Table 1. Current states of clinical candidate pipeline for RNAi therapy

ClinicalTrials.gov identifier	Drug	Route	Delivery	Disease	Target	Phase	States	Company
NCT00499590	Bevasiranib	IVT	Naked siRNA	Wet AMD	VEGF	III	Terminated	Opko Health
NCT00363714, NCT00395057	AGN211745/ Sirna–027	IVT	Naked siRNA	AMD	VEGF-R1	II	Terminated	Allergan/ Sirna
NCT01065935, NCT00658086	ALN-RSV01	Nebulization	Naked siRNA	RSV infection after lung transplantation	RSV Nucleocapsid	II	Completed	Alnylam
NCT00306904	Bevasiranib	IVT	Naked siRNA	DME	VEGF	II	Completed	Opko Health, Inc.
NCT01445899	PF-04523655	IVT	Naked siRNA	DME	RTP801	II	Recruiting	Quark Pharma
NCT01200420	miravirsen	SC	Naked LNA	HCV	miR-122	II	Completed	Santaris Pharma
NCT01551745 NCT01505166	FANG vaccine	*Ex vivo*, Intradermal	Electroporation	Ovarian cancer, colon cancer	Bi-shRNA-Furin and GM-CSF	II, II	Recruiting, Recruiting	Gradalis, Inc.
NCT00802347	I5NP	IV	Naked siRNA	DGF in kidney transplantation	P53	I/II	Recruiting	Quark Pharma
NCT01227291	SYL040012	Ophthalmic drops	Naked siRNA in ophthalmic drops	Glaucoma and ocular hypertension	Adrenergic receptor beta-2 siRNA	I/II	Completed	Sylentis
NCT00725686, NCT00713518	PF-04523655	IVT	Naked siRNA	Wet AMD	RTP801	I, II	Completed	Pfizer/Quark
NCT00716014	TD101	Intralesional	Naked siRNA	Pachyonychia congenita	Keratin 6a N171K mutant mRNA	Ib	Completed	TransDerm/ IPCC
NCT00882180, NCT01158079	ALN-VSP02	IV	SNALP	Liver cancer, solid tumors	KSP and VEGF	I, I	Completed	Alnylam
NCT00554359, NCT00683553	I5NP	IV	Naked siRNA	AKI for major cardiovascular surgery	P53	I, I	Completed, terminated	Quark Pharma
NCT01148953	ALN-TTR01	IV	SNALP	TTR-mediated amyloidosis	Transthyretin	I	Completed	Alnylam
NCT00689065	CALAA-01	IV	RONDEL	Solid cancer	RRM2	I	Active	Calando Pharma
NCT00466583	EZN-2968	IV	Naked LNA	Advanced solid tumor, lymphoma	HIF-1a	I	Completed	Santaris Pharma
NCT01120288	EZN-2968	IV	Naked LNA	Liver metastases	HIF-1a	I	Recruiting	NCI
NCT00672542	siRNA in dendritic cells	*Ex vivo*, Intradermal	Electroporation	Metastatic melanoma	Immunoproteasome subunits LMP2, LMP7, MECL1	I	Active	Duke University
NCT01061840	FANG vaccine	*Ex vivo*, Intradermal	Electroporation	Solid tumors	Bi-shRNA-Furin and GM-CSF	I	Recruiting	Gradalis, Inc.
NCT01064505	QPI-1007	IVT	Naked siRNA	Optic atrophy	Caspase-2	I	Active	Quark Pharma
NCT00938574	Atu027	IV	AtuPLEX	Advanced solid cancer	PKN3	I	Completed	Silence Therapeutics
NCT01188785	siG12D LODER	EUS biopsy needle	LODER polymer	Pancreatic ductal adenocarcinoma	KRASG12D	I	Recruiting	Silenseed Ltd
NCT01262235	TKM-080301	IV	SNALP	Cancer	PLK1	I	Recruiting	Tekmira
NCT00927459	PRO-040201	IV	SNALP	Hypercholesterolemia	Apo B	I	Terminated	Tekmira

