Nanocarriers delivering RNAi to cancer cells: from challenge to cautious optimism

"The use of RNAi in molecular medicine opens opportunities for novel therapeutic strategies to treat cancers."

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Cancer remains one of the most devastating diseases worldwide, with close to 12 million new cancer cases diagnosed each year in the USA alone [1]. Current treatment involves the administration of chemotherapeutic drug(s), often in combination with surgical intervention and/or radiation. Treatment effectiveness is directly related to selective eradication of cancer cells without collateral damage to healthy tissues [2]. Despite the incremental improvements in cytotoxic drug development, patients continue to experience adverse effects and in some cases only marginal improvement in long-term survival [1].

One of the major challenges resides in eradicating cancer stem cells, which are intrinsically resistant to chemotherapy [3,4]. Cancer stem cells utilize pump proteins to efflux chemotherapy out of the cells. Utilizing traditional drugs (which are substrates of the pumps) cannot overcome these hurdles; hence, there is a need for new generations of drugs that will be able to eliminate the resistance machinery and completely eradicate cancer cells. RNA interference (RNAi) might be this Holy Grail.

RNAi is a ubiquitous and highly specific endogenous, evolutionarily conserved mechanism of gene silencing. Since RNAi was demonstrated in mammals [5], the prospect of harnessing RNAi for human therapy has developed rapidly [6,7]. The RNAi machinery probably exists in all cells and marks a new path to silence genes. This discovery opened new avenues for treating various diseases by addressing targets that had otherwise been 'undruggable'; for example, the subunit M of the ribonucleotide reductase (*RRM2*) is a therapeutic target for DNA replication-dependent diseases such as cancer. Although RRM2 has long been considered 'undruggable' using conventional small-molecule approaches, RNAi-based methods have successfully suppressed this target in several different tumors [8,9] and is now under a Phase II clinical evaluation in ovarian cancer patients [7].

RNAi can be activated by expressing short hairpin RNA (shRNA) with viral vectors, or by incorporating small interfering RNAs fragments (siRNAs) into the cell cytoplasm [5]. The latter eliminates clinical safety concerns associated with viral vectors. Therefore, siRNAs represent the most promising type of RNAi-based therapeutics currently advancing in preclinical and clinical trials [6,7].

Despite the promise as a new form of medicine, the prime challenge facing the translation of siRNAs into clinical practice is delivery [6]. The efficiency by which synthetic siRNAs cross the plasma membrane and enter the cytoplasm is usually very low. Unmodified 'naked' siRNAs are subject to rapid renal clearance and are degraded by RNases, shortening their half-life *in vivo*. Furthermore, siRNAs could stimulate the immune system by being recognized via Toll-like receptors (TLRs), thus provoking interferon responses, causing cytokine induction, and activating coagulation cascades. These can cause global suppression of gene expression as well as aberrant immune activation, generating off-target effects and misinterpreted therapeutic outcomes [10]. One recent example of such a case involves the VEGF-siRNAs currently used in clinical trials for age-related macular degradation. The VEGF-siRNAs were found to induce the desired biological effect (i.e., suppression of angiogenesis) not by mediating direct silencing of the VEGF pathway, rather by binding to cell-surface TLR3 and activated TLR3 in a sequence- and target-independent manner, suppressing angiogenesis via off-target effects [11]. This has raised concerns as to the specificity and safety of siRNAs in clinical trials and emphasizes the importance of appropriate delivery systems.

Novel nanotechnologies utilize an interdisciplinary approach to generate nanocarriers and create new opportunities for targeted therapies. Nanocarrier-based approaches typically involve the supramolecular assembly of a carrier (typically constructed from lipids, proteins, carbohydrates or synthetic polymers). Unlike

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conventional therapies, targeted nanocarriers have the potential to offer more effective treatment with significantly reduced adverse effects through precise interactions with membrane receptors by including targeting moieties that can direct drugs in a cell- or tissue-specific manner. The carriers allow the delivery of large amounts of therapeutic payload per recognition event, the capacity to deliver multiple therapeutic agents simultaneously and the potential to overcome physiological barriers [2,12].

