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Review Article

MESENCHYMAL STEM CELLS AND ITS POTENTIAL USE IN CELL THERAPY- AN OVERVIEW

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Stem cells play a central role in the normal growth and development of animals and human having the capacity of self renewal and the potential to differentiate into one or more cell types depending on the *in vivo signals*. Mesenchymal stem cells (MSC) have generated a great amount of interest over the past decade as a novel therapeutic potential for a variety of diseases. Presently MSC based clinical trials have been conducted in many kinds of pathological conditions, like spinal cord injury, traumatic brain injury, Parkinson disease, stroke, bone healing, cardiac repair, tendon healing etc. Many completed trials demonstrated the safety and efficacy. Clinical application of MSC are mainly attributed to their important four biological properties, i.e. ability to home to sites of inflammation following tissue injury; to differentiate into various cell types; to secrete trophic factors capable of stimulating recovery of injured cells and inhibiting inflammation and to perform immunomodulatory functions. Furthermore, genetic modification of MSCs has provided prospects for clinical use in many diseases. Here we are highlighting the importance of mesenchymal stem cells in treatment of different disease conditions.

Key words: Mesenchymal stem cells, Clinical trials, Therapeutic potential, Trophic factors, Tissue injury

Stem cells

Special primal structures in the body with an extraordinary ability to self-renew, the ability to differentiate into a specific cell type, and clonogenic ability (Evans and Kaufman, 1981; Beltrami *et al*., 2003 and Nagy *et al*., 2005). Traditionally, a hierarchical system based on developmental potential has been employed for the classification of vertebrate stem cells. There are two types of stem cells as embryonic and adult stem cells. The embryonic stem cells are further classified as totipotent and pluripotent stem cells, while adult stem cells are classified as unipotent and multipotent stem cells (Leeb *et al*., 2010). Embryonic stem cells (ESCs) derived from the inner cell mass of the blastocyst possess the

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greatest developmental potential with the ability to generate all differentiated cell types in the body, and are termed pluripotent. Whereas, tissue-committed stem cells are restricted to producing cell types found within that tissue, and are termed multipotent.

Totipotent stem cells

Totipotency is the ability of a single cell to divide and differentiate into an entire organism. In mammals (placental animals) totipotent stem cells have the potential to become any type of cells in the adult body and/or cells of the extra embryonic membrane. Only the zygote and the first cleavage stage blastomeres are totipotent in nature (Amabile and Meissner, 2009).

Pluripotent stem cells

Pluripotent stem cells can develop into any of the three major tissue types namely endoderm, ectoderm and mesoderm, but cannot contribute to cells of extra-embryonic membranes. They exist *in vivo* only for a short period of time that includes later blastomeres, inner cell mass(ICM) cells of the blastocyst and the ICM derived epiblast. Under appropriate culture conditions explanted ICM cells give rise to pluripotent embryonic stem (ES) cells. Induced pluripotent stem cells (iPSCs) are adult cells that have been genetically reprogrammed to an embryonic stem cells like state and it express genes important for maintaining the properties of embryonic stem cells. Takahashi and Yamanaka (2006) reported that iPSCs can be generated from mouse embryonic fibroblasts (MEF) and adult mouse tail-tip fibroblasts by the retrovirus-mediated transfection of four transcription factors, namely Oct3/4, Sox2, c-Myc, and Klf4. The iPSCs are useful tools for drug development and modeling of diseases, viruses are currently used to introduce the reprogramming factors into adult cells.

Multipotent stem cells

These are small number of partially undifferentiated stem cells present in the adult tissue and are capable of forming a limited number of specialized cell types, typically those of a closely related family of cells. They are less plastic and more differentiated stem cells. Multipotent stem cells are available from different organs in the body. This offspring of pluripotent cells becomes progenitor of cell line such as blood cells, skin cells, neural cells etc (Leeb *et al*., 2010).

