Regeneration Biology
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W11L54_Types of adult stem cells and trans-differentiation for tissue engineering

Hello everyone, welcome back to another session of regenerative biology, and in today's class, we will learn about the types of adult stem cells and transdifferentiation for tissue engineering. What is their potential for transdifferentiation and tissue engineering? So, we will learn about what they are. Pros and cons: what are the different angles of trans-differentiation to be taken care of? So, adult stem cells are undifferentiated cells found in various tissues after birth that can self-renew and differentiate into specialized cell types. We know that because adult stem cells stay throughout the lifespan of an organism. We have seen this multiple times. We will also see today's class very quickly and will flash through it.

Stem cells are classified based on their differentiation potential, including pluripotent and multipotent stem cells. Pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells, can differentiate into all cell types present in the body. They can give rise to each and every one of the more than 100 different tissue types in our body, so they can give rise to any of them. Multipotent stem cells like the mesenchymal stem cells, short form known as MSCs, can differentiate into a more limited range of cell types within a specific tissue.

Say bone marrow or stem cells; they are multipotent. They can give rise to only RBCs, WBCs, and some immune cells like natural killer cells, blood platelets, macrophages, etc. This is an example: you have bone, and you have bone marrow, and inside the bone marrow, you have got the hematopoietic stem cell, which gives rise to two lineages; one is a lymphoid progenitor cell, so this hematopoietic stem cell gives rise to a multipotent cell, which gives rise to lymphoid lineage and myeloid lineage. The lymphoid lineage gives rise to natural killer cells, T lymphocytes, and B lymphocytes. Whereas myeloid progenitor cells can give rise to neutrophils, basophils, eosinophils, monocytes, macrophages, platelets, RBCs, etc.

And these stromal cells can give rise to bone or cartilage, and there are also adipocytes present that can give rise to fat-storing cells. We can also easily tweak these cells by transdifferentiation into skeletal muscle cells or hepatocytes of the liver, etc. And in some situations, you can make them into bone itself. And it can also be made into other stem cells, hematopoietic stem cells themselves. Some other niche types or unique types of cells can also be made.

Multipotent cells found in the bone marrow usually give rise to blood cells, but they differentiate in a systematic manner and are released into the bloodstream. And that is the routine scenario. But can this rule be violated? That is what we will try to understand. So, types of adult stem cells and their hierarchies will quickly show the principles of renewing tissues. Stem cells are able to make a copy of themselves; they can self-renew, divide rarely, and have a high potency rate.

They are found rarely, and they give rise to committed progenitors, which means they have a defined direction. Now they are called committed progenitors; they are transiently amplifying cells. They are multipotent, divide very rapidly, and have no self-renewal capability. They must come from the stem cells, and they give rise to specialized cells. So this is the simple principle of renewing the tissue.

Hematopoietic stem cells, as we have already seen, give rise to different cells in the bone marrow, such as NK cells, T cells, B cells, dendritic cells, etc. Now, the mesenchymal stem cell, which is basically derived from the transformation of an epithelial characteristic that is static in nature, becomes more mobile once it becomes mesenchymal. So MSCs can make copies of themselves, and once they reach committed progenitors, they can give rise to bone, cartilage, and fat cells. They can give rise to these cells. Natural neural stem cells, also known as NSCs, are derived from stem cells and give rise to committed progenitors, as well as various neurons, interneurons, oligodendrocytes, type 2 astrocytes, type 1 astrocytes, etc.

So gut stem cells are another type of adult stem cells. They can make a copy of themselves, give rise to committed progenitors, and produce paneth cells, goblet cells, endocrine cells, columnar cells, etc., which are specialized cells. So we are trying to understand: can these cells be pushed into any cells of your liking? And there is one more source of cells that we can create, which is called induced pluripotent cells. Pluripotent cells mean that they are not adult stem cells; rather, you can make stem cells from any adult cell or random cell, so induced pluripotent stem cells are made by genetic reprogramming, as we have seen with the Yamanaka factors, Thomson factors, etc.

