REVIEW ARTICLE

A Review on the Discovery of Different Stem Cells and the Potential Therapies based on these Cells

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Abstract

In recent years, stem cell therapy has become a very talented and advanced scientific research topic. The progress of treatment methods has induced great potentials. This paper is a review focused on the discovery of different stem cells and the potential therapies based on these cells. The beginning of stem cells is tracked by laboratory steps of skillful stem cell culturing and origin. Quality control and teratoma development assays are essential procedures in assessing the properties of the stem cells tested. Derivation methods and the application of culturing media are crucial to set correct environmental conditions for controlled distinction. Among many types of stem tissue applications, the use of graphene scaffolds and the potential of extracellular vesicle-based therapies require consideration due to their versatility. Challenges summarize the review that stem cell therapy must overcome to be accepted wide-reaching. A wide variety of possibilities makes this cutting-edge therapy a turning point in modern medicine, providing hope for permanent diseases.

Keywords: Potentials, cutting-edge, Sream cell, Review.

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Introduction

Stem cells are unspecialized cells of the human body. They are able to distinguish into any cell of an organism and have the ability of self-renewal. Stem cells exist both in embryos and adult cells. There are several steps to specialization. Developmental potency is reduced with each step, which means that a unipotent stem cell is not able to separate into as many types of cells as a pluripotent one.

Totipotent stem cells are able to divide and discriminate into cells of the whole organism. Totipotency 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 grow either into any of the three germ layers or form a placenta. After approximately 4 days, the blastocyst's inner cell mass becomes pluripotent.

Pluripotent stem cells (PSCs) form cells of all germ layers but not extraembryonic structures, such as the placenta. Embryonic stem cells (ESCs) are an example. ESCs are consequential from the inner cell mass of preimplantation embryos. Another example is induced pluripotent stem cells (iPSCs) resultant from the epiblast layer of implanted embryos. Their pluripotency is a continuum, opening from completely pluripotent cells such as ESCs and iPSCs and ending on councils with less potencymulti-, oligo- or unipotent cells. One of the methods to evaluate their activity and spectrum is the teratoma formation assay. iPSCs are affectedly generated from somatic cells, and they function similarly to PSCs. Their culturing and utilization are very promising for present and future reformative medicine.

Multipotent stem cells have a thinner spectrum of differentiation than PSCs, but they can specify in separate cells of specific cell lines. One example is a haematopoietic stem cell, which can mature into several types of blood cells. After diversity, a haematopoietic stem cell converts an oligopotent cell. Its variation abilities are then restricted to cells of its lineage. However, some multipotent cells are capable of adaptation into dissimilar cell types, which suggests naming them pluripotent cells. Oligopotent stem cells can separate into several cell types. A myeloid stem cell is an example that can split into white blood cells but not red blood cells. Unipotent stem cells are considered by the narrowest differentiation capabilities and special property of dividing recurrently. Their latter feature makes them a promising candidate for therapeutic use in regenerative medicine. These cells are only able to form one cell type, e.g., dermatophytes.

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Stem cell biology

A blastocyst is designed after the fusion of sperm and ovum fertilization. Its inner wall is lined with passing stem cells, namely, embryonic stem cells. Blastocysts are collected of two distinct cell types: the inner cell mass (ICM), which develops into epiblasts and induces the development of a fetus, and the trophectoderm (TE). Blastocysts are responsible for the regulation of the ICM microenvironment. The TE continues to mature and forms the extraembryonic support structures needed for the successful origin of the embryo, such as the placenta. As the TE begins to form a specialized support structure, the ICM cells remain undifferentiated, fully pluripotent and proliferative.[1] The pluripotency of stem cells allows them to form any cell of the organism. Human embryonic stem cells (hESCs) are derived from the ICM. During the process of embryogenesis, cells form aggregations called germ layers: endoderm, mesoderm and ectoderm, each eventually giving rise to differentiated cells and tissues of the foetus and, later on, the adult animal.[2] After hESCs distinguish into one of the germ layers, they become multipotent stem cells, whose potency is limited to only the cells of the germ layer. This process is short in human development. After that, pluripotent stem cells occur all over the organism as indistinguishable cells, and their key abilities are

proliferation by the formation of the next generation of stem cells and distinction into specialized cells under certain physiological circumstances.

