REVIEW

Natural and artificial small RNAs: a promising avenue of nucleic acid therapeutics for cancer

Sunny Yadav, Mamta Shekhawat, Devashree Jahagirdar, Nilesh Kumar Sharma

Cancer and Translational Research Lab, Dr. D.Y Patil Biotechnology & Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Pune 411033, Maharashtra, India

ABSTRACT   Since the failure of traditional therapy, gene therapy using functional DNA sequence and small RNA/DNA molecules (oligonucleotide) has become a promising avenue for cancer treatment. The discovery of RNA molecules has impelled researchers to investigate small regulatory RNA from various natural and artificial sources and determine a cogent target for controlling tumor progression. Small regulatory RNAs are used for therapeutic silencing of oncogenes and aberrant DNA repair response genes. Despite their advantages, therapies based on small RNAs exhibit limitations in terms of stability of therapeutic drugs, precision-based delivery in tissues, precision-based intercellular and intracellular targeting, and tumor heterogeneity-based responses. In this study, we summarize the potential and drawbacks of small RNAs in nucleic acid therapeutics for cancer.

KEYWORDS   Cancer; drug targeting; gene; intracellular; therapeutics; tumor heterogeneity

Introduction

Cancer is a group of diseases involving uncontrolled and abnormal cell proliferation and can potentially invade or spread to other parts of the body. To date, cancer leads to 168.1 million deaths, of which 3% and 12% of the cases are due to cervical cancer and breast carcinoma, respectively. The number of estimated cancer cases worldwide until 2012 reached 14.1 million, which involved 7.4 million men and 6.7 million women[1].

Traditional therapies for treatment of cancer include surgery, radiation, and chemotherapy[2-4]. Radiation and chemotherapy use non-selective agents, which may cause toxicity to normal tissues[5,6]. In this regard, scholars have developed a promising technology, namely, gene therapy or nucleic acid therapy (NAT), which uses functional genes and small genetic materials, such as RNAs and DNAs[7-13]. In this process, DNA or RNA as coding gene or small non-coding genetic materials is transferred into different cellular compartments of the host (human or animal) to alleviate various pathophysiological conditions, including cancer[12,13]. In addition to their roles in functional gene therapy, small

RNAs have been widely investigated as a tumor suppressor agent to suppress the aberrant expression of oncogenes and DNA repair response genes and control the growth and proliferation of cells[14-20]. Therefore, small RNAs obtained from natural sources and artificial mimetic agents have been increasingly studied to explore new class of anticancer drugs. In this paper, we present the role of small RNAs in cancer therapeutics in terms of pre-clinical and clinical perspectives and the concomitant challenges.

Small RNAs from natural sources

Gene silencing and small RNAs were first studied in the early 1990s[21-23]. The discovery of small ~20–30 nucleotide RNA molecules is an outstanding discovery in biology because of their distinct role in the expression and function of eukaryotic genomes[14-20]. The predominant small RNA classes include short-interfering RNAs (siRNAs) and microRNAs (miRNAs), which function in somatic and germline lineages in a broad range of eukaryotic species[17,19,20,24]. Small RNAs exhibit potential regulatory roles at various cellular strata, including in chromatin structure, chromosome segregation, transcription, and RNA, stability, and translation[17,24]. The regulatory mechanisms of small RNAs in the intra-cellular, intercellular, and extracellular levels are generally inhibitory and interpreted as RNA silencing[22,23]. Small RNAs can also activate gene transcription and are classified as small-

activating RNAs. Natural endogenous small RNAs are found in various organisms including humans, plants, mouse, fungi, bacteria, flies, and worms[17-20]. In addition to their versatile roles in intra-cellular signaling, intercellular communication, and cell growth and development, small RNAs, particularly miRNAs, which are found in biological fluids, such as serum and plasma, function as potential biomarkers for cancer prognosis and detection[25-27]. Various types of small RNAs found in several species of plants and microorganisms exhibit potential as therapeutic agents (**Figure 1**)[17-20].

