Advanced Pharmaceutical Bulletin

Adv Pharm Bull, 2014, 4(4), 313-321
doi: 10.5681/apb.2014.046
http://apb.tbzmed.ac.ir

Review Article

RNA Interference and its Role in Cancer Therapy

Behzad Mansoori, Siamak Sandoghchian Shotorbani, Behzad Baradaran*

Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Article info

Article History:
Received: 30 January 2014
Revised: 16 April 2014
Accepted: 19 April 2014
ePublished: 10 August 2014

Keywords:
· RNAi
· Cancer
· Treatment

Abstract

In todays' environment, it is becoming increasingly difficult to ignore the role of cancer in social health. Although a huge budget is allocated on cancer research every year, cancer remains the second global cause of death. And, exclusively, less than 50% of patients afflicted with advanced cancer live one year subsequent to standard cancer treatments. RNA interference (RNAi) is a mechanism for gene silencing. Such mechanism possesses uncanny ability in targeting cancer-related genes. A majority of gene products involved in tumorigenesis have recently been utilized as targets in RNAi based therapy. The evidence from these studies indicates that RNAi application for targeting functional carcinogenic molecules, tumor resistance to chemotherapy and radiotherapy is required in today's cancer treatment. Knock downing of gene products by RNAi technology exerts antiproliferative and proapoptotic effects upon cell culture systems, animal models and in clinical trials in the most studies. The recognition of RNAi mechanism and the progress in this field leaded several new RNAi-based drugs to Clinical Trial phases. This has also developed genome based personalized cancer therapeutics. Hopefully, this type of treatment will work as one of the efficient one for cancer patients.

Introduction

RNAi, which is commonly understood as RNA interference, refers to a member of non-coding RNA (ncRNA). The term non-coding RNA (ncRNA) is used for RNA that are not translated into protein; however, this does not mean that non-coding RNA delivers no performance.[1] New evidence suggests that a majority of the mammalian genome is transcribed into ncRNA and exclusively 2% of it is transcribed into mRNA and translated into protein.[2-10] RNA sequencing studies showed that the origin of ncRNAs is in the transcript antisense protein-coding genes, bidirectional promoter transcripts, enhancer and repeated sequences areas transcription, Intronic transcripts.[11] Viruses and other double-stranded RNA microorganisms insert their genome into their host cells or artificially synthesize double-stranded RNA.[12-14] Non-coding RNA is divided into two groups: 1- Small regulatory RNA and 2- Long non-coding RNA (Table 1). RNA interference (RNAi) is part of a small regulatory RNA, including siRNA and miRNA.[15] The discovery of RNA interference molecules indebted to Mr. Fire's and Mello's research into the C.elegans in 1998.[12] The advances in RNAi were made in following years leading eventually to the Physiology and Medicine Nobel Prize for Fire and Mello in 2006. Specific gene expression silencing by RNAi is a mechanism of transcriptional regulation in the eukaryotic cell. This is mediated by small RNA with 21-23 nucleotides length called siRNA and is conserved in terms of evolution among eukaryotes. RNAi seems to protect against not only exogenous genes such as microbial organisms genes including viral, bacterial genes, but also endogenous genes such as transposons. The other roles of these molecules in cells involving gene expression regulation and cell growth control have been demonstrated.[16-19] On this regard, 3 mechanisms have been identified embodying: 1) heterochromatin formation changes 2) Inhibition of translation of target mRNA 3) Degradation of the target mRNA. The second and third mechanisms are more divulged. Cancer is one of the main targets for RNAi-based therapy. Several studies conducted invivo and invitro showed that RNAi-based therapy can be used for treating single-gene disorders and those with overexpression of proteins.[20] There are different types of small synthetic RNA used in cancer therapy, that is, siRNA, shRNA and bishRNA. Such cancer therapy outweighs the others due to the silencing mechanism, specificity and lack of side effects.[21]

Mechanisms of gene silencing by siRNA

SiRNA is produced in two stages, that is, starting and effecting stages. In the starting stage, a long double-stranded RNA (500-200 bp) is cleaved into fragments with a length of 23-21 nucleotides by Dicer and siRNA is produced. SiRNA is an functional molecule. The double-stranded RNA can originate from the viral genome, bacterial DNA or synthetic RNA produced by using bioinformatic data (Figure 1).[22,23] In the effecting stage, double-stranded siRNA are separated by helicase and then sense strand is demarcated by endogenous

***Corresponding author:** Behzad Baradaran, Tel: +98 (411) 3364665, Email: baradaranb@tbzmed.ac.ir

endonucleases and antisense strand is directed to the RNA induced silencing complex (RISC). This complex is then directed to the target mRNA. Argonate, as a member of RISC, by its ribonuclease activity from piwi region degrades the target mRNA. Gene expression terminates as long as target mRNA is broken by RISC. There are two pathways through which mRNA is broken. First, they might be broken by ribonuclease. Second, they can be connected to the homologous strand and RNA polymerase forms double stranded RNA leading to the continued interference pathway (Figure 1). By degradation of mRNA, expression of the target gene is suppressed, which is known as post-transcriptional gene silencing (PTGS).[22,23] After the discovery of the fact that siRNA targets gene expression unstably and transiently and finding the evidence that these molecules stimulate the innate immune response such as interferon, another post-transcriptional silencing technique managed by vector called shRNA was developed. This technique functions through frequently gene silencing after transfection into genome by vectors.[24-26]

