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(REVIEW ARTICLE)

The role of siRNA and Argonaute 2 in Cancer Therapy: Molecular mechanisms and drug design perspectives

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Abstract

RNA interference (RNAi) has emerged as one of the key mechanisms of post-transcriptional gene regulation and one of the most promising therapeutic approaches for precision cancer therapy. These include small interfering RNAs (siRNAs) and the catalytic Argonaute 2 (Ago2) protein acting alone or jointly to silence target mRNAs in a sequence-specific way by an irreversible mechanism reminiscent of enzymatic degradation. This study reviews the structural and functional facets of siRNAs and Ago2, along with their synergistic role in targeted cancer gene silencing. We point out the therapeutic use of siRNA-directed oncogene (KRAS, MYC, BCL-2) silencing and explain how Ago2 participates in oncogenic signaling pathways such as MAPK/ERK, PI3K/AKT, and FAK. The chemical modification and nanocarrier-based delivery systems have got siRNA much more stable, specific, and bioavailable, thus virtually solving the major problems of translational research such as nuclease degradation and immune activation. The coupling of artificial intelligence, bioinformatics, and CRISPR technologies has profoundly changed the conception and accuracy of RNAi-based therapeutics. Delivery obstacles, immunogenicity, and large-scale manufacturing problems still exist, but recent inventions like self-delivering siRNAs, circular siRNAs, and hybrid CRISPR-RNAi systems are opening up the way to siRNA-Ago2 clinical applications at a fast pace. Altogether, these breakthroughs make RNAi a revolutionary route in molecularly targeted cancer therapy.

Keywords: siRNA; Argonaute 2 (Ago2); RNA Interference (RNAI); Gene Silencing; Oncogene Targeting; Nanocarrier Delivery; Chemical Modification; KRAS; MYC; BCL-2; PI3K/AKT Pathway; Cancer Therapeutics; CRISPR–RNAI Hybrid

1. Introduction

RNA interference (RNAi) forms a central part of the intrinsic homeostatic mechanism of all cells in nature and has been evolutionarily conserved [1]. Discovered originally in Caenorhabditis elegans as a mechanism for gene-specific silencing induced by double stranded RNA (dsRNA) [2], the phenomenon has become firmly established as a general mechanism of regulation across eukaryotic organisms [3]. The process involves the generation of small RNAs—mainly small interfering RNAs (siRNAs) and microRNAs (miRNAs)—that guide RISC to the target messenger RNA (mRNA) molecules which are then degraded or translation is inhibited [4].

RNAi has emerged as a potent tool in functional genomics and is an efficient and flexible means to uncover gene function, validate targets, and for therapeutic purposes [5,6]. Due to its exceptional sequence specificity, disease-related genes considered previously as “undruggable” can be specifically suppressed by small molecule inhibitor or monoclonal antibody [7].

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The principal effectors of RNAi are short interfering RNAs (siRNA) typically 21–23 nucleotide molecules originating from long dsRNA precursors that upon enzymatic processing by the ribonuclease Dicer [2,6]. This enables incorporation of the resultant siRNAs into RISC, with a guide strand that remains bound and its partner (passenger) strand being degraded [8,9]. Argonaute 2 (Ago2, a principal representative of the argonaute protein family) is the catalytically active core of RISC that mediates endo-nucleolytic cleavage of target mRNAs [8,10]. Supported by the siRNA sequence, Ago2 cleaves its target exactly between nucleotides 10 and 11 of the guide strand, leading to a very rapid degradation of these fragments [7].

Human Ago2 can be divided into four structural domains, N-terminal, PAZ, MID and PIWI that serve to mediate RNA binding and catalysis [10]. The PIWI domain contains the DEDH catalytic tetrad that is required for its slicer activity. A detailed knowledge of these structural and mechanistic characteristics has been crucial in the rational design of siRNAs with preferred strand selection, thermodynamic stability and minimal off-target effects [6].

