Chapter 13
RNA Interference for Oncology: Clinical Prospects Beyond the Hype

Eishi Ashihara and Taira Maekawa

Abstract RNA interference (RNAi) is a process of sequence-specific posttranscriptional gene silencing induced by double-strand RNA, and this phenomenon has been shown to function in higher organisms including mammals, and methods that exploit RNAi mechanisms have been developing. Recently, RNAi induced by short interfering siRNAs has been experimentally introduced as a cancer therapy and is expected to be developed as a nucleic acid-based medicine. Moreover, RNAi technology is used in biomarker-based screening, which is a new screening method based on transcriptional profiling to identify the specific transcriptional activities altered by the compounds of interest. In this chapter, we briefly review the mechanism of RNAi and discuss in detail some of the most recent findings concerning the administration of potential nucleic acid-based drugs. We next discuss several current clinical trials of RNAi therapies against cancers. Finally, we introduce a new high-throughput screening method based on transcriptional profiling for drug discovery. Current studies and clinical trials demonstrate that RNAi technology could establish a novel and promising therapeutic tool against cancers.

Keywords RNA interference • siRNA • microRNA • Cancer • Cancer biomarker • Cancer therapy

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E. Ashihara (✉)
Department of Clinical and Translational Physiology,
Kyoto Pharmaceutical University,
Misasagi-Nakauchicho 5, Kyoto 607-8414, Japan
e-mail: ash@mb.kyoto-phu.ac.jp

T. Maekawa
Department of Transfusion Medicine and Cell Therapy,
Kyoto University Hospital,
Kyoto, Japan

M.K. Danquah, R.I. Mahato (eds.), *Emerging Trends in Cell and Gene Therapy*,
DOI 10.1007/978-1-62703-417-3_13, © Springer Science+Business Media New York 2013

13.1 Introduction

RNA interference (RNAi) is a process of sequence-specific posttranscriptional gene silencing induced by double-strand RNA (dsRNA), and this phenomenon was discovered in *Caenorhabditis elegans* (*C. elegans*) [1]. RNAi has been shown to function in higher organisms including mammals, and methods that exploit RNAi mechanisms have been developing. Aberrant expression of endogenous normal or mutant genes occurs in pathological conditions, resulting in alterations in signal pathways, cellular proliferation, and apoptosis. Posttranscriptional gene regulation by RNAi controls these alterations positively or negatively, and consequently RNAi has now been well established as a method for experimental analyses of gene function in vitro. Recently, short interfering RNA (siRNA), which induces RNAi, has been experimentally introduced as a cancer therapy and is expected to be developed as a nucleic acid-based medicine, and several clinical trials of RNAi therapies against cancers are ongoing. To develop nuclear medicine against cancers, we have two important issues to overcome: one is to select suitable gene targets and another is to develop effective drug delivery systems (DDSs). DDSs are divided into two categories: viral vector-based carriers and nonviral-based carriers. Although viral vectors are the most powerful tools for transfection so far, especially retroviral and lentiviral vectors randomly integrate into host cells' DNA and those might induce insertional mutagenesis [2–4]. The use of nonviral DDSs including cationic liposomes [5, 6] and atelocollagen [7, 8] is preferred because it offers greater safety for clinical application than does the use of viral DDSs.

In addition to the development of a nucleic acid-based medicine, RNAi is put to practical use for a high-throughput screening for development of molecular targeting agents. The alternation of the related gene transcripts which are investigated after the knockdown of the targeted gene transcript by RNAi is compared with that of gene transcripts treated by compounds with unknown functions. The compounds which demonstrate the resemble alternation are recognized as molecular target compounds for the interested gene [9–11]. In this chapter, we discuss the application of RNAi for the development of medicine against cancers.

