Biomaterials 33 (2012) 7138–7150

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Review

A review of nanocarriers for the delivery of small interfering RNA

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ARTICLE INFO

Article history:
Received 21 April 2012
Accepted 25 June 2012
Available online 12 July 2012

Keywords:
siRNA
Nanocarriers
Transfection
Polyplex
Cytotoxicity

ABSTRACT

Increasing knowledge about molecular mechanisms of endogenous RNA interference (RNAi) and small interfering RNAs (siRNAs) has been incorporated into innovative nucleic acid medicines for treatment of diseases such as cancers. Although RNAi and siRNA have the potential to become powerful therapeutic drugs, their delivery to the target site represents a major challenge. The design and creation of nanocarriers for the safe and efficient delivery of siRNA towards their potential applications site is one of the challenging and rapidly growing areas of research since they have to overcome the commonly encountered biological barriers. In this review, we discuss the recent nanotechnological strategies for siRNA delivery by using different carriers such as liposomes, dendrimers and carbon nanotubes.

1. Introduction

The RNAi pathway in the cytoplasm of the cell is divided into two phases. The first phase is the initiation phase in which two effector molecules i.e. small interfering RNA (siRNA) and micro RNA (miRNA) are generated. siRNAs are duplexes of 21–23 nucleotides, approximately 7.5 nm long and 2 nm in diameter [1]. The creation of siRNA commences in the cytoplasm through splitting of long double-stranded RNA by the enzyme Dicer [2,3]. However, the generation of miRNA, commences in the nucleus where endogenously encoded primary miRNA transcripts (pri-miRNA) are processed into precursor miRNA (pre-miRNA). In the cytoplasm, this pre-miRNA is cleaved by Dicer. The second phase is known to be effector phase in which siRNA and miRNA are unwound and then assembled with RNA-induced silencing complexes (RISC). This activated RISC contains only single-stranded (antisense) siRNA or miRNA, which guides RISC to its complementary target mRNA. siRNA generally possess perfect sequence complementary with its target messenger RNA (mRNA) and induces site-specific cleavage of the mRNA. The miRNA has typically imperfect sequence complementary which leads to translational repression without mRNA degradation. Both the pathways result in the inhibition of protein synthesis inside the cell [4–6].

The major advantages of siRNA over small drug molecules are that, the sequences can be rapidly designed for highly specific inhibition of the target of interest and also, the synthesis of siRNAs does not require a cellular expression system, complex protein purification, or refolding schemes, and is relatively uncomplicated [7]. The therapeutic applications of siRNA are as antiviral, as anticancer agents, in central nervous system therapeutics, in inflammation or cardiovascular therapeutics and many more [8].

Although the delivery of siRNA for biomedical applications is highly promising, there are some challenges which restrict its application. First challenge is the potential for an 'off-target' effect. This is the inhibition of a gene, whose expression should not be targeted, because the gene shares partial homology with the siRNA [9]. A latest solution reported by Jackson et al. by 2′-O-methyl ribosyl group substitution at position 2 in the guide strand could reduce silencing of most off-target transcripts with complementarily to the siRNA guide [10]. 'Immune stimulation' is another challenge during siRNA therapy. It is the recognition of a siRNA duplex by the innate immune system [11]. The stimulation of innate immune response by siRNA depends upon the nucleotide sequence [12]. Delivery of siRNA during the therapy is also an important challenge in siRNA therapy. Because of their large molecular weight (MW ~13 kDa), polycationinc and hydrophilic nature, they are unable to enter cells by passive diffusion mechanisms. Also, *in vivo* delivery of naked siRNA to appropriate disease sites remains a considerable hurdle owing to rapid enzymatic digestion in plasma and renal elimination, limited penetration across the capillary endothelium, and inefficient uptake by tissue cells [13]. Cellular uptake and endosomal trapping are significant hurdles, but can be overcome using strategies such as antibody mediated cellular uptake or polyethyleneimine-mediated endosomal escape (Fig. 1) [14]. These hurdles also provoked the development of effective

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http://dx.doi.org/10.1016/j.biomaterials.2012.06.068

