Regeneration Biology
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W9L42_Induced pluripotency and roles of iPFs during tissue Hox genes and homeotic transformation

Hello everyone, welcome back to another class on regenerative biology. In today's class, we will try to understand induced pluripotency and the roles of iPSCs in general during tissue regeneration, as well as Hox genes and homeotic transformation during tissue regeneration. We will also discuss the importance of Hox genes during homeotic transformation. The second part, Hox genes and homeotic transformation, will be minimally represented in today's class because we will address that much later, but we will give more priority to induced pluripotency and its implications in regenerative medicine. So the pluripotency and induced nuclear reprogramming in vertebrates is a very well-established and well-explored research area. You may have heard about different types of animal cloning, such as cloning an animal.

You may have heard that many of you would have seen the movie Jurassic Park, which makes use of this so-called concept of animal cloning. And also you would have heard about the animal, the sheep; the first mammal to be cloned is Dolly. So, with the same concept, you can use it not just for creating an animal; rather, you can use it for making stem cells from animals. Uh, basically, it is related to that organism, but these days we have moved further ahead, and we will see them one by one.

Induced pluripotency refers to a process where differentiated adult cells are reprogrammed to a pluripotent state. So, one option is to have the embryo of that particular species, which has a lot of ethical concerns. Another option is just to have the nucleus from your skin cell or something, take the nucleus and put it into a nucleated embryo of the same species, and then allow it to grow. And now the embryonic cells that are formed are not an embryo; embryonic cells first form in a group of cells, such as the morula or blastula. The inner cell mass of the blastula can be collected and cultured, which is nothing but your stem cell.

This is one approach, but it still raises ethical questions, as you know you created an animal and did not allow it to become an embryo; it could have become an embryo. and now you are killing that blastula so that is basically a technically a genocide or even an abortion is a genocide so ideally that is not the correct way so the accepted way is take a cell say you pluck your hair from your skin it has got the bottom of that hair there are cells which is easy like you will be losing hairs every now and then or you are losing your skin Or you can take your buccal swab, which, when you are doing tongue cleaning, you also lose some cells. Such kinds of cells nobody cares much about, and the cells you collect, you culture in a petri dish and convert them into stem cells—your own stem cells. There is not too much ethical concern; why? Because those cells are never going to be an embryo in any way. They are your stem cells, which are there at the time of your birth; they are in your cord blood cells, etc.

So that is the benefit, or that is the... An interesting aspect of induced pluripotent cells is that you are not pushing your cell into an embryonic stage, nor are you making a clone of yourself; rather, you are making your own skin cell, your own fibroblast cell, into a stem cell and using it for your research. So that is the whole concept of induced pluripotency; that is why we have discussed Yamanaka factors, Thompson factors, etc.

A pluripotent state resembling the embryonic stem cell is achieved by expressing specific transcription factors, opening up the possibilities for regenerative medicine and disease modeling. So why do you need stem cells? To fix a damaged portion of tissue in your body. To make that tissue, which is no longer present in your body, is why you are suffering. So who can address that issue? Your stem cells can make that issue. So, who will give you the stem cell? You have to make that stem cell.

From where? From your skin cells. So that is the whole concept. So induced pluripotent cells. What is pluripotency? Pluripotent cells have the ability to differentiate into any cell type within the body, a characteristic that was previously thought to be exclusive to embryonic stem cells. Now pluripotent stem cells also have the characteristics of an embryonic stem cell, except that an embryonic stem cell, while present in an embryo, can give rise to an organism.

Pluripotent stem cells will never give rise to an organism. Induced pluripotent stem cells, in short called iPSCs, are derived from somatic cells like skin or blood cells, as I mentioned just now, that have been genetically reprogrammed into a pluripotent state. It was your cell, and it is your cell, except that its genetic nature has been changed. The commonly known IPSC generation is via Yamanaka factors. The discovery of IPSCs was made possible by identifying a set of transcription factors, such as OSKM.

They are OCT4. SOX2, KLF4, and CMYK. In short form, they are the Amenaka factors. That, when expressed in differentiated cells such as skin cells, fibroblasts, etc., can induce pluripotency.