AKI, acute kidney injury; AMD, age-related macular edema; DGF, delayed graft function; DME, diabetic molecular edema; HCV, Hepatitis C Virus; IV, intravenous; IVT, intravitreal; KSP, Kinesin Spindle Protein; LNA, locked nucleic acids; NCI, National Cancer Institute; PEG, polyethylene glycol; PLK1, Polo-like Kinase I; RRM2, Ribonucleotide Reductase M2; RSV, respiratory syncytial virus; SNALP, stable nucleic acid lipid particle; TF, transferrin; TTR, transthyretin; VEGF, vascular endothelial growth factor; SC, subcutaneous.
*From ClinicalTrials.gov.

application, such as stable nucleic acid lipid particles (SNALPs) and RNAi/oligonucleotide nanoparticle delivery (RONDEL), are available. These technologies have been shown to be effective *in vivo* (25–27), and progress is being achieved in some clinical trials (ALN-VSP02, ALN-TTR01, CALAA-01, TKM-080301, PRO-040201). In cancer treatment, siRNAs targeting polo-like kinase I (PLK1), kinesin spindle protein (KSP) and vascular endothelial growth factor, which are formulated with SNALP or RONDEL, have been developed as candidate pipelines in Phase I (Table 1).

DRUG DELIVERY SYSTEM FOR SYNTHETIC OLIGONUCLEOTIDE

Nucleic acid medicines, including siRNA, miRNA and anti-miRNA, work only after they penetrate hydrophobic cellular membranes. However, it is not easy for them to go through the lipid bilayer membrane without their carrier because synthetic oligonucleotides are negatively charged. In addition to this, RNAs are very easily degraded by RNase *in vivo*. Accordingly, assisting carriers or chemical modifications for the progression of the transmembrane transport and for the inhibition of the degradation by serum RNases are required. Historically, viral and non-viral delivery has been utilized (Table 2). In a viral delivery system, it was reported that an adenovirus carried short hairpin RNA (shRNA) expression vector targeting angiotensin type 1 (AT1) delivered into the brain intracerebroventricularly (ICV) (28) and that the miR-23b expression vector and miR-23b sponge worked in inflammatory autoimmune diseases *via* intra-articular (IA) infection (29). An adeno-associated virus (AAV) was also successful at carrying a miRNA cluster into the muscle and shRNA vectors targeting mutant huntingtin into the brain by topical administration (30,31). Furthermore, miR-34a treatment prevented lung cancer initiation and progression *via* transtracheal infection, and shRNA targeting superoxide dismutase 1 (SOD1) inhibited amyotrophic lateral sclerosis progression by lentiviral-mediated RNAi (32,33). The herpes simplex virus, which commonly causes an eruption of fluid-containing vesicles on the mouth, lips or face, also has potential for cancer treatment and therapeutic pain relief (34,35). Thus, viral-mediated gene silencing is very useful for local infection, particularly at sites that make frequent administration difficult. Although viral delivery has frequently shown higher efficiency than that by non-viral systems, preliminary clinical studies have shown that it triggered strong inflammatory reactions (36), and these delivery vectors have caused the death of several patients in the clinic (37,38). Thus, understanding the details of the inflammatory mechanism and developing safer viral vehicles are important tasks ahead.

On the other hand, the focus has recently been on the non-viral approach because of its advantages over viral vectors, such as non-immunogenicity, low production cost and easy quality control. This approach requires an optimized delivery reagent, such as a cationic lipid, polymers, nanoparticles, carbon nanotubes and atelocollagen (Table 2). In cancer treatment, atelocollagen or cationic liposome- or polymer-mediated transfection reagents have commonly been used to deliver siRNA or miRNA to cells *in vitro* and *in vivo*. In particular, a number of reports have demonstrated a significant anti-cancer effect caused by systemic delivery of siRNA with cationic liposome (39–41). Similarly, a cationic polymer, polyethyleneimine, was commercialized as *in vivo*-jetPEI™, provided by Life Technologies, and was used to successfully deliver siRNAs to cancer cells in animals (42,43). In addition, atelocollagen can be obtained from type I collagen of calf dermis and has also been expected to be a useful carrier because of its low immunogenicity and efficiency (8,44–46). In case of miRNA therapy, a tumor-suppressive miR-16 mimic was successfully delivered by the systemic approach using atelocollagen, and it dramatically inhibited the growth of metastatic prostate cancer (47). Furthermore, chemically functionalized carbon nanotubes also show potential for novel biological applications for the delivery of Caspase-3 siRNA into the brain by topical injection into the cerebral cortex and reduced neurodegeneration without toxic side effects (48).