Altered gene expression is a well-established characteristic of cancer etiology and progression, resulting from mutations in core signaling machinery as well as disruption of transcriptional and post-transcriptional control [3,11]. siRNA-directed approaches offer an effective way to specifically down-regulate oncogenes, tumor-promoting factors or drug-resistance mediators with great specificity [12,13]. In contrast to classical chemotherapy or targeted inhibitors, siRNA therapeutics act at mRNA level and, therefore, provide potential suppression of pathological proteins independent of their structural draggability [4].

The effectiveness of siRNA-mediated knockdown of oncogenes such as KRAS, MYC and BCL-2 has also been established in multiple studies with induction of cell growth inhibition, apoptotic cell death and abrogated metastatic potential in preclinical models [14]. Moreover, siRNA-mediated therapy in combination with conventional chemotherapeutics sensitizes tumors and abrogates multidrug resistance, substantiating the potential of RNAi as a new generation gene-targeted cancer therapeutic [12].

Chemical modification and nanocarrier-mediated delivery have recently advanced siRNAs to enhance stability and potency. Modifications including 2′-O-methyl (2′-OMe), 2′-fluoro (2′-F) and phosphorothioate bonds increase nuclease resistance, reduce immune activation, whereas lipid and polymeric nanoparticles enables efficient packaging, specific targeting delivery and endosomal escape [4,15].

FDA-approved siRNA drugs, such as Patisiran and Inclisiran have demonstrated clinical success supporting the therapeutic potential of RNAi, and establishing a platform for broader implementation in oncology [16,17]. Innovative delivery systems, for instance self-transporting siRNAs (sd-siRNAs), circular siRNA, and stimuli-sensitive nanocarriers are pushing the research toward enhanced precision of action, stronger therapeutic effect [15].

Nevertheless, siRNA-based therapeutics still remain to address major issues such as nuclease degradation, immune stimulation, rapid systemic clearance and limited intracellular delivery [4]. Overcoming these limitations will necessitate the combination of chemical optimization with targeted delivery that enables improvement in stability, bioavailability and tumor targeting [7].

Novel cross-disciplinary methods are also influencing the development of RNAi-based therapeutics. The combination of AI with bioinformatics and structural biology is facilitating the design of more potent and specific siRNAs beyond chemical enhancement, as well as engineered mutant Ago2. Furthermore, novel hybrid approaches of RNAi with CRISPR-based genome editing are being developed to enable long-term control over programmable and specific gene regulation [1,18]. Collectively, these developments place RNAi as a breakthrough technology for precision oncology and molecular therapy [12,17].

2. siRNA: Structure, Biogenesis, and Mechanism of Action

Small interfering RNAs (siRNAs) are a class of small, double-stranded non-coding RNA molecules that play a significant part in the gene silencing process RNA interference [19–21]. Usually 21–23 nucleotides (nt) long [19,21], these entities are produced by the RNase III enzyme Dicer during the processes of splicing and modification of RNA [20]. Each siRNA is composed of two strands —a guide (antisense) strand, which engages the target mRNA specifically for silencing, and a passenger (sense) strand that is removed during RISC activation [22]. Dicer activity cuts long dsRNA substrates into 21-nt siRNAs with typical structural properties of 2-nt (nucleotide) 3′ overhangs on each strand. Every strand has a 5′ terminus monophosphate and a 3′ terminus hydroxyl [23,24] this specific chemical property reflects genuine Dicer processing and is necessary for efficient silencing by the RNA-induced silencing complex (RISC) [23,24].

Thermodynamic asymmetry determines which of the strands will be assigned as guide: the less stably paired 5′ end of the strand, usually AU-rich rather than GC-rich, is preferentially incorporated into argonaute (Ago) proteins in RISC. The foundational principle of which is well-established in seminal work done by Khvorova et al. (2003) and Schwarz et al. (2003) [23], guarantees directional targeting. Strand selection is also influenced by other factors, such as the nature of the 5′ nucleotide (preferentially U/A) and the presence of loading cofactors for RISC (like TRBP and PACT) [23].

Chemical modifications—such as 2′-O-methyl, locked nucleic acids (LNAs), or phosphorothioate linkages—have the capacity to enhance resistance to nucleases [25,26], reduce immune-stimulation, and affect strand bias, thus making them critical components in the development of therapeutic siRNAs.

