Regeneration Biology
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W9L41_Extracellular matrix and its roles in tissue regeneration

Hello, everyone. Welcome back to another class on regenerative biology. So, in today's class, we will learn about the extracellular matrix and its roles in tissue regeneration. So we have seen the extracellular matrix and its importance in various regeneration aspects. And some emphasis has been given to collagen, especially during fin and limb regeneration, which we discussed earlier. And today we will see an overall view of how the ECM, or extracellular matrix protein, is contributing to regeneration in a nutshell.

So extracellular matrix-mimicking biomaterials are effective tissues for organ culture. They are tissue-engineering scaffolds for regenerative medicine because of their biocompatibility, biodegradability, bioactivity, etc. So we will see some examples in the upcoming classes when we study organ regeneration in vitro. But understand that the use of biomaterials is exclusively dependent on how suitable they are to elicit a regenerative response or maintain a regenerative response, which is similar to that provided by extracellular matrix proteins.

So ECM-mimicking biomaterials preserve natural microstructures and matrix-related bioactive components, and they undergo continuous matrix remodeling upon transplantation. So many of these materials, when we use them, whether they're natural or artificial, once the cells start colonizing on them, then they start secreting their own matrix, which will replace this artificial material, just like you may have heard about absorbable surgical sutures. Which, like internal suturing, when you do not have to go to the hospital to remove those sutures, will get absorbed into the body. The interaction between host immune cells and the transplanted ECM-mimicking biomaterials has attracted considerable attention in the regeneration biology field. Mainly, you have to choose a material that does not have too much immunological implication; it does not attract.

So these are all lessons you are learning from the natural ECM proteins. So the transplantation of biomaterials may initiate injuries and early pro-inflammatory reactions characterized by the infiltration of neutrophils and M1 macrophages, which are both pro-inflammatory and can trigger a wound formation response. And the pro-inflammatory reactions may lead to the degradation of the transplanted biomaterial and drive the matrix

to a fetal-like state. Sometimes the initial pro-inflammation helps in attracting more immune cells, which in turn triggers a lot of protease enzymatic activity. This may help in clearing the ECM in a normal scenario and clearing these biomaterials because of their enzymatic activity in this artificially engineered scenario.

So ECM degradation is a well-studied mechanism that is essential for regeneration. And this degradation also triggers a lot of immunological responses, which are necessary in the initial phases of regeneration. So ECM degradation releases matrix-related bioactive components that act as signals for cell migration, cell proliferation, and cell differentiation. In the late stages, the pro-inflammatory cells fade away. It is a necessity.

Anti-inflammatory cells emerge, which involves macrophage polarization to the M2 phenotype. As we have discussed, M1 is pro-inflammatory. M2 is anti-inflammatory. The M2 phenotype and leukocyte activation to helper T cells, also known as TH2 cells, come into the picture, which we can also refer to as adaptive immune cells. So the anti-inflammatory cells interact with one another to facilitate matrix deposition and tissue reconstruction.

The deposited ECM molecules serve as the vital components of the mature tissue and can also influence homeostasis. However, we should understand that the dysregulation of matrix remodeling can result in severe pathological responses or pathological conditions such as aggressive inflammation and difficulty in healing, as it happens in the case of diabetic individuals. And non-functional fibrosis. Fibrosis is characterized by excessive deposition of ECM proteins, which is mainly triggered by the inflammatory response. Many times, when these M1 macrophages are overly recruited into an injury site, they favor the production of inflammatory cytokines, and as a result, excessive fibrosis may occur, which can sometimes be benign, but at other times, it can even lead to more severe complications.

The debilitation of organ function and the characteristics of the inflammatory response in matrix remodeling after the transplantation of ECM that mimics biomaterials are very important and crucial in organ regeneration; this is what we should keep in mind. The biocompatibility of the ECM material, whether artificial or natural, and how closely it mimics the innate ECM is very important for the success of the cells that are growing or deposited at the site of injury. A brief history of cardiac regeneration and its role in the extracellular matrix. We know cardiac injury is one of the well-studied regeneration scenarios in which ischemia, the lack of blood supply, leads to the formation of scar tissue, which can have serious implications for contraction. So lots of studies are focused on that; if skeletal muscles can regenerate, why can't we determine whether the heart can? And by tweaking that environment, where instead of scar tissue, if you can get some

muscular tissue, cardiac muscle tissue can be replaced, then that will favor the overall pathology or reduce the overall pathology or reduce the complexity because of this ischemia-related pathology of the heart.

So, this is the overall idea. To push the field of cardiac regenerative medicine forward, several hurdles must be overcome before we can develop therapeutic strategies to be implemented in a clinical setting. And every animal, whether it gets cardiac trouble or cardiac ischemia, responds with a regenerative response. Whether it is successful or not is the question. Firstly, we must understand why the extracellular matrix of neonatal mammals or zebrafish, as well as that of adult mammals and adult zebrafish, is able to induce adult cardiac regeneration.