Recognition by morphological differences

The comparability of stem cell lines from different entities is needed for iPSC lines to be used in therapeutics.[3] Among critical quality procedures, the following can be eminent:

Short tandem repeat analysis—This is the comparison of specific loci on the DNA of the samples. It is used in measuring an exact number of repeating units. One unit consists of 2 to 13 nucleotides repeating many times on the DNA strand. A polymerase chain reaction is used to check the lengths of short tandem repeats. The genotyping procedure of source tissue, cells, and iPSC seed and master cell banks is recommended.

Identity analysis—The unintentional switching of lines, resulting in other stem cell line adulteration, requires rigorous assay for cell line documentation.

Residual vector testing—An appearance of reprogramming vectors integrated into the host genome is hazardous, and testing their presence is a mandatory procedure. It is a commonly used procedure for generating high-quality iPSC lines. An acceptable threshold in high-quality research-grade iPSC line collections is ≤ 1 plasmid copies per 100 cells. During the procedure, 2 different regions, common to all plasmids, should be used as specific targets, such as EBNA and CAG sequences.[3] To accurately represent the test reactions, a standard curve needs to be prepared in a carrier of gDNA from a well-characterized hPSC line. For calculations of plasmid copies per cell, it is crucial to incorporate internal reference gDNA sequences to allow the quantification of, for example, ribonuclease P (RNaseP) or human telomerase reverse transcriptase (hTERT).

Karyotype—A long-term culture of hESCs can accumulate culture-driven mutations.[4] Because of that, it is critical to pay additional attention to genomic integrity. Karyotype tests can be performed by resuscitating characteristic aliquots and culturing them for 48–72 h before harvesting cells for karyotypic analysis. If abnormalities are found within the first 20 karyotypes, the analysis must be repeated on a fresh sample. When this situation is repetitive, the line is evaluated as abnormal. Repeated abnormalities must be recorded. Although karyology is a crucial procedure in stem cell quality control, the single nucleotide polymorphism (SNP) array, conversed later, has approximately 50 times higher resolution.

Viral testing—When assessing the quality of stem cells, all tests for harmful human adventitious agents must be performed (e.g. hepatitis C or human immunodeficiency virus). This procedure must be performed in the e of non-xeno-free culture agents.

Bacteriology—Bacterial or fungal sterility tests can be divided into culture- or broth-based tests. All the procedures must be recommended by pharmacopoeia for the jurisdiction in which the work is performed.

Single nucleotide polymorphism arrays—This procedure is a type of DNA microarray that detects population polymorphisms by enabling the detection of subchromosomal changes and the copy-neutral loss of heterozygosity, as well as an indication of cellular conversion. The SNP assay consists of three components. The first is labelling fragmented nucleic acid sequences with fluorescent dyes. The second is an array that contains immobilized allele-specific oligonucleotide (ASO) probes. The last component detects, records, and finally interprets the signal.

Flow cytometry—This is a practice that utilizes light to count and profile cells in a heterogeneous fluid mixture. It allows researchers to accurately and rapidly collect data from heterogeneous fluid mixtures with live cells. Cells are passed through a narrow channel one by one. During light illumination, sensors detect light emitted or refracted from the cells. The last step is data analysis, compilation and integration into a comprehensive picture of the trial.

Phenotypic pluripotency assays—Recognizing undifferentiated cells is crucial in successful stem cell therapy. Among other characteristics, stem cells appear to have a distinct morphology with a high nucleus to cytoplasm ratio and a prominent nucleolus. Cells appear to be flat with defined borders, in contrast to differentiating colonies, which look as loosely located cells with rough borders.[5] It is important that images of ideal and poor-quality colonies for each cell line are kept in laboratories, so whenever there is doubt about the quality of culture, it can always be checked according to the representative image. Embryoid body formation or directed diversity of monolayer cultures to produce cell types representative of all three embryonic germ layers must be performed. It is important to note that colonies cultured under different conditions may have different morphologies.[6]

Histone modification and DNA methylation—Quality control can be achieved by using epigenetic analysis tools such as histone modification or DNA methylation. When stem cells differentiate, the methylation process silences pluripotency genes, which reduces differentiation potential, although other genes may undergo demethylation to become expressed.[7] It is important to emphasize that stem cell identity, together with its morphological characteristics, is also related to its epigenetic profile. [8,9] According to Brindley,[10] there is a relationship between epigenetic changes, pluripotency, and cell expansion conditions, which emphasizes that unmethylated regions appear to be serum-dependent.