Table 1. Various classes of ncRNA in mammalian.[16-19,27-42]

Established ncRNA classes	Common abbreviation	Size in nucleotides	Characteristics
Long non-coding RNAs	lncRNAs	more than 200 nucleotides	The widest class, action as sequence-specific tethers for protein complexes, epigenetic regulation performance and determination of the cell compartments and localization.
Small interfering RNAs	siRNAs	21–22 nucleotides	produced by Dicer cleavage of 200-500 nucleotides length dsRNA duplexes, siRNAs form complexes with RISC involved in gene silencing, viral defense and transposon control.
microRNAs	miRNAs	22 nucleotides	produced by Dicer and drosha cleavage of imperfect RNA hairpins encoded in long primary transcripts. Associated with RISC and primarily involved in post-transcriptional gene regulation.
PIWI-interacting RNAs	piRNAs	26–30 nucleotides	Dicer-independent small RNAs, restricted to the somatic cells and germline bordering the germline, associated with PIWI and Argonaute proteins to regulation of transposon activity and chromatin state.
Promoter-associated RNAs	PARs	-	Inclusion of a set of long and short RNAs, promoter-associated RNAs (PASRs) and transcription initiation RNAs (tiRNAs) that overlap promoters and TSSs, which may regulate gene expression.
Small nucleolar RNAs	snoRNAs	-	This guide rRNA methylation and pseudouridylation, and gene-regulatory roles in some studies.
Small nuclear RNAs	snRNAs	approximately 150 nucleotides	Known as a U-RNA, the primary function of the processing of pre-mRNA in the nucleus, regulation of transcription factors, RNA polymerase II and maintaining the telomeres.
X-inactivation RNAs	xiRNAs	-	Dicer-dependent small RNAs processed from Xist and Tsix the two lncRNAs, responsible for X-chromosome inactivation in placental mammals.
Sno-derived RNAs	sdRNAs	-	Small RNAs, being Dicer-dependent of some of them, and processed from small nucleolar RNAs (snoRNAs), action as miRNA-like and translation regulation by some of them.
microRNA-offset RNAs	moRNAs	20 nucleotides	Small RNAs, derived from the regions related to miRNA , unknown function.
tRNA-derived RNAs	-	-	Small RNAs processed by a conserved RNase (angiogenin) from tRNAs, induction of translational repression.
MSY2-associated RNAs	MSY-RNAs	26-30 nucleotides	associated with the germ cell-specific DNA/RNA binding protein MSY2. restricted to the germline, unknown function.
Telomere small RNAs	tel-sRNAs	22 nucleotides	Dicer-independent RNAs, derived from the G-rich sequence of telomeric repeats. a role in telomere maintenance.
Centrosome-associated RNAs	crasiRNAs	34-42 nucleotides	Small RNAs, derived from centrosomes, a role in local chromatin modifications.

Mechanisms of gene silencing by shRNA

Unlike siRNA, shRNA is synthesized in the nucleus and then is transported into the cytoplasm for final processing; thereafter, it binds to RISC and performs its activities.[43] ShRNA activity within the cell is shown schematically in Figure 2. Processing and maturation of shRNA is similar to those of miRNA.[44] ShRNA is transcribed by RNA polymerase II or III and by the means of RNA polymerase II or III promoters on the expression cassette. Initial transcription of RNA polymerase II promoter is produced by stem-loop-like structure which is processed by complex containing RNaseIII family, Drosha and double-stranded RNA binding protein domain (DGCR8).[45] ShRNA hairpin complex is processed by the abovementioned enzyme and makes individual shRNAs by 2 over hang nucleotide at the 3' terminal.[46] This stage processes a molecule called pre-shRNA that is transported into cytoplasm by

exportin5/RanGTPase.[47,48] In the cytoplasm, pre-shRNA loaded onto RNaseIII complexes containing Dicer, TRBP and PACT and the loop is cleaved and double-stranded siRNA with two nucleotide over hang in the 3' is produced.[48,49] The complex containing Dicer helps loading of siRNA onto the RISCs Argo2 protein. Pre-shRNA is a component of RLC; therefore, pre-shRNA is potentially associated with the RLC. After loaded onto RLC, the passenger strand becomes separated. This also occurs in siRNA. Argonaute family constitutes the major portions of RISC complex.[50,51] from argonaute family, only Ago2 have an endonuclease activity, which is necessary for cleavage and double stranded stem passenger release.[14,52,53] Other members of argonaute are Ago1, Ago3, Ago4, which do not have endonuclease activity, but enters RISC and act independent of the cleavage. RISC complex have two functions, that is to say, function independent of the cleavage and function dependent of the cleavage[52] (Figure 2). Argonaute proteins of RISC complex not only are involved in loading onto siRNA and miRNA, but also in transcriptional and post-transcriptional silencing. Ago protein loading onto siRNA, passenger strand and miRNA are targeted on mRNA and results in of endonuclease activity of Ago2 and cleavage of target mRNA[54,55] (Figure 2).Ago1, Ago3 and Ago4, which do not possess endonuclease activity; are located on 3' UTR of mRNA and inhibit translation by the processing target mRNA in P bodies.[56,57] Details the suppression mechanisms of mRNA translation in the P bodies mRNA release from these bodies are still unknown. Adenylation of target mRNA causing mRNA instability occurs in P bodies. The Coimmunoprecipitation experiments have shown that the RISC complex is strongly associated with polyribosomes and small ribosomal subunits.[58] Cleavage and conformation changes may be made in p bodies.[31,59]

