

**Regeneration Biology**  
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**Lecture: 44**

W9L44\_Embryonic stem cells, cord blood stem cells and adult stem cells

Hello everyone, welcome back to another class on regenerative biology. In today's class, we will learn about embryonic stem cells, how they are used, and the different types of stem cells that are present in the human body. And how can we use them for our own benefit and for regeneration in biology, etc.? So that is the overall theme of today's class. In that, we need to understand first what a stem cell is and what identifies a stem cell from a different cell. It is basically a cell that has the ability to continuously divide and differentiate.

Differentiate basically means developing into a given cell type or into different cell types, which can eventually become a tissue. A group of cells performing a function is called tissue; a group of cells performing a task is also called tissue. And how it can become a different cell type. In this picture, there is a stem cell, and a stem cell must have one feature to make a copy of itself by proliferation, which we refer to as self-renewal.

Normally, self-renewal is a way of sustenance and propagation of the stem cell. Without this property, a stem cell, even if it is present, is not doing justice or fulfilling its adequate role in maintaining a given tissue, and its contribution to the sustenance of a tissue is minimal. And eventually it will become nil if the stem cell population, no matter which stem cell we are discussing, is not self-renewed and then the stem cell must differentiate. Not that a stem cell is constantly renewing and doing anything. It is not going to help.

It should have the ability to differentiate. into any cell provided that it is a perfect stem cell. There are different types. The perfect stem cell I am referring to here is a totipotent cell, which is often later referred to as a pluripotent cell, also with a limited capacity. We will see them one by one.

So stem cells normally give rise to daughter stem cells, and this is self-maintenance. And then a specialized cell, such as a muscle cell, nerve cell, kidney cell, etc. It can differentiate. And this is the process of getting specialized. So a stem cell has varying potential when it comes to its differentiation, and one cell type is called totipotent.

Toti is a representation of total. So totipotent cells can give rise to a complete individual,

as they are all the cells in the body. For example, cells from an early embryo are often referred to as totipotent, meaning they are able to give rise to an entire organism. That is how you end up getting monozygotic twins; monozygotic twins are formed in a womb simply because of this zygote. During division, it got separated, so it was a zygote cell, which was a totipotent cell.

It got separated into two pieces, and these two cells now develop into an embryo. If instead of two, it is split into four pieces, you end up getting four identical twins, so this is what you should understand about the concept of totipotency. When you are referring to potency, it's basically the measure of how many types of specialized cells a stem cell can make. So "toti" means total. Any cell present in an organism should be able to make it so that the entire organism comes out of it; pluripotent means one step below totipotency and can make all types of specialized cells in the body but not a total organism.

For example, if you put a totipotent cell in a womb, it will give rise to a functional, full-fledged organism, but if you put a pluripotent cell... In the uterus or in the womb of a female, you will find a group of tissues that may be teratomas or a group of tissues that means they are a combination of cell types that are formed and form a mass of tissue; every tissue is formed, but it is not an organism. Then come the multipotent and pluripotent cells; examples of pluripotent cells are embryonic stem cells, which are normally pluripotent and are taken from the inner cell mass of the blastula.

When it comes to multipotent cells, they can make multiple types of specialized cells but not all cell types, for example. Tissue stem cells, such as bone marrow stem cells, etc. They are multipotent. Tissue stem cells can give rise to, say, a given tissue that has five types of cells. So the stem cells of that tissue can give rise to all five of these.

But it cannot give rise to another tissue type. Say that if I have liver stem cells, they can give rise to different types of cells present in the liver. If I have a skin stem cell, it can make different cell types present in my skin, but it cannot give rise to blood cells. It cannot give rise to neurons. It cannot give rise to a nephron.

So this is what you should understand about multipotent cells. When it comes to unipotent stem cells, they can produce only one cell type. But they have the property of self-renewal like any other stem cell, which distinguishes them from non-stem cells, because non-stem cells, once they exit the cell cycle, do not normally divide. Fibroblasts are an exception; fibroblasts divide, but they are not considered stem cells because they cannot differentiate into other cell types. Muscle stem cells, for example, cannot give rise to any other cell type; they can give rise only to muscle, so they are examples of

unipotent

cells.

