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Nanoparticle-Mediated Gene Regulation as a Novel Strategy for Cancer Therapy

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In 2014, approximately 14.5 million Americans were either actively fighting cancer or survivors of cancer and this number is expected to increase in the coming years.[1] With this increasing prevalence, research for novel cancer treatments has become of the utmost importance. Current treatments for most forms of cancer include surgery to remove the cancerous cells, and chemotherapy or radiotherapy to cause cancerous cell death. Although these treatments are useful in many cancers, surgery is highly invasive, and chemotherapy and radiotherapy suffer from innate or developed resistance that promotes tumors' continued progression or later recurrence. Researchers are interested in developing new treatments that may be used alone or in combination with conventional therapeutic interventions to more effectively halt tumor growth and prevent recurrence. Gene regulation is a promising new strategy to achieve these goals.

Using gene regulation-based approaches, researchers can deliver nucleic acids into cancer cells to either induce the expression of tumor suppressor genes, which inhibit tumor growth, or suppress the activity of oncogenes, which promote tumor growth. The effective application of gene regulation therapy requires knowledge of the biology involved in each individual patient's tumor. It is believed that providing therapies that are personalized to the complex nature of each type of cancer and to each individual person will improve patient outcomes.[2] In order to deliver this personalized gene regulation-based treatment, several different delivery vectors are currently in preclinical or clinical investigation. Viral vectors remain the most prevalent class of gene regulation agents in development and currently represent ~70% of all gene therapy clinical trials.[3]

Although viral vectors are highly effective as nucleic acid delivery vehicles, several shortcomings limit their success as therapeutics. For example, viral vectors can induce strong immunogenicity, which can produce flu-like side effects and limit the doses that may be administered.[4] Nonviral vectors offer attractive solutions to these limitations. Synthetic, engineered nucleic acid delivery vehicles may be less immunogenic than viral vectors and can be more easily produced on a large scale to contain larger quantities of therapeutic nucleic acids.[5] Such constructs can be engineered to deliver therapeutic nucleic acids including: (i) deoxyribonucleic acids (DNAs), which may either amplify gene expression or suppress the expression of target genes, depending on the nucleic acid design, (ii) messenger ribonucleic acids (mRNAs), which are single-stranded nucleic acids that encode the genetic instructions to produce specific proteins, and (iii) small interfering ribonucleic acids (siRNAs) or micro RNAs (miRNAs), which are short, double-stranded nucleic acids that suppress the expression of target genes in a sequence- specific manner.[5,6] Here, we focus our discussion on the development of non-viral methods to deliver siRNA for RNA interference-based gene therapy, and include specific examples of research occurring at the University of Delaware.

RNA interference (RNAi) has recently emerged as a promising method to suppress the expression of cancer-promoting genes.[7] In RNAi, siRNA that has been introduced into the cell associates with the RNA-induced silencing complex (RISC), which subsequently aligns with perfectly complementary mRNA to facilitate its degradation and prevent protein translation (Figure 1).[8] The RNAi pathway offers several advantages as a strategy for gene regulation.