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Coupling siRNAs to fusogenic peptides, encasing them in lipid complexes, entrapping in liposomes or other types of particles, and linking them to antibodies or cell surface receptor ligands for cell-specific delivery potentially offer methods to protect siRNAs from endogenous RNases and can deliver them into cells [9,13–16]. Unlike delivering multiple drugs, which requires individual optimization, it is possible that the development of nanoscale carriers will enable delivery of different siRNAs with negligible modifications owing to their comparable physicochemical properties [17,18].

Sophisticated delivery strategies are rapidly developing, creating opportunities for cell-type-specific targeting of siRNAs in a safe and efficient manner. The challenges involved in devising such systems include: evading the immune system without inducing cytokine production, elucidating interferon response, or evoking lymphocyte activation; targeting siRNAs to the appropriate cell type with minimal collateral damage; utilizing cellular mechanism for internalization and release of the siRNA payloads into the cell cytoplasm while escaping the endosome; developing a system that will be fully degradable and will mitigate safety concerns.

Liposomes are one of the most studied nanocarriers and the first that have been clinically approved by the FDA [2]. Therefore, many efforts have been made to deliver siRNAs with nanodimension liposomes by exploiting their tendency to passively accumulate in the liver [15,19,20].

Two major types of liposomes focused on passive liver accumulation include: stable nucleic acid-lipid particles (SNALP) [21] and lipidoids [15]. SNALP consists of a liposome containing a mixture of cationic and fusogenic lipids that enables the cellular uptake and endosomal release of a particle's nucleic acid payload. A diffusible polyethylene glycol (PEG)-lipid conjugate coating provides SNALP with a neutral, hydrophilic exterior, which stabilizes the particle during formulation and endows the carriers with long circulating properties (both critical for increasing the chance of finding their target). This surface coating also shields the cationic bilayer *in vivo*, preventing rapid systemic clearance. Recently, SNALP containing siRNAs against essential cell cycle proteins polo-like kinase 1 (PLK1) and kinesin spindle protein (KSP) showed robust systemic delivery to hepatic tumors [21].

Lipidoids are liposomes made from a new class of synthetic lipid-like molecules [15,22]. Cationic lipidoid-containing liposomes have been developed to deliver siRNAs to the liver in an efficient and safe manner [22]. In a recent study, ApoB-siRNAs delivered via lipidoid-containing liposomes induce silencing in the mouse liver, resulting in a more than 80% reduction in mRNA levels [15].

Entrapping siRNAs in liposomes is applicable for tumors in the liver but is not suitable for disseminating tumor cells or hematological cancers. In order to target these cells, there is a need to endow liposomes and other entrapping siRNA nanocarriers with active targeting capabilities that require high specificity between the directing moiety (often on the surface of the nanocarrier) and the receptor or ligand on the target cell [2]. Recently, several elegant approaches have been developed for selective siRNA delivery to particular cell types. Among the most promising are cyclodextrin-containing polycation (CDP), a sugar backbone that self-assembled into colloidal particle (~50–70 nm) upon complexation with siRNAs [8]. The CDP particles are surface decorated with transferrin (Tf)-coupled PEG for targeting. Whereas Tf receptors (TfR) are expressed in many tissues, they are upregulated in cancers. In a recent study, three consecutive daily doses of Tf-targeted CDP nanoparticles carrying two different siRNA sequences targeting RRM2, slowed tumor growth in mice bearing subcutaneous tumors [9]. Tf-coupled CDP-containing RRM2 siRNAs recently entered Phase II clinical trials [7].

