Stem Cells Technology

-For this time the molecular biology is the emerging science. And one of the most important fields under the umbrella of molecular biology is the Stem cells Technology.

-Stem cell: capable of regenerating itself, it's an immortal cell.

-Regenerative Medicine "Human Spare Parts" is a new terminology which means that the Physician can ask for replacement of a damaged organ in a patient ["process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function"].

- We can make so many organs from this "Regenerative medicine"; kidneys, heart valves, trachea, bladder, cornea and skin .We will not face the obstacles that we were facing with other therapeutic methodologies like transplantation; here we have no worries regarding matching, no waiting lists and no legal problems. So the transplantations would disappear with this technology.

♯ They are now able to make from the Stem Cells technology:

-Cartilaginous cells -skin -Heart valves

-Bone -Ear -Ureter

-Bladder -trachea

-But they still have difficulties with making more complex organs.

♯ Types of stem cells:

-Embryonic Stem Cells: More potent but less available & has more ethical problems regarding its use.

-Adult Stem Cells: Less potent and more available (easier to obtain) & has no ethical problems regarding the use.

#Stem Cells and Potency:

1. Totipotent (Fertilized egg; Zygote)

- Can make all embryonic cells and tissue types.

- Can make the supporting tissues; like the placenta.

- Nowadays, scientists can easily create a zygote like cell from a terminally differentiated cell (like a skin cell or a cell from your own blood), how? Through retro-differentiation (reprogramming the cell), and now this created ”zygote like cell” can be a uni-, multi-, pluri- or even toti- potent cell.

2. Pluripotent stem cells

- Give rise to cells of all 3 germ layers (ecto-, meso-, and endoderm)

- Come from embryos and fetal tissue.

- Have active telomerase (maintain long telomeres).

3. Multipotent Stem cells

- Give rise to many but not all tissue types as the totipotent cells.

4. Unipotent stem cells

- Found in adult tissues.

- Cell differentiating along only one lineage (gives rise to only one type of tissues)

→Both Totipotent and Pluripotent stem cells can make all types of tissues, but the totipotent stem cells can also make the supporting tissues like placenta.

Embryonic Stem cells

#Sources Of Embryonic Stem cells:

1. IVF embryos (most important Source).

2. Aborted Fetus

3. Therapeutic cloning

→ How to use IVF to obtain the Stem cells?

-Induce the female to produce so many ova, then fertilize several ova in vitro, then implant limited number of fertilized ova (zygotes) in the uterus. However, there are always additional IVF embryos that remain in IVF labs (not implanted in the mother's uterus) & those are kept so they could be used to extract embryonic stem cells, how? at the 4^{th} or 5^{th} day of the "kept in lab" embryo's life, you get the blastocyst, which consists of inner & outer cell masses, any cell in the inner cell mass is a Pluripotent stem cell; which as we said can make all types of tissues, these pluripotent cells are extracted from the embryo, implanted and frozen in the lab. [Here we have a main ethical dissection; because people who support this argue that "why to dispose the excess IVF embryos while they can be used to treat sick people through embryonic stem cells?!", and till now there is no agreement about the best source of stem cells, may be it would be the cord blood, teeth cells or embryonic cells].

-we have to mention that the development & extraction of these pluripotent cells is easy, however, implantation of these stem cells in growth dishes is difficult; because they need so many nutritional requirements (different from glucose, amino-acids, cytokines, etc…) & these nutritional requirements were granted when scientists used mice fibroblasts; they implanted the embryonic stem cells on a layer of mice fibroblasts which made these embryonic stem cells survive for unknown reason(they don't know what these mice cells have provided for the survival of the embryonic stem cells), but these mice fibroblasts were engineered in a way that they are alive but can't divide.

▪**Advantages of Embryonic stem cells**:

-Main advantage is that they're **Pluripotent stem cells.**

- Immortal: supply endless amount of cells.

- Flexible: can make any type of body cells.

-Available: IVF clinics.

▪**Disadvantages of Embryonic stem cells**:

-Main Disadvantage is the ethical issue.

-Immune rejection because these Embryonic Stem cells have MHCs, but we can avoid this problem by **Therapeutic cloning.**

→The Dr believes that the future will be for the **Therapeutic cloning** to be the source of Embryonic Stem cells. You will be the source of the embryonic stem cells not the embryos of other people; by taking any somatic cell from your body (skin cell for example) and extract its 46-chromosomes and then put these 46-chromosomes of the donor in an ovum of the patient's (donor's) partner, which has been emptied from it's genetic material, now the ovum will think that it got fertilized (they foul it to think so), now, this ovum with the 46 chromosomes of the donor, will start dividing to give rise to an embryo (that has the donor's own genetic make-up, i.e. this embryo is a replicate of the donor).

