CHAPTER 25

CURRENT STATUS AND FUTURE PROSPECTS OF STEM CELL THERAPY IN ANIMAL HEALTH

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INTRODUCTION

Regenerative medicine is a relatively new field in biology that deals with the repair and regeneration of diseased and damaged tissue, as well as to rectify the congenital anomalies. Over time, regenerative medicine has gained much importance because of its promising results and wide-ranging applications. Among the various options for regenerative medicine, stem cells are at the forefront and are comprehensively studied. These cells can repair injured tissue, that the body would otherwise be unable to regenerate. Although regenerative medicine techniques and stem cells have been previously used, however, the concept of stem cell therapy was first coined by Caplan (1991). In his series of studies on stem cells, he suggested isolation and culturing of the stem cells in vivo and in vitro setup. Research has shown that there are many types of stem cells, including embryonic stem cells (ESCs), mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) (Gazit et al. 2019). Amongst, MSCs have been the most studied and used in preclinical and clinical trials because of their invaluable properties such as easy availability, simple culturing techniques and less or no ethical concerns. In addition to their regenerative potential, MSCs can also modulate the recipient's immune system and thus are gaining much recognition and scope in veterinary medicine (Kolios and Moodley 2013). The poor response or unavailability of conventional treatment regimens has shifted the focus of researchers on the use of MSCs in veterinary medicine. MSCs offer potential treatments for many animal diseases including orthopedic, reproductive, dermal, hepato-renal, digestive, cardiovascular, neuromuscular, dental, and respiratory systems. Although, since long, the scientists have been understanding the nature and behavior of MSCs, but still there is a lot to comprehend. This chapter aims to highlight the current status of stem cell applications in regenerative biology in preclinical and clinical trials.

Stem Cells Origin and Types

Stem cells have been defined and redefined many times, however, scientists agree on their undifferentiated nature, self-renewal ability, and plasticity to differentiate into various types of mature cells (Morrison et al. 1997). Depending on the source of stem cells, they can be broadly classified as embryonic stem cells (ESCs), adult stem cells (ASCs) and induced pluripotent stem cells (iPSCs) (Evans and Kaufman 1981; Takahashi and Yamanaka 2006). The ESCs are obtained from early blastomeres before they lose their totipotency. From adult individuals, ASCs are traditionally obtained from bone marrow and adipose tissue. From fetal adnexa, stem cells are routinely retrieved from the amniotic fluid, however, amniotic membrane, Wharton's jelly, cord blood, placenta and other tissues are also rich sources of ASCs (Sarfraz et al. 2021). The discovery of iPSCs is relatively a new addition in stem cell class, obtained by dedifferentiation of adult cells (Takahashi and Yamanaka 2006).

Depending upon their degree of differentiation potential, stem cells are classified as totipotent, pluripotent and multipotent stem cells (Wagers and Weissman 2004). Totipotent stem cells exist only in very early embryonic stages, just before gastrulation, and are able to give rise to a variety of adult body tissues. Furthermore, they are also capable of developing extra-embryonic structures (Evans and Kaufman 1981; Martin 1981). After gastrulation, these cells yield pluripotent stem cells which are proposed to form all types of adult cells except extra-embryonic tissues. The cells produced by successive cell divisions of embryo, having even less differentiation potential than that of pluripotent stem cells are termed as multipotent stem cells. They can differentiate into number of cell types, but their differentiation ability is limited. Other types of stem cells are oligopotent and unipotent stem cells, which can deliver cells of their own lineage only (Thomson and Marshall 1998).

Human ESCs were first reported in 1998, which opened new horizons for gene-expression studies and their functions in early embryonic development and differentiation (Thomson et al. 1998). The studies also focused on development of drugs by identifying and targeting the genes and tissues of interest. However, with the passage of time, the use of ESCs for trials raised huge moral and ethical concerns and cultural predicament which limited their use. Due to these hurdles, the focus of research was shifted to other sources of stem cells for

How to cite this chapter: Sarfraz A, Qureshi AS, Sandhu MA, Masood S, Saba E, Masood A, Hussain M and Salahuddin, 2022. Current status and future prospects of stem cell therapy in animal health. In: Abbas RZ, Khan A, Liu P and Saleemi MK (eds), Animal Health Perspectives, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. I, pp: 194-201. https://doi.org/10.47278/book.ahp/2022.25

stem cell-based treatments. Lately, iPSCs were developed by two Japanese scientists by reprogramming the adult mouse fibroblasts into pluripotent stem cells in 2006 (Takahashi and Yamanaka 2006). This was a huge discovery in the field of regenerative medicine because these cells appeared similar to ESCs in their genotypic, phenotypic and growth kinetics behavior. However, the dedifferentiation of adult cell can create chromosomal changes which may lead to teratological disorders and hence raised a massive concern about their safety and use in the regenerative medicine.

