REVIEW ARTICLE

Stem cell research, regenerative medicine and challenges

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Abstract

Stem cell research and regenerative medicine have exponentially evolved in the past two decades. With the ability of cell renewal, unlimited expression and differentiation, stem cell research can potentially transform medical practice. Stem cells have different sources of origin and a wide range of potency ranging from unipotent to totipotent. Stem cell therapy can modify behavior of cells, generate differentiated tissues, and be used as a pharmacological intervention. Regenerative medicine is an emerging medical endeavor dedicated to reconstructing and repairing of tissues and organs. Several challenges are to be addressed to allow transition of stem cell research from basic sciences to clinical practice. These obstacles include selection of appropriate stem cells with associated ethical concerns, manufacturing and processing of stem cells, genetic instability, lack of complete understanding of their mode of action at target sites, economic concerns, and lack of regulations of stem cell therapy.

Key words: Medicine, Stem Cell, Totipotent, Transplantation.

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Introduction

Since 1950s, work involving stem cells and organ regeneration started with the first attempts at bone marrow transplantation in animal models.[1] With the isolation of the first human embryonic stem cells in 1998, an outbreak of research into stem cells has taken place with the hope and optimism that this research will provide a significantly transform current medical practices.[1] Stem cells have the ability of self-renewal, unlimited expression, and have the potential to generate mature cells of multiple lineages through multipotent differentiation capabilities. [2] Stem cell research is focused not only on finding therapies but understanding the biology and pathogenesis of diseases [2] The application of stem cells has particularly been advanced in the fields of haematology, oncology, ophthalmology, dermatology, and orthopedics.[1] This review aims to describe different types of stem cells, their administration, their role in regenerative medicine and challenges in their clinical application.

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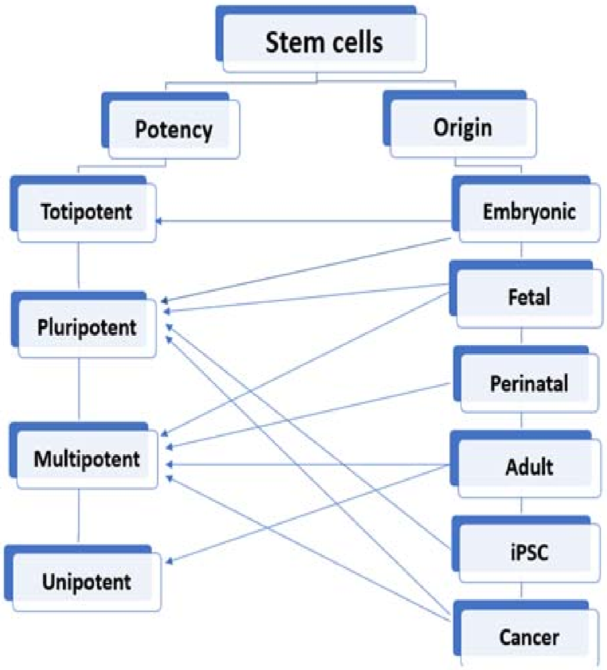

Figure-1: Stem cell classification

Classification of stem cells

Stem cells can be classified based on their origin and potency. (Figure 1)

Based on potency, stem cells can be divided into;[2]

a) *Totipotent cells* can produce all cell types including both embryonic and extra-embryonic tissues.

b) *Pluripotent cells* can form cells of the embryo proper, including germ cells and all cells derived from 3 primary germ layers.

c) *Multipotent cells* can form cells originating from a single germ cell layer.

d) *Unipotent cells* can differentiate into cells of a particular type only.

In terms of origin, stem cells are of 3 major types:[3]

1. Human embryonic stem cells (ES) are undifferentiated cells, derived from the pre-implantation blastocyst.[4] These are pluripotent cells and have not only high proliferative capabilities but can differentiate into lineages of all 3 primary germ cell layers i.e., Ectoderm, Mesoderm and Endoderm and thus potentially can form all cell types.

2. Somatic (adult) stem cells are undifferentiated cells within the differentiated tissues and organs.[5] They are particularly important in tissues with high cell turnover such as blood and intestine and provide the basis for tissue maintenance and regeneration in response to injury.[2] Mesenchymal stem cells (MSC) are extensively studied somatic stem cells derived from hematopoietic and mesenchymal tissues (fat, muscle, skin, brain etc.). Under certain micro-environmental conditions, they can differentiate into multiple cell types including types other than their tissue of origin i.e., trans-differentiation.[6] For example, Haematopoietic Stem Cells (HSC) can generate liver cells and Nerve Stem Cells (NSC) can generate haematopoietic precursors.[6]

Bone marrow represents a major source of its derived adult stem cells containing both HSC and MSC [7] and has been most widely investigated and best understood population of adult stem cells. Mesenchymal stem cells have 'Homing capacity' i.e., the ability to migrate to inflamed/ injured tissue or tumour site by modulating the immune system reaction, by releasing growth factors and by having paracrine effect to create a suitable micro-environment.[6] This property can be used for targeted treatment of various clinical conditions.