In a recent study, the focus was on highly stabilized nanoparticles, and these nanoparticles made the systemic delivery system dramatically more efficient (25,49–52). For example, synthetic miR-34a mimic, which was incorporated into cholesterol, and the cationic liposome *N*-[1-(2,3-dioleoyloxy)]-*N*,*N*,*N*-trimethyl ammonium propane (DOTAP) (1:1 mol/mol) and polyethylene glycol (PEG)-conjugated CG4-targeting single-chain antibody fragment were efficiently delivered into melanoma and inhibited lung metastasis (53). The nanocarrier 'SNALP' by Tekmira pharmaceuticals is one of the technologies with the most potential in the clinical pipeline. SNALP is a PEG-grafted monolamellar liposome that can easily avoid opsonization and subsequent recognition by the macrophages because the hydrophilic nature of PEG constructs an aqueous coating on its particle surface (54). In the work of Judge *et al.*, SNALP-formulated siRNAs against PLK1 and KSP displayed significant anti-tumor effects in liver tumor model mice (26). Successful results have already been reported in the treatment of transthyretin-mediated amyloidosis, hypercholesterolemia, Ebola virus infection (49) and cancer (50). The clinical trials have been identified as NCT00882180, NCT01158079, NCT01148953, NCT01262235 and NCT00927459 in the ClinicalTrials.gov database (http://clinicaltrials.gov).

The effective systemic delivery of siRNA or miRNA toward target cells or tissues has been enormous challenge for RNAi therapy. Indeed, naked siRNAs are rapidly eliminated by the kidneys, and nanoparticle-formulated siRNAs have a tendency to accumulate in the liver. In particular, their suitability for cancer cells depends on the enhanced permeability and retention effect of nanoparticles. To solve these problems, combined use with orienting molecules, such as a cell-specific ligand, can increase the cell or tumor

Table 2. Technologies for drug delivery systems in RNAi therapy

Delivery	Tissue	Route	RNA	References
Viral vector				
Adenovirus	Articulation	IA	miR-23b	(29)
	Brain	ICV	AT1a, AT1b shRNA	(28)
Adeno-associated virus	Muscles	IM	Anti-VEGF miRNA cluster	(30)
	Brain	Intrastriatal	mHTT shRNA	(31)
Lentivirus	Lung	Transtracheal	miR-34a	(32)
	Spinal cord	Intraspinal	SOD1 shRNA	(33)
Herpes simplex virus	Dorsal root ganglia	Injection into the sciatic nerve	Trpv1 shRNA	(34)
	Glioma	IT	EGFR shRNA	(35)
Non-viral reagent				
Liposome				
Oligofectamine	Colon cancer	IP/IV	B-catenin siRNA	(40)
DOTAP	Liver, spleen	IV	GFP siRNA	(39)
LIC-101	Liver metastasis	IV/SC	BCL-2 siRNA	(41)
PEI	Ovarian canner	IP/SC	HER-2 siRNA	(42)
	Glioblastoma	IP/SC	PTN siRNA	(43)
Nanoparticle				
SNALP	Ebola virus	IP/SC	ZEBOV siRNA	(49)
	Lung cancer	IV	miR-34a/let-7	(50)
RONDEL	Melanoma	IV	RRM2 siRNA	(25)
	Ewing's sarcoma	IV	EWS-FLI 1 siRNA	(51)
AtuPLEX	Prostate/pancreatic cancer	IV	PKN3 siRNA	(52)
DOTAP, cholesterol and PEG	Melanoma	IV	c-Myc/MDM2/VEGF siRNA and miR-34a	(53)
Atelocollagen	Testicular cancer	IT	HST-1/FGF-4 siRNA	(45)
	Osteosarcoma	IV	miR-143	(112)
	Prostate cancer		miR-16	(47)
HDI	Liver	IV	HBV siRNA	(113)
Carbon nanotube	Brain	Into the cerebral cortex	Caspase-3	(48)

