RNAi
DELIVERY

Self-Delivering RNAi Compounds

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INTRODUCTION

Introduction of small interfering RNAs (siRNAs) into cells with transfection reagents results in potent and specific gene silencing by RNA interference (RNAi). While siRNA-based drugs represent a potentially significant therapeutic opportunity, the ability to apply this technology to drug development has unfortunately been impeded by the absence of efficient and non-toxic in vivo delivery systems. Currently, delivery is believed to be a major hurdle on the path to wide acceptance and utility of RNAi as a new class of therapeutic modalities. There are two major approaches to enhancing delivery: (1) formulation of oligonucleotides with particles/liposomes or (2) chemical modification of the oligonucleotide itself. RXi pharmaceuticals has recently developed self-delivering rxRNA or sd-rxRNA™, which is a novel, covalently modified RNAi compound configuration that does not require a delivery vehicle to enter cells and has improved pharmacology compared to traditional siRNAs.

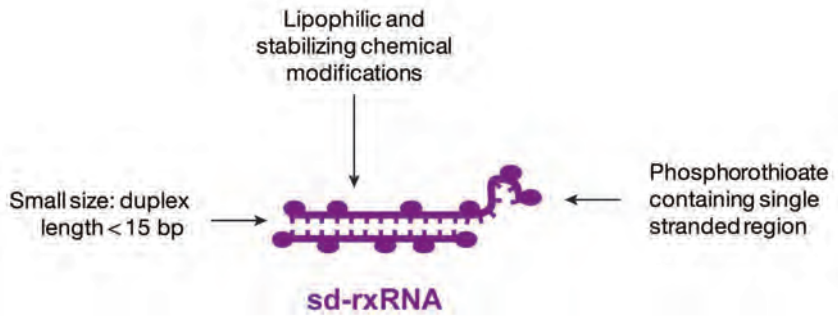
GENE SILENCING BY RNA INTERFERENCE

siRNAs are a promising new class of therapeutic oligonucleotides that target mRNA. In 1998, Andrew Fire and Craig Mello demonstrated that introduction of double-stranded RNA into the cells of a model eukaryotic organism, C. elegans, silenced the expression of complementary target genes by a process of post-transcriptional mRNA cleavage.[1] Introduction of siRNAs into mammalian cells also results in potent and specific gene silencing by the RNAi mechanism.[2] Properly designed RNAi compounds can be 100 to 1000 times more potent than traditional antisense compounds when transfected into cultured cells (EC50 of 5 to 50 pM for RNAi, compared to 1 to 50 nM for antisense).[3] RNAi compounds used in cell culture with cationic lipoplex transfection reagents have become one of the most widely used tools in research biology. In addition, a single administration of RNAi compounds to non-dividing cells results in long-term (up to 15 days) silencing, thus promising patient-friendly administration schedules.[4] Given the high potency of RNAi within the cell, it is generally agreed that RNAi will be broadly developed for therapeutic applications once issues of in vivo delivery are resolved.

CURRENT STAGE OF PARTICLE-BASED APPROACHES TO RNAi DELIVERY

Developers of liposomal and lipoplex formulations have focused on cationic lipid cocktails that mask the high charge-to-mass ratio of siRNAs.[5,6] While these formulations are highly effective in vitro, most are not fully optimized for in vivo applications.

FIGURE 1



sd-rxRNA Structure: Combination of Advanced Features of RNAi & Antisense Technologies
sd-rxRNA compounds have a shorter duplex (< 15 bp), a phosphorothioate containing single-stranded tail, and lipophilic and stabilizing modifications in both strands.

One issue is that the tissue distribution of siRNAs delivered in these formulations is predominantly confined to tissues with discontinuous endothelium, such as the liver. Some of these lipid-based formulations can be efficacious in vivo at very low concentrations (< 0.3 mg/kg) in animal models; however, potential immune-stimulatory side effects, especially upon repeat administration, can still remain an issue. To date, three INDs based on liposome-formulated siRNAs have been filed, one of which has been completed. Immune-stimulatory side effects observed in one patient in this clinical trial resulted in discontinuation of dosing and a revision of the formulation and siRNA.

