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Mini Review

Small interfering RNA-based molecular therapy of cancers

Wei Guo[1,2,3], Wangbing Chen[1,2], Wendan Yu[3], Wenlin Huang[1,2,4] and Wuguo Deng[1,2,3,4]

Abstract

RNA interference (RNAi) has become a gold standard for validating gene function in basic life science research and provides a promising therapeutic modality for cancer and other diseases. This mini-review focuses on the potential of small interfering RNAs (siRNAs) in anticancer treatment, including the establishment and screening of cancer-associated siRNA libraries and their applications in anticancer drug target discovery and cancer therapy. This article also describes the current delivery approaches of siRNAs using lipids, polymers, and, in particular, gold nanoparticles to induce significant gene silencing and tumor growth regression.

Key words Cancer, siRNA, gene delivery, molecular therapy

The discovery of RNA interference (RNAi) as an effective method to knock down the expression of individual genes by double-stranded RNA (dsRNA) has fuelled renewed optimism in cancer therapy. In the future, it will be possible to use gene-specific medicine to treat human diseases, including cancers. Small interfering RNAs (siRNAs), also known as short interfering RNAs or silencing RNAs, are non-coding RNAs that are 20–25 base pairs in length. Endogenously expressed siRNAs have not been found in mammals, but they can be produced from a dsRNA and a small hairpin RNA (shRNA) by Dicer cleavage or produced by RNase III nuclease activity or chemical synthesis. Adenosine deaminase acting on RNA (ADAR), an RNA-editing enzyme, has been shown to compete for dsRNA to make it unfavorable as a substrate for Dicer and thus inhibiting siRNA formation[1]. Dicer delivers the siRNA to a group of proteins called RNA-inducing silencing complex (RISC), where the catalytic component argonaute (Ago) is capable of degrading the siRNA into a single strand to bind to the corresponding mRNA and further degrade the mRNA, resulting in gene silencing (**Figure 1**). ATP is required during the unwinding of the siRNA duplex.

The remarkable ability of siRNA to silence specific genes has been proven useful in dissecting genetic function in plant and mammalian cells. Following the first demonstration of siRNA-mediated gene silencing in mammalian cells[2], it was quickly realized that this highly specific mechanism of sequence-specific gene silencing might be harnessed to develop a new class of drugs that interfere with disease-causing or disease-promoting genes. RNAi holds considerable promise as a therapeutic approach to silence disease-causing genes, particularly those that encode so-called "non-druggable" targets that are not amenable to conventional therapeutics, such as small molecules, proteins, or monoclonal antibodies[3]. The potential of siRNA-based therapeutics in cancer treatment has also been increasingly recognized, as numerous studies have shown that the growth and proliferation of cancer cells can be greatly inhibited by using this approach *in vitro* and *in vivo*[3-5]. Furthermore, siRNA-based therapeutics have shown great potential in sensitizing cancer cells to chemotherapy by silencing genes that contribute to the occurrence of drug resistance during chemotherapy[6,7]. However, the exploration and identification of functional genes that are associated with cancer cell characteristics, such as growth, survival, apoptosis, and drug resistance, have become the important prerequisites for developing and finding effective siRNAs for cancer therapeutics and for advancing the development of targeted and personalized therapeutics. Moreover, the effective delivery of siRNA to the tumor site to achieve *in vivo* gene silencing is another major obstacle.

siRNA Library Screening and Anticancer Drug Target Discovery

Cancer cells undergo numerous genetic changes that drive cellular transformation from normal cell progenitors. Anticancer drug target discovery is now frequently directed toward understanding and

Authors' Affiliations: [1]State Key Laboratory of Oncology in South China, Guangzhou, Guangdong 510060, P. R. China; [2]Research Department, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 510060, P. R. China; [3]Institute of Cancer Stem Cell, Dalian Medical University Cancer Center, Dalian, Liaoning 116044, P. R. China; [4]Key Laboratory of Targeted Drug for Tumors of Guangdong Province, Guangzhou Double Bioproduct Inc., Guangzhou, Guangdong 510060, P. R. China.

Corresponding Author: Wuguo Deng, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 510060, P. R. China. Tel: +86-20-87343066; Email: dengwg@sysucc.org.cn.