Small RNAs from plants as anti-cancer NAT

The plant genome encodes numerous small RNAs that are involved in genetic and epigenetic silencing pathways. The abundance and diversity of small RNA classes differ among plant species[20]. The diversity of small RNAs from plants could be related to coevolution between environmental adaptations and influences from other organisms. Hence, the possibility of cross-kingdom transfer of these small RNAs can be predicted. Small RNAs are commonly found in staple foods, such as rice (*Oryza sativa*) and corn (*Zea mays*). A previous study showed that miRNA plays an important role in cross-kingdom gene regulation, where exogenous plant

miRNAs are found in human serum and possibly acquired through food intake. According to the dietary xenomiRNA hypothesis, miRNAs present in food stuffs may regulate the gene expression in cross-kingdom species[28-30]. Philip et al.[29] demonstrated that plant miRNAs remain intact even after storage, processing, and cooking. Common food materials, such as rice and soybean, contain miRNA that will remain intact after ingestion. Moreover, miRNAs that underwent genetic modifications can be incorporated into the food to protect it from degradation. The regulatory ability of these molecules must be further explored for cancer therapeutics.

MiRNA-168a from rice (*Oryza sativa*) has been found in human serum in its stable form; this miRNA can bind to mRNA encoding for low-density lipoprotein receptor adaptor protein1 (LDLRAP1) and inhibit the protein expression[28,30]. Plant miRNA159, which is abundant in mammalian serum because these organisms consume a variety of food, is inversely correlated to the incidence and progression of breast cancer[31]. Chin et al.[31] reported that miRNA159, encoding for the Wnt signaling transcription factor, can bind to and inhibit the proliferation of TCF7, resulting in reduced MYC protein level.

The potential of plant-based dietary small RNAs in cancer therapeutics have been investigated. Yang et al.[32] reported the presence of plant-specific small RNA MIR2911 in the serum
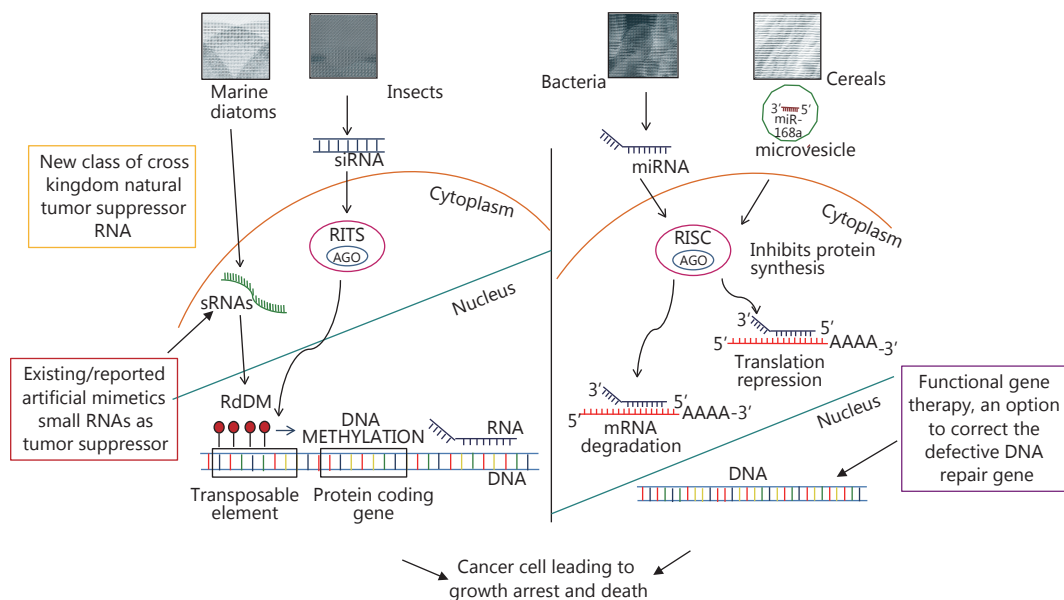
Figure 1 Mechanism of actions of cross kingdom natural small RNAs in cancer. This figure illustrates the mechanism of actions of cross kingdom natural small RNAs to bring cancer cell cycle arrest and death. Further, the mode of growth arrest in cancer cells via translational repression or mRNA degradation has been shown. Small molecular weight RNAs from various natural sources such as marine diatoms, insects, bacteria and cereals RNA have been shown to play a role in cancer. Additionally, pathway has been depicted for diatom encoded RNA directed DNA methylation (RdDM), which methylates the DNA leading to stall in transcription.

of mice fed with diet rich in vegetables. The findings confirm the intrinsic stability of plant-based RNAs for future cancer NAT. The ingested miRNA persists in the gastrointestinal (GI) tract, are packaged into extracellular vesicles (EVs), and are released into circulation to regulate gene expression in animals[31].