Similarities and differences between the siRNA and shRNA

The similarities between these two pathways exist in the function, which is involvement in post-transcriptional silencing. Both siRNA and shRNA use common molecules in their pathways including Dicer, RISC complex and their aim is degradation of target mRNA. However, there are significant differences shown in Table 2.[21]

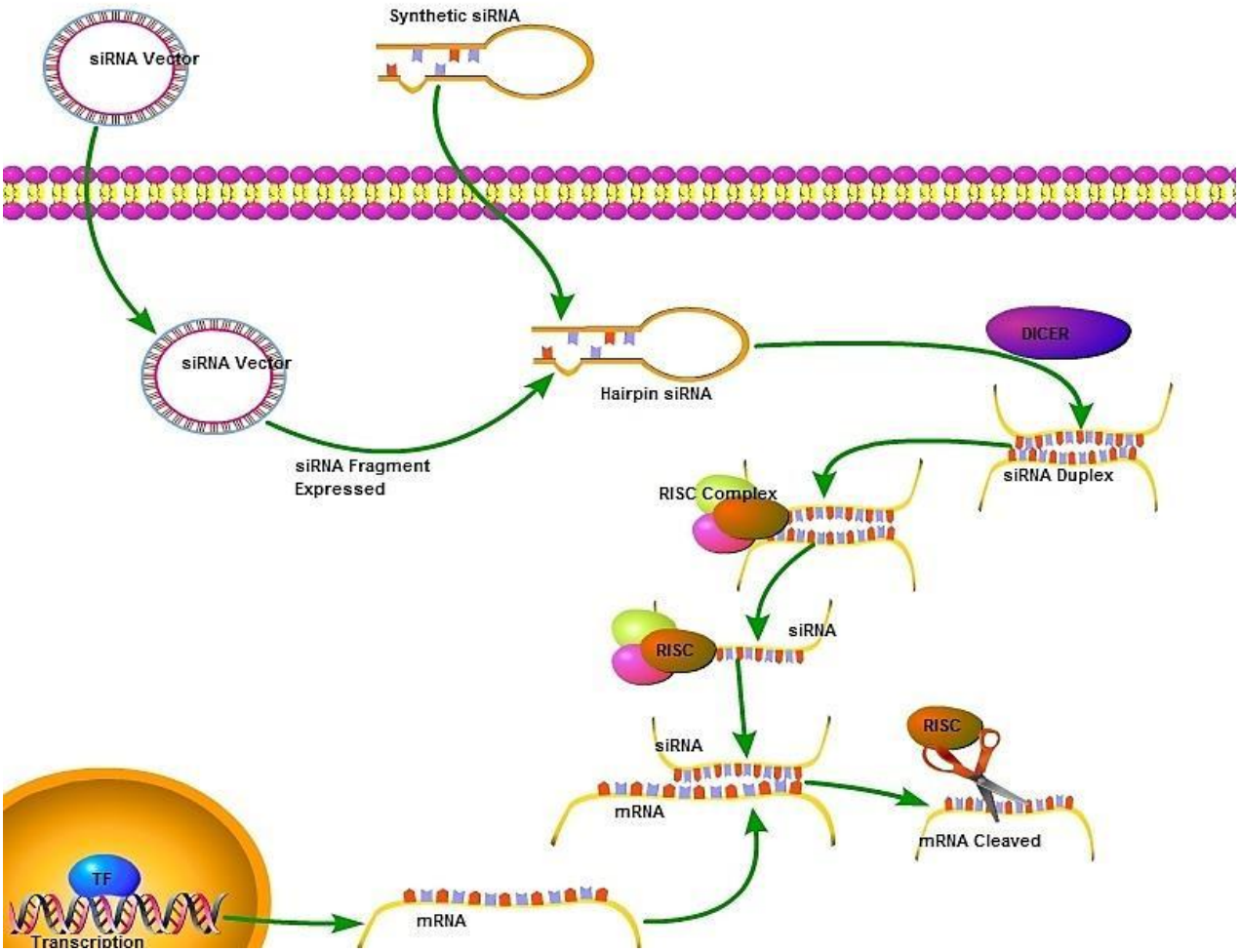

Figure 1. Long double-stranded RNA is cleaved into small interfering RNAs (siRNAs) by Dicer. This reaction needs ATP. SiRNA is then directed to RISC. The separation of the two strands needs ATP. Antisense strand is then directed to RISC