Turning point in stem cell therapy

The turning point in stem cell therapy appeared in 2006, when scientists Shinya Yamanaka, together with Kazutoshi Takahashi, discovered that it is possible to reprogram multipotent adult stem cells to the pluripotent state. This procedure avoided endangering the foetus' life in the process. Retrovirus-mediated transduction of mouse fibroblasts with four transcription factors (Oct-3/4, Sox2, KLF4, and c-Myc)[34] that are mainly stated in embryonic stem cells could induce the fibroblasts to become pluripotent.[35] This new form of stem cells was named iPSCs. One year later, the experiment also succeeded with human cells.[36] After this success, the method opened a new field in stem cell research with a generation of iPSC lines that can be customized and biocompatible with the patient. Recently, studies have focused on reducing carcinogenesis and improving the transfer system.Retroviral-mediated transduction induces pluripotency in isolated patient somatic cells. Target cells lose their role as somatic cells and, once again, become pluripotent and can differentiate into any cell type of human body. The turning point was influenced by former discoveries that happened in 1962 and 1987.The former discovery was about scientist John Gurdon successfully cloning frogs by relocating a nucleus from a frog's somatic cells into an oocyte. This caused a complete reversion of somatic cell development.[37] It is even possible for a somatic cell to again acquire pluripotency.[38] The latter was a discovery made by Davis R.L. that focused on fibroblast DNA subtraction. Three genes were found that originally appeared in myoblasts. The enforced expression of only one of the genes, named myogenic distinction 1 (Myod1), caused the conversion of fibroblasts into myoblasts, showing that reprogramming cells is possible, and it can even be used to transform cells from one lineage to another.[39]

Directed differentiation

To be useful in therapy, stem cells must be changed into desired cell types as necessary or else the whole regenerative medicine process will be pointless. Differentiation of ESCs is crucial because undifferentiated ESCs can cause teratoma formation *in vivo*. Understanding and using signalling pathways for differentiation is an important method in successful regenerative medicine. In directed differentiation, it is likely to mimic signals that are received by cells when they undergo successive stages of development.[51] The extracellular microenvironment plays a significant role in controlling cell behaviour. By manipulating the culture conditions, it is possible to restrict specific differentiation pathways and generate cultures that are enriched in certain precursors *in vitro*. However, achieving a similar effect *in vivo* is challenging. It is crucial to develop culture conditions that will allow the promotion of homogenous and enhanced differentiation of ESCs into functional and desired tissues.

Regarding the self-renewal of embryonic stem cells, Hwang *et al*. [52] noted that the ideal culture method for hESC-based cell and tissue therapy would be a defined culture free of either the feeder layer or animal components. This is because cell and tissue therapy require the maintenance of large quantities of undifferentiated hESCs, which does not make feeder cells suitable for such tasks.

Most directed differentiation protocols are formed to mimic the development of an inner cell mass during gastrulation. During this process, pluripotent stem cells differentiate into ectodermal, mesodermal, or endodermal progenitors. Mall molecules or growth factors induce the conversion of stem cells into appropriate progenitor cells, which will later give rise to the desired cell type. There is a variety of signal intensities and molecular families that may affect the establishment of germ layers *in vivo*, such as fibroblast growth factors (FGFs);[53] the Wnt family[54] or superfamily of transforming growth factors—β(TGFβ); and bone morphogenic proteins (BMP).[55] Each candidate factor must be tested on various concentrations and additionally applied to various durations because the precise concentrations and times during which developing cells in embryos are influenced during differentiation are unknown. For instance, molecular antagonists of endogenous BMP and Wnt signalling can be used for ESC formation of ectoderm.[56] However, transient Wnt and lower concentrations of the TGFβ family trigger mesodermal differentiation.[57] Regarding endoderm formation, a higher activin A concentration may be required.[58,59] There are numerous protocols about the methods of forming progenitors of cells of each of germ layers, such as cardiomyocytes,[60] hepatocytes,[61] renal cells,[62] lung cells,[63,64] motor neurons,[65] intestinal cells,[66] or chondrocytes.[67]

Directed differentiation of either iPSCs or ESCs into, e.g. hepatocytes, could influence and develop the study of the molecular mechanisms in human liver development. In addition, it could also provide the possibility to form exogenous hepatocytes for drug toxicity testing.[68] Levels of concentration and duration of action with a specific signalling molecule can cause a variety of factors. Unfortunately, for now, a high cost of recombinant factors is likely to limit their use on a larger scale in medicine. The more promising technique focuses on the use of small molecules. These can be used for either activating or deactivating specific signalling pathways. They enhance reprogramming efficiency by creating cells that are compatible with the desired type of tissue. It is a cheaper and non-immunogenic method.