So what are the important characteristics of stem cells? One we already discussed is self-renewal. They are unspecialized cells because they are not committed to a function. Stem cells are not committed to performing a given function. They are unspecialized cells capable of renewing themselves through cell division, like any other cell or fibroblast. Sometimes, after long periods of inactivity, stem cell multiplication is demand-based.

Say I have 100 stem cells present in a given area, but none of them are needed because no other tissue types are dying. Like muscle stem cells, one example is that until your muscle is damaged, what is the use of that stem cell? Your muscles can stay for a long time. And if it is damaged, then say 10 muscles have been damaged. Now I need to have 10 new stem cells come into the picture. Then I have 100 cells in stock.

Then, if I take 10 cells from that, the stock will become 90, and you don't want that to happen. So 10 cells will divide and it becomes 20, and it becomes 110 cells now. 190 cells kept quiet. So, 10 cells divided, and it became 110 cells; those 10 will become muscle cells, and the stem cell population returned to 100. So this is the principle on which they maintain.

In a simplistic sense, you can tell that you are taking one glass of milk from a pot and pouring one glass back. Normally, people pour one glass of water into it. But in any case, the milk level will remain the same. It will be diluted. But here, there is no concept of dilution.

It will remain because a properly functioning stem cell is kept. So why I gave this example is that sometimes stem cells won't divide just for the sake of it. It will divide if there is a need for it. By regeneration, they can be induced to become tissue or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn-out damaged tissues.

In other organs, however, such as the pancreas and heart, stem cells only divide under special conditions. Like I told you, in some tissues, tissue erosion is a given, said intestinal stem cells. We cannot imagine that, okay, my intestinal stem cell will stay for a long time. No, because constant wear and tear is happening. Same as your skin cells, whether you walk, run, or do nothing, your skin cells are vulnerable to erosion because they're exposed to the outside.

So it will erode, and the stem cells will replace it. Intestinal is also the same thing. But in some cells, such as the pancreas and heart, etc. The stem cells come into the picture only

if there is damage. Now let us see what the types of stem cells are.

One categorization we have seen is totipotent, pluripotent, multipotent, and unipotent. Another categorization is based on where they are located, regardless of which category they belong to. Embryonic stem cells come from 5 to 6-day-old embryos, which are basically pluripotent. They have the ability to form virtually any type of cell found in the human body, but not a full-fledged human. For that, you need a totipotent cell.

An embryonic germ cell, another group of stem cells, is derived from a part of the human embryo or fetus that will ultimately produce eggs or sperm, which are gametes. They are called gametogenic or germ cells, or germ stem cells, or embryonic germ cells, which basically means they are present in an organism but give rise only to eggs or sperm; that is the purpose of these embryonic germ cells. Then come adult stem cells. They are undifferentiated cells found among specialized or differentiated cells in a tissue or organ after birth. For example, muscle cells have stem cells; your retina has progenitor cells; your skin has stem cells; your liver has some stem cells; and your intestine has stem cells.

So they appear to have a more restricted ability to produce different cell types and self-renew. It doesn't mean that whenever damage happens to that tissue, the stem cells are capable of fixing that damaged area; not always, but there is a day-to-day manner in which some cell death happens in a given tissue that will be taken care of by the stem cells. However, if there is a massive injury or a serious wound, then those adult stem cells are not powerful enough to fix that damage; they are meant only for running day-to-day affairs and are not meant for fixing damage. This scenario is similar in other animals that regenerate efficiently, such as zebrafish. Zebrafish also have adult stem cells, but they are not capable of repairing serious damage to that organ.

That is why differentiated cells present in that tissue have to be reprogrammed to create a large number of stem cells so that they can be repaired; adult stem cells are not able to manage the process. And then comes the induced pluripotent stem cell, called iPS cells, which are, as we discussed in the previous class, also Yamanaka factors. You take a fibroblast cell; they are adult cells of the body that can be reprogrammed to attain or acquire pluripotency. Based on the source, we have classified these types of stem cells according to their origin. So this is an egg, this is a sperm, and you end up making a zygote that divides into two, four, and more.