ApoB, Apolipoprotein B; AT1, Angiotensin type 1; DDAB, dimethyldioctadecylammonium bromide; DOTAP, (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethyl-ammonium methylsulfate; HBV, Hepatitis B Virus; HDI, hydrodynamic tail vein injection; HER-2, human epidermal growth factor receptor 2; IA, intra-articular, ICV, intracerebroventricular; IM, intramuscular; IP, intraperitoneal; IT, intratumor; IV, intravenous; mHTT, mutant huntingtin; PBAVE, poly butyl and amino vinyl ethers; PEI, polyethyleneimine; PKN3, Protein Kinase N3; PPARA, peroxisome proliferator-activated receptor alpha; PTN, pleiotrophin; SC, subcutaneously; SLN, solid lipid nanoparticle; SOD1, superoxide dismutase 1; ZEBOV, The Polymerase (L) Gene of the Zaire Species of Ebola Virus.

specificity and delivery efficiency (55–57). Calando's cyclodextrinpolymer-based delivery platform (RONDEL) consists of cyclodextrin-containing polycation, and adamantine-coupled PEG-stabilized some ligands, such as transferrin (TF), and siRNA or miRNA (Fig. 2). This siRNA delivery platform was conceived by Hu-Lieskovan *et al.* in 2005 (51). The TF receptors are known to be upregulated in malignant cells, and TF-stabilized particles are taken up into cancer cells by TF receptor-mediated endocytosis and subsequent release into the cytoplasm in a pH-dependent manner (25). Phase 1b clinical trials of CALAA-01, including the M2 subunit of ribonucleotide reductase (RRM2) targeting siRNA, are being conducted as a novel RNAi therapy for multiple types of solid tumors.

CHEMICAL MODIFICATIONS FOR OLIGONCLEOTIDES

In addition to the nanocarriers mentioned above, others are being sought through chemical modifications. The purpose of such modifications can be permeability into the cells, specificity for specific tissues and stability against nuclease degradation (Fig. 3 and Table 3). For example, as a

permeability-enhancing factor, the covalent conjugation of the lipophilic molecule assists siRNA or miRNA to penetrate into the cellular cytoplasm and trigger gene silencing *in vivo* (58). In particular, high-density lipoprotein (HDL)-conjugated siRNAs are selectivity taken up by the gut, kidney and steroidogenic organs *via* the HDL receptor, scavenger receptor class B, type I (SR-BI) (59–62). In contrast, low-density lipoprotein (LDL)-conjugated siRNAs are efficiently internalized into the hepatocytes after binding to the LDL receptor (59). The Arrowhead Research Corporation demonstrated that the co-injection of cholesterol-siRNA and hepatocyte-targeted endosomolytic polymer achieved high-level target gene knockdown with low doses of cholesterol-siRNA in non-human primates (63). The company is using this strategy and a polymer-based siRNA delivery platform named dynamic polyconjugate polymer in ARC-520, which is a hepatitis B clinical candidate.

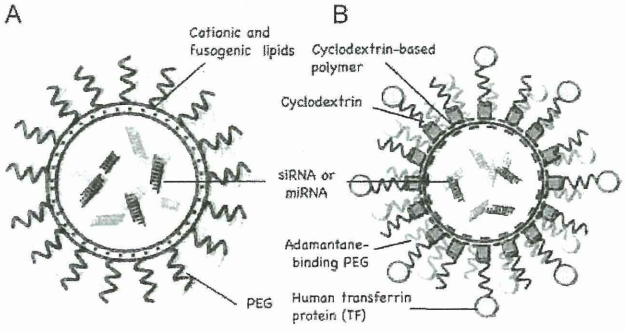

Figure 2. Delivery technology for RNAi therapy. (A) Stable nucleic acid lipid particle (SNALP). The bilayer consists of cationic and neutral lipids and is coated by PEG. The diameter is ~100 nm. (B) RNAi/oligonucleotide nanoparticle delivery (RONDEL). RNAs are protected from degradation in serum by the cyclodextrin-conjugated polymer. The complexes are <100 nm in diameter. In aqueous solution, adamantane easily binds to cyclodextrin as a result of hydrophobic attraction.