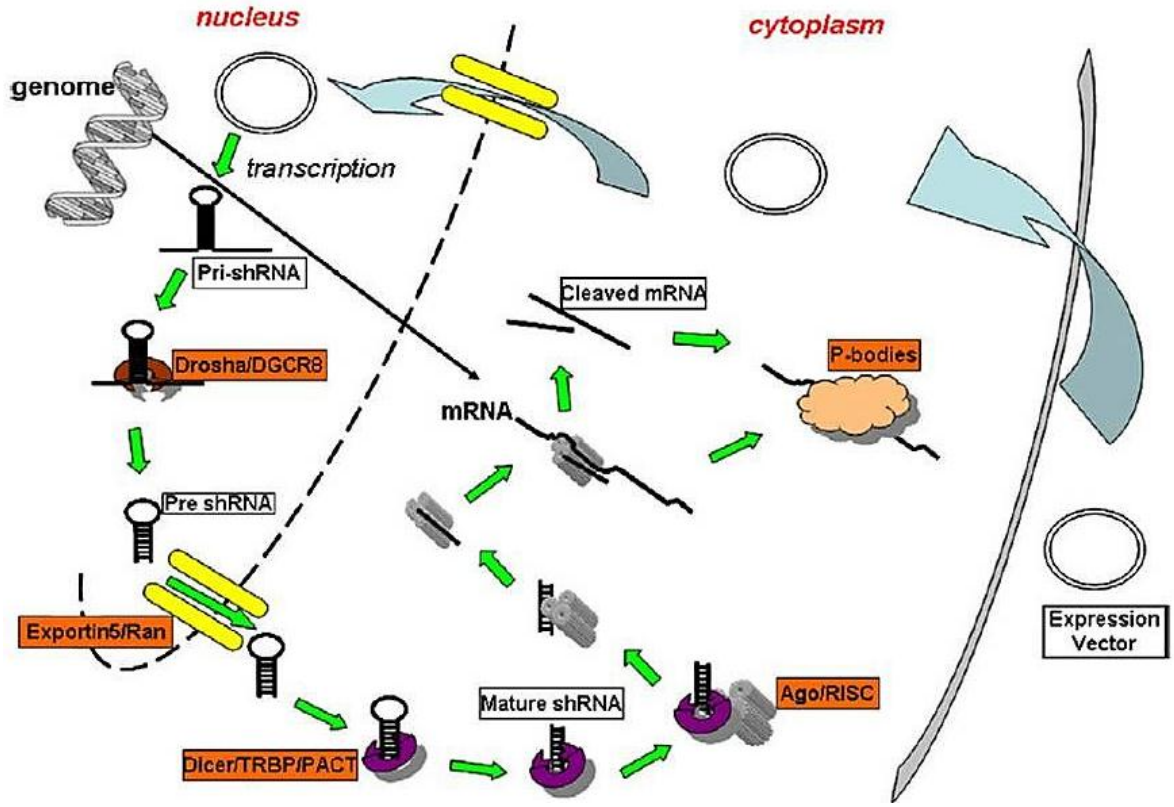

Figure 2. Schematic diagram of the shRNA gene silencing pathway. ShRNA is entered the cytoplasm by using expressing vector. Vector is transferred to the nucleus for transcription. Initial transcription is processed by Drosha-DGCR8 complex. Pre-shRNA is generated from pri-shRNA. Pre-shRNA is transported into cytoplasm by Ran exportin 5. Pre-shRNA is loaded onto Dicer, TRBP, PACT complex. Mature shRNA is generated from Pre-shRNA. The mature shRNA is loaded onto Dicer, TRBP, PACT complex. The complex is joined to argounate protein in RISC, and provides RNA interference (41).

Table 2. Differences between siRNA and shRNA[21]

scale	siRNA	shRNA
Source	Synthetic	Nuclear expression
Delivery	Delivery to the cytoplasm using natural and synthetic polymers or lipids	Delivery to the nuclear using viral or plasmid vectors
Durability	Degradation of 99% of them in 46 hours	Expression up to 3 years
Usage	Limited local or systemic injection	local or systemic injection
Dose	High(nm)	Low(5 copies)
Possibility of Specific Off-target effects	More than shRNA	Lower than siRNA
Possibility of nonspecific Off-target effects	High stimulation of immune system, inflammation, and cytotoxicity	Low stimulation of immune system, inflammation, and cytotoxicity
Application	Acute diseases	Chronic disease

Bifunctional shRNA (bishRNA)

BishRNA, the newly designed RNA interference, is being increasingly used in the post-transcriptional silencing. BishRNA increases efficacy and durability of RNA interference and hastens gene expression silencing. Compared to shRNA, bishRNA is able to loaded onto various RISC complexes. Such property leads to target mRNA degradation and inhibits translation of the mRNA individually.[21] BishRNA shows high efficiency and effectiveness because the leading (antisense) strand in bishRNA is loaded onto at least two RISC complexes, which increases the activity of gene silencing. Moreover, RISC complex loading activates both cleavage dependent and independent pathways, and causes target mRNA degradation and inhibits target mRNA translation[21] (Figure 3).

