from here, although whichever tissue you are talking about, it has the potential to give rise to wandering fibroblasts through EMT transition, but you also have the ability to prevent that formation. So, the contribution of EMT to cancer progression. Let us see how it helps or how it is involved in cancer progression. So progression from normal epithelium to invasive carcinoma goes through several stages. The invasive carcinoma stage involves epithelial cells losing their polarity and detaching from the basement membrane.

That is the initial stage. The composition of the basement membrane also changes, meaning it no longer retains its affinity for the epithelial cell. Altering cell ECM interactions. ECM change happened, cell properties changed, and cell ECM interaction was affected, which in turn will bring about altered signaling networks also because ECM contributes a lot to the gene expression events in that cell. ECM is also a good source of various signaling molecules. Due to the change in the properties of both, the signaling networks also change.

The next step involves EMT, which is epithelial to mesenchymal transition and an angiogenic switch. That means it is favoring the formation of new blood vessels, etc. Because then only the newly formed cells will receive the nutrition. Facilitating the malignant phase of tumor growth requires mesenchymal formation or mesenchymal transition, aided by angiogenesis, and this will also contribute to the EMT process, as well as angiogenesis. The growth of malignant tumors progresses from this stage to metastatic once the tumor is big enough; now it will think of exploring new territory.

The secondary tumor or secondary cancer stage to metastatic cancer also involves EMT, enabling cancer cells to enter the circulation and exit the bloodstream at a remote target site where they may form micro and macro metastases, which may involve MET, that is, mesenchymal to epithelial transition, and thus reversion to the epithelial phenotype. So you can see here that the basement membrane is present, and some of them have changed their properties, and then they have undergone proliferation. And then eventually it is getting into invasive metastatic cells and passing through the blood vessels to a target site. Even in the target site, it will retain its identity.

The cancer cell will not become a new cancer. It will retain its identity as the source of cancer, but it will form a secondary tumor. This is what usually happens. And this kind of migration, which means the first formation of the mesenchyme, if it involves a mutated or troublesome cell that does not follow the cell cycle principle, can become cancerous and must acquire mesenchymal properties to increase in cell number, after which it will start migrating to a target tissue, and all these things orchestrate together. For cancer progression, this mesenchymal cell now undergoes a reversal.

Otherwise, it will be migrating to the target as well. You don't want them to migrate once they have reached the target. Just like when you go on a tour, you take your baggage, and you cannot keep your bag closed even after reaching the destination. Then there is no point in carrying that bag. You carried the bag only to unpack it.

So, this unpacking process is called MET. So this mesenchyme in the target cell becomes epithelium again and becomes part of that tissue, and it will continue to proliferate. It is not going to stop proliferating. It will continue to proliferate, and the cancer will be established in a new section. Why do we study this? Because the same thing happens. During embryonic development and also happening during wound healing or regeneration.

The only difference is that they are normal cells during development and also during wound healing. They are not cancerous cells. They are normal cells. Hence, their proliferation is not unchecked.

They follow this contact inhibition, etc. Rules will be followed. The moment one cell meets another cell, it will not proliferate on top of that because they follow this basement membrane rule. They need to have it. Otherwise, a cancer cell doesn't need. They will climb on top of each other. And this is why cancer becomes a problematic scenario, unlike development, regeneration, or wound healing.

So, in MET, what happened? Mesenchymal-to-epithelial transition is a biological process that changes mesenchymal cells into epithelial cells. It's a key process in development, tissue repair, and disease progression. Disease, here we are referring to cancer. MET is a multi-step process that involves mesenchymal cells becoming tightly linked and polarized into epithelial cells. They have to, from a wandering, what you call a spindle-shaped cell, become a properly shaped cell because cytoskeletal properties change, gene expression even changes, and they get settled, getting attached to the basement membrane.

And it's a reversible process. If a cell became an epithelial cell, it can become mesenchymal again. It is just a question of time and a question of gene expression. It is a mechanical process that involves the interaction between neighboring cells and the microenvironment. The microenvironment mainly refers to the ECM, which you are mentioning. It is orchestrated by transcription factors like SNAIL, TWIST, and zinc finger E-box binding, which are called ZEB proteins.

There are many proteins. Some are listed here. Important ones are listed here. They all contribute to this so-called MET, which is mesenchyme, back to epithelia. MET is critical for the reprogramming of mouse fibroblasts.