Polyplexes are complexes of positively charge polymers that condense siRNAs by charge-to-charge interaction. Polyplexes have been studied as siRNA delivery system; however, their poor pharmacokinetics exclude them from being used systemically. Lately, RGD (Arg-Gly-Asp)

peptide, which binds to α_V integrin, highly expressed in certain cancers and tumor vasculature, was coupled to PEG and attached to polyethylenimine (PEI), a cationic polymer [23]. Intravenous administration of VEGFR-siRNAs formulated in the RGD–PEG–PEI nanoplex given every 3 days significantly suppressed the growth of subcutaneously transplanted tumors in nude mice [23].

Despite the progress in engineering systemic siRNA delivery platforms to the liver and solid tumors, systemic delivery to hematological cells remains challenging and less characterized. Leukocytes continue to be among the most difficult targets for siRNA delivery [24] owing to the fact that they are resistant to conventional transfection reagents, and that they disperse in the body, making it difficult to successfully localize or passively deliver siRNAs via systemic administration [14]. To circumvent these obstacles, we have utilized a leukocyte cell-specific integrin approach and developed integrin-targeted stabilized nanoparticles [7,16]. We employed the integrin LFA-1, which is expressed in all leukocyte subtypes. LFA-1 single chain antibody–protamine fusion protein not only complexes siRNAs via a positively charged protamine moiety, but that also directs the siRNA–fusion protein complex to target cells. Intravenous injection of siRNA fusion proteins targeted against integrin LFA-1, specifically inhibits pulmonary hematopoietic cell tumors [14] and served as a proof of principle that integrins can be used for siRNA delivery to leukocytes. To increase payload and achieve robust targeted gene silencing in leukocytes *in vivo*, we have generated integrin-targeted stabilized nanoparticles and demonstrated that lipid-based nanoparticles decorated with anti-integrin antibody (against β7 integrin) can selectively deliver siRNAs to leukocytes involved in gut inflammation [16]. Since one can potentially change the payloads or the targeting agent (by replacing the antibody or the ligand decorating the surface of the nanoparticle), it is reasonable that this platform might be applicable to other types of tumors outside the hematopoietic system.

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The use of RNAi in molecular medicine opens opportunities for novel therapeutic strategies to treat cancer. The realization of RNAi-based drugs is currently facing a major challenge: systemic and targeted siRNA delivery. To address this challenge, it is important to consider strategies to increase the exposure of siRNAs to target cells. In addition, it is imperative to develop strategies that effectively cross the plasma membrane of different kinds of cells and reach intracellular compartments in which it is possible to utilize endogenous RNAi pathways. Currently, the solution for this challenge is being exploited by an interdisciplinary approach combining nanotechnology, material science, chemistry, and molecular and cellular biology. It is likely that we will witness more technical *tour de force* in the upcoming years that will turn this challenge into reality, applicable for all types of cancer patients. However, it is crucial to monitor the current clinical trials and examine if RNAi will in fact turn into a new therapeutic modality.

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Bibliography

Papers of special note have been highlighted as:
▪ of interest
▪▪ of considerable interest

1 American Cancer Society: *Cancer Facts & Figures 2007*. American Cancer Society (2007).

2 Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R: Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnology* 2(12), 751–760 (2007).

3 Ailles LE, Weissman IL: Cancer stem cells in solid tumors. *Curr. Opin. Biotechnol.* 18(5), 460–466 (2007).

4 Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM: P-glycoprotein: from genomics to mechanism. *Oncogene* 22(47), 7468–7485 (2003).

5 Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411(6836), 494–498 (2001).

▪▪ **Describes the first evidence that RNAi can be activated in mammalian cells.**

6 de Fougerolles A, Vornlocher HP, Maraganore J, Lieberman J: Interfering with disease: a progress report on siRNA-based therapeutics. *Nat. Rev.* 6(6), 443–453 (2007).

7 Peer D, Shimaoka M: Systemic siRNA delivery to leukocyte-implicated diseases. *Cell Cycle* 8(6), 853–859 (2009).

8 Heidel JD, Yu Z, Liu JY *et al.*: Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. *Proc. Natl Acad. Sci. USA* 104(14), 5715–5721 (2007).