The roles of RNA interference in cancer therapy

Effectiveness of RNA interference in cancer therapy Has been characterized by high efficiency and potential, induction of silencing in the advanced stages of growth, transmission of silenced gene to the next generation, low cost compared to the other methods of gene therapy[60,61] and high specificity compared to the other methods of

cancer therapy such as chemotherapy. As mentioned above, cancer is one of the main targets for RNAi-based therapy. Oncogenes, mutated tumor suppressor genes and several other genes involved in tumor progression are good targets for gene silencing by RNAi-based therapy due to the precise functional mechanism, high potential, and high specificity of gene silencing by RNAi, and lack side effects compared to chemotherapies. The major advantage of RNAi in cancer therapy is targeting multiple genes of various cellular pathways involved in tumor progression.[62] Simultaneous inhibition of multiple genes is an effective approach to treat cancer as well as reduction of the possibility of multiple drugs' resistance caused by overdose of chemical drugs. Another advantage of this type of treatment is developing suitable personalized drugs for a specific patient. Personalized drugs are likely to be more effective than others in controlling tumor growth.[62] Many studies have been done in the field of RNAi-based drugs leading to introduction of several RNAi based drugs, which are now studied in the clinical trials. The studies conducted on animal models suggest that targeting essential proteins in the cell cycle, such as kinesin spindle protein (KSP) and polo-like kinase 1 (PLK1) by using specific siRNA have exhibited potent antitumor activity observed potent antitumor activity in both subcutaneous and hepatic tumor models.[58] KSP inhibition causes cell cycle termination and apoptosis induction[63] that is similar to PLK1 inhibition inducing apoptosis.[62] Protein kinase N3 (PKN3) has been introduced as a valid therapeutic target in cancer. The inhibition of PKN3 reduces lymph node metastases in orthotopic prostate cancer models.[64] Atu027 (siRNA-lipoplex targeted against PKN3) has been administrated to mice, rats and non-human primates. And, PKN3 expression silencing resulted in significant tumor growth and lymph node metastasis inhibition.[63] Atu027 were used in animal models of metastatic lung cancer and the results showed the inhibition of metastasis after administration.[63] In addition, Atu027 was evaluated in the animal model of breast cancer metastasis to the lung and the results showed inhibition of lung metastasis.[65] A phase I clinical trial for treatment of solid cancers ended in late 2012 and the next phase of clinical trials has recently been started. Table 3 provides detailed information about the current status of RNAi-based drugs.[66-68] CALAA-01 is another RNAi-based drug that is being evaluated in phase I clinical trial. It is a specific siRNA against transferrin encapsulated by non-chemical nanoparticles and targets the M2 subunit of ribonucleotide reductase (RRM2). The gene is involved in DNA replication. CALAA-01 binds to the transferrin receptor and siRNA releases RRM2-specific after endocytosis, leading eventually to inhibition of RRM2 expression and inhibition of proliferation of tumor cells expressing the transferrin receptor. In a phase I clinical trial of the drug, biopsies from patients with melanoma treated with the drug were collected and the results showed the nanoparticles inside biopsies and a reduction in RRM2 mRNA and RRM2 protein levels. At the end of phase I clinical trial, it was observed that administration of siRNA can systemically silence carcinogenic genes with specific targeting of tumor cells.[69] ATN-RNA, a 160-bp double stranded RNA for targeting Tenasion-c, which is administered locally, is currently in phase I clinical trial.[68] It is directly administrated into the neoplastic tissues during glioma operation. It was found that the survival of patients promoted from 48.2 weeks to 106.8 weeks after treatment. The authors also observed no neurological toxicity for this drug. FANG^{TM} vaccine, designed by bishRNA technology, can be used for ex vivo silencing of Furin. Furin is a non-functional and calcium-dependent proprotein and is essential for proteolytic processing maturation of TGF-β isoform (β1, β2). FANG^{TM} vaccine silences Furin and boosts GM-CSF. The administration of FANG^{TM} vaccine into body causes 3 actions, that is, extensive presentation of antigens, Immune stimulation by GM-CSF, and inhibition of Immunosuppressive protein (TGF-β1, β2). The drug is still in clinical trial studies. In clinical trials, cancer cells are collected from body and GM-CSF/bishRNA furin is transfected into expression plasmid by Electroporation method.[68] bishRNA-STMN1 is a RNAi-based drug, whose phase I clinical trial has recently been started, and targets Stathmin1. Stathmin1 is a 149-amino acid protein that is increased in tumor tissues and is involved in tubulin-microtubule compartmentalization. It plays a role in M-phase entrance and exit, and cell motility. SiRNA and ribozyme can target stathmin1 and it was shown that inhibition of stathmin1 by siRNA and ribozyme increases population of cells arrested in G2/M phase as well as inhibition of cell cloning and apoptosis induction.[68]

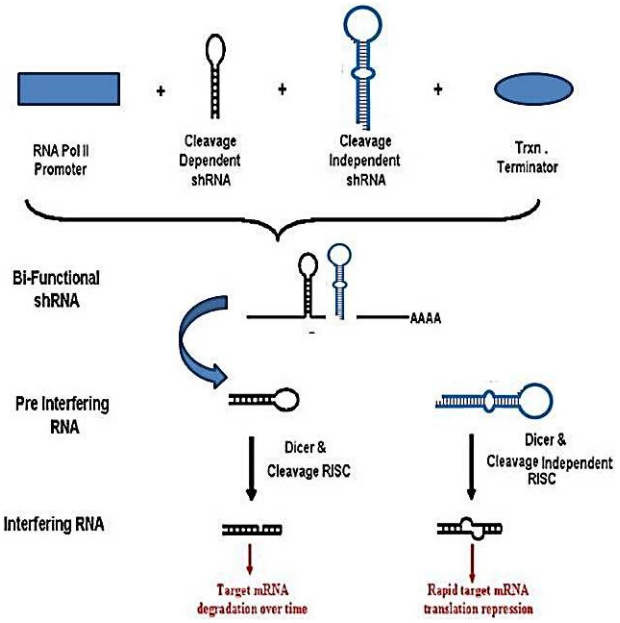

Figure 3. Schematic diagram regarding the bifunctional shRNA. It is designed from two shRNA for an target mRNA, including : 1) one with perfect match and 2) one with mismatches at the central location (bases 9–12). The aim is to activate both RISC dependent cleavage and RISC independent cleavage pathways (41).

