Review Article

Mesenchymal Stem Cells: Angels or Demons?

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Mesenchymal stem cells (MSCs) have been used in cell-based therapy in various disease conditions such as graft-versus-host and heart diseases, osteogenesis imperfecta, and spinal cord injuries, and the results have been encouraging. However, as MSC therapy gains popularity among practitioners and researchers, there have been reports on the adverse effects of MSCs especially in the context of tumour modulation and malignant transformation. These cells have been found to enhance tumour growth and metastasis in some studies and have been related to anticancer-drug resistance in other instances. In addition, various studies have also reported spontaneous malignant transformation of MSCs. The mechanism of the modulatory behaviour and the tumorigenic potential of MSCs, warrant urgent exploration, and the use of MSCs in patients with cancer awaits further evaluation. However, if MSCs truly play a role in tumour modulation, they can also be potential targets of cancer treatment.

1. Introduction

Mesenchymal stem cells (MSCs) are a group of heterogeneous multipotent cells which can be isolated from many tissues throughout the body. The discovery of mesenchymal stem cells can be dated back to the 1960s [1]. In recent years, MSCs have gained popularity among stem cell researchers due to their ability to self-renew and differentiate into many different cell types particularly cells of mesodermal origin such as osteoblasts, chondrocytes, and adipocytes in culture [2–4]. MSCs have also been reported to transdifferentiate into cells of ectodermal [5] and endodermal [6, 7] origins. Besides, MSCs have been applied clinically in patients with severe dilated cardiomyopathy, cartilage disorders, stroke, and autoimmune diseases with very encouraging results [8–11]. However, despite the many potential therapeutic benefits of MSCs, the use of these cells has been reported to bring adverse effects such as an increased recurrence rate of cancer, particularly haematological malignancies. There has been increasing evidence regarding the tumour modulatory effect of MSCs, and it has been shown that MSCs may enhance tumour growth in several studies [12–14]. Besides, MSCs have also been demonstrated to undergo spontaneous malignant transformation *in vitro* [15]. This review therefore gives an overview of the benefits as well as the harmful effects of MSCs with an emphasis on the clinical implications of the use of these cells.

2. What Are Mesenchymal Stem Cells?

The discovery of MSCs can be credited to the work done by A. J. Friedenstein as early as the 1960s during which he observed that the bone marrow is a source of stem cells for mesenchymal tissues in postnatal life [16]. After harvesting bone marrow samples from the iliac crest, Friedenstein and his coworkers plated the suspension on plastic culture dishes. They observed that upon gradual removal of the haematopoeitic counterpart, there existed a population of plastic-adherent, fibroblast-like cells that could differentiate into chondrocytes and osteoblasts and named them colony-forming unit fibroblasts [1, 17]. They were later renamed mesenchymal stem cells due to their ability to differentiate into cells of mesodermal origin [18].

However, it is worth mentioning that A. J. Friedenstein was not the first to propose the existence of stem cells. Prior to his discovery of MSCs, works of several other scientists have marked important milestones in stem cell research and contributed to our current understanding of the important

concepts of stem cells and differentiation. For instance, Alexander A. Maximow was one of the first to introduce the idea of stem cells and their ability to differentiate into other cell types in the context of haematopoiesis. Today, much of our knowledge regarding haematopoiesis originated from Maximow's proposal of the unitarian theory of haematopoiesis in 1906 [19] which he confirmed and updated in his later works and proved that all blood cells originated from a common mother cell [20].

On the other hand, two other scientists, Ernest A. McCulloch and James E. Till were among the first to demonstrate the clonal nature of bone marrow cells through a series of experiments involving bone marrow cell injection into irradiated mice, during which they noticed the spleen of these mice developed lumps or "spleen colonies" in proportion to the number of marrow cells injected. They then linked the observation to the possibility that these colonies originated from a single marrow cell [21, 22].

