Recognition of Exosomes and Their Role in New Technologies

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Abstract: Exosomes or, more broadly, small extracellular vesicles are produced by all cells. They contain an array of biologically active molecules by which exosomes can influence the extracellular environment and affect the properties of other cells. Recognition of their biological role has come a long way from the mere function in the disposal of cellular waste to a concept of universal intercellular vehicle mediating near and long-distance communication in normal and pathological states. As a result, in recent years exosomes have gained much interest in their potential exploitation for therapeutic use. This short review is aimed at presenting a brief exploration of the history of exosome recognition coupled with a snapshot of newly developing exosome-based technologies, touching upon some recent achievements and examples of application.

Keywords: clinical trials; exosome therapy; exosomes; extracellular vesicles; exosome biomarkers

Introduction

Exosomes are a group of small heterologous extracellular vesicles of 30-200 nm in diameter generated by most (if not all) eukaryotic and procaryotic cells. Some authors refer to exosomes as vesicles of denoted size created by the budding of both plasma and endosome membranes[1], while others attribute the name only to the endosome-derived products[2]. In the context of a specific study, it would seem preferable to use stricter terminology in order to clarify the applicability of the findings. However, the nomenclature of extracellular vesicles is still in the state of development, and it was even suggested to use the broader term "small extracellular vesicles" until a definitive consensus on specific biological markers of vesicle groups will be established[3]. Bearing this in mind, the name "exosome" will be used in this review for simplicity and in order to keep consistency with the cited literature. The first part of the review will provide a brief timeline citing major milestones in exosome research. This will be followed by an overview of aspired technological applications of exosomes illustrated by relevant examples.

History of recognition

The term "exosome" (not to be confused with the "exosome complex", a multi-protein structure involved in RNA degradation[4]) was originally suggested in a study of vesicle-associated ecto-enzymes, introducing the concept of vesicle shedding as a biologically meaningful process[5]. Most authors refer to the early 1980s as the initiation stage of exosome research citing the selective shedding of membrane proteins as the first recognized function of exosomes. This is based on studies of transferrin receptor elimination during reticulocyte maturation[6,7], which, indeed, accommodated the term exosome in its current use. However, upon careful analysis descriptions of small vesicles secreted by cells can be found starting at least from the late 1960s, providing early initially unrecognized indications of biological activities other than waste disposal, such as involvement in mineralization, thrombogenesis, and sperm production1.

For almost 30 years (counting from the appearance of 1980s studies) the field developed slowly with exosomes being regarded as no more than a discarded material. A significant change in understanding of exosome biology occurred upon the elucidation of their regulatory role in the immune system. In 1996 Raposo and co-authors published data on the ability of lymphocyte-derived exosomes to carry major histocompatibility (MHC) class II complex and induce T-cell responses

in vivo[8]. In the late 1990s-early 2000s, more data on exosome production by various cells started to accumulate. Studies of dendritic cells-derived exosomes not only confirmed their ability to induce an antigen-depended immune response but also produced detailed analyses of exosomal protein composition delivering evidence of their specific molecular features[9–11].

More than a decade after the discovery of their role in immunity, in 2007, the group of Professor Lötvall demonstrated that exosomes can transport mRNA and microRNA[12]. The idea that RNA molecules can be delivered via an exosomal route to a new cell and exert their biological activity was groundbreaking and resulted in a tremendous growth of scientific and technology-related interest. Over the following years, research has revealed the multiplicity of mechanisms by which exosomes can regulate biological functions, participating in the conditioning of the extracellular milieu, signal transmission, and cargo transportation (reviewed in [13]). The exceedingly fast growth of high-profile journal publications followed by the initiation of the first wave of related clinical trials (Figure 1) indicates that the field of exosome research has achieved a wide interest stretching from basic science to technological developments.

