Journal of Controlled Release 259 (2017) 3–15

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Engineered polymeric nanoparticles to guide the cellular internalization and trafficking of small interfering ribonucleic acids

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ARTICLE INFO

Article history:
Received 1 December 2016
Received in revised form 15 February 2017
Accepted 18 February 2017
Available online 21 February 2017

Keywords:
Nanoparticles
Polymers
Drug delivery
Cancer therapeutics
Trafficking
siRNA

ABSTRACT

Ribonucleic acid interference therapy is a promising cancer treatment, which uses small interfering RNAs (siRNAs) to target and degrade messenger RNAs. Due to endogenous nuclease activity, siRNA is degraded rapidly, resulting in poor cell uptake and hence specificity. Moreover, it will not readily cross the cell membrane by passive diffusion. In order to take advantage of the therapeutic power of siRNA for the treatment of cancer, specialized delivery vehicles have been designed. In this review, we highlight advances in optimizing nanoparticle functionalization for guided siRNA delivery at the cellular level – that is, promoting cell uptake, escaping the endosome, and releasing siRNA from the delivery vehicle.

Contents

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http://dx.doi.org/10.1016/j.jconrel.2017.02.019
0168-3659/

1. Introduction

Ribonucleic acid interference (RNAi) is a powerful tool for the regulation of gene expression, making it an ideal therapeutic for diseases caused by genetic mutations, such as cancer. Often, the cancer cells overexpress oncogenic genes, providing potential targets for gene knockdown [1,2]. Small interfering ribonucleic acids (siRNAs) are short strands of ribonucleic acid typically composed of 21–30 base pairs with overhanging 3′ ends that can induce sequence-specific gene silencing at low (picomolar) concentrations when transfected into cells [3]. Although other silencing technologies are available, including the CRISPR/Cas9 system and antisense oligonucleotides, among others, siRNAs are advantageous due to their high potency and small size. Additionally, delivery of CRISPR/Cas9 components *in vivo* is still a major challenge, whereas there are a plethora of siRNA delivery strategies, as described herein. Naturally occurring small interfering ribonucleic acids (siRNAs) were first reported in 1999 in plants [4], and synthetic siRNAs were used to effect gene knockdown in mammalian cells two years later [5]. "Naked" siRNA therapeutics have been successful in clinical trials for ocular diseases when locally delivered at high concentrations, despite limitations including inflammation and increased ocular pressure [6]. The systemic delivery of these therapeutics presents additional challenges following intravenous injection in order to reach cancerous tissue. When injected, siRNA formulations must (1) evade the immune system, (2) avoid interactions with non-target cells, (3) avoid premature renal clearance, and (4) reach target tissues. These requirements have been reviewed extensively [7–9], thus here we will focus on overcoming further roadblocks once siRNA formulations reach their target tissues, including degradation by extracellular nucleases, poor cell uptake, and trafficking into the lysosomal compartments where the RNA strands are quickly degraded [8,10]. These challenges often require that siRNA therapeutics are combined with specialized delivery materials in order to be effective.

Nanotechnologies, encompassing a wide variety of formulations including metallic nanoparticles, micelles, liposomes, nanocrystals, nanogels/capsules, among others, are important delivery vehicles for a wide range of therapeutics including small molecule drugs, proteins, and siRNAs [11–13]. Advantages of nanoformulations include improved biodistribution and pharmacokinetics, stabilization of therapeutics, solubilization of hydrophobic drugs, and attenuating toxicity to off-target tissues [14–16]. The size, surface charge, and morphology of the delivery vehicle must be considered as they have a significant impact on pharmacokinetics and biodistribution [17–21]. The morphology of the vehicle can also have a significant impact on cellular internalization rates [22]. Moreover, in order for the nanoparticles to respond to both stimuli on the surface of the cell and within intracellular trafficking pathways, they must be flexible in terms of structure, functionalization, and resultant properties [23–25]. Although lipid-based nanoparticles have played an important role in the development of siRNA delivery strategies, lipid-based formulations are limited to a smaller number of well-established lipid components [26], whereas there are numerous monomers for polymer synthesis [27–30]. Therefore, while lipid-based strategies have been extensively studied and reviewed [31–33], this review will focus on fundamental and novel research in polymeric micelles, nanoparticles, and polyplexes for siRNA therapeutic delivery.