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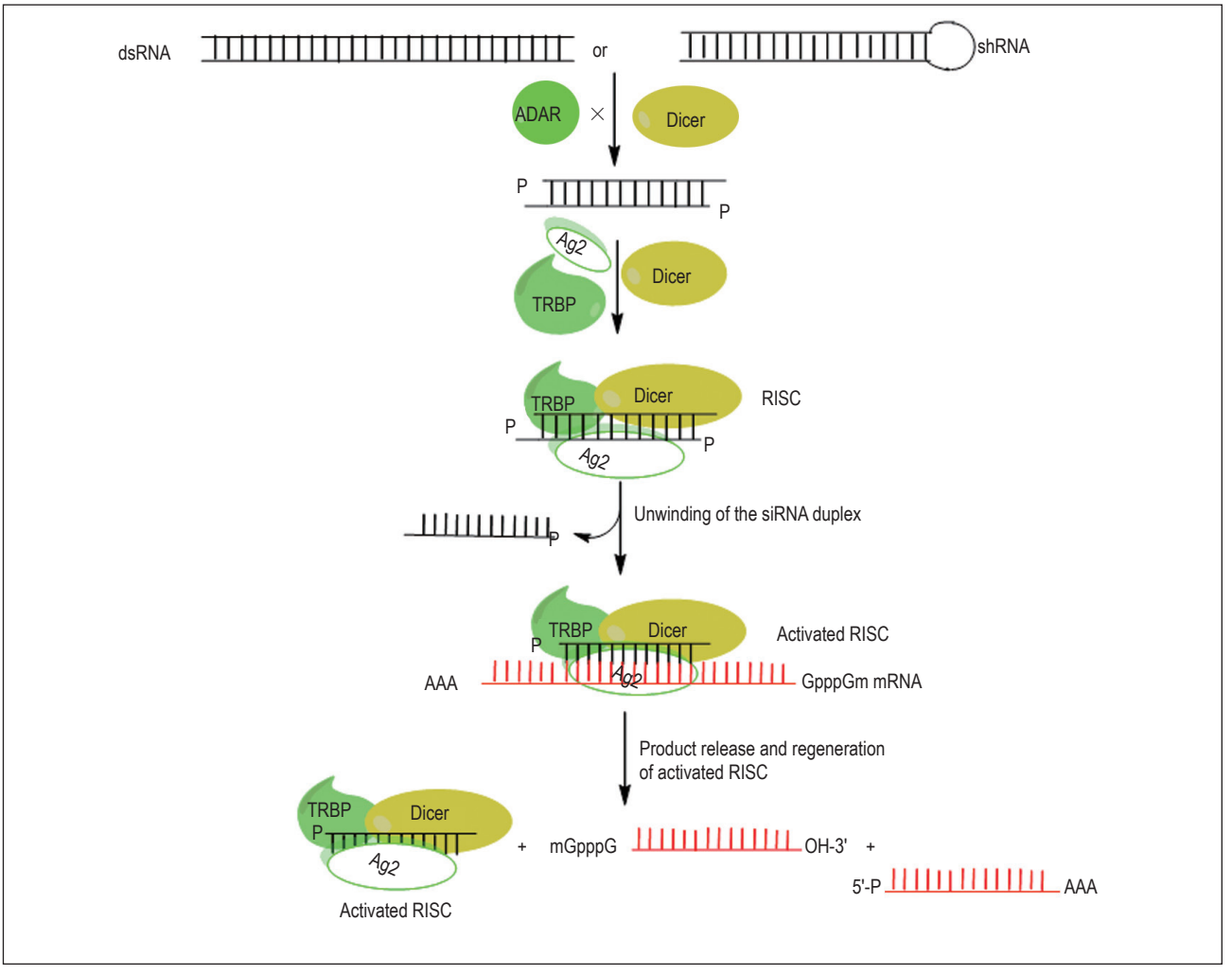

Figure 1. **Mechanism of RNA interference**[1]

exploiting the genetic alterations that exist in tumor cells. Knowledge of the genetic alterations may lead to better use of conventional therapeutics or development of new therapeutics that offer better outcomes. Loss-of-function genetic screens can identify genes whose loss of function inhibit tumor cell growth, promote tumor cell apoptosis, or enhance the cytotoxicity of chemotherapy in tumor cells. The identification of genes that, when silenced, selectively enhance the chemosensitivity of tumor cells would make attractive drug targets. Drugs developed to target these genes have the potential to selectively increase the toxicity of chemotherapy in cancer cells.

Gene silencing by RNAi is a powerful genetic tool for identifying genes involved in specific biological processes in model organisms and human cells. With the advent of large-scale gene knockdown using siRNA libraries, it has become possible to quickly identify new drug targets as well as explore their roles in tumorigenesis[8-12]. Currently, RNAi is being widely used in mammalian cell-based systems to probe known signal transduction pathways for the identification of novel genes. Numerous studies using siRNA libraries have identified Akt-cooperating kinases[13], genes influencing TRAIL-induced cell killing[14], novel regulators of apoptosis and chemoresistant genes[15], products involved in endoplasmic reticulum stress-dependent apoptosis[16], and a novel familial cylindromatosis tumor suppressor gene found to negatively regulate NF-κB signaling[17]. In addition to these smaller scale studies, RNAi has also rapidly expanded to systematic, larger scale gene knockdown studies in mammalian cells[18,19]. Retroviral-based siRNA libraries targeting about one-third of the human genome have successfully identified genes involved in p53-mediated cell cycle arrest[20], human proteasome function[21], and novel tumor suppressor pathways. Large siRNA libraries generated from the processing of long dsRNAs by *Escherichia coli* RNaseIII endoribonuclease have also been screened to successfully identify genes required for mitosis[22]. Recently, siRNA and shRNA screens in human cells have successfully identified genes that are important for cell growth, apoptosis, chemoresistance, and chemosensitivity[23-25]. In our current study, we have established and screened a druggable siRNA library targeting > 6,000 human genes in melanoma cells to identify genes important for cell survival and chemoresistance. The findings of this screen may provide important

