Mesenchymal Stem Cells

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ABSTRACT

Mesenchymal stem cells **(MSCs)** are undifferentiated multipotent stromal cells initially described in BM but later found in almost every tissue. This rare cell population has significant self-renewal capacity and is considered a reservoir of reparative cells lacking tissue specific characteristic. Following signals like injury and inflammation, they have the ability to mobilize and home injured tissue contributing to their regeneration. They can give rise to variety of differentiated cell types including osteoblasts, chondrocytes, adipocytes, myocytes and others. In culture, MSCs can be handled with ease, adhere to tissue culture plastics where they form colonies. They have the ability to modulate the immune system suppressing inflammation and immune reactions while they have low immunogenicity. The aim of this chapter is to present the characteristic features of MSCs and the available clinical and experimental evidence on their applications.

Keywords: Mesenchymal stem cells; Bone marrow; Regeneration

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INTRODUCTION

Mesenchymal Stem Cells **(MSCs)** are undifferentiated non-haematopoietic stromal cells found in almost all tissues. They are multipotent and are considered as a reservoir of reparative cells lacking tissue specific characteristics. Their main feature is their property to mobilise to the site of injury, proliferate and differentiate into committed cellstypes in order to support the overall regeneration process. Thus, MSCs were the recipients of significant interest in the course of the last decades for tissue regeneration approaches. This interest is further strengthened by the fact that MSCs do not pose ethical or tumorigenic concerns unlike the pluripotent embryonic stem cells or the induced pluripotent stem cells. This article aims to present the main characteristics of MSCs and provide an overview of their potential clinical applications.

HISTORY

The concept of stem cells is over 150 years old. In the classical work of Gourjon et al. (1868) the presence of a stem cell population was postulated following the observations demonstrating the development of heterotopic bone formation when bone marrow was transplanted in extra-skeletal sites [1]. At the same time, Cohnheim was the first to articulate the hypothesis that the fibroblastic cells derived from bone marrow were involved in wound healing all through the body [2] Collectively, the works of Gourjon and Cohnheim provided the foundation for all future studies on this bone marrow stromal cell population.

In the 1970s, Friedensteinet al. was the first to provide proof of the presence of a second subpopulation within the bone marrow, notwithstanding the haematopoietic stem cells [3]. These cells were adherent to tissue culture plastic, hada self-renewal capacity, could proliferate in vitro and could produce ectopic bone when transplanted into extra-skeletal areas. They were initially named Colony Forming Unit-Fibroblasts **(CFU-F)** but later several names were given including bone marrow-stromal cells, stromal fibroblasts, osteogenic stem cells, multipotent stem cells and others. Caplan et al. proposed the term Mesenchymal stem cells, which has gained popularity and persisted to date [4].

CHARACTERISTICS OF MSCS

Homing and Differentiation

Since the discovery of MSCs in the 70s, bone marrow **(BM)** was viewed as the fortification of MSCs in the human body. The frequency of MSCs within BM mononucleated cells is about 0.01% to 0.001 %, hence, other tissues were investigated as potential places of MSC residence [5,6]. Throughout the years, it became apparent that many tissues harbour a population of MSCs including trabecular bone, cartilage, synovium, fat, arterial wall, periosteum and others [5]. It was alsodemonstrated that MSCs from different sites had significant differences in their differentiation capacity and regeneration potential. For instance, adipose-derived MSCs had poor capacity to

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differentiate towards osteoblasts when compared to BM-MSCs. To make matters worse, some authors demonstrated differences between same tissues but different sites in the body [7] For example, some authors reported differences in the proliferation and differentiation potential of MSCs derived from the pelvis and spine [7].

MSCs have the ability to mobilise and differentiate into a large number of specialized cell types following specific signals. They can differentiate towards cells of mesodermal (osteoblasts, chondrocytes, adipocytes, myocytes, cardiomyocytes), ectodermal (neuronal cells) and endodermal (hepatic, pancreatic, respiratory epithelium) lineages [Figure 1]. Furthermore, a fully differentiated cell from one lineage can switch into another mature cell type of MSCs; a phenomenon called genetic reprogramming or transdifferentiation. Song & Tuan showed that fully differentiated osteoblasts with detectable Alkaline Phosphatase (ALP) activity and elaboration of calcified extracellular matrix can de-differentiate into either fully functional lipid-producing adipocytes or chondrocytes and vice versa [8].