Small RNAs from bacteria and other lower organisms

Small, regulatory RNAs are present in bacteria. These sRNAs may base pair with target mRNAs by modulating protein activity and function as nucleic acid mimetics[18]. In prokaryotes, such as bacteria, clustered regularly interspersed short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas) are analogous to small RNAs[33].

In addition to various natural sources of small RNAs and other ncRNAs, the former has been identified in marine diatoms and function in gene-silencing activity. Most sRNAs possess length of 25 to 30 nt and target DNA-methylated protein-coding genes; hence, gene-driven methylation in diatoms may be mediated by sRNAs. Moreover, the majority of sRNAs comprise non-coding RNAs, tRNAs, and U2 snRNA, which play important roles in stressful environments like starvation, oxidative stress, etc. Furthermore, miRNAs have been found in two marine diatom species, namely, Thalassiosira pseudonana and P. tricornutum. Nevertheless, none of these species have been experimentally studied[34].

Types of small RNAs in anticancer NAT

Earlier discoveries in molecular biology have resulted in a paradigm shift. Lines of evidence showed that in addition to its function as regulator between DNA and protein, RNA regulates gene expression and genome organization[18-20]. Studies show that the number of genes encoding for RNA is higher than that of genes encoding for proteins[19]. Evolutionary RNA is considered the ancestor of deoxyribonucleic acid (DNA). DNA and RNA exhibit minimal structural differences, but the hydroxyl group at 2′ of the pentose ring significantly affects the interaction and folding properties of RNA. This 2′ hydroxyl allows the interactions between RNA fragments and RNAs and other biomolecules[35,36].

The completion of the human genome project confirmed that all plant and animal species require the same number of genes to produce proteins. Many of these species also produce non-coding regulatory products, such non-protein-coding RNA (npc RNA), which are genes that encode small

RNA molecules[18-20]. Small RNAs constitute different classes of non-protein-coding RNA molecules, such as miRNAs and small interfering RNAs (siRNAs), which function in many physiological and pathological processes. Scholars have revolutionized the understanding of gene performance and the physiology of cells by introducing RNAi[37-39]. With increased knowledge on molecular mechanisms through small-sized RNAs function in normal and malignant cells, researchers can elucidate tumor biology and discover novel therapeutic markers for cancer treatment[10,12,40-46]. In the human genome, many genes produce non-coding regulatory products instead of having 97% of non-coding DNA. Genes that encode for small or short length RNA are members of the non-protein-coding RNA (npcRNA) class, in which miRNAs constitute an important group[37,47].

MiRNAs are small non-protein-coding (~22 nucleotide) RNAs that function in several physiological and pathological processes, such as cell development, evolution, cellular differentiation, proliferation, embryogenesis, cell death, and gene expression[17,19,20,24]. MiRNAs bind to the 3′untranslated regions (UTRs) of mRNA molecules, causing either mRNA degradation or translation repression and eventually resulting in silencing of unwanted genes[47,48]. The suppressed degradation and translation of mRNA could be attributed to imperfect complementary and perfect complementary between miRNA and mRNA, respectively[17,19,20,49]. MiRNAs do not encode any proteins but interfere with protein production by targeting specific mRNA.