2.1. Biogenesis of siRNA

2.1.1. Origins of dsRNA Precursors

In plants, predominantly invertebrates, siRNAs are products of RNA-dependent RNA pols (RdRPs) that synthesize long dsRNAs from RNA templates [27,28]. Dicer processes these dsRNAs in the cytoplasm and then loads it into RISC to mediate sequence-specific gene silencing [28,29]. In plant cells, this is often initiated by environmental stress, transposon activity or viral infection. Unlike in mammals that do not have RdRPs and it was presumed that they only produce exogenous siRNAs [27]. Appreciation of this viewpoint was altered by the discovery of rare endogenous sources of dsRNA as follows [28]: Retrotransposons[30]. For example, LINE-1 elements in humans produce bidirectional polyadenylated RNA transcripts from a double promoter system expressed in both directions oriented within genes and which are therefore copied into complementary RNAs that hybridize to form dsRNA [31]. Pseudogenes containing inverted repeats [32].These structures permit the formation of long intramolecular hairpins [32], reminiscent in structure to shRNA precursors. Transcriptional units overlapped: Antisense transcription from protein-coding loci or noncoding loci may produce two complementary RNAs, suitable for dsRNA synthesis [33].

Endogenous sources of such dsRNA have been found in a number of organisms. AGO2-bound small RNAs in Drosophila contain a unique 21 nt siRNA class that includes pseudogene and inverted repeat derived species [30]. SiRNAs in oocytes have been described in mice, are Dicer-dependent, and occasionally target protein-coding genes [33,34].

2.1.2. Dicer Processing

In the cytoplasm, Dicer digests long double-stranded RNAs (dsRNAs) into small interfering RNA (siRNA) duplexes with specific 2-nucleotide 3′ overhangs and a phosphorylated 5′ termini in the cytoplasm environment [25,26]. An siRNA duplex consists of a guide strand that actively is incorporated into the RNA-induced silencing complex (RISC) and a passenger strand, which will be degraded [35].

RISC Loading and Strand Selection

The siRNA duplex binds to AGO2, the catalytically active mammalian Argonaute protein, and forms the pre-RISC complex [36,37]. The thermodynamic asymmetry between the duplex termini, which drives strand selection [38], generally the less stable 5' end is designated as the guide strand. The passenger strand is subsequently excised and degraded, resulting in the formation of the mature RISC [39].

Target Recognition and Gene Silencing

RISC is targeted to mRNAs with high sequence complementarity by the guide strand. Target RNA cleavage by AGO2 at a single site between nucleotides 10 and 11 relative to the guide strand triggers transcript degradation. Whilst microRNAs frequently have mismatches and mediate translational repression, siRNAs generally lead to efficient mRNA cleavage with near-perfect base pairing [40].

2.1.3. Endogenous vs. Exogenous siRNAs

In vivo, most siRNA experiments in mammals have employed exogenous delivery either using synthetic siRNA duplexes or using short hairpin RNAs (shRNAs) expressed from plasmid or viral vectors [41]. shRNAs, driven by Pol II or Pol III promoter called an RNAi vector [42], are transcribed in nucleus targeted to be processed into pre-shRNA by the Microprocessor (Drosha–DGCR8), then exportin-5 transferred into cytoplasmic space for dicing into siRNA duplexes for RISC complex.

Endogenous mammalian siRNAs are sparsely expressed and less established as regulatory molecules relative to miRNAs and piRNAs [43]. They probably evolved from evolutionary ancient antiviral defense systems and the loci that give rise

to them might still be under selective pressure [43,44]. The functional roles of mammalian endo-siRNAs, particularly those that target protein-coding genes, still need to be elucidated in the course of further research [45].

2.2. Mechanism of mRNA silencing

siRNAs trigger gene silencing by becoming part of the RNA-induced silencing complex (RISC) [19,21]. Artificially synthesized siRNAs, generally 21–23 nucleotides long with 2-nt-long 3′ overhangs [46–48], are delivered to cytoplasm through chemically modified delivery agents such as lipid nanoparticles, conjugates, or viral vectors [19,27,49]. One important step is the endosomal escape as cytoplasmic localization is necessary for RISC formation [49].