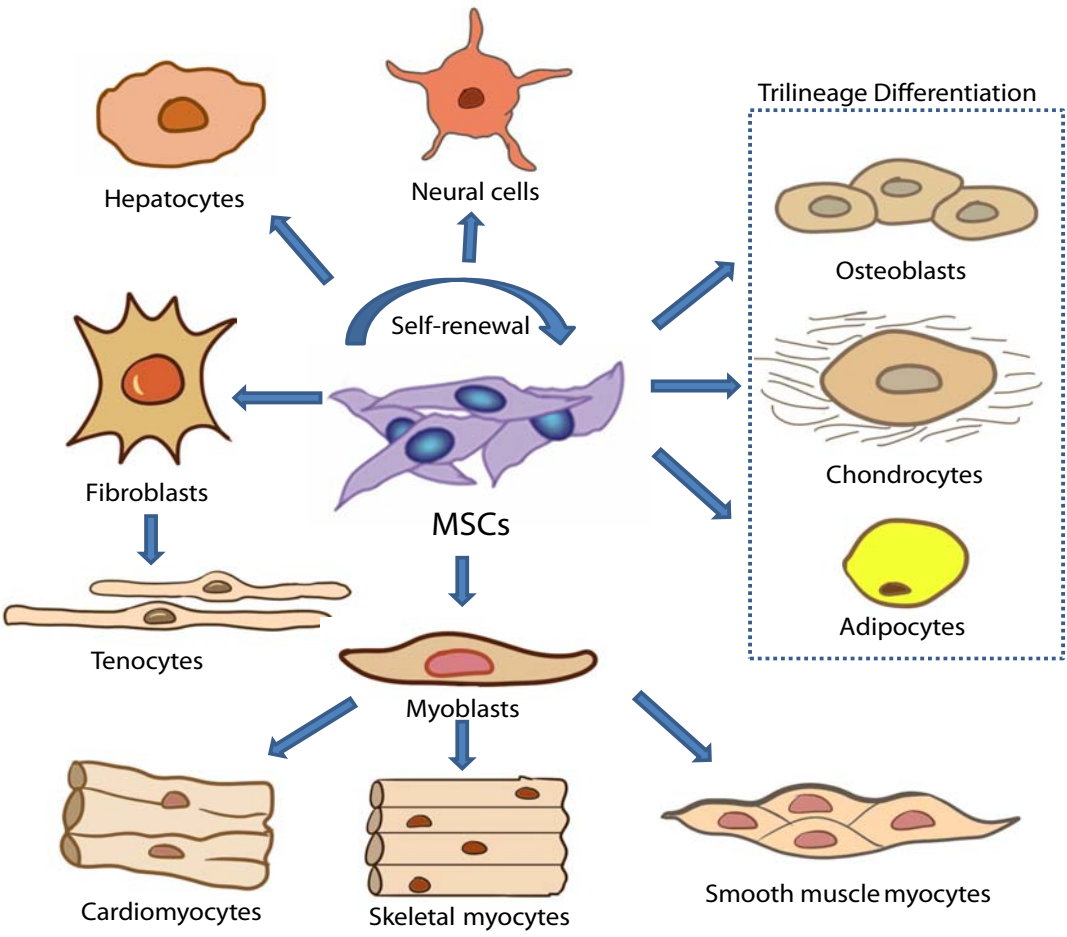

Figure 1: Mesenchymal Stem Cell differentiation potential.

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Msc Morphology and Phenotype

At present robust phenotypic or morphologic criteria to characterise MSCs do not exist. In culture, MSCs remain morphologically heterogeneous, containing cells ranging from narrow spindle-shaped to large polygonal and, in some confluent cultures, tightly packed, slightly cuboidal cells [9]. Sikiya et al. demonstrated that human MSCs in culture undergo a time-dependant transition from small (thin) spindle-shaped cells to wider cells [10]. They showed that once MSCs become wide, they have constrained capacity to proliferate and differentiate.

