Genome Editing and Engineering
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Module - 09
Genome Engineered Disease Modelling
Lecture - 02
IPSc models - Part A

Welcome to my course on Genome Editing and Engineering. We are discussing module 9, where we are going to use genome engineering technologies in disease modeling. In this lecture we are going to discuss about the IPSc models.

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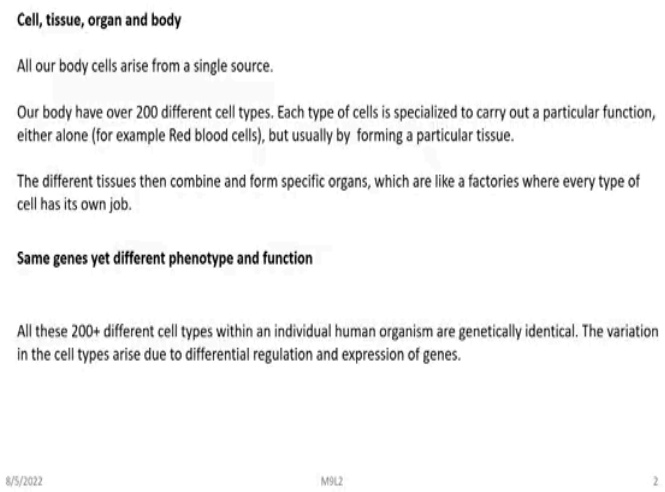

Before that let us briefly discuss about the relationship between cells, tissues, organs and body it is known to you that our body cells arise from a single source.

But over the time our body has over 200 different cell types, and each cell type is specialized to carry out a particular function, either alone for example the red blood cells, but usually the cells form a particular tissue. The different tissues then combine and form specific organs, which are like factories where every type of cell has its own job.

And you know that our body is constituted by various organs which function together. Let us now go to the phenotype and genotype of these 200 different cell types our body have, all

these 200 different cell types because they arise from a single origin within an individual or human are genetically identical.

The variation in these cell types arise due to differential regulation and expression of genes. Certain genes are expressed in certain cell types while they remain silent in others.

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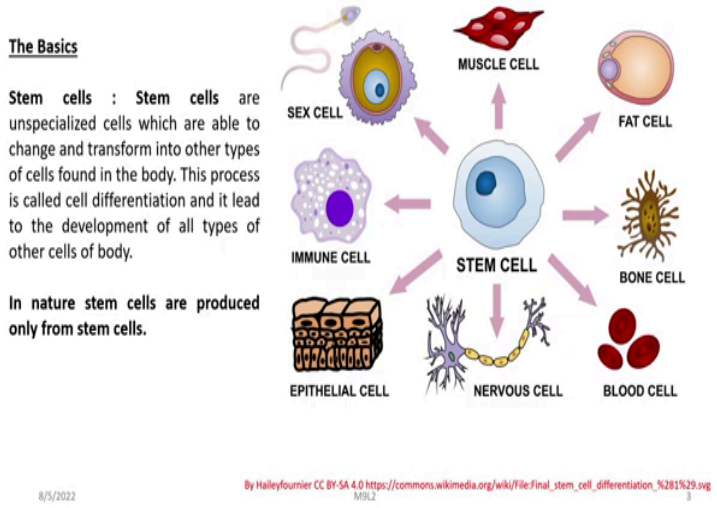

Now, our body has some kind of very special cells which you call as stem cells. These are not specialized cells unlike the other 200 plus different type of cells we have which are specialized cells. Stem cells are unspecialized cells which are able to change and transform into other types found in the body.

This process is called cell differentiation and it leads to the development of all types of other cells of the body. So, you can see a stem cell can give rise to muscle cell, fat cell, bone cell, blood cell, immune cell and so on. Let us now study about a specialized type of cell called stem cell these stem cell is unlike the 200 plus differentiated cells that we have just discussed.

In fact, stem cells are unspecialized cells which are able to change and transform into other types of cells found in the body, this process is called cell differentiation and it leads to the development of all types of other cells of the body from the stem cell. So, a stem cell can give rise to a muscle cell, fat cell, epithelial cell, nervous cell, blood cell etcetera and in nature a stem cell is produced from a stem cell.

Unlike these differentiated cells which are produced from a stem cell, a stem cell gives rise to another stem cell.

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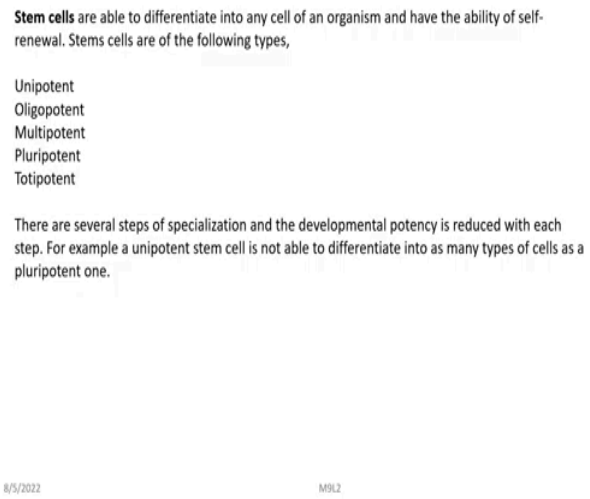

We will discuss about the stem cell division and the differentiation in another slide. A stem cells are able to differentiate into any type of cell of an organism and have the ability of self renewal and they fall into the following classes like unipotent, oligopotent, multipotent, pluripotent and totipotent.

There are several steps of specialization and the developmental potency of a stem cell is reduced with each step. For example a unipotent stem cell is not able to differentiate into as many types of cells as a pluripotent stem cell.

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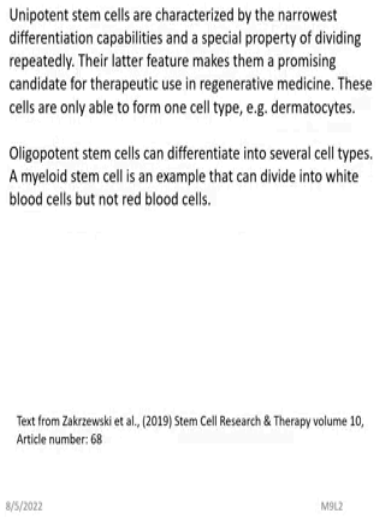

What are unipotent stem cells and what are their characteristics. The unipotent stem cells are characterized by the narrowest differentiation capabilities and a special property of dividing repeatedly. Their latter features make them a promising candidate for therapeutic use in regenerative medicine. These cells are only able to form a one type of cell example dermatocytes.

So, if our target is regenerative medicine using dermatocytes then the unipotent stem cells are the best candidates because we will be sure that these cell type follows a particular a lineage. Oligopotent stem cells however, can differentiate into several cell types unlike unipotent stem cells. A myeloid stem cell is an example that can divide into white blood cells but not red blood cells.

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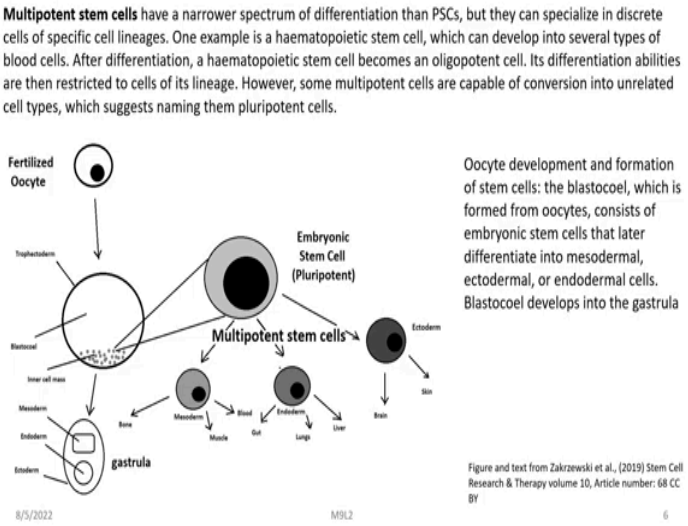

The next type of stem cell we have are the multipotent stem cells. They have a narrower spectrum of differentiations and then the pluripotent stem cells, but they can specialize in discrete cells of specific cell lineages. So, you can see here the multipotent stem cells which can differentiate and become mesoderm from which the blood cells muscle cells and bone cells can be formed, or it can become either gut cell, lung cell or liver cell or it can become the brain and skin cells.