Another source of the stem cells is adult animals, which contain both the hematopoietic (HSCs) and non-hematopoietic stem cells (Non-HSCs). The bone marrow contains both HSCs and non-HSCs or mesenchymal stem cells (MSC). The MSCs are multipotent in nature as they can give rise to diversified cell types including osteo, chondro, adipo, myo and many other cell types. This differentiation is endogenously activated to regenerate the dead, diseased, and injured cells in a tissue (Caplan 1991). The history of MSCs appeared before 1968, when a population of osteoid cells with fibroblastic morphology was extracted from bone marrow (Friedenstein et al. 1968). Studies in the late twenties showed that these cells could differentiate into bone, cartilage and fat-like cells (Dennis et al. 1999). This provided the basis for determining that MSCs exert their healing abilities by differentiating into other tissue types (Miyahara et al. 2006; Quinn and Flake 2008). In many studies, the immune modulation activity of stem cells was probed, and it is hypothesised that MSCs primarily modulate the immune system and are involved in the tissue repair, therefore, they exhibit regenerative ability. Now, it is stated that perivascular MSCs population in the tissues is involved in aiding these cells to sense local or remote tissue injury and riposte to it by focused relocation to the site of damage and involvement in the therapeutic process (Niess et al. 2016). On the basis of this, MSCs should be termed as "medicinal signalling cell" instead of "mesenchymal stem cells" as stated by Caplan (2017).

The MSCs are relatively easy to collect in large number, have good kinetic potential, and their use in not restricted by ethical concerns, therefore, they are considered promising stem cells for therapeutic procedures. With the increased focus of the scientific community of MSC, their exact definition needed to be well elaborated to set a common ground, therefore, The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) formulated a set of canons to define human MSC, which was also comprehended in a position paper (Dominici et al. 2006). The core concepts articulate that; MSCs must adhere to the plastic in standard culture conditions; they must express mesenchymal markers including CD73, CD90, CD105 (Sandhu et al. 2017; Jurek et al. 2020) and not more than 2% of them must have the expression of HLA class II, CD14 or CD11b, CD34, CD79a or CD19, and CD45; and they must yield cells of osteogenic, adipogenic and chondrogenic lineage on appropriate induction in an in vitro setup.

Sources for Isolation of Mesenchymal Stem Cell

There is a long list of tissues and organs used to isolate and characterize MSCs. The results indicate that the cells from each tissue possess a unique set of features besides the basic features of stem cells and these features should be kept in consideration while choosing an appropriate source MSCs for stem cell based therapeutic purposes. In domestic and companion animals, MSCs have been isolated from their adult individuals as well as from their respective fetal annexes including bone marrow (Sasaki et al. 2018; Zhang et al. 2018; Arévalo-Turrubiarte et al. 2019), fat tissue (Sasaki et al. 2018; Arévalo-Turrubiarte et al. 2019; Rashid et al. 2021b), synovial fluid (Bearden et al. 2017; Arévalo-Turrubiarte et al. 2019), patellar fat (Rashid et al. 2021a), umbilical cord (Zhang et al. 2018; Denys et al. 2020), Wharton's Jelly (Sarfraz et al. 2021), cord blood (Kang et al. 2012), periosteum and muscle (Kisiel et al. 2012; Arévalo-Turrubiarte et al. 2019), peripheral blood (Sato et al. 2016; Longhini et al. 2019), periodontal ligament and gingiva (Mensing et al. 2011), placenta (Carrade et al. 2011), amniotic fluid (Sarfraz et al. 2021) and endometrium (Rink et al. 2017). In lab animals, MSCs have also been isolated from soft tissues including the brain, liver, pancreas, kidney, spleen, thymus, lung and muscle (Meirelles et al. 2006). For a long time, the most frequently chosen sources of stem cells have been adipose tissue and bone marrow, because they yield higher numbers of cells as compared to other tissues. Of the two, the former has gained attention due to its minimal invasiveness to the donor, but has similar properties to stem cells isolated from bone marrow, including trilineage differentiation and immunophenotyping (Bearden et al. 2017; Sasaki et al. 2018). They differ in terms of growth kinetics, plasticity and secretory activity (Arévalo-Turrubiarte et al. 2019; Fideles et al. 2019; Villatoro et al. 2019). Even within same type of tissue, for example adipose tissue, the anatomical location for cell isolation matters (Yaneselli et al. 2018; Rashid et al. 2021b).