One of the successful examples of small-molecule cell therapies is antagonists and agonists of the Hedgehog pathway. They show to be very useful in motor neuron regeneration.[69] Endogenous small molecules with their function in embryonic development can also be used in *in vitro* methods to induce the differentiation of cells; for example, retinoic acid, which is responsible for patterning the nervous system *in vivo*,[70] surprisingly induced retinal cell formation when the laboratory procedure involved hESCs.[71]

The efficacy of differentiation factors depends on functional maturity, efficiency, and, finally, introducing produced cells to their *in vivo* equivalent. Topography, shear stress, and substrate rigidity are factors influencing the phenotype of future cells.[72] The control of biophysical and biochemical signals, the biophysical environment, and a proper guide of hESC differentiation are important factors in appropriately cultured stem cells.

Stem cell utilization and their manufacturing standards and culture systems

The European Medicines Agency and the Food and Drug Administration have set Good Manufacturing Practice (GMP) guidelines for safe and appropriate stem cell transplantation. In the past, protocols used for stem cell transplantation required animal-derived products.[73] The risk of introducing animal antigens or pathogens caused a restriction in their use. Due to such limitations, the technique required an obvious update.[74] Now, it is essential to use xeno-free equivalents when establishing cell lines that are derived from fresh embryos and cultured from human feeder cell lines.[75] In this method, it is crucial to replace any non-human materials with xeno-free equivalents.[76] NutriStem with LN-511, TeSR2 with human recombinant laminin (LN-511), and RegES with human foreskin fibroblasts (HFFs) are commonly used xeno-free culture systems.[33] There are many organizations and international initiatives, such as the National Stem Cell Bank, that provide stem cell lines for treatment or medical research.[77]

Stem cell use in medicine

Stem cells have great potential to become one of the most important aspects of medicine. In addition to the fact that they play a large role in developing restorative medicine, their study reveals much information about the complex events that happen during human development.

The difference between a stem cell and a differentiated cell is reflected in the cells' DNA. In the former cell, DNA is arranged loosely with working genes. When signals enter the cell and the differentiation process begins, genes that are no longer needed are shut down, but genes required for the specialized function will remain active. This process can be reversed, and it is known that such pluripotency can be achieved by interaction in gene sequences. Takahashi and Yamanaka[78] and Loh *et al*.[79] discovered that octamer-binding transcription factor 3 and 4 (Oct3/4), sex determining region Y (SRY)-box 2 and Nanog genes function as core transcription factors in maintaining pluripotency. Among them, Oct3/4 and Sox2 are essential for the generation of iPSCs.

Many serious medical conditions, such as birth defects or cancer, are caused by improper differentiation or cell division. Currently, several stem cell therapies are possible, among which are treatments for spinal cord injury, heart failure,[80] retinal and macular degeneration,[81] tendon ruptures, and diabetes type 1.[82] Stem cell research can further help in better understanding stem cell physiology. This may result in finding new ways of treating currently incurable diseases.

Haematopoietic stem cell transplantation

Haematopoietic stem cells are important because they are by far the most thoroughly characterized tissue-specific stem cell; after

all, they have been experimentally studied for more than 50 years. These stem cells appear to provide an accurate paradigm model system to study tissue-specific stem cells, and they have potential in regenerative medicine.

Multipotent haematopoietic stem cell (HSC) transplantation is currently the most popular stem cell therapy. Target cells are usually derived from the bone marrow, peripheral blood, or umbilical cord blood.[83] The procedure can be autologous (when the patient's own cells are used), allogenic (when the stem cell comes from a donor), or syngeneic (from an identical twin). HSCs are responsible for the generation of all functional haematopoietic lineages in blood, including erythrocytes, leukocytes, and platelets. HSC transplantation solves problems that are caused by inappropriate functioning of the haematopoietic system, which includes diseases such as leukaemia and anaemia. However, when conventional sources of HSC are taken into consideration, there are some important limitations. First, there is a limited number of transplantable cells, and an efficient way of gathering them has not yet been found. There is also a problem with finding a fitting antigen-matched donor for transplantation, and viral contamination or any immunoreactions also cause a reduction in efficiency in conventional HSC transplantations. Haematopoietic transplantation should be reserved for patients with life-threatening diseases because it has a multifactorial character and can be a dangerous procedure. iPSC use is crucial in this procedure. The use of a patient's own unspecialized somatic cells as stem cells provides the greatest immunological compatibility and significantly increases the success of the procedure.