Once you have the iPSCs, you can culture them on a layer and force them to differentiate into any cell type, so in this way. We can convert an iPS cell into any cell type of your choice that can be used, and this picture shows that a normal cell you took was converted into a stem cell, and now you can convert the stem cell into any cell type of your choice. And let us see what transdifferentiation is. Transdifferentiation is a very interesting concept in which certain adult stem cell types can differentiate into cell types seen in organs or tissues other than those expected from cells of the predicted lineage. This reported phenomenon is called transdifferentiation.

In a simplistic sense, I can tell you that bone marrow stem cells give rise to all blood-related cells, as we have seen. Now, the bone marrow stem cell, can you make it produce a neuron? Can you make them create a liver cell? Can you make them create a muscle cell? So this is transdifferentiation. Actually, they won't make that. But you can coax them into making it. For example, brain stem cells that differentiate into blood cells or blood-forming cells that differentiate into cardiac muscle cells.

Many such examples can be thought of. So this is the whole concept of transdifferentiation. So, what is the role of transdifferentiation in tissue engineering? This is something that we have to look into. Adult stem cells are clonogenic, self-renewing, and pluripotent cells with the plasticity to differentiate into specific types of tissue in which they reside and often to transdifferentiate into different types of tissues. This property is being used in tissue engineering.

In this sense, it is very easy to collect bone marrow stem cells from a patient. If the patient does not have any genetic issues, you can happily collect the bone marrow cells. Once you collect it, you can program the environment of that cell. You don't need to change the genome of anything. The living conditions of that cell can be changed, and you can now get any cell of your choice.

If you want a kidney cell, you can make it. If you want heart cells, you can make heart cells like that. So which is one of the easiest, least cumbersome, and most cost-effective methods? So, transdifferentiation is also known as metaplasia. In cancer, you may have heard of this as a disease association. Metaplasia basically means standing far away.

That is what metastasis is. Stasis means standing still. Meta means "other place." That is the meaning of it. Normally, if someone has cancer in the liver, this liver cancer cell can go and form a colony in the brain.

And that is usually what we refer to as metastasis or metastasis. a cancer's progression. This transdifferentiation is somewhat related, but it is called metaplasia. So here, what refers to is converting one type of cell to another. It has nothing to do with any cancer formation or something.

Metaplasia basically means you're converting one cell's property into another. How does this happen? An existing differentiated cell can also be converted into another differentiated cell. Not by directly jumping. It will go back one step and from that step it will give rise to another cell type. So a muscle cell went back to its progenitor.

Now instead of becoming a muscle cell, it is now becoming a liver cell. Something like that. In tissue engineering, transdifferentiation induces stem cells to differentiate into the specific cell types needed for tissue repair or regeneration. The sole purpose of using transdifferentiation in tissue engineering is that it can induce stem cells to differentiate not into their natural course of differentiation but into a different course of differentiation; for example, MSCs, mesenchymal stem cells, can be induced to differentiate into bone, cartilage, or muscle cells for tissue engineering applications. So if you have MSCs present from an organism, I can make, oh, I need a bone, I need a muscle cell, so I can make them as I like.

So let us see some examples of transdifferentiation in tissue engineering. Bone tissue engineering MSCs can be induced to differentiate into osteoblasts, which are bone-forming cells, or used for bone regeneration. Cartilage tissue engineering using MSCs can induce them to differentiate into chondrocytes. That is cartilage cells for cartilage repair. Like your ear pinna is cartilage, your nose and many parts of your body have cartilage that is still there, and you can use cartilage synthesis using MSCs.

Muscle tissue engineering. MSCs can be induced to differentiate into myoblasts, that is, muscle-forming cells for muscle regeneration. Skin tissue engineering. Skin stem cells can be induced to differentiate into keratinocytes for skin regeneration. So, the list continues. Don't think that there are only four types of cells that can be produced.

