Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
Week: 4
Lecture: 17

W4L17_Totipotency, pluripotency, multipotency and unipotency, in regeneration context

Hello, everyone. Welcome back to another session of regenerative biology. So today we will learn about different types of cells, which are broadly classified into totipotent, pluripotent, multipotent, and unipotent. And we will try to understand this nomenclature in a regeneration context and why it is named that way. So, what is a stem cell? And we know that a cell that has the ability to continuously divide and differentiate is capable of developing into various other kinds of cells. And that is what is called a stem.

We know the stem, the concept that is able to anchor some groups of branches, etc. That is the whole word stem from which it emerged. Stem cells can make a copy of themselves, which is self-renewal, and then they can give rise to another cell type through differentiation. So if a given tissue has 100 cells, and 30 of them are deciding to differentiate, then the stock should remain at 100.

How is it possible? This 30 will divide and replenish the 30. So 30 will divide into 60. In that 30, I will stay back in the pool. The remaining 30 will differentiate. This is how it is usually done.

So, normally, the stem cell pool has to be maintained perfectly. So stem cells have the potential for differentiation, and based on that potential, we have classified them. Potency is basically a measure of how many types of specialized cells a stem cell can make. Totipotent cells can give rise to a complete individual, not just a complete list of tissues present in an organism; rather, they can form a complete individual. Say, for example, you have a heart, liver, kidney, muscle, brain, etc.

If I take all these tissues and pile them together, putting them in a bucket, it won't become an organism, right? So, forming an organism is slightly different. And we can even call that, you know, that it is a congregation of all these tissues, giving rise to an organism. So the throaty pattern cell is capable of doing that. These cells are from the very early developmental phase; they have this ability, but after a little while, they become pluripotent, which means they can make all cell types or specialize cells in the

body, but they cannot make an entire organism. Embryonic stem cells are normally referred to as pluripotent cells; they are not totipotent and cannot give rise to an organism.

Multipotent stem cells can make multiple types of specialized cells, but not all types of cells present in an organism. A classic example is a tissue-specific stem cell, such as a bone marrow stem cell; they can give rise to WBCs, RBCs, etc., but they cannot make your skin cells. Unipotent stem cells produce one type of cell, are basically capable of renewing themselves, and distinguish themselves from other cell types. For example, muscle stem cells.

If there is tiny damage to the muscle, muscle stem cells come into the picture and give rise to only muscle, not neurons or some other cell type. So important characteristics of stem cells include self-renewal, meaning they are capable of making copies of themselves to maintain the stock, and regeneration. They can be induced to become tissue- or organ-specific cells with specific functions. In some organs, such as the gut and the bone marrow, stem cells regularly divide to repair and replace worn-out or damaged tissue. We have seen that our intestinal epithelium is always under erosion.

And the intestinal stem cells give rise to the intestinal epithelial cells. In other organs, however, such as the pancreas and heart, stem cells only divide under special conditions, and that too in special organisms. Many animals can lead to scar formation. Types of embryonic stem cells come from five or six-day-old embryos. That is basically an embryonic stem cell.

They have the ability to form virtually any cell type of that organism. Embryonic germ cells, which are derived from the part of the human embryo or the fetus, will ultimately produce eggs or sperm. They are called embryonic germ cells. Adult stem cells are undifferentiated cells found among specialized or differentiated cells in a tissue or organ after birth. We know the liver has stem cells, and we also have skin stem cells, muscle stem cells, and even some retinal progenitor cells.

They appear to have a more restricted ability to produce different cell types, and they are also capable of self-renewing. Induced pluripotent cells are the adult cells of the body that are reprogrammed to show pluripotency. That means you take a skin cell and express some transcription factors; now you convert it into a stem cell, which is an induced pluripotent cell, so you can see in this cartoon. The egg and sperm fertilized into two cells, then four cells, and this is a group of cells at the morula stage. You can see this is the blastula stage, and the inner cell mass of the blastula is basically embryonic stem cells that you can collect from here and then culture in a petri dish.

The outer layers of cells usually become the trophectoderm. The inner cell mass is usually used as embryonic stem cells, and tissue stem cells are present in the embryo, while adult stem cells are present in the body. Adult for the maintenance of a given tissue. Embryonic stem cells, where we find them, are in the inner cell mass of the blastula, and they can be separated and cultured on a plate. You have to provide fluid nutrition, and you should have some leukemia inhibitory factor or a feeder layer at the bottom, which has to provide the growth factors that are needed for maintaining the stem cells as stem cells.

