Manipulating EVs for Therapeutic Applications

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Extracellular vesicles (EVs) receive special attention from clinicians due to their assumed usefulness as prognostic markers, immune modulators and physiological delivery tools. The latter application, that supports the reduction of side effects of treatment, is still fraught with many challenges, including established methods for loading EVs with selected cargo and directing them towards target cells. EVs could be loaded with selected cargo either in vitro using several physicochemical techniques, or in vivo by modification of parental cell. Otherwise, EVs may be passively supplemented with selected cargo, such as miRNAs or siRNA. Furthermore, recent findings imply that antigen-specific antibody light chains could coat the surface of EVs to increase the specificity of cell targeting. In addition, the route of EVs' administration also determines their bioavailability and eventually induced therapeutic effect.

Keywords: anti-tumor immune response ; anti-tumor therapy ; drug delivery ; extracellular vesicles ; exosomes ; immune regulation

1. Introduction

Cells of multicellular organisms can communicate with one another through the release of extracellular vesicles (EVs). They are present in virtually all body fluids and can freely circulate between tissues and organs, even crossing the barriers. Thus, EVs mediate intercellular signaling in an auto-, para-, and endocrine manner and induce the eventual biological effect in acceptor cell by receptor–ligand interactions and by delivery of regulatory cargo. In this regard, EVs provide a platform for an exchange of cell-derived constituents, including nucleic acids, proteins, lipids, and metabolites, which physiologically contributes to maintenance of homeostasis. It is worth noting that EVs are multidirectional immune modulators. Accordingly, recent report revealed that the same EV subtype that target antigen-presenting cells can regulate both humoral and cell-mediated immune responses [1].

Consequently, dysregulation of EV-mediated communication drives pathological processes. Simultaneously, substantial changes in cellular origin, composition, and function of EVs are observed under the pathological conditions. This fact made EVs a promising candidate for using as biomarkers of various disorders, including carcinogenesis. Furthermore, growing awareness of factors determining EVs' biogenesis and their eventual function greatly supports the attempts to manipulate EVs for therapeutic application as vaccines and delivery tools. However, inclusion of EV-based therapeutics in clinical practice still requires extensive studies and solving many problems.

This review summarizes the current research findings and discusses the future perspectives in manipulating EVs for further usage as a delivery tool, with the special focus on anti-cancer therapy.

2. Proposed Clinical Applications of EVs in Oncology

Recently, EVs have received special attention from oncologists both due to their physiological capability to deliver signaling molecules and their involvement in carcinogenesis. In addition, EVs play an important role in regulation of innate and adaptive immunity, including anti-tumor responses. Thus, EVs are considered promising tools for diagnostic and therapeutic purposes [2,3,4]. So far, studies on the clinical applicability of EVs proposed their usefulness as prognostic biomarkers, vaccines to induce anti-tumor immune responses, and drug delivery tools [5]. However, due to the limited knowledge on complex EVs' biogenesis and cargo sorting, clinical applications of EVs are still fraught with many challenges.

3. Perspectives in Manipulating EVs for Therapeutic Applications

EV-based delivery systems are promising candidates to significantly improve the efficacy of cancer therapies. However, their future clinical application requires standardization of the following aspects: (i) EVs' generation and isolation methods,

(ii) techniques for selective cargo loading, (iii) strategies for specific cell targeting, and (iv) protocols of their administration, including doses, routes, and timing.

3.1. EVs' Isolation Methods

Methods most commonly used for isolation of EVs have recently been comprehensively reviewed and discussed in the terms of their impact on biological function of EVs [34]. However, there is still a long way to develop a unified protocol for the isolation of a particular EV subtype from a particular biological material. One of the promising approaches to isolate EVs from any biological fluid is based upon size-exclusion chromatography that provides high purity of EV isolate [35,36,37].

In multicellular organisms, EVs are a part of physiological communication system. Therefore, they seem to be much more biocompatible than artificially produced vesicles and liposomes. However, EV populations isolated from body fluids by ultracentrifugation are largely heterogeneous. This prompted researchers to develop the procedures of in vitro stimulation of cultured cells to release EVs. However, cell-released EVs from ultracentrifuged supernatants were found to be heterogeneous as well [38]. Thus, EVs' isolation from both body fluids and cell culture supernatants requires much more precise methods for separation of a particular EV subtype. Those include magnetic bead-based immunoaffinity method [39] and antigen affinity chromatography [40]. By using specific antibodies, both methods should allow the isolation of EVs expressing particular antigen of choice. Otherwise, the use of antigen-coated beads or polymers for chromatography separates EVs that are able to bind the antigen, for example, due to the surface expression of antigen-specific antibody light chains (LCs) [40]. However, the process of EVs' elution from chromatographic columns or their detaching from the magnetic beads may influence their physical and chemical properties and, consequently, their biological activities. Thus, this aspect requires further investigation. Accordingly, our observations suggest that chromatographically-separated EVs eluted with acidic guanidine from column filled with Sepharose linked with either trinitrophenol (TNP) hapten [40], casein hydrolysate [41], or anti-CD9 monoclonal antibodies [41,42] preserved their biological activity.

3.2. Approaches to Loading EVs with Selected Cargo

One can assume that EV-contained cargo is a main determinant of the therapeutic efficacy of their application. As mention above, EVs may carry a great variety of biologically active molecules, including RNAs, proteins and lipids, which makes them a conveyor of virtually unlimited types of cargos. The cargo can be packed into EVs during their intracellular biogenesis or extracellularly after EVs' exocytosis. The latter generally happens in in vitro conditions [43], but some results suggest the possibility of loading of freely circulating RNAs into EVs also in vivo [44]. Accordingly, the methods for loading EVs with selected molecules can be classified into two main groups, i.e., those based on modification of parental cells and those adapting physicochemical techniques enabling in vitro loading [45].