You know the Yamanaka factors. Fibroblasts are nothing but mesenchymal cells. Mesenchyme became a stem cell. Stem cells mean epithelial characteristics in induced pluripotent stem cells. This MET is what is happening. The mesenchymal fibroblasts, with the help of Yamanaka factors, become epithelial cells, which are nothing but iPSCs, as we call them.

MET is a fundamental process to the evolution of multicellular organisms. Multicellular organisms have got orchestration of different cell types and tissues. If EMT and MET are not possible, then you cannot get a multicellular organism. That means development depends a lot on EMT and MET, and regeneration and wound healing depend a lot on them. And naturally, cancer also makes use of this strategy for development and wound healing for cancer cell establishment. MET is associated with morphological and functional changes in multiple endometrial cell types as well.

So you can see EMT and MET in detail here. So you can see that epithelial cells are there. EMT is present, and mesenchymal cells are here, so it can also reverse; the top arrow indicates EMT, while the bottom arrow indicates MET. Stable, intact junctions and apical-basal polarity are present, but that is lost when it becomes a mesenchymal cell in the end product. The mesenchymal state has no junctions or front-back polarities, which means they can go either way; they don't have a proper head or a proper tail. In contrast, an epithelial cell has a head and a tail, which is what we refer to as polarity.

and it can reverse back by retaining these properties. And you can see the markers mentioned here—E-cadherin, N-cadherin, tight junctions, vimentin, extracellular matrix receptors, and extracellular matrix—and how they are contributing. You can see here that the vimentin and N-cadherin levels are high in the mesenchyme, whereas the E-cadherin and tight junctions are high in the epithelium. In the intermediate stage, they are retaining both these properties little by little.

So they are neither mesenchyme nor epithelium. It's a transitional stage. Now, MET during IPS cell reprogramming. Several different cellular processes must take place for somatic cells to undergo reprogramming to induce pluripotent stem cells, or iPS cells. IPS cell reprogramming is also known as somatic cell reprogramming and can be achieved by ectopic expression of Yamanaka factors or OSKM. OKSM, in short form, refers to Oct4, KLF, SOX2, and CMIC. Upon induction, mouse fibroblasts must undergo MET to successfully begin the initiation phase of reprogramming.

That is what MET has to kick-start. Epithelial-associated genes such as E-cadherin, that is, cadherin 1, cadherin 3, 4, 7, up to 11, occludin, epithelial cell adhesion molecule, APICAM, and CRUMS homolog, that is, CRB3, CRUMS homolog 3. And they're also upregulated before another gene that is induced, which is Nanog. Nanog is part of the Thomson factors, another set of proteins used for reprogramming or making iPS cells. A key transcription factor in maintaining pluripotency is Nanog, which serves to retain pluripotent cells as pluripotent, meaning that the epithelial property must be preserved.

was turned on. Additionally, mesenchymal cell-associated genes such as SNAIL, SLUG, ZEB1, ZEB2, and N-cadherin were downregulated. You don't want mesenchymal properties to be retained within the first five days of post-OKSM treatment; that is, Yamanaka factor treatment induction. The addition of exogenous proteins such as TGF beta 1, which blocks MET, decreases IPS reprogramming efficiency significantly, as mesenchymal to epithelial transition is blocked by TGF beta in mammals. So if you put TGF beta, the formation of IPS will be significantly decreased, or rather, it is a MET blocker. These findings are consistent with previous observations that the embryonic stem cells resemble epithelial cells in expressing E-cadherin.

So E-cadherin-expressing cells retain their stem cell or pluripotent characteristics.

Recent studies have suggested that the ectopic expression of KLF4, another protein that is one of the Yamanaka factors in iPS cell reprogramming, may be specifically responsible for the induction of E-cadherin expression by binding to promoter regions of the first intron. The cadherin gene itself, which is the cdh1 gene, indicates that each of these pluripotency factors has its own dedicated role; some of them are listed here. Each of these factors expressed in the mesenchyme contributes, that is, the Yamanaka factors contribute to pushing this mesenchyme back to epithelium. So both EMT and MET happen during regeneration as well.

So it is basically a property of the embryonic stage itself. So we will study more about regeneration biology in the next class. Thank you.