After differentiation, for example a haematopoietic stem cell can become an oligopotent cell. Its differentiation ability is restricted to cells of its lineage. However, some multipotent cells are capable of conversion into unrelated cell types, which suggest naming them as pluripotent cells.

So, here you the you can see the schematics of oocyte development and formation of stem cells in this figure. The blastocoels, which is formed from oocytes, consists of embryonic stem cells that later differentiate into mesoderm, ectoderm, or endoderm cells and which gives rise to different number of cells as already discussed.

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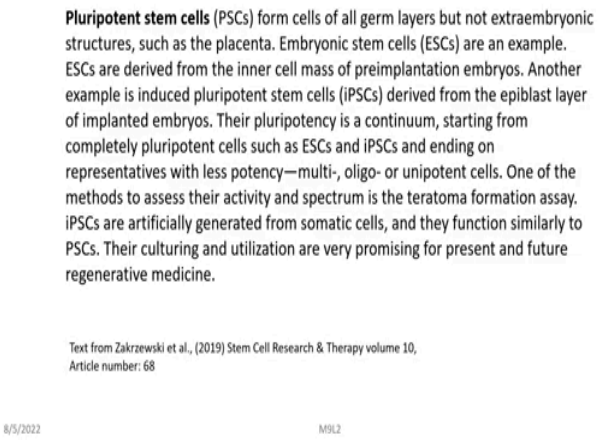

The next type of stem cell are the pluripotent stem cells. They form cells of all germ layers, but not extra embryonic structures, such as the placenta. Embryonic stem cells are an example of these type of PSCs. The ESCs are derived from the inner cell mass of preimplantation embryo.

Another example is induced pluripotent stem cell which is the focus of today's lecture and these are derived from the epiblast layer of implanted embryos, but they are certain chemical treatments or other kind of interventions through which these are generated. The pluripotency is a continuum, starting from completely pluripotent cells such as the ESCs and iPSCs and ending on representatives with less potency multi to oligo or unipotent cells, which we have discussed in the earlier slides.

One of the methods to assess the activity and spectrum is the teratoma formation assay. iPSCs are artificially generated from somatic cells, as I have already indicated and they function similarly to the pluripotent stem cells. Their culturing and utilizations are very promising for present and future regenerative medicine.

In this lecture after the discussion on the basic of stem cells we will be discussing about using genome editing technology in conjunction with iPSC for creating disease models.

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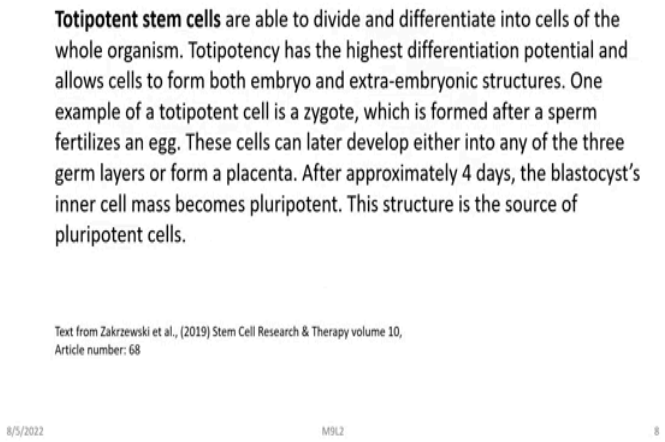

Another type of stem cells are the totipotent stem cells. They are able to divide and differentiate into cells of the whole organism that is why they are called totipotent. It has the highest differentiation potential and allows cells to form both embryo and extra embryonic structures.

One example of a totipotent cell is a zygote, which is formed after a sperm fertilizes an egg. These cells can later develop either into any of the three germ layers or form a placenta. After approximately 4 days, the blastocyst's inner cell mass becomes pluripotent. The structure this structure is the source of pluripotent cells.

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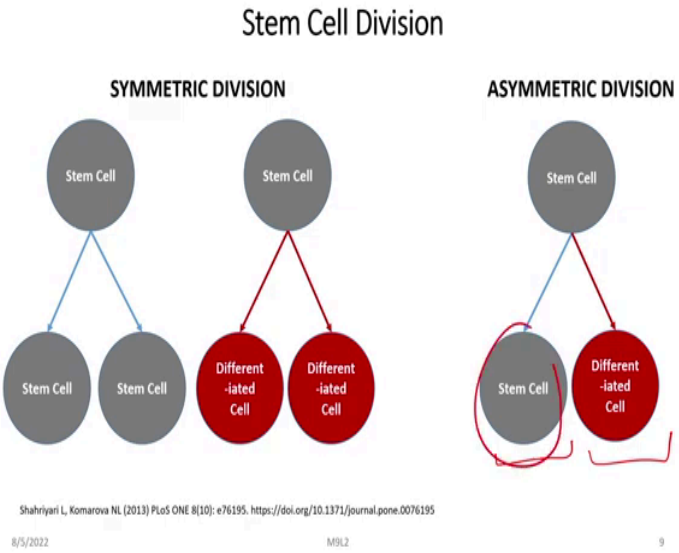

Let us now discuss about one important characteristics of stem cell division. So, like in mitotic division a stem cell gives rise to two daughter cells, but here the division is something very very unique, then somatic cell division. A stem cell gives rise to two daughter cells and in this case both the cells may be either stem cells or either differentiated cells.

In this type of division we call symmetric division has taken place. A stem cell give rise to either two stem cell or two differentiated cells. Stem cells have a very unique type of a division which is known as asymmetric cell division whereby a stem cell give rise to mixed population of cells like it may give rise to both a stem cell and a differentiated cell.

So, this estimated division do not gives rise to two identical daughter cells rather it gives rise to two unidentical daughter cells out of which one is a stem cell, while the other is a differentiated cell.

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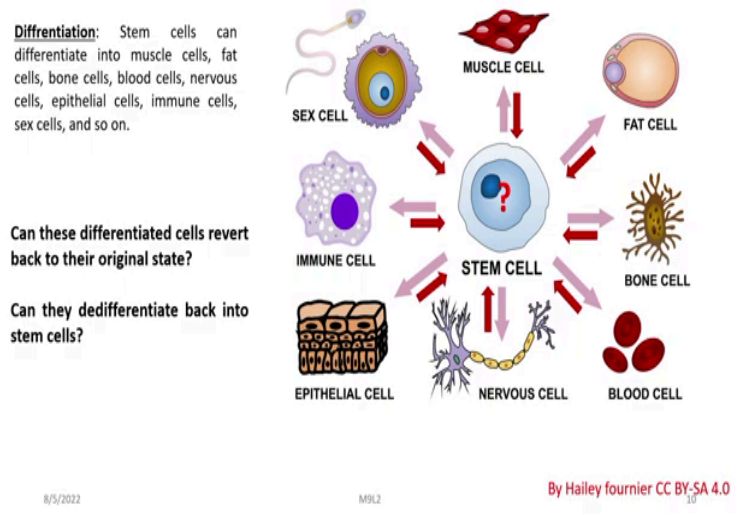

What is a differentiation? Stem cells can differentiate into muscle cells, fat cells, bone cells, blood cells, nervous cells, epithelial cells, immune cells and reproductive cells and so on this has been discussed.

