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ABSTRACT:

Stem cells derived from the embryo (embryonic stem cells), bone marrow or umbilical cord blood (adult stem cells) has the potential to differentiate into many cell types in the human body. The embryonic stem cell are called "pluripotent" because they can become any of the body's 220 cell types including bone, muscle, skin, nervous tissue as well as immune cells. Those derived from bone marrow and cord blood are considered "multipotent" because they can differentiate only to specific families of cell types such as those of muscle and bone and also replenish mature cells within tissue. Despite the potential that they have in repairing or regenerating human tissue damaged by injury or disease, embryonic stem cells have been under a lot of criticisms due to ethical, religious as well as political considerations. The development of induced pluripotent stem cells (iPSCs) from adult stem cells, derived from mouse and man by cellular reprogramming seems to have produced embryonic-like stem cells without destroying the embryo-which critics say it is a deliberate attempt to take away human life because it contains human DNA. As promising as they seem the iPSCs would still have to pass the test of skepticism by certain stem cell scientists and screening tests of functionality when subjected to the usual development and applications cues. These prerequisites are needed before they can be certified as truly pluripotential and therefore could be applied in the treatment of human diseases particularly those that have defied cure such as Parkinson's disease, multiple sclerosis and insulin-dependent diabetes mellitus. Applications of stem cells in rejuvenating diseased or destroyed human parts are largely experimental because of various constraints. However, neurons have been generated from iPSCs made from the skin cells of patients with Parkinson's disease. There are also other success stories in the treatment of degenerative diseases particularly in Germany where stem cells have been employed in treating many patients suffering from diverse diseases. Even if development of iPSCs has not offered much at this time towards repairing of tissues damaged by disease, it has at least circumvented the technical complexities of cloning thus

avoiding most of the ethical and legal constraints associated with human embryo cell research. It has also refuted the old dogma that cells once differentiated cannot be dedifferentiated into primordial stage of development. Embryonic stem cells remain the gold standard in stem cell research while more studies are needed to gain better insight into the pluripotency of iPSCs. Whether derived from the embryo or adult cell, stem cells hold a huge promise in regenerative medicine in the near future.

Key words: Stem cells, research, regenerative medicine

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INTRODUCTION:

There is a long list of controversial topics in medical science today and this includes human cloning-an attempt to reproduce man(thus "imitating" God), cryonics-preserving a dead body waiting for an advance in science to bring it back to life (similar to cell preservation in liquid nitrogen) and euthanasia-mercy killing of terminally ill and helpless patient. Then stem cell research. All these are still under the scourge of political, as well as ethical criticism and bedeviled by superstition, religious and mystical beliefs. As useful as autopsy is today we learn that the first surgeon that performed a post mortem in Italy was publicly executed for interfering with a departed soul! [1] Resistance to blood transfusion by some religious persuasions is too well known to be mentioned and this has been underscored by the Grail message which states inter alia that : "if a different blood group, was used in the case of blood transfusion, then the soul living in such a body would find itself prevented from fully developing its volition, would perhaps be entirely cut off from it because with the blood of different composition the radiation also changes and is then no longer adapted to the soul." Be that as it may blood transfusion has continued to save lives [2]

The term "stem cell" was first proposed for scientific use by the Russian histologist, Alexander Maksimov in 1908. He postulated the existence of haemopoetic stem cells. Research in stem cells was born out of the observations of Ernest McCulloch and James Till of the University of Toronto in the 1960s. Thereafter many other scientists including Joseph Altman and Gopel Das started research on stem cells [3,4] Stem cells consist of two broad types of mammalian cells: Embryonic stem cells, isolated from the inner mass of blastocyts

and adult stem cells found in the adult tissue. The precursor of the embryonic stem cells is the totipotent (omnipotent) morula which can differentiate into many different cell types down to the pluripotent and the unipotent which can produce only one cell type (see figure)We also have oligopotent stem cells having ability to differentiate into only a few cell types such as lymphoid or myeloid cells.

Stem cells research is based mainly on embryonic cells which have the potential to differentiate into any of the 220 cell types in the human body including bone, muscle, skin and nervous tissue as well as blood cells. Because of this they are called pluripotent [5] Man is therefore a progeny (offspring) of stem cells and this is the central logic behind reparative or regenerative medicine using stem cells. One of Buddha's favorite quotations that say it all states "Believe nothing, no matter where you read it or who said it, even if I told you unless it agrees with your reason and common sense". It is therefore sensible to suggest that one day embryonic stem cells will be the answer for the repair of human tissue damaged either by injury or disease. There is of course adult stem cells which are considered "multipotent" because they can give rise only to specific families of cell types such as those in muscle and bone and replenish mature cells within a tissue [5]

RESEARCH:

Work in embryonic stem cells started over 30 years ago in mice particularly the phenomenon of "pluripotency" but it was not until 1998 with the first isolation of human embryonic stem cells that studies commenced in humans [5]. These cells are extracted from the embryo, in the early stage of its development, and cultured in petri dishes in the laboratory thus giving rise to embryonic stem cells. Their pluripotency has to be confirmed when they glow on addition of a fluorescent dye. An alternative source of stem cells has been identified as umbilical cord blood which is capable of differentiating into all blood cell types. Among the advantages is the ease of collection [6].

