Journal of Controlled Release 262 (2017) 104–117

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review article

Mesenchymal stem/stromal cell extracellular vesicles: From active principle to next generation drug delivery system

Barbara Crivelli[a], Theodora Chlapanidas[a], Sara Perteghella[a,*], Enrico Lucarelli[b], Luisa Pascucci[c], Anna Teresa Brini[d,e], Ivana Ferrero[f,g], Mario Marazzi[h], Augusto Pessina[d], Maria Luisa Torre[a], Italian Mesenchymal Stem Cell Group (GISM)[1]

[a] Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy
[b] Osteoarticular Regeneration Laboratory, 3rd Orthopaedic and Traumatologic Clinic, Rizzoli Orthopedic Institute, Via di Barbiano 1/10, 40136 Bologna, Italy
[c] Veterinary Medicine Department, University of Perugia, Via San Costanzo 4, 06126 Perugia, Italy
[d] Department of Biomedical, Surgical and Dental Sciences, University of Milan, Via Pascal 36, 20100 Milan, Italy
[e] I.R.C.C.S. Galeazzi Orthopedic Institute, Via Riccardo Galeazzi 4, 20161 Milan, Italy
[f] Paediatric Onco-Haematology, Stem Cell Transplantation and Cellular Therapy Division, City of Science and Health of Turin, Regina Margherita Children's Hospital, Piazza Polonia 94, 10126 Turin, Italy
[g] Department of Public Health and Paediatrics, University of Turin, Piazza Polonia 94, 10126 Turin, Italy
[h] Tissue Therapy Unit, ASST Niguarda Hospital, Piazza Ospedale Maggiore 3, 20162 Milan, Italy

ARTICLE INFO

Keywords:
Mesenchymal stem/stromal cells
Secretome
Extracellular vesicles
Drug delivery systems

ABSTRACT

It has been demonstrated that the biological effector of mesenchymal stem/stromal cells (MSCs) is their secretome, which is composed of a heterogeneous pool of bioactive molecules, partially enclosed in extracellular vesicles (EVs). Therefore, the MSC secretome (including EVs) has been recently proposed as possible alternative to MSC therapy. The secretome can be considered as a protein-based biotechnological product, it is probably safer compared with living/cycling cells, it presents virtually lower tumorigenic risk, and it can be handled, stored and sterilized as an Active Pharmaceutical/Principle Ingredient (API). EVs retain some structural and technological analogies with synthetic drug delivery systems (DDS), even if their potential clinical application is also limited by the absence of reproducible/scalable isolation methods and Good Manufacturing Practice (GMP)-compliant procedures. Notably, EVs secreted by MSCs preserve some of their parental cell features such as homing, immunomodulatory and regenerative potential. This review focuses on MSCs and their EVs as APIs, as well as DDS, considering their ability to reach inflamed and damaged tissues and to prolong the release of encapsulated drugs. Special attention is devoted to the illustration of innovative therapeutic approaches in which nanomedicine is successfully combined with stem cell therapy, thus creating a novel class of "next generation drug delivery systems."

* Corresponding author.
E-mail addresses: barbara.crivelli@unipv.it (B. Crivelli), theodora.chlapanidas@unipv.it (T. Chlapanidas), sara.perteghella@unipv.it (S. Perteghella), enrico.lucarelli@ior.it (E. Lucarelli), luisa.pascucci@unipg.it (L. Pascucci), anna.brini@unimi.it (A.T. Brini), ivana.ferrero@unito.it (I. Ferrero), mario.marazzi@ospedaleniguarda.it (M. Marazzi), augusto.pessina@unimi.it (A. Pessina), marialuisa.torre@unipv.it (M.L. Torre).

[1] This opinion article reflects the consensus viewpoint of the authors and scientists participating GISM, including the following individual investigators.