That is a very interesting question. But the adult mammalian heart cannot do this successfully. So to this end, advances in omics-based approaches, which can include proteomics, RNAomics, genomics, etc. Omics-based approaches, including proteomics and RNA sequencing, will enable scientists to fully characterize the composition of the ECM and identify which components are no longer present in the adult myocardium. Because adult myocardium of mammals doesn't regenerate, what is the significant change that occurred that does not allow adult cardiomyocytes to regenerate? Next, we must determine how these candidate ECM components, individual candidate members of the ECM, are able to induce repair or regeneration of the injured myocardium.

So, this is another interesting question in vitro. And in vivo, how they are able to induce a regenerative or reparative response is very informative, especially when you are comparing the neonatal versus adult. Finally, the most crucial step will be the development of these ECM components into a synthetic hydrogel; we should bring in all the good stuff that favors regeneration and introduce them. into a synthetic hydrogel to facilitate its movement into clinical application. So it's just like when you get a cut, you'll apply Neosporin powder or Neosporin ointment, which contains antibiotics that will prevent bacterial growth, and the healing has to happen naturally.

In the same way, can you put something that has these matrix components in the injured area that will favor regeneration? So this is the question. So these are all the approaches one has to take. Advances in the field of synthetic biomaterials have demonstrated that the potential for regeneration of such synthetic ECM hydrogels exists. It's a matter of how effectively we can inculcate or how effectively we can introduce this information into an in vivo condition or real animal condition. One recent study has demonstrated the potential of this by developing a degradable elastin-like polypeptide hydrogel.

which mimics that of native ECM. Which ECM? To that of the neonatal heart or the

neonatal heart which regenerates. So the mammalian heart has always been considered a post-mitotic organ without the capacity for self-renewal. Just like your neurons, which are considered post-mitotic, it means they are done and dusted with mitosis; they will not enter the cell cycle where growth is achieved through cellular hypertrophy. It doesn't increase in number, but it increases in the size of a given cell, which is called hypertrophy.

Recently, however, several studies have identified that cardiomyocytes can proliferate to a limited extent in the postnatal heart of mice, not prenatal. Prenatal, naturally it can, but not in adults. Soon after birth, that is postnatal, they still retain the ability, although heart development is completed because there is a size difference between a baby heart and an adult heart, and that size difference is due to hypertrophy. But postnatal hearts of mice, rats, pigs, and even humans. They have the ability to proliferate.

Research has also shown that the immature mammalian heart possesses greater regenerative potential than previously expected. Whatever we know, we know that there is a lot of regenerative potential in the postnatal heart. Embryonic tissue is definitely present in zebrafish throughout the adult stage, and other amphibian models also have the ability to regenerate the heart. Given the similarities between the adult zebrafish heart and the immature mammalian heart, the author, Paolo et al., induced cardiac damage by resectioning 50% of the ventricular apex in one-day-old neonatal mice.

They are done. Surprisingly, what did they find? The apex was progressively regenerated, fully restoring the resected myocardium within 21 days, which is somewhat similar to that of adult zebrafish. So this indicates it has the potential and the ability; it is a question whether this quality can be retained in the adulthood of the mammal. This regenerative ability of the immature heart was lost once the mice became over seven days old, which means something goes wrong. At least from a regeneration point of view within one week of its birth. More recently, similar observations have been made in rats and pigs, not just in mice.

Even more fascinating is that several case reports have also demonstrated a similar phenomenon in newborn human babies. So what should we know? Within the first few days after birth, the changes that occur in the animal tissue make them less regenerative, suggesting that mammals retain a surprising cardiac regenerative ability at birth, not at the time point of birth, which is lost upon maturation. Maturation doesn't mean adulthood; maturation means within the few days after birth. Organ maturation occurs a few days after birth, which we refer to as organ maturation. Understanding which intrinsic mechanisms are involved in neonatal mammalian cardiac regeneration has triggered a new field of research into characterizing this innate ability for cardiac repair.

Lots of people try to hunt for what is lost, what went wrong, and what went missing. in within few days after birth, which may facilitate the identification of novel strategies for cardiac regeneration in a to fix a failing heart or failed heart. So we know ECM components can change after cardiac injury. We know that their components change. This table shows that these are the members of the ECM, such as collagen 1, collagen 3, collagen 4, collagen 5, collagen 6, fibrillin, fibronectin, and hyaluronic acid.

These are all part of the ECM components. There are lots of proteins. And let us see the regenerative situation, non-regenerative situation, and what can be done further. So what are regenerative situations? Adult zebrafish, neonatal rats, neonatal mice, adult rats, and adult mice are non-regenerative. So regenerative are only three: that is, the neonatal of rats, mice, and zebrafish throughout.