It is now clear that there are at least two main types of stem cells in the bone marrow—the haemopoietic stem cells and the nonhaematopoietic MSCs. The latter has been shown to be important in the proliferation, differentiation and survival of haematopoietic stem cells *in vitro* [23]. Traditionally MSCs have been isolated from the bone marrow even though bone marrow MSCs (BM-MSCs) have been shown to represent 0.001% of the whole marrow. Isolation of MSCs from the bone marrow is primarily by adhesion to plastic but these cells can also be concentrated by Percoll gradient centrifugation [24]. While some evidence suggests that BM-MSCs give rise to all mesenchymal lineages in distant tissues [25, 26], these cells have been found to reside in marrow-distant mesenchymal tissues such as skeletal muscles [27] and adipose tissues [28]. Adipose-derived MSCs are found to be similar to BM-MSCs but are easier to produce. These cells have been reported to show a broader therapeutic capacity as compared to BM-MSCs [29]. MSCs have also been located in umbilical cord blood [30], dental tissues [31], synovial fluid [32], palatine tonsil [33], parathyroid gland [34] and fallopian tube [35].

MSCs demonstrate heterogeneity in their morphology. Various terms have been used to describe the appearance of these cells, these include (1) fibroblastoid cells, (2) giant fat cells and blanket cells, (3) spindle shaped, flattened cells, and (4) very small round cells [36]. The morphology of these cells also varies greatly with their seeding density, changing dramatically especially when confluence is reached in cell culture condition. To this end, the relation between the morphology and their cell functions remains unclear.

MSCs express a number of markers phenotypically. However, none of them are specific to these cells. According to the International Society for Cellular Therapy, human MSCs under standard culture conditions must satisfy at least three criteria: (1) they must be plastic-adherent; (2) they must express C105, CD73 and CD90 and not CD45, CD34, CD14, CD11b, CD79 or CD19 and HLA-DR surface molecules by flow cytometry; (3) they must be capable of differentiating into osteoblasts, adipocytes and chondroblasts [37]. However, this set of criteria is not definitive as the expression of cell surface markers can be influenced by extrinsic factors such as those secreted by accessory cells in the initial passages and it is important to note that *in vitro* expression of cell surface markers may not correlate with *in vivo* expression [38]. Other markers that are generally accepted include CD44, CD71, Stro-1, and adhesion molecules such as CD 106, CD166, and CD29 [39].

3. The "Angelic" Side of Mesenchymal Stem Cells

The multilineage differentiation potential is the hallmark of MSCs. One of the criteria a cell has to satisfy before being regarded as an MSC would be its ability to differentiate into bone, cartilage, and fat cells [37]. However, studies have shown that MSCs can be differentiated into other cells such as skeletal cells, cardiomyocytes, endothelial, smooth muscle, and neural cells [40]. There have also been several studies on the transdifferentiation of MSCs into pancreatic beta cells [6, 41]. However, the ability of MSCs to differentiate into cells of all three germ layers must be carefully examined as they have been reported to spontaneously express neural markers even in the undifferentiated state [42].

MSCs are immune privileged cells. The fact that MSCs from children can persist in mothers for decades suggests that these cells can escape immune surveillance for a long period of time [43]. The immune phenotype of MSCs is generally described as major histocompatibility (MHC) I positive and MHC II negative. They also lack the costimulatory molecules CD40, CD80, and CD86. Although expressing low levels of MHC I antigens can activate T cells, the absence of costimulatory molecules cannot initiate secondary signals, thus leaving the T cells anergic [44]. Besides, the expression of low levels of MHC I is important in protecting MSCs from natural killer cell-mediated cytotoxicity. On the other hand, cells that do not express MHC I are targeted and destroyed [45].

