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Controversies in therapeutic application of mesenchymal stem cell-derived secretome

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Abstract: Though mesenchymal stem cells (MSCs) are considered as an important pillar of regenerative medicine, their regenerative potential has been shown to be limited in several pathological conditions. The adverse properties of MSC-based cell therapy have drawn attention to the therapeutic use of MSC-derived secretome. However, MSC-originated exosomes and microvesicles can also possess a significant impact on disease development, including cancer. By interchanging secretome, MSCs can interact with tumor cells and promote mutual exchange/induction of cellular markers. In addition, enzymes secreted into and activated within exosomes can result in the acquisition of new tumor cell properties. Therefore, therapeutic applications of MSC-derived secretome require much caution.

Introduction

Stem cell research and tissue engineering seem to be integral parts in regenerative medicine. According to their ability to differentiate into new cell lines, stem cells can be classified as totipotent, pluripotent, multipotent, and unipotent ones. The use of allogeneic cells may lead to complications, including immunological rejection, but upon administration of autologous cells rejection can be avoided, thus it represents a relatively safer therapeutic form.

Of the adult stem cells, hematopoietic stem cells and mesenchymal stem/stromal cells (MSCs) are the most frequently used ones, mainly because they can be obtained from patients suffering from distinct disease conditions (e.g., aplastic anemia, Duchenne muscular dystrophy, ankylosing spondylitis, etc.) (Vasanthan *et al.*, 2020).

MSCs possess the potential for self-renewal along with limited capacity of differentiation. Their main sources are bone marrow, adipose tissue, skin, liver, lungs, cord blood, and fallopian tube (Mohammadian *et al.*, 2013).

The therapeutical usage of MSCs faces issues like the difficulty of maintaining a homogenous culture and, further, characterizing the cells (Meirelles *et al.*, 2009). Besides cell replacement function, MSCs have a wide range of biological effects (i.e., immunomodulation, anti-apoptotic and anti-fibrotic activity, angiogenesis, chemoattraction, pro-growth and pro-differentiation activity on other stem cells) (Chang *et al.*, 2006; Jones *et al.*, 2007; Saeedi *et al.*, 2019; Patel *et al.*, 2013). One of their most promising properties is the secretion of bioactive components, known as secretome, into the cell culture conditioned medium (González-González *et al.*, 2020). The secretome consists of a soluble and a vesicular fraction. The soluble fraction is rich in cytokines, chemokines, growth factors, and immunomodulatory molecules. The vesicular fraction contains extracellular vesicles, which according to size and synthesis route could be subdivided to exosomes, microvesicles and apoptotic bodies. The MSC-derived secretome consists of exosomes and microvesicles, which in turn contain proteins, lipids, or nucleic acids (González-González *et al.*, 2020). The secretome can directly activate the target cells partly by endocytosis and may exert a broad spectrum of actions, as indicated above (Hassanpour *et al.*, 2020). Nevertheless, it is important to note, that the therapeutic capacity of the secretome may vary depending on the origin of MSCs (Zhao *et al.*, 2019).

Difficulties in Mesenchymal Stem Cell-Based Therapies

Based on their broad spectrum of functional properties, MSCs can be considered as a relevant pillar for regenerative medicine, therefore, their biosafety characteristics during clinical use should be critical in order to eliminate potential functional or genetic changes. According to various studies, however, the regenerative potential of MSCs has been shown to be very limited, especially within pathological

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conditions. Although MSCs are present in several tissues, their number is quite small. In addition, the viability of transplanted cells and their uptake into host tissues are often diminished (Haque *et al.*, 2015). Moreover, many factors, like the age of the donor, the number of passages and culture conditions in the course of *in vitro* expansion, the route of administration, and the pathological host microenvironment confronted by the transplanted MSCs may unfavorably impact on the capacity of these cells to survive and engraft into host tissues (Rezaie *et al.*, 2018). Recent studies have also indicated possible pro-tumorigenic activities of MSCs (Barkholt *et al.*, 2013) along with pro-fibrogenic and pro-coagulant potentials (Russo *et al.*, 2006; Fischer *et al.*, 2009), an increased risk of infections (e.g., zoonotic diseases) during the *in vitro* expansion procedure (Lepperdinger *et al.*, 2008), and the unfavorable heterogeneity of their differentiation potential (Fig. 1) (McLeod and Mauck, 2017).

