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Immune Properties of Mesenchymal Stem Cells in the Translation of Neural Disorders

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Abstract

Mesenchymal Stem cells (MSCs) are in clinical trails for a variety of disorders and thus far, there is no report of deleterious effect. Early studies suggested that MSCs were mesodermal in origin. However, more recent studies indicate that MSCs could be neuroectodermal in origin. This origin might explain why MSCs can efficiently form functional neurons. MSCs are proposed for several medical indications, mostly due to reduced ethical concerns, ease in expansion, ability to be transplanted across allogeneic barrier (off the shelf) and plasticity. Indications include, but are not limited to therapy for inflammation, tissue repair, protection of tissue damage and neuronal disorder. Despite the therapeutic promise for MSCs, there are variations in the data among and within labs. The hindrance appears to be mostly due to the lack of consensus to expand MSCs. We discuss the potential treatment of spinal cord and traumatic brain injury with MSCs and, the utilization of zebrafish as a model system for regenerative medicine. We also discuss the importance of a molecular balance to prevent transformation of MSCs during differentiation to

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neural cells. We explain the over-arching chapter role of the immune properties of MSCs in the translation of MSCs as well as safety issues for clinical application.

Keywords: mesenchymal stem cells, immunosuppression, bone marrow, cytokine, cancer, stem cells.

6.1 Introduction

Stem cells have the potential to self-renew and differentiate into all cell types, making stem cells as the future therapy for tissue regeneration and organogenesis. Embryonic stem cells are linked to ethical concerns and scientifically, in tumor formation. These issues dampen the regenerative potential of embryonic stem cells. Similar arguments can be also made for induced pluripotent stem cells. Thus, clinical application is narrowed to adult stem cells such as those in the brain and in bone marrow, such as mesenchymal stem cells (MSCs). The clinical and experimental data indicated that MSCs can have regenerative/repair potential for several clinical disorders (www.clinicaltrials.gov).

The multipotent mesenchymal stem cells are primordial in origin and can be isolated from fetal and adult tissues such as the placenta, bone marrow and adipose tissues [1–4]. Several membrane proteins have been identified to select and to phenotype MSCs. These include, but not limited to CD73, CD90, CD105. MSCs do not express markers like CD45, CD34, CD14, CD19. In addition to phenotype, other molecules have been proposed as methods to identify MSCs. These include vimentin and fibronectin. In all studies, the function of MSCs need to be validated for multipotency. In general, it is acceptable to induce MSCs to differentiate into osteoblast, adipocyte and chondrocyte [5].

MSCs are attractive for stem cell treatment, mostly due to reduced ethical concerns and ease of *in vitro* expansion. Furthermore, there is no need for a match at the major histocompatibility complex; thus making MSCs available as 'off the shelf' sources in cell therapy. The microenvironment plays an important role in the functional response of MSCs. The immune function of MSCs is particularly relevant. MSCs can be immune enhancer and suppressor cells.The immune function of MSCs depends on the milieu of the microenvironment. Specific cytokines and chemokines can be chemoattractant to facilitate the migration and homing of MSCs and other immune cells to the site of tissue injury.

6.2 MSC Immunology

MSCs show functional plasticity with regards to their immune properties by exerting both immune suppressor and enhancer functions [6], producing various cytokines that can stimulate the cells through autocrine and/or paracrine manner [7]. It has been suggested that major histocompatibility complex-II (MHC-II) expression be included among the minimum requirements for designating a cell as MSC [5]. However, there are several reports of MHC-II-negative cells with a phenotype and multi-lineage capacity similar to MSCs [8], suggesting that a population of MSCs do not express (detectable amounts of) MHC-II.