Table 3. Current Status of cancer siRNA based drug[66-68]

Company	Drug	Target	Vehicle	Disease	Phase
Calando Pharmaceuticals	CALAA-01	RRM2	Cyclodextrin nanoparticle, TF, and PEG	Solid tumors	**I**
Silence Therapeutics AG	Atu027	PKN3	siRNA-lipoplex	Advanced solid cancer	**I**
Alnylam Pharmaceuticals	ALN-VSP02	VEGF, KSP	SNALP	Solid tumors	**I**
Silenseed Ltd.	siG12D LODER	KRAS	LODER polymer	PDAC	**I**
Sataris Pharma and Enzon Pharmaceuticals	EZN-2968	HIF-1, survivin	Nacked	Advanced solid tumor or lymphoma	**I**
Tekmira	SNALP-PLK1	PLK1	SNALP	Solid tumors	**I**
Duisburg University	Bcr-Abl siRNA	Bcr-Abl	Anionic liposome	CML	
Gradalis Inc.	FANG vaccine	Furin and GM-CSF	Electroporation	Solid tumors	**I**
Duke University	iPsiRNA	LMP2, LMP7, MECL1	Transfection	Metastatic melanoma	**I**
Polish academy of sciences	ATN-RNA	Tenascin-c	Nacked	Astrocytic tumor	**I**

KSP, kinesin spindle protein; PKN3, protein kinase N3; RRM2, M2 subunit of ribonucleotide reductase; VEGF, vascular endothelial growth factor; HIF-1, hypoxia-induced factor; KRAS, V-ki-ras2 Kirsten rat sarcoma viral oncogene homolog; PLK1, polo-like kinase 1; IND, investigational new drug. Bcr-Abl, breakpoint cluster region- Abelson; GM-CSF, Granulocyte-macrophage colony-stimulating factor; LMP, *latent membrane protein;* MECL1, Multicatalytic Endopeptidase Complex-Like 1

The future of cancer therapy by RNA interference

Information theory is a new strategic and helpful approach to cancer treatment.[70] Clinical responses by siRNA-based drug were transient and often cause tumor recurrence or progression.[71] Heterogeneity of the tumor, multiple signaling pathways, cross talks and vertical and horizontal feedback loops challenge the gene targeting in most solid tumors. Drug resistance caused by self-treatment is also another problem.[72] In order to minimize drug resistance, at least three different treatments are suggested. With this strategy, an effective therapy by targeting triple factors reduces the risk of cancer.[68] Now researchers are developing an integrated database in a participatory process for the identification of dominant signaling pathways and involved genes based on formulation of targeting three different factors. The next generation of RNAi-based therapies for cancer are based on individual treatments (Figure 4). The approach to cancer will be RNAi based on disruption of signaling pathways and developing of RNAi-based triple vaccine. This approach acts through damages to tumor growth by modifying the ratio of tumor/ T-cell activation, induction of apoptosis to increase antigen presenting, and reducing local and systemic immunosuppression associated with immunotherapy. The effect of these drugs are not limited by time and can provide long time memory T-cell responses.[68]

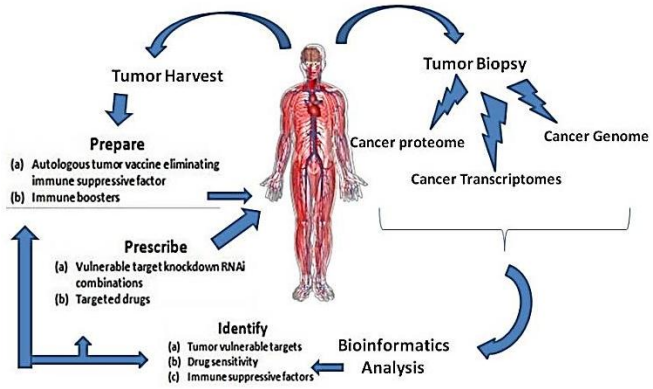

Figure 4. Schematic diagram of the mechanism implementing personalized drugs for cancer. The process is started by collecting a biopsy from the tumor, evaluating DNA, RNA and protein profiles. Tumor and normal tissue profiles are compared. By Using bioinformatic data, genetic abnormalities, impaired tumor-specific pathways are identified. Two-pronged approach (target therapy and vaccine) administered to each patient for having effective treatment (74).

Conclusion

Efficacy and clinical safety of several main components of RNAi technologies such as siRNA, shRNA and bishRNA have been demonstrated. In addition, specify to target and anticancer activity regarding RNAi technologies have been proven in animal models. To date, systemic delivery to tumor targets other than the liver has resulted in many problems; thus, researchers are

now examining safe and effective delivery approaches of these molecules to their target tissues. The results of phase I clinical trial of several RNAi-based drugs are available and shows the safety of treatment. All in all, It is postulated that RNAi-based therapy will be used as a new method of cancer therapy in the near future.

