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MESENCHYMAL STEM CELLS AS AN ATTRACTIVE CANDIDATE FOR EX-VIVO GENE TRANSFER AND REGENERATIVE CELL THERAPY

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ABSTRACT Mesenchymal stem cells (MSCs) possess exceptional abilities of HLA independent universal acceptance, tumor tropism, immunomodulation, ability to be available off-the shelf and flexibility of expression of transgenic proteins whenever desired. MSCs also secrete regenerative and anti-inflammatory factors and MSC-secretome is one of the highly studied emerging therapeutic agents which has led to utilization of conditioned media as a therapeutic agent. MSCs have been studied for the treatment of neurological diseases, cardiovascular ailments, immunological diseases, metabolic disorders and metastatic cancers. It is important to look at the decade long journey of MSCs as therapeutic agents so that the future directions can be confirmed. Takeda pharmaceutical company (after acquisition of TiGenixInc) has successfully received approval in Europe for its first mesenchymal stem cell therapy Alofisel® for the treatment of complex perianal fistulas in Crohn's disease. Mesenchymal stem cells also offer one of the best platforms for the administration of ex-vivo gene therapy as well as exceptional opportunities for development of multifaceted cell therapies for multiple diseases. In this review article, we have attempted to provide a bird's eye view of overall development of MSCs as a therapeutic approach for various diseases including cancer.

KEYWORDS : Mesenchymal Stem Cells, gene therapy, ex-vivo gene transfer, cell therapy, conditioned media, regenerative therapy.

INTRODUCTION

Cell and Gene therapy are the advanced medicinal approaches of the 21st century. Mesenchymal stem cells have been established as immunomodulatory cells that can be obtained from multiple sources with relative ease, which have ability to home to the sites of inflammation and have not shown any oncogenic risk even after use in thousands of patients in several clinical studies and after approval of multiple products worldwide (for example Prochymal®, Alofisel®, Temcell®). Gene therapy (therapy by expression of a therapeutic gene) can be administered in two fundamental ways; in-vivo or ex-vivo. In-vivo gene transfer is conducted directly by virtue of exposing the patient's body to the vectors of gene therapy. There are multiple risks associated with the in-vivo gene transfer; namely immune reaction, minimal control over cell transformation, and uncertainty of efficacy. Ex-vivo gene transfer minimizes the risks associated with immune reaction, provides exceptional control over the gene transfer process and also provides opportunity to test the expression and effectiveness of the gene transfer process. Mesenchymal stem cells offer one of the most ideal candidates for ex-vivo gene transfer as they can express therapeutic proteins, have immunomodulatory properties avoiding immune rejection, and are able to deliver targeted therapies by virtue of homing ability to the disease sites. In this review, we will focus on the use of MSCs with or without genetic enhancement and use of conditioned media derived from MSCs in the treatment of neurological diseases, immunological disorders, cardiac diseases, metabolic diseases gastrointestinal diseases and hematological diseases. In this review, we also address the use of gene modified MSCs as targeted therapies for metastatic cancers.

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) are multi-potent stromal cells that are able to differentiate and mature into different types of cells like osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells), adipocytes (fat cells which give rise to marrow adipose tissue) and even been shown to differentiate into neuron-like cells [1]. The differentiation potential of MSCs is summarized in Figure 1.

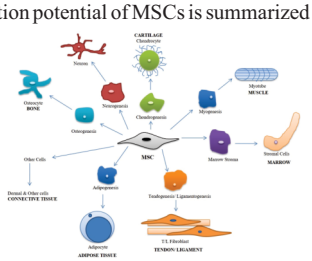

Figure 1: The multipotent differentiation potential of Mesenchymal stem cells. Mesenchymal stem cells are multipotent and possess the ability to proliferate and different cell types based on the environmental condition. MSC can differentiate into osteoblastic, chondrogenic, myogenic, smooth muscle, neurogenic differentiation, adipogenic, fibroblast, stromal cells and other cells connective tissues.

The discovery of MSCs dates back to the early 1900's. Initially in 1924, Alexander Maximow identified them as the precursor cells within mesenchyme that developed into different types of blood cells. Then, Ernest McCulloch and James Till first revealed the clonal nature of marrow cells in the 1960s. An ex vivo assay for examining the clonogenic potential of multipotent marrow cells was later reported in the 1970s by Friedenstein [2]. MSCs have unique ability to be derived from bone marrow cells as well as from other non-marrow tissues, such as placenta, umbilical cord blood, adipose tissue, adult muscle, corneal stroma or the dental pulp of deciduous baby teeth. MSCs are relatively smaller than fibrocytes and are quite difficult to differentiate in histological sections because they appear to have no difference from fibroblasts. The cell body contains a large, round nucleus with a prominent nucleolus, which is surrounded by finely dispersed chromatin particles, giving the nucleus a clear appearance. The remainder of the cell body contains a small amount of Golgi apparatus, rough endoplasmic reticulum, mitochondria and polyribosomes. The cells, which are long and thin, are widely dispersed and the adjacent extracellular matrix is populated by a few reticular fibrils but is devoid of the other types of collagen fibrils [3]. MSCs are plastic adherent cells and express surface markers CD73, CD90 and CD105, STRO-1 and lack of expression of hematopoietic markers CD14, CD34, CD45, CD11b, CD19 and class II human leukocyte antigen – DR [5,6]. MSCs have been the focus of tissue engineering applications and regenerative therapy in part due to their potential to induce tissue repair and unique immunomodulatory capacity. Regardless of the source of the MSCs, their study provides the basis for the emergence of a novel and powerful therapeutic technology of self-repair or self-renewal for the lifetime of an organism by harnessing the healing capacity of these cells. Any organism loses the renewal capacity of stem cells as the age of the organism increases. This is clearly seen from the observation that the capacity of stem cells to proliferate and differentiate decreases with the age of the donor, as well as, the time spent in culture [7]. However, a MSCs can be delivered as allogeneic as well as autologous agents for multiple clinical conditions. MSCs have long been tested in clinical trials for multiple human conditions such as hematological