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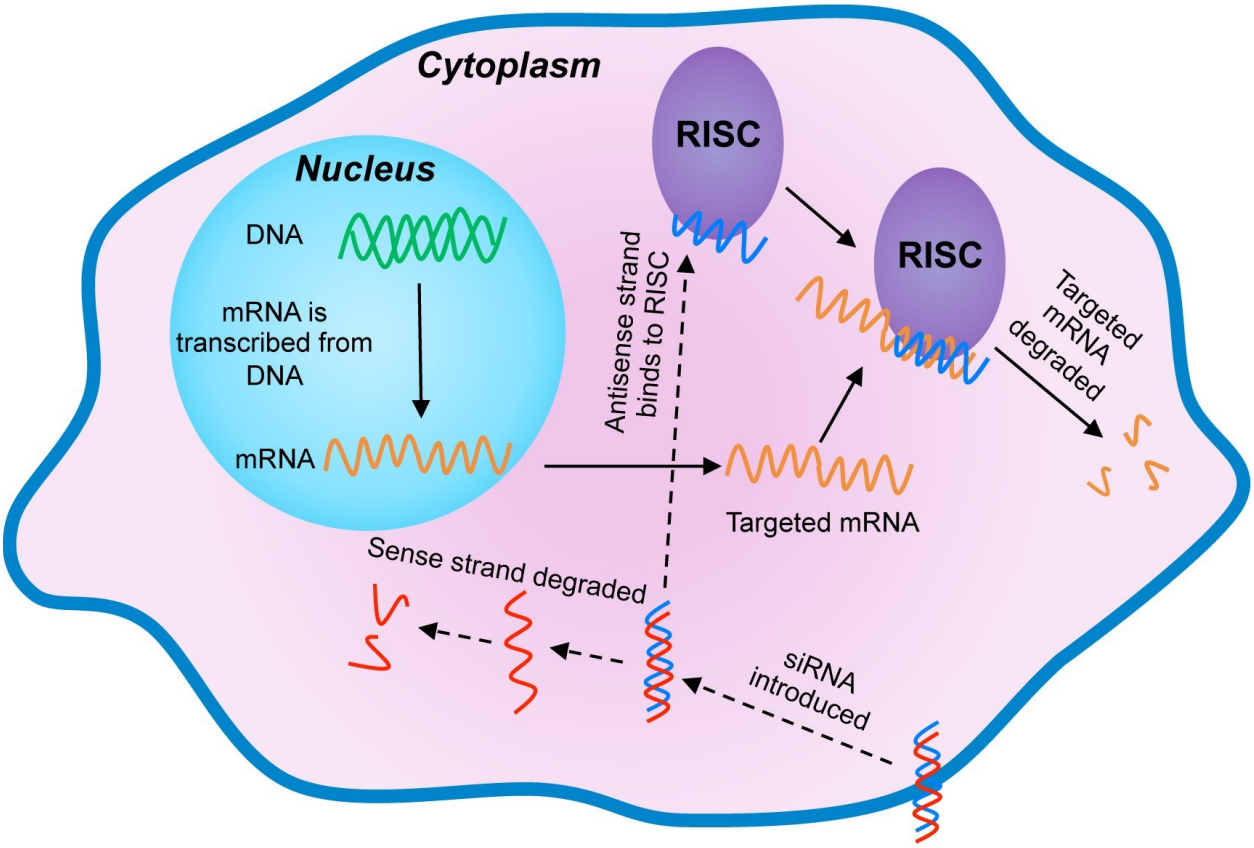
For example, siRNA is highly specific and therefore may have fewer off-target effects than observed with standard chemotherapy and chemical oncogene inhibitors. Further, chemical oncogene inhibitors often rely on the presence of hydrophobic pockets that can be targeted within the protein structure.

Figure 1. Schematic depicting the process of RNA interference therapy.



Ordinarily, DNA is transcribed into mRNA, which is then translated into protein. In RNA interference therapy, siRNA delivered into cells complexes with the RNA- induced silencing complex (RISC) and guides it to perfectly complementary mRNA molecules in the cell cytoplasm, which are subsequently degraded, thereby halting protein production. Using RNA interference to silence the expression of disease- promoting genes has substantial promise as a cancer treatment strategy.

Many oncogenes lack such hydrophobic pockets and are consequently considered "undruggable." Because RNAi inhibits oncogenes at the mRNA level prior to protein translation, these "undruggable" oncogenes can be silenced with siRNA.[9]

Although RNA interference is a promising method to treat cancer, naked siRNA must overcome several delivery barriers to be effective. For example, intravenously injected siRNA must travel through the blood to the desired tissue, penetrate the tissue and extracellular matrix, and enter the desired target cells.[4] Although intravenous injection is an attractive approach to administer siRNA for its simplicity and non-invasiveness, siRNA is rapidly degraded by nucleases in the bloodstream.[7] Additionally, siRNA is relatively large and negatively charged and therefore cannot passively enter cells, which have negatively charged membranes. Further, any siRNA that is taken up by cells faces an additional barrier to therapeutic efficacy. Specifically, endocytosed

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siRNA must escape the endosome to reach the cytoplasm since this is where mRNA resides.4,7 Due to the difficulty associated with delivering bare siRNA to cells, researchers are developing various types of carriers that can improve the circulation time, nuclease stability, cellular uptake, and cytoplasmic delivery of siRNA.[4,7]

Much of the research that aims to improve systemic siRNA delivery focuses on creating nanoscale carriers that contribute to favorable behavior in physiological conditions. These carriers must be carefully designed with optimal physiochemical properties for efficient and tumor-specific siRNA delivery. In terms of size, the particles must be large enough to allow for sufficient doses of siRNA to be encapsulated within or attached to the particle surface while remaining small enough to enter the cell. Typically, nanoparticles on the order of 20-200 nm in diameter are used to meet this criterion.[9,10]

Further, carrier materials must be chosen rationally to promote endosomal escape and gene regulation while minimizing toxicity to noncancerous cells. There are many types of carriers that are being researched that meet these criteria, each with advantages and limitations. We describe some of these below, and we also discuss research occurring at the University of Delaware in the labs of Dr. Emily Day, Dr. Millicent Sullivan, and Dr. Thomas Epps III, who are creating non-viral gene regulatory agents for enhanced cancer treatment.

