Nexus between extracellular vesicles, immunomodulation and tissue remodeling: for good or for bad?

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Introduction

Over the past three decades or so, the field of immunology has advanced hugely with profound understandings on molecular regulation of immune cells and their contribution to various biological processes. The elaborative tools, *in vitro* assays, and refined animal models have favorably anticipated the immunomodulatory (immune suppression or activation) mechanisms elicited during the course of disease progression. The projected roles of immune cells are widely attributed to inflammatory diseases, autoimmune diseases, defense against infections, repairing injuries and progression to cancer among others.

Perhaps, the most widespread explanation to activated immune responses is the pattern recognition (1), generally through surface presentation of receptors and antigen presentation prerequisite to communicate direct messages. Antigen presenting cells (APCs), such as dendritic cells (DCs), B cells, macrophages, and mast cells contribute greatly to antigen presentation through major histocompatibility complexes (MHCs) on their surface which is recognized by T cells and favor a cellular cross-talk conferring immune responses (2,3). The immune responses are not exclusively relied on direct cross-talk, however, cells could also extend their messages through secreted trophic factors, such as cytokines, growth factors, transcriptional factors and non-coding RNAs (4), through extracellular vesicles (EVs)—that all may collectively serve as paracrine messengers of cellular cross-talk (5).

EVs are nanosized membrane vesicles (including exosomes and microvesicles) secreted by virtually all cell types including APCs such as B and T lymphocytes, DCs, and mast cells. Interestingly, EVs from APCs contain MHC class I and II, as well as T-cell costimulatory molecules (6-8). EVs have been thought to play unprecedented role in functional transfer of bioactive molecules such as nucleic acids and proteins between cells (9) and enable cell-to-cell communication (10,11). Largely due to their role in intracellular communication they enable, and due to the exchange of bioactive content between cells, EVs have been implicated in the pathogenesis of variety of diseases such as neurodegenerative and cardiovascular disease, immune diseases, and cancer development.

The immunomodulatory and inflammatory roles of EVs have recently been suggested in huge body of evidence (12-15). The most profound aspect of EVs elicited in triggering immune responses or provoking pro-inflammatory responses owe greatly to the presence of MHC-I and -II complexes. This renowned evidence for the first time came from the description of EVs secreted from APCs, for their extended roles to immune responses (6,7). Interestingly, the MHC-complexes carried by DC-derived EVs were capable for the induction of antitumor immune responses in order to facilitate the eradication of tumor cells in *in vivo* mice models. Such EV-mediated extended functions of APCs, as well as role of EVs in central tolerance, and their