Today, a search of clinical trials at http://www.clinical-trials.gov (a registry of federally and privately supported clinical trials conducted in the United States and around the world) using the key words "mesenchymal stem cells" returns more than a hundred results. The many potential therapeutic uses of MSCs have been related to their multipotent differentiation capacity and unique immunological properties mentioned. However, there are many other reasons why MSCs have gained the interest of researchers in cell-based therapy and tissue engineering. Firstly, MSCs are relatively easy to obtain and maintain compared to other types of stem cells such as embryonic stem cells. BM-MSCs can be readily harvested from the iliac crest, cryopreserved, and expanded many times *in vitro* to increase the number of transplantable cells. It has been shown that these cells could be extensively expanded *in vitro* up to 15 population doublings [46] with minor spontaneous differentiation during *ex vivo* expansion [4]. Other than their ability to differentiate into cells of both mesodermal and nonmesodermal origins, these cells are also immunosuppressive [47]. Besides, MSCs also demonstrate trophic effects via the production of various growth factors and cytokines [48]. Therefore, they have been used for

the purposes of cell replacement, repair and regeneration, immunomodulation, and disease modeling.

Many investigations on the feasibility of the clinical use of MSCs have mushroomed in the 1990s. However, Lazarus and colleagues were among the first to inject autologous cultured MSCs into human subjects intravenously and assessed their safety for cell-based therapy. It was demonstrated that the infusion of autologous MSCs into 15 human subjects with haematological malignancies was a safe treatment with complete remission [49]. Subsequently, MSCs were used in many other clinical trials to treat various diseases. For examples, Horwitz et al. had used MSCs in the treatment of children suffering from osteogenesis imperfecta. The study showed engraftment of MSCs and improvement in these children with a reduced frequency of bone breakages [50].

Besides, MSCs have also been used in clinical trials for the treatment of inflammatory and heart diseases, as well as spinal cord injuries. In 2004, Le Blanc et al. reported successful treatment of steroid-refractory grade IV acute graft-versus-host disease (aGVHD) in a 9-year-old boy [51]. Later in a more recent clinical trial, Le Blanc et al. demonstrated that 6 out of 8 patients with steroid-refractory grade III-IV acute GVHD had complete disappearance of GVHD with the infusion of MSCs [52]. MSCs have also been used in ischaemic cardiomyopathy with promising results [53]. However, the benefits of MSC treatment may be due to paracrine effects of MSCs instead of their capacity to differentiate into cardiomyocytes after injection. It has also been demonstrated that the use of BM-MSCs together with granulocyte macrophage colony-stimulating factors (GM-CSF) improved acute and subacute spinal cord injuries. However, such improvement was not observed in chronic cases of spinal cord injuries [54].

Of particular interest, adipose tissue-derived MSCs or adipose tissue-derived stem cells, ASDCs have gained much popularity lately. Like MSCs of other origins, ASDCs, have been shown to differentiate into various cell types such as adipogenic, chondrogenic, osteogenic, myogenic cells, and cells that adopt a pancreatic phenotype [55, 56]. The list of potential clinical applications using ADSCs is exhaustive. To name a few, disease conditions in which these cells may be useful or potentially useful include intervertebral disc repair, spinal cord injury, stroke, diabetes mellitus, rheumatoid arthritis, and wound healing and repair [57]. ADSCs have also been used in several clinical trials. For example, in a phase II clinical trial by Garcia-Olmo et al., expanded ADSCs were used in the treatment of complex perianal fistula either of crytoglandular origin or associated with Crohn's disease. The study concluded that the use of ADSCs in combination with intralesional fibrin glue treatment achieved higher healing rates than using fibrin glue alone [58]. On the other hand, Fang et al. demonstrated that the use of adipose tissue-derived MSCs successfully suppressed hepatic graft-versus-host disease (GVHD) in a 43-year-old woman after allogeneic haematopoietic stem cell transplantation [59] and the successful treatment of two children with acute GVHD due to allogeneic stem cell transplantation from HLA-mismatched unrelated donors [60]. Other published human trials include the use of adipose tissue-derived MSCs in the treatment of traumatic calvarial defect [61] and urinary incontinence [62].

Another type of MSCs, the muscle-derived MSCs (or muscle-derived stem cells, MDSCs), has also gained much attention recently. Although MSCs can be obtained from the bone marrow, the biopsy procedure is more painful and invasive in comparison to skeletal muscle biopsy, which can be done using a small caliber needle under local anesthesia. Therefore, MDSCs are preferred over BM-MSCs in several studies. Some clinical applications or potential applications include the use of MDSCs in musculoskeletal muscle diseases [63] and urinary incontinence [64].