Mesenchymal Stem Cells' Secretome: Disadvantages in the Shadow of Advantages

The adverse properties of MSC-based cell therapy have drawn attention to the therapeutic use of the MSCs' secretome. Application of the MSC-derived secretome has many meaningful advantages, including the lack of need for surgical intervention to recover cells, the possibility of drug-dose dosage and safety evaluations, the easier way of administration, or the potential manipulation on its composition (Baglio *et al.*, 2012). MSC-derived soluble and vesicular factors represent numerous special properties that could make them an invaluable tool for therapeutic applications (González-González *et al.*, 2020). However, concrete issues arise when working with these entities, whose physical and biochemical characteristics often make them difficult to obtain as pure preparations, and to characterize correctly. Therefore, the International Society for Extracellular Vesicles (ISEV) proposed Minimal Information for Studies of Extracellular Vesicles ("MISEV") guidelines for the field in 2014, which has been recently revised in 2018 (Théry *et al.*, 2018).

On the other hand, exosomes can also possess a significant impact on disease development, including cancer. Tissue damage results in the recruitment of MSCs to support wound healing and regeneration. Similarly, invasive tumor growth also leads to (in part inflammatory) tissue damage with the consequence of intensive attraction and cellular cross-modulation. By interchanging secretome, MSCs can interact with tumor cells and promote mutual exchange/induction of cellular markers (Yang *et al.*, 2015; Nawaz *et al.*, 2018; Mandel *et al.*, 2013; Hass and Otte, 2012; Yang *et al.*, 2015; Salimi *et al.*, 2020).

In addition to the direct effect of soluble fraction secreted by MSCs, enzymes secreted into and activated within exosomes (mainly matrix metalloproteinases /MMP/ and MMP regulators) can result in the acquisition of new tumor cell properties (Yang *et al.*, 2015). The vesicular fraction of secretome plays a role in the construction of pre-metastatic niche and tumor neovascularization. Furthermore, extracellular-matrix-associated abnormalities could affect cancer progression by promoting fibroblastic switching and acquisition of mesenchymal mode (Nawaz *et al.*, 2018).

The incorporation of MSC-derived exosomes has been shown to be associated with acquired ecto-5'-nucleotidase activity by certain tumor cells (Yang *et al.*, 2015). With this new capability tumor cells can suppress and modify pro-inflammatory activities (e.g., tumor-infiltrating T-cell function) via activation of adenosine receptor signaling present on the surface of most immune cells (Ohta and Sitkovsky, 2014; Clayton *et al.*, 2011).

Vice versa, tumor cells can also affect and modify MSCs through their secretome (Nawaz *et al.*, 2018; Ma *et al.*, 2020). Extracellular vesicles secreted by cancerous (stem) cells are able to develop a pre-metastatic niche and induce epidermal-mesenchymal transition, favoring tumor spreading (Fig. 2) (Nawaz *et al.*, 2018).

In addition to unwanted biological properties, vesicular secretome isolation techniques (i.e., ultracentrifugation, membrane filtration, precipitation, size exclusion chromatography, immunoaffinity capture technology) are also currently cumbersome, generally resulting in small amounts of low-purity, sometimes deformed extracellular vesicles. Their subsequent use is therefore difficult to implement (Ma *et al.*, 2020; Ahmadi *et al.*, 2021; Ahmadi and Rezaie, 2021; Babaei and Rezaie, 2021).

Future Perspectives

According to ClinicalTrials.gov the number of studies using MSC-derived secretome is quite low (i.e., 10), and only 3