Parental cells can be passively loaded with chosen molecules. Along these lines, human gingival mesenchymal stromal cells were shown to uptake the chemotherapeutic drugs during standard cell culture [46]. Furthermore, one of these drugs, namely paclitaxel, was then found in cell-secreted EVs that expressed anti-cancer activity in vitro [47]. Similar activity was observed in the case of paclitaxel-carrying EVs released by mouse mesenchymal stromal cells [48]. In addition, mouse and human tumor cell lines were also shown to release drug-containing EVs after simple culturing in the presence of different chemotherapeutics [49]. In such cases, one can speculate that the drug is passively packaged into EVs during their formation. On the other hand, EV-parental cells can be transfected or transduced by non-viral or viral vectors, respectively, to produce the encoded molecules. These would likely be then actively sorted into EVs during their biogenesis. Accordingly, parental cells transfected with different plasmids were shown to secrete EVs that contained the plasmid-encoded products, including antibody protein and mRNA for enzyme that activates the chemotherapeutic prodrug. As a result, EVs were able to deliver mRNA to the cells of HER2-positive human breast tumor xenografts in a targeted manner due to the surface-expressed anti-HER2 antibody, which inhibited the growth of the xenografts in mice [50]. Interestingly, the later results suggested that EVs may deliver in vitro transcribed enzyme-encoding mRNA, which allows to eliminate the potentially harmful plasmid transfection of EV-parental cells [51]. Another interesting possibility was proposed by Sancho-Albero et al. [31]. The authors reported that hollow gold nanoparticles incubated with EV-parental cells are much more efficiently incorporated into EVs after their PEGylation. Delivery by EVs may greatly improve the accumulation of PEGylated gold nanoparticles in tumors [31]. However, many variables have to be taken into account while using these strategies. Therefore, at present, much more commonly used strategies are based on loading EVs with selected cargo after their isolation [45]. Maintaining the EVs' integrity, allowing to protect the incorporated cargo from extracellular degradation or inactivation, is one of the features that should be considered while choosing the loading method.

3.3. Directing EVs towards Desired Target

Loading EVs with selected cargo seems to be crucial for induction of expected biological effect. However, directing EVs towards desired target cells is likely the most important step to achieve the highest efficacy of EV-mediated therapeutic effect. Directed targeting greatly increases the dose of EVs that reach the desired cells and tissues and, simultaneously, limits the unwanted engulfment of EVs by other cells, including phagocytes.

Currently, some researchers attempt to genetically modify the parental cells to facilitate the selective tissue targeting by derived EVs. Along these lines, EVs generated by engineered immature DCs expressed membrane protein (Lamp2b) that was fused to αv integrin-specific iRGD peptide, which mediates tumor homing [95]. Otherwise, EV-parental cells were transfected with plasmid containing cDNA sequence for anti-HER2 antibody single-chain variable fragment (scFv) of ML39 clone, which allowed generation of EVs that expressed the antibody. After in vivo administration into mice with implanted HER2-positive breast tumor, these directed EVs, additionally loaded with mRNA for enzyme that activates chemotherapeutic prodrug, were found most effective [50]. In another study, AS1411 DNA aptamer that binds to nucleolin abundantly expressed on breast cancer cells was used as tumor targeting ligand. Its conjugation to cholesterol in EV membrane ensured selective, tumor cell-targeted EV action [96]. Future perspectives in cell targeting may be based on interactions between receptors and ligands as well as on specific binding of antigen by antibodies.

3.4. Selecting the Optimal Route of EVs' Administration

Depending on the route of administration, antigens may be either immunogenic or tolerogenic [118]. Analogously, one can speculate that the route of EVs' administration may either increase or diminish their eventual effect. Furthermore, it also determines the biodistribution and bioavailability of EVs as well as may facilitate their barrier-crossing ability. Thus, delivery route is one of the essential factors determining the overall efficiency of EVs' therapeutic activity [119]. On the other hand, route of therapeutic EVs' administration should be accepted by patients.

So far, various routes of EVs' administration have been experimentally examined. Some showed that intravenous route is more efficient than intraperitoneal injection [120], and that intradermal application has an advantage over subcutaneous treatment [121]. Interestingly, intravenously infused EVs were shown to co-localize with microglia in injured spinal cord of contused rats [122]. Furthermore, intranasally administered EVs can be incorporated by neurons and microglia [123]. Moreover, orally administered EVs from bovine milk were found to ameliorate arthritis in mice [124]. Similarly, we have observed that EVs released by suppressor T cells from mice tolerized to casein, suppress casein-induced delayed-type hypersensitivity response after administration via intravenous, intraperitoneal, intradermal and oral routes into actively immunized mice [41]. Several other studies also suggested the functional activity of EVs delivered via oral route [125,126]. Therefore, oral route of treatment seems to be promising approach, firstly due to its accessibility and well acceptance by patients, and secondly, as it is amenable for repetitions. However, EVs' formulations and dosing protocols for oral treatment must be well established to avoid variability in therapeutic efficacy.

4. Conclusions

The goal of this review was to comprehensively discuss the knowledge on currently available methods as well as future perspectives in manipulating EVs for therapeutic applications with a special emphasis on cancer treatment. EVs' biology and their clinical applications are tremendously complex research areas. We hope that this review provides some useful insights for possible strategies and innovation in EVs' applications, while being aware of the virtually inexhaustible nature of the undertaken topic [127]. Extracellular vesicle research can be compared to exploring a newly discovered cave. The deeper you enter the cave, the more new side corridors you will find for exploration.