13.2 Mechanisms of RNAi

RNAi processes can be roughly divided into the initiation phase and the effector phase. In the initiation phase, following introduction of dsRNA into a target cell, dsRNA encounters a dsDNA-specific RNAse III family ribonuclease Dicer. Dicer is a modular enzyme and is composed of an N-terminal helicase domain, an RNA-binding Piwi/Argonaute/Zwille (PAZ) domain, two tandem RNAse III domains, and a dsRNA-binding domain [12]. Dicer acts to produce both siRNAs and microRNAs (miRNAs) [13–16]. dsRNA is processed into shorter lengths of 21–23 nucleotides (nts) dsRNAs, termed siRNAs by the ribonuclease activity of Dicer. dsRNA precursors are sequentially processed by the two RNAse III domains of Dicer and

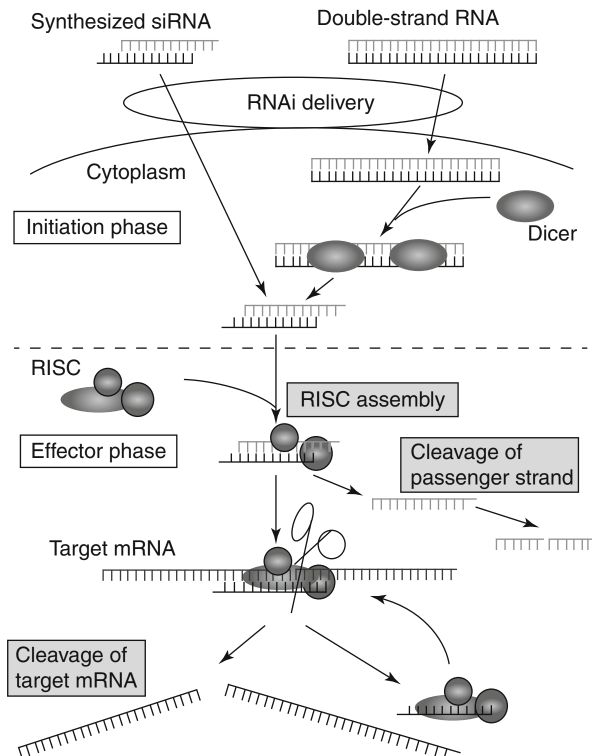

Fig. 13.1 Mechanisms of RNA interference. Synthesized short interference RNA (*siRNA*) or double-strand (ds) RNA is introduced into a target cell. The dsRNA is processed into siRNA length of 21–23 nucleotides by Dicer (initiation phase). siRNA then enters an RNA-induced silencing complex (*RISC*) assembly pathway. The dsRNA unwinds to form two single strands of RNA. The passenger strand rapidly degrades and the guide strand binds and cleaves the target mRNA, resulting in mRNA degradation (effector phase)

cleaved into smaller dsRNAs with 3′ dinucleotide overhangs [12]. In the biogenesis of miRNA, pre-miRNA is also processed into a miRNA duplex (Biogenesis of miRNA is discussed below).

In the second effector phase, smaller dsRNAs enter into an RNA-induced silencing complex (RISC) assembly pathway [17]. RISC is ribonucleoprotein complex that contains Argonaute (Ago) proteins, siRNAs or miRNAs, and complementary mRNAs. Ago is a family of proteins characterized by the presence of a PAZ domain and a PIWI domain [18]. The PAZ domain of Ago protein is likely to engage siRNA or miRNA, and the PIWI domain adopts an RNAse H-like structure that can catalyze the cleavage of the guide strand. The dsRNA is unwound by ATP-dependent RNA helicase activity to form two single strands of RNA. dsRNA is unwounded by ATP-dependent RNA helicase activity to form two single strands of RNA. The guide (antisense) strand directs silencing targeted mRNA, and the other strand is called the passenger (sense) strand. Ago2 protein binds the guide strand and cleaves its targeted RNA at the phosphodiester bond which is positioned between nucleotides 10 and 11. The cleaved products are rapidly degraded because of its unprotected ends, and the passenger strand is also degraded. After dissociation of cleaved mRNAs from siRNA, the RISC encounters and cleaves mRNA, resulting in decrease of expression of the target gene (Fig. 13.1).