Small temporal RNA (stRNA) and siRNAs are categorized as two classes of miRNA. Only 1% of human genes encode for miRNAs. The first miRNA discovered in *Caenorhabditis elegans* in 1993 is Lin-4, which controls the development of the organism[15,50-52]. Thereafter, let-7 was discovered in 2000 and found to have similar effects to those of Lin-4[51]. Researchers have found the homologs of let-7 in many species, such as frog, mice, and humans, indicating the sequence similarity of let-7 among these species[15]. Tumor suppressor miRNAs, such as let-7[53] and miR-34[54], exhibit low expression levels in many cancers, leading to enhanced cell proliferation. Overexpressing or repressing miRNA expression, depending on the type of disease, and suppressing or replacing miRNA are promising areas for study in therapeutics. The overexpression of miRNA can be observed in many diseases. For example, miR-21 is overexpressed in many cancers, leading to the progression of cell cycle and enhanced proliferation[49,55]. Studies have mainly focused on the inhibition and replacement of miRNA for therapeutic purposes[49]. SiRNAs, which are a group of small dsRNA consisting of 21–23 nucleotides, can cleave the RNA

molecule as mediated by RNA inducing silencing complex (RISC), ultimately resulting in the disruption of translation[48].

Small nuclear RNAs (snRNAs), which are members of small RNAs, play an important role in splicing and are also known as spliceosomal RNA. As another class of small RNAs, small nucleolar RNAs (snoRNAs), which are found in the nucleolus, are involved in methylation and pseudouridylation of snRNAs, tRNAs, and rRNAs[37,56]. Cajal bodies, also called small cajal body-specific RNA (ScaRNAs) are small RNAs found in the subnuclear region[19]. Recently, two other classes of small RNAs have been identified in animals[19]; these classes include transcription initiation RNAs (tiRNAs)[57], which initiate the RNA transcription and splice-site RNAs (spliRNAs)[58]. These small RNAs are involved in nucleosome positioning and chromatin organization. Scholars have also reported the presence of less distinct classes, namely, promoter-associated RNAs (PASRs)[59], transcription start site-associated RNAs (TSSa-RNAs), and promoter upstream transcripts (PROMPTS)[60]. A summary of the information on different types of small RNAs is given in **Table 1**.

Obstacles and bottlenecks encountered in RNA delivery

One of the major challenges in small RNA therapy (RNAi technology) is the delivery of these molecules into the cell[7-13]. The delivery of nucleic acids (i.e., DNA, RNA, siRNA, shRNA, and antisense oligonucleotides) can down-regulate and silence unwanted gene expression and thus suppress tumor growth and invasion[3,48,77]. However, the delivery of these molecules remains challenging because of their large size and negative charges; the main obstacles are related to stability of small RNAs as therapeutic drug, controlled intra-cellular and inter-cellular release, unwanted inflammation due to immune responses, and precision to the target genes[7-13]. Therefore, the delivery system must be modified to achieve stability against serum nucleases, evade the immune system, avoid non-specific interactions with serum proteins and non-target cells, prevent renal clearance, and minimize off target effects[41,78-80].

Synthetic small RNAs as mimetic agent

The two major types of small dsRNAs, namely, siRNAs and miRNAs, participate in RNA interference (RNAi), which involves several mechanisms, including gene silencing by remodeling chromatin to suppress transcription; degrading complementary mRNA; or blocking protein translation[12,44-46]. The development and design of synthetic small RNAs in cancer therapeutics have gained increasing research attention. Considering the different methods available for synthetic development of small RNAs, Yoo et al.[81] reported that synthetic small RNAs can be constructed through conventional gene cloning. The available different therapeutic options using drugs containing small RNAs are listed in **Figure 2**[18-20].

Chemically synthesized siRNAs have been developed to target mammalian cells without any innate immune responses to improve their inherent properties, such as *in vivo* instability, off-target effects, and immunogenicity. The most widely used chemically synthesized siRNA is (19+2