4. The "Demonic" Side of Mesenchymal Stem Cells

Despite the many potential therapeutic benefits of MSCs, these cells have been found to have various adverse effects, especially in the context of their direct and indirect involvement in cancer. Basically, the role of MSCs in cancer can be divided into (1) indirect involvement via the tumour modulatory effect of MSCs and (2) direct involvement via malignant transformation of the MSCs themselves.

4.1. Tumour Modulatory Effect of Mesenchymal Stem Cells. There has been a constant debate on the role of MSCs in tumour modulation. A few studies have supported that MSCs may suppress tumour growth [65–67] while others believe that MSCs may contribute to tumour protection via antiapoptotic effect, tumour progression, metastasis, and drug-resistance of cancer cells [12–14, 68–75]. Although many have demonstrated an antiproliferative effect exerted by MSCs in cancer cells, the set back is that such effect is often accompanied by an antiapoptotic effect. For instance, Ramasamy et al. reported that MSCs inhibited proliferation and apoptosis of tumour cells of haemopoeitic and non-haemopoeitic origins, which was related to a transient arrest of the tumour cells in G1 phase of the cell cycle *in vitro*. On the other hand, when tumour cells were injected with MSCs *in vivo*, the former demonstrated a faster growth when compared to injection of tumour cells alone [12]. The finding was supported by another study by Wei et al. who reported that BM-MSCs from leukaemia patients inhibited growth and apoptosis in serum-deprived K562 cells *in vitro* [69]. Interestingly, Li et al. reported that human MSCs played a dual role in tumour cell growth *in vitro* and *in vivo*. It was found that human MSCs inhibited the proliferation of A549 (lung cancer) and Eca-109 (esophageal cancer) cell lines and caused G1 phase cell cycle arrest and apoptosis *in vitro*. However, human MSCs were also found to enhance tumour formation and growth *in vivo* [70]. The tumour protective effect of MSCs was also observed in melanoma A375 cells but was absent in glioblastoma 8MGGBA cells as described by Kucerova et al. [71]. Moreover, MSCs have also been found to protect breast cancer cells through regulatory T cells [14], prevent apoptosis of acute myeloid leukaemia cells by upregulation of antiapoptotic proteins [68], and
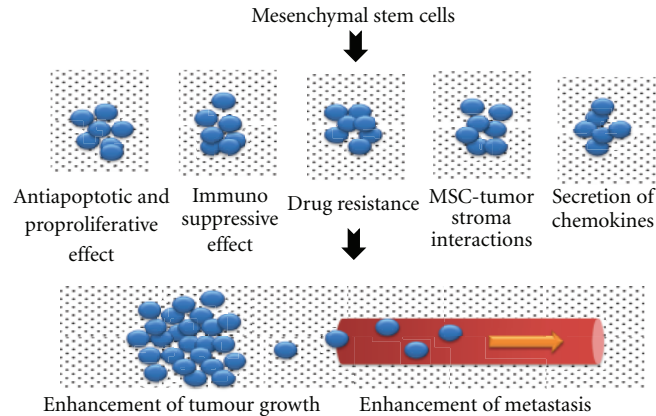
FIGURE 1: Indirect involvement of mesenchymal stem cells in cancer through tumour modulatory and other effects.

exert antiapoptotic effect in U266 and H929 melanoma cells mediated by upregulation of survivin [13].

Besides preventing apoptosis and enhancing tumour growth, MSCs have also been related to the promotion of metastasis. Recent evidence suggests that MSCs could migrate towards primary tumours and metastatic sites [76]. Chemokines secreted by MSCs have been shown to enhance the emergence of pulmonary metastases and such secretion has a strong interaction between breast cancer cells and MSCs [72]. In addition, MSCs have also been found to play a role in drug resistance in various cancer cells. Kurtova et al. showed that MSCs protect chronic lymphocytic leukaemic cells from fludarabine-, dexamethosone-, and cyclophosphamide-induced apoptosis [74]. Lin et al. demonstrated that BM-MSCs not only modulated the proliferation of U937 but also the response of U937 cells to daunoblastina and that Bcl-XL apoptosis inhibiting gene may be important in determining the sensitivity of leukaemic cells to treatment [73]. In another study, Vianello et al. showed that BM-MSCs nonselectively protected chronic myeloid leukaemia cells from imatinib-induced apoptosis. It was proposed that the effect was mediated via the CXCR4/CXCL12 axis and that the disruption of this axis could restore the leukaemic cells' sensitivity to imatinib [75].

