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Review Article

A NOVEL CLASS OF GENE DELIVERY SYSTEMS: EXOSOMES

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ABSTRACT

Biological therapeutics, including short interfering RNA and recombinant proteins, are prone to degradation, have limited ability to cross biological membranes, and may elicit immune responses. Therefore, delivery systems for such drugs are under intensive investigation. Exploiting extracellular vesicles as carriers for biological therapeutics is a promising strategy to overcome these issues and to achieve efficient delivery to the cytosol of target cells. Exosomes are biological membrane vesicles measuring 30 to 100 nm. They contain an abundance of small molecules like tetraspanins, receptors for targeting and adhesion, lipids, and RNA. They are secreted by most biological cells, and are involved in a plethora of physiological functions including, but not limited to, transport of genetic material, modulation of the immune system, and cell-to-cell communication. Due to their viral-like transfection efficiency and inherent biological function, compelling evidence indicates that exosomes can be used as novel delivery platforms for gene therapy. This review provides insights into the composition and functional properties of exosomes, and focuses on therapeutic, diagnostic and gene delivery potential of exosomes.

Keywords: Exosomes, RNA, Gene therapy, extracellular vesicles.

INTRODUCTION

Cells are well known to communicate via soluble mediators or cell-cell contact, but in recent decades, intercellular communication through extracellular vesicles has also increasingly gained attention. The first notion of such vesicles arose when Wolf described the formation of “platelet dust” upon storage of blood platelets[1]. These phospholipid-rich particles were shown to exert coagulant activity and were later determined to be actively shed membrane-derived vesicles[2]. Since then our knowledge about such vesicles has expanded dramatically, and vesicle secretion is now widely accepted to occur in most, if not all, cell types. Characterization studies identified three main populations of extracellular vesicles, which are commonly classified based on their intracellular origin. Cells that undergo apoptosis fractionate their cellular content into subcellular apoptotic bodies in order to prevent leakage of possibly toxic or immunogenic cellular contents into the extracellular matrix[3]. Apoptotic bodies appear as a heterogeneous group of vesicles, with sizes ranging from 50 nm to 5 µm and a buoyant density of 1.16-1.28 g/mL[4-7]. They contain a variety of cellular contents, including DNA, RNA, and histones, and display “eat-me” signaling molecules, causing them to be rapidly cleared by macrophages[8, 9]. Due to their specific cellular content and high density, they may be distinguished from two other major vesicle populations, which show considerably more overlap. One of these populations originates from budding and fission from the plasma membrane into the extracellular space and contains vesicles of about 50-1000 nm in size. Such vesicles are interchangeably referred to as microvesicles[10], ectosomes[11], shedding vesicles[12], icroparticles[13,14], plasma membrane-derived vesicles[15] or even exovesicles[16].

A microvesicles are supposedly generated by budding from the plasma membrane, exosomes appear to be formed by tightly controlled inward budding into large multi vesicular bodies in the cytosol. These multi vesicular bodies are able to fuse with the plasma membrane, causing the release of exosomes into the extracellular space. In theory, exosomes and microvesicles are clearly distinguishable by their origin, but in practice such a distinction is seldom possible [17]. Exosomes are bio-nanoparticles, measuring 30 to 100 nm in diameter, and are secreted by most biological cells[18]. They are responsible for a plethora of biological functions, including but not limited to, cell-to-cell communication, signal transduction, transport of genetic materials, and modulation of immune responses[19]. Alluding to their nanometer size and inherent biological role[20], compelling evidence indicates that exosomes can be utilized as a novel nanoscale delivery platform for gene therapy[21], as well as a diagnostic tool for biomarkers of disease[22]. This dual functionality of therapeutics and diagnostics is termed “theranostics”, and is a new and emerging field of nanomedicine[23]. Gene therapy is a technique by which an abnormal gene is replaced with a normal one to correct the disease manifestation, and to restore biological function. However, the field of gene therapy is still in its infancy, and it has been fraught with setbacks in the past[24]. At the time of writing, the US Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale[25]. Interestingly, China has sought to lead the way by approving the world's first gene therapy-based drug, Gendicine™ (a recombinant adenovirus) in 2004 for use in cancer patients [26], with moderate success[27].

