Research Article

Stem cell therapy as a potential therapy for Duchenne muscular dystrophy.

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Introduction

Mesenchymal stem cells (MSCs) are a population of adult stem cells with many properties that make them attractive for use in the fields of tissue engineering and regenerative medicine[1]. These cells are inherently plastic, enabling them to differentiate along different lineages under the appropriate induction conditions. They also appear to exhibit a number of trophic properties that promote regeneration in the surrounding tissue[2]. MSCs can be harvested from a variety of adult tissues including the bone marrow, adipose tissue and wharton's jelly of the umbilical cord and unlike embryonic stem cells, their use is not restricted by ethical considerations or legal restrictions. As a result, there is considerable hope that MSCs will be incorporated in a variety of clinical interventions, either as a cellular therapy to improve the natural history of diseases or as a component of engineered tissue constructs that can replace diseased or damaged tissues. More recently, it has been noted that the regenerative benefit of MSCs does not appear to correlate solely or directly with their ability to differentiate into the diseased tissue type[3]. In a number of studies, MSCs have been injected into diseased tissue, such as the heart[4] and brain[5]. Although there was functional improvement to these tissues following MSC injection, there was little evidence of the MSCs differentiating into the surrounding cell types, as was expected. Instead, these studies have led to the discovery that MSCs promote wound healing and regeneration in the surrounding tissues by modulating the local inflammatory responses [6] and by limiting fibrosis of the functional tissues [1]. It has since been shown that MSCs also promote angiogenesis and secrete trophic (i.e., pro-growth and pro-survival) factors that augment the endogenous regeneration process[7]. Although substantial investigation is still needed to elucidate fully the mechanisms of their trophic behavior. Despite the potentially far-reaching promise of MSCs in many aspects of regenerative medicine and tissue engineering, any approaches using these cells are limited by the availability of a suitable MSC population in a clinical setting [1]. The most common source of MSCs for clinical use is the bone marrow. However, the low concentration of MSCs in the bone marrow necessitates the application of specialized equipment to concentrate the MSCs prior to use [8], and may still yield too few cells for many tissue engineering applications [9].

Similar limitations also exist for MSCs derived from adipose tissue, which is another commonly used source of MSCs[10]. As a result, there is considerable interest into alternative sources of MSCs that may be able to overcome these clinical limitations like induction of MSCs from induced pluripotent stem cell or embryonic stem cells. Through the years, significant progress has been made toward MSC characterization, and now days the International Society for Cellular Therapy (ISCT) gathered a series of recommendations regarding the minimal criteria for the definition of "mesenchymal stem cell" populations.

Specifically, MSCs are determined to be characterized by (i) plastic adherent ability; (ii) absence of definitive hematopoietic lineage markers, such as CD45, CD34, CD14, CD11b, CD79-α, CD19, and class II major histocompatibility complex (MHC) molecules B7-1, B7-2, CD40 and CD40L molecules, specially human leukocyte antigen (HLA)-DR, and expression of nonspecific markers CD105, CD90, and CD73; and express the major

histocompatibility complex (MHC) class I (iii) MSCs can be expanded more than 104-fold in culture without loss of their multilineage differentiation potential ability and to differentiate into mesodermal lineage cells, osteocytes, chondrocytes, and adipocytes[11].
MSCs secrete a complex set of multiple soluble biologically active molecules, the secretome, composition of which varies significantly, depending on the age of the host and niches where the cells reside(12,13). The MSCs secretome in general consists of such biologically active factors as chemokines and cytokines, cell adhesion molecules, lipid mediators, interleukins (ILs), growth factors (GFs), hormones, micro RNAs (miRNAs), long non-coding RNAs (lncRNAs), messenger RNAs (mRNAs), exosomes, as well as microvesicles(13). It is revealed that MSC secretion include in particular vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), transforming growth factor beta 1 (TGF-b1), nerve growth factor (NGF), placental growth factor (PGF), stromal-derived growth factor (SDF-1/CXCL12), monocyte chemo-attractant protein-1 (MCP-1/CCL2), IL-6, IL-8, IL-10 and IL-13(14). Also MSCs were found to secrete bone morphogenetic proteins (BMP), CC chemokine ligand 5/ Regulated on activation, normal T cell expressed and secreted (CCL5/ RANTES), epidermal growth factor (EGF), granulocyte colony-stimulating factor (G-CSF) granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), inter-cellular adhesion molecules (ICAM), indoleamine-2,3-dioxygenase (IDO), leukemia inhibitory factor (LIF), matrix metalloproteases (MMP-1, MMP-2, MMP-3, MMP-7), platelet-derived growth factor (PDGF), metalloproteinase inhibitors (TIMP-1, TIMP-2)(13,15). Different MSCs from different sources may show different secretomes, For instance, ADSCs have higher expression of mRNA, VEGF-D, IGF-1 and IL-8, while dermal sheath and dermal-papilla-derived cells secrete higher concentrations of CCL2 and leptin(16). It is known that placenta derived MSCs are characterized with increased expression levels of HGF, bFGF, IL-6, IL-8, IL-1a and IL-1b, while in secretome obtained from bone marrow-derived MSCs the levels of VEGF-A, NGF and angiogenin are higher(17).