▪▪ **Introduces a new cancer target RRM2, and show safety in nonhuman primates.**

9 Bartlett DW, Davis ME: Impact of tumor-specific targeting and dosing schedule on tumor growth inhibition after intravenous administration of siRNA-containing nanoparticles. *Biotechnol. Bioeng.* 99(4), 975–985 (2008).

10 Robbins M, Judge A, Ambegia E *et al.*: Misinterpreting the therapeutic effects of siRNA caused by immune stimulation. *Human Gene Ther.* 19(10), 991–999 (2008).

11 Kleinman ME, Yamada K, Takeda A *et al.*: Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. *Nature* 452(7187), 591–597 (2008).

▪▪ **Introduces the first doubt that siRNAs can also antagonize, nonspecifically, Toll-like receptors.**

12 Allen TM, Cullis PR: Drug delivery systems: entering the mainstream. *Science* 303(5665), 1818–1822 (2004).

13 Song E, Zhu P, Lee SK *et al.*: Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. *Nat. Biotechnol.* 23(6), 709–717 (2005).

▪ **Introduces the use of protamine–antibody fusion proteins for siRNA delivery to cell type specific receptors.**

14 Peer D, Zhu P, Carman CV, Lieberman J, Shimaoka M: Selective gene silencing in activated leukocytes by targeting siRNAs to the integrin lymphocyte function-associated antigen-1. *Proc. Nat. Acad. Sci. USA* 104(10), 4095–4100 (2007).

▪ **Describes the use of protamine antibody technology to silence immune cells, and demonstrates the ability to target activated leukocytes selectively.**

15 Akinc A, Zumbuehl A, Goldberg M *et al.*: A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. *Nat. Biotechnol.* 26(5), 561–569 (2008).

▪ **Introduces the use of lipidioids as new siRNA delivery platform to the liver.**

16 Peer D, Park EJ, Morishita Y, Carman CV, Shimaoka M: Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target. *Science* 319(5863), 627–630 (2008).

▪▪ **Introduces the first targeted nanoparticles made from neutral materials that selectively target leukocytes, and demonstrates using this technology that cyclin D1 is a potential anti-inflammation target.**

17 Bumcrot D, Manoharan M, Koteliansky V, Sah DW: RNAi therapeutics: a potential new class of pharmaceutical drugs. *Nat. Chem. Biol.* 2(12), 711–719 (2006).

18 Root-Bernstein RS, Dillon PF: Small molecule complementarity as a source of novel pharmaceutical agents and combination therapies. *Curr. Pharm. Des.* 14(1), 55–62 (2008).

19 Morrissey DV, Lockridge JA, Shaw L *et al.*: Potent and persistent *in vivo* anti-HBV activity of chemically modified siRNAs. *Nat. Biotechnol.* 23(8), 1002–1007 (2005).

20 Zimmermann TS, Lee AC, Akinc A *et al.*: RNAi-mediated gene silencing in non-human primates. *Nature* 441(7089), 111–114 (2006).

▪▪ **Describes a study demonstrating the silencing of an endogenous liver gene in non-human primates at doses of 1 mg/kg using a liposomal system.**

21 Judge AD, Robbins M, Tavakoli I *et al.*: Confirming the RNAi-mediated mechanism of action of siRNA-based cancer therapeutics in mice. *J. Clin. Invest.* 119(3), 661–673 (2009).

22 Akinc A, Goldberg M, Qin J *et al.*: Development of lipidoid-siRNA formulations for systemic delivery to the liver. *Mol. Ther.* (2009) (Epub ahead of print).

23 Schiffelers RM, Ansari A, Xu J *et al.*: Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. *Nucleic Acids Res.* 32(19), E149 (2004).

24 Goffinet C, Keppler OT: Efficient nonviral gene delivery into primary lymphocytes from rats and mice. *FASEB J.* 20(3), 500–502 (2006).