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REVIEW

15 years on siRNA delivery: Beyond the State-of-the-Art on inorganic nanoparticles for RNAi therapeutics

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Summary RNAi has always captivated scientists due to its tremendous power to modulate the phenotype of living organisms. This natural and powerful biological mechanism can now be harnessed to downregulate specific gene expression in diseased cells, opening up endless opportunities. Since most of the conventional siRNA delivery methods are limited by a narrow therapeutic index and significant side and off-target effects, we are now in the dawn of a new age in gene therapy driven by nanotechnology vehicles for RNAi therapeutics. Here, we outlook

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the ''do's and dont's'' of the inorganic RNAi nanomaterials developed in the last 15 years and the different strategies employed are compared and scrutinized, offering important suggestions for the next 15.

Introduction

During the last 15 years we assisted to a fast and significant revolution in the RNA world. One of the most astonishing milestones was the discovery of RNA interference (RNAi), a regulatory mechanism of gene expression widely diffused in eukaryotes, including fungi, plants, and animals. Overall, based on noncoding double-stranded RNA (dsRNA) molecules, RNAi drives homology-dependent degradation of target mRNA leading to specific gene silencing [1]. The discovery of RNAi has expanded our knowledge of gene regulation since Fire, Mello and colleagues demonstrated that long dsRNA mixtures were 10—100-fold more efficient at triggering gene silencing than single strand RNAs in *Caenorhabditis elegans*. However, the use of RNAi as a potent tool for gene regulation came when Elbashir and co-workers proved that synthetic small interfering RNAs (siRNAs) enabled sequence specific gene knockdown in a mammalian cell line [2]. These observations laid the foundations to employ RNAi as a key tool for gene functional analysis as well as a therapeutic tool.

So far, four major types of noncoding RNAs have been identified as RNAi effectors: siRNAs, microRNAs (miRNAs), piwi-interacting RNAs (piRNAs) and long intervening noncoding RNAs (lincRNAs) [3]. While the main goal of piRNA goes from transcriptional gene silencing and genome defence to transgenerational epigenetic mechanisms [4], miRNA and siRNA act more specifically as triggering molecules of gene silencing. Specifically, miRNAs are a large class of endogenous small regulatory molecules, derived from imperfectly paired hairpin RNA structures naturally encoded in the genome [5]. They prevalently act to control translational repression or mRNA degradation. Instead, siRNAs represent a heterogeneous class of noncoding RNAs typically including exogenous synthetic or viral inducers of RNAi (Fig. 1). Despite their different biogenesis, miRNA and siRNA once into the cytoplasm share common molecular machineries as Dicer enzymes for precursors excision, and Ago proteins, which vehicle their silencing functions [6,7]. Consequently, the enzymes Dicer and Ago, together with the 21—23 nt duplex-derived RNAs represent the key components of the RNA silencing pathway.

For a long time, RNAi has been considered a regulatory mechanisms merely controlling gene expression at the post-transcriptional level. Recent findings have now established that RNAi also plays a central role in transcriptional repression (RNA-induced transcriptional silencing, RITS). Nevertheless, a growing body of evidence supports RNAi regulating transcription through interactions with the transcriptional machinery. In light of this paramount potential, RNAi approaches are tremendously appealing for developing new therapies [8]. In fact, it has been shown that many human developmental and degenerative disorders as well as cancers encompass some form of aberrant gene regulation. One of the first clinical approaches aiming to harness the RNAi pathway for gene silencing employed siRNA by intravenous administration in patients with age-related macular degeneration (AMD) to downregulate the vascular endothelial growth factor transcript [9]. Beside this and other ocular diseases, ongoing clinical trials using RNAi-based strategies hold promise for treating fatal disorders (viral infections, neurodegenerative diseases, inflammatory diseases, cancer) [9,10] or provide alternatives to traditional small molecule therapies [11—13]. However, several hurdles must be overcome before RNAi technology can be translated from an effective research tool into a feasible clinical practice. In this respect, one of the primary obstacles remains the efficient *in vivo* delivery of these small molecules to the target cell type. Depending on the mode of administration, siRNA must cross many biological barriers before reaching the target cells, facing degradation by nucleases, issues related to their relative instability and half-life, short-lived nature of their transient gene silencing, sequestration by the immune system and elicitation of an immune response [14]. Upon reaching the targeted cells, siRNA molecules cannot readily diffuse across cellular membranes due to their anionic backbone and hydrophilic nature. Thus, delivery vehicles must be used to protect/conceal the siRNA within biological fluids, while facilitating its transfection to the cytoplasm of the target cells. The different strategies developed for efficient siRNA delivery can be grouped in two categories: those involving a chemical modification of the siRNA and those mediating the delivery by exogenous compounds, such as aptamers, liposomes, nanoparticles (NPs), polymers, dendrimers, all requiring a specific chemistry to preserve the biological activity of the siRNA upon conjugation. In fact, the therapeutic efficiency of delivery vehicles and a specific siRNA can be increased by modifications in key characteristics such as charge, size, shape, composition, surface chemistry and targeting motifs. DNA/RNA nanomaterials have also been developed in the last year for miRNA sensing and delivery [15]. Probably those relying on nanoparticles hold the best promise to improving stability, cell penetration, increasing administration dose, while enabling the specificity and/or self-tracking properties (*via* conjugation to antibodies and/or fluorophores) or other nanoparticle dependent properties (magnetic, electric, optical properties). Among the numerous nanoparticle formulations employed for siRNA delivery, here we will focus on inorganic nanoparticles [16], *i.e.* nanosized structures made by an inorganic material (*e.g.* silica, gold, iron oxide, quantum dots, carbon nanotubes, calcium phosphate), coated by polymeric layers and conjugated to siRNA through specific approaches including covalent binding, electrostatic absorption and encapsulation, depending on the material [17—22]. Compared to conventional transfection agents, nanoparticle-conjugated siRNAs have been shown to be less susceptible to degradation by nuclease