The established fact is that the phenotypic and genotypic characteristics of the stem cells are greatly influenced by the type and location of the tissue, therefore, it is of utmost importance to consider these properties while choosing the cells for regenerative and therapeutic purposes. Furthermore, there is no general rule of thumb to prefer one type of tissue over the other for stem cell isolation. Apart from the in vitro culture conditions, the cellular properties are partly influenced by the donor's conditions like species, age, health etc. Because of different variations, there is no single yard stick for comprehensively comparing the results of one study with another (Dominici et al. 2006). Though all the MSCs isolated from different species and sources show plastic adhesion and differentiation, yet their extent of surface antigen expression is highly variable and is no way to compare these results with the criteria described specially for CD73, CD90, CD105 and other hematopoietic lineage markers (Boxall and Jones 2012).

Donor-recipient Relationship for Therapeutic Applications

Since stem cells are aimed to treat injured, diseased, and degenerated tissues, it is important to understand the relationship between donor and recipient. This relationship can be one of three types, xenogeneic, allogeneic, or autologous (Rashid et al. 2021a). Xenogeneic stem cells are cells used across species, the term allogenic means to use stem cells from the same species, while the term autologous is used to use cells from the same individual. An autologous use of stem cell seems the most convenient and efficient than allogeneic, however, both have some advantages over the other. The limiting factors for the use of autologous stem cells include the time for in vitro cell expansion, health status and age of patient (Zajic et al. 2017; Fideles et al. 2019; Taguchi et al. 2019). Contrarily, the

allogeneic stem cells are ready to use, obtained from a young and healthy individual as well as passed a number of safety and scrutiny tests.

The limitation in the use of allogeneic stem cell therapy is the likelihood that major histocompatibility complex class-I on a donor's MSCs is identified by the recipient's T cells, leading to immediate cytotoxicity of donor cells. Moreover, major histocompatibility complex class-II molecules can be identified by recipient's T cells, leading to humoral or cytotoxic immune reactions. Major histocompatibility complex molecules may also be indirectly recognised by antigen presenting cells (APCs) to produce antibodies in B cells (Wieczorek et al. 2017). Numerous studies using allogeneic cells in preclinical and clinical trials show immune responses in in vivo and in vitro settings (Joswig et al. 2017; Oliveira et al. 2017; Cabon et al. 2019; Ursini et al. 2019). These immune reactions have raised questions about the immune-privileged status of MSCs in allogeneic use. Even with the repeated exposure of allogeneic stem cells, the immune reaction was not diminished, contrarily, severe local side effects have been reported in the host's immune response (Joswig et al. 2017; Bertoni et al. 2019; Cabon et al. 2019). Still, some studies suggest non-statistical difference in host's response on repeated exposures (Magri et al. 2019). Not only the immunogenic properties of clinically tested MSCs vary, their MSC expression also vary which seems largely depended on the species and breed of origin, tissue, culture environment and even on the individuality of the donor (Ménard et al. 2020). Although many studies have demonstrated the immunomodulatory properties of allogeneic mesenchymal stem cells, the issue of immunogenic response has plagued the use of these cells, making autologous cells still the best choice for regenerative biology in contemporary settings.

Therapeutic Potentials of Mesenchymal Stem Cells

More recently, stem cells have been thought to heal diseased and damaged tissues by differentiating and replacing these cells. MSCs are now found to be involved in complex immune regulatory mechanisms, including paracrine, vesicle release, immune regulation, and cell-to-cell transfer of organelles.