Table 1 List of small RNAs and their function

Type	Role	Size (nt)	Reference
Scan RNAs (scnRNA)	Genome rearrangement, chromosome segregation, meiotic prophase	~29	61, 62
Small nuclear RNA (snRNA)	Splicing (removal of introns from genes)	120–300	63
Small nucleolar RNA (snoRNA)	rRNA processing	Variable 87–275	64-66
Repeat-associated siRNA (rasiRNA)	Silencing of genetic repeat	~24–26	67-69
Transacting short interference RNA (tasiRNA)	Post -transcriptional gene regulation	~21	67, 70
Natural antisense transcript-associated siRNA (natsiRNA)	Derived from antisense transcript region	20–25	67, 70
Piwi -intracting RNAs(piwiRNA)	Fertility of male mammals, male fish or fly of either sex	26–31	24, 71-73
X- inactive specific transcript RNAs (xistRNAs)	Inactivation of X chromosome, more severe in females	16500	14, 74-76
Pregnancy- induced non-coding RNA (pincRNA)	Effective during pregnancy	22–25	37
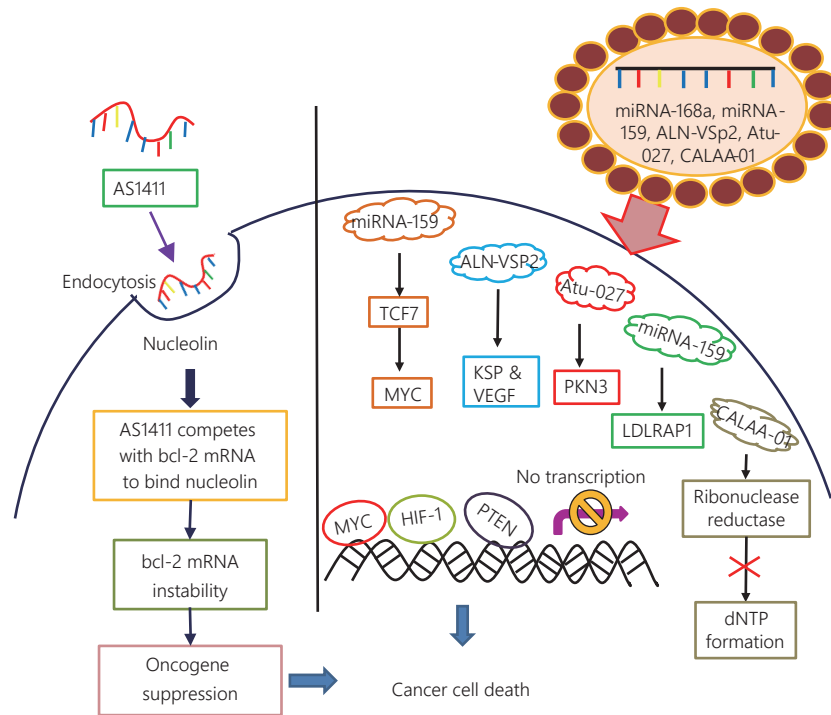
Figure 2 Various RNA based drugs to target cancer cell. This figure depicts the newly synthesized RNA and drugs, which targets the progression of cancer cell by inhibiting the transition from epithelial to mesenchymal cells and also by preventing angiogenesis. An aptamer drug AS1411 has been depicted to block the nucleolin receptor based signaling and inhibit the oncogene expression for cancer survival. On the other hand, this diagram shows the use of small RNAs as a cancer therapeutic approach including ALN-VSP2, miRNA-159, CALAA-01 etc.

traditional siRNA), which is similar to the nature's dicer product[13,82]. Chemically synthesized siRNAs with two nucleotides overhanging at 3' can efficiently cleave siRNA, and the cleavage site is located near the region covered by the guiding siRNA[83]. Major challenges associated with RNAi mechanism exist due to the naked RNA structure of naturally available small RNAs; such challenges include rapid RNA degradation in biological fluids, poor cellular uptake, and off-target effects. In this regard, synthetic RNA mimetics have been developed for RNAi- based medicine[11-13].

Synthetic gRNAs induce a site-specific mutation in the target sequence. The site-specific mutation mediated by gRNA modifies pre-miRNA without synthesizing particular miRNAs[84]. Cancer cells possess the ability to modify pre-miRNA alternative splicing[85]. The gRNA lentiviral CRISPR/Cas9 vector can interrupt miR-21, which inhibits epithelial-to-mesenchymal transition (EMT)[84].

Synthetically created mRNAs exhibit strong therapeutic potential after its introduction into mammalian cells. These mRNAs are stable and can encode desired proteins[86]. Such mRNAs are known as naked mRNA because they elicit immune responses around the tumor cells. Naked mRNAs do not need any carriers for transport and require short period of time to show their effectiveness; hence, these mRNAs are important in the field of modern therapy[87].