Abbreviations

CaP	calcium phosphate
CNTs	carbon nanotubes
DOPC	1,2-dioleoyl sn-glycero-3-phosphatidylcholine
DOPE	dioleoyl hosphatidylethanolamine
EEA1	early endosome antigen 1
EPR	enhanced permeability and retention
ERK	extracellular signal-regulated kinase
FACS	fluorescence-activated cell sorting
FITC	fluoroisothiocynate
GC	glycol chitosan polymer
HPV	human papilloma virus
IL	immunoliposomes
i.p.	intraperitoneal
ILP	immunolipoplexes
LCP	liposome/calcium/phosphate
LEsiRNA	liposome-entrapped siRNA
LHRH	luteinizing hormone-releasing hormone
MDA	hexamethylenediamine
MDR	multi-drug resistance
MENPs	multifunctional envelope-type NPs
MMP	matrix metalloproteinase
miRNA	micro RNA
mRNA	messenger RNA
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
MWCNTs	multiwalled CNTs
NPs	nanoparticles
OLO	oligolysinoyl oleylamides
PBMCs	receptors on human T cells and peripheral blood mononuclear cells
PDDA	poly(diallyldimethylammonium) chloride
PAMAM	poly(amidoamine)
PEG	poly(ethylene glycol)
PEI	complexed with polyethylenimine
P-gp	P-glycoprotein
PIHCA	polyisohexylcyanoacrylate
PPD	PEG-peptide-DOPE
PPI	poly(propylenimine)
PRDM14	proline-rich domain proteins
pre-miRNA	precursor miRNA
pri-miRNA	primary miRNA transcripts
RES	reticuloendothelial system
RISC	RNA-induced silencing complexes
RNAi	RNA interference
SAHA	suberoylanilide hydroxamic acid
siRNAs	small interfering RNAs
SLNs	solid lipid nanoparticles
SWCNTs	single walled CNTs
TNF-α	tumor necrosis factor-α
TLO	trilysinoyl oleylamide
TLPD	tumor specific antibody conjugated liposome-polycation-DNA complex

in vivo delivery systems. Several types of synthetic vectors have been scrutinized for gene silencing applications, including the development of cationic lipids, liposomes; cationic polymers; cationic cell-penetrating peptides; cationic dendrimers and carbon nanotubes (CNTs). These carriers protect siRNA from degradation and facilitate uptake by target cells [15]. This review highlights the recent approaches for siRNA delivery using different nanocarriers like liposomes, nanoparticles, dendrimes and carbon nanotubes.

2. Liposome based siRNA delivery

Various lipid-based delivery systems have been developed for *in vivo* delivery application of siRNA (Table 1). Lipid-based systems include liposomes, micelles and emulsions. Among these, liposomes are non-toxic carrier systems widely used in drug delivery. Liposomes are vesicles composed of a phospholipid bilayer with an aqueous core, and for this reason hydrophilic as well as hydrophobic materials could be packaged into liposomes. The surface charge and the size of liposomes can regulate their *in vivo* stability and can modify the pharmacokinetic properties of the encapsulated drugs [16,17]. Liposomes are attractive carrier for gene delivery because they can be formulated as ~100 nm in size and their non-cytotoxic by-products. As a result of electrostatic interactions, cationic liposomes spontaneously form complex with anionic liposomes with siRNA. Lipid complexation with the payload (i.e. siRNA) simply involves mixing and incubation. Moreover, the targeted therapeutic efficacy of liposomes can be achieved by conjugating specific ligands to the lipid molecule that enhances their selective interaction with tumors [18]. For example, siRNA-loaded immunoliposomes targeted with anti-transferrin antibody produced specific inhibition of Human Epidermal growth factor Receptor 2 (Her-2) expression in breast cancer animal models and inhibited tumor growth in pancreatic cancer animal model [19]. Among various liposomal nanocarrier systems, PEGylated-stealth liposomes may be considered one of the ideal carriers for siRNA delivery, mainly due to their biological inertness and protection of siRNAs from nucleases [20]. Rothdiener et al. reported the 'anti-CD33 single-chain Fv fragment' conjugated PEGylated liposomal carrier systems for targeted delivery of anti-leukemic siRNA into CD33-positive myeloid tumor cells *in vitro*. Further modification of 'anti-CD33 single-chain Fv fragment,' with C-terminal cysteine residue allowed site specific targeting of PEGylated liposomes as well as the effective siRNA delivery and gene silencing. The siRNA was encapsulated into PEGylated liposomes either in free form (immunoliposomes; IL) or complexed with polyethylenimine (PEI) (immunolipoplexes, ILP). The resulting siRNA-loaded immunoliposomes (IL) and immunolipoplexes (ILP) showed specific binding and internalization by CD33-expressing myeloid leukemia cell lines (SKNO-1, Kasumi-1) [21]. Attachment of cell-penetrating peptides (CPP a family of peptides able to translocate across the cell membrane), to the liposomes also delivered siRNA into cancer cells [22].

Among liposomal delivery systems for siRNA, cationic liposomes have emerged as one of the most attractive vehicles owing to the simple manner in which such liposomes form complexes with negatively charged siRNA, their high transfection efficiency, their enhanced pharmacokinetic properties, and their relatively low toxicity and immunogenicity. Moreover, cationic liposomes can protect siRNA from enzymatic degradation, and provide reduced siRNA renal clearance. To assess or evaluate the efficiency of cationic liposome-mediated systemic delivery of siRNAs, Sorensen et al. delivered fluoroisothiocynate (FITC)-labeled siRNA i.v. and intraperitoneal (i.p.) directed against tumor necrosis factor -α (TNF-α). Distribution of a FITC-labeled siRNA in mouse spleen after i.v. injection was found to be localized around the vessels (Fig. 2). Authors suggested that the *in vivo* uptake of siRNA may vary with the cell types as well as with the status of cell differentiation. They reported that a large proportion of adherent peritoneal cells were