Embryonic stem cell research continues to be regarded by critics as a deliberate taking away of human life by destroying its embryo. Which they say contains human DNA. With the recent breakthrough in stem cell research this criticism will be a thing of the past soon. In 2006 Yamanaka and his colleagues of the University of Kyoto published a groundbreaking study in which they created what they called induced pluripotent stem cells (iPSCs) from the skin cells of mice [5]. As should be expected this has to pass the test of skepticism by other stem cell scientists. The good news is that

today thousands of them, using the formula developed by the Japanese researchers as a paradigm, are working to understand how they can translate the study and apply it in the treatment of human diseases that have defied treatment.

What did the Japanese scientists do? They reversed the development of mouse cells by returning them to the embryonic state! This is called cellular reprogramming. It is analogous to reversing the biological clock or what chemists call back titration. Mammalian cells under normal circumstances can only differentiate progressively with time but they never dedifferentiate or revert into a more primitive type. This was what the Japanese did by using a few select genes to change adult cells' identity and making them embryonic and therefore truly pluripotent! Before the ingenious approach of the Japanese scientists cellular reprogramming has been on but with a different technology.

This was done by injecting the genetic material from an adult cell into an egg devoid of its DNA. The resultant DNA-egg hybrid develops into an early- stage embryo from which pluripotent stem cell can be extracted. Dolly the sheep was created in 1997 by this nuclear transfer method otherwise known as cloning. Yamanaka and his colleagues circumvented this approach by directly changing adult cells into pluripotent cells without the use of eggs or embryos. Rather "they reasoned that introducing the genes normally active only in embryos into the adult cell might be sufficient to reprogram that cell into an embryo-like state"[5] They screened a total of twenty- four genes that are turned on in pluripotent cells but silent in adult cells and came up with four*: Oct 4,Sox2,Klf4, and c-Myc* that are actually necessary to produce iPSCs.They then used a modified retrovirus as a delivery vehicle to inject the four genes into the DNA of mouse skin cells.

Other researchers were able to replicate this study in mouse and human cells and even with the proteins encoded by the four reprogramming genes directly into the cells. One of such studies was conducted by Sommer and Mostaslavsky [7] in which they produced transgene-free induced pluripotent stem cells. Another approach that was contemplated was to reprogram body cells into iPSCs without using viruses just as the Japanese group circumvented the nuclear DNA-egg hybrid technique. Pioneers in this alternative approach used a cocktail of four drugs that can mimic the effect of the reprogramming genes but they were unable to generate pluripotent cells. So far about a dozen adult cell types have been reprogrammed into iPSCs from both man and animals.

Next to the promulgation of a new truth the second best thing I think scientists should do is to appraise their work in light of foreseeable problems. Konrad Hochedlinger of the Department of Stem Cell and Regenerative Biology at Harvard University has done that quite eloquently.

Some of the new pluripotent stem cells though look like embryonic stem cells microscopically, do not pass screening tests of functionality to which the embryonic stem cells have also been subjected. These tests are "the ability to produce a wide variety of body cells types in petri dish when exposed to the appropriate developmental cues; the ability of stem cells to produce a teratoma (a type of tumour containing cells from all embryonic tissue lineages) when injected under the skin of mouse; and the capacity, when injected into an early-stage mouse embryo, to contribute to the development of all tissue lineages, including germ cells, in the resulting new born mouse"[5] Another problem observed was that the retroviruses used to deliver the four reprogramming genes are not completely "silenced" or properly shut off, and the important genes in the cells' original DNA are not properly turned on.

This results in cells that have lost both sides: losing their identity without gaining their pluripotency. All viruses are intracellular pathogens and utilize the host genome for their replication. It is not therefore surprising that the Japanese group was able to discover that some of the mice that they injected with iPSCs in their developing embryos formed cancers as a consequence of DNA damage caused by a residual effect of retroviral activity. Even if all these problems are overcome another particular one that is glaring in the face is the issue of rejection of transplanted stem cells because of histo-incompatibility [8].

Therapy with stem cells

It was Paracelsus, renowned as father of pharmacology that aptly said that "all that man needs for health and healing has been provided by God and nature, the challenge of science is to find it."Applications of stem cells to treat or cure diseases are progressing in two areas: diseases modeling and cell therapy. The latter in mouse and the former in man [5].