diseases, graft-versus-host disease, organ transplantation, diabetes, inflammatory diseases, hepatic autoimmune or inflammatory conditions, chronic renal disease, pulmonary as well as cardiovascular conditions, degenerative diseases of bone and cartilage, neurological diseases, or autoimmune diseases [8]. MSCs can also be delivered together with other natural and synthetic biomaterial scaffolds which provide three-dimensional surrounding environment that provide a mechanical support to promote cell adhesion, migration and differentiation in vivo, all of which improve the bioavailability of MSCs for imparting the regenerative potential in vivo. MSCs have tendency to home to the damaged and injured tissues mediated via the inflammatory cytokines, adhesion molecules, and matrix metalloproteinases (MMPs). Such homed MSCs interact with the local stimuli and inflammatory cytokines that leads to their own subsequent stimulation to produce beneficial growth factors involved with tissue regeneration [9,10]. More recently preclinical studies have shown that MSC functionalization in vitro through cell priming drastically increases their immunomodulatory, reparative, trophic and overall regenerative capacities in vivo [6]. The immunomodulatory effect of MSCs has been summarized in Figure 2.

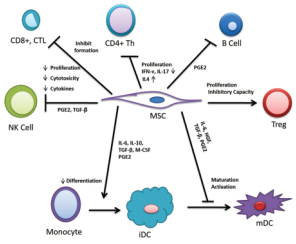

Figure 2: MSCs impart immunomodulatory effects on various cells.

MESENCHYMAL STEM CELLS–UNIQUE CELL THERAPY AGENT

It has been more than two decades since MSCs were first tested as the agents for cell therapy in human subjects in 1995 by Hillard Lazarus [11,12]. Since then several trials have been conducted to evaluate the functional potential in human population for various diseases [8]. Despite of worldwide efforts in establishing the MSCs as a regenerative therapy, MSCs have only received conditional approval in 2012 to treat children with graft versus host disease (GvHD) in Canada, New Zealand and Japan (under various trade names) after successful phase III clinical trial by Osiris Therapeutics (United States) for Prochymal derived from bone marrow from healthy donors (Martin et al, 2010). In India, an ex-vivo cultured adult allogeneic MSC therapy has been conditionally approved in 2017 for the treatment of Critical Limb Ischemia. South Korea has multiple MSC products derived from different human sources for the treatment of Amyotrophic Lateral Sclerosis (ALS), acute myocardial infarction, knee cartilage defects such as traumatic articular cartilage and degenerative arthritis and rheumatoid arthritis (https://alliancerm.org/available-products/). In March, 2018 the European Commission MSC pharmaceutical product, Alofisel was approved to treat Crohn's-related enterocutaneous fistular disease. MSCs have also been explored as the vehicle to carry the modified molecular agents. Preconditioning and genetic modification of MSCs has shown great benefit in augmenting MSC-based therapeutic approach [6]. Tumor tropism of MSCs results from crucial molecular mechanisms that regulate the mobilization of MSC to tumors. Tumor tropism of MSCs offers an exceptional 'targeted therapy delivery mechanism (TTDM)' and has led to attempts of generation of modified MSCs equipped with the enhanced ability to delivery of specific anti-cancer agents to develop an effective therapy against cancer [13]. MSCs can be genetically modified virally or non-virally to overexpress therapeutic proteins that synergistically or complementarily potentiate their innate properties. MSC can also be primed with non-peptide based drugs or magnetic nanoparticles for improved efficacy and precise targeting [14].

MESENCHYMAL STEM CELLS AND THEIR USES

In this review article, we have attempted to discuss and highlight the therapeutic benefits of not only MSCs but also conditioned media derived from MSCs, for various human diseases with emphasis on the mechanism through which they impart their effects. Furthermore, we have shed some light on the current regulatory and ethical aspects related to MSCs as pharmaceutical products.

a.Neurological diseases

Neurological disorders are diseases that affect the central or the peripheral nervous systems where the function of the brain, spinal cord, peripheral nerve or neuromuscular system is impaired. Although there are more than 500 neurological diseases, the most common ones are stroke, brain tumors, migraine, multiple sclerosis and spinal cord disorders. Many of the neurological diseases have no definitive cure. Due to their unique ability to decrease inflammation, repair damaged tissue via neurotrophic factors and paracrine activity, capacity to transdifferentiate into neural, glial and astrocytic-like cells in vitro, ability of homing and tumor tropism and immunomodulation, the MSCs and other stem cell based therapies are focus of multiple exploratory studies.