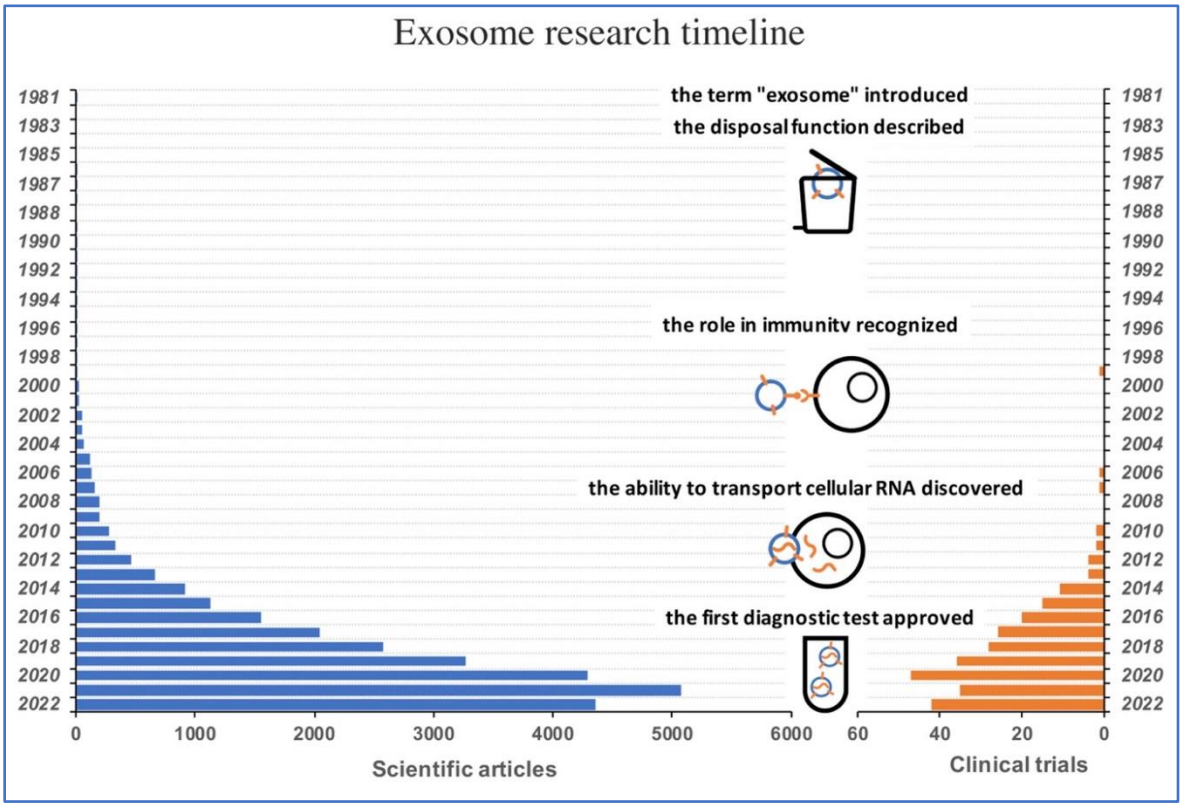

Figure 1 Timeline of progress in exosome research: number of scientific articles (blue) and clinical trials (orange), which include the term "exosome", distributed by year from January 1981 to October 2022. The articles are sorted by the date of publication, clinical trials - by the starting date.
Data sources: https://pubmed.ncbi.nlm.nih.gov/ and https://beta.clinicaltrials.gov/

Application approaches

It is now established that exosomes are associated with a broad spectrum of biological processes. They take part in healthy functions – such as immune modulation, tissue growth and regeneration, inflammation, antiviral activity, and reproduction – and in pathological states – including cancer progression, neuropathology, immune escape and propagation of pathogens, diabetes, obesity, cardiovascular diseases, and other[1,13]. This diversity underlines the universal role of exosomes as intercellular messengers, which relies on their ability to transport multiple cargoes. The basic properties of exosomes – enrichment in components for targeting the recipient cell and retained features of the source cell – produced two perspective paths for exosome exploitation in biotechnology and

medicine: as therapeutic formulations and as carriers of diagnostic markers.

Exosome therapy and exoengineering

Exosomes were found to shuttle diverse cargo, including proteins, lipids, nucleic acids, and metabolites[13,14]. This suggests not only the prospect of using exosomes as multicomponent regulators of biological processes but also as potential delivery systems. Representing a naturally occurring communication route exosomes are likely to have better biocompatibility and exhibit fewer side effects than synthetic delivery formulations. Similarly, exosomes may possess higher bioavailability and consequently give more promise in accommodating different administration routes[15,16]. Exosomes have the intrinsic ability to pass the blood-brain barrier[17,18] which alleviates a major hurdle in the development of treatments for neuropathological conditions. In spite of playing a distinctive role in immune modulation exosomes do not seem to induce severe immune reactions when introduced systemically[19]. Taken together these characteristics present exosomes as a uniquely versatile platform suitable for engineering a new class of therapeutics.