In another example, nanoparticles composed of poly (lactic-*co*-glycolic acid) were modified with a cell-penetrating peptide, penetratin, and used for the systemic delivery of the miR-155 inhibitor in the mouse model of lymphoma (64).

On the other hand, cell-specific factors, such as aptamers (65,66), peptide (64,67), antibodies (68,69) and agonists (56), can enhance cell specificity in cases of systemic administration into experimental animals. For example, octaarginine-modified liposomal particles were used to suppress an endogenous gene in the liver at low concentrations of siRNA without any toxicity (67). Usually, targeting proteins were conjugated to cationic bridges, such as polylysine or protamine, which can mediate uptake of nucleic acids, to link targeting proteins to effector oligonucleotide (68,70–72). In contrast, the siRNA-aptamer chimeras have also been of interest because a completely RNA-based approach may have important advantages over other methods for targeted delivery of siRNAs in terms of cost, productivity, safety and flexibility regarding chemical modification. RNA aptamers are single-stranded oligonucleotides and bind with high affinity to specific molecular targets, such as small molecules, proteins and nucleic acids, with their 3D structure (65,66). Here, although antibody-mediated siRNA delivery is required for the biological production of antibodies and antibody-siRNA conjugations by using a linker such as PEG, chimeric aptamer-siRNA can be synthesized as a single unit at once. However, for the utilization of chimeric aptamer-siRNA, more structured RNAs capable of binding with higher affinity and specificity have been required.

Stabilization in serum has been developed for the inhibition of the nuclease activity. Indeed, the backbone linkage introduced phosphorothioate (PS) or the sugar conjugated with protecting groups such as 2′-*O*-methyl (2′-*O*-Me), 2′-fluoro (2′-F), 2′-*O*-(2-methoxyethil) (2′-*O*-MOE), 5′-methylenephosphonate (5′-MP) and 5′-(E)-vinyl-phosphonate (5′-VP)

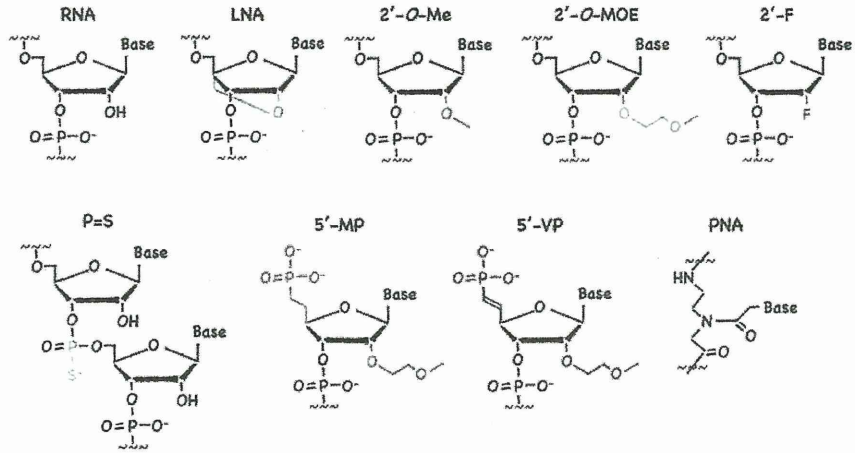

Figure 3. Chemical modifications for stability. Sugar, backbone and base modifications are illustrated. Shown are locked nucleic acid (LNA), phosphorothioate (P = S), 2′-*O*-methyl (2′-*O*-Me), 2′-fluoro (2′-F), 2′-*O*-(2-methoxyethil) (2′-*O*-MOE), 5′-methylenephosphonate (5′-MP), 5′-(*E*)-vinyl-phosphonate (5′-VP) and peptide nucleic acid (PNA).