MHC-II allows cells to act as antigen presenting cells (APCs), with the cell mounting the antigen within the MHC-II grove to activate CD4+ T cells. The MSCs expressing MHC-II may then be able to act as APCs. However, unlike most APCs, MSCs express MHC-II in a bimodal fashion, with high MHC-II densities at low levels of interferon gamma (IFNγ), and low MHC-II density at high IFNγ levels [9]. This is highly significant when considering MSCs as a therapeutic tool, as the MSCs would be in an inflammatory microenvironment, in the presence of a milieu of inflammatory cytokines – including IFNγ. This bimodal activity has been observed *in vitro* in MSC-derived neurons, whereby the neurons expressed low levels of MHC-II, but MHC-II level could be restored by stimulation with IFNγ [10]. Thus MHC-II expression has the potential to be problematic in case the MHC-II is re-expressed. If so, this could result in rejection of the transplanted MSCs.

Since the majority of MSCs show low to undetectable expression of MHC-II molecules on their cell surface they fail to activate T-cell response [5, 8]. In the absence of an allogeneic response, MSCs are suitable candidates for allogeneic transplant [6]. In the absence of pro- and anti-inflammatory cytokines, MSCs are further immunoprivileged in that they interfere with other immune cell functions, such as inhibiting B-cell proliferation and chemotaxis [11], suppressing the activity of dendritic and natural killer cells [12], and triggering the proliferation of regulatory T-cells to suppress an immune response [13]. It has been suggested that these immunosuppressive properties may play a role in tissue repair, modulating the other immune cells to prevent immune inflicted tissue injury and promote healing [14]. An equilibrium of these two properties must be understood and balanced for normal MSC function, as well as for MSC function in regeneration and repair [6, 15].

6.3 MSCs and Cancer

As a consideration to the translational potential of MSCs, one must examine the long-term effects of their presence in patients. We have discussed the immunomodulatory properties of MSCs and now must consider what may happen when the cellular signals go awry. This brings us to the role of MSCs in cancer; the current research has found them to be both tumor suppressive and enhancing, depending on the microenvironment. This section reviews the supportive and inhibitory properties of MSC in cancer and then takes a closer look at MSCs and cancer of the brain. In this special case we will consider how MSCs can be used for treatment of this deadly disease.

The association of MSCs with respect to tumors is of great interest from the past decade. MSCs have a contradictory role in cancer, in some studies it is found that MSCs can promote tumor progression through immune modulation, increase in metastasis, or increasing tumor cells, while in others a tumor suppressive role of MSCs was described. This contradicting behavior may be due to difference in the source of tissue, method of cell administration, individual donor variability, and injection timing of MSCs.

6.3.1 Role in Tumor Growth

MSCs, irrespective of their origin, were shown to have a role in tumor progression by initiation, growth and metastasis of the cancer or tumor cells [16–20]. Function and properties of MSCs can also vary depending on the origin of MSCs. Although MSCs are ubiquitous, there are two major organs of MSC. In the adult, the bone marrow and adipose tissues are the major sources of MSCs. Other sources of MSCs include the skin, muscle, lung, tendons and periodontal ligament. MSCs from peripheral origin exhibit greater tumor tropism than the MSCs from bone marrow. The immune suppressive function of MSCs is relevant for tumor initiation and growth. This was observed in an experimental transplantation model of B16 melanoma cells, which formed tumors only when co-injected with MSCs into allogeneic mice [16]. MSCs role in metastasis of cancer was demonstrated by co-injecting human breast cancer cells with human MSCs derived from bone marrow into immune compromised mice [18].

Growth-promoting effect of MSCs was shown in an *invivo* model of colon cancer with co-injecting of adult and fetal MSCs [21]. The role of MSCs in tumor survival was shown *invitro* with human B-cell lymphoma. [22].Tumor promoting properties of MSCs were also shown with MSCs derived from peripheral tissues like adipose tissue [23]. These MSCs were also responsible

for the increase in the tumor size and the viable tumor cells count when co-injected with lung cancer or glioma cells [24].

The research studies that showed MSCs aiding tumor growth needs careful evaluation as MSCs have the tendency to proliferate in the presence of tumor cells. Hence, the increase in tumor mass could be due to the ability of MSCs forming carcinoma associated fibroblasts to support tumor growth.