Studies focusing on therapeutic applications are using exosomes in their naïve state (upon naturally occurring production in cell culture or purification from biological liquids), or as "engineered" products that are modified to express specific functions and/or loaded with therapeutic agents. Among naïve exosomes, mesenchymal stem cell-derived vesicles received attention due to their immunomodulating and regenerative effects. Some of these studies have already been transposed into human clinical trials, testing exosome potential in diabetes and chronic kidney disease[20]. Similarly, the availability of MHC-peptide complexes and immunostimulatory proteins in dendritic cell-derived exosomes led to their recruitment as anti-cancer vaccines[21]. New commercially developed products employing naïve exosomes have entered the clinical trial stage for the treatment of various skin conditions. Aegle (http://www.aegletherapeutics.com/), has announced two clinical trials aimed at the analysis of allogeneic mesenchymal stem cells-derived exosomes in the treatment of severe burn patients and management of pathological inherited skin condition (epidermolysis bullosa). ExoPharm (https://exopharm.com/) is using its platelet-derived exosomes for wound healing. Aside from the direct therapeutic application, there are other opportunities for naïve (and, in perspective, engineered) exosomes. One such possibility is the exosome-conditioning of bioartificial products. The construction of encapsulated pancreatic islet grafts as an insulin delivery platform is explored for the management of Type 1 diabetes patients, and exosomes may play a valuable role in the improvement of these bioartificial constructs[22].

While naïve exosomes rely on their intrinsic properties determined mostly by the source cells, specific features can also be selectively accommodated by pre- and post-release engineering procedures. Several approaches have been tested, including genetic modification of exosome-associated proteins in the producing cells, the direct introduction of functional moieties, and the loading of exosomes with a therapeutic cargo. These modifications extend the field of exosome-derived drugs into the area of targeted and highly selective nano-medicines.

Directed modification of exosome composition can be achieved by virtue of manipulating the producing cell. This process mainly relies on the knowledge of exosome-specific proteome – i.e. proteins that are enriched on or within the vesicles. These include tetraspanins, ESCRT-related proteins, lysosome-associated membrane proteins (LAMP), MHC, members of EWI immunoglobulin superfamily (namely, PTGRFN), MARCKS protein family (MARCKS, MARKCSL1, and BASP1), and others[23–26]. Transfection of the source cells with genetic constructs enables the expression of functional peptides as a fusion with proteins normally present in exosomes[16,27]. This universal strategy has allowed the introduction of various features permitting specific targeting, surface display, protection, and selective cargo loading.

Exosome engineering for tissue targeting relies on the positioning of peptides with known tropism on their surface. For example, expression of rabies virus glycoprotein, selectively recognized by receptors in the brain, in fusion with exosome-associated LAMP 2B resulted in the delivery of exosome-transported siRNA to the designated tissues and subsequent suppression of BACE1 – a therapeutic target in Alzheimer's disease17. On the other hand, the expression of a regulatory ligand can prevent undesirable exosome interactions. The latter was nicely illustrated by CD47-mediated "don't eat me" signal, which upon incorporation

into exosomes allowed evasion from phagocytic degradation[28]. This example also demonstrates that the enrichment of exosomes with ligands can be used in signaling.

Expression of cytokines on exosome surface can serve for targeting corresponding signaling pathways. This principle was employed in model studies of inflammation and as anti-cancer treatment[29,30]. It is currently being tested in a clinical trial (Phase I) for T-cell lymphoma therapy with commercial exosome product exoIL-12 from Codiak Biosciences (https://www.codiakbio.com/), which is designed to display PTGFRN-scaffolded cytokine IL-12.

The engineering of exosome proteins was also exploited for intraluminal loading. For example, fusion to an N-terminal fragment of exosome BASP1 enabled luminal incorporation of broad classes of proteins of different sizes and complexities, including the RNA-binding MS2 bacteriophage major coat protein, chicken ovalbumin, and enzyme Cas924. RNA-guided DNA nuclease Cas9 is widely used for direct genome editing[31,32] and has a tremendous therapeutic potential that could be further unraveled with exosome-guided delivery. In its turn, exosome modification with bacteriophage MS2 protein represents an example of an elegant approach to endogenous loading of therapeutic RNAs. While exosomes show the intrinsic ability to transfer RNA molecules from the producing cell, selective incorporation is desirable and especially relevant for larger mRNAs[33].