Another valuable property, but not a defining feature of MSC populations in-vitro, is their ability to adhere to plastic and form colonies after low-density plating although it is unknown what the relation is between MSCs and CFU-Fs [3]

In addition to the morphological irregularities, MSCs express a number of nonspecific cell surface markers, none of which individually or in combination, has been shown to be a specific marker forMSCs. MSCs share phenotypic features with other types of cells including endothelial, epithelial and muscle cells [11]. At present a loose phenotypic definition is achieved with positive and negative phenotypic staining. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy **(ISCT)** has defined three minimum criteria to define MSCs: i. Adherence to tissue culture plastics, ii. Trilineage differentiation capacity (differentiation towards osteogenic, chondrogenic and adipogenic lineages) and iii. Display of specific surface antigens (negative for CD45, CD34, CD11b, CD14, CD19, CD79a, HLA-DR and positive for CD73, CD90 and CD105) [12].

Tropism of MSCs towards Injury or Tumour

An emerging property of MSCs is that they tend to mobilise towards the injury and home damaged tissues. Signals responsible for their mobilisationinclude damage in the tissues like trauma, fracture, inflammation, necrosis and tumours [13,14]. MSCs are known to express integrins, adhesion molecules and chemokines that regulate their capacity of migration and homing [15]. Due to their ability to express these molecules, MSCs can reach distant tissues and participate in the replenishment of the local microenvironment and tissue regeneration. An example includes the ability of these cells to migrate and colonise the injured site after intravenous injections [16,17]. Myocardial infarction, ischaemic cerebral disease, fracture and spinal cord injury are conditions where these beneficial properties are demonstrated [16,17]. Similarly, suspended MSCs injected intra-articularly into the knee joint following injury appeared to engraft and regenerate damaged meniscus and cartilage [18].

MSCs and the Immune System

Mesenchymal stem cells are not immunogenic and have the ability to modulate an immune reaction in accordance to the local environment. Natural killer cells **(NKs)** are not capable of recognizing MSCs as they express human leukocyte antigen class I molecule [19]. The IFN-γ

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secreted by the monocytes and the MHC class I chain-related protein A could also protect MSCs from NKs aggression [20]. MSCs were shown to inhibit the proliferation, development and cytokine productionof T cells [21]. The MSC induced anergy of T cells can be induced by several pathways including the inhibition of cyclin D2 expression and the release of nitric oxide. Similarly, MSCs can modulate the proliferation, differentiation and activation of B cells via inducing cycle arrest at GO/G1 phase and through the release of soluble molecules like the Blimp-1, which is necessary for the Ig production [21]. Likewise, MSCs have shown to regulate some immune cells including macrophages, dendritic cells, T helper and Treg cells, leading to an overall control of the immune response [22]. Notably, MSCs can suppress these cells independently of the major histocompatibility complex (MHC) system as they have a low expression of the MHC-II and other stimulatory molecules [23]. MSCs' effect is achieved mainly through cell-to-cell contact and the secretion of a number of molecules including the transforming growth factor-β, prostaglandins, IDO, hepatocyte growth factor and nitric oxide [24].

ISOLATION, EXPANSION AND IMPLANTATION OF MSCS

Based on the aforementioned properties, MSCs based regeneration approaches have attracted significant interest. Current potential pitfalls for the development of their clinical utility include the source of MSCs, the isolation protocols and the need for expansion as they represent a rare population in bone marrow and other tissues. As far as the available sources are concerned, bone marrow and adipose tissue are the most common ones. However, MSC expression and differentiation capacity varies between these two sources. Adipose-derived MSCs express higher levels of CD49a, CD34 and CD54 while BM-derived MSCs have a higher expression of CD106. Besides, poor osteogenic and chondrogenic potential has been reported with adipose-derived MSCs. Other factors that can influence MSCs output include the age of the patient, co-morbidities, the initial MSC yield and even the location of the source in the body. The significance of these findings is still to be explained.

Isolation of MSCs

At present there isn't a well-established procedure for the isolation of MSCs but a variety of protocols exists providing non-comparable data. The first and simplest method used utilises the adherence properties of MSCs, which were first identified by the pioneer work of Friedenstein, et al [3]. Seeding BM cells on tissue plastic dishes and washing out the non-adherent cells is the simplest method of obtaining MSCs. This technique can be used for solid tissues, expecting MSCs to grow out of the tissue onto the plastic substratum. Alternatively, extraction of the monocytes from BM through gradient centrifugation or the collagenase digestion technique for solid tissues can be used to increase the yield of MSCs. These techniques will not result in a pure MSC population but a collection of different cells including MSCs.