Unipotent stem cells

Stem cells which are available in the specific part of an organ like retina, striated muscle, dermal papillae are called unipotent. These cells on development cannot produce any cell type other than itself, but have the ability to self renew, such as limbic stem cells that help in repair of retina (Guan *et al*., 2006).

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent cells able to differentiate into several mesenchymal lineages, classically

derived from bone marrow (Azouna *et al*., 2012). MSCs represent a rare population ~ 0.001 to 0.01% of the total nucleated cells in bone marrow (BM) (Pittenger *et al*., 1999 and Le Blanc and Pittenger, 2005), giving rise to adipocytes, osteoblasts, chondrocytes, and vascular smooth muscle (VSM) like hematopoietic supportive stromal cells (Caplan, 1991 and Prockop, 1997). Evidence for the existence of a cell population within the bone marrow that produced non-hematopoietic progeny emerged in the mid-1960 with the pioneering work of Friedenstein and colleagues (Friedenstein *et al*., 1966 and Friedenstein *et al*., 1970). Thereafter numbers of independent studies were carried out by different researchers, who employed the isolation and culture techniques of Friedenstein *et al*. (1987) and together these studies demonstrated that MSCs were multipotential and differentiated into cells of the mesodermal lineage, including osteoblasts (Friedenstein *et al*., 1987), chondroblasts (Mackay *et al*., 1998), adipocytes (Beresford *et al*., 1992) and myoblasts (Prockop, 1997). The marrow stromal stem cells concept was proposed in 1988 by Owen and Friedenstein (Owen and Friedenstein, 1988), whereas the currently popular term "MSC" was later coined by Caplan (Caplan, 1991). All these initial studies have paved the way for the development of the field of MSC research which we know today. Exponential increase in scientific and clinical research in the field of MSC was observed since then. It is now generally recognized that MSCs possess the *in vitro* characteristics of stem cells with the ability to proliferate, symmetrically divide, and produce multi-lineage mesodermal derivatives, making MSCs an attractive candidate for use in potential cellular therapies. In addition, MSCs exhibit further promising qualities for regenerative medicine, including relatively easy isolation from small aspirates of BM, as well as relatively easy expansion in culture with low tumorigenicity and teratoma formation.

MSCs have been reported to display immunosuppressive properties that are advantageous for allogeneic transplantation, and in ideal settings, autologous transplantation is also possible. Subpopulations of MSCs have also been reported to be capable of differentiation to non-mesodermal lineages, indicating the potential application of MSCs in a wider range of diseases. Mesenchymal stem cells (MSCs) have been referred to other names such as colony-forming fibroblastic cells (Friedenstein *et al*., 1976), BM stromal stem cells (Bianco *et al*., 2001) mesenchymal progenitor cells (Sun, *et al*., 2003) and BM stromal cells (Prockop, 1997).

International Society for Cellular Therapy has proposed three minimal criteria for defining human MSCs (hMSCs) (Dominici *et al*., 2006), were; 1) MSCs must be adherent to plastic when cultured in standard conditions; 2) MSC populations must express specific surface antigens CD105, CD73 and CD90, but lack

expression of haematopoietic surface antigens, such as pan-leukocyte marker CD45, primitive haematopoietic progenitor and endothelial marker CD34, monocyte and macrophage markers CD14 and CD11b, B lymphocyte markers CD79á and CD19, and human leukocyte antigen (HLA)-DR molecules; and 3) MSCs must exhibit multipotent differentiation potential by differentiating to osteoblasts, adipocytes and chondroblasts under standard in *vitro tissue* culture-differentiating conditions with confirmation of differentiation using histochemical and immunohistochemical staining (Dominici *et al*., 2006).