Paracrine Secretions

The immune cells including natural killer cells (Spaggiari et al. 2006), dendritic cells (Gao et al. 2017), macrophages, as well as B and T cells to be affected by MSC paracrine signalling. Likewise, many factors and cytokines are thought to have immunomodulatory effects, among them are tumor necrosis factor (TNF) stimulated gene-6 (TSG-6), interleukin 10 (IL-10), transforming growth factor-beta (TGF-β), prostaglandin E2 (PGE2) and indolamine-2,3-dioxygenase (IDO).

TSG-6 is an inflammation-related protein that is also involved in anti-inflammatory and protective functions (Day and Milner 2019). TSG-6 is released by MSC and is involved in cellular structure, vesicle size, growth kinetics, plasticity and survival, hence is vital for MSC stemness (Romano et al. 2019). TSG-6 triggers the switch from M1 to M2 phase, which alleviates the signs of inflammation in many diseases (Wang et al. 2015b; Um et al. 2017; Song et al. 2018; An et al. 2020).

IL-10 is a known anti-inflammatory factor that limits Th1 and Th2 responses and the axillary roles of dendritic cells and macrophage; as well as inhibits T-cell proliferation (De Vries 1995). IL-10 is secreted by the contact of T-cells and inflammatory milieu (Najar et al. 2015; Um et al. 2017).

TGF-β is an important growth factor that contributes to cell propagation, plasticity, angiogenesis, wound healing and embryonic development (Gordon and Blobe 2008). The homing and migration of MSCs are also affected by TGF-β (Deng et al. 2017a; Dubon et al. 2018). TGF-β, like TSG-6, triggers the transition of macrophages from an inflammatory (M1) to an anti-inflammatory/regulatory (M2) state, thus helping to regulate T cells (Schmidt et al. 2016; Gazdic et al. 2018; Liu et al. 2019; Wu et al. 2020).

PGE2 is a major prostaglandin that blocks pro-inflammatory cell migration, modulates chemokine production, and promotes regulated cell differentiation (Kalinski 2012). It is important in NK-cell inhibition (Spaggiari et al. 2008) and in M2 conversion of macrophage polarization (Jin et al. 2019). Recently, it was reported to aid in the clearance of apoptotic cells by MSC (Zhang et al. 2019).

IDO is involved in multiple roles, including lymphocyte arrest (Spaggiari et al. 2008; Franquesa et al. 2015) and M2 transformation of macrophage, (François et al. 2012). This enzyme is secreted in an inflammatory milieu by MSCs (Luk et al. 2017).

The above discussion suggests that MSCs can alter the progression of events through paracrine responses, thereby altering and regulating local niches.

Release of MSC-derived Extracellular Vesicles (MSC-EVs)

The role of MSCs in regulating inflammatory cells is not limited to affecting cells through a paracrine mode but can also modulate niche by secreting vesicles in the extracellular environment. These vesicles are enveloped and protected by components of the plasma membrane, so they can be transported over long distances in the body (Jung et al. 2013; Mäkelä et al. 2015).

The vesicles are involved in M2 conversion of macrophages (Hyvärinen et al. 2018), T-cell suppression (Crain et al. 2019) and upregulation of IL-10 (Park et al. 2019). Vesicles have shown therapeutic potential in respiratory (Khatri et al. 2018), renal (Eirin et al. 2017), neurological disorders (Deng et al. 2017b; Ruppert et al. 2018) hepatic (Haga et al. 2017) and cardiac cell damage (Liu et al. 2017). They help to promote healing through formation of new blood vessels and the production of extracellular connective tissue matrix (El-Tookhy et al. 2017).

Studies have shown that vesicles can function in a cell-free environment, thereby avoiding the possible side effects of MSC immune response elicitation (Mäkelä et al. 2015). Nonetheless, cell-to-cell interactions are still required to confer immunomodulatory properties (Luk et al. 2016 & 2017; Gao et al. 2017).

Major hurdle, so far, is the lack of a gold standard technique for the isolation and standardization of MSC-EVs. The most commonly used techniques to isolate MSC-EVs include ultracentrifugation, isolation kits, ultrafiltration, and chromatography. Different techniques yield vesicles of different sizes, characteristics and degree of purity. Therefore, it is believed that each type of MSC-EV has its own unique function. Furthermore, there are many discrepancies and ambiguities in the available literature on MSC-EVs (Reiner et al. 2017; Toh et al. 2018) leading the International Society for Extracellular

Vesicles to require the use of generic terms for such vesicles, unless they are fully defined. Moreover, the society urged to explain the methods of isolation and characterization of MSC-EVs in detail so that the similar results could be reproduced.