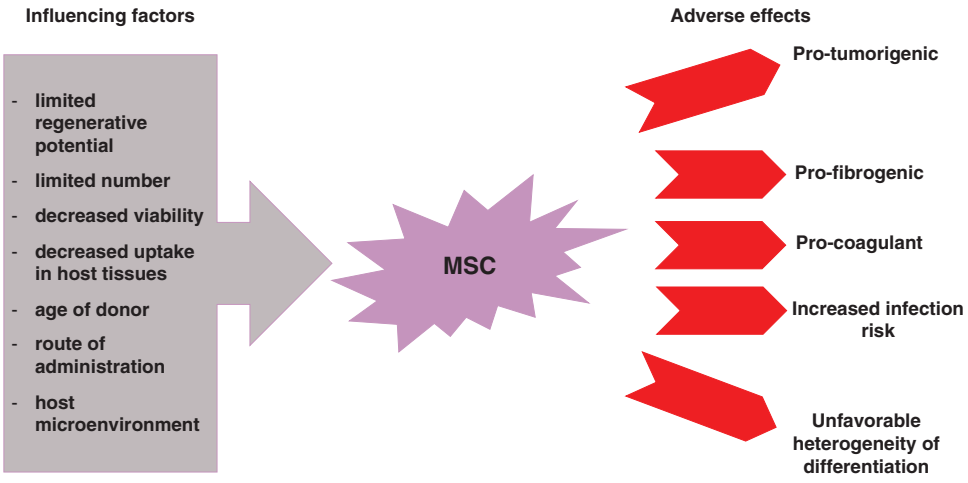

FIGURE 1. Factors influencing the therapeutic potential of mesenchymal stem cells and the resulting adverse effects.

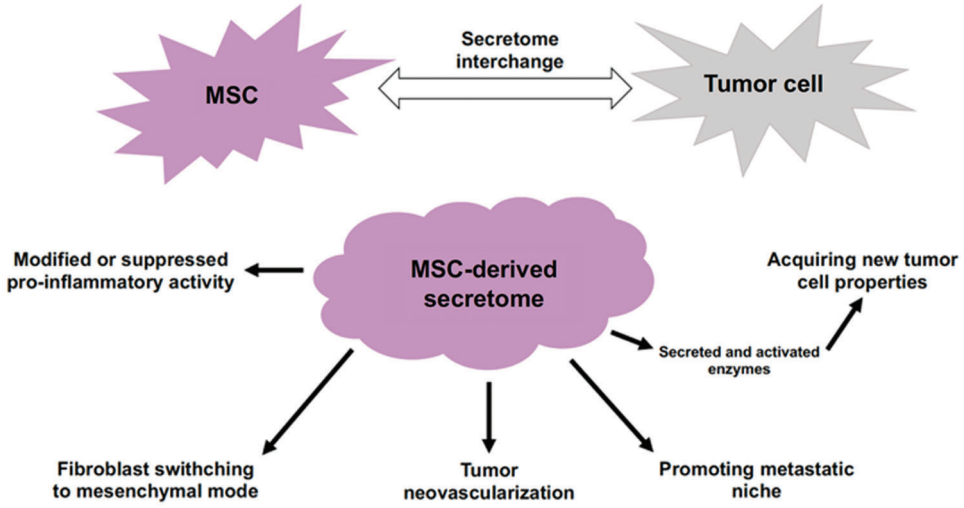

FIGURE 2. Unfavorable effects of secretome interchange between mesenchymal stem cell and tumor cells.

have been completed up-to-date. The therapeutic application of MSC-derived secretome looks promising, yet caution is required. It is not just about that the composition and function of the MSC-derived secretome are highly dependent on the origin of the MSCs (i.e., healthy, inflammatory or tumorous environment), but for the time being, the therapeutic targeting of the secretome used is also difficult (Phelps *et al.*, 2018). Whichever route of administration is used, it is not yet possible to be absolutely certain that the bioactive substance will act on a particular cell type, nor is it entirely possible to determine how the expected biological effect is affected by the milieu to which the secretome is added.

Currently we also lack knowledge of how the MSCs and their secretome can be affected by the combinations of drugs used in the disease conditions. Further researches are also needed to explore how possible genetic or epigenetic changes in MSCs affect the composition and biological effects of the secretome. This is especially important to avoid possible tumorigenic side-effects (Hassanzadeh *et al.*, 2021).

In addition to the technical difficulties of finding and extracting MSCs, new and efficient laboratory techniques are also needed to extract the MSC-derived secretome in the right quantity and quality, making it easier to apply in daily practice. It would be useful to reduce the time and cost of these new techniques as well, thus efficiently promoting their spread. Overall, there is no doubt that besides the cellular-based strategies cell-free bioactive materials, like the secretome may represent a considerable alternative in translational medicine.

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