Table 13.1 Target genes for experimental RNA interference cancer therapies

Target genes	Cancers
1. Proliferation/anti-apoptosis	
BCL-2	Lung cancer, prostate cancer, fibrosarcoma
VEGF	Ewing's sarcoma, prostate cancer
PLK-1	Urinary bladder cancer, lung cancer (liver metastasis)
Survivin	Glioblastoma, rhabdomyosarcoma
CDC25B	Hepatocellular carcinoma
EGFR	Glioblastoma
Telomerase	Malignant melanoma
EZH2	Prostate cancer (bone metastasis)
FGF-4	Germinoma
2. Signal transduction	
ERK1/2	Hepatocellular carcinoma
STAT3	Colon cancer, prostate cancer, breast cancer
β-catenin	Colon cancer, multiple myeloma
BCR-ABL	Chronic myelogenous leukemia
LYN	Chronic myelogenous leukemia
3. Drug resistance	
MDR1	Colon cancer
MRP7/ABCC10	Non-small cell lung cancer
RPN2	Breast cancer
ABCG10	Gastric cancer
FGFR1	Breast cancer
4. Metastasis/angiogenesis	
VEGF/VEGFR	Ewing's sarcoma, breast cancer, colon cancer, prostate cancer
u-PA/u-PAR	Squamous carcinoma
CCR7	Colon cancer
LYN	Ewing's sarcoma
RhoC	Hepatocellular carcinoma

13.3 Target Genes for Cancer Therapy

The RNAi technology in the clinical setting has relied on localized drug delivery first. This reason is that the localized administration could maintain higher concentrations of siRNAs in the targeted diseases. However, thanks to the development of DDSs (see Refs. [19, 20]), RNAi has recently been evaluated as a therapeutic strategy for cancer treatment. To develop nuclear medicine against cancers, suitable gene targets should be selected (Table 13.1). The definition of cancers is cell proliferation without normal regulation, and one of the most important characteristics of cancers is to bereave the host's life with their malignant behaviors. Such targets include antiapoptotic proteins, cell cycle regulators, transcription factors, signal transduction proteins, and factors associated with malignant biological behaviors of cancer cells, all of these genes are associated with the poor prognosis of cancer patients.

Among such suitable genes, BCL2 protein is one of the anti-apoptotic members of BCL family proteins and contributes to resistance to apoptosis against external stimuli, including cytotoxic agents. BCL2 participates in tumorigenesis and progression and its overexpression in tumor cells correlates with the poor prognosis of the cancer patients [21–24]. Many studies have demonstrated that siRNA treatment against BCL2 inhibited the proliferation of tumor cells [5, 25–27]. Intravenous administration of synthetic BCL2 siRNA, using a cationic or pegylated cationic liposome, suppressed tumor progression in a xenograft mouse model, and BCL2 siRNA treatment significantly elongated the survival of cancer-bearing mice [5, 27]. Oblimersen sodium is a 18-mer phosphorothioate antisense oligonucleotide designed to bind to the first six codons of the human BCL2 mRNA [28]. Though this nucleic acid medicine is an antisense oligonucleotide, it has been also used in a substantial number of clinical trials against several types of cancers [29–33]. These observations indicate that BCL2 is a suitable target for cancer therapy.

Signal transduction molecules are other candidates for RNAi. Member of the signal transduces and activator of transcription (STAT) family act as key components of cytokine signaling pathways that regulate gene expression. Among STAT family, STAT3 is most strongly implicated in carcinogenesis. Its constitutively active form is detected in variety of cancers and dysregulates the downstream target genes of cell proliferation [34] and survival [35, 36]. RNAi therapy against STAT3 demonstrates the inhibition of tumor progression as well as invasion [37–40].

Bcr-Abl fusion protein, which is created by the molecular consequence of the *t*(9; 22) translocation, is a constitutively active tyrosine kinase that causes Philadelphia (Ph)-positive leukemias [41]. Imatinib mesylate (IM; Gleevec™, Glivec™) was developed as a first-generation tyrosine kinase inhibitor (TKI), and its emergence has dramatically changed the outcomes of therapies against Ph-positive leukemia, especially chronic myelogenous leukemia (CML) [42–45]. Moreover, several second generation TKIs developed to overcome resistance to IM have yielded excellent outcomes [46–49]. These clinical observations demonstrated that targeting Bcr-Abl protein is a promising strategy to eliminate Bcr-Abl-positive leukemic cells. The approach to downregulate the expression of Bcr-Abl mRNA by RNAi was investigated in vitro [50–53]. Koldehoff et al. reported the in vivo administration of synthetic Bcr-Abl siRNA with cationic liposomes in a patient with recurrent Ph-positive CML resistant to IM [54]. This patient had a high level of Bcr-Abl transcripts and subcutaneous nodule, and she was treated with 10 μg/kg of Bcr-Abl siRNA intravenously by a bolus injection and 300 μg iRNA was directly injected into CML node. The level of Bcr-Abl mRNA transcript was drastically decreased; however, no obvious effects were observed after the second and third courses. Although this report was not constructed as a clinical trial, these observations are worth noting for developing nuclear medicine against CML.