Now, these differentiation pathways are actually influenced by many molecular and genetic factors and they are also the main source of specialized cells for particular tissues and finally, they give rise to specialized organ in the body. So, the stem cells are very very important for the survival of an organism, and if the organism is having any kind of disease the stem cells can be used as a therapy.

Stem cells also can be modulated and we can also form disease models out of this. In this lecture we are going to deal with that to a large extent particularly from the point of view of genome editing. Now, once these stem cells a stem cell differentiate into any of these cells that we have listed.

Can these differentiated cells revert back to their original state or simply if they can differentiated back into a stem cell. Can a muscle cell become a stem cell? The answer is no, as far as the natural condition prevails, but today we have the technology of reverting back differentiated cells into stem cells and that is what we are going to discuss in this lecture again.

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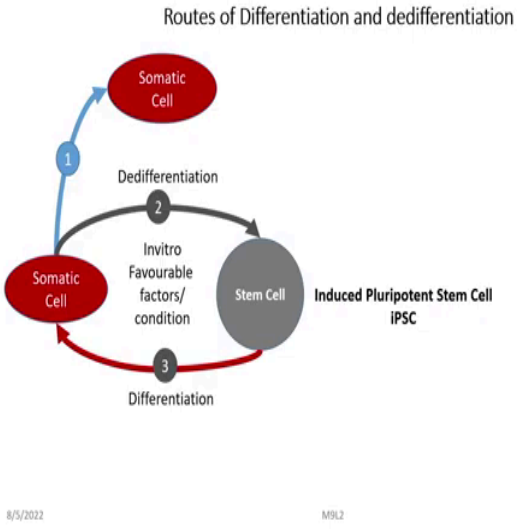

Let us look into the roots of differentiation and dedifferentiation. We know that stem cells give rise to different kinds of tissue specific cells and somatic cells. However, the somatic cell division gives rise only to somatic cell inside the body under normal conditions, but we can also dedifferentiate the somatic cells following dedifferentiation part as shown in part number 2.

Under invitro favourable factors and conditions and convert these stem cells somatic cells into stem cells. The stem cells which are produced from somatic cells under invitro favourable conditions are known as the induced pluripotent stem cells because we have induced the somatic cells to dedifferentiate back into a stem cell and we simply name them as iPSC and these iPSCs or stem cells can again be dedifferentiated sorry differentiated and form into somatic cells.

But using these pathway a muscle cell can be dedifferentiated back into stem cell and once it becomes a stem cell it can become any of the cells as shown in this picture including becoming a muscle cells again. So, this is in brief the iPSC technology we will be discussing how this was developed. One thing we need to remember is that induced pluripotent stem cells are pluripotent.

And we know the potency of a pluripotent stem cell is quite wide and large and it will actually generate lot of tissue specific cells and these iPSCs are generated artificially from somatic cells.

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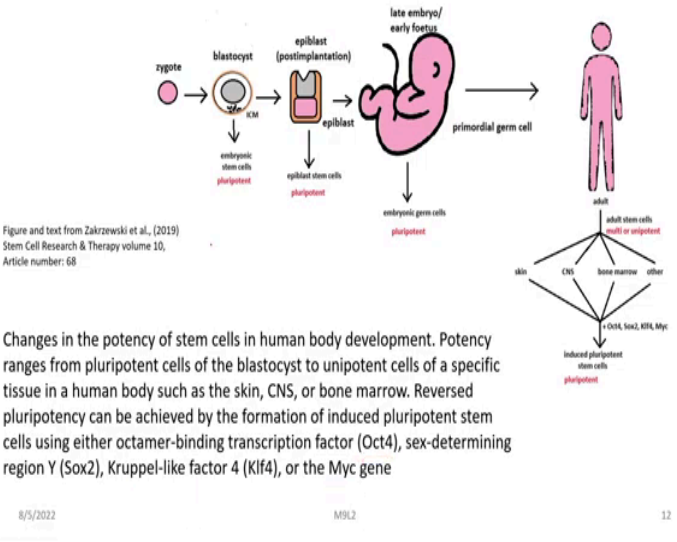

So, these pictures tell us about the changes in the potency of stem cells in human body development, and we already have discussed about the various potencies starting from unipotent to oligopotent to multipotent to pluripotent to totipotent.

The potency ranges from the pluripotent cells of the blastocyst to unipotent cells of a specific tissue in human body such as the skin, central nervous system, or bone marrow. A reversed pluripotency can be achieved by the formation of induced pluripotent stem cells using either octamer binding transcription factor Oct4, sex determining region Y Sox 2, Kruppel like factor 4 Klf4, or the Myc gene.

So, you can see here zygote giving rise to the embryonic stem cells which are pluripotent and as time progresses and differentiation stages progresses we have different kinds of cells and tissues and organs being formed in these developmental process which gives rise to a complete adult from a zygote.

Now, these adult has a various kinds of adult stem cells which may be multipotent or pluripotent and with these particular factors like Oct4 Sox2, Klf4 and Myc we can reconvert them back into induced pluripotent cells. So, this is an overview of these iPSC technology.

So, the original discussion that whether stem cells which differentiate into various kinds of somatic cells and if the somatic cells can be formed back into stem cells is now clear to you

with these certain factors like these we can reverse these differentiated cell into a stem cell by the process of dedifferentiation.

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So, how these technology got developed step by step starting from cloned frogs to the development of iPSCs, it took many years and many interesting research to happen which was happening phase wise and in a in over a long duration of time in the way these mega city was built over historical time points.

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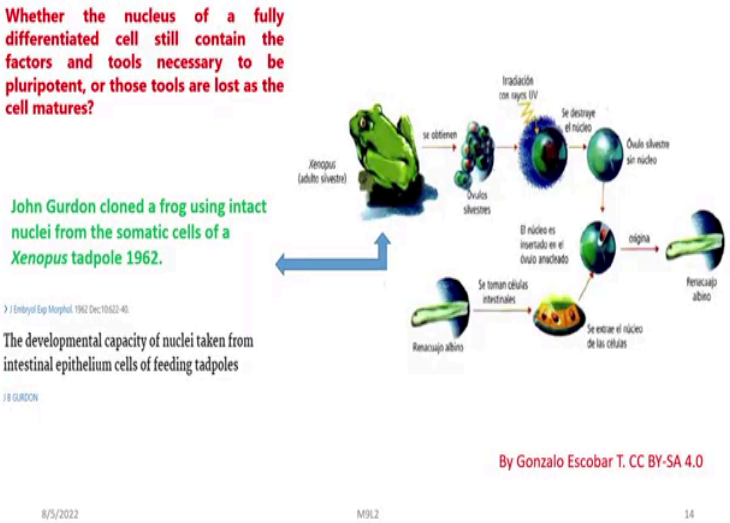

This is the experiment of John Gurdon who cloned a frog using intact nuclei from the somatic cells of a Xenopus tadpole in 1962. The question here was, whether the nucleus of a fully differentiated cell a still contain the factors and tools necessary to be pluripotent, or those tools are lost as the cell matures.

And these paper published in journal of embryology and experimental morphology by the title the developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles is considered a landmark paper in this progress.

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Thirty-five years later, Sir Ian Wilmut and his team used the same SCNT strategy of cellular reprogramming in the cloning of Dolly the sheep, the first mammalian to be generated by somatic cloning.