And upon some insult or some differentiation cues, if you provide them, it can give rise to muscle, neurons, skin, etc. Whatever you want, you can differentiate it into all possible types of specialized cells. Embryonic stem cells are pluripotent cells derived from the inner cell mass. And the blastocysts are derived at a particular stage of embryonic development. You cannot go to any stage of embryonic development.

Details about the blastocyst and the embryonic stem cell source are also included. The blastocyst is a structure formed during the early development of mammals. possesses a part called the inner cell mass, or ICM, which subsequently forms the actual embryo of that particular species. The outer layer of the blastocyst consists of cells collectively called trophoblast, and this layer surrounds the inner cell mass, while a fluid-filled cavity, known as the blastocele, is present in the blastula; the trophoblast eventually gives rise to the placenta for an internally developing organism because the uterus and placenta are important for the development of the embryo in humans. The blastocyst formation begins about five days after fertilization when a fluid-filled cavity opens up in the morula.

A ball consisting of a few dozen cells exists in that phase. The blastocyst has a diameter of about 0.1 to 0.2 mm and contains around 200 to 300 cells. Following rapid cleavage, it forms at a rapid pace compared to normal skin or any other cell type.

After about one day, the blastocyst embeds itself into the endometrium. That is the lining of the uterine wall where it undergoes later developmental processes, including gastrulation. And this is supported by the formation of the placenta at a later stage. The inner cell mass of the blastocyst is a source of embryonic stem cells if you want to create them from that particular species. Embryonic stem cells have unique characteristics.

They have self-renewal in an undifferentiated state for a long period and maintain stemness. What does stemness mean? The ability to give rise to any cell type or pluripotent markers. They will express like SOX2, OCT4, KLF, and cMYC; these are all called Thomson factors or Yamanaka factors. They are used to make stem cells from

differentiated cells. So they are hallmarks of embryonic stem cells.

The formation of teratoma is induced in severe combined immunodeficiency mice called SCID mice. So, what is a teratoma? Any cell you implant into an immunocompromised mouse in the uterine environment, that cell is basically a cancer that will have all the tissues that are formed, which include teeth, nails, skin, bone, etc. But it's not an organism. Why? Because it is not formed from a zygote. It is formed from a malformed cell in the uterine walls.

So, that is called a teratoma. And this is done in skid mice mainly because you don't want your immune system to get rid of the unwanted tumor. Normally, a malformed embryo is taken care of. Taken care of means expulsion by the mother's immune system. So if that has to be compromised, then a teratoma can develop. Maintenance of the normal karyotype, which means the number of chromosomes (46 chromosomes, or 23 pairs), should be preserved, as it is due to division that we cannot change.

and clonality. Clonality means the ability to make an exact copy of themselves. So stem cell marker expressions, NaNOG and Oct4, don't think these are the two markers. There are many markers, but Oct4, NANOG, and SOX2 are all seen as markers of stem cells. So clinical research has huge implications in clinical research. Myocardium disease, which is the regeneration of the damaged heart muscle by injecting human embryonic stem cell-derived cardiomyocytes directly into the site of infarction, can have some beneficial traits that develop because of the stem cell delivery.

Lung disease involves alveolar type 2 epithelial cells. Derived from human embryonic stem cells and in a nude mice model, nude mice do not have immunity, which means they do not reject foreign cells. This is called a nude mice model of acute lung injury. The method has also been published. Oligodendrocyte progenitor cells derived from human embryonic stem cells can improve functional locomotor behavior after cell implantation at the damaged site seven days after the injury, basically in the animal model, so there are a lot of implications.

For this stem cell research. Human embryonic stem cells are generated by transferring stem cells from the pre-implantation stage embryo. Preimplantation means before it gets attached to the uterine wall or endometrium. into the plastic laboratory culture medium and dish that contain a sufficient quality of culture medium. The inner surface of the culture dish is coated with mouse embryonic skin cells, specifically treated so that they do not divide.

They will simply survive. They produce the nutrients or secret growth factors for the

stem cells to thrive. This coating layer of cells is called the feeder layer. On top of that, you can grow stem cells. Cells divide and spread over the surface of the dish, and the plated cells divide and multiply, crowding the dish. Then, they are removed gently and plated into several fresh culture dishes, which also have this feeder layer.

This process of replating or subculturing the cells is often referred to as passage, not passage passage; that is what it is referred to as. You can keep going. And once the cell lines are established, the original cells yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in a culture for a prolonged period of time without differentiating are being studied. And the pluripotent cells are often referred to as embryonic stem cell lines.

