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Targeted electro-delivery of oligonucleotides for RNA interference: siRNA and antimiR☆

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ABSTRACT

For more than a decade, the understanding of RNA interference (RNAi) has been a growing field of interest. MicroRNAs (miRNAs) are small regulatory RNAs that play an important role in disease development and progression and therefore represent a potential new class of therapeutic targets. However, delivery of RNAi-based oligonucleotides is one of the most challenging hurdles to RNAi-based drug development. Electropermeabilization (EP) is recognized as a successful non-viral method to transfer nucleic acids into living cells both *in vitro* and *in vivo*. EP is the direct application of electric pulses to cells or tissues that transiently permeabilize plasma membranes, allowing the efficient delivery of exogenous molecules. The present review focused on the mechanism of RNAi-based oligonucleotides electrotransfer, from cellular uptake to intracellular distribution. Biophysical theories on oligonucleotide electrotransfer will be also presented. The advantages and few drawbacks of EP-mediated delivery will also be discussed.

Contents

Abbreviations: RNAi, RNA interference; 2′-O-Me, RNA with methylated 2′-hydroxyl group; miRNA, microRNA; mRNA, messenger RNA; LNA, locked nucleic acid; siRNA, small interfering RNA.

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1. Introduction

RNA interference (RNAi) was characterized as a novel and essential biological process more than 10 years ago [1]. RNAi offers the possibility of targeting and silencing any pathological protein in a specific way [2]. RNAi is mediated endogenously by microRNAs (miRNAs) [3] and experimentally by small silencing RNAs (siRNAs) [4]. Both are small (~22 nt) noncoding RNAs that, once loaded into the cytoplasmic RNA-induced silencing complex (RISC), bind to their target messenger RNA (mRNA) and impair translation. As a result, gene expression is inhibited [5,6].

More precisely, miRNAs post-transcriptionally regulate gene expression by repressing translation or accelerating mRNA decay [5]. miRNAs play crucial roles in the control of critical biological processes, including development, cell differentiation, proliferation and apoptosis [7]. miRNAs may also positively regulate gene expression by targeting promoter sequences [8]. miRNAs are involved in a wide variety of human diseases, such as diabetes [9], cardiovascular disease [10] and cancer [11,12] and, thus, have rapidly emerged as a new class of potential therapeutic targets [13]. The binding of miRNA to its target mRNA by Watson–Crick base-pairing is important for its biological function [14]. Since miRNA target recognition does not require perfect complementarity, a single miRNA can regulate multiple messenger RNAs; this is in contrast to siRNAs which mediate sequence-specific gene silencing [1,2]. siRNAs are not used for specific miRNA inhibition but they can silence unintended transcripts. If siRNAs match the guide-strand "seed region" similar to the way miRNAs match their target sites, off-target effects or potential toxicities can be caused by these off-target gene regulations [15,16].

With the intensifying research in miRNA field, molecular tools have been developed for specific inhibition of miRNA function such as synthetic antisense molecules of different chemistry that are complementary to the targeted miRNA or DNA-encoded miRNA inhibitors. Among the various strategies to inhibit miRNA, the most widely used approach is by using antisense oligonucleotides (antimiR). An efficient antimiR is an oligonucleotide that is complementary to its target miRNA and binds to it with high affinity and specificity to achieve efficient antisense oligonucleotide properties [13]. A vector-encoded miRNA inhibitor that is a vector expressing miRNA target sites (miRNA sponge) can be used to scavenge an endogenous miRNA and prevent it from regulating its natural targets. This type of construct is expressed *via* a strong promoter element and a bulge in the central part of the target sites prevents endonucleolytic target cleavage [17,18]. With these technologies, inhibition is obtained by preventing the miRNA from doing its job by competing with its endogenous targets. As opposed to such decoy approaches, the RNAi machinery can be employed to trigger the degradation of miRNA. For instance, the short hairpin RNA (shRNA) contains a predesigned (to target the sequence of the mature miRNA) antisense and sense sequence separated by a loop structure. The shRNA product is loaded into the RISC and the sense strand is degraded. The antisense strand directs RISC to the targeted miRNA leading to its degradation [19]. For these DNA-encoded miRNA inhibitors, as far as gene transfer is concerned, a major issue is the safety or the efficiency of the transfer methods. An approach using a direct transfer of the oligonucleotides appears more suitable for further clinical development. Indeed RNAi-based experiments can suffer from a lack of specificity due to the silencing of non-targeted genes unless a well-designed sequence is used [20]. To overcome these limitations, progress has been made in the development of new technologies optimizing oligonucleotide chemistry.