Table 3. Chemical modifications for permeability and specificity

Chemical modification	Tissue	Factor	Route	RNA	References
PEG, PBAVE and ligand	Liver	NAG	IV	ApoB and PPARA siRNA	(114)
Aptamer	PSMA-positive prostate cancer	Anti-PSMA aptamer	IT	PLK-1/BCL-2 siRNA	(65)
	HIV-infected T cells	Anti-gp120 aptamer	IV	tat/rev siRNA	(66)
Cholesterol	Colon adenocarcinoma	Cholesteryl oligo-D-arginine	IT	VEGF siRNA	(58)
	Liver	HDL/LDL	IV	ApoB siRNA	(59)
Antibody	HIV-infected T cells	Anti-HIV Envelope Fab	IV	gag siRNA	(68)
	Hepatocellular carcinoma	Anti-EGFR Fab	IV	Luciferase siRNA	(69)
Peptide	Liver	Octaarginine	IV	SR-B 1 siRNA	(67)
	Lymphoma	Penetratin	IV/IT	Anti-miR-155-PNA	(64)
Agonist	TLR9 + myeloid cells and B cells	Anti-TLR9 agonist	IV/IT	Stat3	(56)

NAG, *N*-acetylgalactosamine; TLR, toll-like receptor.

enhance the resistance against exonuclease or endonuclease activity (73,74) (Fig. 3). Currently, the most consequential modification is the PS inter-nucleotide linkages that have been developed in the history of anti-sense oligonucleotides and have contributed to remarkable stabilization of double-strand RNA as well as the single-strand oligonucleotide (75,76). However, the influence of chirality in the phosphorus atom on the stability and the activity of duplexes is not entirely understood. Therefore, further investigation of the thermodynamic features and physiological activity with regard to the assignment of the absolute configuration will be required for therapeutic applications.

As reported above, a number of chemical modifications have been produced, which have enhanced the potential of siRNA, miRNA, miRNA inhibitors and anti-sense oligonucleotides. However, it has been required that the optimization of the modifications need to be optimized, as their efficiency depends on the position and combination. In 2012, chemical modifications were optimized for single-stranded siRNAs (ss-siRNAs), and the change was an important advancement for the practical application of RNAi therapeutics. It was shown that ss-siRNA with a number of chemical modifications, such as 5′-phosphonate and 2′-MOE-modified 5′-terminal nucleotide, 2′-F and 2′-*O*-Me motifs with contiguous PS modifications and 2′-MOE-modified adenosine dinucleotide at the 3′ terminus and C16 modification, brought about significant and efficient target gene silencing *in vivo via* the Ago2-mediated RNAi pathway (74). Furthermore, chemically modified ss-siRNAs targeting mutant huntingtin mRNAs have been employed as a novel nucleic acid drug for therapeutic application for Huntington's disease (77). Although single-stranded RNAs (ssRNAs) have been shown to have extremely rapid degradation in serum and poor activities so far (78,79), they have advantages, such as the absence of risk of undesirable off-target effects by passenger strand and the potential of systemic delivery without complex lipid formulations that sometimes trigger the inflammatory toxicities (80). Hence, these stabilized ssRNAs are expected to place RNAi therapy in a prominent class of nucleic acid drugs.

DYSREGULATED MIRNA AS THERAPEUTIC TARGET IN CANCER TREATMENT

The alterations of miRNA expression profiling are significantly related with cancer initiation and progression. To identify dysregulated miRNAs in the physiological and pathological pathway of cancer malignancy is the first step for therapeutic applications. Generally, the widespread disruption of miRNAs is caused by at least three different mechanisms: the loss, amplification or mutation of a fragile cancer-related genomic region; the change of epigenetic control; and the abnormality of miRNA-processing steps. The genetic change has the potential to affect radically the abundance of miRNA, and it was reported that >50% of miRNAs locate on the fragile genomic region in cancer (81–83). For instance, a significant downregulation of miR-15 and miR-16, which is caused by deletion or mutation in chromosome 13q14.3, was observed in 70% of patients with chronic lymphocytic leukemia.