One class of agents being investigated for siRNA delivery is “soft” materials, which include peptides, polymers, and lipids. For example, cationic cell- penetrating peptides (CPPs), such as TAT and Transportan, may be electrostatically complexed with negatively charged siRNA to create particles that are able to avoid endocytosis by forming pores in the cell membrane to deliver siRNA to the cytoplasm.[10–12] Similarly, positively charged polymers can bind and condense negatively charged siRNA to protect it from degradation and facilitate cell uptake by endocytosis (Figure 2a).[10,13] The mechanisms by which cationic polymer/siRNA complexes escape endosomes remain poorly understood, but may rely on endosomal osmotic swelling and subsequent rupture, releasing the endocytosed cargo into the cytosol.[14] Within this carrier subtype, dendrimers are polymers with a highly organized branched structure that extends from a central core molecule, and they offer similar advantages in siRNA encapsulation and delivery.[7] Finally, lipid-based particles such as liposomes (Figure 2b) and micelles are biocompatible and biodegradable, and are currently the furthest along in clinical development. Positively charged lipid-based carriers can be tailored to effectively protect siRNA and promote endosome escape.[7] For example, much recent research has focused on developing ionizable lipids, which become protonated to carry a net positive charge only in low pH conditions such as the interior of endosomes. Upon protonation, these ionizable lipid-based particles can fuse with the endosomal membrane to release their cargo into the cytosol and enable gene silencing.[15]

Figure 2. Examples of the types of nanoparticles used for siRNA delivery.

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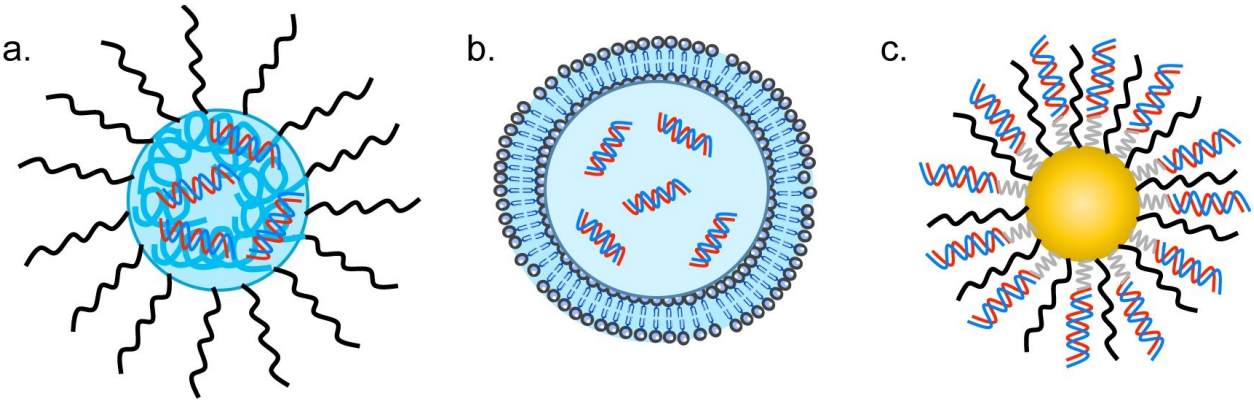

(A) Polymers (light blue) can complex with and encapsulate siRNA (dark blue/red). Additionally, polymeric nanocarriers can be coated with stealthing agents such as polyethylene glycol (PEG, black) to enhance their stability and extend their circulation time in the blood. (B) Lipsomal siRNA carriers consist of lipid bilayers that enclose siRNA inside the particle. (C) Spherical nucleic acids (SNAs) consist of gold nanoparticles densely coated with siRNA that is radially oriented away from the particle surface. SNAs are also coated with PEG for stability.

At the University of Delaware, Dr. Millicent Sullivan's lab is working in collaboration with Dr. Thomas Epps III's research group to design novel polymer structures to successfully deliver siRNA to cells.[16–18] For example, they have developed a block co-polymer (BCP) formulation that can complex with siRNA and degrade in response to light to trigger intracellular release of the siRNA cargo.[16] This BCP contains a cationic polymer region that electrostatically interacts with the siRNA, a photocleavable region that will cleave in response to ultraviolet light, and a polyethylene glycol region to impart stability and biocompatibility.