Some examples are mentioned here. And let us understand what the growth and isolation of stem cells are and test to confirm the presence of stem cells. So what are the ways in which people approach the transdifferentiation potential of adult stem cells, and their capacity for tissue renewal and damage repair has attracted too much attention from biotechnologists and clinicians, because transdifferentiation, like I told you, collecting bone marrow cells is very, very easy and there are plenty of stem cells out there. Of course, you can get a stem cell from the skin, or you can get one from the intestine. You can collect it wherever you want. But bone marrow and stem cell collection, we have a lot of bone marrow throughout our bodies.

So tissue is not in short supply. So you can easily collect it. People even do donate like someone who has leukemia; you can take bone marrow from the sibling or the genotypically closer individual, and you can even donate to a recipient. So, it is very, very less problematic. Stem cell isolation and in vitro maintenance are important for the applied application of this technology. for implementation in an applied biology field.

Take the stem cells and you should be able to culture them without losing their properties. And how do you isolate a stem cell? When you collect bone marrow, you cannot use one needle to take the cells and say, "Okay, now I have collected all the stem cells." It will be a mixture of cells. So you should be able to isolate the stem cells from that. Flow cytometric separations of stem cells, as well as positive and negative selections using magnetic beads tagged with antibodies targeting specific markers on the surface of stem cells, are now used routinely in many such applications.

So you can have a mixture of, say, I have 100 cells in which only 5 are stem cells. I will be able to identify that 5 by making use of the marker. Alternative approaches to stem cell identification have also been proposed based on the specific behavior of individual stem cells. Every stem cell has got a unique set of markers. And if you want to identify that stem cell from a group of cells, you should go after those markers, which are unique to that particular cell.

One of these approaches exploits stem cell homing characteristics, which means its identity is retained in these homing or marker genes. These and other principles effectively isolate stem cells for research and biological applications. Based on these approaches, we will see one example, though there are multiple methods. The prevalent approach is the isolation of stem cells by flow cytometry; flow cytometry passes a mixture of cells tagged by appropriate fluorochrome-labeled stem cell markers through a laser beam, so the stem cell has a marker, and now you are putting an antibody against that marker, which has a fluorophore.

Antibodies will not bind to every cell. It will bind only to that cell that has this marker expressed. And this marker-bearing cell is nothing but the stem cell that you are hunting for. You have 100 cells or maybe 1,000 cells, of which maybe 10 cells are the stem cells you are looking for, and this antibody goes unbound. Now, this antibody has a fluorophore, which is a fluorescent tag; the cells scatter the fluorescence, which provides information on the cells' morphology and composition of surface proteins.

DNA content and cytoplasmic processes. So you will be able to measure many properties of the cells based on the scattering, fluorescent scattering, which is derived from the fluorophore attached to the antibody that is already bound to the functional marker present on the surface of this stem cell. So the advantage of flow cytometry is its flow speed, which allows for quick processing of a large population of cells. The quick processing of a large population of cells is possible only if you have the flow cytometric approach and adopt it for separating the cells. The cytometric analysis goes one step further and allows cell sorting, which breaks the fluid stream containing the cells into droplets by piezoelectric perturbation.

So this is the technology that is being applied. Cytometric analysis can be further refined to improve it. This also leads to cell sorting, which breaks the fluid stream containing the cells into droplets through piezoelectric perturbation. Slight electric charges will be applied accordingly. It will have a different behavior. It is then possible to detect a selected droplet with precise timing on a charge, and this droplet has a charge that can be detected as it passes through an electric field, allowing the cells to be separated at a given electric charge, with the droplets containing the stem cells and every cell present in a given droplet.

And that droplet has stem cells as well, and these stem cells of interest-bearing droplets are deflected in the electric field, which can be collected, and you can culture them or observe them, etc. So now what do you do for the growth and validation of stem cell presence? Like other mammalian cells, stem cells are grown and maintained at 37 degrees Celsius in humidified cell culture incubators under a 5% CO_2 atmosphere. Like any other cell culture, stem cells also need the same kind of environment; the media requirements vary among individual stem cell types, and it is important not to induce differentiation of the cultured stem cells, as we have seen. A feeder layer, leukemia inhibitory factor, etc.