Several preclinical studies for neurological diseases have validated the neuroprotective and neuo-regenerative role of MSCs. MSCs lead to improvement in the cognitive function, decline in the neuronal loss and overall increase in the survival of animals[15]. The number and scope of MSC-based clinical trials continues to demonstrate that the beneficial results of MSCs are reproducible. The strategies to enhance the neuroprotective effects via genetically modified MSCs are on the rise as long-term engraftment of MSCs has shown varying success[16,17]. The neuroprotective effects of MSCs on immunomodulation and remyelination are largely mediated by the paracrine signals induced by the MSCs, and several secreted soluble molecules; TGF-β1, IFN-γ, indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2. MSCs can also promote an anti-inflammatory effect through promotion of proliferation of T-reg cells. In addition, MSCs also positively modulate the functions of astrocytes, oligodendrocytes, and neuronal axons[18,19].

Moving the discussion to specific neurological diseases, we can look at the case of Parkinson's disease (PD), which is a long-term degenerative disorder of the central nervous system that mainly affects the motor system. PD is a neurodegenerative disorder that affects predominately dopamine-producing ("dopaminergic") neurons in substantia nigra. In a 6-OHDA- induced Parkinson's disease model, damage in the striatum was not prevented by MSCs but the MSC therapy significantly improved the behavioral stereotypes triggered by apomorphine. This indicated that injected MSCs induced functional compensatory changes in the nigrostriatal system possibly through modulation of the responsiveness of striatal neurons to dopaminergic stimulation[20]. Riecke at el performed a meta-analysis to estimate the treatment effect of unmodified MSCs on behavioral outcomes in preclinical studies of PD and concluded that the intravenous administration and higher dose of MSCs had a greater effect on limb function and MSCs improved the behavioral outcomes in PD models[21].

Second prominent example of neurological diseases is stroke, which is a major cause of death and long-term disability worldwide. Cell-based therapies improve neural functional recovery in pre-clinical studies and clinical trials for stroke where results are encouraging. MSCs based interventions with and without genetic modification limit the brain damage and functional deficits after ischemic stroke through the secretion of trophic, protective, neurogenic and angiogenic factors[22,23]. Multiple proteins have been considered as targets of transgenic expression to genetically enhance the neuro-regenerative capacity of the MSCs. The MSCs enhanced by expression of one or more factors like brain-derived neurotrophic factor (BDNF), angiopoietin-1, ciliary neurotrophic factor (CNTF), vascular endothelial growth factor (VEGF) and Neurogenin-1 have shown exceptional therapeutic benefits in animal model for stroke by inducing secretion of various trophic factors, enhancing angiogenesis and decreasing infarct volume, leading to overall improvement in the recovery of neurological function[24,25].

Multiple sclerosis (MS) is another example of leading neurological disorders. It is a demyelinating disease where myelin sheath covering the spinal and cerebral neurons is progressively damaged. Therapy with MSCs mediates immunomodulatory, immunosuppressive, neurotrophic, and repair-promoting properties and thus improves the disease condition. MSCs have been proposed as a treatment for autoimmune diseases, including MS, because of their immunomodulatory properties and neural repair function. In an Experimental Autoimmune Encephalomyelitis (EAE) induced MS model, MSCs also increase number of inflammatory myelin-specific Th1 cells and astrocytes as well as an increased number of

inflammation inhibiting Th2 cells, oligodendrocytes and neurons[26,27]. Similarly, intrathecal as well as intravenous treatment of MSCs in in-vivo models of Amyotrophic Lateral Sclerosis (ALS), demonstrated delay in the death of compromised motor neurons, decrease in the inflammatory response and prolonged survival of the diseased animals[28]. In a spinal cord injury (SCI) model, damage to the spinal cord causes loss of motor, sensory or autonomic functions in parts of the body served by the spinal cord below the level of the lesion. Current treatment of SCI is limited to early administration of high dose steroids to reduce the harmful effect of post-injury cord edema and to reduce the cascade of secondary delayed SCI. In the animal models of SCI, MSCs-based therapy provided both structural and functional benefits. The structural benefits included stabilization of the blood-spinal cord barrier (BSCB), axonal sprouting/regeneration, and remyelination. The therapy with MSCs was explored in a clinical trial involving the local administration of autologous bone marrow derived MSCs in subjects with the chronic SCI. In 50% of patients, there was augmentation in sexual function and sphincter control along with reduction in spasticity due to on-going improvement in the intra-lesional motor activity evident by neurophysiological studies[29]. A recent study utilized the advanced cell transplantation technology in which three-dimensional complexes of brain-derived neurotrophic factor (BDNF)-MSC spheroids were transplanted at SCI at the thoracic level (Th9) in mice and led to a significantly enhanced recovery of hind-limb motor function mostly due to synergistic effect of BDNF and the anti-inflammatory factors secreted by the MSCs in acute phase of SCI[30].