information for understanding the molecular mechanisms involved in melanoma tumorigenesis and drug resistance. In addition, these identified genes may constitute a novel set of targets for melanoma therapy.

Tumor-targeting Delivery of Functional siRNAs Based on Gold Nanoparticles

The specific delivery of therapeutic siRNAs to the tumor parenchyma remains an intractable problem. The initial therapeutic applications of siRNAs only relied on local delivery into the specific tissue or tumor site, but for the true therapeutic value and clinical benefit to cancer therapy, siRNAs need to be introduced systemically. The systemic delivery of siRNAs is becoming a major topic in cancer therapy but also facing many challenges, such as how to get siRNAs to interfere with specific gene targets in the correct tissue and cell types at a safe and pharmacologically effective level and how to maintain the stability of siRNAs in circulation, enhance the cellular uptake, and monitor their distributions and therapeutic efficacies. These challenges need to be addressed for the successful development of novel delivery vehicles and targeting strategies. Lipid-, polymer-, and nanoparticle-based vehicles for the systemic delivery of siRNAs have been developed and tested for delivery to the lung, liver, and other local tumors in animal models, including non-human primates. These different approaches for siRNA delivery exhibit various advantages and disadvantages (**Table 1**). Moreover, these siRNA delivery vehicles also present a variety of potential problems regarding toxicity, immune and inflammatory responses, gene-control and gene-targeting issues. To realize the full potential of siRNA-based therapeutics, new strategies are required to substantially improve their delivery efficiency, toxicity profiles, monitoring techniques, and pharmacologic and therapeutic efficacy.

Gold nanoshells (GNS), specially designed and optically responsive nanoparticles, have been developed and applied in biomedical applications[26-30]. These nanoshells, usually consisting of a silica core and a gold shell, can be designed to absorb specific

Table 1. Comparison of different approaches for small interfering RNA (siRNA) delivery

Approach for siRNA delivery	Examples	Advantages	Disadvantages
Liposome-based delivery systems	DOTAP cationic liposomes; PEGylated immunoliposome; RGD-modified DOTAP cationic liposomes; PEGylated DOTAP cationic liposomes	High transfection efficiency; enhanced pharmacokinetic properties; relatively low toxicity and immunogenicity; protect siRNAs from enzymatic degradation, and provide reduced siRNA renal clearance; targeted therapeutic efficacy of liposomes can be achieved by conjugating specific ligands to the lipid molecule	Cell toxicity caused by cationic lipid is still a major concern; the *in vivo* therapeutic effect is still not ideal.
Nanoparticle-based delivery systems	PEGylated nanoparticles; calcium phosphate (LCP) nanoparticle; chitosan/polyethylenimine nanoparticles; magnetic resonance (MR)-sensitive liposome-entrapped siRNA NPs	Long circulation time due to the hydrophilic shell; improve the pharmacokinetics, pharmacodynamics, biodistribution, and toxicology; promote desired tissue distribution profiles through EPR effect or linking targeting moieties	NPs with a diameter greater than 100 nm are recognized by the RES, and have a short half-life.
Dendrimer-based delivery systems	PPI; PAMAM; dendrosomes; PAMAM-PEG-PLL	Controllable molecular weight; large number of readily accessible terminal functional groups to conjugate ligands; ability to encapsulate siRNA within internal cavities; higher branched dendrimers exhibit longer circulation half–lives; owes the proton sponge effect	Cytotoxicity augments as their generation increases; still very limited for clinical use
Carbon nanotube-based delivery systems	Single-walled; multi-walled	Ability to perform controlled and targeted RNA delivery; ability to penetrate cells because of needle-mechanism	Have been introduced to gene delivery research for a limited number of years and more research needs to be explored

NP, nanoparticles; PPI, polypropylene imine; PAMAM, polyamidoamine; PAMAM-PEG-PLL, multifunctional triblock nanocarrier; PEG, polyethylene glycol; PLL, PEGylated poly(l-lysine).

wavelengths of light, which is determined by the relative size of the core and shell layers[31]. They can be tuned to absorb light in the infrared region of the spectrum, known as the "water window," where light penetrates tissue by several inches; virtually all soft tissues of the body are optically accessible by the infrared region of the spectrum.