Professor Sir John Gurdon, winner of Nobel Prize for Physiology or Medicine 2012, at the annual Scholar's Dinner of Magdalene College, Cambridge, with several other members of the college, 10 October 2012. CC BY-SA 3.0

35 years later, Sir Ian Wilmut and his team use the same SCNT strategy of cellular programming in the cloning of Dolly the sheep, the first mammalian to be generated by somatic cloning. And Sir John Gurdon was awarded the Nobel Prize for Physiology and Medicine in 2012, and here you can see his picture at the annual Scholar's Dinner of Magdalene College, Cambridge.

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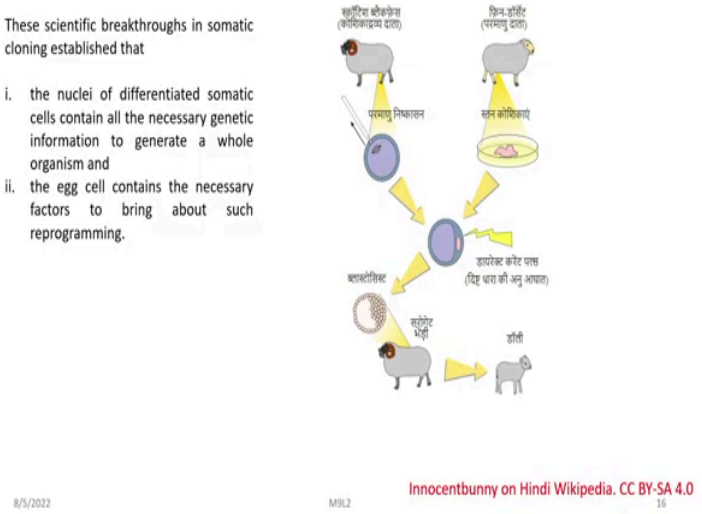

The scientific breakthroughs in somatic cloning established that the nuclei of differentiated somatic cells contain all the necessary genetic information to generate a whole organism. Thereby answering the question whether the nucleus of a fully differentiated cell still contain the factors and tools necessary to be pluripotent or whether these tools are lost.

The tools are not lost they have the information generating information to generate a whole organism. And the egg cell contains the necessary factors to bring about such reprogramming. This picture is a schematic picture because of copyright issues I could not get a picture labeled in English you can try to find out a picture of corresponding similar representation.

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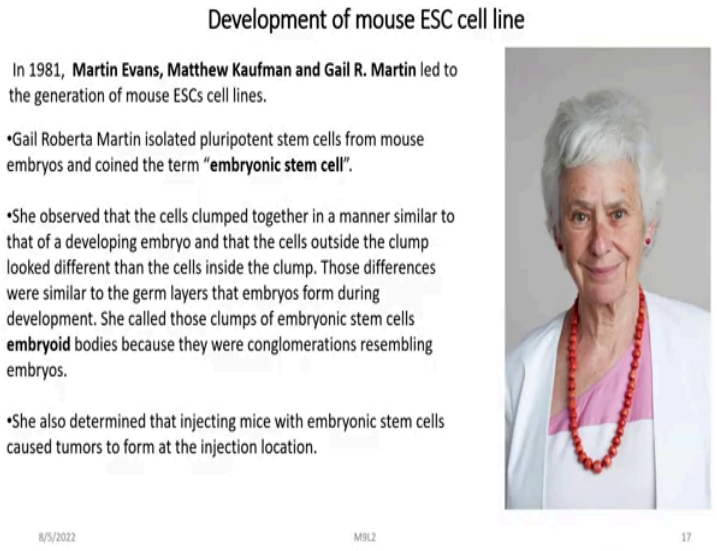

Let us now discuss about the development of mouse embryonic stem cell stem cells and cell lines. So, in 1981 Martin Evans, Matthew Kaufman and Gail R. Martin led to the generation of mouse embryonic stem cell lines. Gail Roberta isolated pluripotent stem cells from mouse embryos and coined the term embryonic stem cell and which is now simply referred to as ESCs.

She observed that the cells clumped together in a manner similar to that of an developing embryo and that the cells outside the clump looked different than the cells inside the clump. Those differences were similar to the germ layers that embryos form during development.

She called those clumps of embryonic stem cells embryoid bodies because they were conglomerations resembling embryos. She also determined that injecting mice with embryonic stem cells cause tumors to form at the injection location.

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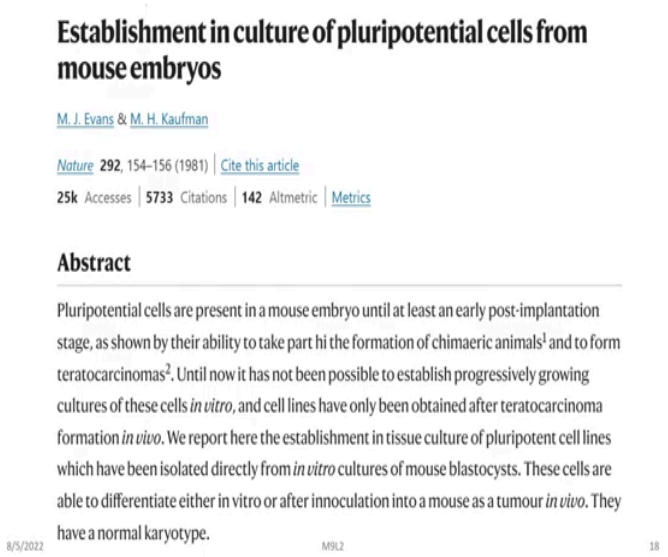

So, this is the paper on establishment in culture of pluripotent pluripotential cells from mouse embryos by Evans and Kaufman.

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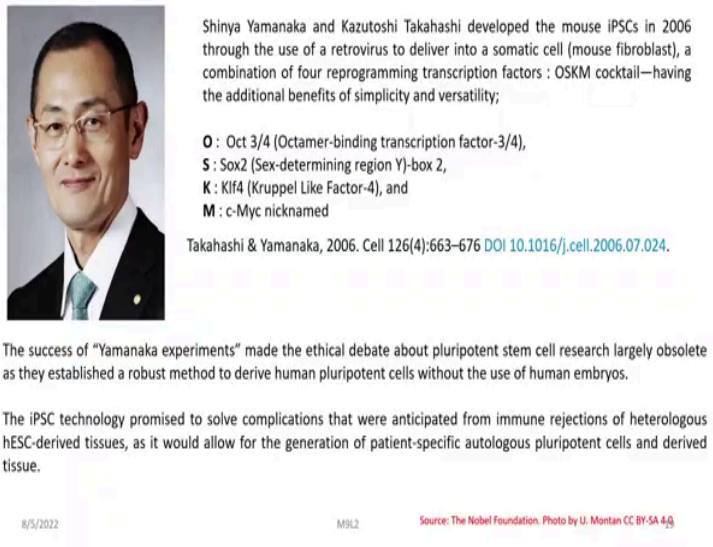

Later Shinya Yamanaka and Kazutoshi Takahashi developed the mouse induced pluripotent stem cells in 2006 through the use of a retrovirus to deliver into a somatic cell which was a mouse fibroblast, a combination of four reprogramming transcription factors. These are known as the OSKM cocktail and these are already discussed earlier you have the octamer

binding transcription factor 3 by 4. Sox2 the sex determining region Y box 2, Klf 4 or Kruppel like factor, and c Myc which has been nicknamed.

And these remarkable work was published in the journal cell and the success of these experiment Yamanaka experiments made the ethical debate about pluripotent stem cell research largely obsolete as they establish a robust method to derive human pluripotent cells without the use of use of human embryos.

Earlier to obtain the human pluripotent stem cells the embryos were considered as the important or primary source, and handling or working with human embryos was very very controversial and having many ethical and legal issues. Now, since these development of the Yamanaka technique or experiments those kind of issues were settled for once and all. We can take any somatic cell like a mouse fibroblast or a skin cell and then can dedifferentiated them with reprogramming transcription factors called OSKM factors and generate pluripotent stem cells.