b.Immunological diseases

Immunological disorders are diseases or conditions caused by a dysfunction of the immune system. Some examples of the immunological disorders are allergy, asthma, autoimmune diseases, auto inflammatory syndromes and immunological deficiency syndromes. These immunological disorders are either caused due to compromised immune system or due to over-active immune system. MSCs are beneficial in hyperactive immunological conditions due to their immunomodulatory as well as immunosuppressive properties[31]. The immunomodulatory functions of human MSCs (hMSCs) have been demonstrated by co-culturing them with purified subpopulations of immune cells where hMSCs altered the cytokine secretion profile of dendritic cells (DCs), naive and effector T cells (T helper 1 and 2 [TH1, TH2]), and natural killer (NK) cells to induce an anti-inflammatory or tolerant phenotype[32]. Specifically, hMSCs caused mature DCs type 1 (DC1) to decrease tumor necrosis factor α (TNF-α) secretion and induced mature DC type 2 (DC2) to increase interleukin-10 (IL-10) secretion; hMSCs caused TH1 cells to decrease interferon γ (IFN-γ) and caused the TH2 cells to increase secretion of IL-4; hMSCs caused an increase in the proportion of regulatory T cells (TRegs); and hMSCs decreased secretion of IFN-γ from the NK cells. Mechanistically, the hMSCs produced elevated prostaglandin E2 (PGE2) in co-cultures and inhibition of PGE2 production mitigated hMSC-mediated immune modulation[32]. Mesenchymal stem cells (MSC) possess a range of immunomodulatory properties which they exert through soluble mediators and through direct cell–cell contact. Considering the therapeutic potential of immunoregulatory properties, the safety and clinical efficacy of MSC treatment has been tested in a number of autoimmune disorders[33]. MSCs alone can act as an immunomodulatory therapy; it can regulate several immune cells such as T cell, B cell, Natural killer cells and dendritic cells, DCs affect significantly the balance between helper and regulatory T cell and establish tolerance to self-antigen[23]. The main features of immune disease are mediated by T cell response and include cell proliferation and cytokines secretion. MSCs cause inhibition of T cell proliferation. The implantation of allogenic MSCs has shown to inhibit the T cell response and altered cytokine production in vitro[34]. MSCs also suppress the T cell proliferation by secreting transforming growth factor beta (TGFβ) and hepatocyte growth factor (HGF) as well as by decreasing cytokines like Tumor necrosis factor alpha (TNFα)[34]. MSCs have also been shown to possess broad immune regulatory capabilities and are capable of influencing both adaptive and innate immune responses. MSCs inhibit T- cell proliferation, allogenic T-cell response and cytotoxicity. They also inhibit cytotoxic T-cells (CTLs), dendritic cells (IL-6 PGE-2) and β cells. As stated above, multiple factors like nitric oxide (NO), hepatocyte growth factor (HGF), interleukin-6 (IL-6) and IL-10 are responsible for inducing immune-suppressive effects by MSC[23]. The clinical trials using MSCs for immune disease have been promising[35]. MSCs have been explored in several immune disorder diseases, including multiple sclerosis, Crohn's disease, GVHD, systemic lupus erythematosus (SLE), and type 1 diabetes[8]. Moreover, Prochymal® and Cupistem® products marketed by Osiris Therapeutics Inc. and Anterogen respectively are based on the immunomodulatory capabilities of MSCs. There have been several articles providing the updated clinical trial data on the therapeutic application of MSCs for various diseases [8,36,37]. A six year follow-up published in 2017, showed that the MSCs are safe and posed no risks in the SLE patients who received two MSC infusions in 2009[38]. In 2018 it was announced that the Prochymal® achieved the primary endpoint in clinical trials for GVHD in USA; among patients who received at least one treatment infusion and were followed for 100 days (n=50), the mortality was 22% in comparison to mortality rate as high as 70% at 100 days in steroid resistant GVHD patients. Prochymal® has been approved in Canada and New Zealand.