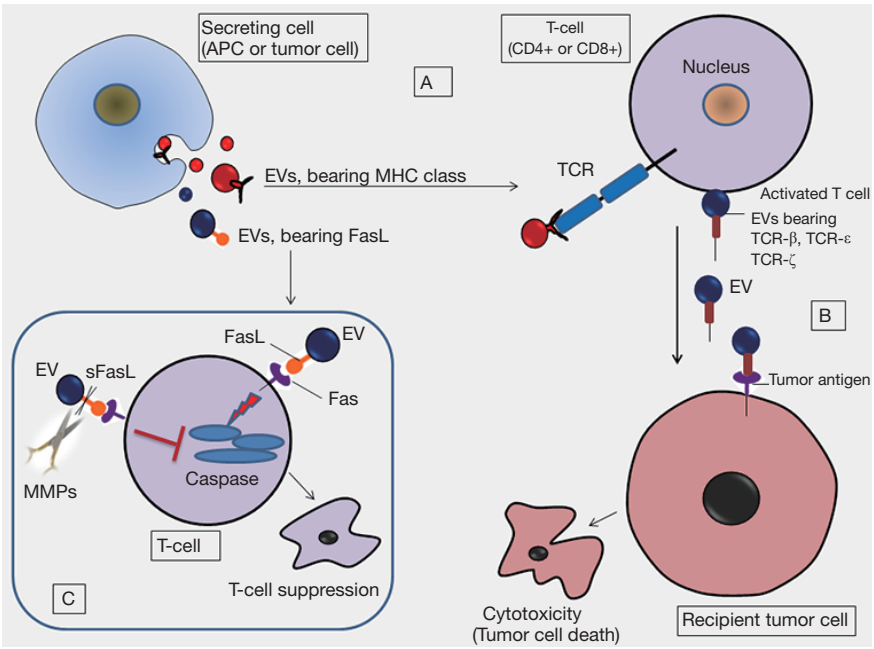

Figure 1 Extracellular vesicle mediated immune activation and immune suppression. (A) EVs containing MHC class from donor cells (either from tumor cells or from APCs) could bind with T cell receptor thereby inducing downstream signals to the T cell; (B) the activated T cells are further capable to release EVs loaded with TCR-β, TCR-ε, TCR-ζ that promote enhanced immune action and cytotoxicity in recipient cells; (C) EVs harboring FasL may have inherent ability to interact with corresponding Fas receptor present on T cells and induce signals down to T cell allowing the activation of downstream caspases which ultimately result into caspase induced T cell apoptosis. (C) Conversely, the caspases activity could be minimizing by EV-associated MMPs which convert membranous FasL into soluble FasL. This inhibits the interaction of FasL to Fas receptor, thus blocking the signals to caspases (17). EVs, extracellular vesicles; MHC, major histocompatibility complex; APC, antigen presenting cell; TCR, T cell receptor; FasL, Fas ligand; sFasL, soluble Fas ligand; MMPs, matrix metalloproteinases.

contribution to activation or suppression of the immune responses could be exploited for developing prospective immunotherapies (16,17).

The immunomodulatory features of EVs elicited in the context of regenerative processes are scarce in the literature, and are only more recently started being explored (18). Silva *et al.*, in a recent issue published in *Eur J Pharm Sci* (19), demonstrated the immunomodulatory features of EVs in the context of tissue reparative programs through their ability to participate in immune regulation and inflammation resolution. These features of EVs allow injured tissue to undergo tissue remodeling phase that is perquisite for reparative process. The current study serves as source of valuable knowledge for tissue regenerative biology; however translating this knowledge into therapeutic applications will require deeper understandings on such mechanisms. Moreover, in a certain resident tissue the detrimental effects of EVs conferred through their immunomodulatory properties making EVs good or bad, must also be determined.

This is important to consider that EVs by themselves do not show a uniform molecular pattern; instead they act as conveners and mediators of cellular responses through their cargo shipping ability. This implies that the molecular patterns contained by EVs and the cargo strictly depend on the external conditions, cell state as well as nature and type of the secreting cells which allow immunoreactive or immuno-suppressive consequences in several different ways (*Figure 1*) (17). Therefore, the immune regulatory features of EVs could be considered in both good and bad. Silva and colleagues propose that knowing the conditions linked to the production of EVs which foster inflammation resolution, could allow manipulation of the inflammatory processes to benefit tissue repair programs (19).

The authors of this study anticipate that EV-mediated transmission of damage-associated molecular patterns to the injury zone could activate certain immune cell populations thereby allowing the onset of the inflammatory

response against the injury (19). This proposition supports the idea that the activation of resident immune cells or the homing of additional immune cells at the site of injury/infection could provoke an inflammatory response, which is considered the seeding phase of tissue repair. In fact, tissue repair and regeneration develop along three major phases which are dependent on each other and include stepwise: inflammation resolve → repair → remodeling. During the course of infection or injury the exposed cells of the local tissue get activated that may induce local inflammation. These may include exposed resident epithelial cells, activated immune cells and the fibroblasts that further may promote the recruitment of circulating immune cells as well as growth factors to the site of injury. This reaction is thought to be the first phase in removing pathogens and washing out damaged cells from the injury zone.

The inflammation resolve allows reparative process to progress into the remodeling phase, which is characterized by enhanced angiogenesis facilitated by growth factors, and fibroblast activation. These processes are functionally linked with extracellular matrix (ECM) turnover and the deposition of new ECM, as well as damaged tissue cell replacement that is facilitated by cell proliferation and differentiation. Keeping in view the aforementioned three phases; authors arguably count on the fact that the inflammation resolve is a key phase in the context of tissue repair and regeneration whereby EVs are playing key role. Interestingly, the evolving roles of EVs in tissue repair and regeneration are mainly reliant on their features mimicking stem cell properties and promoting tissue's intrinsic regenerative programs within recipient cells in a paracrine manner (5). The most profound and relevant therapeutic implications in regenerative medicine that the past two decades have witnessed are those achieved through stem cell assisted tissue regeneration. In this context, stem cell-derived secreted trophic factors such as growth factors, cytokines and EVs could contribute greatly to inducing tissues intrinsic regenerative programs (5). Moreover, the tissue undergoing reparative program requires population equilibrium between cells, which could be accomplished by EV-assisted stem cell proliferation, differentiation and bi-directional communication established between injured tissues and stem cells i.e., injured cells send signals back to stem cells for producing more progenies (5). In this context, authors elaborate that EVs may influence the repopulation of regenerated tissue and functional differentiation of cells. What more can be expected on the beneficial effect of EVs, is their ability to promote angiogenesis—an integral element in healing process, which can be promoted by EV-mediated transportation of pro-angiogenic growth factors to the injury site. Of particular note, the stem cell-derived EVs such as those secreted from mesenchymal stem cells (MSCs) might have immunosuppressive role (16). In this regard Silva *et al*. envisaged such role of EVs as a major cause of inflammation resolution (19).

