Transplantation of Fetal Stem Cells: a New Horizon for Treatment of Degenerative Diseases

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Abstract

Background: The purpose of the current study was to present an overview of different types of stem-cells and their application for treatment different degenerative disorders with specific focus on ongoing clinical trials.

Methods: For the purpose of the current narrative review article, a comprehensive search was carried out on the existing literature in Google Scholar, PubMed and Scopus using the keywords: stem-cell (fetal and mesenchymal), regenerative. Relevant articles published from 1957 to 2013 are extracted and presented.

Results: During the past decades, different types of stem-cells (including adult and fetal) have been used for treatment of a wide range of immunologic (Severe Combined Immunodeficiency, Di George syndrome), neurologic (Parkinson's disease, Huntington Chorea, Cerebral Palsy), musculoskeletal (ALS), and cardiovascular diseases (heart failure and cardiomyopathies) as well as chronic and acute ulcers, and diabetes.

Conclusion: The results of our study demonstrated that during the past decades, stem-cell technology has been applied for treatment of a wide range of degenerative disorders with considerable success. The current ongoing clinical trials clearly demonstrate a great potential and a promising future for the technology in terms of offering curative treatment for a wide range of hitherto-incurable diseases.

Keywords: Stem cell, Transplantation, Treatment, Review Article

Introduction

Stem cells are undifferentiated cells with the potential of proliferation, regeneration, and transformation into differentiated cells, which results in tissue production. It is demonstrated that their plasticity or potency is hierarchical which ranges from totipotent (capability of differentiation into all cell types) to pluripotent (capability of differentiation into cells which are placed in fetal layers), multipotent (capability of differentiation into cells of specific categories in fetal layers), and unipotent (capability of differentiation into only one type of cell) (1).

Stem cells can broadly be classified into three different categories based on the time they are isolated during ontogenesis: embryonic, fetal and adult stem-cells (2). Embryonic stem cells (ESC) can be isolated from the inner cell mass of the embryo in pre-implantation phase. It is noteworthy, however, that safety concerns in terms of tumorigenicity of ES cells are quite intense. Adult stem cells can be found in almost all adult tissues and have been the focus of great interest in terms of their therapeutic potential for degenerative disorders in the recent years. Adult stem cells can be

also generally classified into two different categories: multi-potent and unipotent. Multi-potent stem cells, themselves, are categorized into hematopoietic stem cells (HSCs) which differentiate into blood cells and mesenchymal stem cells (MSCs) that give rise to bone, fat, cartilage and muscle (3).

Application of fetal stem cells for therapeutic purposes is a novel and promising treatment option in different fields of medicine. Fetal stem-cells can be derived either from the fetus itself or supportive extra embryonic structures (not from fetal origin) such as umbilical cord blood (UCB), amniotic fluid (AF), Wharton's jelly, amniotic membrane andplacenta which are all disposed following birth. These types of stem-cells are greatly favored for transplantation purposes because of their being extra embryonic, containing of a considerable volume of tissue mass, and possibility of easy access for future uses. Moreover, their harvesting is not hindered by ethical concerns and legal limitations which other types of stem-cells are faced with (4).

The primitive origin, potential for differentiation, and no reported risk of tumorigenicity make fetal stem cells an attractive option for treatment of different degenerative medical conditions. However, considering the novelty of the technology, there exist little evidence regarding different aspects of the potentials and risks involved in their application. Therefore, to address the current scarcity of literature this review article aims to shed more light on the characteristics of fetal stem cells as well as their extraction methods and the opportunity they provide for treatment of different degenerative diseases.

Fetal tissues stem cells

Fetal stem cells can be harvested from fetal blood and bone marrow tissues as well as other fetal organs such as the liver and kidney. Fetal stem cells are generally classified into two major categories: Hematopoietic Stem Cell (HSC) and Mesenchymal Stem Cells (MSC). Both fetal HSCs and MSCs are considered advantageous over their adult counterparts because of their superior intrinsic homing and engraftment, greater multipotentiality

and lower immunogenicity. Moreover, application of fetal stem cells is less ethically controversial in comparison with embryonic stem cells and they have demonstrated superior differentiation potentials in compared to adult stem cells (5).