Purification techniques using fluorescence-activated cell sorting or magnetic beads can be used in conjunction with a variety of positive and negative cell surface markers including, CD45,

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CD56, CD105, CD146, Stro-3, MSCA-1, and CD271 [25]. The results of several studies highlighted that the purity of the resulted MSCs yields can be improved significantly through these techniques. However, it is possible that the differences in MSC expression are a result of their anatomical location and environment. For example, Tormin, et al. showed that MSCs in perivascular regions were CD271+CD146+ while the ones found at the endosteal surfaces were CD271+CD146− [26]. At present, the significant heterogeneity of stromal cell populations and the lack of specific markers that will enable prospective isolation has triggered a large number of different approaches for their isolation.

Ex-vivo Expansion of MSCs

Ex-vivo expansion and manipulation of MSCs is the only way to safeguard significant numbers of MSCs for clinical applications. MSCs can be expanded tremendously within a relatively short period. This rapid proliferation could result in an expansion of thousand-fold in two to three week's time [27]. Also, MSCs could proliferate for about 19 doublings in culture without losing their property to proliferate and differentiate [16]. Having to isolate only a limited number of cells from the body, and the site morbidity is minimal with a final MSCs yield higher than extensive bone marrow aspiration.

However, expansion was shown to reduce the maximal differentiation potential of MSCs [28]. Extensive subcultivation impairs the cells' function resulting in cellular senescence that is associated with growth arrest and apoptosis [29]. However, retroviral transduction with human telomerase gene can extend MSCs' lifespan to more than 260 doublings without losing multilineage capacity [30]. On the other hand, data are suggesting that prolonged culture could result in spontaneous transformation acquiring tumorigenic potential [31]. It was also shown that particular properties of MSCs are lost during culture. For instance, the cardioprotective effect of MSCs is reduced in cells at passage 5 and 10 compared to those of passage 3 [32]. This could be explained by the reduced vascular endothelial growth factor release potential [32]. Therefore, a compromise should be made between the yield and the quality of the expanded cells.

The most important component of successful ex-vivo expansion of MSCs is the culture conditions. Culture media consist of a basal medium containing glucose, amino acids and ions including calcium, magnesium, potassium, sodium, and phosphate, as well as fetal heat inactivated animal sera in concentrations of 10% or 20% [33]. Two main issues are arising from their use in culture media. The first is their efficacy compared to human serum, and secondly their potential side effects such as transmission of disease and immune reactions. Both basal media and sera can result in a significant difference in the MSC yield after expansion. Sotiropoulou, et al. has shown considerable variability between media used in terms of adherence efficacy, growth index and the final number of cells obtained in culture [34]. Serum free media are an alternative. However, most of them have demonstrated only limited performance.

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Implantation of MSCs

Once ex-vivo expanded MSCs are harvested, the next frontier for providing clinical application lies in the successful delivery of these cells at the site of interest. Briefly, a variety of approaches have been studied, but in principle all utilised a scaffold loaded with the expanded cells. By this approach, un-manipulated culture expanded MSCs or even adenoviral transfected MSCs, as well as already differentiated cells, have been uploaded on scaffolds targeting the healing of bone defects [35]. Concerning the scaffold, a huge variety exists but it is important to note that the ideal scaffold has to mimic the native characteristics of the tissue, providing a source of cells capable of promoting regeneration, as well as, a biodegradable matrix that would act as scaffolding for vasculogenesis, cell migration and attachment [36,37].

CLINICAL APPLICATIONS

Musculoskeletal Pathologies

Since the discovery of MSCs their relation to Bone Morphogenetic Proteins (BMPs), the treatment of a variety of musculoskeletal conditions was revolutionised. Orthopaedic surgeons, not infrequently, used these two elements together to create a powerful tool for bone regeneration. As a result of successful outcomes of several preclinical experimental studies, a large number clinical studies currently exists including cases of bone defects and non-unions, avascular necrosis of the hip, bone cysts, cartilage lesions and spinal fusion.

