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Polycation-based nanoparticle delivery of RNAi therapeutics: Adverse effects and solutions ☆

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ABSTRACT

Small interfering RNA (siRNA) that silence genes by the process of RNA interference offers a new therapeutic modality for disease treatment. Polycation-based nanoparticles termed polyplexes have been developed to maximise extracellular and intracellular siRNA delivery, a key requirement for enabling the clinical translation of RNAi-based drugs. Medical applications are dependent on safety; therefore, detailed investigation into potential toxicity to the cell or organism is required. This review addresses potential adverse effects arising from cellular and tissue interactions, immune stimulation and altered gene expression that can be associated with the assembled polyplex or the polycation and siRNA component parts. A greater understanding of the cellular mechanisms involved allows design-based solutions for rationale development of safe, effective and clinically relevant polyplex-based RNAi drugs.

Contents

1. Introduction

The capability to silence genes by the natural process of RNA interference (RNAi) offers a new therapeutic modality for disease treatment. Post-transcriptional gene silencing occurs by mRNA interaction with the RNA-induced silencing complex (RISC) mediated by small interfering RNA duplex (siRNA) recruitment, disassembly and subsequent complementary base pairing of the guide strand to the mRNA [1–4].

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This sequence specific nature and ability for exogenous siRNA to enter the RNAi pathways has promoted the development of siRNA-based drugs [5,6]. Therapeutic exploitation depends on stability within the bloodstream, dissemination to the target site and cellular entry. The nucleic acid composition, polyanionic and macromolecular characteristics, however, render siRNA susceptible to serum nucleases and rapid renal clearance and restrict entry across biological and cellular membranes.

Naked siRNA-based treatments applied local to the disease site have now reached clinical trials [7]; however, overcoming extracellular and intracellular delivery requirements is crucial to achieve the full potential clinical potential of RNAi [8]. Polymer-based nanoparticles have been used to maximise siRNA delivery. Among them, polyplexes are a promising class that have now reached clinical trials [9,10]. These are submicron spherical nanoparticles formed by electrostatic self-assembly of siRNA with cationic polymers [11,12] (Fig. 1). The ability to incorporate functional groups during polymer synthesis offers the versatility to design nanoparticles that can overcome different extracellular and intracellular barriers such as transport across biological membranes and intracellular trafficking. The potential of siRNA polyplexes to modulate biological membrane interactions and cellular gene expression requires a detailed understanding of the biological effects of the component parts and the assembly in order to design non-toxic clinical therapeutics. This work addresses potential adverse effects arising from cellular and tissue interactions, immune stimulation and altered gene expression and describes design-based solutions.

2. RNAi effector molecules

Possible adverse effects of polyplex-based RNAi therapeutics can be induced by the effector molecule itself. In general, these effects derive from the undesirable engagement with the RNAi machinery or mRNA such as saturation or unintentional targeting and/or activation of the immune system. However, these issues may be resolved by siRNA design and chemical modification (Table 1).

2.1. The RNAi pathway

RNA interference constitutes a fundamental pathway in the eukaryotic organism that allows selective transcriptional [13,14] or post-transcriptional gene silencing (PTGS) [3]. The PTGS process has been extensively studied and shown to be mediated by double stranded small interfering RNA (siRNA) that, following incorporation into the protein complex RISC, directs the cleavage, destabilization or translational repression of the targeted mRNA [1,15]. Target selectivity in this pathway is determined by the partial or total sequence complementarity between the siRNA strand retained in the active RISC, the so-called guide strand, and the messenger RNA.

Exogenous RNA enters the RNAi pathway at the level of RISC either directly as synthetic 21–22 nt duplex RNA or following cleavage of longer double stranded RNA by the cytoplasmic RNAse III enzyme Dicer. Since Dicer processing seems to favour effective RISC incorporation, 25–27 nt in length synthetic RNAi triggers, the so-called Dicer substrates, have also been developed as potential RNAi therapeutics [16,17] in addition to the conventional 21–22 nt siRNA.

In higher eukaryotes, an alternative RNAi pathway occurs in which endogenous small non-coding RNA known as microRNAs (miRNAs) regulate cellular gene expression. These effector molecules are normally transcribed by RNA polymerase II as long primary miRNA (pri-miRNA), processed by the enzyme Drosha into precursor miRNA (pre-miRNA) (50–70 nt stem-loop structures) and transported to the cytoplasm in an exportin 5 dependent manner [18]. They are subsequently processed into 21–22 nt duplex RNA by Dicer, converging at this level with the exogenous pathway. In contrast to siRNA that requires perfect guide strand/mRNA complementarity to activate cleavage, mature miRNA incorporated into RISC will induce translational repression by partial annealing to the 3′ untranslated region (UTR) of the target mRNA. In this process, only perfect complementarity between the miRNA seed sequence, that is, position 2–8 from the 5′ terminus of the guide strand and the 3′ UTR is required. Due to the involvement of some miRNAs in tumour promotion [19,20] selective miRNA inhibition through homologous antisense oligonucleotides (termed Antagomirs) [21,22] may have significant therapeutic potential. As an alternative approach, anti-neoplastic activity may be achieved by restoring the expression of miRNAs with tumour suppressor functions (such as let-7). Synthetic miRNA mimics whose natural counterpart are commonly under expressed in cancers, could, therefore, be delivered as "processed" triggers at the cytoplasm, as pri-miRNAs structures at the nucleus [23] or be transcribed in the cells from plasmids or viral vectors [24,25]. There is, therefore, a wide repertoire of potential RNAi-based drugs that engage at different points within the RNAi pathway. Owing to the pivotal role of the RNAi pathways in gene expression and regulation of vital cellular processes such as cell division, differentiation or apoptosis, it should be stressed that engagement into these pathways in a specific and controlled manner is important to avoid disturbance of miRNA activity or unintended gene silencing through siRNA off-targeting.

2.2. MicroRNA-like off-target effects

Compiling evidence have demonstrated that RNAi activity is not as specific in nature as suggested in early reports [26,27]. Significantly, mRNA targeting can occur not only by the annealing of a given siRNA guide strand with perfect or near-perfect complementarity, but also through base pairing of much shorter sequences (6 or 7 nucleotides in length) [28,29]. In fact, perfect match of the siRNA seed sequence to the 3′ UTR of mRNAs often results in gene silencing [29,30] in a manner resembling miRNA translational blockage [30]. This off-target

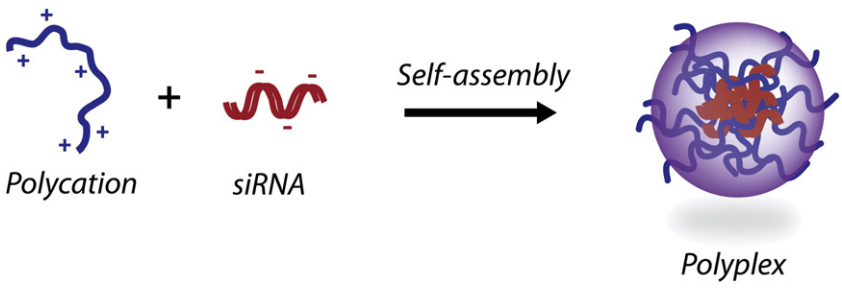

Fig. 1. Polyplex formation. Entropy-driven self-assembly of submicron polyplexes results from ionic interaction between polycationic amines and small interfering RNA (siRNA) anionic phosphates (graphic by Troels Bo Thomsen).