6.3.2 MSCs in Tumor Suppression

MSCs can be used as tumor suppressors by secreting anti-tumor molecules like TNF, IFN-ß and Dickkopf-related protein-1 (DKK-1) [25–27]. These molecules will modify pathways that include Akt signaling, ß-catenin and c-Myc, which are linked to tumor progression [25, 26, 28, 29].

The tumor suppressive effect of MSCs was shown when they were co-administered with glioma cells, resulting in modified Akt signaling [26]. Glioma cells secrete VEGF and other soluble factors to facilitate the invasion and migration of the transplanted MSCs to the site of the tumor. Human fetal skin-derived MSCs inhibited human liver cancer cell lines, with reduced proliferation, colony formation, and oncogene expression both *in vitro* and in vivo [30]. When these cell lines were co-injected with equal ratio of MSCs, tumor development was delayed and tumor size decreased. MSCs were shown to inhibit the growth of rat colon cancer when equal number of MSCs and tumor cells were co-injected [31]. The pro-inflammatory role of MSCs was suggested by the infiltration by Macrophages and granulocytes when co-injected with tumors. The anti-tumor effect of MSCs was noted when ß-catenin signaling was inhibited through DKK-1 with solid and hematological tumors [25, 32].

6.3.3 MSC and Brain Cancer

The most common type of primary brain tumor is glioblastoma multiforme (GBM). GBM is a very aggressive and invasive cancer with an extremely poor prognosis. Current therapy involves tumor resection followed by both radio- and chemotherapy and these results in a median survival of less than 15 months [33]. Due to an urgent need for new treatments, scientists have begun to look at MSCs as tumor targeting drug delivery vehicles. This is partly due to the many characteristics that make MSCs a great transplant option. This topic is discussed above, describing the ease of access, production, and the donor of MSCs as universal. In addition to these benefits, MSCs also showed tumor tropism.

An increase in the interest of the mechanisms of homing and migration of MSCs to tumors, has led to a better understanding of this process. There are several different molecules that have been found to be involved. Although the candidate molecules vary with the cancer type, they include growth factors, chemokines, and cytokines that are released from the tumor or surrounding stroma. One example is stromal cell derived factor 1α (SDF-1α) and its receptor chemokine (C-X-C motif) receptor 4 (CXCR4) commonly expressed on cancer cells. MSCs have been found to use SDF-1α-CXCR4 signaling for migration to areas of inflammation, which is often common in the tumor microevironment [34].

Other factors, such as VEGF, can enhance tumor tropism of MSCs to tumors. Breast cancer and gliomas have been reported to express high levels of VEGF, which induces the migration and invasion of MSCs to tumors [35]. MSC migration may be also increased in response to irradiation and hypoxia. Radiation may lead to increased expression of inflammatory mediators to enhance the migration of MSC to the tumor [35]. Hypoxia is often associated with tumor progression and can lead to the production of IL-6 which acts in a paracrine fashion on MSCs, causing increased migration to the tumor [36]. The mechanism of MSC homing and migration to the tumor site continues to be elucidated. However, the clinical and experimental evidence provided information on the tropism of MSC to brain tumors.

One of the earlier reports showing MSCs migrating to the region of gliomas was indicated with an experimental model using rats [37]. Autologous MSCs were intracranially implanted into rats that developed gliomas. The MSCs migrated and dispersed within the tumor mass [37]. Subsequent studies with immunocompromised mice showed human MSCs migrating to the region of human gliomas [38]. The MSCs were injected into the ipsilateral and contralateral carotid arteries of the mice [38]. In other studies, rat MSCs were injected intratumorally and this resulted in the migration to the invasive rat glioma and to the distant tumor microsatellites [39]. The investigators also observed that the implanted MSCs avoided the normal brain gray matter [40].