Conflict of interest

The authors report no conflicts of interest.

References

1. Gesteland RF, Cech TR, Atkins JF. The RNA World. 3rd ed. New York: Cold Spring Harbor Laboratory Press; 2006.
2. Consortium EP, Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 2007;447(7146):799-816.
3. Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, et al. The transcriptional landscape of the mammalian genome. *Science* 2005;309(5740):1559-63.
4. Carninci P, Sandelin A, Lenhard B, Katayama S, Shimokawa K, Ponjavic J, et al. Genome-wide analysis of mammalian promoter architecture and evolution. *Nat Genet* 2006;38(6):626-35.
5. Cheng J, Kapranov P, Drenkow J, Dike S, Brubaker S, Patel S, et al. Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. *Science* 2005;308(5725):1149-54.
6. Cloonan N, Forrest AR, Kolle G, Gardiner BB, Faulkner GJ, Brown MK, et al. Stem cell transcriptome profiling via massive-scale mRNA sequencing. *Nat Methods* 2008;5(7):613-9.
7. Core LJ, Waterfall JJ, Lis JT. Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. *Science* 2008;322(5909):1845-8.
8. Johnson JM, Edwards S, Shoemaker D, Schadt EE. Dark matter in the genome: evidence of widespread transcription detected by microarray tiling experiments. *Trends Genet* 2005;21(2):93-102.
9. Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, et al. RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science* 2007;316(5830):1484-8.
10. Seila AC, Calabrese JM, Levine SS, Yeo GW, Rahl PB, Flynn RA, et al. Divergent transcription from active promoters. *Science* 2008;322(5909):1849-51.
11. Levin JZ, Yassour M, Adiconis X, Nusbaum C, Thompson DA, Friedman N, et al. Comprehensive comparative analysis of strand-specific RNA sequencing methods. *Nat Methods* 2010;7(9):709-15.
12. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 1998;391(6669):806-11.
13. Kim DH, Behlke MA, Rose SD, Chang MS, Choi S, Rossi JJ. Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy. *Nat Biotechnol* 2005;23(2):222-6.
14. Matranga C, Tomari Y, Shin C, Bartel DP, Zamore PD. Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell* 2005;123(4):607-20.
15. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. *Cell* 2003;115(2):209-16.
16. Brodersen P, Voinnet O. The diversity of RNA silencing pathways in plants. *Trends Genet* 2006;22(5):268-80.
17. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. *Cell* 2009;136(4):642-55.
18. Ghildiyal M, Zamore PD. Small silencing RNAs: an expanding universe. *Nat Rev Genet* 2009;10(2):94-108.
19. Malone CD, Hannon GJ. Small RNAs as guardians of the genome. *Cell* 2009;136(4):656-68.
20. Dykxhoorn DM, Lieberman J. Knocking down disease with siRNAs. *Cell* 2006;126(2):231-5.
21. Rao DD, Vorhies JS, Senzer N, Nemunaitis J. siRNA vs. shRNA: similarities and differences. *Adv Drug Deliv Rev* 2009;61(9):746-59.
22. Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: biology, mechanism, and applications. *Microbiol Mol Biol Rev* 2003;67(4):657-85.
23. Matzke MA, Birchler JA. RNAi-mediated pathways in the nucleus. *Nat Rev Genet* 2005;6(1):24-35.
24. Cullen BR. Induction of stable RNA interference in mammalian cells. *Gene Ther* 2006;13(6):503-8.
25. Hemann MT, Fridman JS, Zilfou JT, Hernando E, Paddison PJ, Cordon-Cardo C, et al. An epi-allelic series of p53 hypomorphs created by stable RNAi produces distinct tumor phenotypes in vivo. *Nat Genet* 2003;33(3):396-400.
26. Rubinson DA, Dillon CP, Kwiatkowski AV, Sievers C, Yang L, Kopinja J, et al. A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference. *Nat Genet* 2003;33(3):401-6.
27. Belostotsky D. Exosome complex and pervasive transcription in eukaryotic genomes. *Curr Opin Cell Biol* 2009;21(3):352-8.
28. Bortoluzzi S, Biasiolo M, Bisognin A. MicroRNA-offset RNAs (moRNAs): by-product spectators or functional players? *Trends Mol Med* 2011;17(9):473-4.
29. Ender C, Krek A, Friedländer MR, Beitzinger M, Weinmann L, Chen W, et al. A human snoRNA with microRNA-like functions. *Mol Cell* 2008;32(4):519-28.
30. Gerbi SA. Small nucleolar RNA. *Biochem Cell Biol* 1995;73(11-12):845-58.