So these genes, when expressed, can make a differentiated cell into a stem cell. So let us learn about induced pluripotent stem cells. So induced pluripotency starts with a cell from the body. This is the body, and one cell is plugged in; then you are doing genetic reprogramming, and certain genes, such as the Yamanaka factors, need to be expressed, and you end up getting induced pluripotent cells. And behaves like an embryonic stem cell, and this culturing also has requirements; what are the requirements to grow embryonic stem cells needed for this pluripotent cells? Also, to culture iPSC in the lab, you need a feeder layer, leukemia-inhibiting factor, etc.

And they can be pushed into differentiation into neurons, muscles, or fibroblasts themselves, or maybe some other specialized gland cells, etc. All possible types of specialized cells can be derived from or formed from this embryonic cell. Or the so-called IPS cells, which mimic the embryonic stem cell. The advantage is that there is no need for embryos. That is the greatest advantage.

And another advantage is that there is no ethical concern associated with that. Embryonic pluripotent stem cells, IPS cells, like you can see here, are cells from the body that can be converted into pluripotent cells by genetic reprogramming. And this is a picture shown in a stepwise manner. This is fibroblast, and you end up getting an embryonic stem cell-like pluripotent cell, which can be pushed into differentiation into cartilage, neural tissue, muscle, and whatnot; you can make them into specific cell types. So what are the applications of iPS? Why would someone make iPS? What was the purpose? As we already told you, it's for regenerative medicine.

Let us Learn more specifically. iPSCs can be differentiated into specific cell types to replace damaged or diseased tissues, offering the potential for treating conditions like Parkinson's, Alzheimer's, and spinal cord injuries. Parkinson's, you know, people have less dopaminergic neuron being produced from the substantia nigra of your brain. Because of this, they will have uncontrolled muscle movements. You may have sometimes seen aged people whose hands may be shaking like this; it will be shaking like this, not because you are shivering with fear, but like this.

It will be moving like this; you know they do not have good control of their hands. This is mainly because not every old person whose hands are moving like this should be pictured as a Parkinson's patient; it needs diagnosis. So, in general, the dopaminergic neurons are.

.. Not producing an adequate amount of dopamine or the number of dopaminergic neurons is depleted. So dopamine is very important, and that can also lead to depression, etc. And in Alzheimer's units, because of the neuronal damage, your short-term memory is lost faster, and your old long-term memories remain lost much longer. And then spinal cord injury because of rod accidents, etc. We have also seen some spinal cord injury regeneration in the previous class, but understand the absence of a specific type of, say, dopaminergic neuron or some of these alchemist cases; some specific types of neurons when they are.

.. Affected, you can supply stem cells or differentiated neurons to that affected area so that now you have more dopamine or more neuronal communications, etc. This is the approach; that is why you need stem cell disease modeling. Another application is that IPSCs derived from patients with specific diseases can be used to study the underlying mechanisms of these diseases in a controlled environment. Say a person has a disease due to a gene mutation, and you have no idea what is happening, so you can take that person's cells from the body and make an IPSC out of them. And you can see how that cell is behaving, or you can correct it into a normal cell and see how it is supposed to behave from that particular person's cell, or maybe because of this mutation, this particular cell is not able to produce an enzyme.

Then what you can do is in that patient. Sometimes you cannot replace a cell type; it may not be in a place where you can replace the cell type, but you understood the etiology and what is missing. It's like a small kid, a very small baby, who is three months old or four months old. The baby cannot speak and is crying. Crying can be for various reasons: sometimes, if they are sleepy, they will cry; if they are scared, they will cry; if they are hungry, they will cry; and if they have stomach pain, they will cry.

You can't ask if it is a grown-up person; you can ask why you are crying. What is your problem? In the same way, if a cell has a mutation, why are some activities not happening? That may not be simply direct because the mutation may not be in the gene at all. Mutation may be in a non-coding region. So how will you decipher it? So understanding this mechanism, you have the stem cell, and you differentiate it into different types of cells to see which cell type is going to be affected. Can we tweak the scenario so medicines can be designed for that patient? That is the whole idea in disease modeling.

And drug discovery. iPSCs can screen potential drugs and assess their efficacy and safety before clinical trials because iPSCs have a lot in common with an entire embryo. Although it is not an embryo, it has similarities to those of an embryo. So they can be screened if a drug is toxic or a drug is accelerating cell proliferation. Some drugs favor a particular cell type being formed from the stem cells; they can all be categorized. Sometimes it may be anti-cancer treatment, etc.