Exosomes

Exosomes are nanoscale membrane vesicles first described in the 1980s[28], but the word “exosome” was (confusingly and rather unfortunately) re-used in 1997 to refer to exoribonuclease complexes in RNA processing [29]. Exosomes are secreted by cells from multivesicular endosomes, and transport various biological molecules, ranging from membrane receptors, proteins, to mRNA and (microRNA) miRNA for maintenance of biological homeostasis[30], as well as epigenetic reprogramming[31]. Exosomes are constitutively generated (a process which is not calcium triggered) from late endosomes, which subsequently form multivesicular bodies, and it has been found that this mechanism is ceramide-dependent[32]. Upon release, exosomes can transport materials to neighboring cells via clathrin mediated endocytosis[33]. However, there is also evidence to suggest that the uptake of exosome relies on specific surface molecules on the exosome itself, as well as the presence of specific receptors on the recipient cell membrane[34]. Due to the similarity in size and composition of retroviruses and exosomes, it has been postulated that exosomes can serve as the ultimate “Trojan horse” for delivering small molecules into cells (similar to how viruses infect cells)[35], with a potential for being gene delivery vehicles.

Functions of exosomes

- **Physiological**

  Exosomes are involved in the modulation of the immune system by functioning as shuttles for antigen presentation[36]. Upon internalization of exosomes, antigen-specific immune responses can then be appropriately mounted [37]. Dendritic cells can also secrete exosomes to communicate with T cells and B cells to mount an immune response or to mediate immune tolerance[38]. Thus, exosomes can either have a stimulatory or inhibitory effect on the immune system, although its exact mechanism has not been fully elucidated. Apart from its role in the immune system, it has also been demonstrated that exosomes serve other physiological purposes as well. Evidence suggests that mesenchymal stem cells secrete exosomes, which can attenuate ischemia reperfusion injury in myocardial infarction[39]. In addition, exosomes are also thought to be involved in the regulation of neuronal cell function[40,41] as well as communication between cells [42].

- **Pathological**

  Recent evidence suggests a stark similarity in both composition, as well as mechanism of action of material transfer in exosomes and retroviruses[43,44]. Both exosomes and retroviruses have a lipid bilayer membrane, share a common glycan coat, and are enriched with similar proteins and genetic materials. It has been hypothesized that exosomes and retroviruses share a common ancestry, merely differing by a mutation of a single structural gag gene. Indeed, it has been shown that exosomes are involved in the functional delivery of viral miRNA, as a potent mechanism of infectivity[45]. It has been experimentally demonstrated that exosomes are associated with the

transmission of prion proteins via intercellular membrane exchange in Creuzfeldt-Jakob disease[46-48].

- **Gene therapy**

  The concept of gene therapy is attractive to both the scientific community as well as patients, because the idea of replacing a defective gene with a corrected one appears simple and elegant. However, the gene therapy fraternity was forced to re-evaluate its protocols with the sudden death of Jesse Gelsinger, the first patient in a gene therapy clinical trial to succumb to an acute immunological reaction to the adenovirus, which was used as a vector for delivering the gene[49]. Nevertheless, much progress has been achieved in the realm of gene therapy. Clinical trials have shown that gene therapy is safe and effective in treating diseases in humans such as severe combined immunodeficiency (SCID)[50], β-thalassemia[51], inflammatory bowel disease[52], Duchenne muscular dystrophy[53], and chronic lymphoid leukemia [54].

CONCLUSION

The safe and effective delivery of drug molecules to their target site is a field which has increasingly gained attention in drug design and development. In recent decades, the focus has shifted from synthetic drug compounds to the delivery of biological drugs (ie, proteins and nucleic acids), which are very prone to immune effects and degradation. In this regard, exosome mimetics are promising candidate delivery vehicles, given that they mimic nature's delivery vehicles of biologicals, but are not as complex as their biological counterparts. These characteristics may allow them to deliver biological in an effective and safe manner, with high pharmaceutical acceptability due to their well characterized components. This review provides insights into the composition and functional properties of exosomes, and focuses on therapeutic, diagnostic and gene delivery potential of exosomes. The similar mechanism of actions for gene transfection of both exosomes and viruses highlights the potency of exosomes in gene therapy. As exosomes can be derived from the patient's own cells, the issue of immunogenicity can be circumvented. Nevertheless, the concept of utilizing exosomes as a gene delivery vehicle is an attractive and promising technique in gene therapy.

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