Nat delivery system

Considering the potential mechanisms of small RNAs in gene therapy against cancer, scholars have performed gene silencing by decreasing the expression of the target genes, which are mostly oncogenes, in cancer[10,12,42,44-46]. These interfering RNAs can be synthetic (i.e., oligonucleotide therapy) or encoded in novel genes; as such, the sequences are the inverse of the normal sequence (i.e., antisense) and can thus hybridize to the message and prevent its translation[10,12,42,44-46]. Systemic gene delivery is one of the major challenges in modern cancer gene therapy and is a limiting factor in experimental and clinical approaches[9,40,88,89]. Synthetic siRNAs are poly-anionic macromolecules that do not readily enter the cell but require a delivery system for effective gene silencing[90,91]. Previous

studies attempted the precise and effective delivery of these potential NAT agents by using several targeted delivery systems, such as exosomes, nanocarriers, and aptamers[48,92]. First, naked therapeutic DNA or RNA can be transferred into cells by using high voltage (i.e., electroporation), through uptake by invaginating vesicles (i.e., endocytosis), or by sheer mechanical force using a gene gun instrument. DNA or RNA can be packaged into liposomes (i.e., membrane bound vesicles) and naturally released exosomes, which can be more easily absorbed by the cells than naked DNA/RNA[87,93,94]. Different types of liposomes have been developed to preferentially bind to specific tissues and modify protein or RNA at different levels. Third, DNA or RNA can be packaged into virus-like particles by using a modified viral vector. Finally, DNA or RNA can be combined with cell therapy protocols[95]. Chemically synthesized siRNAs can be encapsulated within the nanocarriers and may be administered with chemotherapeutic drugs[96]. **Figure 3** shows the schematic of therapeutic delivery options based on small RNAs for cancer treatment[12,44-46].

With the advancements in nanotechnology, cancer therapy has considerably progressed. In nanomedicine, a wide variety of nano-carriers containing polysaccharides have been developed. Nanoparticle carriers, which are non-toxic, biocompatible, biodegradable, and immunoefficient, can be potentially used for cancer therapy[97]. Ideal polymers for

preparing nano-systems include anionic polymers, such as hyaluronic acid, heparin, or alginate, which exhibit anticancer property[98]. Modified delivery systems include lipid carriers and polymers aptamers. Nanotechnology and modern cancer research have allowed the development of highly safe medicine with reduced toxicity and ability to carry large payload and multivalent ligand targeting and the improvements in cancer diagnostics[7]. Yuan et al.[99] showed that nanoparticles carrying three different siRNAs can be delivered to tumor xenografts. The simultaneous delivery of *KRAS-*, *PIK3CA-*, and *PIK3CB-*targeting siRNAs improved the therapeutic efficacy but did not increase the toxicity of the drug. Therefore, this approach can be used to develop nontoxic drugs for tumor suppression.

Exosomes as NAT cargo system

Exosomes are naturally occurring nano-sized vesicles released by all mammalian cells to facilitate cell-to-cell communication[44]. The delivery of exogenous siRNAs to the target cells poses many obstacles for effective gene therapy. The unmodified siRNA is unstable in the bloodstream, is immunogenic, and cannot easily cross the membrane and enter the cell. Therefore, a siRNA-based therapeutic approach requires a safe and reliable delivery method[80]. Hence, natural exosomes are used to deliver therapeutic
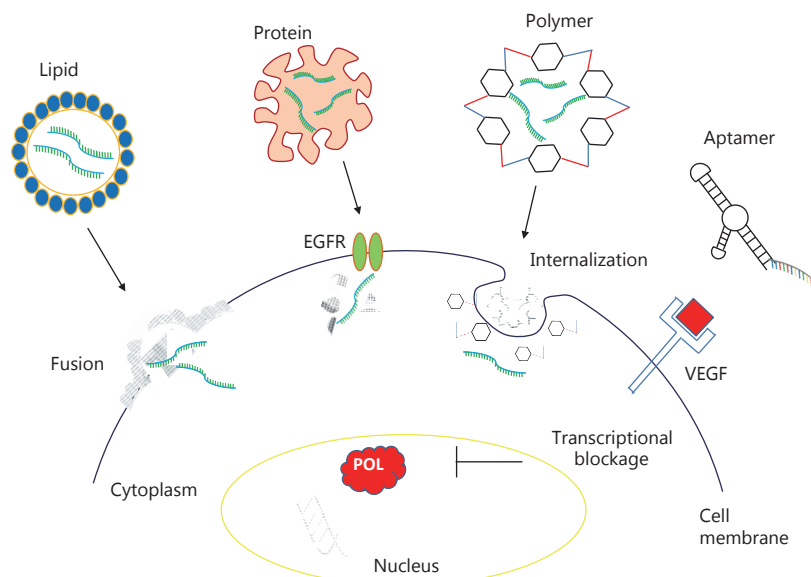
Figure 3   Import pathways of small molecular weight RNAs. This figure shows the diagrammatic representation of import of small molecular weight RNA through cellular or non-cellular contents. Small molecular weight RNAs have been shown to enter either through lipid vesicles, protein conjugates or through a polymer. These carriers are either embedded or engulfed by the cell membrane and RNA is released into the cells. After entry into cell, these small RNAs facilitate the blockage of transcription.