Sources of Mesenchymal stem cells (MSCs)

These cells are characterized morphologically by a small cell body with a few long and thin cell processes. MSCs which reside within the stromal compartment of bone marrow were first identified in the pioneering studies of Friedenstein and colleagues in the 1970s as cells that are capable of self-renewal and possess multipotency (Friedenstein *et al*., 1970). Besides from bone marrow, MSC-like cells have been isolated from many other organ and tissue including brain (Uchida *et al*., 2000), skeletal muscle (Williams *et al*., 1999), umbilical cord blood (Erices *et al*., 2000 and Peters *et al*., 2010), dental pulp (Gronthos *et al*., 2000), adipose tissue (Zuk *et al*., 2001 and Lopatina *et al*., 2011) and amniotic fluid (Moorefield *et al*., 2011) etc. Bone marrow derived MSCs have been successfully isolated and expanded mostly from human (Pittenger *et al*., 1999), rat (Yoshimura *et al*., 2007), rabbit (Lapi *et al*., 2008), canine (Yoshimura *et al*., 2007) and mouse (Peister *et al*., 2004), but except in pig (Zeng *et al*., 2006), now from domestic animals also being isolated like from goat (Kumar *et al*., 2013). The youngest, most primitive MSCs can be obtained from the umbilical cord tissues, namely Wharton's Jelly and the umbilical cord blood. However, the MSCs are found in much higher concentration in the Wharton's Jelly compared to the umbilical cord blood, which is a rich source of hematopoeitic stem cells. The umbilical cord MSCs have more primitive properties than other adult MSCs obtained later in life, which might make them a useful source of MSCs for clinical applications (Fu *et al*., 2006 and Wang *et al*., 2004). Today, BM remains the principal source of MSCs in studies investigating their potential use in cell therapy.

Surface markers of MSCs

Considerable effort has been made on the identification of specific surface markers for selection, detection and testing of MSC preparations. It is widely accepted that bone marrow derived MSCs (BM-MSC) express SH2 (CD105), H3/SH4 (CD73), integrin b1 (CD29), CD44, Thy-1 (CD90), CD71, vascular cell adhesion molecule-1 (CD106), activated leukocyte cell adhesion molecule (CD166), STRO-1, GD2, and melanoma cell adhesion molecule (CD146) (Haynesworth *et al*., 1992; Galmiche *et al*., 1993; Sordi *et al*., 2005 and Sacchetti *et*

al., 2007) and believed that BM-MSCs lack expression of hematopoietic surface molecules including CD45, CD34, CD14 or CD11b, CD79a, or CD19, and HLA-DR (Dominici *et al*., 2006). However, recent studies have implied that mouse BMMSCs might express the hematopoietic surface molecules, CD45 (Chen *et al*., 2007) and CD34 (Copland *et al*., 2008).

Immuno- modulatory effect of MSCs

MSCs possess remarkable immunosuppressive properties and can inhibit the proliferation and function of the major immune cell populations, including T cells, B cells and natural killer (NK) cells; modulate the activities of dendritic cells (DCs); and induce regulatory T cells both *in vivo* and *in vitro* (Sotiropoulou *et al*., 2006; Ryan *et al*., 2007 and Miller *et al*., 2010). The *in vivo* immunomodulatory properties of MSCs were first described in a baboon model of skin transplantation (Bartholomew *et al.*, 2002). MSCs inhibit T-cell proliferation stimulated by polyclonal activators, cognate antigen, and allogeneic mixed lymphocyte reaction (Darlington *et al.*, 2010). MSCs also inhibit B-cell proliferation, expression of chemokine receptors, differentiation and production of IgM, IgG, and IgA (Corcione *et al.*, 2006). In acute (Zappia *et al.*, 2005) and chronic (Karussis *et al*., 2005 and Gerdoni *et al*., 2007) experimental autoimmune encephalomyelitis (EAE) mice, intravenous MSC administration ameliorated clinical manifestations, CNS inflammatory infiltration, demyelination, and axonal damage. The potent immunomodulatory properties of MSCs are particularly relevant for multiple sclerosis (MS) (Uccelli *et al*., 2006 and Newman *et al*., 2009). These unique properties make MSCs ideal candidates for clinical application as immunosuppressant.