Different approaches based on the introduction of chemical modifications in the sequence of RNA or DNA oligonucleotides have been evaluated for efficient delivery (see [21] for review). A variety of chemical modifications can be used to improve the performance and potency of antimiR oligonucleotides. In general, modifications that confer nuclease stability and increase binding affinity improve antimiR oligonucleotide performance. Thus, new generations of chemically modified oligonucleotides have been developed [22,23], including 2′-O-methyl, 2′-methoxyethyl, locked nucleic acids (LNAs), and phosphorothiate linkages [24,25]. AntagomiRs are 2′-O-Me oligonucleotide phosphorothioated substitution (PS) linkages with the addition of a 3′-cholesterol moiety. AntagomiRs were among the first antimiRs to show *in vivo* efficacy [26]. LNA oligonucleotides exhibit strong thermal stability when hybridized with their RNA target molecule [27,28], and incorporation into a DNA oligomer (LNA/DNA oligomer) significantly improves mismatch discrimination compared to unmodified reference oligonucleotides [29]. This is due to the constraint on the sugar moiety that results in a locked C3′-endo/N-type conformation that pre-organizes the base for hybridization [30]. Furthermore, an LNA oligonucleotide is highly resistant to nuclease degradation and displays low toxicity in biological systems [31,32]. Therefore, LNA-based molecules appear to be promising therapeutic tools for developing miRNA mimics or inhibitors.

The development of therapeutic RNAi-based oligonucleotides is now moving to the next step, which involves efficient tissue delivery for siRNA ongoing clinical trials [33] for recent preclinical and clinical trials based on miRNA therapeutics [31,34]. In fact, their physicochemical characteristics (*i.e.* large molecular weight and anionic charge) prevent passive diffusion across the plasma membrane into the cytoplasm in most cell types. Therefore, enhanced delivery methods are required to allow therapeutic oligonucleotides to enter cells whilst being biocompatible, safe, and targeted.

In this context, electropermeabilization (EP) is a non-viral method for the *in vitro* and *in vivo* delivery of various molecules such as drugs [35] and nucleic acids [36,37]. EP was introduced in the 1960s [38]. It consists of the application of an external electric field pulse to target cells or tissues. Under calibrated electric conditions, it transiently destabilizes the plasma membrane, causing its permeabilization [39]. The convenience (*i.e.* ease of procedure, relatively low cost and speed) and the efficacy of this biophysical technique have led to its *in vivo* use for the treatment of both internal and surface organs [40,41]. Only few side-effects have been reported (such as superficial burns in the vicinity of the electrodes), supporting the innocuousness of this method for clinical use. In addition, no change in the expression profile of major tumor suppressor genes, oncogenes, or genes involved in the stability of DNA has been detected [42]. To date, several clinical studies using EP for cancer treatment have given positive results demonstrating antitumor effectiveness [43–46].

This review focuses on the mechanism of oligonucleotide electrotransfer: from their cellular uptake to their intracellular distribution. AntimiR oligonucleotide electrotransfer and siRNA electrotransfer will be compared. Biophysical theories on oligonucleotide electrotransfer will also be presented. Finally, the advantages and drawbacks of EP-mediated delivery will be discussed.

2. Electrotransfer of RNAi-based oligonucleotides

To be effective, therapeutic oligonucleotides have to find their target, meaning that cellular and sub-cellular localizations of oligonucleotides are the determinant for their biological effects. For instance, numerous reports have demonstrated that naked oligonucleotides are poorly internalized by cells and tend to localize in the endosomes/lysosomes, where they are unavailable for their targets [47,48]. In addition, a strong correlation between oligonucleotide nuclear localization and its specific efficiency has been reported [49,50]. Thus, knowledge of the dynamics and distribution of oligonucleotides in live cells is important for their transfer optimization.

2.1. Cellular uptake

EP represents a very attractive delivery method that is widely used, but only a few papers have described the mechanism of delivery [51]. We have shown that the electrotransfer of large molecules such as plasmid DNA (pDNA) is a multistep process with electrophoretic migration toward the permeabilized membrane and insertion into the membrane