c.Cardiac diseases

Cardiovascular diseases (CVD) comprise of all diseases of the heart and circulation and mainly include coronary heart disease, angina, myocardial infarction, and stroke. MSCs have been well known to exert therapeutic potential for patients with myocardial infarction (MI) and repair infarcted hearts mainly through paracrine mechanisms.Three main mechanisms of action through which MSCs impart their beneficial effects in MI include 1) reduction of MI induced fibrosis, 2) stimulation of new vessel formation or angiogenesis and 3) restoration of contractile function through the proliferation and differentiation of cells into cardiac stem cells[39]. These effects together lead to reduction of formation of the scarred or dysfunctional myocardial tissue along with preservation and proliferation of the contractile and perfused tissue thereby restoring the ejection fraction after MI. The delivery of MSCs at the MI site could be achieved via intracoronary, intramyocardial, intramuscular or intravenous injection though prolonged survival of MSCs at the site of infarct is crucial for their therapeutic effect. Multiple studies have been designed to study the effect of MSCs in MI[40]. The combination of implanted MSCs with anti-inflammatory factor IL10 has shown to attenuate MI by suppressing MI associated inflammation[35]. Bian et al. concluded that intramyocardial injection of MSCs derived extracellular vesicles (MSC-EVs) markedly enhanced blood flow recovery, reduced infarct size and preserved cardiac performance (systolic and diastolic) as compared to those treated with phosphate buffered saline (PBS) in an acute myocardial infarction rat model[41]. The potential of therapy with MSCs can also be improved via genetic manipulation of MSCs to express therapeutic genes that enhance the efficacy of MSCs against MI and even other CVDs. Preclinical MI models have been successfully tested with MSC enhanced to express VEGF, NO, BDNF alone or in combination with other biomaterials[42]. Interestingly, bone marrow MSCs (BM-MSCs) co-transduced with the engineered lentiviruses co-overexpressing both BDNF and VEGF along with corresponding fluorescent protein reporters led to recovery from a cardiac arrest induced global ischemia upon injecting into jugular vein of rats[43]. Apart from MSCs, MSCs derived conditioned media (MSC-CM) has been successful in preserving cardiac function after MI[44].Timmers et al. injected MSC conditioned mediaintravenously for 7 days in pigs in which MI was induced by surgically ligating left circumflex coronary artery and showed that the MSC-CM was able to reduce myocardial infarct size and preserve systolic and diastolic performance[44]. More recently, a novel technique has been utilized where MSC-conditioned media comprised of secretome was incorporated in Poly-lactic-co-glycolic acid (PLGA) to form microparticles (MP), and the MP were coated with MSC cell membrane to form synthetic mesenchymal stem cells (synMSC)[45]. Such synMSC demonstrated beneficial effect in mice with acute myocardial infarction to promote angiogenesis, improve cell proliferation and halted left ventricle remodeling in the infarcted heart.

The regenerative potential of MSCs in other CVD conditions has been studied in Phase II clinical trials in subjects with non-ischemic cardiomyopathy upon intravenously administered ischemia-tolerant MSCs (itMSCs) where the treatment increased six minute walk test (SMWT) performance, improved Kansas City Cardiomyopathy clinical summary and functional status scores, thereby indicating the overall improvement in left ventricular ejection fraction[46]. A comparative study evaluating the beneficial role of BM-MSCs, bone marrow-mononuclear cells (BM-MNCs) and adipose tissue MSCs (AD-MSCs) in a mice model of dilated cardiomyopathy showed that BM-MSC treatment not only induced significant improvement in ejection fraction (EF), fractional shortening (FS), left ventricular end-systolic dimension (LVESD) and left ventricular end-systolic volume

(LVESV) but also exerted more anti-fibrotic effects and more ability to reorganize myocardial tissue compared to AT-MSCs. The mononuclear cells from bone marrow (BM-MNCs) failed to show any such benefit[47]. Moreover, study of safety and efficacy of allo-hMSCs versus auto-hMSCs in non-ischemic DCM patients proved the superiority of allo-hMSCs over auto-hMSCs in regards to efficacy, including EF, SMWT, Minnesota Livingwith Heart Failure questionnaire (MLHFQ) and endothelial function and remodeling abilities [48].

Heart failure (HF) is defined as a clinical syndrome caused by structural and functional defects in myocardium resulting in impairment of ventricular filling or the ejection of blood. Some of the major pathogenic mechanisms for HF include increased hemodynamic overload, ischemia-related dysfunction, ventricular remodeling, excessive neuro-humoral stimulation, abnormal myocyte calcium cycling, excessive or inadequate proliferation of the extracellular matrix, accelerated apoptosis and in some rare cases genetic mutations [5,49]. In preclinical models for HF, MSCs have shown to improve LV contractile and diastolic function, reverse or attenuate LV remodeling, reduce collagen deposition and myocardial fibrosis, and increase vascularity and perfusion. These effects are proposed to be mediated via paracrine effects[50]or by differentiating into cardiac stem cells[51]. The promising results from preclinical studies led to clinical trials using MSCs injections given via different routes and at optimal dosage and all trials have shown an improvement in the clinical scores and parameter used to evaluate the beneficial effects of MSCs[52].

d.Metabolic diseases

The diseases or disorders that disrupt normal metabolism are coined as metabolic diseases. Metabolic disorders could be inherited as a single gene anomaly, most of which are autosomal recessive or could be caused due to abnormal chemical reactions in the body disrupting the normal metabolic processes[53]. The disease syndrome due to deficiency of enzymes required for metabolic activity in the body is called as inborn errors of metabolism. Obesity, liver diseases and diabetes mellitus are some of the most common metabolic diseases.