The iPSC technology promised to solve complications to that were anticipated from immune rejections as well of heterologous human embryonic stem cells derived tissues, as it would allow for the generation of patient specific autologous pluripotent cells and the derived tissues. Not only the legal or ethical problems were solved by this technology. But it is also addressed the biological problems like immune rejection. So, it is a really a landmark discovery and it change the course of science in a big way.

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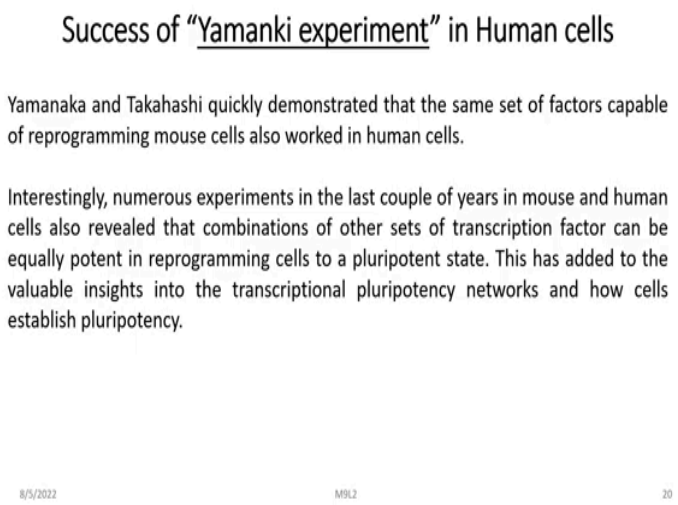

Yamanaka and Takahashi quickly demonstrated that the same set of factors capable of reprogramming mouse cells also worked in human cells. So, this was one of the biggest discovery. So, we can dedifferentiate our somatic cells into the stem cells and from those stem cells we can generate multiple tissue specific cells.

And those tissue specific cells can be of various kinds can be assembled together to form different organs. So, the science of regenerative medicine got a big boost by the by this kind of disruptive innovation or technology. Interestingly, numerous experiments in the last couple of years in mouse and human cells also revealed that combinations of other sets of transcription factors can be equally potent in reprogramming cells to a pluripotent state.

This is added to the valuable insights into the transcriptional pluripotency networks and how cells establish pluripotency.

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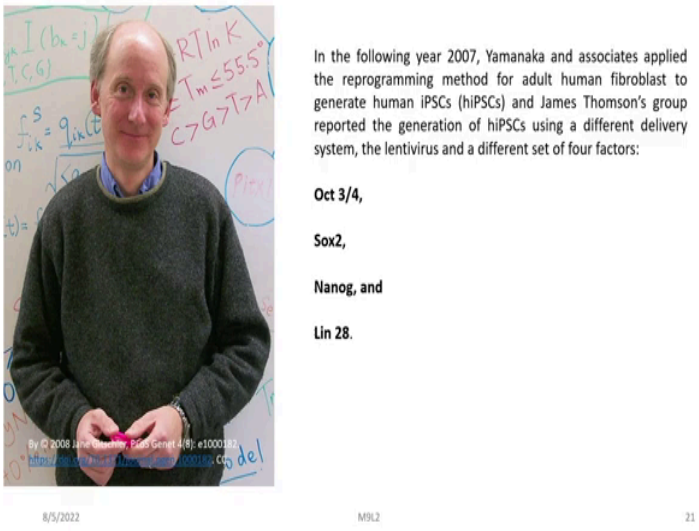

In 2007 Yamanaka and associates applied the reprogramming method for adult human fibroblast to generate human iPSCs and James Thomson's group reported the generation of a human induced pluripotent stem cells using a different delivery system, the lentivirus and a different set of four factors: Oct 3 by 4, Sox 2, Nanog, and Lin 28.

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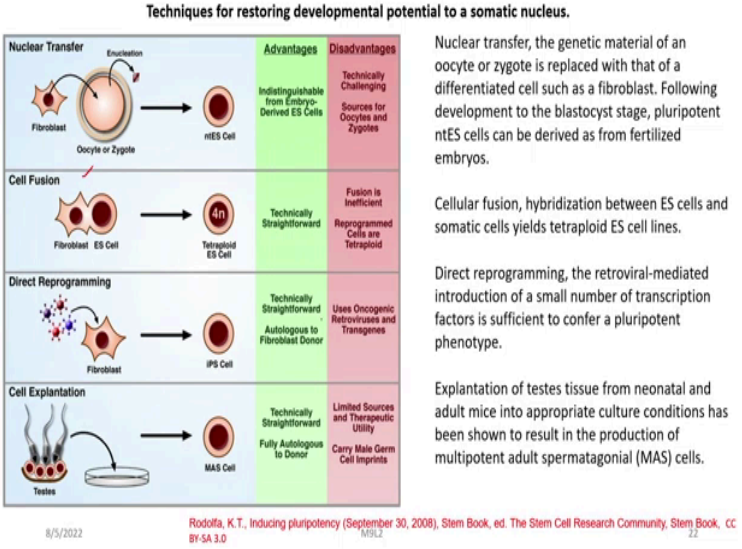

So, what are the techniques for restoring developmental potential to a somatic nucleus. You have various techniques for this like nuclear transfer, cell fusion, direct reprogramming, and cell explantation. In nuclear transfer the genetic material of an oocyte or zygote is replaced with that of a differentiated cell such as a fibroblast. Following development to the blastocyst stage, pluripotent ntES cells can be derived as far as from the fertilized embryos.

So, the advantage here is indistinguishable from embryo derived embryonic stem cell. The disadvantage is that technically quite challenging sources for oocytes and zygotes also having certain disadvantages. The next technique is cell fusion. So, here you have a fibroblast and a embryonic stem cell, if we fuse it they become a tetraploid embryonic stem cell. This is a technically very straight forward method.

And the disadvantage is that the fusion is sometimes inefficient and the reprogram cells are not diploid, but tetraploid. So, hybridization between embryonic stem cell and the somatic cells yield these tetraploid embryonic stem cell lines, but they are not diploid that is the biggest problem.

In the direct reprogramming, the retroviral mediated introduction of a small number of transcription factors is sufficient to confer a pluripotent stem cell somatic cell into a pluripotent phenotype. So, here this fibroblast got converted due to this direct reprogramming through retroviral delivery of factors into a induced pluripotent cell. This is also technically a very straightforward or simple and it gives autologous to fibroblast donor.

So, the problems of immune rejection and incompatibility is addressed straight forward here. However, disadvantage is that it uses oncogenic retroviruses and transgenes. So, it may have some kind of potential harm in the future. Another method of restoring developmental potential to a somatic nucleus is through cell explantation.

For example, explantation of testes tissue from neonatal and adult mice into appropriate culture conditions has been shown to result in the production of multipotent and spermatogonial cells or MAS cells. This method also technically straightforward gives fully autologous to donor and disadvantage is that limited sources and therapeutic utility and it carry male germ cell imprints.

