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Exosomes for targeted siRNA delivery across biological barriers ☆

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ABSTRACT

Using oligonucleotide-based drugs to modulate gene expression has opened a new avenue for drug discovery. In particular small interfering RNAs (siRNAs) are being rapidly recognized as promising therapeutic tools, but their poor bioavailability limits the full realization of their clinical potential. In recent years, cumulating evidence has emerged for the role of membrane vesicles, secreted by most cells and found in all body fluids, as key mediators of information transmission between cells. Importantly, a sub-group of these termed exosomes, have recently been shown to contain various RNA species and to mediate their horizontal transfer to neighbouring- or distant recipient cells. Here, we provide a brief overview on membrane vesicles and their role in exchange of genetic information. We also describe how these natural carriers of genetic material can be harnessed to overcome the obstacle of poor delivery and allow efficient systemic delivery of exogenous siRNA across biological barriers such as the blood–brain barrier.

Contents

1. Introduction

Cells communicate and exchange information via different mechanisms. These include secretion of soluble factors and direct cell-to-cell contact. In addition, cells communicate through membrane transfer by the secretion of membrane vesicles [1]. These vesicles are secreted by most, if not all, normal and malignant cells and are found in most body fluids. Initially thought of as cell debris with no significant biological role, mounting evidence is now implicating these vesicles as key components in the transfer of information between cells. They exert their biological effects in a pleiotropic manner through i) directly activating cell surface receptors on target cells, ii) the transfer of receptors from the cell of origin to the recipient cell, and iii) the transfer of effectors such as oncogenes, transcription factors or infectious particles [2]. Furthermore, numerous studies are now suggesting that membrane vesicles, and in particular a sub-group termed exosomes, play a crucial role in the horizontal transfer of RNA between cells [3–9]. Of particular interest, in the context of this review, is their ability to deliver small regulatory

Abbreviations: miRNA, microRNA; siRNA, small interfering RNA; RNAi, RNA interference; BBB, blood–brain barrier; MV, microvesicle; MVB, multivesicular body; MSC, mesenchymal stem cell; HSPC, hematopoietic stem progenitor cell; shRNA, small hairpin RNA; RISC, RNA-induced silencing complex dendritic cells (DC).

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micro RNAs (miRNAs) into recipient cells to induce gene silencing [5,7,10–17].

Exploiting RNA interference (RNAi) to induce gene silencing has enormous clinical potential for treatment of various disorders and has opened a new avenue in personalized medicine to target disease-causing mRNA transcripts [18,19]. The majority of RNAi trials are making use of small interfering RNAs (siRNAs) that, similar to many miRNAs, target complementary mRNAs for degradation in a sequence-dependent manner [19]. Despite their great therapeutic potential, successful implementation of siRNAs in vivo is hampered by the low bioavailability of these hydrophilic compounds and their inability to cross key biological barriers such as the blood–brain barrier (BBB). Despite the enormous investments into RNAi, to date only 24 clinical trials have applied siRNA-mediated therapy [20]. This is primarily a consequence of inefficient in vivo delivery. Potent synthetic delivery vectors have lately been developed that improve systemic delivery of siRNAs to the liver and to different tumours. However, efficient vectors with the ability to target other tissues such as the brain and muscle are still lacking [21,22]. Thus, there is an urgent need to find efficient and biocompatible vectors that can overcome the hurdles of targeted RNAi delivery [23]. Here we provide an overview into membrane vesicle-mediated RNA delivery and discuss how these natural information transmitters could be harnessed for RNAi therapy.

2. Membrane vesicles: characteristics and biological role

Membrane vesicles are a heterogeneous group of intercellular messengers. While they share the common characteristic of an internal microenvironment protected from the extracellular milieu by a lipid bilayer with the same membrane topology as that of the cell of origin, they can be divided into distinct subgroups based on their biogenesis, biophysical properties and functions. These are: i) apoptotic bodies, ii) microvesicles or MVs (also referred to as ectosomes, microparticles or exovesicles depending on their cellular origin) and iii) exosomes. They vary significantly in size from as small as 50 nm to 5 μM in diameter and although sharing some features with other membrane vesicles, apoptotic vesicles can contain cytosolic organelles and nuclear fragments, which are absent in MVs and exosomes [24–26]. The biology and function of apoptotic bodies are outside the scope of this review but it should be emphasized that apoptotic bodies have been reported to participate in the transfer of miRNAs between cells [27].