MSCs are considered as one of the most attractive cell sources for treatment of diabetes mellitus. Due to their self-renewal ability, pluripotency, low antigenicity, ease of culture and ease expansion in vitro to obtain sufficient cells for treatment, MSCs have been explored as a potential preferred therapeutic agent against common metabolic diseases such as DM. Type 1 diabetes is associated with a progressive loss of β cells and pancreatic islet transplantation could represent a cure for this disease. The transplantation of bone marrow–derived mesenchymal stem cells (BM-MSCs) allowed a reduced number of pancreatic islets to improve glycemic control in diabetic rats by promoting islet vascularization. It has been shown that co-transplantation of MSCs with pancreatic islets improved islet graft function by promoting graft vascularization [54]. BM-MSC as well AT-MSCs are found to be valuable source of autologous cell transplantation for treatment of DM type 1, DM type 2 and diabetic complications[55,56]. Similarly, umbilical cord and human placental derived MSCs upon differentiation into functional islet cells serve as the therapeutic agent for both Type 1 and 2 DM as well as an in vitro screening platform for hypoglycemics/insulin secretagogues[57]. More recently, BM-MSCs have been fortified with MSCs derived from Wharton's Jelly (WJs) or human Umbilical cord (UC-MSCs). Such MSCs secrete cocktail of growth factors, extracellular matrixes and exosomes, and upon co-culturing ameliorate proliferative capacity, mitochondrial degeneration, and induced exosome secretions leading to significant increase in the therapeutic effect of BM-MSCs, which may allow effective autologous cell transplantation[58]. Both BM-MSCs and UC-MSCs have shown safety and efficacy in the patients with DM Type 1[59]. Due to their anti-fibrotic, immune-modulatory and anti-inflammatory properties, MSCs could be used in a variety of liver diseases including hereditary liver diseases, cirrhosis and liver failure [60]. The therapeutic potential of mesenchymal stromal cells (MSC) in the treatment of liver fibrosis is predominantly based on their immunosuppressive properties, and their ability to secrete various trophic factors. Various trophic factors secreted by MSC play key therapeutic roles in regeneration by alleviating inflammation, apoptosis, and fibrosis as well as stimulating angiogenesis and tissue regeneration in damaged liver[61].

e.Hematological diseases

Hematologic diseases are benign or malignant disorders primarily affecting the blood components and bone marrow. Common blood disorders include anemia, bleeding disorders such as hemophilia and other malignant blood disorders such as leukemia, lymphoma, and myeloma. MSCs indeed have impacted the success rate of hematopoietic stem cell transplantation by minimizing the risk and incidence of graft versus host disease (GVHD). GVHD is a severe inflammatory condition resulting from immune-mediated attack of recipient tissue by donor T cells present in the allogeneic graft and occurs in acute and chronic phases [62]. Acute GVHD (aGVHD) seen in 40-60 % cases within first 100 days after transplant, occurs due to direct cytotoxic effects of donor T cells on recipient tissues and involves activation of antigen-presenting cells and associated inflammatory cascade that produces cytokines, including IL-1, IL-6, IL-12, IFN-γ and tumor necrosis factor-α. Chronic GVHD (cGVHD) mainly occurs after the first 100 days of HSCT and is characterized by autoimmune-like dysregulation. While aGVHD involves mainly the skin, liver, and GI tract, cGVHD is a multi-organ disorder and is the major cause of late non-relapse mortality after allogeneic hematopoietic SCT[63].

MSCs play a vital role in modulating BM microenvironment and promoting hematological engraftment and preventing engraftment failure and poor graft function associated with GVHD. MSCs act on the hematopoietic microenvironment of ablated bone marrow and help to reconstitute the damaged stroma by secreting the hematopoietic cytokines, including IL-6, IL-7, IL-8, IL-11, Flt-3 ligand, and stem cell factor (SCF) to promote self-renew and differentiation of HSCs[64]. As evaluated from the clinical trial results, it is interesting to observe that a single infusion of MSCs given at the time of the transplant did not prevent the development of aGVHD [65]. However, not only BM-MSCs but also UC-MSCs were capable to impart their immunomodulatory properties against pathological factors involved in GVHD when infused in multiple doses given at weekly intervals subsequent to transplantation[66,67]. Especially, MSCs activated with IFN-gamma have been reported to increase the overall success rate in preventing and treating the GVHD [68,69]. Even though the success rate of MSCs to aGVHD patients is superior to that of cGVHD [70], still MSCs infusion serves as an effective salvage therapy for patients with steroid-resistant, cGVHD where high overall survival rate has been shown in multiple resistant cases[71,72]. Based on the successful clinical trial reports, some MSC products have been developed or are in process of receiving the approval from the regulatory authorities to be available as off-the-shelf agents in some countries[73].

f.Gastrointestinal diseases

After conducting a European Phase III clinical trial (ADMIRE-CD) in August 2015 in which both the primary endpoint and the safety and efficacy profile were met, the Alofisel was approved for the treatment of complex perianal fistulas in adult Crohn's disease patients that have previously shown an inadequate response to at least one conventional therapy or biologic therapy. The treatment group with Alofisel showed a 44% greater probability of achieving combined remission compared to control (placebo). The 24-week results of the Phase III ADMIRE-CD trial were published in The Lancet in July 2016[74]. The 52-week results were published in the Journal of Gastroenterology in 2017[74]. The product Alofisel was given orphan drug approval by the EMA in 2009 and by the FDA in 2017. The product received marketing approval in EU in 2018. In addition to treatment of complicated fistuals in Crohn's disease patients, the MSCs have been successfully tested in the treatment of refractory luminal Crohn's disease.

g.Conditional media from MSCs

Conditioned media refers to cell secretome comprised of small secreted proteins, molecules and cytokines [75]. Despite of the differences in the source cells, culture conditions, harvest time and passage number of the cells utilized to collect CM, multiple studies have confirmed the beneficial effect of CM derived from MSCs for various diseases conditions [76].