The primary aim in bone defects, spinal fusion and non-unions is the successful consolidation of the bone [Figure 2]. MSCs through their ability to drive the bone healing process are the ideal candidate to enhance the healing potential in such scenarios. The majority of the clinical studies have utilised whole bone marrow aspirates, avoiding the excessive manipulation and handling of MSCs. Such simplified approach minimises the donor site morbidity, does not induce excessive new bone formation (unlike BMP application) and no induction of tumor formation was reported [38]. Hernigou et al. have shown a positive correlation between the actual volume of the mineralised callous and the number of CFU-Fs in the 60 patients treated with autologous bone marrow injections for atrophic non-unions [39]. The authors also revealed that the CFU-Fs counts in the patients where the procedure was unsuccessful were significantly lower. As far as the studies that used culture expanded MSCs are concerned, the results are limited but promising. Quarto et al can be considered one of the pioneer in treating non-unions with culture expanded MSCs [40]. They presented a case series of 3 patients suffering from long bone defects treated with cultures expanded MSCs loaded onto a macroporous hydroxyapatite scaffold. The results revealed abundant callus formation along the implants and good integration at the interfaces with the host bones. Culture expanded MSCs were also used in two studies of distraction osteogenesis [41,42]. These studies reported a shorter treatment period and reduced associated complications by accelerating new bone formation in distraction osteogenesis.

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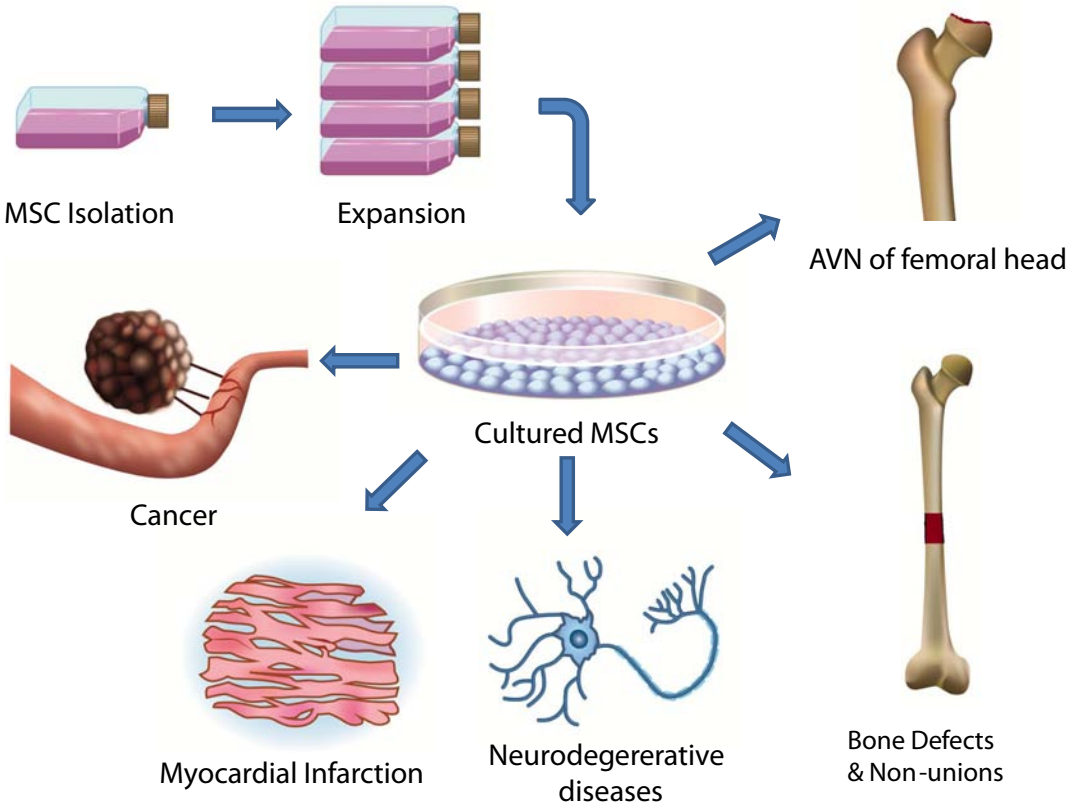

Figure 2: Clinical applications of MSCs.