siRNAs to target cells[42]. O'Loughlin et al.[46] reported the use of extracellular vesicles to deliver small RNAs as therapeutic RNA cargo. Cholesterol-conjugated siRNAs (cc-siRNAs) should be used to develop EV-based therapeutics with increased loading of small RNAs to extracellular vesicles. Lunavat et al.[44] proposed a new class of extracellular vesicles to efficiently transport the RNA molecule into the cell cytoplasm. The authors provide evidence on the effective use of exosome-mimetic nanovesicles (NV) to reduce the expression of target genes, such as c-Myc, in cancer cells.

Aptamers and NAT

The conjugation of siRNA to delivery vehicles is a promising anticancer therapy. This single-component system uses equimolar concentration of the delivery system and siRNA. Several conjugate delivery systems have been developed by coupling siRNA to polymers, such as aptamers, peptides, and proteins[80,100,101]. Several researchers have transformed molecular recognition by fabricating synthetic RNA motifs that bind to specific targets. These molecules are called RNA aptamers[102-104]. These aptamers are selected through *in vivo* selection method called the systematic evolution of ligands by exponential enrichment (SELEX)[103-105]. These synthetically derived molecules are selective and demonstrate high affinity toward the target[8,104]. RNA aptamers are a special class of nucleic acids that can fold into composite structures[106] and possess pockets and hands for binding specific molecules. AS1411 is the first aptamer approved for clinical trial for treatment of different types of cancer. Wang et al.[101] demonstrated the application of aptamer AS1411-modified extracellular vesicles as RNAi nanoplatform for breast cancer therapeutics. Chen et al.[107] used a novel aptamer-siRNA chimera delivery system mediated by cationic Au-Fe3O4 nanoparticles (NPs) to reverse the multi-drug resistance (MDR) of ovarian cancer cells. Considering their development for systemic delivery, RNA aptamers have become active therapeutic agents particularly for blood stream and NAT[104,108].

Liposome- and polymer-assisted delivery of NAT agents

Lipids are involved in the survival, proliferation, and death of cells and in cell-cell interaction[45]. Lipids, particularly phospholipids, function in cellular processes, such as signal transduction, post-translational modification, homeostasis, adhesion, migration, apoptosis, neurotransmission, vesicular trafficking, and energy storage[109,110]. A combination of cationic polymers, polyethyleneimines (PEIs), and liposomes can facilitate the formation of lipopolyplexes, which are used to deliver nucleic acids with improved efficacy and biocompatibility[111]. Many liposomes, such as cationic lipids, can be used to successfully deliver small RNAs. Positively charged lipids can improve the entrapment of small RNAs, increase the cellular uptake, and provide protection from endosomal escape. Examples of cationic lipids are Lipofectamine 2000 and RNAiMAX transfection reagent, which is a recently developed compound with high efficiency[80,101]. The modified cationic lipids do not act as carrier in the delivery of small RNA, but provide stability against serum and access to RNAi machinery[101,112-114].

