Extracellular Vesicles of MSCs

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Definition

Mesenchymal stem cells (MSCs), the cells distributed in the stromas of the body, are known for various properties including replication, the potential of various differentiations, the immune-related processes including inflammation. These cells were shown to play relevant roles in the therapy of numerous diseases, dependent on their immune regulation and their release of cytokines and growth factors, with ensuing activation of favorable enzymes and processes. Soon thereafter, it became clear that therapeutic actions of MSCs are risky, accompanied by serious drawbacks and defects. MSC therapy has been therefore reduced to a few diseases, replaced for the others by their extracellular vesicles, the MSC-EVs. The latter vesicles recapitulate most therapeutic actions of MSCs, with equal or even better efficacies and without the serious drawbacks of the parent cells. In addition, MSC-EVs are characterized by many advantages, among which are their heterogeneities dependent on the stromas of origin, the alleviation of cell aging, the regulation of immune responses and inflammation.

1. Introduction

During the studies of the last two decades it has become clear that all cells of the various organs, and their extracellular vesicles (EVs), do not share the same states and functions. In all animal organs including man the tissues, abundant of common cells, include also small, specialized areas called stromas. In addition to some common cells, the stromas include cells of a special family, the mesenchymal stem cells (MSCs), characterized by peculiar properties, essential for the development of the organs and for the acquisition of their structure and critical functions. The study of MSCs and EVs has revealed their extraordinary role in the therapy of many diseases. Moreover in cancers, considered some sort of pathological organs, stem cells are present in areas similar to stromas where they exhibit unique properties, essential for their existence and aggressive activities. Recent reviews, appeared in Biomedicines [1] and other Journals, have reported about the distinct mechanisms and effects induced by stem cells from normal organs and cancers. Important aspects of these processes are summarized in the present article.

2. Mesenchymal Stem Cells

Discovery of the stroma cells, started a few decades ago, was pursued in the following years, with identification of the peculiar MSC properties, including continuous self-replication and multidirectional differentiation. MSCs from different organs share many properties, however they are never identical. From the functional points of view, commonalities are frequent, heterogeneities rare but relevant. Distinctions of diseases include manipulations of immunological processes and attenuations of inflammatory processes. These and many other properties, initially considered as simple curiosities, with time started to stimulate interest, initially because of their apparent functional similarity to recombinant proteins involved in bone and cartilage healing [2].

Shortly thereafter, additional results were obtained concerning local processes, such as self-renewal and repair of organs, followed by protective effects over diseases of lung, heart, kidney, brain, and many other organs [3][4][5]. Such effects were thought to start by paracrine fusions of MSCs to their specific target cells, locally reinforced by release of soluble and bioactive factors, such as cytokines and growth factors [6][7]. The identification, around 2010, of another potentially important process, secretion of EVs, suggested their possible participation in the effects of their parental cells, confirmed by results of experiments in which cells and vesicles were administered together [8][9].

In most initial experiments, carried out on animals, MSCs were shown to induce positive therapeutic

results against various types of diseases. Their extension to human diseases was therefore considered of potential interest and intensely investigated. Yet, despite encouraging preclinical outcomes, human MSC infusion and transplantation therapies were found to induce also considerable risks, including immune rejections and various types of cancer promotion [9]. Because of these negative results, the enthusiasm about MSC decreased rapidly, with ensuing reduction of its employment in therapy. In parallel, the majority of registered clinical trials applying MSC therapy to diverse human diseases did fall short of expectations. At present, therefore, the study of these therapies remains of interest, however its clinical perspectives are limited to only a few types of disease.

3. Extracellular Vesicles of MSCs

The risky problems recognized to MSC employment in human diseases opened the way to corresponding studies about their EVs. Unexpectedly, the role of these vesicles did not consist only in strengthening effects. EVs were found to recapitulate many of the MSC therapeutic effects with equal or even better efficacies. For many studies, therefore, these EVs, named as MSC-EVs, opened a new type of therapy [10]. Their advantages are considerable. The inability of MSC-EVs to self-replicate greatly reduces the risk of tumors and expansions, typical of MSCs; the limited potential to trigger the immune system prevents disappointments; easier transport and storage makes the potential of EV therapy optimal when compared to cell-based approaches [10][11][12]. In view of the multiplicity and the differences of the organs, the isolation of many MSCs and their MSC-EVs was difficult. The present studies, therefore, are mostly carried out not with all, or with only a single, but with four types, easy to use: those from bone marrow, adipose tissue, umbilical cord and blood from that cord. The discovery of MSC-EV properties, confirmed in a variety of experimental conditions, have lead to an explosion of interest about these vesicles, with over 20 fold increase of their publications during the last 5 years. Clinical studies, however, are still early, meaning that their work is encouraging, but not yet carried out in detail.