The third phase, in the context of repair and regeneration is the ECM turn over and tissue remodeling—that overlaps with above mentioned tissue repair phase. Authors continue to assess the tendency of EVs harboring matrix remodeling molecules which modulate the extracellular environment, as well as matrix deposition at the site of injury (19). One of the class of vesicles known as matrix vesicles have been reported previously for their selective distribution at the sites of initial calcification in cartilage, bone, and predentin and are thought to have role in mineralizing of vertebrate tissues during bone development (20). This indicates the importance of calcification and mineralization in developing matrix. Considering the fibroblasts activity in ECM environment, it is also notable that fibroblasts could be differentiated into cancer associated fibroblasts (CAFs) [reviewed elsewhere (16)]. However, despite the evidences for the involvement of EVs in ECM degradation, the relevance of their enzymatic activity in ECM turnover during tissue repair has not been fully explored.

Discrepancy of EVs in eliciting immune responses and immunotherapy

Hitherto, the concept of EV-mediated immune exploitation of target cells is extremely attractive, nevertheless several questions of such process requires critical considerations. The most important consideration would be to determine the relationship between two different aspects of immune responses such as immunosuppressive features versus immune provoking potentials of EVs which depend on several factors (*Box 1*). These discrepancies may represent EVs with variable outcomes in therapeutic perspectives. Presumably, EV-mediated overwhelming immune activation and pro-inflammatory cascades occurring at the site of injury may have undesirable and devastated effects. An example could be seen in the down-regulation of NK and B-cell proliferation by inflammatory cytokines (21). It is anticipated that a pro-inflammatory environment could not only modify the composition of EVs but also the consequent biological activities of immune effector cells, with possibility of increased risks of unpredictable effects (14).

Box 1 EV-mediated immune modulation: for good or for bad?

The nexus of EVs in stimulating immune responses largely depends on type and state of secreting cells e.g., cancer cells or immune cells, maturation state of APCs, as well as the content of EVs
Presumably, EVs may educate immune cells to be recruited at injured niche and further stimulation of local immune cells. This increased number of immune cells will foster local inflammation
EVs secreted from APCs or immune cells could stimulate the secretion of inflammatory or anti-inflammatory cytokines that may have opposite roles to inflammation resolve
EVs bearing (matrix metalloproteinases) MMPs may have a role in matrix remodeling that may either favor the tissue repair or cancer metastasis. Similarly, EV-mediated activation of fibroblasts and epithelial or endothelial cells may either have role in repairing tissue or may differentiate into cancer associated fibroblasts that may initial cancer instead of repair
EV-assisted recruitment of pro-angiogenic growth factors may have role for angiogenesis required for healing process, may also favor tumor angiogenesis
Activated T-cells may secrete EVs, bearing TCR-β, TCR-ε, and TCR-ζ that may enhance activities of NK cells or T- cell effectors; can foster cytotoxicity to kill cancer cells (*Figure 1*). If similar mechanism may apply to cytotoxicity at injured cites it may reflect adverse effects of EVs
EVs could inhibit and impair the maturation of B and T lymphocytes or natural killer (NK) cells: for this cancer cells use EVs to inhibit or suppress immune cells and evade immune surveillance
Apoptosis of T-cells: for their survival, the cancer cells secrete EVs with apoptotic molecules (such as those bearing FasL) which stimulate intrinsic or extrinsic apoptosis cascades in T-cells (*Figure 1*)
Cancer cell-derive EVs may have role in suppression or down regulation of T cell receptors (TCRs): this inhibits the recognition of MHCs by T cells
EVs stimulate the secretion of cytokines that may suppress or activate immune response presumably conferring different roles in tumor environment as compared to injured tissue environment
Collectively, these observations indicative that EV-mediated modulation of cellular responses could be considered both good and bad

APC, antigen presenting cell; EV, extracellular vesicle; MMP, matrix metalloproteinase; MHC, major histocompatibility complex.