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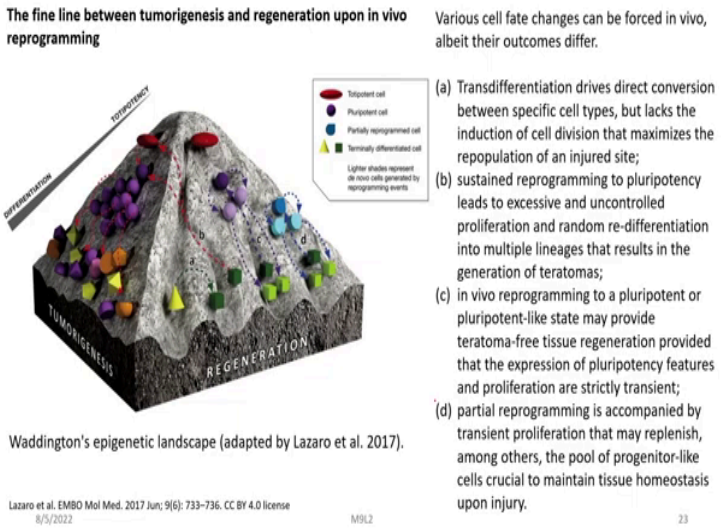

Look into this picture which is giving a symmetric representation of Waddington's epigenetic landscape and there is a fine line between tumorigenesis and regeneration upon in vivo reprogramming. So, we have cells here which are totipotent and then we have these cells which are pluripotent and then we have partially reprogram the cells and then we have terminally differentiated cells over here.

The lighter sets represent de novo cells generated by reprogramming events. So, this is the fine line which divides the regeneration from tumoro genesis and transdifferentiation drives direct conversion between specific cell types, but lacks the induction of cell division that maximizes the repopulation of an injured site.

Here you can see in b sustained reprogramming to pluripotency leads to excessive and uncontrolled proliferation and random redifferentiation into multiple lineages that results in the generation of teratomas. In c we have in vivo reprogramming to a pluripotent or pluripotent like state may provide teratoma free tissue regeneration provided that the expression of pluripotency features and proliferations are strictly transient.

And in d we have partial reprogramming accompanied by transient proliferation that may replenish, among others, the pool of progenitor like cells crucial to maintain tissue homeostasis upon injury. So, whenever there is a regeneration phenomena occurring if any kind of imbalance occurs and it crosses the fine line the body may end up in promoting tumoro genesis.

So, this is important from the translational point of view whenever we are considering iPSCs which will be later differentiated into various tissue types and along with it we are going to use the CRISPR Cas9 or any of the ZFN or TALEN genome editing technologies.

So, any of these technologies having some kind of element of factor which can derive a iPSC towards the tumorogenesis has to be considered in the overall scheme of things, and now there are lot of work going on in that area to make these kind of therapies safer.

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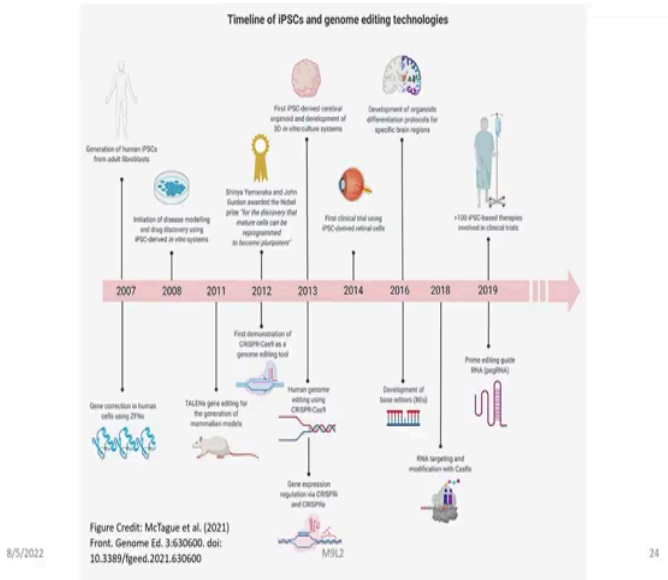

So, let us look into timelines of development of two things. The development of iPSCs and the development of genome editing technologies. More or less you know about both the

timelines separately when we look into them together an interesting scenario emerges and in 2007 this is the year where the generation of human iPSCs from adult fibroblast took place ok, and then same here we have the gene correction in human cells using zinc finger nucleases happening.

And, in 2008 the initiation of disease modelling and drug discovery using iPSC derived in vitro systems was started, and in 2011 TALENs used for gene editing for the generation of mammalian models started. In 2012 Yamanaka and Gurdon were awarded the Nobel Prize for their discoveries which we have already discussed, same year the first demonstration of CRISPR Cas9 as a genome editing tool was demonstrated.

The following year first iPSC derived cerebral organoid and development of 3D in vitro cultures was reported. While, in the genome editing side human genome editing using CRISPR Cas9 and gene expression regulation via CRISPR I and CRISPR A was were reported.

In 2014 the first clinical trial using iPSC derived retinal cells was reported and in 2016 the development of organoids differentiation protocol for specific brain regions was reported and the same year development of base editors were reported in the genome editing domain.

In 2018 we had a report of the RNA targeting and modification with CRISPR Cas9 and in 2019 more than 100 iPSC based therapies involved in the clinical trials, and the prime editing guide RNA peg RNA was reported the same year. In 2021 also there were some similar developments in both of the fields which has not been included in this timeline.

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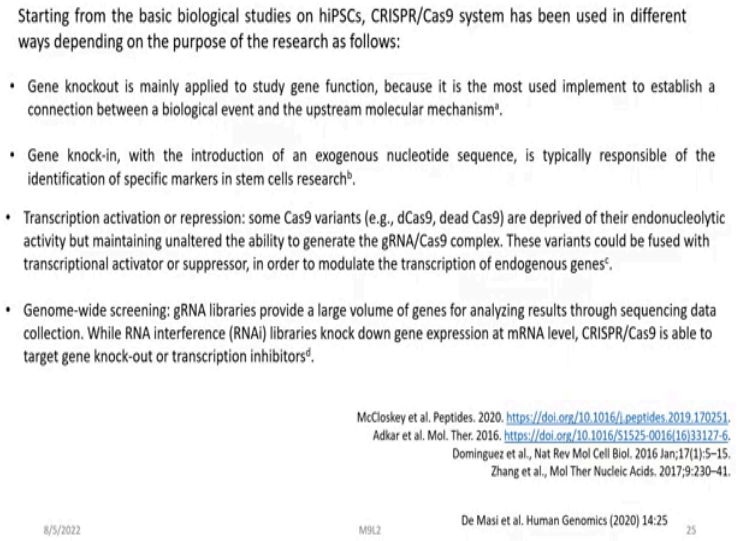

So, starting from the basic biological studies on human induced pluripotent stem cells CRISPR Cas9 system has been used in different ways depending on the purpose of the research as follows.

Gene knockout is mainly applied to study gene function because it is the most used tool to establish a connection between a biological event and the upstream molecular mechanism. Starting from the basic biological studies on human induced pluripotent stem cells a CRISPR Cas9 system has been used in different ways depending on the purpose of the research as follows particularly in gene knockout and gene knock in.

In gene knockout mainly it is applied to study the gene function because it is the most used tool to establish a connection between a biological event and the upstream molecular mechanism. Gene knock in with the introduction of an exogenous nucleotide sequence, is typically responsible for the identification of specific markers in stem cells research.

Transcription activation or repression, with Cas9 variants like dCas9 are derived deprived of their endonucleolytic activity, but maintain unaltered ability to generate the gRNA Cas9 complex. These variants could be fused with transcriptional activator or suppressor, in order to modulate the transcription of endogenous genes.

In the case of genome wide screening gRNA libraries provide a large volume of genes for analyzing results through sequencing data. While, RNA interference libraries knock down

gene expression at the mRNA level, CRISPR Cas9 is able to target gene knockout or the transcription inhibitors.