As already mentioned, a critical property of MSC-EVs is their heterogeneity. Variability among EVs is significant, concerning components such as proteins and non-coding RNAs that participate in the generation of properties such as potency, inflammatory resolution and tissue regeneration [13][14][15]. Diseases differentially affected by various MSC-EVs are numerous. For example the vesicles from the umbilical cord affect acute diseases and operate well in their damage repair; those from adipose tissue are more active against prolonged diseases with immune responses, including Alzheimer's disease and multiple sclerosis; those from bone marrow are especially active in tissue regeneration [15,17]. The relation of the effects on diseases, useful also for preclinical analyses, stimulated the use of MSC-EVs as therapeutic tools of translational potential [16][17][18].

4. Mechanisms of MSC-EVs Against Diseases

The therapeutic effects of MSC-EVs depend on signaling pathways altered by diseases and modulated by vesicles. Diseases are quite different from each other. The therapeutic effects of MSC-EVs can be explained by their cargo components, i.e. proteins, lipids and RNAs. While the identification of the proteins involved is often difficult, the identification of non-coding micro RNAs (miRNAs) is easy. Its therapeutic action has been shown to depend on their activation of signaling cascades involving enzymes and other proteins. Here we report short data concerning MSC-EV actions against diseases of three important organs (bones, heart and vessels and brain), followed by Table 1, specifying the diseases and the miRNAs governing their therapy. Details about Therapies and Clinical Medicine are missing here. They can be found in [1].

Bones. MSC-EVs are widely employed in osteoblastogenesis and rheumatisms, the latter also for clinical application.

Heart and Vessels. Various MSC-EVs are protective of myocaradial infarction and other heart diseases. In vessels MSC-EVs have been shown to favor growth, prevent the formation of atherosclerotic plaques and other various functions.

Brain. Most intensely investigated are neurodegenerative Alzheimer's and Parkinson's diseases, for which future developments are promising. The same occurs for multiple sclerosis. Positive results have been obtained also with post-stroke neurodegeneration, ischemic immunosuppression and other diseases.

Table 1. MSC-EVs affecting diseases in various organs by specific cargo miRNAs.

Organs (Years of the First Report)	Diseases & Processes	Examples of Active miRNAs within the MSC-EVs Involved
Bones (2011)	osteoblastogenesis	miR-133-3p
	rheumatic diseases	miR-483-5p
Heart & Vessels (2012)	infarction cardio-protection atherosclerosis	miR-125b
		miR-182
		miR-22-3p
Brain (2013)	neurodegeneration multiple sclerosis stroke circulation arrest	miR-221
		miR-467f
		miR-466q
		miR-124

5. Stem cells and extracellular vesicles in cancers

The data presented so far about MSCs and their EVs refer to both physiology and pathology of the numerous organs present in animal bodies. Such data, however, do not cover the whole problems in the field. In fact, animal bodies may include additional organs, however of exclusive pathological nature, i.e. many types of cancer. Similar to non-cancer organs, cancers include stroma-like areas, named the niches that, together with normal cancer cells, include stem cells of two different families. Cells of the MSC family, as well as their EVs, regulate cancer growth. However, their results are quite variable. In glioma and breast cancers they induce positive therapy, i.e. reductions of cell proliferation together with reinforced effects of anti-cancer drugs; in multiple myeloma and gastric cancers they induce no reduction but stimulation of cancer proliferation [19][20][21]. Employment of MSCs and EVs in cancer therapy is very limited.

The role played by the stem cells of the secondfamily, i.e. those named cancer stem cells (CSCs), is much more important. Immunomodulation of these cells appears largely proficient at evading immune surveillance. Various malignant components of cancers are therefore resistant to immunotherapy [22]. Moreover, CSCs and their EVs govern many critical processes of cancer life: from their initiation and progression to their formation of metastases, therapy resistance, and cancer relapse [23][24]. In addition, CSCs are needed for every day cancer functions, including drug efflux, autophagy and immune suppressions. Due to their proliferation, providing multi-lineage differentiations, CSCs induce stable generation and activities from initiation, distinction and development of cancers. In case of elimination, CSCs are rapidly regenerated by changes of normal cancer and MSCs cells, a form of replacement that protects cancers against therapies against CSCs that are now being developed.

6. Conclusion

Stem cells play critical roles in all organs of animals and man. In the MSC family the heterogeneity, considerable and significant for their distinctions, accounts for the regulation with specificities of distinct organs. The combination of anti-disease therapies with risky dangers has eliminated most therapeutic uses of these cells. The unexpected replacement of MSC by their EVs has validated important properties

of these therapies, expected to become soon relevant also for clinical medicine.

Promising is also the state of stem cells of cancer. These pathological organs are the only ones in which two families of these cells coexist and develop distinct functions in parallel. MSCs and their EVs carry out functions analogous to those of non-cancer organs but often with different results. Highly important, in contrast, are the cancer processes governed by CSCs. Without them, cancers could not exist. For all their properties, CSCs are being considered as tools essential for future development of new, operational therapies, critical also for their expected medical success in many cancers.

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Keywords

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