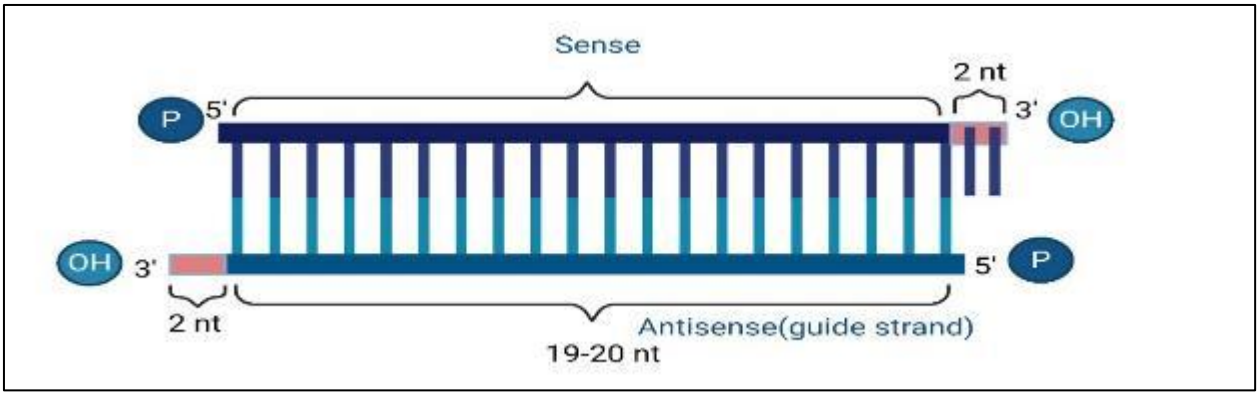

Figure 1 Structural features of exogenous siRNA. Exogenous siRNAs are typically designed as 19-20 bp RNA duplexes with two-nucleotide 3' overhangs composed of DNA nucleotides (red #E07F80). The canonical 5' monophosphate and 3' hydroxyl termini are preserved to ensure proper argonaute loading and gene-silencing activity

In RISC, the double-stranded siRNA is subject to strand selection, with the passenger strand eliminated, primarily via Ago2-catalysed cleavage or by dissociation with the help of accessory factors [50]. The passenger strand is thermodynamically locked onto Ago2, the catalytic engine of the complex [51]. The guide strand guides RISC to mRNA sequences that are complementary via Watson–Crick base pairing [52]. Recognition is directed by the seed region (positions 2–8 from the 5′ end of the guide strand), and silencing efficiency correlates with the size of base pairing [4].