3. Induced pluripotent stem cells (iPSC) are adult somatic cells which can be reverted to pluripotent stem cells. In 2006, Takahashi et al.[8] were able to transform adult differentiated cells (by using certain transcription factors (Oct 4, Sox 2, Klf 4 and Myc), derived from genes expressed in pluripotent ES cells) into highly immature cells resembling ES cells in terms of morphology and differentiation potential. This process is now called as 'reprogramming'. (Figure 2).

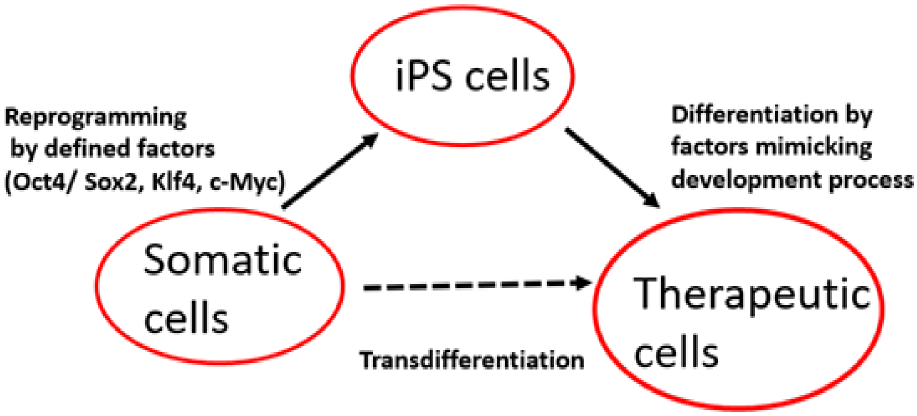

Figure-2: Strategies for inducing cells for therapeutic use; Differentiation,Trans differentiation and Reprogramming.

In clinical medicine, the role of stem cells has evolved in 3 ways;[5]

- Stem cell therapy to modify behaviour of other cells or to replace cell lines (lost or damaged by ageing, diseases, genetic modification, injury etc.) with healthy ones.
- Stem cells to generate differentiated tissues for studying in-vitro disease models to improve molecular understanding of diseases and their treatment.
- Stem cells as targets of pharmacological intervention and drug therapy.

Routes of administration of stem cells

Local injection and transplantation of stem cells has been effective in models of chronic inflammatory and degenerative diseases. For cancer models, intravenous administration of stem cells is given; however, systemic administration can lead to potential problems as stem cells can get localized in pulmonary capillary bed leading to clogging and impaired gas exchange, respiratory distress, and haemodynamic compromise.[4]

Regenerative medicine

Stem cells have the ability to self-renewal and differentiation into different cell lines. In recent times, these characteristics of stem cells have been utilised as active research in Regenerative Medicine (RM). The research work in RM is dedicated to the reconstruction and repair of tissues and organs damaged by various congenital disorders, malignancies, and trauma.[3] Biological therapies, stem cells, genes and medical devices are used for regeneration. This field is different from conventional treatment as the focus is on maintaining the function of an organ or tissue rather than controlling the process of illness. The development of iPS cell technology by reprogramming has also stimulated scientists to describe various pathways involved in organogenesis.[4]

Tissue engineering (TE) combines the field of cell biology and material science with the aim of generating tissues and organs to be used for regeneration, reconstruction, and replacement in the areas of unfulfilled clinical need. This is particularly important when conventional reconstruction is not suitable or where transplantation facilities are not available due to technical considerations &/ or limited availability. (9) The biomaterials used for TE can be divided into naturally derived materials, natural acellular (biologic) scaffolds and synthetic materials[9] which provide 3-D structure supporting cell engraftment and tissue growth.

Due to scarcity of organ donation, RM offers an alternative, viable substitute by using stem cells for tissue engineering. Using autologous stem cells generated materials, the problem of tissue rejection and/ or use of immunosuppressants with their unwanted side effects can be avoided.

Challenges in Clinical Application of Stem Cell and RM

Selection: There are numerous challenges towards stem cell treatment such as selection and choice of stem cells i.e., embryonic, adult or induced. Additionally, ethical, and political debates have raised in both scientific and public forums on the use of embryonic stem cells and destruction of human embryos after their harvestment.[1] The issue of development of 'human embryonic stem cells (hESC) lines' from 'excess' embryo found in in vitro fertilization (IVF) clinics has also been rigorously criticized.[10] However, this issue has largely been mitigated due to the availability of inducible (somatic) pluripotent stem cells.