β-catenin is a downstream protein of the canonical Wnt signaling pathway that has been shown to play an important role in the process of development, proliferation, and differentiation [55]. In the absence of Wnt signals, adenomatous polyposis coli (APC), Axin, glycogen synthase kinase-3β (GSK3β), and casein kinase 1α

(CK1α) form a complex called the "β-catenin destruction complex." GSK3β and CK1α target serine/threonine residues at the N terminus of β-catenin for phosphorylation [56]. Phosphorylated β-catenin is recognized and polyubiquitinated by β-transducin repeat-containing protein (β-TrCP), a component of a ubiquitin ligase complex, targeting β-catenin for degradation by the 26S proteasome [57, 58]. On the other hand, the binding of Wnt ligands to Frizzled (Fz) receptors and the low-density lipoprotein receptor-related protein 5/6 (LRP5/6) co-receptors induces the phosphorylation of Disheveled (Dvl) and prevents GSK3β-dependent phosphorylation of β-catenin. Stabilized β-catenin translocates into the nucleus and interacts with T cell factor (TCF)/lymphocyte enhancer factor (LEF). In the absence of β-catenin, TCF/LEF, which interacts with Groucho and histone deacetylase (HDAC), acts as a repressor of the transcription [59]. The β-catenin/TCF complex regulates the transcription of a number of genes associated with cell proliferation and apoptosis, as well as the expression of growth factors. Typical β-catenin/TCF target genes that are associated with cell proliferation are c-myc and cyclin D1. The c-myc oncogene regulates cell cycle progression and apoptosis. Cyclin D1 activates cyclin-dependent kinases leading to cell cycle progression. Recently, this pathway has been focused on as it is involved in cancer development. Aberrant activation of Wnt/β-catenin signaling is observed in many human cancers. Genetic mutations of Wnt signaling pathway components are primarily responsible for this aberrant activation and cause β-catenin to escape the degradation process and lead to nuclear stabilized β-catenin accumulation [60]. Treatment of siRNAs against β-catenin successfully suppressed the proliferation of colon cancer cells and myeloma cells by inducing caspase-dependent apoptosis [61–63]. Thus, β-catenin represents a suitable target for RNAi therapy.

Molecules controlling cell division are also useful targets for cancer therapy. Polo-like kinases (PLKs) belong to the family of serine/threonine kinases. PLK family has identified PLK-1, PLK-2 (SNK), PLK-3 (FNK), and PLK-4 (SAK) in mammalians so far and PLKs function as regulators of both cell cycle progression and cellular response to DNA damage. PLK-1 is the best characterized among them to date. PLK-1 regulates cell division at several points in the mitotic phase: mitotic entry through CDK1 activation, bipolar spindle formation, chromosome alignment, segregation of chromosomes, and cytokinesis [64]. Whereas PLK-1 is scarcely detectable in most adult tissues [65, 66], PLK-1 is overexpressed in cancerous tissues [65], and many reports have described that PLK-1 is overexpressed in cancerous tissues and that PLK-1 expression levels were tightly correlated with histological grades of tumors, clinical stages, and prognosis of the patients.

Inhibition of PLK-1 activity in cancer cells induces mitotic arrest and tumor cell apoptosis. Depletion of PLK-1 mRNA also inhibits the functions of PLK-1 protein in DNA damages and spindle formation and causes the inhibition of the cell proliferation in a time- and a dose-dependent manner. PLK-1 siRNA treatment induces an arrest at the G2/M phase in the cell cycle with the increase of CDC2/Cyclin B1 and the transfected cells had dumbbell-like and misaligned nuclei. Moreover, the caspase activation was induced in these cells [6, 67, 68]. These observations indicate that PLK-1 could be an excellent target for cancer therapy.