It can be beneficial in personalized medicine. iPS cells made from patient-derived cells can be used to create disease models or for cell-based therapies, offering the potential for personalized treatment. Like many medicines, allopathic or any ayurvedic or homeopathic, any medicine you take can be effective. If they cannot, a given medicine will not work in ten different people. That's why you would have seen that during the time of COVID, doctors normally gave a cocktail of medicine.

For some people, it clicks, and they survive. For other people, that medicine doesn't work. There's nothing wrong with the medicine. Medicine was fine; the treatment regime was fine. But somehow it was not giving them the desired result as they had obtained in another patient.

So the patient-specific medicine is a necessity, which medicine works for whom. So that has to be tailor-made, just like if a dress is made for you, your best-fitting dress may not fit another person the way it fits you. It may not be a serious thing for gents who wear loose dresses, but for ladies who care too much about the fit, they are very particular. So you can understand that one person's body fitting may not fit another person the same way; the same goes for drugs. Not every drug, including paracetamol or some painkillers, may give the expected results for specific diseases in every person.

So maybe slight tweaking of dosage and slight tweaking of a drug's nature are necessary, especially since it has huge implications in psychiatric treatment, etc. So another point we should understand is transdifferentiation. Transdifferentiation, certain stem cell types can differentiate into cell types that are seen in organs or tissues other than those expected from the cells' predicted lineage. Say, for example, in some families you can see the grandfather is a doctor, and the son is a doctor. The grandson is a doctor, and the great-grandson is a doctor.

So, if one more kid is born, you will think, oh, this person is also going to be a doctor. So, this is the assumption about the lineage. But suddenly, one person in the family became a social worker. His father, grandfather, and mother are not social workers. From the doctor's lineage, you got a social worker from that family.

Similarly, if a muscle-specific lineage or neuronal-specific lineage gives rise to another cell type that is not expected from that lineage, we usually refer to it as transdifferentiation. This reported phenomenon of transdifferentiation is very important and useful in regenerative medicine. Why? Say I want my kidney cells. To be formed, I am not interested in making IPS cells from my skin cells because it's very costly. Instead, I took bone marrow stem cells; I have bone marrow stem cells available in me now.

I am saying that instead of making blood cells, can you make kidney cells? This is the whole concept of transdifferentiation. For example, brain stem cells can differentiate into blood cells, or blood-forming cells can differentiate into cardiac muscle cells. These are some examples. Stem cells at home, that is the environment, the niche. There is a popular saying: "When you are in Rome, do as the Romans do.

" We have heard about that, or rather I can say the other way around: if you are in Rome, you will automatically do as the Romans do. This is much better; if you are a sensible person, you will do as the Romans do. It's like if you go to a function or if you go to the Western world, where people are wearing formal dress in a different way than Indians. Formal dress in India is different from formal dress in the western world. Both are formal dress, but if you are in India, you will wear Indian formal dress; when you are in the US or other European countries, you will wear that country's formal dress.

In the same way, stem cells behave according to the tissue they are in. For example, if I take iPS and put them into the kidney, they will not make liver cells; instead, they will try to make kidney-like cells as they look at the surroundings. So, stem cells have specific niches. Niche is basically the microenvironment around stem cells that provides support and signals.

Both are needed. Support and signal. Signal means chemical signals that regulate cell renewal and differentiation. So stem cells should be able to make copies of themselves, and they should be able to differentiate when needed according to what is required. Not that I randomly have a pool of stem cells and keep making neurons today, muscles tomorrow, and liver cells the day after tomorrow. It cannot work that way. It can create a given type of cell based on the signals coming from the microenvironment.

So, stem cells are there. They can influence the niche, and they are influenced by the niche. So, they need to have direct contact, like we have discussed about the feeder layer in a petri dish. You need to have leukemia inhibitory factor present to maintain the pluripotent stem cells, as pluripotent stem cells and a lot of soluble factors. Direct contact is one feature that comes into the picture, along with soluble factors released from the microenvironment and also intermediate cells. The niche cell will guide another cell to influence the stem cells in a particular manner.

It is like making a phone call to a person who is sitting far away from you versus sending a message with a person to convey to that person. So, that is what you are seeing. So in either case, these stem cells are too responsive to the immediate neighborhood, which we refer to as the microenvironment or niche. So, when we use stem cells for tissue engineering, we need to follow certain steps.