Another tumour enhancing property of MSCs is their ability to suppress the immune system [47]. While this immunosuppressive property of MSCs is good news to patients with immune disorders, it is indeed a double-edged sword, causing harm to those with cancer, as a suppressed immune system may encourage tumour growth. In addition, MSCs have been shown to migrate to the tumour stroma and resulted in enhanced tumour growth and metastasis. For example, Shinagawa et al. reported that MSCs moved toward and interacted with tumour stroma of colon cancer cells. They also differentiated into carcinoma-associated fibroblasts and promoted angiogenesis, tumour growth, migration, invasion, and metastasis [77]. Figure 1 gives a summary of the indirect involvement of MSCs in cancer via their tumour modulatory and other effects.

4.2. Malignant Transformation of Mesenchymal Stem Cells. The involvement of MSCs in cancer is not restricted to their

ability to enhance proliferation of cancer cells and to cause drug-resistance in various cancer cell types via antiapoptotic and other mechanisms. The MSCs themselves have also been reported to contribute to malignant transformation in several studies. Malignant transformation of MSCs used in cell-based therapy can take place under three conditions: (1) during *in vitro* expansion of MSCs, (2) malignant transformation as a result of MSC interaction with the tumour stroma, and (3) genetic manipulation of MSCs which turn these cells cancerous (Figure 2).

In order to produce enough MSCs for clinical use, massive *in vitro* expansion is often necessary. This in turn renders MSCs susceptible to malignant transformation. In 2005, Rubio et al. first reported that adipose tissue-derived MSCs could immortalize and transform spontaneously as a result of long-term *in vitro* expansion [15]. This was followed by the demonstration of chromosomal instability in long-term cultures of MSCs in later studies [78, 79]. Subsequent molecular characterization of MSC malignant transformation by Rubio et al. demonstrated that these cells were able to bypass senescence by upregulating c-myc and repressing p16 levels. These cells were also shown to bypass cell crisis through acquisition of telomerase activity, InK4a/Arf locus deletion, and Rb hyperphosphorylation. In addition, modulation of mitochondrial metabolism, DNA damage-repair proteins, and cell cycle regulators were also found to play a role in malignant transformation [80]. Besides, it was also reported that alterations in the p21/p53 pathway resulted in MSCs bypassing senescence *in vitro* with the generation of tumours resembling mesenchymal sarcomas *in vivo*. The study concluded that a single mutation was insufficient to cause malignant transformation and that such transformation was a result of multiple genetic alterations [81]. In addition, malignant transformation of MSCs has also been demonstrated *in vitro* by indirect evidence of the derivation of malignant populations from *in vitro* culture of MSCs, suggesting that MSCs could be the origins of various cancers [82, 83].

The homing effect allows MSCs to migrate towards tumour cells. This migration not only allows MSCs to interact with the tumour stroma and enhance tumour growth as mentioned in Section 4.1, it also leads to malignant