, must also be included. In the medium, so that they will not differentiate, stem cells are very easy to differentiate. If you like, there is a joke people say: if you take a stem cell petri dish and simply stare at it, it will differentiate. You don't need to do anything; it is that sensitive. It will not die; it is itching; it is waiting to differentiate. You take a petri dish, a stem cell petri dish, keep it on your bench for 10 minutes, and then put it back.

Because your bench is not at 37 degrees, it is at room temperature, enough. They would have become neurons or some other cell types. So it has to be; it is almost like you have been given a soap bubble, and I'm telling you that for the next five hours, the soap bubble should not break. It is sensitive.

Cells will not die, but cells will differentiate. So maintaining the stemness or maintaining stem cells as stem cells is the challenge. But growing, culturing, etc. It is easy if you follow the protocol as it should be. Any insult you provide, it can differentiate. HSCs are obtained from bone marrow aspirates, placental, or umbilical cord blood, and their high growth rate makes them prone to differentiation in culture.

This is a challenge that you should be aware of. Bone marrow-derived stem cells are grown in culture media supplemented with serum, while HSCs usually require a co-culture system. Hematopoietic stem cells require a co-culturing system containing fibroblasts and feeder layers that support growth and differentiation. Normally, it supports growth and differentiation when required. So you have to give, sometimes it may change the serum concentration; sometimes you are turning on certain genes or inducing some growth factors, etc. Differentiation is very much possible, so the change of cytokine conditions in such culture systems affects the differentiation of stem cells.

Any cytokine concentration will affect either concentration or the addition of a new cytokine; any change can. have huge implications in the differentiation of stem cells. The effects of specific culture conditions and differentiation speeds vary among different stem cell populations. It keeps varying. So, bone marrow stromal cells attach to culture dishes and grow slowly for weeks before differentiation.

First, the stem cells should increase in number, and then they will tend to differentiate. Neural stem cells from fetal or adult brain tissue can grow suspended in a culture medium without additional serum supplementation. This depends on neural stem cells. If every stem cell has its own unique properties, unique niche, unique pros and cons, etc. Neural stem cells are like that, and if you isolate them from fetal or adult brain tissue, they can grow in suspended culture medium, which is something unique.

Why it is mentioned again is that normally cells will grow in attachment only, but they can go into suspended culture medium without any specific serum supplement, and then comes the validation of these cells, validation of the presence of stem cells in a culture system with histochemical methods, antigenic markers, and morphogenetic studies. Primarily depends on the surface morphology and immunophenotype of the stem cells of interest. Like when you say bone marrow stem cells, or when you say kidney stem cells, or when you say heart stem cells, or liver stem cells, each of them has the potential to give rise to the respective cell types. However, sometimes, as you saw in the previous slide, there are stem cells and a committed progenitor. So what is the difference between a stem cell and a committed progenitor? The difference will be reflected in its surface marker.

So based on the markers, you can identify whether it is a committed progenitor or a core stem cell? So that is why the markers come into the picture. These are used in their validation. For in vitro studies, cytogenetic analysis or RT-PCR, reverse transcription, and quantitative PCR are commonly applied. So let us see the plasticity of adult stem cells. Adult stem cell plasticity and its implications in regenerative medicine.

Cell-based therapy may soon represent a new strategy for treating a wide array of clinical conditions. It is very, very promising. Using adult stem cells instead of human embryonic stem cells for therapy avoids all kinds of ethical problems because you are not damaging any embryos.

No questions will be asked. It has two additional advantages. Advantage A: adult stem cells can be isolated from patients, and this overcomes the problem of immunological rejection because they are from the self. There is no question of a non-self. The advantage is that the risk of tumor formation is greatly reduced compared to the use of embryonic stem cells, because embryonic stem cells, even if they are iPSC cells, are tweaked. But when you take a bone marrow stem cell, when you take your own adult cell, it is from you.