When you can go into different passages, we often refer to them as human embryonic stem cell lines. The importance of a feeder layer. The mouse cells at the bottom of the culture dish provide the cells with a sticky surface to which they can attach. Also, the feeder layers release nutrients into the culture medium, which are beneficial for the embryonic stem cells.

Disadvantages of the feeder layer. What are the disadvantages? There is always a risk that viruses or other macromolecules in the mouse cells may be transmitted into the human cells because even though they are not dividing, they are vulnerable to releasing some unwanted biohazard into the stem cell. So if you are using it for human treatment, one should be wary about that. Differentiation of embryonic stem cells. So fertilization leads to the embryo, which is a group of cells known as the inner cell mass. Collect it, culture it, and keep passaging it, and you can give rise to ectoderm, brain skin, mesoderm, muscle, blood, endoderm, lung, gut, and germline, sperm, egg.

You can make anything. Just your imagination is enough to make anything happen. And three germ layers can give rise to the three germ layers, which is one example of a cell type derived from each layer: ectoderm-derived, mesoderm-derived, endoderm-derived. We have three main germ layers, and they can give rise to each and every cell type that is present in our body. Differentiation of the embryonic stem cell, when removed from the factors that maintain it as a stem cell, occurs when those factors that help it maintain its stemness are removed; it will differentiate. Under appropriate conditions, any insult is enough: just take the petri dish and keep it outside for some time; a slight increase or decrease in temperature is good enough.

Any insult given to the embryonic stem cell will cause it to differentiate. Generate progeny consisting of derivatives from the three germ layers, namely mesoderm, endoderm, and ectoderm, which can be formed from the stem cell if you stop producing

the factors or feeder layer factors that allow the stem cells to remain stem cells. General approaches to differentiation. ES cells, which are embryonic stem cells, are allowed to aggregate and form three-dimensional colonies known as embryoid bodies. Embryoid bodies are a unique technique used in some stem cell culture fields because they can be fused into another developing embryo, allowing the creation of chimeric mice or chimeric animals, which are called EB's.

Embryonic stem (ES) cells are cultured directly on stromal cells, and differentiation takes place in contact with these cells, involving the differentiation of ES cells into a monolayer or extracellular matrix protein, as they need to have some attachment; without that, they can't grow in suspended culture like bacteria. And yes, embryonic stem cell differentiation. So, you can have different methods of differentiation there. The initiation of differentiation involves getting rid of the feeder layer and also eliminating the leukemia inhibitory factor, LIF, which is a protein that allows stem cells to continue to be in a stem cell state. So feeder cells also do more or less the same function.

So, you remove the LIF and feeder layer. They are now ready for differentiation. Here, cells differentiate into embryoid bodies; they look like this: spherical, and cells differentiate into stromal cells using another method. Cells differentiate into extracellular matrix proteins, and all cells eventually give rise to differentiated cells, which we can analyze. And tissue stem cells or adult stem cells.

We have seen it. Every tissue has stem cells that are for maintaining that tissue or organ. So we can see where all the tissue stem cells are. Where do we find them? The surface of the eye has structures such as the cornea and conjunctiva.

We have brain stem cells. We have skin stem cells. We have breast stem cells. We have testicles because the germs you have produce a stem cell. The intestine has intestinal stem cells. Bone marrow has bone marrow stem cells, and muscles have stem cells.

So these are all running in the steady state of air. They are not meant just for making a new set of muscle, but for running the homeostasis of that organ. Adult stem cells, in short form, are called ASCs. And they have certain characteristics. They have the potential to self-renew for a long period of time. They can give rise to mature cell types that have characteristic morphologies and specialized functions along multiple lineages.

So they are quite unique in that respect. They will continue to give rise to that cell type, which it is supposed to make throughout the lifespan of that organism. Types of ASCs. Hematopoietic stem cells, mesenchymal stem cells, and other stem cells such as neural stem cells, endothelial stem cells, intestinal stem cells, olfactory stem cells, adult stem

cells, and mammary stem cells. Even your hair follicles have stem cells so that they can give rise to new hair strands.

Sources. They are bone marrow, umbilical cord, cord blood, adipose tissue, a lot of tissues, deciduous teeth, brain, and peripheral blood. They all have a good source of stem cells, and whether we can harness them and give rise to that tissue is limited by the availability of suitable techniques. Adult stem cells, you can see like this.