This carrier is advantageous in that it can bind siRNA molecules to keep them inactive until they are released within the cell cytoplasm by triggering the polymer's degradation with ultraviolet light. By increasing light exposure time, the researchers have been able to tune the levels of mRNA knockdown achieved upon light- triggered siRNA release from 0 to 86 percent.[16] In the future, the ability to provide cell-specific delivery of siRNA at specific points in time may enable improved treatment with reduced off-target effects. However, in vivo utility of these constructs may require the polymer design to be tailored to degrade in response to near-infrared (NIR) light, rather than ultraviolet light, since NIR light penetrates tissue more deeply than other wavelengths.[19]

The second major class of materials that is being widely investigated for siRNA delivery is metallic nanoparticles. Specifically, gold-based nanoparticles are of great interest due to their biocompatible, bioinert nature and ease of synthesis.[20] Unlike other materials, which may encapsulate siRNA, most gold nanoparticles (AuNPs) that are used as siRNA carriers are designed to display the siRNA on their surface since AuNPs are not typically porous. siRNA is typically loaded onto AuNPs by gold-thiol bonding.[20] Within this class of materials, spherical nucleic acid nanoconjugates (SNAs) have recently received much attention as siRNA delivery vehicles. SNAs consist of a gold core densely coated with a radially-oriented shell of siRNA (Figure 2c).[21] This conformation promotes siRNA stability, reduces immunogenicity, and enhances cellular uptake despite the SNAs having a net negative charge.[22,23] To date, SNAs have been used to suppress target gene expression in glioblastoma tumors[24] and in non-cancerous applications such as diabetic wound healing.[25] Additionally, SNAs have been found to be

nontoxic in animal trials,[24] validating their continued development as effective siRNA delivery vehicles.

Notably, SNAs targeting Bcl2L12, an oncogene that inhibits apoptosis, have recently been approved for human clinical trials in glioblastoma multiforme.[26] Preclinical studies performed by Dr. Emily Day, currently an Assistant Professor at the University of Delaware, with colleagues at Northwestern University, showed that SNAs that silence Bcl2L12 reduce the growth of orthotopic glioblastoma tumors in mice.24 The planned clinical trial will be invaluable for demonstrating whether the promising effects observed in murine cancer models are consistent in human tumors.

In the past five years, research has demonstrated that there are sequence-specific design rules that must be considered to develop effective SNAs. For example, the cellular uptake of SNAs and the stability of siRNA on SNAs are influenced by the nucleotide content.[27,28] Additionally, studies have shown that while SNAs can enter cells to regulate gene expression, they are relatively inefficient because a large fraction of endocytosed SNAs remain trapped in endosomes and are unable to escape into the cytoplasm to promote maximum gene silencing.[29] Dr. Emily Day's lab at the University of Delaware is actively investigating different methods to trigger SNAs' endosome escape, which would increase SNAs' potency. These investigations have revealed that coating SNAs with positively charged polymers such as polyethylenimine improves their endosome escape relative to uncoated SNAs, and these modified SNAs can enable gene silencing at low particle doses.

Beyond SNAs, a separate class of gold-based siRNA carriers that is being developed by the Day lab and by other researchers is photothermally active siRNA nanocarriers. These nanocarriers consist of nanoparticles such as nanorods or silica core/gold shell nanoshells that are densely coated with siRNA. Upon activation with near- infrared light, these nanoparticles convert the absorbed energy to heat, which both ruptures endosomes and releases siRNA from the nanoparticles' surface to enable cytoplasmic siRNA delivery.[30–32] The ability to precisely control the exact timing and location of siRNA delivery with photothermally active nanoparticles holds much potential as a new area of investigation in the realm of gene regulation.

Overall, siRNA-mediated gene regulation has substantial promise as a treatment for many types of cancer. Although there are unresolved challenges to effective siRNA delivery, recent research with nanoscale carriers has demonstrated the ability to overcome these challenges, and we have highlighted a few types of these carriers here. While some siRNA nanocarriers are now transitioning into clinical trials,[26,33] ongoing studies will continue to optimize material design to improve siRNA delivery to tumors for potent gene silencing. In the future these siRNA nanocarriers may ultimately be used to improve tumor eradication and extend patient survival.

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