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Extracellular Vesicles: Immunomodulatory messengers in the context of tissue repair/regeneration

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ABSTRACT

Inflammation is a complex and highly regulated biological process, crucial for a variety of functions in the human body, from host response against infectious agents to initiation of repair/regeneration of injured tissues. In the context of tissue repair, the action of different immune cell populations and their interplay with tissue specific cells, including stem cells, is still being uncovered. Extracellular Vesicles (EV) are small membrane vesicles secreted by cells in a controlled manner, which can act locally and systemically. The ability of EV to influence tissue repair and regeneration has been proposed as a physiologically intelligent and targeted strategy of cell communication. Herein, the role of EV in tissue repair is reviewed, summarising first their contribution to the regulation of immune cell function, and discussing the implications for the resolution of inflammation during repair. Next, the impact of EV on cell proliferation and differentiation, and on extracellular matrix remodelling, key aspects of the subsequent phases of tissue repair, is addressed. Finally, EV-based therapies are discussed, focusing on the application of naturally produced EV, and the use of EV as delivery vehicles.

1. Introduction

In the last decade, the importance of inflammation and immune cells action in triggering tissue repair or regeneration has been increasingly recognized in different systems, such as bone (Loi et al., 2016; Claes et al., 2012), skin (Eming et al., 2014), skeletal muscle (Tidball et al., 2014), cardiac muscle (Frangogiannis, 2015), and the peripheral and central nervous system (Mietto et al., 2015). Also, a very recent report elegantly demonstrates the importance of a mature peritoneal macrophage population for tissue repair, in a model of liver injury (Wang and Kubes, 2016). A sequential arrival of immune cells at the injury location and their concerted action, tightly regulated in time, appear to be required for successful regeneration, namely in bone (reviewed in Claes et al., 2012). Unbalanced immune responses are considered deleterious for tissue regeneration. Taking bone as a model, it was reported that regeneration was delayed in the absence of effective TNF-α signalling, in a mouse model of tibia fracture (Gerstenfeld et al., 2003). Moreover, bone loss is a common symptom in patients with chronic inflammatory disorders, such rheumatoid arthritis (Crotti et al., 2015). Thus, while the inflammatory response is necessary for complete tissue repair, it has to be tightly and timely controlled, and that likely relies on close cell-to-cell communication.

The concerted action of immune cells and tissue cells may be achieved not only by contact-dependent cell-to-cell communication, but also in a paracrine manner, *via* secreted immune mediators, which include free proteins (cytokines, chemokines, growth factors) and Extracellular Vesicles (EV) (Murray and Stow, 2014). The designation of EV encompasses different populations of vesicles, mainly exosomes, microvesicles and apoptotic bodies, distinguishable by their size, composition and origin in the cell (György et al., 2011): exosomes are smaller (50–200 nm), formed in multivesicular bodies and seem to benefit from a more controlled release mechanism; microvesicles are bigger vesicles (200 nm–1 μm) and originate by budding from the plasma membrane; and apoptotic bodies correspond to portions of cells (> 1 μm) that disintegrate from the parent cell during cell apoptosis (for a review of EV nomenclature and characterization please see Lötvall et al., 2014). Although several attempts of standardization have been made in this field (Witwer et al., 2013; Lötvall et al., 2014), the methods currently employed to isolate each population have varying degrees of success in terms of population purity. Also, not all studies follow the nomenclature guidelines proposed by the International Society for

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Extracellular Vesicles, which makes the real identity of EV populations found in different reports sometimes still unclear. Thus, in this article the designation "EV" will be preferred.

In broad terms, EV are composed by a lipid bilayer that encloses and protects biomolecules such as proteins, nucleic acids (both RNA and DNA) and bioactive lipids, but their detailed composition depends on their biogenesis mechanism (revised in Lo Cicero et al., 2015). In general, different types of phospholipids, cholesterol and sphingomyelin compose the lipid fraction of EV's membrane, with different types of vesicles carrying distinct proportions of each component (revised in Record et al., 2014). EV membrane is further decorated with proteins involved in membrane trafficking, adhesion and cell communication (*e.g.*, tetraspanins, ESCRT proteins, Rab proteins, integrins, ligands for cell surface receptors). In their lumen, EV have been reported to enclose a wide variety of proteins, such as enzymes (*e.g.*, GAPDH, pyruvate kinase, phospholipases), signalling and signal transduction proteins (*e.g.*, cytokines, growth factors, 14-3-3 proteins) and cytoskeleton proteins (*e.g.*, actin, tubulin), among others (Pocsfalvi et al., 2016; Record et al., 2014). Moreover, several kinds of RNA molecules can also be found inside EV, with an enrichment of small non-coding RNAs, mainly vault RNA, Y-RNA, transfer RNA (Nolte-'t Hoen et al., 2012), and miRNA (Valadi et al., 2007). Messenger RNA (Valadi et al., 2007), long non-coding RNA (Takahashi et al., 2014) and fragments of DNA (Thakur et al., 2014) have also been found inside EV.

EV are secreted by virtually all cell types and can be found in circulation, likely mediating biological actions in locations distant from their origin (Teixeira et al., 2016). These vesicles are reported to interact with target cells by different mechanisms. They can either bind receptors on the cell membrane, triggering intracellular signalling or internalization and, when internalized (either receptor mediated or not), EV release their content in the endocytic pathway. Alternatively EV can also fuse with the plasma membrane and deliver their content to the cell cytosol directly (Mathivanan, 2012).