This is the bone. Bone has bone marrow stem cells. And it can give rise to the hematopoietic stem cells and multipotent stem cells. And it gives rise to either myeloid lineage or lymphoid lineage. The lymphoid lineage gives rise to a bunch of cells. That includes natural killer cells, T lymphocytes, B lymphocytes, etc. And the myeloid lineage gives rise to neutrophils, basophils, eosinophils, monocytes, macrophages, platelets, and RBCs.

And on the other hand, if you have a stromal stem cell from the bone marrow, it can give rise to lining cells and also osteocytes. And it can give rise to dedicated cell types, as you can see here: hematopoietic cells and marrow adipocytes, etc. Because the bone marrow is not just for producing blood alone. It has adipose tissue, it has calcium-producing cells, etc. Blood stem cells are a classic example of multipotentiality because they can give rise to a group of cell types; they undergo differentiation, and you can see here that only specialized types of blood cells—red blood cells, white blood cells, and platelets—are usually kept in circulation, but their original source is the bone marrow stem cell and types of adult stem cells and their hierarchies.

The principles of renewing tissues. So, what is the principle? How do we decide? Oh, it is time to renew, or it is time to make it. It is purely demand-based. Say you donated blood to someone. They took 300 mL of blood from you.

So there is this, this is basically nothing but an injury. Injury means that although there is no physical injury, there is a depletion. In every injury, say that your liver got damaged. What is happening? Tissue loss.

Your leg was hurt. So basically there is tissue damage. So the removal of blood from your body is nothing but the depletion of tissue. Immediately, it will be sensed by your bone marrow cells, and they will start overproducing. The rate of synthesis will increase. In the same way, females who have this menstrual cycle lose blood from their bodies, although it is not a large quantity. But again, it will immediately start producing more blood in their body just to balance out the lost blood during their menstrual cycle.

So the stem cells have to self-renew, divide rarely, basically based on demand, and have a high potency rate. High potency rate means they can divide efficiently and completely with the adequate number. It cannot happen if there are 100 stem cells or if only 30 are dividing and the remaining 70 are just lethargic.

They may or may not divide. It doesn't happen. They are highly potent. They are capable of dividing. In the earlier example, I told you 30 cells because you only need 30; if only 30 cells are damaged, you don't need 100 stem cells to divide. Now, only 30 cells will divide, make a copy of themselves, and then the remaining newly formed 30 will give rise to the differentiated cells. And the committed progenitors are the ones that are transiently amplifying cells. They are multipotent in nature, and they divide rapidly because the actual stem cells make a copy if needed.

But once they made a copy, that is when the committed progenitors are made. Now they have to divide rapidly. Like when someone removes blood through blood donation, you have a depletion of cells. Your bone marrow cells give rise to some of the committed cells, which are capable of giving rise to WBCs and RBCs. Once it has been given rise, they will divide quickly.

They won't say, "Okay, I will divide it in the next 10 days." No. Within two to three days, the lost blood is restored, and the cells divide rapidly. They do not have the ability to self-renew because they must come from stem cells, and committed progenitors are meant only for dividing as progenitors; they continue to differentiate. They will not make a copy of themselves and keep it. If 100 committed progenitors are formed, they will become 200.

But of the 200, the entire 200 gives rise to the differentiated cell type. They will not keep a 100 copies like a stem cell is doing. So this is a difference that you should keep in mind. And then they give rise to specialized cells. That is, they are meant for work, and many of them will not divide at all.

And hematopoietic stem cells, if you see here, this is exactly what happens. They have different lineages. Major lineages are the lymphoid and myeloid lineages. You can see here in one group, they give rise to NK cells, T cells, and B cells. And another group gives rise to dendritic cells and then megakaryocytes, platelets, erythrocytes, macrophages, neutrophils, eosinophils, and basophils.

And this group is called committed progenitors. And bone marrow stem cells are the actual stem cells; as you can see here, this is the real stem cell, and these committed progenitors you are seeing here are committed progenitors, and they can expand in

number. Usually, if you have taken a vaccination and if it is staying in the memory, then... If you encounter that pathogen again, there will be a sudden surge of committed progenitors that will kick-start the process so that they can give rise to the specific cell type needed for producing the antibody against that pathogen, which is normally the B cell that produces the antibody.