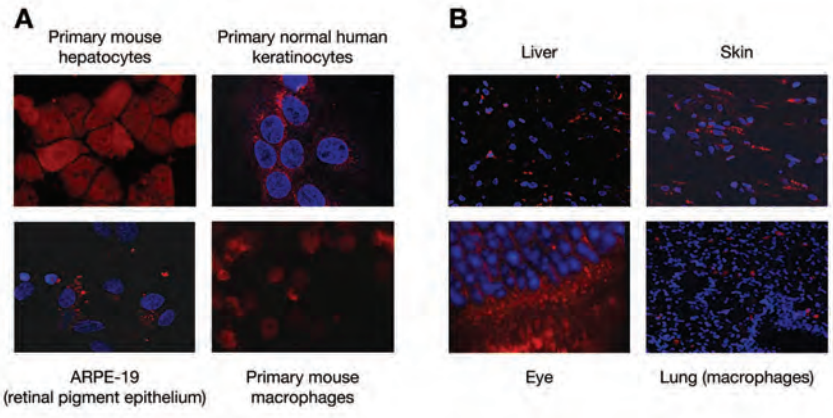
In an alternative delivery approach, significant progress has been achieved with a tumor-targeted, dextran-based siRNA formulation. Evidence of RNAi efficacy (mRNA knock-down) was seen in patients receiving repetitive, high doses (17 to 35 mg/kg) of this type of particle in a Phase I clinical study.[7] This work presented one of the first indications of RNAi compound efficacy in man and proved general applicability of RNAi technology for development of human therapeutics. While oligo formulation is one approach to achieve in vivo efficacy, development of siRNAs with improved drug-like properties will significantly expand RNAi clinical utility.

CHEMICALLY MODIFYING RNAi COMPOUNDS TO ENHANCE DELIVERY

In principle, it would be advantageous if chemical modification of the RNAi compound itself could facilitate delivery in a manner analogous to that achieved with antisense therapeutics. Early on, Alnylam Pharmaceuticals demonstrated that simple cholesterol conjugation to an siRNA compound improved PK/PD properties and enabled gene silencing in the liver.[8,9] Although the observed silencing effect required repetitive administration of high doses of oligonucleotide (3 x 50 to 80 mg/kg), this work provided the first evidence that medicinal chemistry can significantly impact in vivo efficacy of an oligonucleotide. RXi has recently developed a different type of chemically modified RNAi compound with improved drug-like properties. These chemically modified RNAi compounds are referred to as "self-delivering," defined as chemically modified RNAi compounds that do not require a delivery vehicle to efficiently enter target cells in vitro and in vivo.

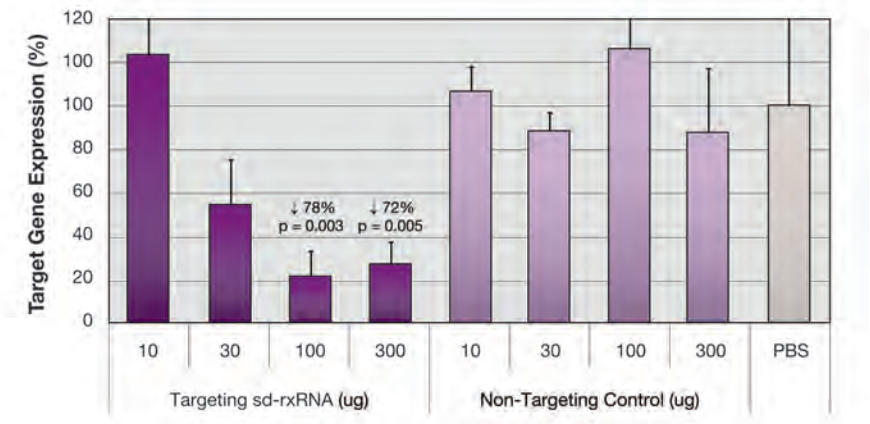
The self-delivering RNAi approach would obviate the need for potentially complex and toxic delivery vehicles. This approach should also reduce the expense and complexity of clinical development and commercialization because it requires the manufacture of the compound alone without the development of a complex formulation. Self-delivering RNAi compounds are much smaller than liposome or lipoplex complexes (~ 10 nm for sd-rxRNA compared to > 80 nm for liposomes or lipoplexes), and their reduced size may allow for broader tissue distribution, better tissue penetration, and the ability to use subcutaneous administration routes.

FIGURE 2



sd-rxRNA Uptake in Diversity of Cells & Tissues
A) DY547 fluorescently labeled sd-rxRNA (red) was added to cells in culture without a transfection reagent for 24 to 48 hours, nuclei were stained with Hoechst dye (blue), and cells were visualized with confocal microscopy. Silencing (> 90%) was observed by assaying MAP4K4 expression normalized to housekeeping genes PPIB and ACTB using a branched DNA assay (data not shown). B) DY547 fluorescently labeled sd-rxRNA compounds were administered by IV (liver); intradermal injection (skin), intravitreal injection (eye), and instillation (lung macrophages).