Non-neonatal, as well as neonatal and adult, they behave the same. If you see one ECM component called agrin studied in mice, it increases. And remember, neonatal mice regenerate; you can see collagen one in zebrafish goes up, whereas in rats it goes down. That is a conundrum because neonatal rats regenerate, but collagen one goes down. Now, collagen three in neonatal rats goes down; expression levels go down, but it regenerates.

In mice, it goes down even for collagen one, but it regenerates, and in zebrafish, collagen four goes up. In neonatal rats, collagen 5 goes up, but in zebrafish, it goes up; however, in neonatal rats, where it can regenerate, collagen 5 goes down, so these are some opposing results also seen. A lot of them, like fibrillin and fibronectin, in zebrafish goes up, in rats it goes up, and even in mice it goes up. When it comes to the adult non-regenerative scenario, that is, the adult rat and adult mice, you can see a lot of them, like collagen 1, goes down in both mice and rats, but it goes up. In the case of adult rats, it doesn't regenerate, so this table in general tells you what the situations are or what the factors that are contributing to a regenerative as well as non-regenerative environment are, so studies demonstrating the components' involvement in cardiac regeneration can be seen.

Recombinant agrin promotes cardiac regeneration. There is a research publication available. And fibronectin is important for zebrafish cardiac regeneration. There is a research study available. Hyaluronic acid inhibition blocks zebrafish cardiac regeneration.

Research study is available. Like hyaluronic acid, you can see in zebrafish, it goes up. And in neonatal mice, it increases. Both situations, regeneration happens. And in adult mice, soon after cardiac injury, the hyaluronic acid levels go down.

No regeneration happens. So these are all things that you should keep in mind: ECM components have a majority of similarities among different species; in a regenerating scenario, such as neonatal mammals and adult zebrafish, a somewhat similar response is seen, while in a non-regenerating scenario, the levels are opposite. You can also see other components like laminin, periostin, and perlecan. Thrombospondin and versican are different ECM components; you can observe their levels, and periostin is involved in neonatal but not adult cardiac regeneration. A published research study is available, so we should keep in mind that the ECM components are indeed regulated soon after injury. How are they regulated? Are they going up or down? It depends on the nature of that protein and also the nature of the tissue, whether it's adult or neonatal, or whether they can be regenerative or non-regenerative.

Accordingly, they have some common ways of responding, and these are all the ways in which you can strategize. Can we think of some of these components introduced into a matrix protein, artificially put into the matrix protein, and can we facilitate a regenerative response? For example, if collagen one is downregulated in rats and in mice, and it is going up in adult rats which do not regenerate, then when it is going down, can we think of introducing something into that material that will downregulate collagen one to favor the regenerative response? So now, lessons learned from innate cardiac regeneration will teach us that innate means the native regenerative ability. A brief history from the studied literature within the animal kingdom shows numerous examples of spontaneous organ regeneration following injury; we have many examples. In terms of cardiac regeneration, the zebrafish is the most well-studied and one of the best-suited organisms for studying regeneration biology. Zebrafish can undergo complete cardiac regeneration without any scar being formed after resectioning up to 20% of the ventricular apex.

It's a two-chambered heart, and the ventricular apex is very much accessible and visible. That is the place people use, and they also understand that ventricular wall fibrillation is more problematic when it comes to cardiac dysfunction than the atrial wall because the ventricles supply blood to the rest of the body. Complete regrowth of the amputated region, including the coronary vasculature, myocardium, and endothelium, is achieved within around 60 days post-resection. Sixty days means about two months of post-damage. This results in a fully functional heart in the case of zebrafish.

This model for cardiac regeneration has helped. To unravel certain aspects of the regenerative mechanism, we can hunt for them in the mammalian scenario to determine whether a given pathway is on or off; in the case of mammals, neonatal mice or rats also come into the picture for this kind of analysis. you directly compare into a non-regenerative model. Of course, in a non-regenerative model, you can always compare it to a regenerative model irrespective of the adult or non-adult situation. But what is most

important is to compare a well-studied pathway that has serious implications for regeneration. Say, for example, in zebrafish, and look at the status of that pathway in an adult.

So this is the overall theme of the research that has been carried out in the field. Still, since it is based on removing heart tissue, you are normally cutting 20% of the ventricular apex surgically, rather than damaging the tissue that naturally occurs because someone has heart failure; no one is going to chip off their heart. There is damage that is occurring due to ischemia. It is not the most accurate model for studying human cardiac damage and repair. So to this end, a recent study has characterized the cellular response and the ability for functional repair, which is the regenerative capacity in general, of the zebrafish heart after a cryo-injury, where a small piece of dry ice is applied to stop the blood supply and oxygen supply.