One thing is isolation. Whichever tissue or stem cell you are dealing with, you need to isolate it, characterize it, and expand it. And then you should learn how to differentiate between them. So isolate individual stem cell populations from an adult stem cell or any part of the body. Ensure that the cells retain their functionality and potential to differentiate.

You don't want a dead cell isolated from people. You want healthy cells. And then characterize and track the stem cell population during characterization because you need to be sure that I took this stem cell; I did not take one random stem cell, and ensure that the cells are transplant-ready in the sense that they are ready for culturing, ready for putting into a petri dish, and ready for expansion in number. Culture stem cell lines into a stable multi- or pluripotent state based on the type of stem cell you harvested to start with, free from mutations and of sufficient quantity. You need to have a good number when expanding. You cannot perform a stem cell transplantation with the five cells.

You need whatever it takes or whatever target tissue you are going to push into. It should have that much good quality, numbering in the thousands or millions, that enables economical expansion to make cell therapy a reality. This expansion requires around 10 million dollars, or about 100 crore rupees; no patient will be able to afford this. Therefore, a therapeutic approach should consider that it should be viable and allow people to access it. Handle that economic burden behind it and then control and activate stem cells to differentiate into desired lineages.

I have a stem cell, but it will differentiate only into neurons, not into any other cell types. Then, basically, whatever effort I put in is a waste. Although I claim it as an iPS, oh, I made an iPS; it's very nice. It's like, you know, I'm a singer, but I know only one song. I'm a great singer of one song, but after that song is done, I don't know what to do.

So this is what we don't want in a stem cell. And functionally active differentiated cells. Now I differentiated, but then it's not doing the job that a cell is supposed to do. I made a neuron, but it's a dummy piece, just like in some dining tables you would have seen; they have apples, bananas, or other types of brightly colored fruits that look nice, but you can't eat them because they are made of wax. They are for decorative purposes; they are there to satisfy your eyes and fill the void. You don't want a cell made like that; you want a cell that is functional.

You don't want a dummy piece, so one has to make sure that a perfectly functional cell is being made. So, what are the challenges in stem cell research? It is uncertain whether the human embryonic stem cells can give rise to all the different cell types of the adult body in vitro. Although it can give rise to 10 different, 20 different, or 50 different types, I am not interested in that 50; I am interested in that 51st type of cell, which the stem cell is not able to give rise to. Stem cells have some lacunae; they are not perfect, so this is what one has to take care of. It is unknown if stem cells cultured in vitro, apart from the embryo, will function as the cells do when they are part of a developing embryo.

This is a big question: will they? As they are supposed to do. Like you are very comfortable in your house, dining room, or living room. But if I drop you on the moon without oxygen and with too much radiation, will you be as comfortable? Because the environment can matter. So can stem cells do the same job? A simple appearance is not good enough. Stem cell development or proliferation must be controlled once placed into patients. Now, I put stem cells, which can give rise to any cell type and can proliferate into everything.

What if it becomes cancer? One has to be concerned about the possibility of rejection of a stem cell transplant. As foreign tissues are very high when you transplant them, stem cells can have too much MHC class 1 expression, and sometimes this class 1 expression goes beyond normal levels. Normally, the class 1 MHC proteins are less in differentiated cells, while class 2 are more in differentiated cells. So all of a sudden, your immune system is detecting class 1 expressing cells, and the body's immune system is not responding to that much.

Stem cells are around, and they may be vulnerable to immunological attack. So the chances of tissue rejection, although it is made from your own body, are due to it being from a differentiated cell. Stem cells are not common in your body. Your body saw stem cells during your embryonic development. So contamination by viruses, bacteria, fungi, and mycoplasma is possible.

See, because you grew them in a petri dish, you cultured them. Sometimes epigenetic changes are also possible in those genotypes. Maybe some genes will not be expressed at all. And during this process, there may be some fungus, mycoplasma, etc. Are there inbuilt features in that cell type? Once you transplant, you not only cure the disease, but you also create a new disease in the patient.