- The point where ethical complains rise, is when this embryo is left to keep dividing until making up a whole complete being that's a replicate of the donor, SO this is where therapeutic cloning stops, it's only allowed for the embryo to keep dividing until the 4^{th} or 5^{th} day when it develops into the blastocyst, at that point, a pluripotent cell(s) is/ are extracted from the blastocyst and frozen.

- The whole world agrees that it's totally unethical to grow that embryo into a human clone.

- However, the debate is still there, whether from a country to another, or from a religion to another; for example, the Vatican's say that there is a human life even in the zygote, so you can't touch it, on the other side, there are other groups/ countries that actually support the whole idea to the furthest extremes & there are other countries like Jordan, that had a balanced point of view regarding therapeutic cloning (there's a new law that has been set about therapeutic cloning, with certain conditions, most important of which are the source of the ova, the kind of disease intended to be treated (whether there's another treatment for the disease, etc…) & the duration intended to keep the embryo dividing).

#Advantages of the Therapeutic cloning:

-No Immune Rejection because they're your own cells.

-You can get the embryonic stem cells; which are the most potent.

-No one's embryo (from fertilization) will be used.

"It's all about you being made again!!"

→The Dr said that there are ways to know if the cells are stem cells or they've started to differentiate (tests to identify the Stem cells).

Adult Stem Cells

#Sources of adult Stem cells (we have so many sources but the main ones are):

-Bone marrow (Hematopoietic stem cells) - Blood stream

-Umbilical cord blood

-Dental pulp of the tooth (deciduous teeth have stem cells that are one of the few stem cells found in adult tissue that are as near as possible to the pluripotent stage of the embryonic stem cells; you can make lots of tissues out of them).

→These are most commonly used because they are *accessible* .But remember every tissue has its own stem cells that have been there since the early formation of tissues during embryogenesis … Remember they're different types in different tissues.

-Hematopoietic stem cells of the bone marrow have been used for treating leukemia through bone marrow transplantation, so it's actually a form of stem cell therapy.

#Adult stem cell plasticity:

-stem cell from one adult tissue can generate different cell types of other tissues IF these cells are put in the right conditions &media resembling the tissue these cells are desired to differentiate to, i.e. adult stem cells are not as rigid as we think:

EX. Hematopoietic stem cells can make neurons in addition to their ability to make different blood cells.

- Due to this, some scientists now argue about the need of embryonic stem cells if the adult stem cells have the "plasticity" advantage.

-They were able to take a hematopoietic stem cell from the BM and implant it into an injured heart. Also they made bone cells, skin cells, cartilaginous cells ((trachea)) and cornea.

→ **No Immune Rejection here** (the main advantage for the Adult Stem Cells; because they're from your own tissue).

→ **Limited Potency** (the main disadvantage of the Adult Stem Cells; it's not easy to make any adult stem cell capable of producing other tissue cells). Also we add the limited availability to get some adult stem cells, like neural stem cells in comparison with hematopoietic stem cells which are easy to get.

Induced pluripotent stem cells (IPSCs)

- The one who invented it (Yamanaka) has got a Nobel Prize.

- Very promising technique.

- It's all about: Reprogramming the cells.

#All the cells in our body have the same genes, but the genes that will be activated will determine the type of the cell (skin cell, neural cell, etc…). So when the cell starts to differentiate, it will lose its interest in a large pool of the genes and only activate the genes that are important to its function and specialty. Each cell has what is called "**Master transcription factors"** and these master transcription factors are the key control of the cell's function and specialty, so if I can regain the control on these master transcription factor, I can open up the whole genes and retro differentiate the cell into a stem cell.

-The special thing about these master transcriptional factors is their ability to activate so many genes; so if I want to activate 25,000 genes, I do not need 25,000 Transcription factors, I only need these Master transcriptional factors.

#The most important Master transcriptional factors are:

-Oct-4 -Sox2

-Nanog

-We can add C-Myc but the main problem with C-Myc is that it's an oncogenic transcription factor.

→Some cells need 3 Master transcription Factors, some cells need 4 Master transcription Factors. So it's variable.

- Yamanaka added these Master transcriptional factors into the cells by integrating them into a plasmid/victor ((External foreign DNA)), then add the plasmid/Victor into the cell. After these Master transcriptional factors got translated into proteins, they retro-differentiated into stem cells.

-Another way used to integrate these Master transcription factors into the cell is to translate these factors' sequence outside the cell into proteins, then put the proteins themselves inside the cell. Why not integrate the genes themselves as Yamanaka did? Because in order to integrate the genes inside the cell, we need a victor, the vector has foreign DNA sequence (majorly the vector is a virus), so some people fear the risk of integrating part of a viral genome into the cell. This way (inserting proteins into the cell instead of the DNA) is safer; because once the Master transcription factors (as proteins) activate and retro-differentiate the cell, they will be lysed and broken down, so nothing foreign remained inside the cell after all…however, this procedure is more costly (synthesizing proteins) & the likelihood of transformation of the cells is less, but after all, it's safer.