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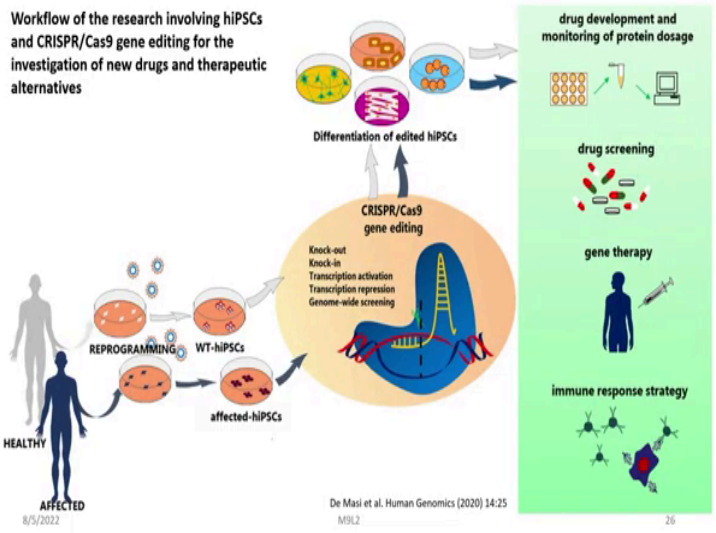

Let us see the workflow of research involving human induced pluripotent stem cells and CRISPR Cas9 gene editing for the investigation of new drugs and therapeutic alternatives. So, we have a healthy person here, and an affected person here cells are taken from the healthy persons as well as the diseased persons and after reprogramming we have the wild type human iPSCs and affected human iPSCs.

And these are used for CRISPR Cas9 gene editing experimentation where we go for knockout knock in transcription activation or repression or genome wide screening as discussed in the earlier slide, and they are used for drug development and monitoring of the protein doses in drug screening, then in gene therapy, and also for immune response strategy.

So, this is overall is workflow of the research involving human iPSCs and CRISPR Cas9 gene editing system. The many applications of CRISPR Cas9 on human induced pluripotent stem cells have already been developed.

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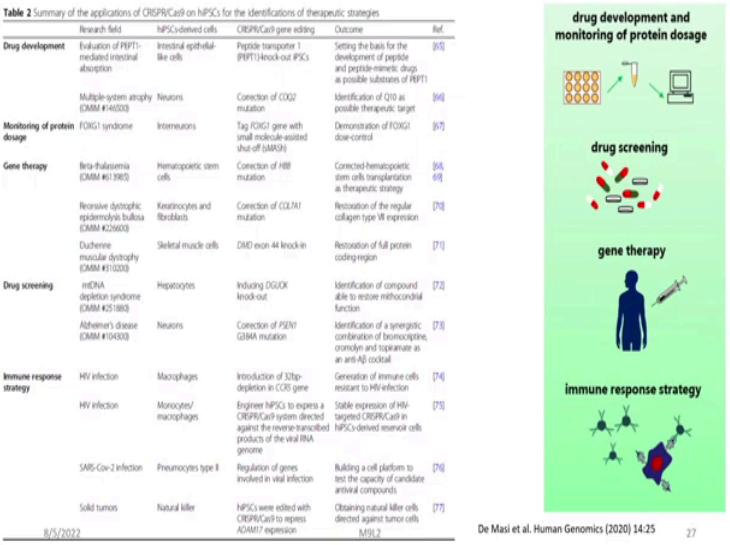

And there are many therapeutic strategies already reported for a detailed discussion on these type of developments you may read De Masi et al human genomics 2020 and briefly we can see what are the progress happening in the area of drug development, or gene therapy, or drug screening. In the case of drug development, evaluation of PEPT1 mediated intestinal absorption has been studied.

And intestinal epithelial like cells has been used for the human iPSC derivation. In other cases they have used neurons and inter neurons or even in certain cases hepatocytes then macrophages and natural killer cells. So, this is the genome editing that was done in this column you can see peptide transporter 1 P PEPT1 knockout iPSCs were developed. Here correction of COQ2 mutation was done in these particular case for example, correction of COL7A1 mutation was done.

And in this case the Duchene muscular dystrophy which we have discussed several times earlier DMD exon 44 knock in was conducted. So, these are the various genome editing being done in the in these type of systems and you can see the corresponding outcome for example, in PEPT1 knockout iPSCs its setting the basis for the development of peptide and peptide mimic drugs as possible substrates of PEPT1.

In the case of DMD exon 44 knock in with respect to Duchene muscular dystrophy restoration of full protein coding e region was done with this CRISPR Cas9 editing. Similarly, in immune response strategy against HIV infection you can see we have discussed

in length about the 32 base pair deletion in CCR5 gene both in the case of ZFN, TALEN and also in the CRISPR Cas9.

Here in this CRISPR Cas9 genome editing the outcome was the generation of immune cells which are resistant to HIV infection and this is all known to you and then there are other applications like building a cell platform to test the capacity of candidate antiviral compounds in the case of SARS Cov 2 infection which is the current challenge to this world.

So, you can see that this technology which involves CRISPR Cas9 application on human induced pluripotent stem cells has a big potential and not only the other cases even current challenges like SARS Cov 2 infection has been attempted to be you know solved through this kind of technological approach.

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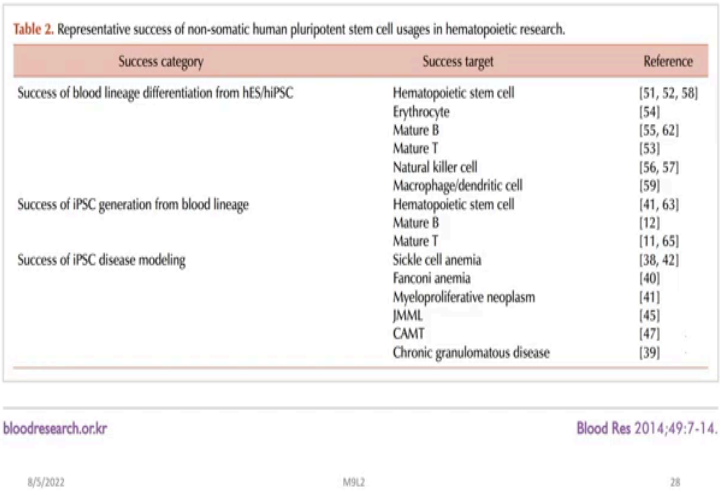
Table 2. Representative success of non-somatic human pluripotent stem cell usages in hematopoietic research.

Success category	Success target	Reference
Success of blood lineage differentiation from hES/hiPSC	Hematopoietic stem cell	[51, 52, 58]
	Erythrocyte	[54]
	Mature B	[55, 62]
	Mature T	[53]
	Natural killer cell	[56, 57]
	Macrophage/dendritic cell	[59]
Success of iPSC generation from blood lineage	Hematopoietic stem cell	[41, 63]
	Mature B	[12]
	Mature T	[11, 65]
Success of iPSC disease modeling	Sickle cell anemia	[38, 42]
	Fanconi anemia	[40]
	Myeloproliferative neoplasm	[41]
	JMML	[45]
	CAMT	[47]
	Chronic granulomatous disease	[39]

So, these tables show some success of non somatic human pluripotent stem cell usage in hematopoietic research for full details you have to refer to this paper in Blood research published in 2014 and the corresponding references against each and every success story is listed over here which can be easily assessed in the article published in this research paper in Blood research.

So, success of blood lineage differentiation from human embryonic stem cell and human induced pluripotent stem cells and the success target has been a hematopoietic stem cell, erythrocyte, mature B cells, T cells, natural killer cells, macrophages dendritic cells and this

tells us about the success of iPSC generation from blood lineages with respect to the targets hematopoietic stem cell, mature B T cells, and in the case of disease modelling which is the main focus of these lecture we have quite a number of success stories for example the sickle cell anemia, fanconi anemia, JMML CAMT and so on and full details available in these reference cells.