Polymer-assisted delivery vehicles comprise polymeric nanoparticles and are characterized as biodegradable; these vehicles prevent aggregation during storage, increase the circulation time, and reduce the off-target effects[80,101,115]. Cyclodextrin and polyethylenimine (PEI) are the most commonly used polymers for siRNA delivery[101,116-118]. Other polymers used for NAT delivery are polycaprolactone (PCL), poly (D, L-lactide) (PLA), poly (D, L-lactide-co-glycolide) (PLGA), chitosan, poly-L-lysine, dextran, polyglutamic acid, hyaluronic acid, and gelatin[48,101,119]. Magnetic molecularly imprinted polymers (MMIPs) are another form of delivery system and are synthesized through photo-polymerization of methacrylic acid and ethylene glycol dimethacrylate around the template molecule and in the presence of magnetite. These polymers exhibit controlled release and high magnetic properties[120].

Clinical evaluation of therapeutic small RNAs

Various small RNAs have been reported by preclinical and clinical interventional studies[7-13,46]. Since the discovery of RNAi, more than 50 clinical trials involving 26 different siRNAs have been documented[8-13]. A previous study reported the use of siRNAs with liposome as a delivery vehicle to treat a patient with chronic myeloid leukemia[121]. Zou et al.[122] reported the potential of vascular endothelial growth factor siRNA (VEGF-siRNA) for treatment of hepatocellular carcinoma. RNAi is a potent tool in gene regulation, and the first in-man trial of ALN-VSP02, an RNAi therapy, has been reported in patients with advanced solid tumors[123,124]. Finally, preclinical and clinical trials and investigations have been conducted on the use of Atu027, a siRNA-lipoplex directed against protein kinase N3 (PKN3), for treatment of advanced solid cancers, including pancreatic cancer[125,126].

CALAA-01, a type of siRNA targeted to curb the

expression of M2 sub-unit of ribonucleotide reductase (R2), can be used for siRNA-based therapies for cancer[127]. This drug is formulated in a stabilized nanoparticle to prevent nuclease-mediated degradation within tumor cells. The drug ALN-VSP02 is a lipid nanoparticle formulation containing two siRNAs targeting kinesin spindle protein (KSP) and VEGF with potential antitumor activity[123]. A phase 1 clinical study was conducted to investigate the use of TKM 080301, a type of lipid nanoparticles encapsulating siRNA targeted to the PLK1 gene for treatment of primary or secondary liver cancer[128]. Furthermore, the drug ALN-VSP02 demonstrated siRNA-mediated mRNA cleavage in the liver and exhibited antitumor activity[123].

Another phase 1 interventional study evaluated the immunotherapy of melanoma by using tumor antigen RNA and small inhibitory RNA loaded into dendritic cells to target immunoproteasome beta subunits LMP2, LMP7, and MECL1[129]. Another clinical study evaluated the efficacy and safety of single-dose siG12D LODER administered with chemotherapy drugs to patients with unresectable locally advanced pancreatic cancer[130]. An ongoing clinical study reported on the use of siRNA-transfected peripheral blood mononuclear cells APN401 for treatment of pancreatic cancer, colorectal cancer, and other solid tumors that spread to other places in the body or have relapsed[131].

Conclusions

Non-coding small RNAs have been increasingly investigated as NAT to repress pivotal specific genes related to tumor progression and drug resistance. These new class of drugs should be viewed from the perspective of combinatorial options as a cocktail of cancer drug therapy. Hence, the use of these small RNAs as NAT in cancer treatment should be encouraged in conjunction with other drug options, such as epigenetic, signaling, growth, and metastasis blockers. Such combinations may contribute to completely eradicate issues with regard to inherent tumor heterogeneity and drug resistance in clinical settings. In addition to new findings and the development of NAT based on small RNAs from natural and artificial sources, further studies should evaluate the stability and precision of NAT for targeting tumor tissues and analyze specific compartments of intercellular and intracellular locations. To achieve therapeutic approaches with effective targeting at the site of action, scientists, clinicians, and industry researchers should collaborate to develop an efficient drug delivery system and nano-imaging system for improved monitoring of cancer treatment. Overall, small-RNAs-based NAT is more effective and precise and poses less risks to healthy tissues than existing traditional therapeutic regimens for cancer.

Conflict of interest statement

No potential conflicts of interest are disclosed.

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