Based on the above findings, MSCs show promise as a delivery system for toxic substances to the tumor while being able to avoid adverse effects of the drug on normal brain tissue. Given the dire need for improved therapy for GBM researchers have also started looking at ways to increase the efficacy of current treatments by sensitizing the cells. Our group has recently published on the chemosensitization of GBM cells through the transfer of functional anti-miR-9 within MSCs, by packaging it within the exosomes [41]. As a cellular vehicle, MSCs could deliver chemosensitizing reagents as adjuvant to other treatments.

As an example, TRAIL-secreting MSCs in combination with lipoxygenase inhibitor, MK886, enhanced apoptosis of the resistant cancer *in vitro* and, also increased tumor regression in an orthotopic mouse model of glioma [42].

The reports are not consistent with regards to favoring the use of MSCs as cellular targets for brain cancer. Rather, there is some evidence of a supportive role for MSCs in brain tumors [43, 44]. In a murine model of GBM, MSCs were shown to infiltrate the tumor and this correlated with tumor progression [43]. Similarly, *in vitro* studies with the tumor-associated MSCs led to increased proliferation of the GBM cells [43]. One must also consider the source of the MSCs that will be used for cellular based therapy. Akimoto *et al* examined the effect of MSCs from different sources on primary GBM cells and found that umbilical cord blood-derived MSCs inhibited the proliferation of the GBM cells while the adipose tissue-derived MSCs supported proliferation of the cells' proliferation [44]. Although there is substantial data to support the use of MSCs as cellular vehicle of drugs to brain cancers there are still some reports that should not be ignored.

A major concern with using MSCs in the treatment of cancer is their potential to become immunosuppressive, which could become a survival advantage for the very GBM cells that are being targeted. One must also consider the long-term fate of the transplanted MSCs in patients after the treatment has been completed. There are two major reasons for this, first would be to avoid continuous exposure of normal tissue to anti-tumor agents delivered by the MSCs. Second, MSCs respond strongly to their microenvironment, which may change dramatically once the cancer is eliminated and could lead to malignant transformation. To avoid these issues, scientists have developed suicide gene therapy. This method involves the transfer of a gene encoding a suicide protein into the MSCs for selective elimination. Herpes simplex virus thymidine kinase (HSV-TK) is the most commonly used. Expression of HSV-TK in the cells sensitizes it to the prodrug ganciclovir (GCV) by phosphorylating GCV into its toxic form. This will allow targeting of only the cells producing thymidine kinase. The activated form of GCV inhibits DNA synthesis, which leads to cells death of the MSCs but also has a significant bystander effect that will cause the death of neighboring cells [40]. There is continued research to increase the efficacy of the suicide gene engineered MSCs, by co-expressing other proteins known to target cancer cells. One example is a group that co-expressed a potent and secretable variant of tumor necrosis factor apoptosis-inducing ligand (S-TRAIL) in addition to HSV-TK. This caused caspase-mediated GBM cell death and selective sensitization of the MSCs to the prodrug GCV [45].

6.4 Regenarative Potential

MSC was discovered by Friedenstein *et al.* and was referred as CFU-F (colony forming unit-fibroblasts). Similar cells were isolated from the bone marrow and with similar formation of colonies [46, 47]. MSCs have several functions, including support of hematopoiesis during transplantation with simultaneous decrease of graft versus host through veto property [48].

MSCs have been shown to restore heart function [49, 50]. At a high dose of MSC when injected into the intracoronary region of left ventricle showed significant improvement in the normal function of the heart [49, 50]. This is a highly significant property of MSCs because cardiac failure is the leading cause of death in USA. The use of MSCs for neurological disorder has not yet reached the patients. However, the use of MSCs for neural disorder is widely accepted. Several animal models have shown full recovery of the damaged neurons. There are three possible explanations why MSCs might be important for neural repair. MSCs can differentiate into neurons, undergo cell fusion, release neurotropic factors to maintain the survival of the neurons and/or the release of non-neurotropic factors to promote the tissue repair [51]. Despite MSC is able to introduce repair and regeneration in the brain, it is still unclear if it is able to cross the blood brain barrier [52].