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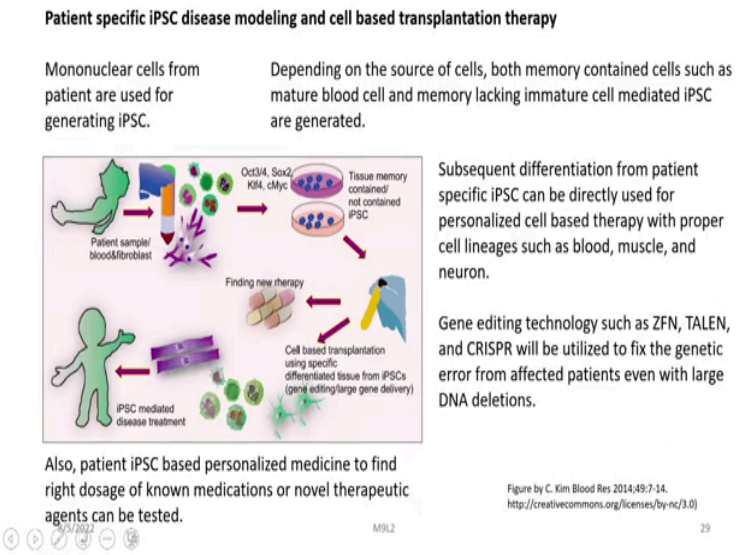

Let us now have a discussion on the patient specific iPSC disease modelling and cell based transplantation therapy. Here you can see the mono nuclear cells from patients are used for generating iPSC. So, you have a patient over here and blood and fibroblast samples for example are taken and then depending on the source of cells both memory contain cells such as mature blood cell and memory lacking immature cells mediated iPSCs are generated.

And these are the reprogramming factors OSCM which we have discussed earlier, and you can see here tissue memory contained not contained in the iPSCs ok. The subsequent dedifferentiation from patient specific iPSCs can be directly used for personalized cell based therapy with proper cell lineages such as blood, muscle, and neuron and in at this stage gene editing technologies such as ZFN TALEN and CRISPR will be utilized to fix the genetic error from the affected patients with large, even with large DNA deletions.

And, we know that CRISPR Cas9 has been now can be modified and developed into dCas 9 onto which various functional modules can be loaded and then convert it into different functionalities like editing functionalities and so on. So, this picture overall gives us a idea

how both the technologies can be merged together and then its potential can be exploited to the fullest extent.

So, here we have the iPSC mediated disease treatment. Now, in the case of disease modelling we may take a normal patient cell line, normal person cell line, and then in a similar way dedifferentiate it into a iPSC and through the power of genome editing create the errors which causes the disease. So, this is thus that would be the just a reverse approach.

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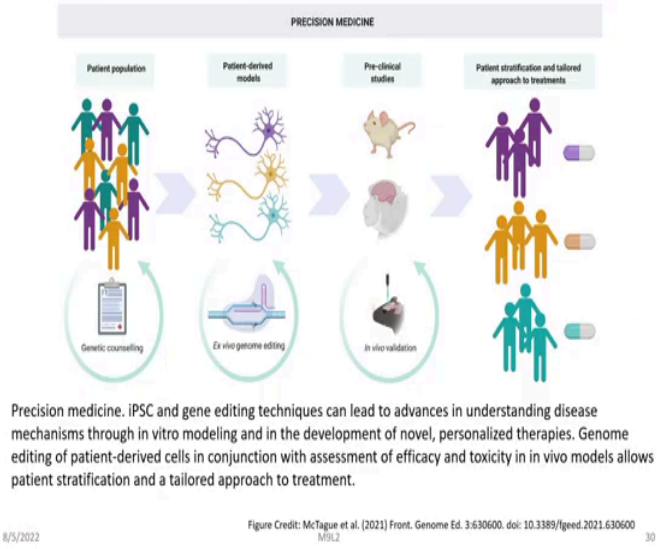

Precision medicine. iPSC and gene editing techniques can lead to advances in understanding disease mechanisms through in vitro modeling and in the development of novel, personalized therapies. Genome editing of patient-derived cells in conjunction with assessment of efficacy and toxicity in in vivo models allows patient stratification and a tailored approach to treatment.

Figure Credit: McTague et al. (2021) Front. Genome Ed. 3:630600. doi: 10.3389/fgeed.2021.630600

Now, let us discuss something about precision medicine. So, we have a patient population and they have certain genetic defects maybe or diversity. So, there is a kind of a genetic counseling being done and then from these particular population we get patient derived models which we may go for ex vivo genome editing and then these are subjected to pre clinical studies for in vivo validation.

And then the patient stratification and tailored approach to the treatments is the key in these particular cases because you can see here these population is not a homogeneous, it is heterogeneous as represented by various colors color coded, and then every type of patient depicted by the color coded may have a different kind of a resulting model for a particular disease for example here it is a symbolic for the neuronal disease in a way.

And then when finally, the treatment is developed you can see the corresponding treatments which are tailor made or customized for the particular population as per the color code for

symbolic representation here soon are developed. So, here that the patient are different divided into various classes or stratified and the treatments are also accordingly tailored for each and every patient.

So, these treatment is not going to be used here and in this case here, but in the conventional treatment approach we do not take care of these diversity we just develop a single type of a therapy either one of them, and we try to force that particular therapy into all the patients. So, in such cases only some population in which they have the compatible therapy would respond positively while the others will not respond at all.

So, in iPSC precision medicine iPSC and gene editing techniques can lead to advances in understanding disease mechanisms through in vitro modelling and in the development of novel personalized therapies. Genome editing of patient cells in conjunction with assessment of efficacy and toxicity in vivo models allows the patients patient stratification and a tailored approach to treatment.

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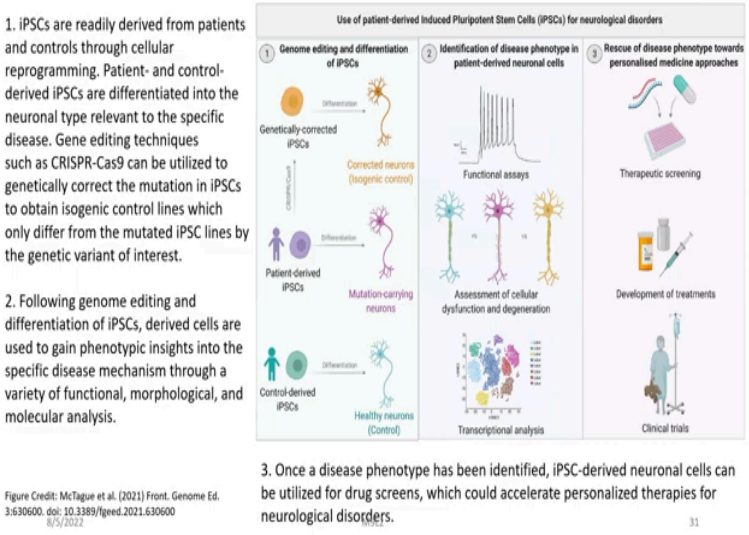

Figure Credit: McTague et al. (2021) Front. Genome Ed. 3:630600. doi: 10.3389/fgeed.2021.630600

So, use of patient derive induced pluripotent stem cells for neurological disorders this is a example being given over here, how do we use these technologies the iPSCs are readily derived from patients and controls through cellular reprogramming patient and control derived iPSCs are differentiated into the neuronal type relevant to the specific disease gene editing techniques such as CRISPR Cas9 can be utilized to genetically correct the mutations

in the iPSCs to obtain isogenic control lines which only differ from the mutated iPSC lines by the genetic variation of interest.

Following the genome editing and differentiation of iPSCs derived cells are used to gain phenotypic insights into the specific disease mechanism through a variety of functional morphological and molecular analysis. Once a disease phenotype has been identified the iPSC derived neuronal cells can be utilized for drug screenings which could accelerate personalized therapies for a neurological disorders. So, with this we come to the end of part A of iPSCs in disease modeling. We will continue these lecture in part B.

Thank you.