Homing of MSCs

MSC have the tendency to home at the site of injury. Homing is the term used when cells are delivered to the site of injury. Most of the time local delivery and homing of cells are found beneficial due to interaction with the host tissues, accompanied by the secretion of trophic factors (Figueroa *et al.*, 2012). There are a number of factors like cells age, culturing conditions, cell passage number and the delivery method, which influence the homing ability of MSCs to the injured site. Freshly isolated MSCs will have greater homing efficiency than the cultured cells. Matrix metallo-proteases (MMPs), the important proteases which are involve in the cell migration also plays important role in the MSCs migration (Ries *et al*., 2007). The hypoxic condition of the culturing environment influences the expression of these MMPs (De Becker *et al*., 2007). The next important factor is delivery method via which the MSCs are administered to the desired tissue. The most convenient and feasible way of MSCs transplantation is local injection to the site of injury or near the site of injury which provides more number of cells and increases its functional capacity. The exact mechanism of cell delivery is still unknown.

Mesenchymal stem cells as cell therapy

MSCs have a promising future in the world of cell therapy and the number of clinical trials has been rising since the last decade. Along with preclinical studies, MSCs have been found to be convincing in the treatment of many diseases (Wei *et al*., 2013). Currently, there are more than 463 registered clinical trials in different clinical phases, evaluating the potential of MSC-based cell therapy throughout the world. Among 463 registered trials, 264 trials are in open status which is open for recruitment whereas 199 trials are closed; out of which 106 studies are completed whereas the rest are in active phases (Ullah *et al*., 2015). Due to immunomodulatory properties, MSCs have been used in many human autoimmune disease clinical trials. However, the exact mechanism by which MSCs regulate the immune response is unclear (Baker and Issacs, 2014). Although the progress of clinical studies so far registered is slow, but the efficient use of MSCs in large clinical trials with upcoming promising results have proven MSCs as boon for regenerative medicine both in human and animals and it hold great promise for the future regenerative therapy (Ullah *et al*., 2015).

Mesenchymal stem cells and neuroprotective potential

Despite evidence showing that MSCs can transdifferentiate into multiple cell types *in vitro* and *in vivo*, the real contribution of MSCs to tissue repair-through significant engraftment and differentiation into biologically and functionally relevant tissue-specific cell types is still elusive (Phinney and Prockop, 2007). Experimental autoimmune encephalo myelitis (EAE), a model for multiple sclerosis, has been the first experimental autoimmune disease successfully treated with MSCs (Zappia *et al*., 2005). Several lines of evidence suggest that, somehow, MSCs have a direct effect on neural cells. They have been shown to enhance remyelination *in vivo* (Bai *et al*., 2009 and Constantin *et al*., 2009), provide *in vitro* soluble cues that influence fate determination of neural cells (Rivera *et al*., 2006 and Bai *et al*., 2009), display a potent antioxidant effect *in vivo* (Lanza *et al*., 2009 and Ripoll *et al*., 2011) and display a neuroprotective effect (Stemberger *et al*., 2011) mediated by the release of anti apoptotic molecules *in vitro* (Kemp *et al*., 2010) and *in vivo* (Ohtaki *et al*., 2008). Irrespective of these aspects, the current view suggests that MSCs may exert their neuroprotective effect at distance through the release of trophic molecules, possibly affecting microglia activation (Ohtaki *et al*., 2008) and inducing local neurogenesis (Bai *et al*., 2009 and Constantin *et al*., 2009). MSC transplantation has shown great promise in the treatment of many neurological disorders including spinal cord injury (McDonald *et al*., 2003), traumatic brain injury (Reiss *et al*., 2002), Parkinson disease (Bjorklund *et al*., 2003) and stroke (Savitz, 2002).

