Rationally Designed Tumor-Penetrating Nanocomplexes

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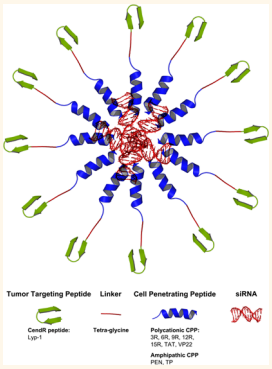
ABSTRACT Small interfering RNA (siRNA) therapeutics have broad potential uses in medicine but require safe and effective delivery vehicles to function. An ideal delivery system should encapsulate and protect the siRNA cargo from serum proteins, exhibit target tissue and cell specificity, penetrate the cell surface, and release its cargo in the desired intracellular compartment. One approach to the design of delivery vehicles that meets all of these requirements utilizes the systematic assembly of multiple components that can address each barrier. This rational approach was adopted by Ren *et al.*, who designed novel myristoylated tandem peptides that consist of a tumor-targeting module and a cell-penetrating module, as described in this issue of *ACS Nano*. These tandem peptides were formulated with siRNAs into nanocomplexes for cell-specific delivery to a variety of tumor cell lines. The correlation of the structural properties of the nanocomplex to cell-type-specific activity *via* a computational approach identified the valence of the tumor-targeting ligand and overall nanocomplex charge as important parameters for the activity of the formulations. The *in vivo* gene silencing potency of these peptide-based nanocomplex formulations was demonstrated by Ren *et al.* in an ovarian cancer model. Tumor-penetrating nanocomplexes carrying a siRNA sequence against a novel oncogene (ID4) led to a significant reduction in tumor burden and an 80% increase in mouse survival. As such, the combination of a systematic approach with computational modeling can be advantageous for improving the delivery and potency of siRNA therapeutics.



The recent accumulation of large biological data sets describing complex biological systems has provided new insight into the genetic control of a wide variety of multiscale systems from the cellular, tissue, and whole organism levels.[1] The advancement of high-throughput screening and computational modeling, especially toward signaling pathways, has extended our understanding of complex biochemical interactions. This, in turn, has led to the identification of extracellular and intracellular targets for various diseases. In order to develop potent small interfering RNA (siRNA) therapeutics against these targets, the therapeutic cargo needs to be encapsulated in a delivery vehicle that can bypass or overcome numerous biological barriers. The delivery vehicle should encapsulate and protect its cargo from serum proteins, exhibit target tissue and cell specificity, penetrate the cell surface, and release its cargo in the desired intracellular compartment.[2] The combination of rational design with computational modeling is a promising approach for the development and optimization of these delivery vehicles and future therapies. The use of computational modeling that incorporates experimental data sets can help generate an *in silico* framework for the hypothesis-driven prediction of parameters to improve the performance of these therapeutic modalities. For example, *in silico* nanomaterial design can provide a template for the development of drug-delivery vehicles with tailored pharmacodynamics, as well as pharmacokinetic and drug-releasing properties.[3] In addition, by taking advantage of the information describing certain signal transduction pathways, computational methods can be used to identify combinations of targets at the molecular level, or even to design optimal drug-delivery strategies computationally at a systemic level.[4]

Computational modeling can also be used to correlate the sequence and structural information of proteins and peptides with bioactivity. These correlations can help identify novel bioactive peptides that interact with intracellular proteins, cell surface receptors, lipid membranes, and nucleic acids.[5] This information can be used toward the development of novel protein-targeting agents for cell-specific delivery and the

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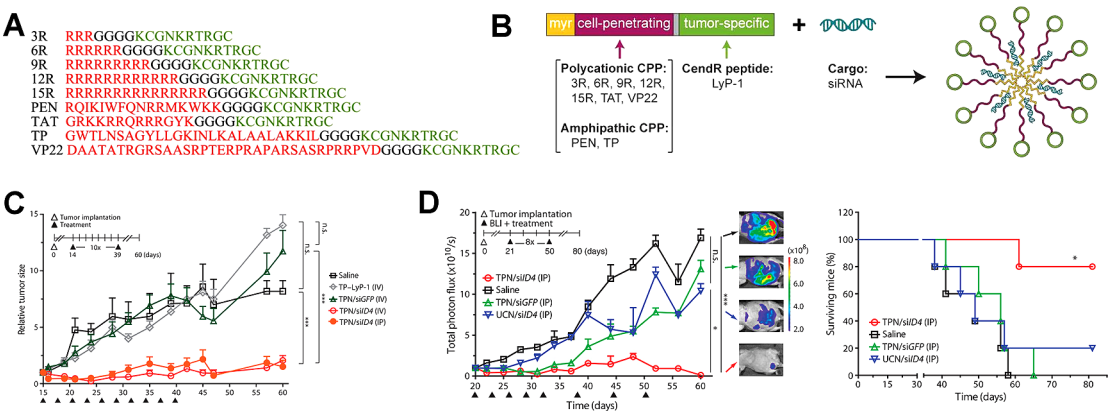