Stem cells as a target for pharmacological testing

Stem cells can be used in new drug tests. Each experiment on living tissue can be performed safely on specific differentiated cells from pluripotent cells. If any undesirable effect appears, drug formulas can be changed until they reach a sufficient level of effectiveness. The drug can enter the pharmacological market without harming any live testers. However, to test the drugs properly, the conditions must be equal when comparing the effects of two drugs. To achieve this goal, researchers need to gain full control of the differentiation process to generate pure populations of differentiated cells.

Stem cells as an alternative for arthroplasty

One of the biggest fears of professional sportsmen is getting an injury, which most often signifies the end of their professional career. This applies especially to tendon injuries, which, due to current treatment options focusing either on conservative or surgical treatment, often do not provide acceptable outcomes. Problems with the tendons start with their regeneration capabilities. Instead of functionally regenerating after an injury, tendons merely heal by forming scar tissues that lack the functionality of healthy tissues. Factors that may cause this failed healing response include hypervascularization, deposition of calcific materials, pain, or swelling.[84]

Additionally, in addition to problems with tendons, there is a high probability of acquiring a pathological condition of joints called osteoarthritis (OA).[85] OA is common due to the avascular nature of articular cartilage and its low regenerative capabilities.[86] Although arthroplasty is currently a common procedure in treating OA, it is not ideal for younger patients because they can outlive the implant and will require several surgical procedures in the future. These are situations where stem cell therapy can help by stopping the onset of OA.[87] However, these procedures are not well developed, and the long-term maintenance of hyaline cartilage requires further research.

Osteonecrosis of the femoral hip (ONFH) is a refractory disease associated with the collapse of the femoral head and risk of hip arthroplasty in younger populations.[88] Although total hip arthroplasty (THA) is clinically successful, it is not ideal for young patients, mostly due to the limited lifetime of the prosthesis. An increasing number of clinical studies have evaluated the therapeutic effect of stem cells on ONFH. Most of the authors demonstrated positive outcomes, with reduced pain, improved function, or avoidance of THA.[89,90,91]

Rejuvenation by cell programming

Ageing is a reversible epigenetic process. The first cell rejuvenation study was published in 2011.[92] Cells from aged individuals have different transcriptional signatures, high levels of oxidative stress, dysfunctional mitochondria, and shorter telomeres than in young cells.[93] There is a hypothesis that when human or mouse adult somatic cells are reprogrammed to iPSCs, their epigenetic age is virtually reset to zero.[94] This was based on an epigenetic model, which explains that at the time of fertilization, all marks of parenteral ageing are erased from the zygote's genome and its ageing clock is reset to zero.[95]

In their study, Ocampo *et al*.[96] used Oct4, Sox2, Klf4, and C-myc genes (OSKM genes) and affected pancreas and skeletal muscle cells, which have poor regenerative capacity. Their procedure revealed that these genes can also be used for effective regenerative treatment.[97] The main challenge of their method was the need to employ an approach that does not use transgenic animals and does not require an indefinitely long application. The first clinical approach would be preventive, focused on stopping or slowing the ageing rate. Later, progressive rejuvenation of old individuals can be attempted. In the future, this method may raise some ethical issues, such as overpopulation, leading to lower availability of food and energy.

For now, it is important to learn how to implement cell reprogramming technology in non-transgenic elder animals and humans to erase marks of ageing without removing the epigenetic marks of cell identity.

Conclusion

After several decades of experiments, stem cell therapy is becoming a wonderful game changer for medicine. With each experiment, the capabilities of stem cells are growing, although there are still many obstacles to overcome. Regardless, the influence of stem cells in regenerative medicine and transplantology is immense. Currently, permanent neurodegenerative diseases have the possibility of becoming treatable with stem cell therapy. Induced pluripotency enables the use of a patient's own cells. Tissue banks are becoming progressively popular, as they gather cells that are the source of regenerative medicine in a struggle against present and future diseases. With stem cell therapy and all its regenerative benefits, we are improved able to prolong human life than at any time in history.

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