Human MSCs injected at the site of brain injury release neurotropic factors to induce endogenous recovery of damaged neurons [53]. This occurred by reduced inflammation, inhibition of apoptosis and increased proliferation and differentiation of neural stem cells. An early set of studies [54, 55] used a co-culture technique to shown that brain derived neurotropic factor (BDNF), glial derived neurotropic factor secreted by MSCs, induced neurite formation in neuroblastoma cell line. The role of BDNF was demonstrated with neutralizing antibodies, which prevented the regenerative potential of MSCs [54, 55]. In a spinal cord injury model with zebrafish, *TAC1* expression in MSCs improved the sensory and locomotors recovery by releasing some neurotropic factor [56]. Other examples of neurotropic factors can be nerve growth factor, neurotropin-3, ciliary neurotropic factor and vascular endothelial factor. In addition to neurotropic factors, *in vitro* studies showed that the extracellular matrix from MSCs can have a positive effect on the adhesion of neuronal cells by inducing neurite growth and astrocyte proliferation [57].

Both *in vitro* and *in vivo* studies suggest that MSCs can transdifferentiate into neurons, thereby providing these cells with the potential to promote neuronal repair. MSCs can be induced with defined condition and with cytokines to differentiate into neurons [56]. There are occasions if transdifferentiation of

MSC to neural cells can occur within a few hours and, whether the formation of neurons can be solely dependant on morphology [58, 59]. Subsequent studies indicated that transdifferentiation could happen only under stress and not under normal conditions [58–61]. In 2001, noggin, which can induce neural formation by inhibiting BMP2/4, TGF-ß, was used to induce neuron formation by transfecting MSCs. The noggin-transfectants expressed neuronal and astrocyte markers [62]. Retinoic acid (RA) was identified as an inducer of MSCs to form neurons with the expression of the neurotransmitter gene, *TAC1* with synaptic transmission [63]. There are several induction factors that can up regulate transdiffentiation of MSCs into neural cells such as matrigel [64]. The logic behind transdifferentiation of MSCs is not based solely on *in vitro* studies, as the conditions provided are totally artificial. When MSCs were injected to rat it migrated to the brain in the way neural stem cell (astrocyte engraft) migrated further losing MSC marker [65]. This showed the engrafted MSCs was well supported by the microenvironment of the rodents. The gradual increase in the neural marker in these MSCs showed its ability to transdifferentiate.

In Parkinson's disease (PD) rat model when hMSCs were injected it improved the motor function [66]. This was followed by clinical trial of using hMSCs in PD patient and it eventually lead to improvement in motor function with no significant side effect [67]. Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease caused by the death of motor neurons in cerebral cortex, brain stem and spinal cord. Human MSCs when transplanted in transgenic ALS mouse model with spinal cord injury improved the motor activity [68].

Patients with stroke who were injected with autologous MSCs showed transient improvement [69]. A subsequent repeat of the trial indicated that the transdifferentiated MSCs were able to retain their function for a prolonged period [69]. A recent *in vitro* study generated neurosphere-like aggregates from MSCs and then when injected them into a rat model of ischemic stroke to show that this method induced neuroprotection [70].

Other than neurotropic factor and transdifferentiaton, it is possible for the injected MSCs might fuse with the neural cells at the site of injury to cause functional improvement. Although evidence for this mechanism is limited there was a report in which MSCs were introduced into the rodent model [71, 72]. There was fusion with the neural cells and epigenetic changes with complete recovery of neuronal function [71, 72].

MSCs can be obtained and expanded easily without any ethical issues. These cells can differentiate into both mesenchymal and non-mesenchymal

types of cell depending on the microenvironment. There are several successful clinical trials with MSCs. There are concerns associated with these successes. Long-term follow up are needed with the MSC transplantation since MSCs can also exert immune regulation. Also, as discussed above, MSC therapy has to cautiously examined for patients who have survived cancer since this could reactive the tumor. Hence, long-term follow up is necessary for any patient receiving MSC or regeneration of the damaged tissue.

