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Review

Circulating extracellular vesicles: Their role in tissue repair and regeneration

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ABSTRACT

Extracellular vesicles (EVs) have been a growing interest of the scientific community in recent years due to the wide possibilities of their evaluation as biomarkers of disease, and their potential to be used as therapeutic agents or vehicles. EVs that circulate in plasma carry proteins and nucleic acids, potentially to distant locations in the body where they can interfere with several cellular processes. To aid understanding of this rapidly evolving field, circulating EVs, including immune cell-derived ones, are reviewed here. Their cellular origins and described functions are discussed in a perspective of their contribution to regenerative processes. Different techniques for EV engineering and examples of their application are reviewed as a strong future direction of EV research. A summary of important aspects yet to be addressed ties up this review.

Contents

1. Introduction

Paracrine communication between cells of the same tissue and across different tissues is of utmost importance to maintain body homeostasis, playing also a role on disease onset and dissemination. For a long time, soluble factors such as cytokines and growth factors, secreted by cells, were considered the main effectors of paracrine signaling.

This vision is evolving, due to great advances, partly in the study of extracellular vesicles (EVs), a major component of what is now called the cell's secretome. The exponential growth of publications in the field of EV research, in the past 10–15 years, evidences their growing importance for the scientific community [1].

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Exosomes, microvesicles and apoptotic bodies comprise the main types of EVs secreted by cells [2], but the first two have been attracting the most attention from researchers. Exosomes and microvesicles are mainly distinguished based on their properties and origin within the cell. Exosomes are nanovesicles with a size ranging from 50 to 100 nm. They form within the endosomal compartment, through the invagination of the membrane, in multivesicular bodies (MVBs), being released upon MVBs fusion with the cell membrane [2,3]. This type of EVs is composed of a lipid bilayer enriched in specific types of lipids comparing to the parental cell, namely cholesterol, sphingomyelin and ceramide, which are implicated in EV formation [3,4].

Their membrane is enriched also in some proteins such as tetraspanins (e.g. CD63 and CD81), which constitute the most consensual markers for exosomal identification, and transmembrane proteins characteristic of the parental cells, which can be used to characterize their origin and have functional relevance [5]. Similarly, exosomes' internal content is enriched in particular biomolecules compared to their cell origin [6], carrying functional proteins and nucleic acids, namely microRNAs (miRNAs), messenger RNAs (mRNAs) and even DNA [7], with miRNAs being particularly explored in terms of the potential functional roles of exosomes. Most interestingly, the whole pool of exosomes secreted by a cell seems to be actually composed of several subpopulations of exosomes, differently enriched in specific components, and thus with potential to target different cells types and to have distinct biological functions [8,9].

Microvesicles are larger EVs with a size from 200 nm to 1 μm, formed by direct cell membrane budding [2,10]. Interestingly, they originate from lipid raft areas of the cell membrane, enriched mainly in the same type of lipids found in exosomes membrane.

Similarly to exosomes, microvesicles also carry functional miRNAs, mRNAs and proteins [11]. In either case, the content of both vesicles depends on the biomolecules available at each moment in the secreting cells, thus reflecting, at least in part, their physiological status [12].

Upon release, EVs may act upon specific target cells in the vicinity of the secreting cells, or may enter biological fluids, such as plasma [13], or urine [14], among many others [15], acting as endocrine-like messengers and potentially mediating numerous biological processes [15]. EVs popularity among the scientific, medical and pharmaceutical communities mainly arose from the functions and potential applications that they have been ascribed, namely: (i) their capacity to transfer active biomolecules to target cells, modulating their behavior [16]; (ii) their composition in terms of biomolecules, with biomarker potential for disease diagnosis and prognosis [17]; (iii) their natural therapeutic and targeting potential [18]; (iv) the possibility of engineering their content relatively easily for numerous biomedical applications, including drug delivery [19].

Among the EVs functions reported so far, their role in cancer progression and their immunoregulatory actions are perhaps the ones most characterized. In fact, cancer cells-derived EVs have been shown to act upon multiple pathways, promoting cancer progression and metastasis, by transferring oncogenic cues to normal cells in the tumor microenvironment, promoting extracellular matrix remodeling, mediating evasion from the immune system, among other aspects that have been recently reviewed in Reference [20]. Furthermore, EVs may act either as pro-inflammatory, immunosupressor or tolerogenic stimuli, transferring immunomodulatory cytokines, miRNAs and other mediators among immune cells, and between immune cells and other cell populations (and *vice-versa*) [21]. From a clinical point of view, EVs have been particularly explored as a source of biomarkers for cancer diagnosis and prognosis [17], and as therapeutic vehicles for the treatment of cancer and other pathologies, with a special emphasis on immune cell-derived EVs for the latter [22].

Although widely less explored, EVs potential for modulating tissue repair and regeneration has also not gone unnoticed. EVs are interesting candidates to considerably replace a wide range of current cell and tissue engineering approaches.

One of the most striking observations supporting this vision comes from studies on mesenchymal stem/stromal cell (MSC) transplantation for immunomodulation and tissue repair, which have shown that their action is mainly through paracrine factors [23]. Thus, one can envisage "a cell therapy without the cell", using "paracrine factors" such as EVs, to promote tissue repair/regeneration. Considering the participation of immune cells and their by-products in the injury microenvironment, together with their long-distance range of action throughout the body, immune cell-derived EVs and those that circulate and/or originate in the blood compartment are particularly interesting players for the development of such cell-free therapies. Therefore, the next section reviews current evidence on the potential of circulating EVs, particularly immune cell-derived ones, for promoting tissue repair and regeneration. The contribution of these vesicles for cell-to-cell communication both locally and systemically is discussed, as well as emerging strategies for vesicle modification.

2. Circulating EVs: cellular origin, markers and functions

One of the richest and most easily accessible biological sources of EVs is plasma, which is considered to be an important physiological medium for circulating EVs [24–26]. Nonetheless, given the nature of plasma, protein aggregates and lipoprotein contaminants are a common problem for EVs functional characterization that is still not completely solved. Conventional ultracentrifugation protocols can yield EVs with high levels of protein contaminants, so there is a critical need for further processing, incorporating filtration, chromatography, and gradient centrifugation steps in the protocols used. Fig. 1 shows EVs from human plasma separated by conventional ultracentrifugation protocol, compared to PBS washed and gradient centrifugation. The trade-off of purification is yield, and contaminant proteins heavily influence protein quantification methods, which are often used to estimate the amount of EVs in a given sample. This reinforces the need for a combination of approaches also to evaluate the amount of EVs in a sample.

Plasma-derived EVs contain a heterogeneous mixture of vesicles mainly derived from: endothelial cells; cell populations that circulate in the blood (red blood cells, white blood cells and platelets); megakaryocytes; and different

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