We begin with a brief discussion of stability of siRNA in the extracellular environment, and then examine some of the key challenges of siRNA delivery and trafficking in the target tissues using polymeric delivery vehicles, including: enabling cellular uptake, avoiding degradation within the cell, and successfully releasing the therapeutic payload. While there are many parameters that influence the success of an siRNA nanoparticle delivery system, including uptake specificity, rate of clearance and degradation, the key parameter is efficiency of knockdown and it is this parameter on which we have based our review.

2. Stability of siRNA in the extracellular environment

In order to increase the delivery efficiency of siRNA payloads, siRNAs are often conjugated or complexed to nanoparticles that protect them from nucleases and rapid clearance (Fig. 1). Within 15 min of injection in mice, >90% of standard 21-mer siRNAs are degraded by serum nucleases or lost *via* renal or lymphatic clearance [34], underlining the importance of the delivery vehicle. Polymeric nanoparticles can increase the stability of siRNAs against degradation: Raja et al. demonstrated that crosslinked chitosan nanoparticles increased the stability of siRNAs against serum during a 15 day storage at 4 °C [35] while Zhu et al. increased the half-life of siRNA in the blood to approximately 8 h by encapsulating it within a PLGA-based delivery vehicle, resulting in better tumor accumulation [36]. It is hypothesized that the nuclease resistance conferred by nanoparticle formulations is due to the steric bulk of the polymeric corona, preventing nucleases from reaching the siRNA. Therefore, with increasing density of the polymeric corona, the stability of siRNA in biologically relevant conditions is increased [37,38].

SiRNAs can also be chemically modified in order to increase their stability against nucleases. These modifications include any change to the native siRNA structure, typically employed on the phosphodiester bond or sugar ring (Fig. 2). These modifications enhance siRNA stability and potency, provide longer knockdown duration, reduced off-target effects, and lower immunostimulatory effects [39–42]. Modified siRNAs are now commonly used in research [43–45]. As shown in Fig. 2, some of the most common modifications of oligonucleotides include modifications to the backbone or nucleosides. For example, backbone modifications include phosphorothioate [46] and boranophosphonate [47] linkages, which increase nuclease resistance, while nucleoside modifications include 2′-*O*-methyl [48,49], 2′-deoxy-2′-fluoro [50], and locked nucleic acids [51], which increase stability and target binding affinity. Chemical modification of oligonucleotides and the effect on potency have been extensively reviewed by Deleavey et al. [40].

3. Cellular internalization

Most clinically relevant hydrophobic small molecule drugs can passively diffuse through the cell membrane. SiRNAs are large, hydrophilic, and negatively charged, so their passage across the cell membrane in the absence of a specialized carrier is hindered or blocked entirely. In a nanoparticle formulation, several different internalization pathways

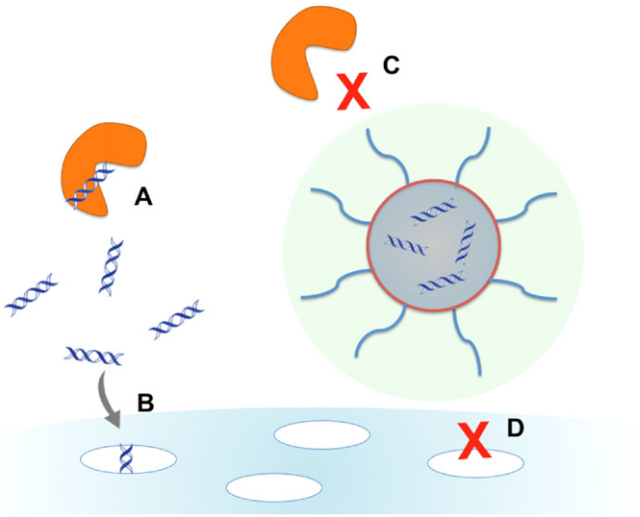

Fig. 1. SiRNA nanocarriers protect it from nuclease degradation. (A) Free siRNA (blue double helix) is rapidly degraded by nucleases (orange semi-circle) and (B) cleared by lymphatic drainage (pale blue ovals). (C) Nanoparticles may protect siRNA from nucleases and (D) reduce clearance.