Apoptosis-Derived Immunosuppression

Phagocytosis is not only involved in the clearance of dead and dying cells, but also contributes to the immune response, therefore, have immunomodulatory functions. This effect was recoded in an experiment in which heat inactivated MSCs increased IL-10 and decreased interferon, suggesting that the elicitation of immune function is independent of MSCs (Luk et al. 2016).

Contemporary studies suggest that cells of the innate immune system mediate MSC immunomodulatory effects. MSCs have been shown to undergo phagocytosis when physically interact with T-cytotoxic cells and monocytes/ macrophages. The macrophages/monocytes that engulf MSC subsequently exhibit indolamine-2,3-dioxygenase activity (Galleu et al. 2017) to inhibit T-cell proliferation (Cheung et al. 2019).

Transfer of Organelles

In addition to the mechanisms described above, MSCs were also observed to transfer their mitochondria and other organelles through tunnels (Spees et al. 2006). The transfer facilitates in respiration in the recipient cell. When MSCs transferred their mitochondrial contents to immune cells, they showed better phagocytic and antimicrobial activity (Jackson et al. 2016). Other similar studies have shown that organelle transfer mechanisms can be used to pave the way for the treatment of physiological disorders and pathological conditions.

Homing of the Damaged Tissue by MSCs

In addition to their potential to modulate the immune system through direct interaction and release of extracellular vesicles, MSCs home the damaged tissue and release the growth factors, chemokines and cytokines. The chemokines upon activation, are involved in the cell migration (Wynn et al. 2004; Chamberlain et al. 2008; Zou et al. 2011). MSCs homing also originates from different growth factors, including TGF-β1 (Gao et al. 2014), vascular endothelial growth factor (Ball et al. 2007), insulin-like growth factor-1 (Xinaris et al. 2013), fibroblast growth factor (Wang et al. 2015c) and hepatocyte growth factor (Forte et al. 2006). MSCs homing is also influenced by physical stimuli like stress and strain (Xiaorong et al. 2019). It is preferred that the stem cells should be administered in the parenchyma of the desired tissue, however, it is not always possible (Nowakowski et al. 2016), therefore, general administration is performed.

In general, IV administrations, MSCs face many challenges, including migration from the systemic circulation to desired tissues (Nowakowski et al. 2016) mainly due to entrapment in the lung (Gao et al. 2001; Eggenhofer et al. 2012; Jasmin et al. 2014). In the lung tissue, integrins are over activated resulting into cellular interactions (Wang et al. 2015a). Another obstacle to systemic infusion of MSCs is their short lifespan, because they disappear 24 hours after infusion (Eggenhofer et al. 2012; de Witte et al. 2018), therefore no long-term benefits can be obtained, yet some effects can be attained with their apoptosis-linked immunomodulation (de Witte et al. 2018).

Alternatives to IV, intra-arterial (IA) and intraperitoneal routes are experimented because they bypass filtering organs and tissue entrapment, thus providing better tissue distribution. In the IA injection of MSCs, the organs showed better cellular uptake, especially in the liver (Mäkelä et al. 2015). Contrarily, IA administration is complicated by challenging procedures and possible intravascular occlusion and thrombosis (Sole et al. 2013). It was later shown that the problem of thrombosis could be avoided by injecting cells without a tourniquet (Trela et al. 2014). Similar to IA, intraperitoneal administration of cells showed favourable results because the cells housed the desired tissues and did not induce immune reactions (Gooch et al. 2019).

In the recent years, stem cells have been widely studied and used in clinical and preclinical trials which lead to a better understanding of their mode of action, therapeutic activity and healing power. Modern understanding redefines their nature and role in regenerative medicine, thus opening up new horizons and perspectives in the field of regenerative medicine. Many questions about MSC implantation have already been answered that allow us to use these cells effectively, but at the same time, new problems have arisen that need to be addressed. However, with current knowledge about the role of stem cells in veterinary regenerative medicine, we can better address animal diseases and pathologies.

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