Figure 1. Ren *et al.*[1] constructed 18 different tandem peptides for creating nanoparticles that deliver small interfering RNAs to tumor cells. (A) The authors tested nine different peptides with a free primary N-terminus amine or they were myristoylated; such modification is shown to promote peptide association to lipid membranes. The amino acid sequences of the tested peptides are shown in A. Such peptide sequences were composed of a tumor-targeting module combined with a cell-penetrating module. The tumor-targeting module located at the C-terminus of the tandem sequences was the peptide Lyp-1 (shown in green), while the cell-penetrating module located at the N-terminus consisted of known positively charged cell-penetrating peptides (shown in red). The two modules were separated by a tetraglycine linker. (B) Nanocomplexes are formed through electrostatic interactions of the negatively charged siRNA molecules with the positively charged peptides with the tumor-targeting moiety located at the outer, solvent-accessible, surface of the particles. Reproduced from ref 14. Copyright 2012 American Chemical Society. (C,D) Such nanocomplexes can be proven useful for translating findings describing the molecular mechanisms of tumor development to actual clinical applications. For example, they can deliver siRNA for novel oncogenes like ID4 and inhibit tumor growth in subcutaneous (C) and orthotopic (D) ovarian tumor animal models. (C) Nanocomplexes injected either intrevenously (TPN/siID4 (iv)) or intraperitoneally (TPN/siID4 (ip)) significantly reduce the tumor size of an ovarian cancer xenograft for more than 60 days, whereas control vehicles containing siRNA for GFP have no effect. Reproduced with permission from ref 2. Copyright 2012 American Association for the Advancement of Science. (D) Nanocomplexes injected intraperitonealy (TPN/siID4 (ip)) also inhibit tumor growth in animals developing orthotopic ovarian tumors and prolong their survival. Reproduced with permission from ref 3. Copyright 2012 Elsevier.

identification of novel biomarkers and targets for the detection and treatment of diseased cells.

Tandem Peptides for the Delivery of Therapeutics. Cell-penetrating peptides (CPPs) are a class of peptides that hold great potential for the delivery of therapeutic molecules to a variety of cell types.[6] Over the last 30 years, numerous reports have revealed insights into the mechanism of CPP-mediated cellular entry,[7] and various CPP formulations have been utilized in biomedical applications as drug-delivery vehicles. In general, cell-penetrating or transduction domain tides possess the ability to transverse the cellular membranes and transport their cargo intracellularly.[8] Mechanistically, it is believed that CPPs undergo endocytosis either by fusing with lipids in the cell membranes which leads to a vacuole-based endocytic route or by creating pores in the cellular membrane.[9] The majority of CPPs are derived from bacterial or viral proteins, although some are of synthetic origin. Typical physicochemical features that characterize such sequences are their high positive charge and amphiphilicity, which have been identified as critical parameters for cell penetration.

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Cell-penetrating peptides have been utilized for the intracellular delivery of a variety of therapeutic cargo. The most abundantly used CPPs are positively charged polyarginine (poly-R)[10] or polylysine (poly-K)[11] based peptides (6−12 residues). By modifying the number of residues, one can control the total charge of the peptide. In addition to poly-R and poly-K sequences, there are an increasing number of peptides derived from naturally occurring protein sequences (mostly viral or bacterial) that are also positively charged, amphiphilic, and possess cell-penetrating properties. Typical examples include the TAT peptides derived from the HIV-1 virus sequence; penetratin, a peptide derived from the *Drosophila melanogaster* antennapedia homeodomain protein; the VP22 protein-based peptide from herpes simplex virus; and the MPG peptide, which contains a hydrophobic domain derived from the fusion sequence of HIV glycoprotein 41 and a hydrophilic domain derived from the nuclear localization sequence of the SV40 T-antigen.[12] When used with drug-delivery vehicles, these peptides can be either covalently attached to the therapeutic molecules or noncovalently used to create nanocomplexes *via* electrostatic interactions. A few of these CPP-based therapeutics, such as TAT-based peptides,

have reached phase I and phase II clinical trials for the delivery of proteins and other cargo.[13]