And as you can see here, these are specialized cells that are not going to divide. They are going to perform their duties. Committed progenitors can divide, and they won't make a copy of themselves. Of course, they will make a copy of themselves, but they will not. Store them; they are meant for differentiating, and then comes the mesenchymal stem cell, which is also present in the bone marrow. They also have a group of committed progenitors that specialize in giving rise to the cells needed for the maintenance of the bone.

And it also needs to maintain the cartilage that is called chondrocytes. And then the fat-storing adipocytes. So the mesenchymal stem cells of the bone marrow give rise mainly to bone, cartilage, and fat-producing cells. So these are all the main jobs of mesenchymal stem cells. Hematopoietic stem cells are the ones that give rise to this bone marrow. Bone marrow hematopoietic stem cells give rise to blood cells, which are meant for both oxygen transport and immunity.

Then comes the neural stem cell, which is present in the brain, abbreviated as NSC. And they also have a phase of committed progenitor phase. And then they can give rise to neurons, interneurons, oligodendrocytes, type 2 astrocytes, and type 1 astrocytes.

All of these are specialized cells. And we know... Neurons do not divide, so oligodendrocytes, which are all glial cells, of course, the peripheral nervous system has Schwann cells, but we are talking about brain stem cells; they are called neural stem cells. Hence, oligodendrocytes are there; they are not neurons; they are glia, whereas neurons and interneurons, which are usually found in the spinal cord. The neurons are present both in the brain and spinal cord, and outside of the brain and spinal cord, we usually refer to them as the peripheral nervous system; they are not part of the central nervous system. Oligodendrocytes are seen only in the central nervous system, but they are not neurons; they are glia. What you should understand is that neural stem cells are capable of giving rise to both neurons and glia, which are terminally differentiated, as well as astrocytes, which are also a glial cell population.

And then you can see the gut stem cell; its short form is called GSC, and gut stem cells are mainly present in the small intestine because that is one organ that has maximum wear and tear. Wnt signaling comes into the picture; Wnt signaling maintains it and is

strictly controlled as well; otherwise, you can have over-proliferation. Proliferation is badly needed, but you don't want over-proliferation. Intestinal stem cells are very strictly controlled stem cells as well as a group of proliferating cells. So here you have committed progenitors, and they give rise to paneth cells because every cell present in the intestinal epithelium, the gut stem cells, should be able to supply paneth cells and goblet cells.

Endocrine cells are columnar cells, which are mainly specialized cells; each cell has its own function, like endocrine cells that produce lots of hormones, and many intestinal cells that produce serotonin, while goblet cells produce mucus. Here, they have dedicated biological functions. Now comes induced pluripotent cells, a very exciting topic that has fetched a Nobel Prize. Yamanaka received the Nobel Prize for the discovery of it, and the question was very simple.

Can I make a stem cell on demand? I have a cell. I'm ready to share my skin or my buccal swab, and I'm ready to donate. Anyway, it doesn't cost me anything. Just swab your buccal cavity.

You get it yourself. Can you make that into stem cells? So, this was a very interesting question. They have done extensive research. They narrowed down from 256 to four factors.

Just four factors are enough. And Oct4, SOX2, KLF, and MYC. So, these are all the Yamanaka factors. So what you can see here is an induced pluripotent stem cell. What they do is take a cell from the body, preferably fibroblasts, which are easily available. Genetic reprogramming is the process of making the cell go all the way back to the embryonic stage. And add a certain gene to that particular cell, and you end up getting an induced pluripotent cell that behaves like an embryonic stem cell, which can be cultured just like the use of either layer or leaf, etc., and that has been trained to differentiate into any cell type—neuron, muscle, or skin, anything you name, you can give rise to.

Induced pluripotent stem cells, or IPCs, are cultured cells from the body. You collect them, and then you perform genetic reprogramming through transfection. Like I told you, OCT4, SOX2, KLF, and MYC, usually KLF4. And make these express in the cell by transfection; then it is going to behave like a stem cell. You end up getting a stem cell, and that can differentiate into bone, skin, and muscle.

Only three are shown here, but don't think only three can be given; you can give rise to any type of cell. So, what do you understand about stem cells so far? Stem cells have the inherent capacity to differentiate into any cell type in your body. Say a person has a

damaged tissue, and now a scar has formed due to fibrosis. But you want a proper tissue that is present in that organ. Stem cells have the capacity provided the environment supports that. If the environment is not supporting the stem cells' ability to form, then it will fail because stem cells cannot do the job of that particular tissue; stem cells can give rise to that particular tissue, so the environment should be supportive of that.

Therefore, we will study more about stem cells and also about regeneration biology in the next class. Thank you.