What is being manipulated here is the potential ability of embryonic stem cells (ESC) and iPSCs to produce "spare parts" for organs or systems damaged by injury or disease as can happen in myocardial infarction, neurological disorder such as multiple sclerosis [9] or spinal cord injury, cancer, sickle cell anaemia or insulin-dependent diabetes mellitus. Recent studies have shown that delivering bone-marrow-derived stem cells to infarcted patients

could reduce mortality rates and clinical symptom, but improvement s in the myocardial delivery and stem cell optimization are required to realize this potential [10] Also by use of reprogrammed skin cells from mice into iPSCs it was possible to cure sickle cell anaemia in mice with iPSC –derived blood progenitor cells with corrected sickle cell anaemia genes [5]. The healthy precursors now produced normal red blood cells. A version of Parkinson's disease in rats has also been treated by transplanting iPSC-derived neurons, but not without complications for as much as 20% of them died from brain tumours (David Prentice, personal communication). In a span of ten years when Noseworthy and colleagues speculated that it may be possible to enhance remyelination by transplanting oligodendroglial precursor cells into discrete, clinically important lesions in multiple sclerosis [9]. Sundberg and coworkers have shown that both oligodendrocyte precursor cells and oligodendrocytes can be used in repairing myelin in multiple sclerosis and spinal cord injury [11].

Applications of stem cell in regenerating diseased or destroyed human parts are largely experimental because of various constraints already mentioned. Skin cells as well as blood cells are easily accessible and could be transformed into iPSCs and later into any cell type to be used as "repair kits" to cure disease. If that happens adult cells from patient can be turned into pluripotent cells and then tailored into desired cells into the ailing tissue of that patient. This is analogous to auto transfusion and therefore there will be a perfect match both genetically and immunologically. As good and promising as this technique sounds it is a daunting task because the precursor cells are difficult to grow or manipulate outside the body. However, neurons have been generated from iPSCs made from the skin cells of patients with Parkinson's disease. Cancers can be caused by stem cells gone berserk because they have lost contact inhibition. If that proves to be correct it should revolutionize cancer therapy by using replacement cell transplant. So far the best known stem cell therapy to date is bone marrow transplant used to treat leukaemia which is a cancer of white blood cells.

Doctors in the X-Cell Centre at the Institute of Regenerative Medicine in Germany have employed stem cells in treating more than 3500 patients suffering from diverse diseases. They treated patients with their own (autologous) adult stem cells. These included Alzheimer disease (a brain disorder causing intellectual impairment and memory loss), Liver disease, stroke,

multiple sclerosis, muscular dystrophy and cerebral palsy [12]. Also more than 1500 patients received either intravenous or subcutaneous transplantation of fetal stem cells for the treatment of stroke, epilepsy as well as multiple sclerosis [13]

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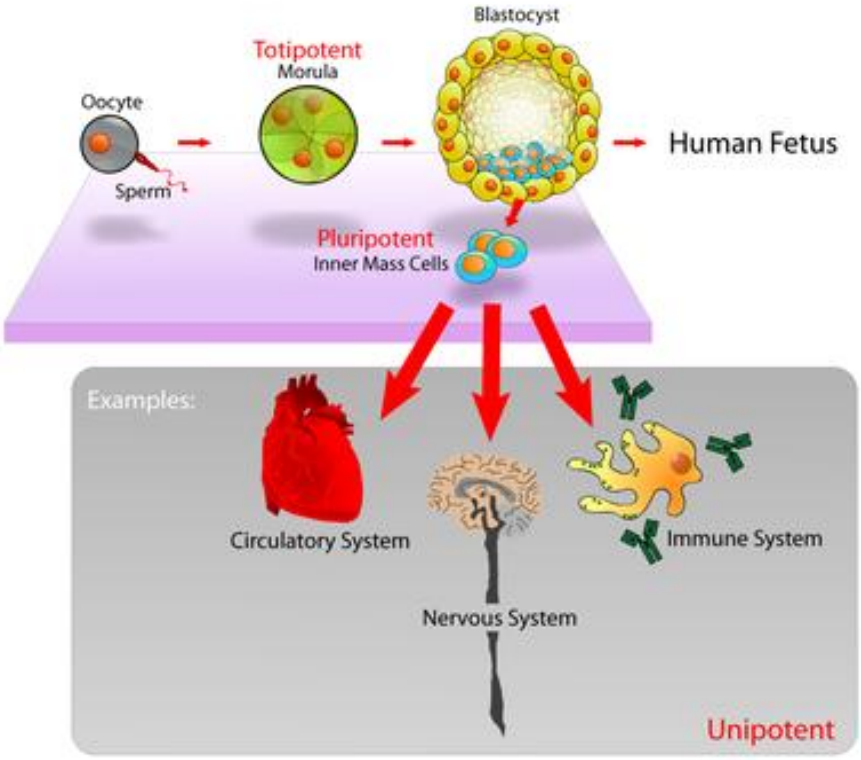
Figure: Diagram downloaded from Wikipaedia showing embryonic stem cells isolated from inner mass of blastocyts {www.Wikipaedia.com}