So these are all the things, the challenges that stem cells are facing. Some challenges and future directions regarding safety concerns, which we already mentioned, arise because many of these Yamanaka factors are expressed using viral vectors to deliver reprogramming factors. Researchers are exploring alternatives due to the safety concerns associated with this method. Methods for reprogramming, such as chemical reprogramming, use some drugs with no need to introduce any genes; just administer some drugs so that they can be reprogrammed efficiently and reproducibly. For example, I gave my skin cells to a researcher and said, "Okay, please make my iPSCs and give them to me.

" And I gave 100 cells; he still, you know, all died. I cannot give them to me, so I cannot keep supplying kilograms of cells for that purpose. I can give only x amount of cells, so the efficiency is a major concern. If the efficiency is not good, it is not a good strategy. Like, if you are planning to cook some food, oh, if I make a thousand dosas, then only one will be edible and 999 have to be discarded, then I am not a good cook. So, epigenetic memory in IPSCs may retain some epigenetic memory from their original cell state, from where they came, such as skin cells or something, affecting their differentiation potential.

Although you are applying Yamanaka factors, they may not forget. Like you know, old memories die hard. There is a saying: "So immunogenicity, iPSC derived from one individual may be rejected by another," which is normal. Raising concerns about using iPSC-derived cells for transplantation to another cell transplantation is allowed. And there are also ethical considerations.

Development of iPSCs has reduced the need for embryonic stem cells. And addressing some ethical concerns associated with embryonic research, so if anyone publishes embryonic research data, people may ask, "Oh, but this has no relevance because people are doing this with the iPSCs, so what is the point of this kind of research?" So these are all some concerns. Now we will move on to Hox genes and homeotic transformation. Hox genes are A family of homeotic genes, because these Hox genes are necessary for differentiation or even the formation of a whole embryo itself. They are crucial for establishing the body plan. A body plan basically means you have only one nose, two ears, two eyes, two hands, and two legs.

Why can't you have three hands? Why can't you have five hands instead of one? Why don't you have three noses? These are all fixed because of the homeotic transformation happening at the right time. So, during development, mutations on any of these genes can lead to homeotic transformation, where one body part develops into another. Like in the place of my nose, I now have a hand, or in the place of my nose, I have an ear, not a nose. In place of an ear, I have two eyes, something like that. Homeotic transformation is a phenomenon in developmental biology that involves the alteration of one body part into another, often caused by mutations or misexpression of homeotic genes.

These genes are basically Hox genes, which are crucial for establishing the body plan and segment identity. So, homeotic transformation by definition, homeosis, is a homeotic transformation that refers to the developmental process where one body structure develops into a structure normally found in a different location or a different segment. How does this mechanism? This transformation is often triggered by mutations or altered expression of homeotic genes, also known as Hox genes. These genes are master regulators that control the development of specific body segments and structures. And for example, some examples include Drosophila; a classic example that has been worked out in Drosophila is the transformation of antennae.

You know, Drosophila has a sensory organ that represents antennae in the legs instead of antennae or something equivalent, I can tell. In place of a nose, you have got a leg; something like that, because antennae are a sense organ for Drosophila, and that has been appearing in the fruit flies. It means not naturally; you can make it by homeotic transformation due to mutations in specific homeotic genes.

There are different odd shapes. Even skipped, different types of jeans are there. They are all Hawk's jeans, and Drosophila has a segmented body pattern. Even our bodies are segmented. You may not agree, but actually, we have segmented the body pattern. That is why in some anterior segments you end up getting, you know, the shoulder, the pectoral girdle, the pelvic girdle, etc.

Not that you have the pectoral or pelvic girdle right in your belly region. It is this girdle that is here and not above your head. Our head is in the anterior portion. So this kind of identity is due to the segmentation in your body. And anurans, the vitamin A treatment during tail regeneration in anuran tadpoles in amphibians can lead to homeotic transformation of tails into limbs.

In place of tails, you end up getting a leg. All you did was treat them with vitamin A, and in vertebrates, homeotic transformations of vertebrae have been observed in murine embryos that are exposed to teratogenic doses of retinoic acid, leading to posterior transformation along the body axis. That means you end up getting legs in by the shoulder or you end up getting hands in the pelvic girdle, etc. So this kind of positional identity is maintained by Hox genes. And they have huge implications for the development of an entire organism.

And these Hox genes are responded to by the stem cells. So I am not going into developmental biology. But understand that Hox genes decide how an organ is formed from the stem cell during embryology. We will study more about regenerative biology in the next class. Thank you.