In the last stage, you end up getting a blastula, and the cells inside are called the inner cell mass. The inner cell mass of the blastula is called embryonic stem cells, and that can... give rest; the blastocyst, a very early stage of the embryo, is called the blastocyst,

and then it has an outer layer of cells called trophoctoderm.

Trophoctoderm will not become the animal, whereas the inner cell mass, which is called the blastocyst, is the one that gives rise to the animal. Then there is a full-fledged animal that grows into an adult animal; in this case, it is. Human fetal cells and throughout life, these stem cells continue to be present in different parts of the body as adult stem cells. Not every differentiated tissue has adult stem cells. Now let us understand a little bit more about embryonic stem cells and where we find them.

One, we already know they are the inner cell mass of the blastocyst, and they can be taken and dissociated into individual isolated cells. They can be taken from the inner cell mass and placed onto a feeder layer that provides nutrition, and they will grow in culture. You can differentiate them into all possible cell types or specialize the cells. Embryonic stem cells are pluripotent in nature and are derived from the blastula stage, or they can be made from induced pluripotency by transfecting Yamanaka factors. These cultured cells, when allowed to differentiate, can give rise to unipotent stem cells.

Not all stem cells always differentiate into pluripotent cells, nor do embryonic stem cells always differentiate into tissue. Sometimes they can differentiate into another tissue-specific stem cell, which also means it is less capable of giving rise to all cell types. So it is moved away from pluripotency. But it is not differentiated either. So it is what you should understand that the circulatory system cells, nervous system cells, and immune system cells are a group of cells that can be seen in the early embryos as well.

They are capable of differentiating into a fully committed or fully differentiated cell, but they can also have an intermediate stage. So if you look at more details about the blastocyst or the embryonic stem cell source, the blastocyst is a structure formed in the early development of mammals, and it is the inner cell mass. In short, it is called ICM. which subsequently forms the embryo. The outer layer of the blastocyst consists of cells collectively called trophoblasts.

This layer surrounds the inner cell mass and the fluid-filled cavity known as the blastocele. The trophoblasts give rise to the placenta that attaches to the uterine wall. In humans, blastocyst formation begins around 5 days after fertilization. When a fluid-filled cavity opens up in the morula, a ball consisting of a few dozen cells is formed, and that is what is used for blastocyst formation, which occurs around five days post-fertilization.

The blastocyst has a diameter of 0.1 to 0.2 millimeters. One millimeter, how small do we know? It is around 1/10 to 1/5 of a millimeter. It compresses around 200 to 300 cells following rapid cleavage; that is, the cell division. After about one day, the blastocyst

embeds itself into the endometrium, which is the uterine blood vasculature present in the uterine wall. Of the uterine wall, where it will undergo later developmental processes including gastrulation, the blastula matures into a gastrula where three germ layers are formed.

The inner cell mass of the blastocyst is a source of embryonic stem cells usually used in culture. If you look further, the characteristics of self-renewal are an undifferentiated state for a long period during which it can stay, and the maintenance of stemness, the so-called stemness or pluripotency. Mark pluripotency has to be maintained, and this is a characteristic feature of the stem cell. It also has the potential to form a teratoma when induced in SCID mice or severe combined immunodeficient mice, which can cause the pluripotent cell to differentiate randomly and become a mass of tissue, a meatball, which contains all the tissues present in the human body. Maintenance of a normal karyotype is a necessary feature.

That is the number of chromosomes and clonality, the ability to make an identical copy of itself. Stem cell marker expression, such as Nanog or Oct4, has to be constantly maintained in them. Otherwise, they can lose their stemness or pluripotency in clinical research. A lot of myocardium diseases exist; myocardium is the muscle of the heart. Regeneration of damaged heart muscle by injecting human embryonic stem cell-derived cardiomyocytes directly into the site of infarct is a published paper you can read.