It is normally present in you. There is no great modification happening. While pluripotency and plasticity are considered properties of early embryonic stem cells, adult stem cells are traditionally thought to be restricted in their differentiation potential to the progeny of the tissue in which they reside. Means they are already tamed, properly tamed cells. When parts of an organ or tissue are transplanted to a new site, the transplanted tissue maintains its original character. So this is one good advantage of this transdifferentiation potential when you use it. Similarly, when dissociated cells from an organ or tissue are cultured, they also tend to maintain their original phenotype despite losing some differentiation properties.

They do not normally acquire differentiated characteristics of a different cell lineage. They can be forced to acquire different cell characteristics. Normally, it will retain its normalcy. So this is a diagram that illustrates the plasticity of bone marrow-derived cells. Like you have bone marrow-derived cells, and this arrow indicates fusion; the second indicates true transdifferentiation.

Fusion means you are taking the stem cell and putting it in an environment, expecting the stem cells to become that tissue. It's like you are an Indian, and I put you in Rome, expecting you to become like Romans. Or another option is while in India itself, I train you how to behave and work like Romans and just put you. So that is transdifferentiation. So normally, the hematopoietic stem cells and mesenchymal stromal cells are vulnerable to both.

Vulnerable in the sense that they are amenable to both. That is, they can be fused, transdifferentiated, and become skeletal myocytes, cardiomyocytes, pancreatic beta cells, and hepatocytes. Neural and glial cells mean that, depending on the need or the decision made by the researcher or the clinicians, you can either dump these cells into the tissue, such as hematopoietic stem cells or mesenchymal stromal cells, put them into a tissue and expect these cells to become that, or convert them into liver cells or cardiomyocytes in a petri dish and then place them into the respective tissues. So this is a diagram illustrating the different mechanisms of cell fate switching in adult stem cells. So in this diagram, you can see how the starting material becomes terminally differentiated. Differentiated cells become terminally differentiated, which can occur through either transdifferentiation, dedifferentiation, or cell fusion.

So you have one cell; this is a stem cell, and it became, say, if I name this A, it became B. B is terminally differentiated, but that is done via transdifferentiation. But then there is the same cell. It can have an intermediate progenitor cell, and it gives rise to cell A and cell B, two different cell types.

And this is called de-differentiation. And this happens in the retina. The mullerglia de-differentiate and then give rise to the other neurons. It's not transdifferentiation. So here is what happened: this existing stem cell now fused with another cell, which means a cytoplasmic mixing occurred, and you end up getting a heterokaryon. This heterokaryon now gives rise to the nuclear mixing, and resolution happens, resulting in a stable heterokaryon polyploid, leading to cell fusion and ultimately a unique fate.

So these are all the different mechanisms of fate switching. All are trans-differentiation only. In the net outcome, there is only transdifferentiation. Only the roots are different. So, what are the challenges and future directions? Having said that, it's a very promising approach.

Trans differentiation is excellent, etc. Some troubles, like any other system, it also learns. Ensuring transdifferentiated cells' long-term stability and functionality is a major challenge in tissue engineering. Okay, I put the bone marrow stem cells into the liver, and the liver is fine. Will it stay like that forever, or will it start showing its original color? This is one question. Factors like epigenetic memory and incomplete conversion can affect the stability and function of transdifferentiated cells.

Say I took my bone marrow cells and put it into my brain. It made a neuron and it was all fine. Now will it switch back into bone marrow or some other cell types? Because it started to memorize its old lineage. This is one problem. Further research is needed to understand these factors and develop strategies to improve transdifferentiated cells' long-term stability and functionality in tissue engineering applications.

So the promise is there, but time testing cannot be done overnight. Time tests only when you see, "Oh, it stayed for several decades," then we can get the true benefit out of it. So these are all the points one has to keep in mind while considering transdifferentiation as a stable option for fixing damaged tissue. So we will learn more about regenerative biology in the next class. Thank you.