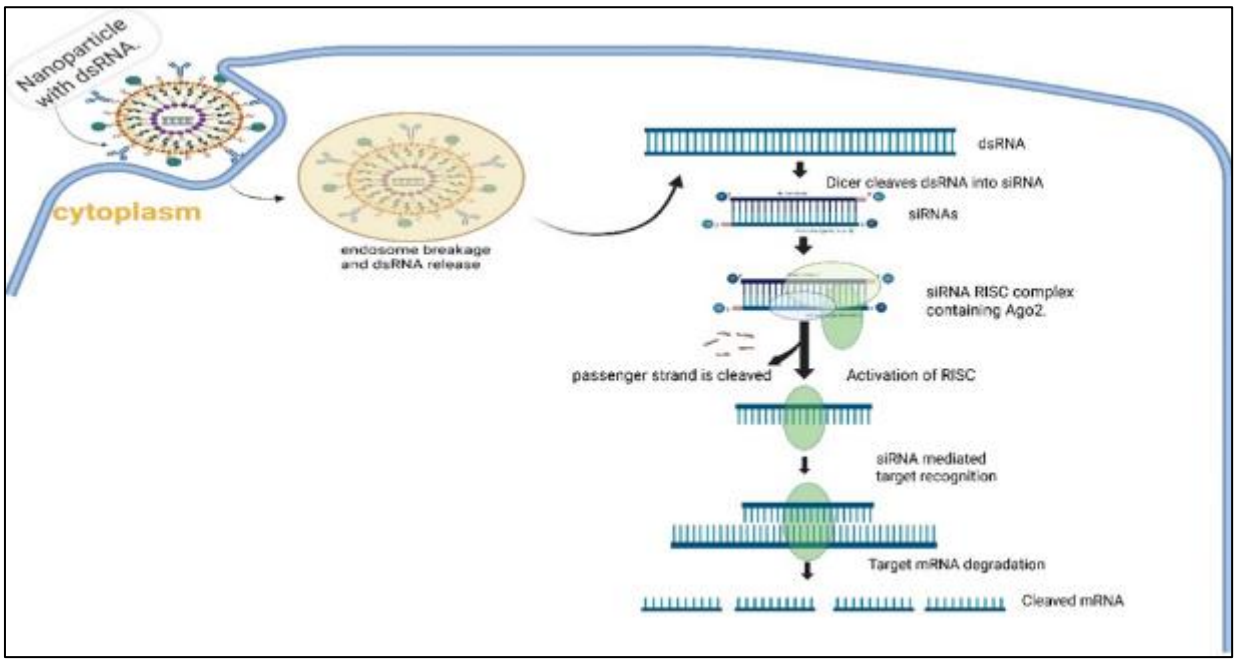

Figure 2 Schematic illustration of the small interfering RNA (siRNA) mediated gene silencing

In cases of nearly perfect base pairing, typically in a coding region, Ago2 catalyzes endonucleolytic cleavage of the target mRNA between the nucleotides opposite the guide positions 10 and 11. These resulting RNA fragments are then

degraded rapidly by cellular exonucleases, including XRN1 and the exosome complex [53], thereby inducing fast, irreversible, and potent silencing of gene expression. This cleavage-based pathway is the major form of action for siRNA therapeutics and designed to be fully homologous to their targets. In contrast, in the case of only partial complementarity, usually in the 3′UTR, slicing activity of Ago2 is not involved. Instead, RISC acts to recruit effector proteins, which block translation initiation, induce ribosome disassembly, and promote mRNA decay at the level of deadenylation and decapping, frequently within processing bodies (P-bodies) [25,26,54]. Even though this mode of action seems to be more typical of miRNA-mediated regulation [55], off-target effects in the form of non-cleavage can be elicited by siRNAs under limiting complementarity. Therefore, siRNA-induced gene silencing is mainly initiated via Ago2-cleavage of the mRNA [54,56], leading to the subsequent secondary events such as translation repression and target mRNA degradation [48]. The siRNA therapeutic opportunity emanates from their ability to manipulate this endogenous system with specificity and potency [57], depending on the successful addressing of delivery, stability and endosome escape hurdles.

3. Argonaute 2: Structure, Function, and Role in RNAi

3.1. The argonaute Protein Family

The Argonaute (Ago) proteins are a group of specialized small-RNA-binding proteins that act as the main effectors in RNA-silencing pathways [58]. Small RNAs must be incorporated into Argonaute-associated ribonucleoprotein complexes, collectively known as RNA-induced silencing complexes (RISCs) [59], in order to carry out their regulatory functions. Argonautes are processed into complexes that use small RNA guides to identify complementary nucleic acid sequences, allowing for post-transcriptional gene regulation, transcriptional silencing or genomic defense within these complexes [59].