On the other hand, CpG-island hypermethylation and histone modification as good markers for functional miRNA have also been investigated by using 5-aza-2′-deoxycytidine and a histone decetylase inhibitor, such as 4-phenylbutyric acid or trichostatin A (84–86). For example, miR-124a that regulates the expression of cyclin D kinase 6 was located in three chromosome loci, 8p23.1, 8q12.3 and 20q13.33, and these regions were hypermethylated in 75% of patients with primary colorectal tumors (87). In addition to genetic and epigenetic validation, alterations of the protein machinery related to the biogenesis of miRNA might impair global

miRNA expression. Indeed, a copy number change of DICER1 and Ago2 is frequently observed in melanoma, breast and brain cancer (88). Especially, TAR RNA-binding protein 2 (TARBP2), in the DICER-containing complex, showed frameshift mutations and caused a destabilization of DICER1 protein, resulting in global downregulation of mature miRNA in colorectal and gastric tumors (89). According to one estimate, the widespread downregulation of the miRNA expression levels is prevalent in several cancer types (90,91). In contrast, a kind of multi-functional polyphenolic compound, resveratrol, which is present in red wine, induced widespread upregulation of miRNAs and inhibited tumor growth through the acceleration of the expression and activity of Ago2 (92). Thus, the observation and management of the total balance of miRNAs are important for cancer diagnosis and treatment.

INHIBITION OF MIRNA EXPRESSION AND FUNCTIONS

For the therapeutic applications of miRNA, the intracellular expression levels of miRNAs have to be artificially controlled. Although it is relatively easy to upregulate miRNAs, the strategy for the downregulation of miRNAs requires a refined miRNA inhibitor such as a chemically modified antisense oligonucleotide. As the inhibitor against endogenous miRNA, locked nucleic acid (LNA), which has a methylene bridge connecting 2′ and 4′ carbons, is one of the most widely used platforms. LNA nucleotide organizes the phosphate backbone in the *N*-type (C3′-endo) conformation, whereas, in general, the conformations of DNA or RNA duplexes are flexible between *N*-type and S-type (C2′-endo). This conformational change contributes to a more efficient stacking of the nucleobases and functional inhibition of target miRNAs (93). In therapeutic applications, LNA against the liver-expressed miR-122, which is a potential therapeutic target in the hepatitis C virus (HCV), accomplished the long-lasting reduction of mature miR-122 and suppression of HCV viremia (94,95). Furthermore, LNA against hypoxia inducible factor 1α, the primary transcription factor activated by hypoxia that allows glycolysis and angiogenesis to progress, provides significant lowering of the expression of HIF1-α and suppression of tumor growth. Clinical trials of these LNA against miR-122 (SPC3649) and HIF1-α (EZN-2968) have progressed to Phases I and II by Santaris Pharma.

In addition to this, as competitive inhibitors of miRNAs, the miRNA sponge (96), the tough decoy (TuD) RNA (97), antagomirs (98), peptide nucleic acids (PNAs) (99) and anti-miRNA oligonucleotides (AMOs) (100) have also been developed toward medical practice targeting onco-miRNA as well as LNA. Antagomirs composed of 2′-*O*-Me, PS and cholesterol modification were the first miRNA inhibitors that provided a significant reduction in mammals (98,101). However, antagomirs were excluded as clinical candidates because they were less effective than other miRNA inhibitors. PNAs are replaced its sugar-phosphate backbone to