Fetal Mesenchymal stem cells (MSC)

Mesenchymal stem cells (MSC) are multipotent non-hematopoietic cells capable of differentiation into different mesenchymal lineages, including adipose tissue, bone, cartilage, muscle, stromaland also nerve cells both in vitro and in vivo. They were first identified in adult bone marrow where they constitute approximately 0.001–0.01% of total nucleated cells. Fetal MSCs can be isolated from fetal blood, liver and bone marrow (6), as well as kidney (7), pancreas (8) and lung (9). MSCs are formed in the fetal blood from early gestation time (first trimester) when they constitute approximately 0.4 per cent of total nucleated cells. It is demonstrated, however, that the numbers of MSCs decline to very low levels after 13 weeks of gestation (6). MSCs which are derived from fetal bone marrow also decline with age to the extent that one MSC is found among 10000 in mid-trimester compared with one MSC per 250 000 cells in adult marrow (10).

It is demonstrated that adult MSCs show increased immunosuppressive characteristics by the mechanism of decreasing alloreactivity as well as contribution to formation of cytotoxic lymphocytes (in vitro). The immunosuppressive potentials of the first trimester fetal MSC are lower although may be induced with interferon (INF γ). Adult and fetal MSCs differ in their expression of alloantigens despite the fact that they both express HLA class I. In adult MSCs, however, intracellular deposits of HLA class IIare present as well and cell surface expression which may be facilitated by the application of INF γ to the cells. Fetal MSCs, on the other hand, contain no intracellular HLA class II and require seven days of INF γ exposure for de novo synthesis of it and consequent cell surface expression. However, neither fetal nor adult MSC appear to be inherently immunogenic as lymphocytes are incapable of recognizing them (11).

Hematopoietic or endothelial differentiation associated markers are not expressed by MSCs. However, according to their site of derivation there might be some differences in expression of extracellular matrix protein. Fetal MSCs are demonstrated to be CD45-, CD34-, CD14-, CD31- and vWF negative. Moreover, many different adhesion molecules including CD44, VCAM-1 and CD29 are expressed by them and they are uniformly positive for intracellular markers such as fibronectin, laminin, vimentin and for mesenchymal markers such as SH2, SH3 and SH4 when they are in their undifferentiated state (5, 6).

In contrast to adult bone marrow MSCs, first-trimester fetal blood, liver and bone marrow MSCs express baseline levels of the pluripotency stem cell markers such as Oct-4, Nanog, Rex-1, SSEA-3, SSEA-4, Tra-1-60 and Tra-1-81. It is demonstrated that regardless of their tissue of origin, first-trimester fetal MSCs regenerate themselves in a faster pace in culture in comparison with adult MSCsand undergo aging process later while maintaining their phenotype. In addition, it is demonstrated that fetal MSCs, generally, possess longer telomeres, have greater telomerase activity and express more human telomerase reverse transcriptase (12).

MSCs cells express α2, α4 and α5β1 integrin profile which renders them superior in terms of homing and engraftment, and therefore, demonstrate significantly higher binding potential to their respective extracellular matrix ligands in comparison with adult MSCs (3). In terms of cell surface markers, significant differences have been demonstrated among fetal-tissue cells. For instance, fetal lung tissue has a higher level of CD34$^+$/CD45$^-$ cells compared to bone marrow, spleen and liver. Moreover, there exist differences among the cells in regards with to differentiation capacity. For instance, MSCs derived from fetal liver during the first and second trimester demonstrate a reduced osteogenic differentiation potential in comparison with the first-trimester fetal blood as well as with second-trimester spleen, lung and bone marrow MSCs. This might be due to a reduction in the number of osteogenic progenitors (13). These findings cumulatively indicate a considerable degree of heterogeneity among different fetal stem cell population.

Fetal Hematopoietic Stem cells (HSCs)

HSCs are multi-potent stem cells that perform their functional haematopoiesis by generation of all hematopoietic lineages throughout fetal and adult life. During ontogeny, hematopoiesis accures in the yolk sac and then are transferred to spleen and liver to eventually located in the bone marrow (14). HSC originates from haemangioblasts, which is suggested to be the common precursor for blood and endothelial cells. Fetal HSC can be obtained from blood, liver and bone marrow. Because of cells migration from the fetal liver to fetal bone marrow, the number of circulating HSCs increases from the first trimester to reach a peak in the second trimester *in utero*. These cells are characterized by CD34 and CD45 markers which they present on their surfaces. Moreover, the number of CD38- and HLA-Dr-cells within the CD34Cpopulation is demonstrated to be higher in early fetal blood which indicates that these cells are more primitive and may have greater potential than HSC circulating later in ontogeny (5). It is also demonstrated that first-trimester fetal blood contains greater proportions of primitive CD34$^+$ cells in comparison with term gestation blood (15).