Several studies have confirmed that functional activity of MSCs is largely dependent on factors that MSCs release in the nearby environment in vivo as well as in conditioned medium[77]. The secretome consists of myriads of growth factors such as vascular endothelial derived growth factor (VEGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II), hepatocyte growth factor (HGF), fibroblast growth factor 2/basic fibroblast growth factor (FGF-2/bFGF), keratinocyte growth factor/fibroblast

growth factor 7 (KGF/FGF-7), platelet derived endothelial cell growth factor (PDEGF), heparin binding epidermal growth factor (HEGF), placenta growth factor (PlGF), neural growth factor (NGF), and brain derived neurotrophic factor (BDNF); anti-inflammatory cytokines such as TGFβ1, IL-6, IL-10, IL-27, IL-17E, IL-13, IL-12p70, and IL-1 receptor antagonist (IL-1ra) as well as proinflammatory cytokines such as IL-8/CXCL-8, IL-9, and IL-1b[78].

Other secreted factors such as leptin, angiogenin, granulocyte colony stimulating factor (GCSF), granulocyte macrophage CSF (GM-CSF), macrophage CSF (MCSF), fractalkine, monocyte chemotactic protein (MCP-1), serpin E-1, endostatin/collegen XVIII, UPA, thrombospondins 1 and 2, tissue inhibitor of metalloproteinase-1 (TIMP-1), IGF binding protein (IGFBP), stem cell-derived factor 1 (SDF-1)/CXCL-12, adrenomedullin (ADM), Dickkopf-1 (DKK-1), and few receptors such as MCSF receptor (MCSFR) and PDGF receptor (PDGFR) have also been identified in the CM secretome[77].
CM derived from tissue sources such as BM, UC, AT and even embryonic stem cells all have potential to facilitate recovery after injury, and maintenance of healthy aging and homeostasis of organ and tissues. The combination of secretory factors leads to immunomodulatory, anti-apoptotic, homing and paracrine effects which subsequently coordinate to improve the overall disease process. MSC derived CM media have been explored against almost all disease conditions, including cardiovascular diseases, acute liver injury/failure, cerebral injury/ischemia/stroke, spinal cord injury, lung injury, COPD, GVHD, bone defects, anti-aging, wound healing, alopecia, diabetes, orthodontic defects, autoimmune and connective tissue disorders. Few studies have explored the preconditioning strategies to improve the therapeutic potential of MSCs and MSC derived CM [79]. One such modification includes use of tri-dimensional spheroid culture method instead of traditional monolayer culture method. CM from human MSC spheroids shown to stimulate trophic factors secretion and also been found to inhibit TNF-α, CXCL2, IL6, IL12p40, and IL23 production from LPS-stimulated macrophages and stimulate higher production of prostaglandin E2 (PGE2)[80].

Overall CM derived from MSCs has a few competitive advantages over MSCs as CM can be manufactured in massive amount, freeze-dried or concentrated, packaged, and transported more easily. Moreover, the time and cost of expansion and maintenance of cultured stem cells is greatly reduced and off-the-shelf secretome therapies could be immediately available for treatment of acute conditions such as acute demyelination, cerebral ischemia, myocardial infarction, or military trauma. Finally, the biological product obtained for therapeutic applications could be modified or potentiated for their therapeutic effects[81].

More recently, clones of immortalized MSCs (iMSCs) from human dental pulp-derived primary MSCs have been developed. Each clone of iMSCs produced constant amounts of bioactive molecules which can be harvested for their immunoregulatory and tissue regeneration properties. The conditioned media offers safe and efficient therapeutic strategies to treat various diseases and is being explored in the clinical trials against diabetes and liver cirrhosis[82].

h.MSCs as vectors for anti-cancer agents

MSCs are rapidly emerging as promising vectors to carry anticancer agents due to their ability to migrate to the tumor microenvironment (tumor tropism) and to produce interferons IFN-α and IFN–β. So far MSCs have shown to impart role in management of a number of different cancer types, including glioblastoma and metastatic breast carcinoma, ovarian carcinoma and hepatic carcinoma[83].

MSCs have ability to migrate to the damaged tissues and act against any degeneration occurred due to the pathological conditions such as ischemia, cerebral injury or malignancies based on the effective signaling cues which are mostly chemokines or growth factors secreted by the inflamed tissues. This signaling leads to effective recruitment of the MSCs and possibly enhanced survival of MSCs in the damaged tissue. MSCs express a broad spectrum of receptors for all four chemokine sub-families CC, CXC, C and CX3C [84]. This unique property of MSCs has been explored in MSC based gene therapies where MSCs function as vectors to overexpress the small molecules and proteins such as tissue growth factor TGF-beta, fibroblast growth factor FGF2, and BDNF (Ozawa et al., 2008). An example of signaling molecule is CXCL12 which exerts significant chemotactic activity on MSCs by interacting with its receptor CXCR4 [85]. In addition to CXCL12, other chemokines such as CXCL8, CCL5 and CCL22 and growth factors such as the bone morphogenetic proteins (BMPs; BMP-2 and BMP-4), platelet derived growth factor (PDGF-AB, PDGF-BB), epidermal growth factor (EGF), hepatocyte growth factor (HGF) as well as insulin-like growth factor (IGF-I and IGF-II) have also been shown to act as potent chemo-attractants for MSCs in in vitro studies[83,86]. Interestingly, under inflammatory conditions when MSCs were pre-treated with tissue necrosis factor-α (TNF-α), CCL5, CXCL12 and CCL22 may be dramatically increased suggesting a role for inflammatory signals in the mobilization of MSCs and their subsequent homing to injured tissues [87]. Such property of MSCs has been explored to dispense anti-cancer agents at the tumor and metastatic sites especially for the deep-seated tumors and the micro-metastatic tumors which are inaccessible via surgery.