The avascular necrosis of the hip is a pathologic process that results from interruption of blood supply to the femoral head. MSCs can be used to augment the core decompression procedure, which aims to relieve the intraosseous pressure and enhance new bone formation and angiogenesis. Some early studies utilising whole bone marrow transplantation have shown a positive correlation between the number of osteoprogenitor cells and the outcome [43]. A significant delay in the time to collapse was also noted with improved functional outcomes [44]. In two studies where culture expanded MSCs were used in a total of 6 patients, promising results were presented with no complications and an enhanced new bone formation [45,46].

The clinical studies involving the treatment of bone cysts have exclusively used intralesional injections whole bone marrow. While an early study presented less favourable outcomes [47], healing rates similar to those achieved through open procedures have been reported in the majority of the clinical studies [48,49].

The treatment of articular cartilage defects through MSC-based tissue engineering approaches has attracted significant interest over the last decades. Similarly to autologous chondrocyte implantation **(ACI)** where chondrocytes are ex-vivo cultured before re-implantation, MSCs based approaches were evaluated over the years. Ex-vivo expanded MSCs seeded on the collagen gel

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scaffold were used for patella defects in two patients with good outcomes [50]. Similar outcomes were reported in comparable studies afterwards [51,52]. A biopsy of the regenerated cartilage performed in one of the patients previously treated with this approach revealed the formation of a proteoglycan rich fibrocartilage [51]. In a recent randomised trial comparing ACI with BM-MSCs have reported similar functional outcome in the 2-year of follow-up [52].

Cardiovascular Disease

MSCs are an attractive candidate for cellular therapy of ischemic heart disease and congestive heart failure. Many studies have shown that MSCs can engraft to the affected myocardium following administration and improve its regeneration. Animal studies have demonstrated that MSCs injections have the ability to partially repair the infarcted myocardium, increase the capillary density and decrease the fibrosis of the myocardium [53].

Clinical trials utilising MSCs to improve cardiac function have shown encouraging results. Chen et al. conducted a pilot randomised clinical trial to study the effect of percutaneous coronary injection of MSCs or saline in 69 patients suffering from acute myocardial infarction [54]. The authors reported no adverse effects with the MSC-treated groups showing significant improvement in left ventricular ejection fraction as compared to control. Thus, Katritsis et al. treated 11 patients suffering from acute myocardial infarction with autologous MSCs together with endothelial progenitor cells and reported partial improvement of the myocardial contractility [55]. In addition to autologous MSCs, allogeneic MSCs have also been used in this clinical setting. The administration of a commercially available off-the-self allogeneic MSCs product has shown to improve cardiac function and reduce the incidence of arrhythmias and chest pain. In spite of the fact that the exact mechanism for these results is obscure, some authors suggested that MSC-excreted cytokines could inhibit the apoptosis of the cardiomyocytes facilitating the formation of new blood vessels within the affected tissue [56].

Cancer

MSCs have great potential for cancer therapy due to their inherent ability to migrate towards tumours. In such scenarios, MSCs can be used in their pure non-manipulated form or can be genetically modified to promote specific anticancer genes.

There is significant controversy on whether unmodified MSCs have anti-tumorigenic capabilities. In co-cultures with MSCs, tumour cells decreased their proliferation, increased their apoptosis and their malignant phenotype was inhibited [57,58]. It was hypothesised that MSCs could modulate the pathways involved in the apoptosis of cancer cells through direct cell-to-cell contact [58]. On the contrary, in the coexistence of MSCs, tumour cells found to exhibitan elevated capability of proliferation, rich angiogenesis in tumour tissues and highly metastatic ability [59]. Furthermore, the immunosuppressive effect of MSCs was reported to be in favour of tumour growth [60].

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There is a large number of experimental studies in which MSCs have been modified to express various anticancer molecules. These molecules include interleukins, oncolytic viruses, interferons, antagonists of several growth factors and drugs. These studies have shown a potent antineoplastic effect but the clinical usefulness is yet to be elucidated. Among the fundamental challenges is to characterise the safety of such approaches [61,62]. Some authors highlighted the potential of malignant transformation of these cells due to the lack of control of the extent and length of their action [61,62].