Extraembrionic stem cells

Umbilical cord blood stem cells: Umbilical cord blood (UCB) has been advocated as a rich source of hemopoietic stem cells (HSCs) as well as mesenchymal stem cells (MSC). Hemopoietic and progenitor stem cells produce more effective hemopoiesis (in term of their treatment potential), both for malignant and non-malignant disorders (16). Approximately, 1% of the mononuclear cord blood cells express the CD34 antigen which is demonstrated to be the most important marker for hemopoietic stem cells. In spite of the fact that HSCs from cord blood have been successfully expanded *ex vivo* prior to transplantation procedure, because of their relatively limited capacity for homing and engraftment, they have generally exhibited limited long-term engrafting potential (4).

Similar to the cord blood MSCs, HSC derived from cord blood also express neural proteins which enables them to differentiate into neural cells (17) rendering them suitablefor treatment of neurological disorders (18). The second major cell population of cord blood is UCB-MSCs although its frequency is too low (16). UCB-derived MSC, however, have the potential to support the in- vivo expansion of HSCs and function as an accessory cell mass for engraftment (19).Cord blood stem cells, which express baseline levels of ES cell markers such as Oct-4, Nanog and SSEA-4, have also been reported (Zhao et al. 2006). Subpopulations of CXCR4+, CD133+, CD34+, Lin2- and CD45 - cells were also isolated and enriched using a two-stage isolation approach. These cells have been described as very small embryonic-like (VSEL) stem cells because they are only 3–5 mm in diameter (20).

Wharton's jelly stem cells: These stroma cells can be isolated from the mucoid connective tissue surrounding the two arteries and the single vein of the umbilical cord. MSCs derived from Wharton's jelly can express a typical pattern of mesenchymal (not hematopoietic) markers and can differentiate into adipocytes, osteogenic and chondrogenic cells, cardiomyocytes, neurons and glia (19) and dopaminergic neurons (21).

Amniotic membrane stem cells: Amniotic membrane is derived from the epiblast by day 8, and is consisted of three layers: inner epithelial layer, which is formed of epithelial cells, termed amniotic epithelial cells (AEC), an acellular basement membrane in the middle, and an outer layer juxtaposed to the chorion consisting of mesenchymal cells called amniotic membrane mesenchymal stromal cells (AM-MSC). Amniotic epithelial cells are known to have unique characteristics, such as low-level expression of major histocompatibility complex antigens, and lower restricted differentiation potency. The differentiation of the AEC to the neural lineage is well documented and explained.

These cells express various stem cell surface markers, which are usually found on pluripotent stem cells such as embryonic stem cells. It is noteworthy that in the culture, AE cells can differentiate into all cell types from three germ layers (22). However, it should be noted that these amnion stem cells, in contrast to human ESCs, do not lead to formation of teratomas*in vivo* (at least for the 10-week period of monitoring) (23).

The outer layer of amniotic membrane has recently been demonstrated to be a rich source for MSCs.In vitro, it is demonstrated that these cells are plastic-adherent, spindle-shaped cells which can produce fibroblast colony-forming units and display a specific pattern of cell surface antigens. Although they do not express the hematopoietic markers, they express different levels of CD90, CD73, CD105, CD29, CD44, CD49d, CD49e, CD56, and CD166.These cells are capable of differentiation into several cell lineages such as neurogenic, osteogenic, chondrogenic, and adipogenic cells (24).

Placenta stem cells: The placenta provides an ample and valuable source of stem cells with different potencies. The expression of CD105/endoglin/SH-2 as well as many other MSC markers—including SH-3, SH-4, and a number of integrins and matrix receptors on placenta derived MC, along with a fibroblastoid morphology, plastic-adherance nature, and mesodermal differentiation capabilities, is highly suggestive that these cells are similar to MSCs from the bone marrow. It should be noted that in addition to MSC markers, three cell surface markers—SSEA-4, TRA-1-61, and TRA-1-80—found only in ESCs and embryonic germ cells, are also found on Placenta MC (25).