Mesenchymal stem cells

Spotaneous malignant transformation during *in vitro* expansion

Tumour stroma-induced transformation into cancer-associated fibroblasts

Malignant transformation due to genetic manipulations
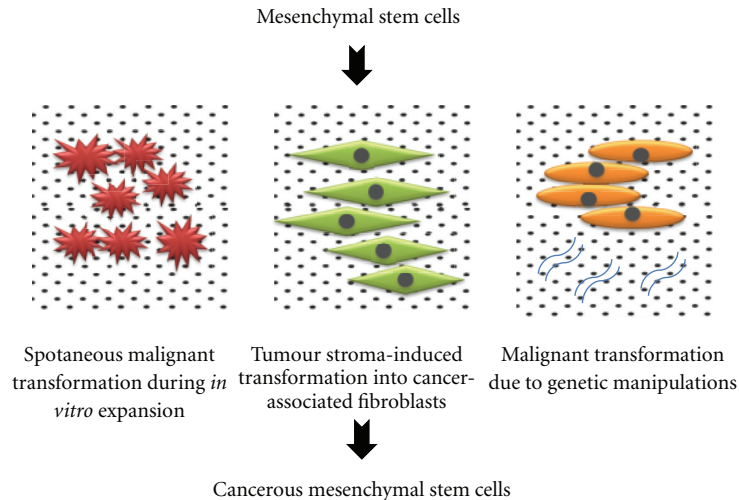
Cancerous mesenchymal stem cells

FIGURE 2: Direct involvement of mesenchymal stem cells in cancer through malignant transformation.

transformation of MSCs at the tumour site [77]. Exposure of MSC to local stroma environment of a tumour can lead to differentiation of these cells into cancer-associated fibroblasts (CAF) or tumour-associated fibroblasts (TAF) [84]. This transformation of MSCs into CAF or TAF becomes an important part of the tumour, contributing to fibrovascular network expansion and tumour progression as described by Spaeth et al. [85].

Other than *in vitro* spontaneous transformation and stroma-induced transformation, a third possible way MSCs can also undergo malignant transformation is by genetic manipulations. Genetic manipulations of MSCs either by viral or nonviral transgene delivery methods have been extensively explored in many studies for the purpose of immortalizing MSCs for long-term cultures and maintenance as well as for the treatment of various diseases such as neurological, blood, vascular, musculoskeletal disorders, and cancer (reviewed by Resier et al., [86]). However, such genetic manipulations are not without risks and disadvantages—either the transgene may be tumorigenic or the insertion of transgenes causes disruption to MSC's genome and lead to malignant transformation. Lessons from spontaneous malignant transformation of MSCs in long-term cultures show that immortalizing MSCs by genetic manipulations may increase the oncogenic potential of these cells as MSCs tend to accumulate chromosomal instability during long-term cultures [79, 82]. Literature on the potential dangers and tumorigenic capacity of genetically manipulated MSCs is scarce and further exploration is necessary.

5. Clinical Implications and Future Directions

In conclusion, the implications of the clinical use of MSCs are at least five-fold.

(1) The use of MSCs for disease treatment in general: the vast number of clinical trials and the abundance of published literature suggest that MSC treatment is feasible. Ongoing translational research may bring new hopes to sufferers of many diseases which can lead to improvement or cure.

(2) The use of MSCs in noncancer patients: there should be long-term followups of patients in this category with respect to the incidence of cancer. While MSCs have been shown to be useful or potentially beneficial in many pathological conditions, care must be taken not to introduce cancer to patients who receive MSC therapy years down the line.

(3) The use of MSCs in cancer patients: further exploration of the use of MSCs in cancer patients, especially those with haematological malignancies and those requiring MSCs for GVHD, is necessary. The benefits of treatment should outweigh the adverse effects that these cells could bring to the patient. This is especially true if the patient is on chemotherapy and the interactions between anticancer-drug-treated cancer cells and MSCs need to be established.

(4) MSCs as vehicles of targeted therapy: if MSCs truly play a role in tumour modulation, the mechanisms by which they exert their modulatory effect should be well elucidated. This includes investigations on the signaling pathways or regulatory proteins involved in the tumour modulatory behaviour of MSCs. On the other hand, such tumour modulatory effect of MSCs can be viewed as a double-edged sword, that is, if they are the cause of the problem, they could also be the solution for being vehicles of targeted delivery of anticancer drugs.

(5) Stricter control and safety measures in the production of MSCs for cell-based therapy: taking into consideration that MSCs can turn malignant as a result of long-term culture and genetic manipulation, there is a need for stricter control in cell handling procedures to minimize the risk of malignant transformation.

Conflict of Interests

The author declares that there are no competing interests.

Acknowledgments

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