The name of this family originates from a phenotype described in Arabidopsis thaliana of a mutant with knockout of AGO1 that had abnormal leaf shape similar to tentacles of the octopus Argonauta Argo [60]. Argonautes are divided into two major subclasses, the Ago subfamily, which is similar to Arabidopsis AGO1, and the Piwi subfamily, which is homologous to Drosophila PIWI proteins [8]. Ago subfamily is widely expressed in somatic cell types, and mainly functions by associating with miRNAs and siRNAs. The Ago subfamily contains AGO1, AGO2, AGO3, and AGO4 in the human genome [61]. Of these, AGO2 is unique in that it retains endonucleolytic "slicer" activity to cleave target RNAs while the other paralogs mainly exert translational repression and mRNA destabilization activity [62]. Despite a wide conservation of Ago proteins between species, the copy number is highly variable: from one in Schizosaccharomyces pombe [60], five in Drosophila, eight in humans, ten in Arabidopsis thaliana, and as many as twenty-seven in Caenorhabditis elegans. Interestingly, although several emergent yeasts, including Saccharomyces Castellii, have functional argonaute genes and generate siRNAs by means of a divergent Dicer enzyme, the widely used model organism Saccharomyces cerevisiae lacks Argonautes altogether, along with canonical RNAi pathways. On the other hand, the Piwi subfamily has a much more limited expression pattern, mainly expressed in germline cells, where Piwi proteins bound to Piwi-interacting RNAs (piRNAs) silence transposable elements in the germline to maintain genomic integrity [63]. Although four piwi proteins (HIWI (PIWIL1), HILI (PIWIL2), HIWI3 (PIWIL3) and HIWI2 (PIWIL4)) are encoded in the human genome [64], most studies in mammals have focused on HIWI and HILI [63]. Three Piwi proteins, MIWI, MILI, and MIWI2, are expressed during spermatogenesis of mice and play a key role in transposon regulation. Likewise, argonaute proteins have been detected in various lineages of bacteria and archaea but, here, the distribution seems rather erratic. Comprising a highly conserved four-domain architecture (N, PAZ, MID, and PIWI) [65,66], these prokaryotic Argonautes (pAgos) frequently employ DNA as a guide rather than RNA [67], using a variety of foreign DNA and RNA substrates as part of their defense systems against invading elements (phage and plasmids) [68], although Pagos have so far received much less attention relative to eukaryotic Argonautes [69].

Table 1 Classification: Ago vs. Piwi clades. [8,70,71]

Feature	AGO Clade	PIWI Clade
Small RNA Guides	Binds siRNAs (~21 nt) and miRNAs (~22 nt) to regulate gene expression via RISC.	Binds piRNAs (~24–31 nt) to silence transposons and preserve genome integrity, especially in the germline.
Expression Pattern	Ubiquitously expressed across somatic cells in most organisms.	Primarily restricted to germline cells in animals
Function	Facilitates post-transcriptional gene silencing via mRNA cleavage or translational repression.	Mediates transposon silencing through cytoplasmic cleavage and/or nuclear silencing, often involving heterochromatin formation.
Target Recognition Rules	Requires strict seed pairing (guide nucleotides g2–g8) for target recognition and silencing.	Displays relaxed complementarity requirements, tolerating mismatches even near the cleavage site—advantageous for combating rapidly diverging transposon.
Slicer Activity	Many AGO proteins have endonucleolytic ("slicer") activity, e.g., human AGO2 cleaves target mRNAs directly.	Some PIWIs (like Aub and Ago3 in Drosophila) are slicers in the cytoplasm, but others (like Piwi) lack slicer activity and act as a scaffold for co-transcriptional silencing.
Evolutionary Conservation	AGO and PIWI are two phylogenetically distinct subfamilies within the Argonaute superfamily	PIWI proteins are animal-specific, evolving unique functions in germline defense against transposons

3.2. Structural domains of Ago2

Recent structural observations support a bi-lobe architecture of human AGO2 as defined by the N–PAZ lobe connected through two flexible linkers (L1 and L2) to the MID–PIWI lobe (in what is referred to as mid-kernel exchange) [72]. Collectively, these lobes create a central cleft that binds the guide RNA, as well as its complementary target strand, allowing AGO2 to serve as the catalytic engine of RNA-induced silencing complexes (RISCs) [72].

Figure 3 Linear representation of human AGO2, illustrating its four major domains along with their core functions.

Domain	Function
N	Unwinds RNA duplex
PAZ	Binds 3′ overhang of guide RNA
MID	Anchors 5′ phosphate of guide strand
PIWI	Catalytic activity (endonuclease/slicer)
C-terminal	Structural extension (no defined domain)

3.2.1. N-terminal Domain

The N-terminal domain plays an essential role in RISC complex assembly, particularly in facilitating the unwinding of the small RNA duplex and passenger strand elimination. This component represents a rate-limiting step in RISC activation. The N-domain, although not essential for RNA loading and slicing, is required for assembly and functional maturation of RISC [73]. In addition, the N-domain makes a second contribution to guide-target pairing, by limiting pairing outside of the 16th nucleotide of the guide RNA, thus ensuring that only a specific location is cleaved [72,74], although this function is not yet definitively shown for eukaryotic AGO2.