Amniotic fluid (AF) stem cells: The population of cells in AF is heterogeneous which originate from all three germ layers and are consisted of different partially differentiated cell types with progenitor characteristics. These cells are mainly epithelial which are derived from either the developing fetus or the inner surface of the amniotic membrane. The cellular composition changes of AF changes during gestation coincide with maturation of the fetus (4).

Various types of stem cells have been isolated and characterized from amniotic fluid. These cells include those found before 12th weeks of gestation which express the hematopoietic marker CD34

(26) and mesenchymal features. Multipotent mesenchymal stem cell which can be isolated from AF show the ability to proliferate *in vitro* more rapidly in comparison with fetal and adult cells. In spite of their high proliferation rate, these cells are of normal karyotype when expanded *in vitro* and are not tumogenic *in vivo* (27).

About 1 per cent of the cells present in second-trimester amniotic fluid are c-kit-positive (CD117) cells. They can be cultured without feeders, double in 36 h, are not tumorigenic, and possess long telomeres and maintain a normal karyotype for more than 250 population doublings. Human AFS cells, in culture, are positive for ES cell (e.g. Oct-4, Nanog and SSEA-4) and mesenchymal cell markers such as CD90, CD105, CD 73 as well as several adhesion molecules (28).

Fetal tissue stem-cell transplantation procedures

Fetal tissue can be provided following spontaneous abortion, stillbirth, or surgery for ectopic pregnancy in obstetrics and gynecology departments. Such tissue may also be derived from elective abortions. The fetal tissue obtained may be processed and utilized for transplantation in the form of a cell suspension, which is usually injected intravenously or intraperitoneally. It can be transplanted into predefined implantation sites in surgery as well (29).

After obtaining the tissue, it is necessary to accurate define and implement all procedures and resources in a quality management and assurance system including extensive validation, process control and documentation (30, 31). Accordingly, MSCs categorized as advanced therapy medicinal products (ATMPs) by the European Medicines Agency (EMEA) must undergo a manufacturing process in compliance with the current Good Manufacturing Practices (cGMP) as a quality assurance system in order to meet the sterility, safety, potency, quality, and all of preset specifications required (31).

Moreover, for regulation of cell-based products, a risk-based approach is taken by the FDA to in two classes: minimally manipulated (cryopreservation, thawing, density gradient isolation, washing,

and dilution) and effectively or optimally manipulated (ex vivo expansion and genetic modification). For minimally manipulated production, it is necessary that the process follows the current Good Tissue Practice (cGTP) guidelines. On the other hand, for optimally process, cell manufacturing need to also follow the cGMP guidelines in addition to cGTP (32). cGMP includes all stem cell manufacturing requirements such as organization, facilities, machines and instruments, man power and staff, standard operating procedures (SOPs), environmental control and monitoring, equipment maintenance, supplements and reagents, process controls, validation, labeling and tracking design and control, storage requirements, documentation and records, non-conformances and complaints management, risk management, reporting and reviewing, audit plan, education and training (33, 34). It is noteworthy however, that labeling, coding, and traceability system must be implemented in the cGMP facility (35).

Clinical applications of stem-cell therapy

Stem-cell therapy has been utilized for treatment of different disease. In hematologic disorders, this technique has been used as a curative intervention for different types of post-radiation leucemia, aplastic anemia with quite favorable outcomes (36-49). Moreover, several types of impairments of the immune system have been successfully treated with the use of stem-cell technology. Severe Combined Immunodeficiency for instance, which is characterised with a genetic impairment in B-cells and T-cells, has been successfully treated by stem-cell transplantation of liver-derived fetal stem cells of 8 weeks embrio. In several different studies, similarly, favoralbe outcomes are reported from application of stem-cell technology using a suspen-sion of cells extracted from liver or thymus fetal tissue (8, 9, 10 weeks) with different number of cells ($6*10^8$, $9*10^7$) which not only resulted in improvement in the function of the immune cells, but also contributed to antibody production and secretion.

Di George syndrome is a genetic disorder causing malfunction of immune-related T-cell function which leads to recurrent eposiodes of infection. Several studies have demonstrated that transplant-

ation of stem-cell harvested form 13 to 16 weeks fetus can result in significant improvement in T-cell function (50-54).