This citation also applies to lung disease, specifically alveolar type 2 epithelial cell-derived treatments. From human embryonic stem cells and in a nude mice model that is immunocompromised, a model of acute lung injury is also possible, and oligodendrocyte progenitor cells derived from human embryonic stem cells can improve functional locomotor behavior after cell implantation at the damaged site. So, seven days after injury in the animal model, these are all the different research studies that have been done using human embryonic stem cell culture in the laboratory. Human embryonic stem cells are generated by transferring cells from the pre-implantation stage of the embryo into a plastic laboratory culture dish that contains the culture medium and the feeder layer. The inner surface of the culture dish is coated with mouse embryonic skin cells and specially treated so that they do not divide.

They will be alive, but they will not divide. This coating layer of cells is normally referred to as the feeder layer. And cells divide and spread over the surface of the dish. Some of you may be thinking about why a feeder layer is needed, because for stem cells to grow, they need lots of growth factors. So when you feed them into this feeder layer and inoculate them into the feeder layer, this feeder layer will provide the nutrients, growth factors, etc. that will allow the stem cells to be stem cells and maintain

themselves as stem cells.

So the plated cells divide, multiply, and crowd the dish. Then they are removed gently and plated onto several fresh culture dishes. This process of replating and subculturing the cells is often referred to as passage. So that is cell passage. It is often referred to as. Once the cell line is established, the original cells yield millions of embryonic stem cells.

Embryonic stem cells that have proliferated in culture for a prolonged period of time without differentiating and are still pluripotent are often referred to as embryonic stem cell lines. It's a cell line. The importance of the feeder layer. The mouse cells are at the bottom of the culture, which provides the cells with a sticky surface to which they can attach.

The feeder cells also release nutrients into the culture medium. Disadvantages of the feeder layer. There is always a risk of viruses and other macromolecules in the mouse cells that may be transmitted into human cells, and in the future, it can get into humans. That is a challenge. So the differentiation of embryonic stem cells, blastocysts, fertilization, and inner cell mass is collected, plated, and they are self-renewing, and you can make them into ectoderm cells, mesoderm cells, endoderm cells, germline cells, etc.

You can differentiate between them. So, three germ layers can be formed easily: ectoderm-derived neurons, mesoderm-derived blood cells, and endoderm-derived liver cells. So the ectoderm gives rise to a lot of tissues that include the spinal cord, nerve cells, hair, skin, teeth, sensory cells, eyes, ears, and nose. To fix all these troubles from a regeneration point of view, you can use stem cells that are initially pushed into the ectoderm. Some of them are mesoderm-derived cells, including their muscle, blood vessels, connective tissues, and heart, etc. Endoderm-derived cells include the gut, pancreas, stomach, liver, lungs, urinary bladder, and germ cells, etc.

Come under endoderm-derived cells. So, how do we differentiate embryonic stem cells? When removed from the factors that maintain them as stem cells, such as the feeder layer, ES cells will differentiate, and under appropriate conditions, they can generate progeny consisting of derivatives of the three embryonic germ layers, namely mesoderm, ectoderm, and endoderm. So general approaches of differentiation include that ESCs are allowed to aggregate and form three-dimensional colonies known as embryoid bodies, or short form called EBs. ESCs are cultured directly on stromal cells, and differentiation takes place in contact with these cells; another approach. It involves differentiating embryonic stem cells into a monolayer on an extracellular matrix protein. So there are different approaches through which you can help them differentiate.

So for ES differentiation, you have got undifferentiated ES cells. Initiate the differentiation. There is a leukemia inhibitory factor known as LIF, and there are also embryonic feeders. And these are embryoid bodies, and these are the formation of incubating them with the stromal cells and cells differentiated on an extracellular matrix. In all three situations, they can give rise to different types of differentiated cells. So, tissue stem cells, or adult stem cells, this is what we should understand: tissue stem cells, where we can find them, are present in the eye, brain, skin, breast, testicles, intestine, muscles, and bone marrow.

All these tissues have adult stem cells present, and they will be maintaining homeostasis or performing day-to-day functions, so adult stem cells. Adult stem cells have unique characteristics. They are a short form; they are known as ASCs. Adult stem cells have characteristics.

They have the potential to self-renew for a long time. So we should understand that older people have wrinkled skin, and you may notice that wrinkles don't appear randomly, especially around your mouth. The people often refer to them as laughing lines. Some people get foreheads, and some people get wrinkles on the edges. So in some places, you get wrinkles. And if you think logically, they are the places where the skin is constantly put under stress.