2.1. Microvesicles and exosomes

MVs, or shedding vesicles, are a heterogeneous group of spherical vesicles with a diameter ranging from 100 to 1000 nm that are formed by budding of the cell plasma membrane producing small cytoplasmic protrusions that are shredded in response to calcium influx [28,29]. MVs are abundant in the circulation where they are predominantly platelet-derived (so-called microparticles), with smaller amounts originating from other blood cells and endothelial cells [30,31]. However, many other cell types including embryonic stem (ES) cells and tumour cells secrete large quantities of MVs and exosomes [3,9,32].

Exosomes are more homogenous and generally smaller than MVs, with a diameter size ranging from 40 to 120 nm. In contrast to MVs, exosomes are derived from the internal endo-lysosomal compartments of cells. Endocytic vesicles form at the plasma membrane and fuse to generate the early endosomes [33]. Late endosomes/multivesicular bodies situated close to the nucleus develop from early endosomes by acidification, via altered protein content and with fusion of vesicles [34]. The limiting membrane continuously buds into the lumen, thereby trapping cytosol and generating a population of intraluminal vesicles (ILVs) that upon exocytosis are released extracellularly as exosomes [35]. Hence, exosomes are highly enriched in proteins found within MVBs such as CD63, CD9, Alix and Lamp1. The mechanism of assembly and sorting of exosomes is largely unknown but stimulation of sphingomyelinase and subsequent formation of ceramide are known to promote exosome formation and release [36]. Importantly, ceramide formation seems crucial also for functional miRNA transfer through exosomes [37].

2.2. Biological role of MVs/exosomes

Most cell types release MVs and exosomes that may remain in the proximity of the cell of origin or enter biological fluids, thus allowing long-range exchange of information. There is an ever growing literature on the role of these vesicles both for maintaining normal physiology and in pathological conditions where they can modulate various disease processes such as angiogenesis, cell proliferation and apoptosis, inflammatory mechanisms, blood clotting, and tumour cell invasion etc. [38,39]. MVs and exosomes might directly stimulate recipient cells by cell surface interactions. For example platelet-derived MVs are able to interact with receptors expressed on platelets and macrophages and provide a surface for assembly of clotting factors [40]. Similarly, MVs directly activate endothelial cells through bioactive lipids [41] and exosomes from antigen presenting cells appear to display MHC complexes that directly participate in antigen presentation [42]. Another level of communication involves transfer of receptors from vesicles to recipient cells. For example, the transfer of Fas ligand on MVs secreted from tumour cells to activated T cells have been shown to induce apoptosis [43]. Similarly, the chemokine receptors CXCR4 and CCR5 have been reported to be secreted on MVs and can potentially facilitate HIV-1 virus infection in non lymphatic or haematopoietic cell lineages [44]. Yet another level of information transfer involves direct delivery of vesicle-encapsulated proteins into target cells. Prion protein has been shown to be released in exosomes [45] and tumour-derived MVs were elegantly demonstrated to convey oncogene products into neighbouring cells [46]. Hence, these multifaceted vesicles are being increasingly recognized as a prime source of potential biomarkers for a wide range of different disorders since they are easily obtainable from biological fluids, and very likely contain unique protein and RNA signatures reflective of the cell of origin and its state of health or disease [9,47]. This biomarker approach has already been particularly useful for diagnosing survival and therapeutic outcome of some human cancers [48].

Finally, the therapeutic potential of membrane vesicles has recently been affirmed within the field of regenerative medicine with a growing number of reports suggesting that MVs and exosomes secreted by mesenchymal stem cells (MSCs), hematopoietic stem progenitor cells (HSPCs), and multipotent stromal cells possess cytoprotective properties mediated by inhibiting apoptosis and stimulating proliferation of residing cells and promoting neovascularization [39,49]. These pro-regenerative effects mediated by MVs can be explained by the fact that they i) are enriched in bioactive lipids, ii) contain anti-apoptotic and pro-stimulatory growth factors and cytokines on their surface and iii) deliver proteins that improve cell function. Furthermore, these MVs contain mRNAs and several species of miRNAs that may also function to regulate cell survival and angiogenesis [50].

3. Exosomes and MVs as carriers of RNA

Perhaps the most intriguing property of MVs/exosomes, from a gene therapy point of view, is their ability to mediate the horizontal transfer of genetic material [3,38,39,51,52]. In 2006, the group of Ratajczak and colleagues demonstrated that ES cell-derived MVs are enriched in mRNAs encoding pluripotency proteins and that these can be used to epigenetically reprogram HSPCs by transferring these mRNAs [53]. Similarly, Bauj-Krzyworzeka et al. described the presence of tumour cell markers and mRNAs in tumour cell-derived MVs, and demonstrated their transfer in vitro into monocytes [4]. Others have shown that MVs from human endothelial progenitor cells can activate angiogenic programmes in quiescent endothelial cells through selective mRNA transfer and glioblastoma derived exosomes have been reported to