When these nanoparticles are illuminated with infrared light at their resonant wavelength, their local photothermal response can be used to trigger drug release from a carrier in the local vicinity of the nanoparticles[32]. Several novel and further modified GNS nanocarriers have been developed and applied in recent years to mediate siRNA delivery and have achieved efficient silencing of target genes *in vitro* and *in vivo*[33,34]. GNS-based RNA delivery systems potentially represent a powerful nanotechnology for cancer therapy[35]. By collaborating with other research groups, we proposed and developed novel siRNA-GNS complexes that combine the molecular targeting of cancer cells with the light-triggered release of therapeutic siRNA. Functional siRNAs identified from our established siRNA library, directed against genes required for melanoma cell growth, proliferation, and chemoresistance, could be bound to the nanoparticle surface and delivered in a protective complex. This approach will offer a novel systemic siRNA delivery strategy that couples the molecular targeting of cancer cells with the infrared-triggered local release of functional siRNAs for human melanoma cancer therapy. If successful, this GNS-siRNA delivery system will be a major advancement in siRNA delivery technology and tumor-targeted therapy and may have immediate translational applications for systemic treatment of melanoma.

Preclinical Studies and Clinical Trials of siRNAs for Cancer Treatment

The effectiveness of gene-silencing approaches in suppressing oncogenes for cancer therapy has been demonstrated by many preclinical studies. Some of the approaches have entered clinical trials. siG12D, an siRNA drug encapsulated within a miniature biodegradable polymeric matrix and targeting the *KRAS* oncogene, is in a phase I trial for pancreatic ductal adenocarcinoma treatment with intratumoral injection. It has been proved to be active, but its combination with chemotherapy in patients with unresectable, locally advanced pancreatic cancer has not yet been shown to be successful in a phase II trial by Silenseed Ltd[36]. Tekmira Ltd developed another siRNA drug, TKM-PLK1, which is encapsulated in a lipid particle and targets polo-like kinase 1 (PLK1), a validated oncology target involved in tumor cell proliferation, and began a phase I trial for patients with advanced solid tumors. The previous research showed that TKM-PLK1 might be effective in treating colorectal, breast, non–small cell lung, and ovarian cancers. ALN-VSP02, a therapeutic agent with two distinct siRNAs encapsulated within a lipid nanoparticle and that targets vascular endothelial growth factor (VEGF) and kinesin spindle protein (KSP), was generated by Alnylam Pharmaceuticals and was well tolerated in a phase I trial of liver cancer treatment[37]. The long-term follow-up of the patients treated with ALN-VSP02 is continuing in a second phase I trial (NCT01158079). The University of Duisburg-Essen also sponsored a phase I trial of an siRNA drug against Bcr-Abl with anionic liposomes, but the inhibition of the oncogene mRNA was not stable[38]. The first receptor-mediated delivery of siRNA nanoparticles to treat relapsed/refractory cancers was developed by Calando Pharmaceuticals[39]. The siRNA was condensed into cyclodextrin nanoparticles coated with polyethyleneglycol, and the human transferrin protein functioned as the ligand to the transferrin receptor, which is often highly expressed in tumor cells. Such nanoparticles have also entered phase I trial (NCT00689065) in adults with solid tumors[39].

Perspectives of siRNAs in Molecular Cancer Therapy

A large number of *in vitro*, animal, preclinical, and clinical studies have proven the specificity and efficiency of siRNAs to induce the cleavage of mRNA transcripts, resulting in the down-regulation of oncogenes and associated genes in cancer treatment. However, siRNA-based therapies have encountered many obstacles in clinical trials, including the stability of the RNA molecule itself, minimization of nonspecific inflammation, controlled release of the RNA molecule, and specificity and efficiency of the delivery vehicles. These barriers must be overcome for the future success of clinical application of siRNAs. Chemical modifications might be required to improve the stability of the siRNA molecules and minimize the non-specific immunogenicity. Tailored carriers should also be developed for efficient and specific delivery. Many established improvements have shown the great potential of siRNA therapeutics in clinical trials of cancer therapy, but more advanced delivery strategies are needed for siRNAs to fully play their roles in cancer therapy. A multi-component design, such as using a PEGylated, tumor-specific ligand-decorated nanoparticles combined with other light-, thermal-, pH-, or magnetic-sensitive components, might improve the precision, specificity, and efficiency of siRNA delivery to tumor sites and into tumor cells through systemic delivery, especially for those sites unsuitable for local treatments. In general, siRNA-based treatment has opened a new window for cancer therapy. However, biochemical modifications of siRNAs have been and will continue to be made to maximize their potency, minimize their off-target effects, and minimize their other adverse effects to accelerate the translation of siRNA drugs from the bench to clinical application.

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