Other candidate siRNA targets are molecules that define the malignant behavior of cancerous cells. The vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) axis plays an important role in angio- and lymphangiogenesis. VEGF family has seven members. Among them, VEGF-A stimulates angiogenesis in tumor masses, enhances the permeability of the blood vessels, and promotes the motility of cancer cells, which results in metastases [69, 70]. The previous investigations reveal that VEGF-A depletion successfully prevents metastasis of cancers [71, 72]. In contrast to VEGF-A, VEGF-C and VEGF-D are associated with tumor lymphangiogenesis and lymph node metastasis. Depletion of VEGF-C/D inhibits metastasis of cancers [73, 74]. Another example of the molecule associated with metastasis is the urokinase-type plasminogen activator (u-PA). u-PA binds to u-PA receptor (u-PAR), and this molecule activates plasminogen and matrix metalloproteases, which enhances the degradation of basement membranes and extracellular matrices and promotes metastases [75, 76]. Data using a mouse model demonstrated that the administration of u-PAR inhibited metastasis and progression of oral squamous cell carcinoma [77]. These molecules associated with metastasis will also be attractive targets of RNAi therapy.

13.4 microRNAs

microRNAs (miRNAs), as the name suggests, are very short RNAs consisted of 21 nts. Those short RNAs regulate target gene expression through translation repression or mRNA degradation, and consequently miRNAs involve diverse biological processes in eukaryocytes. miRNAs are derived from stem-loop-structured primary miRNAs (pri-miRNAs) by the cleavage activity of Drocha, a nuclear-localized member of the RNAse III family, to yield short precursor miRNAs called pre-miRNAs. Pre-miRNAs comprising 70–90 nts exhibit a hairpin structure with a 5′-phosphate and a 3′-2 nts overhang. After translocation from the nucleus to the cytoplasm by Exprtin-5 pre-miRNAs are processed by Dicer into miRNAs of 21 nts. miRNAs as well as siRNAs enter into RISC assembly pathway. Unlike siRNAs, the mature miRNAs often have a partially complementary sequence to the target mRNAs, and a single miRNA might bind to numerous target genes. Therefore, a single miRNA has diverse functions including proliferation, differentiation, and apoptosis [78].

One of the mechanisms of carcinogenesis is the imbalance of oncogenes and tumor suppressor genes caused by several factors including carcinogen. miRNAs affect gene expression by regulating the translation of mRNAs into proteins. In many cancers, some kinds of miRNAs negatively regulate tumor suppressor. miRs-15/16 are downregulated in chronic lymphocytic leukemia (CLL). miR-15a and mir16-2 recognize target sites on the 3′UTR of BCL-2, an anti-apoptotic oncogene [79]. These miRNAs regulate BCL-2 expression in normal cells. However, these are deleted in patients with CLL. On the contrary, other kinds of miRNAs regulate carcinogenesis and tumor progression. Mir-17-92 cluster is overexpressed in lung

cancer tissues [80] and its target genes are PTEN and RB2 [81]. These observations indicate that the overexpression of this miR-17-93 cluster induces the carcinogenesis in lung tissues. Anti-miRNA oligonucleotides (AMOs) can suppress the miRNA activity [82], and recently MAOs are developed as nucleic acid medicines [83–86]. miRNAs regulating anti-apoptosis and cell proliferation are also suitable target molecules against cancers.

13.5 Preclinical Application of RNAi

Before the clinical trials for RNAi therapy, preclinical studies are performed. We introduce two applications of PLK-1 siRNA for cancer therapy. One application is an intravesical treatment against urinary bladder cancers. PLK-1 protein is overexpressed in urinary bladder tumors, and moreover PLK-1 expression levels are correlated with histological grades of tumors, clinical stages, and prognosis of the patients [6]. Superficial urinary bladder cancers are approximately 70 % of urinary bladder cancers at initial diagnosis. After resected transurethrally, Bacillus Calmette-Guerin (BCG), mitomycin C, and Adriamycin are administered intravesically to prevent the recurrence of or diminish the residual cancers [87]. However, half of superficial cancers recur, and consequently novel intravesical treatment should be developed. Clinical trials of RNAi therapy often rely on localized drug delivery because maintenance of higher siRNAs concentrations is necessary for efficacy against the targeted diseases. The urinary bladder which is closed to the urethra is considered as a "putative" in vitro space. In accordance with the unique idea, the efficacy of intravesical therapy of PLK-1 siRNA against urinary bladder cancers was investigated. Bladder cancer-bearing mice were established by the implantation of luciferase (Luc)-labeled UM-UC-3 bladder cancer cells into the murine bladder cavity through the urethra. After the engraftment of cancer cells in the bladder was evaluated by using the in vivo imaging system (IVIS) of bioluminescence imaging (BLI) [88], cancer-bearing mice were treated with PLK-1 siRNA/cationic liposome complexes. Tumor progression was significantly suppressed by the intravesical treatment of PLK-1 siRNA [6].