Giulio Alessandri, Cellular Neurobiology Laboratory, Department of Cerebrovascular Diseases, IRCCS Neurological Institute C. Besta, Milan, Italy, giulio.alessandri@istituto-besta.it.
Laura de Girolamo, Orthopaedic Biotechnology Laboratory, Galeazzi Orthopaedic Institute, Milan, Italy, laura.degirolamo@grupposandonato.it.
Davide Maria Donati, Orthopaedic, Musculoskeletal Oncology Department, Rizzoli Orthopaedic Institute, Bologna, Italy, davide.donati@ior.it.
Maura Ferrari, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy, maura.ferrari@izsler.it.
Massimiliano Gnecchi, Laboratory of Experimental Cardiology for Cell and Molecular Therapy, Fondazione IRCCS, Policlinico San Matteo, Pavia, Italy; Coronary Care Unit and Laboratory of Clinical and Experimental Cardiology, Fondazione IRCCS, Policlinico San Matteo, Pavia, Italy; Department of Molecular Medicine, Unit of Cardiology, University of Pavia, Pavia, Italy; Department of Medicine, University of Cape Town, Cape Town, South Africa, m.gnecchi@unipv.it.
Mauro Krampera, Stem Cell Research Laboratory, Section of Hematology, Department of Medicine, University of Verona, Italy, mauro.krampera@univr.it.
Katia Mareschi, Stem Cell Transplantation and Cellular Therapy Laboratory, Paediatric Onco-Haematology, Department of Public Health and Paediatrics, Turin, Italy, katia.mareschi@unito.it.
Maurizio Muraca, Department of Women's and Children's Health, University of Padova, Padova, Italy, muraca@unipd.it.
Lorenzo Piemonti, Vita-Salute San Raffaele University, Milan, Italy, piemonti.lorenzo@hsr.it.

http://dx.doi.org/10.1016/j.jconrel.2017.07.023
Received 6 June 2017; Received in revised form 12 July 2017; Accepted 15 July 2017
Available online 20 July 2017

1. MSCs: therapeutic agents and drug carriers

The idea to employ MSC-derived EVs instead of their parent cells in clinical practice derives from their therapeutic efficacy [1–3]. MSC paternity can be attributed to the pathologist Julius Cohnheim, who, in 1867, was the first to hypothesize that non-hematopoietic bone marrow cells migrate to far injured tissues to take part in regenerative processes [4]. In 1966, Alexander Friedenstein observed the development of reticular tissue from a heterotopic transplantation of bone marrow fragments and cell suspensions [5]. Later, he reported the presence of fibroblastoid cells in rodent bone marrow, early named Colony Forming Unit Fibroblasts (CFU-F). These cells were morphologically different from marrow hematopoietic cells with in vitro clonogenic potential that had previously never been attributed to other lineages [6]. In 1976, Friedenstein described the isolation procedure to separate CFU-F from whole bone marrow aspirate based on differential adhesion properties [7]. Over the years, other researchers confirmed his observations by demonstrating CFU-F ability to differentiate into osteoblast, chondrocyte, adipocyte and myoblast lineages [8–12]. In 1991, Caplan renamed these CFU-F cells into “Mesenchymal Stem Cells” (MSCs) to emphasize their ability to theoretically differentiate into end-stage cells of mesodermal tissues such as bone, cartilage, bone marrow stroma, adipose tissue, muscle, ligaments, and dermis. Two decades later, in an opinion paper, Caplan proposed to change their name to “Medicinal Signaling Cells” due to the secretion of a heterogeneous pool of bioactive compounds with an immunoregulatory and regenerative potential [13].

MSCs were first isolated from the bone marrow [7], then from several other adult tissues such as fat [14–16], dental pulp [17], synovial membrane [18] and tendons [19]. Moreover, MSCs populate lymphoid tissues [20] such as spleen and thymus [21], and perinatal sources including cord blood [22,23], Wharton jelly [24], placenta [25] and amniotic fluid [26].

Currently, bone marrow still represents the most popular source of MSCs for clinical applications, even if the collection is painful for the patient and results in a low yield of recovered cells [27]. On the contrary, fat tissue represents a minimally-invasive source of MSCs, symbolized by stromal vascular fraction (SVF). Some authors have proposed the use of SVF instead of MSCs due to its heterogeneity reducing manufacturing times and preserving the regenerative potential [28–31]. The MSCs derived from adipose tissue and other sources such as deciduous teeth, placenta or umbilical cord are characterized by features similar to bone marrow-derived MSCs in terms of morphology, multilineage potency and cell yield, despite specific differences in transcriptional and proteomic expression [14,32–35].