Depending on the type of cancer, MSCs can be engineered to carry various therapeutic agents alone or in combination. Some of the common therapeutic agents include IFN-β, IFN-γ, interleukins (IL-12, IL-24), Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), herpes simplex virus thymidine kinase (HSV-tk) and cytosine deaminase (CD)[88]. Upon activation of the pro-apoptotic death receptors (DRs) 4 and 5, TRAIL gene leads to induction of caspase-8-dependent apoptosis without causing any apoptosis in the non-malignant cells[89,90]. Several studies have confirmed the anti-tumor effect of TRAIL expressing MSCs against glioblastoma [91,92]. In addition to viral vectors, the therapeutic effect of MSCs carrying oncolytic viruses has been tested in animal models for glioma, melanoma and breast cancer [93]. Especially, oncolytic measles virus bearing MSCs prolonged the survival of animals harboring the ovarian cancer indicating the presence of immunity against MV in vivo [94]. Similarly, MSCs equipped with HSV-tk and CD as a part of suicide gene/enzyme prodrug system where HSV-tk and CD can convert non-toxic prodrug into toxic anti-metabolites and kill tumor cells selectively and induce killing effect. These studies indicate the therapeutic potential for both suicidal genes and furthermore, the synergistic cytotoxic effect which enhances the high therapeutic efficacy against metastatic lesion induced by tumor in an in vivo model [95,96]. MSCs expressing HSV-tk genes can further be modified to express the fluorescent or radiolabeled markers to monitor the engraftment as well as therapeutic effect as evaluated in glioblastoma model [95].

MSCs derived from the gingival papilla (GinPa-MSCs) used to incorporate three anticancer drugs (Paclitaxel, Doxorubicin and Gemcitabine) efficiently released them in active form in sufficient amount and produced significant inhibition of squamous cell carcinoma growth in vitro [97]. Similarly, MSCs loaded with nanoparticle carrying anti-cancer drugs such as paclitaxel, doxorubicin, cisplatin and gemcitabine have been shown to accumulate at the tumor site and release the therapeutic drugs with high efficiency and low systematic toxicity[88,98]. Similarly, MSCs derived microvesicles and exosomes have been successfully tested to mediate the paclitaxel induced strong anti-proliferative activity against human pancreatic cell line CFPAC-1[99]. Recently, He et al (2018) showed that CM derived from MSC could inhibit activated Stat3, suppress cancer growth, and improve the sensitivity of breast cancer to radiotherapy using in vitro and in vivo studies. CM-MSCs was also found to reduce ALDH-positive cancer stem cells, modulated stem cell markers and decreased tumor migration as well as metastasis [100].

Overall, MSCs can be effectively used as a unique cell-mediated drug delivery system and by virtue of their tumor tropism (homing and cell-cell crosstalk between tumor site and MSCs) they are capable of imparting anti-tumor effects at the tumor site, when equipped with anti-cancer agents. Modified MSCs as well as modified MSCs derived conditioned media successfully and efficiently carry various anti-tumor cytokines, viruses and drugs leading to anti-proliferative and apoptotic effect against various cancers.

CONCLUSION

Considering the rapid progress in the field of cell therapy in the past decade MSCs offer an excellent therapeutic option as a regenerative therapy. Mesenchymal stem cells offer an excellent tool to fulfil a 21st century vision of extending human life span beyond 100 years because they can control an overactive immune system, they can be administered universally and do not need HLA matching. MSCs can be

easily enhanced to express desired genes. Prochymal®, the first MSC product to be approved for clinical use for the treatment of acute graft versus host disease (GVHD) has been approved in Canada, New Zealand, and Japan (under trade name Temcell®). Alofisel® (darvadstrocel), has been approved in EU for the treatment of complex perianal fistulas in adult patients with nonactive/mildly active luminal Crohn's disease, when fistulas have shown an inadequate response to at least one conventional or biologic therapy. Several clinical trials have already demonstrated regenerative potential of MSCs. There have been more clinical trials in the completion and recruitment phase (clinicaltrials.gov) designed to further explore the multifunctional benefits of MSCs for human diseases. Rapid advancement in the understanding of gene therapy has raised the prospects of the MSCs based ex-vivo gene therapy for multiple chronic conditions which are usually very hard to treat and lower the quality of life of patients.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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