31. Kiss T. Small nucleolar RNA-guided post-transcriptional modification of cellular RNAs. *EMBO J* 2001;20(14):3617-22.
32. Langenberger D, Bermudez-Santana C, Hertel J, Hoffmann S, Khaitovich P, Stadler PF. Evidence for human microRNA-offset RNAs in small RNA sequencing data. *Bioinformatics* 2009;25(18):2298-301.
33. Lee JT, Davidow LS, Warshawsky D. Tsix, a gene antisense to Xist at the X-inactivation centre. *Nat Genet* 1999;21(4):400-4.
34. Matera AG, Terns RM, Terns MP. Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. *Nat Rev Mol Cell Biol* 2007;8(3):209-20.
35. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009;10(3):155-9.
36. Ogawa Y, Sun BK, Lee JT. Intersection of the RNA interference and X-inactivation pathways. *Science* 2008;320(5881):1336-41.
37. Shi W, Hendrix D, Levine M, Haley B. A distinct class of small RNAs arises from pre-miRNA-proximal regions in a simple chordate. *Nat Struct Mol Biol* 2009;16(2):183-9.
38. Taft RJ, Glazov EA, Lassmann T, Hayashizaki Y, Carninci P, Mattick JS. Small RNAs derived from snoRNAs. *RNA* 2009;15(7):1233-40.
39. Taft RJ, Kaplan CD, Simons C, Mattick JS. Evolution, biogenesis and function of promoter-associated RNAs. *Cell Cycle* 2009;8(15):2332-8.
40. Thompson DM, Parker R. Stressing out over tRNA cleavage. *Cell* 2009;138(2):215-9.
41. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. *Genes Dev* 2009;23(13):1494-504.
42. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol* 2009;11(3):228-34.
43. Cullen BR. RNAi the natural way. *Nat Genet* 2005;37(11):1163-5.
44. Zeng Y, Yi R, Cullen BR. Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. *EMBO J* 2005;24(1):138-48.
45. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003;425(6956):415-9.
46. Zhang H, Kolb FA, Brondani V, Billy E, Filipowicz W. Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP. *EMBO J* 2002;21(21):5875-85.
47. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J* 2002;21(17):4663-70.
48. Lee YS, Nakahara K, Pham JW, Kim K, He Z, Sontheimer EJ, et al. Distinct roles for Drosophila Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* 2004;117(1):69-81.
49. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 2003;17(24):3011-6.
50. Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ. Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science* 2001;293(5532):1146-50.
51. Sontheimer EJ, Carthew RW. Molecular biology. Argonaute journeys into the heart of RISC. *Science* 2004;305 (5689):1409-10.
52. Preall JB, Sontheimer EJ. RNAi: RISC gets loaded. *Cell* 2005;123(4):543-5.
53. Rand TA, Petersen S, Du F, Wang X. Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation. *Cell* 2005;123(4):621-9.
54. Hutvágner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. *Science* 2002;297(5589):2056-60.
55. Yekta S, Shih IH, Bartel DP. MicroRNA-directed cleavage of HOXB8 mRNA. *Science* 2004;304(5670):594-6.
56. Humphreys DT, Westman BJ, Martin DI, Preiss T. MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc Natl Acad Sci U S A* 2005;102(47):16961-6.
57. Thermann R, Hentze MW. Drosophila miR2 induces pseudo-polysomes and inhibits translation initiation. *Nature* 2007;447(7146):875-8.
58. Judge AD, Robbins M, Tavakoli I, Levi J, Hu L, Fronda A, et al. Confirming the RNAi-mediated mechanism of action of siRNA-based cancer therapeutics in mice. *J Clin Invest* 2009;119(3):661-73.
59. Parker R, Sheth U. P bodies and the control of mRNA translation and degradation. *Mol Cell* 2007;25(5):635-46.
60. Yazdi Samadi B, Valizadeh M. Genetics A molecular approach. Iran: Tehran Univercity Publishers; 1388.
61. Fire AZ. Gene silencing by double-stranded RNA. *Cell Death Differ* 2007;14(12):1998-2012.
62. Bora RS, Gupta D, Mukkur TK, Saini KS. RNA interference therapeutics for cancer: challenges and opportunities (review). *Mol Med Rep* 2012;6(1):9-15.
63. Tao W, South VJ, Zhang Y, Davide JP, Farrell L, Kohl NE, et al. Induction of apoptosis by an inhibitor of the mitotic kinesin KSP requires both activation of the spindle assembly checkpoint and mitotic slippage. *Cancer cell* 2005;8(1):49-59.
64. Leenders F, Mopert K, Schmiedeknecht A, Santel A, Czauderna F, Aleku M, et al. PKN3 is required for malignant prostate cell growth downstream of activated PI 3-kinase. *EMBO J* 2004;23(16):3303-13.
65. Santel A, Aleku M, Roder N, Mopert K, Durieux B, Janke O, et al. Atu027 prevents pulmonary metastasis in experimental and spontaneous mouse metastasis models. *Clin Cancer Res* 2010;16(22):5469-80.

66. Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. *Chem Biol* 2012;19(1):60-71.
67. Burnett JC, Rossi JJ, Tiemann K. Current progress of siRNA/shRNA therapeutics in clinical trials. *Biotechnol J* 2011;6(9):1130-46.
68. Rao DD, Wang Z, Senzer N, Nemunaitis J. RNA interference and personalized cancer therapy. *Discov Med* 2013;15(81):101-10.
69. Davis ME, Zuckerman JE, Choi CH, Seligson D, Tolcher A, Alabi CA, et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* 2010;464(7291):1067-70.
70. Paninski L. Estimation of entropy and mutual information. *Neural Comput* 2003;15(6):1191-253.
71. Duxbury MS, Whang EE. RNA interference: a practical approach. *J Surg Res* 2004;117(2):339-44.
72. Kerbel RS. A cancer therapy resistant to resistance. *Nature* 1997;390(6658):335-6.