DISCUSSION AND CONCLUSION:

Embryonic stem cells (ESC) as well as iPSC research have a lot to offer in rejuvenating the body which hitherto has been done by people through cosmetic surgery, balanced nutritious diet and physical exercise. Unfortunately resentment towards ESC research is still on. Recently in the United States, following a federal judge's decision to temporarily block federal funding for embryonic stem cell research, the Obama administration appealed the judge's decision, and got the injunction lifted, because it issued guidelines in 2009 permitting the National Institutes of Health to conduct embryonic stem cell research and allowed federal funding for it. If this injunction is not lifted very promising research studies that are potentially life-saving for millions of people around the

world will be halted. The law suit was against National Institutes of Health and filed by researchers opposed to the use of ESC, a group that seeks adoptive parents for human embryos created by in-vitro fertilization and a non -profit Christian Medical Association. The ruling said that all ESC research involves destroying embryos, which violates the Dickey-Wicker Amendment which unambiguously prohibits the use of federal funds for all research in which human embryo is destroyed [14].

The good news is the discovery of iPSCs which circumvents the technical complexities of cloning and avoids most of the ethical and legal constraints associated with human embryo stem cell research. However the problems with reprogramming genes using retroviruses still remain. Because these viruses integrate themselves permanently and remain active in the host genome they can cause DNA damage which may trigger cancerous changes in the cells as mentioned earlier. Many scientists including researchers in Harvard University have developed methods that avoid permanent genetic manipulation of cells including the use of a modified type of adenovirus, which resides inside the cells for a short time enough to convert them into iPSCs, to deliver the four reprogramming genes mentioned earlier. It is believed that this discovery will be a stepping stone towards the application of virus-free and tumour-free iPSCs for human therapy- thus passing the test of quality control. Another source of encouragement is the umbilical cord blood which is an alternative source of haemoepoetic stem cells discovered in 1978. They have the advantage of higher chances of finding donors and lower risk to recipients since cord blood does not have to match recipient's tissue being immature. But its limitation is that it is only capable of differentiating into blood cell types including those of the immune system [6]. Also stem cells have been found in amniotic fluid. They are multipotent and can differentiate into cells of adipogenic (fat cells), osteogenic (bone cells), myogenic (muscle cells),endothelial, hepatic and neuronal cells (nerve cells) [15]. A Vatican paper " Osservatore Romano" called stem cells from the amniotic fluid the "Future of Medicine" because they can be harvested without destroying the embryo [16].

Two more hurdles facing ESCs or iPSCs are whether the conversion of body cells into iPSCs and the subsequent conversion of iPSCs into therapeutically relevant cell types can be made efficient enough for widespread use. The other hurdle is whether iPSCs retain any memory of the body cell type from which they are derived. This is reminiscent of anamnestic response exhibited by T and B lymphocytes. The

question here is, for example, will it be possible to produce a custom-made cardiac muscle tissue to replace the myocardium in case of myocardial infarct? The risk here is that a iatrogenic disease may ensue due to graft-versus-host reaction because of an immunological attack mounted by donor cells on the host lymphoid tissue [17]. The observation of Cowan [18] that ESC stored for a long time have been shown to create the type of chromosome anomalies that lead to cancer is yet another rider to worry about. I am aware that there is now a commercially marketed product made from human iPSCs-a heart cell line called iCell Cardiomyocytes intended for use by pharmaceutical companies to test the effect of heart drugs. But not to replace the damaged heart muscle yet.

Because of all the problems with iPSCs mentioned above, embryonic stem cells remain the reference point in stem cell research while more research is needed to gain more insight into the pluripotency of iPSCs and answer questions surrounding their applications in treating many human diseases. Having said that, the discovery of iPSCs by cellular reprogramming is a great breakthrough that has refuted the accepted dogma that cells once differentiated cannot be dedifferentiated into primordial stage. Critics of embryonic stem cell research-the bone of contention- have to rethink. Blastocyte-the stage before embryo's cells begin to differentiate and from which the so-called embryonic cells are derived has no human features and so this procedure cannot be tantamount to taking away human life. Also new stem cell lines already exist due to the common practice of in-vitro fertilization. Addressing the other unexpressed concern of the critics, advocates of embryonic stem cell research guarantee that new human life will not be created even for the purposes of experimentation- the type that created Dolly the sheep in 1997.

The concern is genuine because embryonic stem cells, theoretically speaking, can be conditioned to generate sperm and ovum to produce human embryo through the traditional in-vitro fertilization. Stem cells, whether from the adult or embryo, umbilicus or amnion, hold a huge promise in regenerative medicine in years to come.

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