Another application is a systemic administration of siRNAs against liver metastatic tumors of lung cancers. Distant metastasis is one of the life-threatening factors in lung cancer patients. Despite the development of new molecular targeting agents [89, 90], current therapies are not sufficient to cure or manage the patients with distant metastasis [91, 92]. Therefore, novel therapies should be developed. Kawata et al. investigated the effects of PLK-1 siRNA on the liver metastasis of lung cancers in an orthotopic liver metastatic mouse model. Spleens were exposed to allow direct intrasplenic injections of Luc-labeled A549 non-small cell lung cancer cells. After the removal of spleens, the Luc-labeled A549 cell engraftment was confirmed by using IVIS, and then PLK-1 siRNA/atelocollagen complexes were administered by intravenous injection for 10 days. On day 35, mice treated with PLK-1 siRNA/atelocollagen complex showed the significant suppression of tumor

growth compared to mice treated with nonsense siRNA/atelocollagen complex or PBS/atelocollagen complex which showed extensive metastases in the livers. These findings indicate that PLK-1 siRNA/atelocollagen complex is an attractive therapeutic tool for further development as a treatment against liver metastasis of lung cancer [8].

13.6 Adverse Effects of RNAi

Although RNAi shows excellent specificity in gene silencing, several adverse effects are brought in in vivo application. One probable adverse effect is activation of immune reaction. Mammalian immune cells express family of Toll-like receptors (TLRs), which play an essential role in innate immune responses. TLRs recognize microbial ligands including bacterial lipopolysaccharide, lipopeptides, or viral and bacterial RNA and DNA. Among 13 TLRs, TLR7 and TLR8 recognize ssRNA sequence-dependently and produce interferons (IFNs) and inflammatory cytokines such as IL-12 and TNF-α through the activation of NF-κB and IFN regulatory factor (IRF)-7. For this immune response, the length of single-strand RNA (ssRNA) is important and 16–19 nt ssRNA induces IFN production although 12 nt ssRNAs contains the immunostimulatory motif (GUCCUUAA) [93]. The administration of siRNAs into mammalian cells activates the immune systems also sequence-independently. siRNAs induce dsRNA-activated protein kinase (PKR) autophosphorylation and PKR produces IFNs through the activation of NK-κB and IRF-3. TLR3 recognizes unmethylated CpG DNA but not ssRNA. dsRNA directly binds to TLR3 and this signaling pathway is activated sequence-independently [94]. Interestingly, although the receptors recognizing a ssRNA containing a CpG motif and a 6 nt poly-(G) run at the 3′ end are still unknown, a ssRNA activates monocytes [95]. TLR 9, which expresses in endosomes, recognizes CpG oligodeoxynucleotides (ODNs). Purified recombinant TLR 9 binds CpG ODNs directly in a sequence- and pH-dependent manner [96]. Thus, the activation of immune response by siRNAs is dependent on their sequence and chemical nature, implying that chemical modifications of siRNAs might prevent the immune activation. The 2′ position of nucleotides is within TLR-7-interacting sequences and 2′ O-methyl or 2′ fluoro modification abrogate immune response. Furthermore, the uridine or guanosine modification is most effective [97]. Locked nucleic acid modifications of the 3′ of 5′ termini of the sense strand of siRNAs can reduce the immunostimulatory effects [93]. siRNAs conjugated to cholesterol have no significant activation of immune system and improve the distribution of siRNA to the targeted organ including the liver. Systemic administration of cholesterol-conjugated apolipoprotein B siRNAs induces a decrease of apolipoprotein B expression in liver and jejunum of mice, resulting in a decrease in cholesterol levels without the activation of immune systems [98].