Duchenne muscular dystrophy (DMD)

Duchenne muscular dystrophy (DMD) is an X-linked progressive muscle wasting disorder caused by mutations in the DMD gene (18), affecting 1 in 3,500 - 5,000 male birth. Serum creatine kinase (CK) levels are elevated at birth, and motor milestones are delayed. Reduced motor skills between age 3–5 years provoke diagnostic evaluation. Quality of life for DMD boys is further affected early in life, with inability to keep up with peers in early school age, and loss of ambulation by 12 years of age, with premature death at 20–30 years of age due to respiratory and cardiac complications. Mutations of the DMD gene cause complete (Duchenne) or partial (Becker) loss of dystrophin protein at the sarcolemma[19]. In normal muscle cells, dystrophin forms a complex with glycoproteins at the sarcolemma, forming a critical link between the extracellular matrix (ECM) and cytoskeleton [20]. Without the complex, the sarcolemma becomes fragile and is easily ruptured by mechanical stress [20].

Dystrophin deficiency is the primary mechanisms underlying DMD but other secondary pathological mechanisms rapidly develop after dystrophin deficiency including disturbed immune response, ischemia and marked fibrosis. Fibrosis is defined as the excessive or unregulated deposition of extracellular matrix (ECM) components and is a particular hallmark of DMD. Controlled deposition of ECM components during growth and repair is critical for providing a scaffold to build and structure new tissue, but alterations in the timing, the intensity, and/or the components of this process can lead to excessive ECM deposition (fibrosis) and loss of tissue function. A longitudinal study of 25 DMD patients with an average follow-up time of over 10 years examined the correlation of the severity of the pathology and different pathological features, including myofibres atrophy, necrosis, and fatty degeneration. Severity was gauged by muscle strength and age at loss of ambulation. The study concluded that endomysial fibrosis was the only myopathologic parameter that significantly correlated with poor motor outcome.

MSCs and anti-fibrosis effect

An anti-fibrotic effect of stem cells conditioned medium is mediated by bioactive molecules in

MSCs secretome which decrease accumulation of extracellular proteins and, therefore, lead to reduced scar formation. An et al., (2017) studied the influence of umbilical cord-derived mesenchymal cells (UCMSC) secretome on formation of fibrotic areas in mice with hepatic fibrosis. A decrease in the number of activated hepatic stellate cells (HSCs) ex-pressing α-smooth muscle actin (α-SMA) was shown after an injection of the UCMSC-CM in the diseased mice, which was accompanied by reducing fibrotic areas.

The researchers analysed the UCMSCs secretome using nano chip-LC/QTOF-MS and discovered the presence of milk fat globule EGF factor 8 (MFGE8), an anti-fibrotic protein known to down-regulate the expression of TGF-βR1 (transforming growth factor β type 1 receptor) at the mRNA and protein level, thereby decreasing the activation of human hepatic stellate cells. For muscle disease Nakamura et al. reported that MSC-exosomes containing miRNAs promoted muscle regeneration and reduced the fibrotic area [Nakamura et al., 2015].

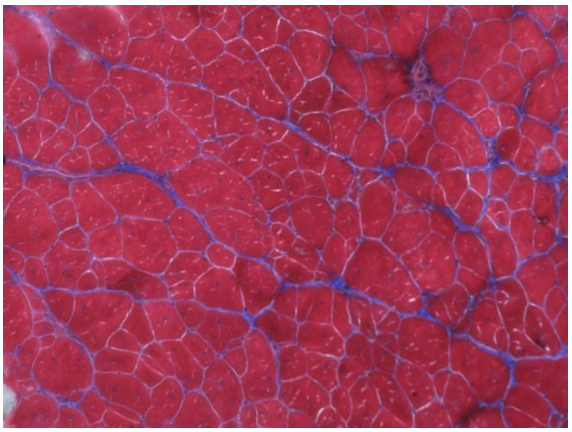
Material and methods

In our study we use bone marrow mesenchymal stem cell to see its effect on muscle fibrosis in our DMD animal models. We inject half million of MSCs into tibialia anterior muscle of DMD animal model.

We repeat injection of MSCs for four times and by using Masson trichrome stain we evaluate of MScs injection on degree of fibrosis.

Results

We can see decrease degree of fibrosis in injected tibialis anterior muscle of injected animals.



We can see that the degree of blue color has decreased indicating decrease degree of fibrosis.

Conclusion

Regenerative Medicine or stem cell therapy aims to restore the loss of function in tissues and organs due to any cause by the replacement of dysfunctional structures with competent cells or tissues. Medical researchers believe that stem cell treatments have the potential to change the face of human disease and alleviate suffering.

In order to achieve this goal regenerative medicine takes advantage of the use of mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) in untreatable neurological disease as in Duchenne muscular dystrophy, a progressive fatal muscle disease with patients usually die at the age of 20 years due to severe muscle weakness and wasting.

Stem cell therapy is considered to be one of the most promising strategies for treating muscular dystrophies and the use of the induction abilities of human induced pluripotent stem cells (hiPSCs) with the abilities of obtaining unlimited numbers of myogenic and non-myogenic as MSCs are a promising strategies for stem cell therapy to regenerate skeletal muscle with a potential for more self-renewal and regenerative capacity.

Mesenchymal stem cells (MSCs) secrete soluble factors necessary for skeletal muscle growth and regeneration. MSCs are also reported to regulate immune responses and can decrease the degree of fibrosis.

DMD have no cure until now and most patients die by thirty from cardiac and respiratory problems only genetic interventions and stem cell therapy can provide hope for the treatment of Duchenne muscular dystrophy. MSCs can provide a therapeutic option for treatment of Duchenne muscular dystrophy

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