When you laugh, a fold will be created. Another thing is that you will get here because whenever you smile, a folding happens on your cheek. So these are all the places where the damage is happening more than the rest of the places. You often don't see wrinkles here. A place where the chances of folding are less, so what happens then is that a skin cell gets damaged more frequently and is replaced by a stem cell. So, what happens with old age is that the damage occurs at the same rate as it did in young age; slowly, your stem cell population in that area, not elsewhere, just beneath that damaged area, is not sufficient if it is a skin stem cell.

It is present wherever there is skin, underneath. So the stem cells are depleted only in that area. It is not depleted here because the cells were not dying here. Hence, I was not replacing. Here, cells were dying more. Hence, I was replacing, but now the stem cell population is not as adequate because of age.

Although they are dividing, they are not dividing as fast as the damage; hence, you see a dent. You see a dip just like you may have seen on the road. People see a gutter, and in that gutter, you put some soil. Immediately, it is sealed; after two or three times some vehicles go over that.

The gutter will show like a ditch. So that kind of ditch that you see is called wrinkled lines. So they have the potential to renew for a long time, but long does not mean that. It's a given. So they can give rise to mature cell types that have characteristics, morphologies, and specialized functions across multiple lineages.

Types of adult stem cells. They are hematopoietic stem cells because your RBCs last only four months. They have to be constantly replaced because they all have a short lifespan, and that's why in old age sometimes your RBC number decreases and WBC number decreases, because the same bone marrow is not producing as fast as it did in your young age: mesenchymal stem cells, other stem cells such as neural stem cells, endothelial stem cells, intestinal stem cells, olfactory adult stem cells, and mammary stem cells. And the sources' locations include bone marrow, umbilical cord, and cord blood. Cord blood is another thing that is present from the embryo to the mother, the connection of cord blood. They have lots of stem cells because the umbilical cord is formed as a remnant of the embryo.

So the cord blood cells have lots of embryonic stem cells present. Adipose tissue, deciduous teeth, brain, peripheral blood, amniotic fluid, amniotic membrane, synovial fluid, synovium, and placenta all have a lot of stem cells, so if you see adult stem cells, this is bone, and inside the bone, there is a bone matrix; bone marrow is present, and there are a lot of different types of cells. RBCs have their own lineage; there is a lymphoid lineage and a myeloid lineage of cells, and they give rise to different types of cell types that have been produced today. You can see. Hematopoietic stem cells give rise to multipotential stem cells that are able to give rise to lymphoid progenitor cells, which give rise to natural killer cells, T lymphocytes, and B lymphocytes.

They are called lymphoid progenitors. And then there is another called a myeloid progenitor. Myeloid progenitors give rise to neutrophils, basophils, eosinophils, and also give rise to platelets and RBCs, as well as monocytes and macrophage cells; they come from the myeloid lineage, and this stromal stem cell present in the bone marrow can give rise to bone or cartilage. It can also give rise to other hematopoietic stem cells and can also give rise to adipocytes, which are fat-storing cells, and some of these. Stem cells can give rise to the skeletal muscle stem cell and hepatocyte stem cell that are coaxing them.

Normally, they don't produce. Can we take these bone marrow cells and force them to make a skeletal muscle stem cell? Can we make them into hepatocyte stem cells? Technically, it is possible through transdifferentiation, and that kind of research helps them fix damaged tissue. So this is a blood stem cell found in the bone marrow. They are multipotent cells. They usually differentiate into specialized types of blood cells. Like I mentioned, myeloid and lymphoid lineages, as well as red blood cells, white blood cells,

and platelets, are usually involved.

But they can be forced to give rise to, as it is mentioned, skeletal muscle and hepatocytes, or various other cell types if you provide a proper atmosphere. So these are all the approaches that are done to make use of stem cells in regenerative medicine. So we should understand that establishing these cells, isolating them, propagating them, and implementing them in the right place is the future of regenerative medicine. We will study other aspects of tissue regeneration in more detail in the next class. Thank you.