Tissue regeneration therapy generally requires an immunosuppressive environment, in particular during organ implants; whereas the cancer immunotherapy largely relies on evoking the host immune system to fight against cancer cells (16). This reflects that for the purpose of repair programs the EV-mediated immune responses will need to be manipulated differently from those manipulated for the purpose of tumor eradication. The immunosuppressive tumor microenvironment is considered a major barrier to the effectiveness of anti-tumor immune activities, since it offers lower immunogenicity of immune cells against the cancer cells. However, growing literature on EVs functional roles continue to provide us with new insights in understanding such discrepancies.

Other therapeutic applications of EVs

In parallel to other beneficial effects resulted from transport of bioactive molecules and intercellular communication—EVs could also be applied as drug delivery vehicles. This is largely due to their natural tendency to transport biological molecules as well as their biocompatibility with the target cells. In the context of drug delivery vehicles, a relatively different but potentiating proposition of EVs—is their pharmacokinetics and pharmacodynamics (19), which could be tailored for pharmaceutical purposes in *in vivo* animal studies.

However, there remain several potent issues to be solved. For instance, loading efficacy and stability of a certain drug is a major concern, as has been observed with other delivery vectors. The additional consideration is the specific targeting issues, since EVs having surface chemistries compatible with cell receptors, could interact with unpredicted cells/tissues that may give undesired results/effects. Moreover, donor cell derived EV-cargo could provoke immune responses in recipient cells with a possibility to confer cross-reactivity.

Considering these facts, one of the important aspect

of the description by Silva and colleagues could be *in vivo* administration of EVs, biodistribution and the delivery of EV-cargo to targeted destinations (19). However, the targeted uptake and internalization of EVs by proposed target recipient cells remains an impeding question. Some of the strong clues provided by Hoshino *et al.*, offer interesting information with the arguments that EVs could seek target organs through different forms of surface integrin's presented on their surface (22). This knowledge could guide researchers for *in vivo* delivery of EV-loaded drugs, however, further studies will warrant translating this knowledge into targeted and organ guided drug delivery.

From bench to bedside

Pertaining to therapeutic applications in the context of tissue regeneration—the feasibility of EV-based therapies have not been eventuated in clinical trials (19). However, there is initial evidence for applying EVs to tissue healing process in an individual patient case. Kordelas *et al.* showed that MSC-derived EVs are well tolerated in patients during the treatment of graft-versus-host disease (GVHD) (23). Moreover, MSC-derived EVs treatment significantly reduced the pro-inflammatory cytokine response in patients' peripheral blood mononuclear cells (PBMCs) *in vitro*, as well as the clinical symptoms of GVHD were improved significantly shortly after the start of MSC-derived EV therapy to the patient (23). It was proposed that the donor derived EVs could recapitulate the immunomodulatory properties of MSCs. Therefore, the applications of immunosuppressive EVs could be of great therapeutic value for future clinical consideration due to the fact that such EVs are well tolerated in patients.

Despite improvements, both in the clinical procedures intended for tissue repairs, organ transplantation and cell-based therapies over the last decade, current methods present potential complications (for example, an increased risk of infection, toxicity, and graft rejection). In this context, compared with traditional stem cell therapies, EV-based cell-free therapies may improve patients' outcomes considerably with reduced complications as compared to cell-based therapy (16). However, stem cell-based therapies also need to consider potent risk factors and technical complications such as: culture-induced senescence, genetic instability, loss of functional properties, immune-mediated rejection, and the risk of transformation of resident cells into malignant phenotypes which presumably limit the applications of stem cells in tissue regeneration (5). Therefore, steering traditional stem cell-based therapy toward EV-based therapy is still a debated issue. In this regard, this could be of interest to applying combination of EV-based therapies with existing approaches in order to improve the therapeutic benefits.

Parallel to clinical trials on tissue regeneration, the evaluation of EVs for clinical trials in other human diseases is also very limited (24,25). However, this interesting to consider that in spite of very small number of patients included in these clinical trials, yet the potential of EVs for their prospective translation from bench to bedside is thought be promising. However, several technical hurdles still require an explicit attention. A potential challenge in the field exists largely due to the limitations of standardizing the existing technologies. In particular, standard protocols for EV isolation, purification and characterization are still a debated issue (9,26). It has been argued that the development of high-throughput approaches and robust capture platforms will warrant the implications of EVs in routine biomarker development, and therapeutic implications with a proposed workflow sheet to applying for USA food and drug administration (FDA) approval (27). Since there is intensive interest in the field both in basic research as well as therapeutic point of view—it is anticipated that in the next decade, EVs arena will see significant advances in clinical pipelines.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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