Table 4. Programs of clinical/pre-clinical study in miRNA therapeutics

miRNA	Therapy	Disease	Phase	Company
miR-208/499	Inhibitor	Chronic heart failure	Pre-clinical	MiRagen
miR-15/195	Inhibitor	Post-MI remodeling	Pre-clinical	Therapeutics
miR-451	Inhibitor	Polycythemia vera	Pre-clinical	
miR-122	Inhibitor	HCV	Pre-clinical	
miR-21	Inhibitor	HCC, cancer, fibrosis	Pre-clinical	
miR-10b	Inhibitor	Glioblastoma	Pre-clinical	Regulus
miR-33a/b	Inhibitor	Atherosclerosis	Pre-clinical	Therapeutics
miR-155	Inhibitor	Immuno-inflammatory diseases	Pre-clinical	
miR-122	Inhibitor	HCV	Phase II	Santaris Pharma
miR-29	Mimic	Cardiac fibrosis	Pre-clinical	MiRagen Therapeutics
let-7	Mimic	Lung cancer	Pre-clinical	Mirna Therapeutics
miR-34a	Mimic	Solid tumors	Pre-clinical	
miR-16	Mimic	Cancer	Pre-clinical	
miR-34a	Mimic	Hepatocellular carcinoma	Pre-clinical	Regulus
miR-146a	Mimic	Autoimmunity, cancer	Pre-clinical	Therapeutics

From MiRagen Therapeutics (http://www.miragentherapeutics.com), Regulus Therapeutics (http://www.regulusrx.com), Santaris Pharma (http://www.santaris.com), Mirna Therapeutics (http://www.mirnatherapeutics.com).

N-(2-aminoethyl)glycine units, also have a potential to inhibit miRNA activities. Reports indicate that PNA-DNA chimeras have the potential to inhibit miRNA *in vitro* and *in vivo* (99). On the other hand, unlike chemically modified ASOs, a miRNA decoy can be stably integrated into the chromosomes and degrade miRNA targets. The stable suppression of miR-301a by a miRNA decoy was reported to have inhibited tumor growth by the upregulation of NF-κB-repressing factor in pancreatic cancer (102), and TuD-RNA against miR-122a showed a significant suppression of the HCV replication in liver hepatocytes (103).

PIPELINE OF MIRNA IN CANCER TREATMENT

In a recent study, onco-miRs or tumor-suppressive miRs that work as master regulators in cellular processes have been identified, and a number of pre-clinical trials have been conducted by firms such as MiRagen Therapeutics, Regulus Therapeutics, Santaris Pharma and Mirna Therapeutics (Table 4). For example, miR-34a, which is one of the best-studied tumor-suppressive miRNAs, was a therapeutic target in solid tumor treatment by Mirna Therapeutics and Regulus Therapeutics. miR-34a is commonly downregulated in human cancer, such as prostate, breast, lung, kidney, bladder, ovary and skin cancer (104–106), and was identified as a target of the tumor suppressor gene p53. The reduction of miR-34a by CpG methylation is observed in multiple types of cancer. The restoration of miR-34s has the potential to cause cell cycle arrest, senescence and apoptosis (107). Mirna Therapeutics has also been conducting pre-clinical trials with miR-16 and let-7 mimics, which are potent tumor-suppressive miRNAs (47,108,109). Furthermore, pre-clinical trials of miRNA inhibitors against miR-21 and miR-10b, which are targeted as onco-miRs in hepatocellular carcinoma and glioblastoma, are being conducted. In addition to these developments, a number of non-public candidates for miRNA therapy are being considered by Mirna Therapeutics; they include miR-Rx01, 02, 03, 06 and 07. Thus, miRNA therapeutics using miRNA mimics or inhibitors has been growing in pre-clinical studies and might appear in clinical trials over the next several years.

CONCLUSION

RNAi is one of the most versatile knockdown tools in recent biotechnology, and the potential of RNAi therapeutics using miRNA for cancer treatment has been rapidly expanding. In particular, unlike siRNAs as a tool that specifically impairs the function of a target gene, miRNAs work as key regulators that control target genes and establish balanced cellular organization. Indeed, the disruption of such a balance leads to the possibility of a tumor to become malignant (110,111). To utilize these discoveries of cellular biological basic research for clinical investigation, further innovations in the field of the delivery systemic and chemical modifying strategies are desired. Indeed, although chemically modified ASOs and ss-siRNAs are potentially promising nucleic acid drugs that can efficiently manage RNAi in animals, immeasurable synthesis costs and technical difficulties for bulk production remain. In addition, safer and more effective delivery systems, including a viral approach, are needed. However, the progression of RNAi technology over the past decade has been remarkable, and the hope is that ongoing investigations will result in the use of RNAi therapeutics as a prominent cancer treatment.

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Conflict of interest statement
None declared.

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