-Imagine that you can make stem cells from lymphocytes, cells from buccal smear and skin cells by only activating these Master transcription factors… no need to do therapeutic cloning at all!

-They could make a whole mouse by this mechanism (through implantation of the retro-differentiating cells into a female mouse uterus). So you can reproduce yourself theoretically.

-The Dr talked here about the automated synthesis of the genes, you can sequence whatever gene that you want; since after the Human Genome Project (genomic mapping), sequences of all genes are known now.

-Also, the Dr talked about the Next generation sequencing (if you'd like to read about "Oxford Nanopore sequencing", it's very interesting)

Stem Cells For Neurological Disorders:

- Why the sudden urge to treat neural disorders by stem cells? Because the western world is an aging population, so the urge is in order to reduce the economical costs spent yearly for treatment of such disorders.

- What are the sources for the stem cells:

- Adult Neural Stem cells taken from the neural tissue itself ((Most common used)).

-Hematopoietic and mesenchymal stem cells: those are the main adult stem cells; i.e. most accessible ones, even mesenchymal stem cells are easier to make it differentiate to other types of tissues than hematopoietic stem cells.

- ***Umbilical cord blood cells.***

-***Embryonic stem cells*** ((not yet used)).

- ***IPSCs*** ((used on a minimal scale)).

→We Know that the neural tissue has limited capacity to regenerate itself to do limited repair in certain areas, however, there are two regions in brain where we can find concentrated adult neural stem cells, these cells are capable of repairing neural damages, so, these cells are taken from these regions (where they're present) & induced to proliferate in the lab & then put back in other regions of CNS where there's a noticeable damage, or –for another solution- in case of CNS damage, there's the use of mesenchymal stem cells (MSC), because it's even easier to make them neural cells than the use of hematopoietic stem cells.

→What it is the mechanism by which we will deliver the proliferated adult neural stem cells from the lab to the damaged area in the CNS?

- Through Blood {IV-Injection} is easier than the other ways, but it's not easy for the cells to reach their target.

- Direct injection into the CNS.

→ We have several options of how to deal with these stem cells before delivering them to the patient, & our choice depends on the type of disease I'm handling with: (graft types):

- Stem cell without editing.

-Genetically modified neural stem cell.

-Neural stem cells with scaffold or without.

→There's a comparison in the slides, between different types of stem cells; advantages, disadvantages, sort of diseases each type can be used for.

Diseases that can be treated by Neural Stem Cells:

- ***Spinal cord injury***; physical damage = trauma ((the most common disorder that can benefit from neural stem cells technology)), but we have some abuse here; crude experiments were done to the patients with no results, so we have to apply the main methodologies not our own methodologies. But also we have some patients who could walk again after the neural stem cell therapy (i.e. it's the only resort for patients with such injury, it may work & it may not).

-***Parkinson*** (Parkinson disease patients will greatly benefit from the neural stem cells; because the degeneration is localized; degeneration of substantia nigra, so, easily, the neural stem cells can be injected into substantia nigra).

-Alzheimer (widespread neuro-degeneration, so not all patients will benefit from this technology during the initial phase of therapy, besides, the earlier the stage of the disease, the more localized the damage, the better the results are… results depend on the stage of the disease).

-***Huntington disease***; good target for therapy, since it's a single gene disorder and it's localized (So we use neural stem cells with gene therapy; stem cells with genetic modifications).

- ***Stroke*** (to replace (functionally) the damaged areas in the brain by neural stem cells).

-Multiple sclerosis: trials are being held in UJH, & the initial results are promising ☺

→There are so many successful trials, but still, they can not make it as an official treatment.

Where can we find considerable amounts of these neural stem cells?

- Lateral ventricles (sub ventricular zone of lateral ventricles).

-Hippocampus (dentate gyrus).

- From these 2 areas, neural stem cells have been caught migrating out of their center; to repair distant damages!

→The Dr talked about the fact that the process of taking neural stem cells from a patient & inducing their proliferation & putting them back to the patient will not be a common future practice (taking the cells requires a head incision, so it's not a preferred procedure), however, the common future practice will be about the use of universal stem cells ((embryonic or most probably IPSCs)); these universal stem cells will undergo a complete removal of all antigenic determinants by certain companies, so that they can be used by anybody in this world, and then will be sold to hospitals/ centers with recipes to differentiate these universal stem cells into different mature cells.

-Also, there's a new developing technology where scientists can convert mature differentiated cells into other differentiated cells, without the need of retro-differentiation. This is more complicated; because for each conversion of the cell, we will need different mechanisms (different genes & different transcription factors).

Import ant debatable things to remember about the Stem Cells Therapy:

- Best type of stem cells to be used.

- Route of injection.

- Dose of injection (how many stem cells to be injected)

- Give one dose or multiple doses with an interval time in between.