6.5 Safety

The safety of MSCs has been addressed throughout the text above. However, this section discusses that issue of care for tumor formation by the MSC itself. Here we discuss the differentiation of MSCs to neurons. REST, which is a tumor suppressor gene, is expressed in MSCs [73]. During differentiation to dopamine neurons, *REST* is decreased [74]. Thus, there is point where the balance between neuronal formation and the decrease of a tumor suppressor gene in stem cells could become tumorigenic (Figure 6.1). Similarly, the

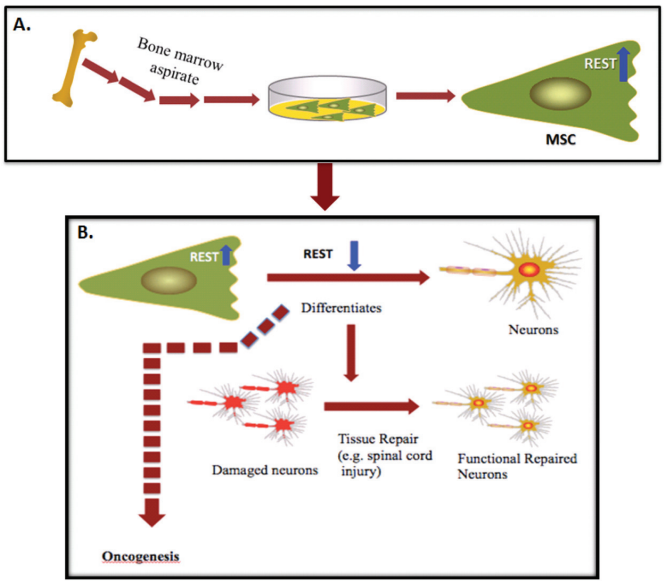

Figure 6.1 Shown is the differentiation of MSCs into neurons. **A.** MSCs are cultured from bone marrow aspirates and then subjected to differentiation to neurons. **B**. As the MSCs differentiate, *REST* expression is decreased. If REST protein is rapidly decreased this could cause cell transformation.

decrease in REST during neuronal differentiation can cause the oncogenic *TAC1* gene to be expressed [75]. This shows a scenario in which a tumor suppress gene (*REST*) is decreased with concomitant increase in the oncogenic *TAC1*. Going forward, studies are needed to carefully determine how the balance occurs and the rate of differentiation for safe trials.

6.6 Conclusion

MSCs have a dual role in regulating the immune response, depending on the microenvironment where the MSCs are placed. MSCs have the potential to introduce tissue repair and regeneration in brain and other organs. The differentiation of MSCs to neurons is mostly regulated by transcriptional repressor RE1- silencing transcription factor (REST; also known as neuron restrictive silencer factor-NRSF). REST regulates neurogenesis negatively and is degraded during normal neural differentiation. Its reduction induces MSCs to differentiate into neuron and repopulate the damaged neurons. (Figure 6.1), which under normal condition is highly expressed MSC, as it is tumor suppressor protein. Thus, the level of REST protein needs to be downregulated for efficient differentiation to neurons. A rapid decrease in REST protein in MSCs can also be oncogenic. Therefore, before using MSCs as a clinical therapeutic in neuronal damaged tissue for its repair, there is a need to figure out ways to balance REST protein.

Translation of this exciting research to the clinic will take time but the supportive results seen thus far have promise. MSCs are an ideal candidate for cellular therapy, their ease of isolation and lack of ethical concerns, along with tumor targeting properties make them a model transplant cell. Development of MSCs that release more potent tumor destroying molecules, addressing safety concerns of MSC transformation with improved therapeutic transgenes, and identifying the best route of administration are areas of continued research. However, a scenario where MSCs can be genetically modified and implanted into inoperable or partially resected invasive tumors, giving patients much needed treatment options is definitely on the horizon.

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