Due to the variability among MSC populations, a consensus document was written by the International Society for Cellular Therapy in order to establish minimal criteria to define MSCs and to facilitate their clinical application. Three minimal criteria were proposed: 1) adherence to plastic supports when cultured in standard conditions; 2) expression of specific surface markers, including positivity for CD105, CD73, CD90 and negativity for CD34, CD45, CD14 or CD11b, CD79α or CD19 and HLA-Class II markers; 3) in vitro differentiation into osteoblasts, chondrocytes and adipocytes, named multipotency [36]. In addition, MSCs may express other positive markers such as embryonic stem cell markers, i.e. Oct-4, Rex-1, and Sox-2, although their expression is strictly influenced by the source of the MSCs method of isolation and different culture stages [37].

MSCs are attractive candidates for several clinical applications, including oncohaematology and regenerative medicine, and currently several trials are recruiting patients in different countries to test the efficacy of MSCs in the treatment of several diseases. The translational application of MSCs means considering them as Advanced Therapy Medicinal Products, or drugs, thus their manufacturing process for clinical purposes should comply with Good Manufacturing Practice (GMP) to preserve the quality and safety standards of the final product. For this reason, all manufacturing steps, ranging from their collection to their clinical application, must be controlled and validated, as recently reported by Torre et al., on behalf of the Italian Mesenchymal Stem Cell Group (GISM) [38,39].

In clinical practice, MSCs retain several drawbacks that can be classified into different categories. The first category is represented by risks associated with the intrinsic cellular properties mainly related to cell differentiation status, tumorigenic potential, proliferation ability and life span in culture. The second category comprises risks associated with extrinsic factors introduced by cell collection, handling, culturing and storage. Finally, MSC therapy suffers from risks associated with clinical features including exposure duration and administration route [40]. The biodistribution of the cells is a critical step because the majority of the systemically injected MSCs are trapped by the lung and liver microvasculature, and the consequent failure to reach the target site [41]. This limit may be overcome by tissue engineering approaches, either by implanting cells within a three-dimensional scaffold [42–45] or by engineering their surface, resulting in an improvement in their ability to reach their native niche or injured tissues [46].

Due to their plasticity, MSCs were considered “ideal tools” for regenerative medicine, since originally it was assumed that their therapeutic potential depended upon their differentiation ability [47]. It is currently believed that the therapeutic potential of MSCs does not depend only by their regenerative capacity, but largely by the release of molecules possessing paracrine activity that are partially delivered by extracellular vesicles (EVs) [48–50].

1.1. Therapeutic effects of MSCs

MSCs are currently employed as “eclectic key players” in several clinical trials (730 clinical trials are available online on June 5th 2017; http://www.clinicaltrials.gov) either alone or in combination with scaffolds or biomolecules of different natures. However, it is still unclear how injected cells interact with the target site while maintaining their viability and phenotype [51]. Some authors suggested a strict interaction between exogenous and resident MSCs that would switch off the immune surveillance thus allowing tissue regeneration [47,52]. In fact, MSCs suppress T cell proliferation and differentiation per se or by regulating the Treg cells activation by secreting immunosuppressive factors and chemokines [53,54].

Nearly half of all registered clinical trials exploits the MSCs' immune-modulatory and/or anti-inflammatory properties for the treatment of severe autoimmune diseases, organ transplantation and rejection, inflammatory conditions such as multiple sclerosis [55], diabetes [56], osteoarthritis [57], inflammatory bowel disease [58] and osteogenesis imperfecta [59,60]. In particular, Graft versus Host Disease (GvHD) represents one of the major fields of application for MSCs, the data suggest that MSC therapy results in effective remission of the symptoms without serious side effects [61–63]. Similarly, MSCs have been tested in several other autoimmune disorders such as systemic lupus erythematosus (SLE), rheumatoid arthritis and Crohn's disease [64–66].

The MSCs used in clinical trials have been isolated from different sources: the most common being bone marrow, but also adipose tissue, umbilical cord and placenta. Notably, even though both autologous and allogeneic MSC transplantations have shown to be safe, the latter is preferred due to the isolation source (young and healthy donors), availability off the shelf and the higher cost-effectiveness [67,68].

Albeit clinical results are encouraging several concerns about MSC-based therapy including safety, long-term efficacy, route of administration, and cost-effectiveness still represent issues that need to be addressed. MSC engraftment at the target site also represents a limit and many studies support the lack of long-term MSC engraftment; this evidence lead to the hypothesis that the positive effects exerted by MSC therapy were mediated by paracrine mechanisms [69]. The use of tissue engineering approaches such as 3D scaffolds or injectable hydrogels has