And that will lead to a result similar to that of producing an injury that more closely models the pathophysiological processes undergone by the human heart, especially in an ischemic condition. So, damage was induced to 25% of the ventricle using cryo-cauterization. That is, you are causing a cryo injury or a low temperature injury, resulting in massive cardiomyocyte cell death within the injured area and also near the coronary vasculature. So this is the overall idea of a cryoinjury. Cell death, which occurs because of this cryo injury, induces a proliferative response in the endocardium, epicardium, and myocardium.

There are two layers and three layers for the heart, ultimately resulting in the formation of a scar at the site of injury in a normal scenario. However, what we see, unlike in the injured human heart, is that the fibrotic scar tissue was degraded. First, a scar is formed, even in zebrafish, but the scar doesn't stay. In humans and other mammals, the scars remain.

That is the issue. The tissue was degraded and replaced by functional cardiac tissue in the case of zebrafish, suggesting that myocardial regeneration can occur even in the presence of scar tissue. means scar tissue is not debilitating the regenerative response, so if you have scar tissue in the mammalian heart, scar tissue is not to be blamed because in zebrafish, also in cryo-injury, there is scar tissue formation which is removed. Although the damage of this tissue composition in terms of matrix and cellular components was not fully characterized, these results indicate that the scar can be removed and suggest certain endogenous mechanisms are involved with scar tissue regression and cardiac tissue replacement. So we should understand it's not a given that if scar tissue is there, it will be replaced; scar is formed maybe at a makeshift arrangement and which is slowly replaced by a regenerative response.

Or the bloodshed is not needed. You don't have to have an amputation. There is no need for a wound signaling cardiac regeneration. Characterizing these mechanisms underlying heart regeneration in such animals will offer a way to identify novel strategies to overcome the limited regenerative response in mammals. So this is the overall idea: we should understand if a given animal's tissue can have scar formation, which is not a debilitating scenario to start with; it is a debilitating scenario, but it can be overcome slowly. And why should such a strategy not be adopted in another animal, no matter whether it is neonatal or adult? So here you can see an overview.

It is being given a developmental ECM selection. How do you select? That is different sequencing: work on proteomics or cardiac development and maturation. Everything has to be looked into, and you take different stages, from a small heart to a big heart, during the developmental stage. You do sequencing proteomics and identify unique features, and in vitro cardiomyocyte proliferation can be studied very effectively in the development of ECM components. You can also pursue strategy development, which means you can design a hydrogel that includes these unique developmental components that are identified; you can infuse them into it, or you can even provide a recombinant ECM component into the system so that it will be favored. And then comes the cardiac regeneration that is in vivo; cardiac regeneration can be introduced either by delivering the key components needed soon after an injury into the site.

And that is what has been mentioned. And we can also think of translation into clinical cardiac regenerative therapy, where you can get a cocktail just like how you deliver a vaccine to a host to protect against infection. To advance the field of cardiac regeneration, it is essential to take advantage of the innate regenerative ability of the fetal mammalian heart, which can regenerate effectively. So the developmental ECM section will require, first, the utilization of advancements in the omics-based approach. such as RNA sequencing, proteomics, etc.

To identify key ECM components. And that is essential for a developing heart. And then comes the developmental ECM component that will need to be screened in vitro for its capacity to influence cardiac regeneration; just identifying it doesn't make any sense unless it can do a similar job in an adult heart, which is a key component for which the induction of cardiomyocyte proliferation must be obtained in the adult heart; then only is it useful; otherwise, it is a useless thing. Say, for example, if a French or a German person is able to read that manuscript in that language, it doesn't mean that if it is put in front of you and you don't know that language, you will read as efficiently as that person. Knowledge is important; thus, it varies: the embryonic heart is different from the adult heart. So, the delivery of the candidate developmental ECM components will have to be

determined either by means of naturally produced ECM hydrogel, by synthetically produced ECM hydrogel, or by direct administration of these components in recombinant form by injection into the matrix ECM of the injured or damaged heart.

Having selected the most suitable ECM component for cardiac regeneration and the appropriate delivery system, these will have to be tested for their in vivo capacity to induce cardiac regeneration. The first step will be to look around the animal's model or different model organisms, such as adult mice or rats, either in a rodent model or in a large mammal model, such as pigs, for heart failure. Here, what we have learned so far is the development of an approach that will be implemented or administered in the animal after an injury to determine its efficiency in inducing cardiac regeneration and improving overall cardiac function. As the final step, the most crucial will be to translate this ECM-based therapeutic strategy into the clinical side. So what we have learned so far is that there is potential and there is possibility.

And because the ECM can hold the key to bringing in a regenerative scenario, as you saw in the previous table, there are components that can mimic a regenerative environment, but getting the correct cocktail holds the key, so we should understand these angles or approaches that will enable a researcher to come up with the most suited composition of the ECM that will allow the regeneration of any tissue. We will learn more about regenerative biology in the next class. Thank you.