Neural stem cells exist in the developing and adult mammalian nervous system are

capable of undergoing expansion and differentiation into neurons, astrocytes, and oligodendrocytes *in vitro* (Reynolds and Weiss, 1992) and after transplantation *in vivo* (Svendsen *et al*., 1997). Treatment with MSCs appears to enhance functional recovery in the absence of combinatorial treatments. Studies with other stem cell populations suggest that they antagonize the negative effects of immune cells (Busch *et al*., 2010) while MSCs appear to release trophic factors that promote axonal regeneration and may also enhance the survival of damaged neurons (Cho *et al*., 2009). Mesenchymal stem cells also release signals that modulate host tissue responses. Through the release of trophic factors MSCs are capable of enhancing the endogenous repair potential of many tissues (Miller *et al*., 2010). Transplantation of MSC in spinal cord injury of rabbit model has shown the recovery in hind limb paralysis (Kumar, 2013).

Cardiovascular diseases

For myocardial repair, cardiac cells transplantation is a new strategy which is now applied in animal models (Singh, 2013). MSCs are considered as good source for cardiomyocytes differentiation. However, *in vivo* occurrence of cardiomyocytes differentiation is very rare and *in vitro* differentiation is found effective only from young cell sources (Noort *et al*., 2010 and Ramkisoensing *et al*., 2011). The systematic injection of BM-MSCs into diseased rodent models partially recompensed the infracted myocardium (Nagaya *et al*., 2005). Although MSCs are effective in myocardial infarction and related problems, but still cell retentivity in the heart is rapidly decreased, after 4 h of cells injection only 10% and after 24 h it was found approximately 1% cell retention (Freyman *et al*., 2006 and Van Der Spoel *et al*., 2011). Following this study, Roura *et al*. (2012) reported that UCB-MSCs retained for several weeks in acute myocardial infarction mice, proliferated early and then differentiated into endothelial lineage. Most recently, transplantation of UCB-MSCs into myocardial infarction animal model along with fibronectin-immobilized polycaprolactone nanofibres were found very effective (Kang *et al*., 2014).

Tendons and ligaments

Tendons and ligaments connects muscle-to-bone and bone-to-bone, respectively, they share a similar hierarchical structure of collagen fibers and resulting mechanical properties. The interface between tendon, ligament and bone are otherwise indistinguishable, although a large degree of variability exists depending on the location of the attachment site. Tendons are poorly vascularized, are relatively acellular, and have limited potential for regeneration (Avella *et al*., 2009). Injuries to tendon and ligaments are common and are traditionally classified as either acute ruptures or chronic degenerative changes (Benjamin *et al*., 2002). Kannus and Josza reported that nearly all (97%) of spontaneously ruptured tendons showed histopathological changes

indicative of chronic tendon degeneration (Kannus and Jozsa, 1991). Likewise, acutely ruptured tendons are significantly more degenerated than tendinopathic tendons (Tallon *et al*., 2001). These tissues heal with inferior 'scar'-type tissue, with the risk of subsequent rupture at the repair site or formation of fibrous adhesions.

MSCs have emerged as the gold standard for cellular therapies in musculoskeletal diseases (Tuan *et al*., 2003). MSCs are capable of differentiating into tenocytes, chondrocytes, and osteocytes (Awad *et al*., 1999; Tuan *et al*., 2003 and Kuo and Tuan, 2008), thereby potentially aiding in restoration of the native structure of the healing tissues. Beyond differentiating into site-appropriate epithelial lineages, MSCs secrete bioactive molecules that provide a regenerative microenvironment for a variety of injured adult tissues (Caplan, 2007). Experiments investigating the utility of MSCs in augmenting bone-tendon healing were first performed in rabbit models (Lim *et al*., 2004 and Ouyang *et al*., 2004). Mesenchymal stem cells (MSCs) transplantation in the therapy of equine tendonitis has been reported beneficial effects by many researchers (Nixon *et al*., 2008; Crovace *et al*., 2010 and Carvalho *et al*., 2011). BMSCs can also be used clinically to augment healing at the bone-tendon interface after procedures such as anterior cruciate ligament reconstruction (Lim *et al*., 2004). There are now several studies illustrating the potential for the use of stem cells in tendon repair, and also their use in other tissue engineering applications (Rayanmarakkar *et al*., 2009; Rayanmarakkar *et al*., 2010; Oragui *et al*., 2011 and Gerdoni *et al*., 2012).

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