FIGURE 3



sdRNA In Vivo Efficacy

Intradermal injection of sd-rxRNA results in robust and potent silencing. 10 to 300 micrograms of a targeting or a non-targeting sd-rxRNA were administered by a single intradermal injection to the dorsum of a rat. At 48 hrs, 3-mm punch biopsies were harvested, and target gene expression was determined on the purified RNA by qPCR. Data are presented as target gene level normalized to a housekeeping gene and expressed relative to the PBS treated control group (± stdev).

sd-rxRNA COMPOUNDS

sd-rxRNA is a novel class of proprietary, lipophilically modified RNAi compound that does not require a delivery vehicle for cellular uptake and efficacy. It incorporates advanced features of both RNAi and antisense technologies (Figure 1A). Traditional, single-stranded antisense compounds have favorable tissue distribution and cellular uptake properties; however, they do not have the intracellular potency that is a hallmark of double-stranded RNAi compounds.[3] Conversely, the duplex structure and hydrophilic character of traditional siRNA results in poor tissue distribution and cellular uptake. In an attempt to combine the best properties of both oligonucleotide classes, sd-rxRNA has a single-stranded region and a shorter duplex region[10] and contains a variety of nuclease-stabilizing and lipophilic chemical modifications (RXi, Manuscript in preparation).[10] The combination of these features allows sd-rxRNA to achieve more favorable tissue distribution, efficient spontaneous cellular uptake, and potent long-lasting intracellular activity.

EFFICIENT CELLULAR UPTAKE IN VITRO & IN VIVO

Treatment of multiple cell types with fluorescently labeled sd-rxRNAs results in efficient and universal cellular uptake (Figure 2). In contrast to lipid-mediated delivery, in which transfection efficiency can be seen to be highly variable within a field of cells, sd-rxRNA is uniformly taken by cells upon contact. All cell types tested to date (including primary, neuronal, and non-adherent) internalize sd-rxRNA compounds efficiently, resulting in significant target silencing activity (data not shown). In addition to efficient cellular uptake in vitro, sd-rxRNAs demonstrate good tissue penetration and silencing activity following local and systemic administration in rodents. Efficient liver uptake has been observed with both IV and SC administration, in which high doses were required for silencing. While achievement of systemic clinically relevant efficacy requires further technology optimization, it is immediately applicable for gene silencing in tissues, where local administration is an option (Figure 2B). Local delivery to the desired site of action avoids the issues of kidney clearance and vasculature escape, allowing focus on the challenge of tissue penetration and spontaneous cellular uptake in vivo. As shown in Figure 2B, efficient in vivo cellular uptake can be seen in the liver following IV dosing, and in the skin, the retina of the eye, and alveolar macrophages following local administration.

EFFICIENT IN VIVO SILENCING UPON LOCAL ADMINISTRATION

Using intradermal administration as a model for local delivery, sd-rxRNAs were demonstrated to efficiently induce gene silencing. Maximum mRNA target level knock-down was achieved with a single administration of 100 micrograms. A minimal amount of compound was in the extracellular matrix, with the majority present in cellular cytoplasm 24 hours post-injection. Similar levels of silencing were demonstrated for multiple genes, and the effect persisted for more than a week (following 2 injections on days 1 and 2).

THERAPEUTIC POTENTIAL

Based on the promising data to date, sd-rxRNA and other chemically modified siRNA approaches may offer a key advance for use in future clinical development. These results may support clinical development in a number of dermatological indications, such as wound healing, scar reduction, and other indications where local injection is feasible or in which the skin is compromised. In addition, sd-rxRNA shows complete retinal penetration and efficient silencing upon intravitreal administration to the eye (data not shown), efficient delivery to alveolar macrophages (lung), and spinal cord, and may be applicable for treatment of a variety of ocular, respiratory, and CNS diseases. Thus, sd-rxRNA may have near-term clinical utility for local administation, with the potential for systemic therapeutic applications based on further technology optimization.

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BIOGRAPHIES

Dr. James Cardia is currently working as a Scientist at RXi Pharmaceuticals. His interests include applications of RNA biochemistry and chemistry to therapeutics. He was awarded the Donald White Teaching Award in 2004 and the Ruth Kirschstein National Research Service Award in 2007. He earned his PhD in Chemistry at Boston College in 2006 and performed his post-doctoral fellowship at Harvard University from 2006-2009.

Dr. Tod Woolf is a Scientific Advisory Board Member at RXi Pharmaceuticals. His research is focused on the use of chemical modifications to enhance oligonucleotides (synthetic DNA and RNA) for therapeutic and research applications. He co-founded and served as CEO of Sequitur and RXi Pharmaceuticals. He has co-authored over 50 scientific papers and patent applications. He served on scientific advisory boards of Signet Labs, ProNai, and TriLink Biotechnology. Dr. Woolf earned his PhD in Molecular Biology from Harvard University.