EV have been most studied so far in the fields of cancer research and immune response (Fais et al., 2016). In fact, EV are being regarded as important regulators of immune cell activity, and consequently capable of modulating inflammatory responses, which are crucial for the outcome of tissue repair and regeneration processes. At the same time, a limited number of *in vivo* studies have been associating the administration of EV with tissue function recovery in different models of injury (Yáñez-Mó et al., 2015), but the mechanisms underlying this beneficial effects have not yet been revealed.

In this work we review the potential contribution of EV for tissue repair and regeneration, in terms of their described roles and therapeutic potential. We start by addressing described EV functions that are relevant for the different stages of tissue regeneration. Then, the therapeutic potential of natural, modified and artificial EV for inflammation control and enhanced tissue repair and regeneration is discussed.

2. EV in Tissue Repair and Regeneration

Tissue repair and regeneration develop along three main phases: inflammation, repair and remodelling, which partially overlap, and are dependent on each other (Stroncek and Reichert, 2008). Injury activates tissue cells, including epithelial, stromal and resident immune cells that induce recruitment of other circulating immune cells, starting an inflammatory response, the first phase of tissue repair/regeneration. This reaction is crucial for removal of pathogens and damaged cells from the injury site, as well as for extracellular matrix (ECM) turnover. After clearance of dead and/or infected cells inflammation needs to resolve (Atala et al., 2010), in order to allow for tissue regeneration to progress into the remodelling and repair phases, mainly characterized by enhanced angiogenesis, fibroblast activation and deposition of new ECM, and tissue cell replacement by proliferation and differentiation. Interestingly, EV have been assigned with functions that might play important roles in the different processes taking place in each of the main stages of tissue repair/regeneration (Yáñez-Mó et al., 2015) (Fig. 1).

2.1. EV as Immunomodulatory Messengers: Implications for Tissue Repair and Regeneration

Immune cells are able to modulate the activity of other immune and tissue-resident cells. This has been reported to take place by direct contact, and by the action of paracrine mediators, some likely contained in EV. In fact, EV have been reported both as immune activators and immune suppressors (Table 1).

The most described function of EV in immunity is the triggering of pro-inflammatory responses, as they have been reported to transport antigens loaded onto MHC class I and II complexes. Dendritic cell (DC)-derived exosomes loaded with viral antigens are capable of activating $CD8^+$ T cells (Admyre et al., 2006), and EV from infected cells contain bacterial and viral antigens, eliciting macrophage and T cell activation (Bhatnagar and Schorey, 2007; Walker et al., 2009). The Antigen Presenting Cell (APC) function of EV has been explored to develop anti-cancer therapies for about 20 years (Zitvogel et al., 1998; Raposo et al., 1996), including in a few clinical trials. However, this is not a focus of the current work, and has been reviewed recently elsewhere (Pitt et al., 2014; Fais et al., 2016). Similarly, although largely unexplored so far, it is likely that EV are involved in the transmission of Damage-Associated Molecular Patterns, activating specific immune cell populations (Elsner et al., 2007), and thus modulating the onset of the inflammatory response to injury. On the other hand, EV from some body fluids and immune cells have been shown to have tolerogenic and immunosuppressive roles in different contexts, such as in pregnancy (Admyre et al., 2007; Hedlund et al., 2009). In particular, EV from amniotic fluid were reported to modulate cytokine production by monocytic cells (Bretz et al., 2013). Also, EV from immature DC were shown to have a tolerogenic effect in allogeneic transplantations in different animal models. Importantly, this effect was correlated with an increase of $CD4^+CD25^+$ Treg cells, suggesting DC-EV capacity to regulate their activity (Yang et al., 2011; Li et al., 2012). Interestingly, EV are capable of transferring tolerance against a specific antigen, as demonstrated for EV from ovalbumin challenged mice that were then transferred to naive syngeneic mice (Ostman et al., 2005). Furthermore, EV derived from IL-10-overexpressing DC inhibited T cell proliferation in a mixed lymphocyte reaction *in vitro*, delayed the onset of collagen-induced arthritis in mice and suppressed its progression in animals where the disease was previously established (Kim et al., 2005). The same group also demonstrated that EV originated from myeloid cells ($MHCII^+CD11b^+$ EV), circulating in the blood of mice immunized with keyhole limpet hemocyanin antigen, had anti-inflammatory properties, suppressing hypersensitivity inflammatory reaction upon a second challenge with this specific antigen (Kim et al., 2007). Interestingly, EV miRNAs are known to be responsible for regulating immune cell maturation and activation (Davidson-Moncada et al., 2010). MiR-155 and miR-146a were shown to be transferred between bone marrow-DC and to be functionally active in the recipient cells (Alexander et al., 2015). These studies suggest that exosomes may be involved in inflammation modulation through influencing immunological memory, and could potentially be used to promote tolerance in new tissue engineering strategies.

In the context of tissue repair and regeneration, inflammation resolution is a key step where EV have been reported to be involved. A recent study from Saha et al. demonstrates that EV have the capacity to elicit inflammation resolution after cell exposure to a tissue injury-like scenario (Saha et al., 2016), instead of an infection-like scenario. The authors demonstrated that exposure of THP-1 monocyte cell line to alcohol promotes secretion of EV that transfer molecules, such as miR-27a, into naïve THP-1 cells, stimulating their differentiation into M2-like macrophages, which is important for inflammation resolution in the context of tissue repair (Saha et al., 2016). This work unravels important clues regarding physiological mechanisms of the body to resolve tissue