Besides perfect complementarity of siRNAs in target RNA sequence, partially complementary sequences in unintended RNAs induce gene silencing (off-target effect). This effect is induced by the sequence complementarity in the seed region

of siRNAs or short-hairpin RNAs (shRNAs) [99]. Moreover, the 7 nt motif complementary to 2–8 nt at the 5′ end of antisense strands of siRNAs has been shown to be a key determinant in directing off-target effects [100]. There are several ways to control the off-target effects. The in silico screening of siRNA constructs are useful for optimization to prevent the off-target effects, and several groups have been developing algorithm [101, 102]. Chemical modification is also useful. For example, the O-methyl modification of the 2′-position of the ribose within the seed region of siRNAs reduces the off-target effect [103]. Asymmetrically designed siRNAs reduce off-target effects compared to symmetric siRNAs. Sun et al. designed asymmetric RNA duplexes of various lengths with overhangs at the 3′ and 5′ ends of the antisense strand to target genes. All siRNAs against target genes were designed to match the same 19 nt sequence. The asymmetric siRNAs effectively induced gene silencing of targeted genes without silencing of nontargeted genes [104].

shRNAs can also induce stable gene silencing. Consequently, it is possible that long-term silencing by shRAN overexpression causes fatal adverse effects. Because shRNA is processed through the miRNA pathway, the miRNA maturation is blocked in response to shRNA concentration. Grimm et al. demonstrated that the sustained high-level shRNA expression in the liver of mice by AAV vector downregulated liver-derived miRNAs, resulting in hepatic injury and death. Morbidity was associated with the downregulation of liver-derived miRNAs [105]. They speculated that saturation of Exportin-5 whose function is nuclear transport inhibited the miRNA maturation pathway. On the contrary, Constein et al. demonstrated that the administration of synthesized siRNAs induced acute and long-term gene silencing without interrupting the endogenous miRNA biogenesis [106]. As mentioned by Grimm et al. [105], higher expression of shRNAs by viral vector might influence the miRNA biogenesis. Considering these findings, careful modification and formulation of siRNAs could avoid the competition between siRNA and miRNA.

13.7 Clinical Trials of RNAi Towards Cancer Therapies

siRNA cancer therapies have been conducted in clinical settings, but few clinical trials for cancer therapy are ongoing (Table 13.2). Alnylam Pharmaceuticals is developing ALN-VSP01 targeting kinase spindle protein and VEGF, and conducting a Phase I study in patients with advanced tumors with liver involvement. Calando Pharmaceuticals is conducting a Phase I study of CALAA-01 in patients with solid tumors refractory to standard-of-care therapies. CALAA-01 is composed of RRM2 siRNA and CDP nanoparticles called Rondel™, and CALAA-01 has been proven safe and effective in mice and nonhuman primates' studies. Clinical studies using LNAs are also ongoing. Santaris Pharma has developed LNA against Bcl-2, SPC2996, for use in an ongoing Phase I/II study in patients with relapsed or refractory chronic lymphocytic leukemia is ongoing. Enzon Pharmaceuticals has developed a LNA against hypoxia-inducible factor-1α and a Phase I/II study in patients with advanced solid tumors or lymphoma is ongoing. National Cancer Institute and

Table 13.2 Clinical trials of RNAi towards cancer therapies

Sponsor	siRNA	Target genes	Disease	Root	Phase	Year
Santaris	SPC2996[a]	Bcl-2	Chronic lymphocytic leukemia	i.v.	Phase I/II	2005
Santaris&Enzon	ENZ-2968[a]	HIF-1α	Metastatic liver tumors	i.v.	Phase I/II	2007
Calando	CALLA-01	RRM2	Relapsed or refractory solid cancers	i.v.	Phase I	2008
Alnylam	ALN-VSP01	KSP + VEGF	Metastatic liver tumors	i.v.	Phase I	2009
Silence Therapeutics	Atu027	PKN-3	Advanced solid cancers	i.v.	Phase I	2009
Silenseed	siG12D LODER	KRAS[b]	Locally advanced adenocarcinoma of pancreas	Local[c]	Phase 0	2010
National Cancer Institute	TKM080301	PLK-1	Metastatic liver tumors	i.v.	Phase I	2012
Tekmira Pharmaceuticals	TKM080301	PLK-1	Solid tumors or lymphomas	i.v.	Phase I	2012

HIF-1α hypoxia-inducible factor-1 α, *RRM2* ribonucleotide reductase M2 subunit, *KSP* kinase spindle protein, *PKN-3* protein kinase N3, *PLK-1* polo-like kinase 1