Isolation, manufacturing, and pharmacokinetics

Another challenge is identifying and properly isolatiing of stem cells from a patient's tissues. Although not very common, immunological rejection, is also a barrier to successful stem cell transplantation. The manufacturing process of stem cells is challenging and demanding to ensure that the 'product' is consistent, fully characterized, safe and free from contamination.[11] Quality control assays are important to assess properties of the stem cells tested.[2] The pharmacokinetic behavior of stem cells must be fully analyzed in pre-clinical studies.[12] Stem cells are highly sensitive to biopharmaceuticals and low - molecular- weight drugs. Harvesting and culturing protocols for stem cells, optimal dose & route of administration, frequency of treatment and metabolic memory of stem cell are concerning problems. The food and drug administration (FDA) issued guidelines in 2017 regarding use of stem cells which are now classified as drugs and are subject to scrutiny with the aim to establish a 'clear and modern' framework for regenerative medicine.[13]

Mechanism of action

For a particular condition, the exact mechanism of action of stem cells is not fully known. The proposed mechanism includes cell contact, paracrine signalling systems, neo-vascularization and differentiation into a particular cell type. Stem cells secrete growth factors to aid their function the environments where stem cells go initiate a specific differentiation pathway.[14] Adult multi-potent stem cells have several disadvantages due to the lack of unique cell surface markers, preventing the isolation of homogenous, well-defined populations of stem cells.[15] The integration of transplanted cells into the surrounding tissues, particularly in complex areas such as brain and heart is also challenging.[2]

Genetic instability

Adult stem cells also risk of epigenetic modification and senescence, which could alter the fate of these cells.[16] Stem cells can exhibit genetic instability with a risk of chromosomal aberrations and subsequent tumorigenesis. [16] Teratoma formation has been reported in immunodeficient mice transplanted with pluripotent stem cells.[17] It is also difficult to define the genetic integrity of the manipulated cells; however, detailed genetic analysis can overcome this problem prior to start of any cell-based therapy. [18] Cancer stem cells can form a nascent tumour or can facilitate growth of preexisting tumors.[18] Risk of mutagenesis by integration of viral genome into iPS is also a concern as Retro-virus and Lenti-virus based re-programming of these cells is being carried out.[19] For significant medical conditions, the potential benefits of stem cell therapy may outweigh the risks, but for less severe diseases, this could be a major concern.

Stem cell regulation

Regulation of stem cell therapy and products is very crucial. Despite a lack of robust evidence, many stem cell clinics offer poorly scrutinized dubious therapies without clear understanding and unrealistic hopes.[9] The only stem cell therapies approved by FDA in the US are haematopoietic progenitor cells to treat blood and immune disorders and certain cancers. Holoclar R, an eye stem cell-based therapy was approved in Europe for cornea damage while others stem cell therapies have remained experimental.[20] The hype regarding stem cell therapy has resulted in serious scientific frauds and in the last decade many scientific papers published in leading medical journals like Lancet have been retracted.[21] To overcome some of the issues, Lancet commission on Stem cells and regenerative medicine has made an executive summary and has put forward some recommendations.[9] (Table 1)

Table-1: Lancet commission recommendations for stem cell and regenerative medicine. [9]

• Rigorous experimentation, evaluation, and reporting of preclinical animal work to allow smooth transition to Phase 2 and beyond.
• Investment by institutions to develop good medical practice facilities, transition from preclinical to clinical work and making clinical trials affordable.
• Priority setting for making stem cell and gene therapy more cost effective and achievable.
• Creation of international register of cell and biological experimental interventions with securing sustainable funding from FDA and European medicines agency.
• Trials reporting policy should be developed by international committee of Medical Journal Editors for stem cell research.
• Review of experimental stem cell therapy and RM under relevant social and regulatory context.
• Appropriate use of dissemination forums including internet and social media for communicating stem cell research to public including source of funding and publications.

Cost efficacy is another concern as funding for large and robust clinical trials is difficult to find. [22] Stem cell therapy or bio-engineered products have not been proven to be economical and/ or better alternatives to conventional treatment.

Stem cell and RM; Prospects in Pakistan

Developing countries like Pakistan are facing issues precluding the use of stem cell technology. These include poor socioeconomic status & financial limitations, lack of governmental support, lack of skilled human resources, ethical issues and absence of regulatory body and policies. There is also lack of international collaboration and partnership by the private sector.

The Aga Khan University's 'centre for regenerative medicine and stem cell research' (CRM) was established in 2016 as a pioneer in initiating research of stem cell biology and tissue regeneration in Pakistan. [23] This centre contributes research capacity building in collaboration with University of California San Francisco (UCSF). State-of-the-art facilities are available to study biology of stem cells, stem cells niche and different disease models including cancer and tissue repair. The centre is also striving to develop economically viable diagnostic methods and therapies.

Conclusion

In recent years, significant research has explored the value of stem cells in various clinical conditions with promising results. However, their therapeutic applications have progressed very slowly. Although the results of various trials are encouraging, several challenges, as highlighted above remain, preventing wider clinical utilization of stem cells in current medical practice. However, the future looks bright and stem cell therapy will find its place in the managing various medical conditions. It seems likely that stem cell therapy protocols will become the standard of care in medical practice.

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