Liver Diseases

Liver cirrhosis and hepatic failure result in hepatocellular death and fibrosis. Such conditions are conventionally treated with liver transplantation, which is associated with a high cost, permanent immunosuppression and a high rejection risk. Several animal studies have shown that MSCs administration in cases of hepatic failure can facilitate the repair of the infarcted tissue and prevent further damage of the liver parenchyma [63]. In a rat model of fulminant hepatic failure, the systemic administration of MSCs resulted in a 90% reduction of apoptotic hepatocellular death and a significant upregulation of the number of proliferating hepatocytes [64]. A dramatic increase in the expression levels of several genes known to be up regulated during hepatocyte replication was also noted.

In a phase I trial, 4 patients with decompensated liver cirrhosis were treated with ex-vivo expanded MSCs [65]. The authors reported no side effects in the patients during follow-up. The end-stage liver disease scores for some of the patients and the quality of life of all four patients improved by the end of follow-up. In a phase I-II trial including 8 patients with end-stage liver disease autologous ex-vivo expanded MSC were injected into the portal vein [66]. The treatment was well tolerated by all patients and the liver function significantly improved. An improvement of clinical indices of liver function was noted with the prothrombin complex, serum creatinine and bilirubin been improved significantly. Similar results were reported when pure CD133 and CD34 ex-vivo differentiated MCs were in 40 patients with post-hepatitis C virus **(HCV)** end-stage liver disease [67].

Autoimmune Disease

The role of MSCs to reduce the inflammation through immunomodulation has been investigated for potential treatment therapies in a variety of autoimmune diseases including rheumatoid arthritis, Crohn's disease and systemic lupus erythematosus (SLE).

Rheumatoid arthritis is an autoimmune disease characterised by chronic inflammation in the affected joints leading to cartilage and bone destruction. The therapeutic potential of MSCs in rheumatoid arthritis is rather controversial. Animal studies of collagen-induced arthritis have shown a reduction in the levels of inflammatory cytokines with inhibition of T-cells proliferation [68]. Unfortunately, several authors who failed to share the same findings, instead, suggested that MSCs can in fact attenuated joint damage through the over expression of interleukin-10 [69,70].

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Crohn's disease is a chronic inflammatory condition of the bowel. Following promising preclinical findings, a pilot study was conducted on 5 patients with Crohn's who received intraregional injections of MSCs [71]. Normal healing was observed in two patients with 75% of the fistulas closed. Similar promising results were reported from a phase II clinical trial from the same group [72].

Systemic lupus erythematosus is characterised by immune system attacks towards healthy tissue in the body. Animal studies have shown that transplantation of human MSCs in animals with SLE can significantly inhibit the autoimmune progression [73]. In particular, MSCs can reduce the proliferation and number of T lymphocytes, reduce serum levels of anti ds-DNA antibodies and increase the Th1 subpopulation. Besides, MSC administration can lessen the renal pathology and reduce the expression of TGF-beta, FN, VEGF and the deposition of complement C3 in renal tissue.

Neurodegenerative Diseases

Some authors have evaluated the clinical utility of MSCs to treat a number of neurodegenerative conditions including multiple sclerosis, Alzheimer's and Parkinson's disease.

Multiple sclerosis is a condition characterised by an aberrant immune-mediated response leading to myelin and axonal damage, and chronic axonal loss attributable to the absence of myelin sheaths [74]. It has hypothesised that MSCs through their immunomodulatory capacities they can inhibit the disease progress. Literature has shown that MSCs administration can in fact promote self-repair, reduce the formation of scar, promote angiogenesis and protect from further damage through secretion of molecules like the superoxide dismutase-3 [75,76]. Also, due the nature of the disease with multiple foci of lesions, MSCs are anideal candidate as they can infiltrate the CNS and getwidely distributed.

Parkinson's disease is a neurodegenerative disease characterised by a progressive loss of the dopaminergic neurons. Experimental models have shown that the levels of tyrosine hydroxylase and dopamine can be enhanced after MSC administration [77]. In a similar study, a neuroprotective effect was reported through anti-apoptotic effects and the secretions of growth factors including the vascular endothelial growth factor, fibroblast growth factor andthe brain-derived neurotrophic factor [78].

Alzheimer's disease is characterised by the degeneration of the cholinergic neurons through the deposition of β-amyloid and the formation of neurofibrillary tangles. MSCs have shown to enhance the clearance of the amyloid plaques increasing neural survival [79]. Animal studies have shown that MSC transplantation could modulate microglial activation, alleviate symptoms, and delay the cognitive decline [80].

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