Apart from protein and RNA shuttling exosomes have been explored for delivery of oligonucleotides, plasmid DNA, chemotherapeutic drugs, fluorescent probes, nanoparticles, small-molecule agonists, and others, using a variety of physical and chemical loading methods[16,34–37]. In addition to single macromolecules or their complexes application of exosomes has also found promise in incorporating whole viral particles. Elaboration of efficient and safe delivery methods for adeno-associated viruses (AAV) is of interest due to their wide use in gene therapy. Combining AAV with exosomes may overcome several important limitations, in particular – to surpass immunogenicity and achieve conditions for multiple low-dose administrations – and it has already shown promise in animal model studies. For example, exosome-incorporated AAV was found to be well tolerated and demonstrated more efficient delivery of transgene compared to conventional AAV allowing the partial rescue of hearing in a mouse model of human deafness[38].

Listed above are only a few examples of the diversification modality of exosome-based drugs. In addition to genetic engineering and functional cargo loading, exosomes can be altered by other methods, such as chemical modifications or liposome fusion[16,34,39,40]. The diversity of exosome properties is also dependent on the source of production. While most studies recruit allogenic or allogeneic vesicles from donor material or adopt existing mammalian cell cultures, an interesting deviation in exosome manufacturing is the employment of agricultural products (plant-derived or "food exosomes"). Mainly aimed at overcoming the issues of limited production as economically practical sources these are also envisaged to have a promising safety profile, being commonly ingested in everyday life[16,41,42]. Ongoing clinical trials include drug-loaded plant-derived exosomes tested in cancer, inflammation, and prevention of oral mucositis[43,44].

Exosome diagnostics

Exosomes are present in all biological fluids and, as a consequence, can be found in many routinely collected medicinal samples[45]. There is growing evidence that exosome composition reflects the state of the source cell and, in the case of pathology, it includes proteins and nucleic acids associated with the disease[13,46]. Protected and selectively enriched in the exosome carrier these potential biomarkers are likely to provide a more stable and reliable source of information compared to those in free circulation. Characteristic proteins present on the exosome surface can be used for further enriched sampling by virtue of immune capture methods[13]. On top of that, exosomes are sufficiently stable and can be lyophilized and frozen for up to 2 years of storage[47,48]. These prerequisites make the idea of exosome-based liquid biopsy, allowing multifactorial testing with minimal invasiveness and repetitive sampling, extremely attractive.

In recent years exosomes were under intensive investigation for use in disease diagnosis. They were found to contain proteins and nucleic acids associated with cancer, liver, kidney, neurodegenerative, infectious, and metabolic diseases, and about half of the exosome-related clinical trials belong to biomarker applications[49].

The world`s first exosome test ExoDx™ Prostate IntelliScore EPI CE-IVD developed by ExosomeDx

became available in 2016 and has since covered more than 60 million patients in the U.S. (https://www.exosomedx.com/europe/physicians/exodx-prostate-test). This non-invasive at-home collection urine test helps assess the risk of prostate cancer thus allowing to avoid unnecessary biopsies in more than 30% of cases. Other companies are also actively involving in the creation of pipeline systems and instruments for exosome-based diagnostic tests in diabetes, Alzheimer's disease, and various cancers.

Conclusions

Scientific understanding of exosomes went a long way from the position of cellular waste to the concept of extracellular organelles heavily involved in a multiplicity of normal and pathological functions. However, once this shift of paradigm was established, the exosome field met an extreme level of enthusiasm and an ongoing influx of new developments. This is clearly illustrated by the surging numbers of scientific publications, clinical trials, and dedicated biotechnological companies. The potential implementation of exosomes in liquid biopsy, precision medicine, and regenerative treatment is the subject of more than 200 clinical trials (according to https://beta.clinicaltrials.gov/). As with any other quickly evolving field, exosome-derived technology faces many challenges, especially in scaling, standardization, and safety control of exosome manufacturing and its compliance with good manufacturing practices[43,50]. An increasing body of scientific evidence indicates tremendous heterogenicity of exosomes as a group[51], and a major part of the biology and the functional essence of this diversity remains to be investigated. With multiple small and big companies all over the world jumping into the exosome innovation studies, and startups emerging to specifically target exosomes for medical use the technology is rushing forward, sometimes preceding the science. This is not at all unusual nor unreasonable, and the history of medicine can provide examples of efficient treatments emerging before the detailed elucidation of underlying mechanisms. Yet, it is clear that an actual understanding of exosome biology would promote the arising technologies to a completely new level both in terms of efficiency and biosafety. While these innovations stand at a very early stage of development and yet need to demonstrate efficiency in advanced clinical trials, the overall promise of the field is tremendous and, hence, encouraging.

Abbreviations

AAV, adeno-associated virus; MHC, major histocompatibility complex; RNA, ribonucleic acid; mRNA, messenger RNA; miRNA, microRNA; siRNA, small interfering RNA

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