Stem-cell therapy has also been applied for treatment of different degenerative neurological disorders such as Parkinson's disease. Parkinson is a progressive impediment of CNS which is charcterized by destruction of mesencephalic dopaminergic neurons via an obscure mechanism which results in motor malfanction. From 1993, utilization of stem-cell technology has been the focus of manyclinical trials desinged for treatment of Parkinson's disease in animal studies (55, 56). Subsequently, several studies have been carried out with the primary objective of treatment of Parkinson's disease using fetal mesensephalic dopaminergic neurons with different degrees of favorable therapuetic outcomes such as reported significant improvements in tremor and diskenisia. Moreover, findings of imaging techniques have demonstreated persistent fetal graft in the recepient which further supports the success of the procedure. Currently, different ongoing clinical trials are investigating the effectiveness of stem-cell technologyfor treatment of neurological disorders (57-75). Huntington Chorea is another field where therapeutic application of stem-cell transplantation is being investigated (76-78). Cerebral Palsy (CP) has also been the focus of great attention in terms of application of olfactory ensheating and neural progenitor cells in children suffering form the disease. In this procedure, stem-cells were injected into the lateral ventricles of the patients and significant motor development followed after the first month of the intervention. By the end of the first year, gross motor, fine motor, and cognitive function of the intervention group signifiantly improved without any reported side effect.

Stem-cell transplantation is also applied for treatment of Hereditory Cerebral Atrophy. In several studied, stem-cells were extracted from cerebellum of 8-10 weeks fetus and were subsequently implanted in dentate nuclei of the patients using stereotactic procedure. In 3, 6, 12 and 24 months follow up, significant improvements were observed in an slowly increasing rate

(79). In head truma, stem-cells extracted from neurons and hematopoetic tissues are used in patients with low Glasgow Coma Scale. The results indicated 2.5times superior outcomes in patients who undervent the procedure in comparison with the control group.

ALS is yet another musculoskeletal disorder in which application of stem-cells is studied with the use of the spinal cord derived cells using microinjection method (a phase 1 study). The therapeutic outcome of this procedure is still under investigation (80, 81).

Moreover, transplantation of another type of neural stem-cells (fetal olfactory ensheating glia) is utilized for treatment of patients with spinal cord injuries. In these patients, considerable improvement in sensory function was reported 14 months after the first injection (82). Pigmentosa Retinitis is a hereditary degenerative disease which leads to severe visual defects as a result of progressive degeneration of the rod phosphoreceptor cells in retina. Therapeutic potential of application ofof fetal stem-cells is investigated for treatment of the condition which following confirmation of safety, the efficacy is under investigation (83). In several other occular disorders such as macular dystrophy, the effectiveness of stem-cell transplantation is also under investigation.

In heart failure, application of a cell suspension derived from the fetus is investigated with some considerable improvements in Exercise Tolerance Test (84). Further studies are being carried out to demonstrate neomyogenesis in cardimyopathies with heart failure presentation.

The application of fetalliver-derived stem-cells are also studied in diabetes and its safety is demonstrated (85).

The effectiveness of stem-cell therapy for treatment of chronic and acute ulcers are also investigated. In a phase 1 safety study, these cells were used for treatment of leg ulcer where fetal cells are used in the form of fetal biological bandage. No side effects are reported form application of stem-cells for treatment of any type of ulcer. In genetic investigations, the presence of these cells in fibroblasts of the ulcer is reported (84).

Osteogenesis Imperfecta is another field where stem-cell therapy is applied in both prenatal and postnatal patients. In these patients, Mesenchymal stem-cell suspension have demonstrated favorable clinical outcomes (86).

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Conclusion

As discussed in details, transplantation of fetal stem-cells can be considered as one of the most representative benchmarks of the progress made in the field of stem-cell therapy considering the significant attainments and low level of controversy surrounding the issue. It is expected that, in the future, in countries where there exist no ethical and legal challenges to stem-cell therapy such as Iran, this novel treatment option will flourish and there is the possibility that technology will be widely used for treatment of different diseases. Obviously, this necessitates financial and technical for ongoing research. In this regard, selection of patients in certain stages of the disease when the intervention is most likely to be effective is of utmost importance.

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