In this issue of *ACS Nano*, Ren *et al.*[14] describe the combination of rational nanomaterial design with computational modeling to understand the mechanism of tandem CPP-mediated siRNA delivery. Ren *et al.*[14] designed and tested 18 different tandem peptides for creating nanocomplexes that deliver siRNAs to tumor cells (Figure 1). The tandem peptide sequences were composed of a tumor-targeting domain combined with a cell-penetrating domain. The tumor-targeting domain, located at the C-terminus of the tandem sequence, is a CendR (C-terminus-Rule) peptide Lyp-1.[15] This peptide sequence was previously identified[15] to target tumor cells and tumor lymphatics *via* binding to p32, a tumor mitochondrial protein expressed at the cell surface. The cell-penetrating module, located at the N-terminus, consists of known positively charged cell-penetrating peptides. The authors tested nine different peptides with either a free or myristoylated N-terminus. The latter has been shown to promote peptide association to lipid membranes as well as to enhance nanocomplex formation and stability. A tetraglycine linker was embedded between the two domains in order to reduce the risk of siRNA complexation interfering with receptor targeting. Nanocomplexes were formed by combining positively charged tandem peptides with negatively charged siRNA molecules. The diameters of the nanocomplexes ranged from 150 to 500 nm, primarily depending on the charge of the cell-penetrating sequences. In addition to achieving cell-specific gene silencing, the authors probed the mechanism of siRNA delivery by carrying out linear regression modeling of the physiochemical characteristics of the nanocomplexes. This analysis led to the identification of valency and overall peptide charge as critical parameters to the cell-specific silencing activity of the formulations. These parameters were shown to affect the endocytic performance and endosomal release capability of the nanocomplexes.

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The ability to target tumor cells and to deliver siRNA efficiently provides an opportunity to treat several *in vivo* targets that are responsible for potentiating tumor development. In an analogous study, Ren *et al.*[16] screened genes that were overexpressed in human ovarian cancer cells and identified ID4 as a novel oncogene. The authors showed that ID4 is amplified in human primary high-grade serous ovarian tumors and is essential for the anchorage independent proliferation of ovarian cancer cells. Furthermore, they showed that this oncogene induces tumorigenicity by activation of the HOXA9 gene, making it a potential therapeutic target for silencing with siRNA. Tumor-penetrating nanocomplexes carrying a siRNA sequence against ID4 reduced the expression of ID4 by 90% in mice bearing ovarian tumor cells after subcutaneous and orthotopic administration. The latter led to tumor suppression for 60 days and an 80% mouse survival.[16] In another example, Nijhawan *et al.*[17] identified 56 genes with partial copy number loss, which when suppressed inhibited tumor cell proliferation. Genes with such copy number losses leading to cancer cell vulnerabilities are termed CYCLOPS (copy number alterations yielding cancer liabilities owing to partial loss). The PSMC2 gene encoding a protein of the 19S cell proteasome is a CYCLOPS gene for OVCAR8 ovarian cancer cells and provides a target for therapeutic intervention. Subcutaneous injection of tandem CPP-based nanocomplexes carrying a siRNA sequence against PSMC2 in mice bearing OVCAR8 reduced their tumor burden and prolonged their survival time.[17]

Advancing Therapeutic Potency with Computation Modeling. The ultimate contribution of computational modeling to the advancement of siRNA therapeutics is the development of accurate *in silico* models that can inform the design of nanocomplex formulations for efficient siRNA delivery to the desired target tissue. Although we do not yet have complete predictability, mathematical and computational modeling have begun to reveal how modifications in nanocomplex formulations can lead to significant differences in performance and activity. For example, Decuzzi *et al.* utilized mathematical modeling to generate design maps that predict how differences in size, shape, charge, and chemical composition affect the adhesive and endocytic performance of nanoparticles.[18] Given the large number of biophysical nanocomplex descriptors and the infinitely possible formulation combinations, *in silico* modeling and, at the very least, regression analyses are becoming necessities for understanding the mechanism of nanocomplex activity and thereby optimizing its performance. Despite its importance, computational modeling should not be limited to optimizing the nanocomplex alone because the pharmacokinetic properties of the nanocomplex are dependent on the microenvironment of the target tissue. As a result, future computational models should also account for properties specific to the target

tissue's microenvironment such as its extracellular matrix composition, pressure distribution, rate of cell proliferation, and the degree of vasculature permeability.

Conflict of Interest: The authors declare no competing financial interest.

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