[a]ENZ-2968 is LNA (locked nucleic acid), KRAS

[b]KRAS with G12D mutation

[c]siRNA is administered using an endoscopic ultrasound biopsy needle

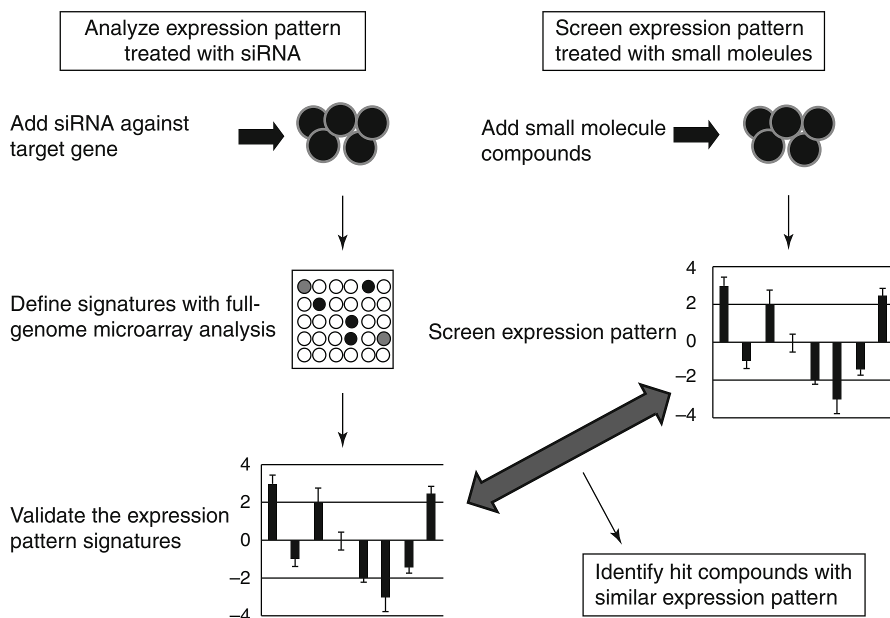

Fig. 13.2 Biomarker-based screening using RNA interference. This assay proceeds in two steps: the first step consists of setting up the signature of siRNA against target gene. The second step involves screening for compounds with the similar expression patterns. Consequently, hit compounds that inhibit the downstream signal of the target gene

Tekmira Pharmaceuticals are conducting clinical trials on PLK-1 RNA interference against solid tumor or lymphoma. As clinical trials of cancer therapies have just started, their outcomes are expected.

13.8 Biomarker-Based Screening

RNA interference technology is also used in the field of drug discovery. The biomarker-based screening is a new high-throughput screening method based on transcriptional profiling and identifies the specific transcriptional activities altered by the compounds of interest. PGX Health, A division of Clinical Data Inc. (formerly Avalon Pharmaceuticals, MD, USA) assessed the transcriptional response of a colon cancer cell line to treatment with β-catenin siRNA using full-genome microarray analysis [9]. Nine biomarkers were selected for their potential as indicators for cancer therapy. A library of 90,000 individual compounds was screened to identify compounds that showed a similar expression pattern to the siRNA (Fig. 13.2). Finally, the compound LC-363 was detected based on its ability to mimic the effect of β-catenin knockdown. The effect of AV-65, one of LC-363 compound series, on MM cells and CML cells was investigated. AV-65 inhibited the proliferation of MM

and CML cells by promoting the degradation of β-catenin and inhibiting β-catenin/TCF transcriptional activity. AV-65 decreased the expression of c-myc, cyclin D1, and survivin, which resulted in the inhibition of tumor cell proliferation through the apoptotic pathway [10, 11]. Moreover, AV-65 treatment prolonged the survival of orthotopic MM-bearing mice [11]. A clinical study with this compound series in solid and hematopoietic malignancies will be carried out in the future.

13.9 Conclusion

RNAi therapy against cancers has just started and the outcomes are expected. However, it should be warranted to establish the pharmacokinetics and pharmacodynamics of siRNAs on the administration for the potential approval of siRNA as a tool for cancer therapy. Moreover, to maximize efficacy and to minimize adverse effects of RNAi, it should be determined whether siRNAs are best administered alone or in combination with chemotherapeutic agents [107], and whether it is better to administer a single specific siRNA or multiple specific siRNAs [108–110].

In conclusion, RNAi therapy represents a powerful strategy against cancers and may offer a novel and attractive therapeutic option. The success of RNAi depends on the suitable selection of target genes. Besides developing nucleic acid-based medicine, RNAi technology is applied into the field of drug discovery. We anticipate that RNAi technology could establish a novel and promising therapeutic tool against cancers.

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