3.2.2. PAZ Domain

The PAZ domain shared between AGO2 and Dicer has the oligonucleotide/oligosaccharide-binding (OB) fold structure that specifically binds to the 3′ two-base overhang of guide RNAs. This allows for increased stability of the guide RNA within the assembly and ensures that they are orientated correctly [72,75].Studies using mutagenesis show that mutations in the PAZ domain of AGO2 proteins allow binding of small RNAs, but loss the duplex unwinding ability and the ability to form effector cognate RISC, indicating an important role for the PAZ domain in RISC assembly [76,77].

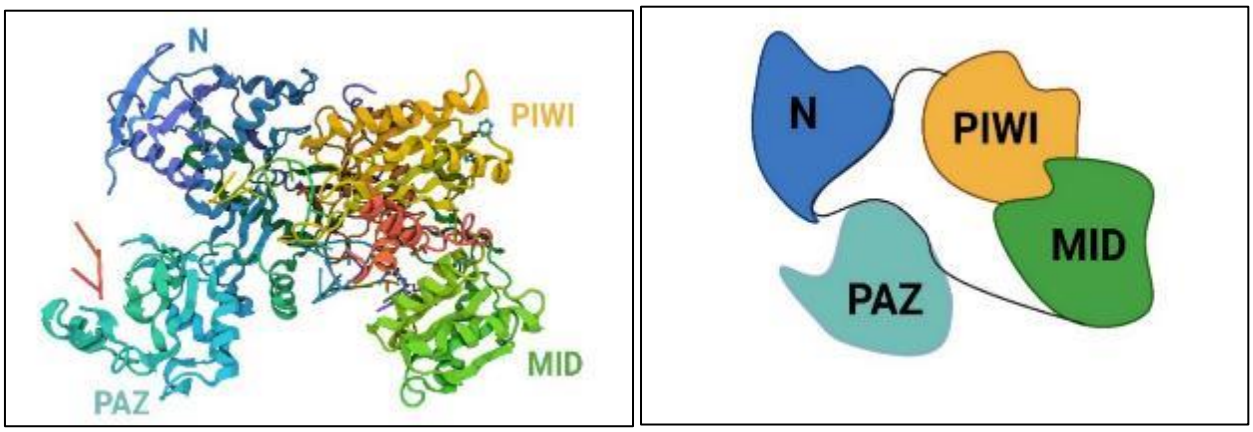

Figure 4 Structure and schematic of human argonaute 2 (Ago2, PDB ID: 4Z4F), highlighting the N, PAZ, MID, and PIWI domains that mediate small RNA binding and target RNA cleavage

3.2.3. MID Domain

The MID domain has a Rossmann-like tertiary structure and creates a highly conserved pocket that binds the 5′ phosphate of guide RNAs, thus enforcing polarity and strand selection [72,78]. Structural studies have defined additional binding sites near the core active site stabilized by sulfate ions and conserved lysine residues (e.g., Lys599, Lys638) and could play a role in binding the m7GpppG cap of target mRNAs. This suggests that the MID domain provides structural and regulatory functions to AGO2 activity [79].

3.2.4. PIWI Domain

The PIWI domain acts as the catalytic center of AGO2, displays structural resemblance to RNase H, and contains a conserved DEDH catalytic tetrad that mediates endo-nucleolytic "slicer" activity. It is known that this domain cleaves target RNAs 10 and 11 nucleotides from the guide strand [80]. In addition to its catalytic function, the PIWI domain contains tryptophan-binding pockets that are required for the recruitment of GW182 and other tryptophan-rich cofactors essential for translational repression and mRNA decay. In addition, the PIWI domain strengthens the contacts between the MID–PIWI lobe and DNA or RNA substrates, and some specific residues such as Asp603 are crucial for structural integrity [72,81] .

3.3. Mechanism of AGO2-Mediated Slicing

Recent biochemical and structural studies have provided an integrative model for how human AGO2 mediates RNA cleavage (slicing). This can be systematically classified into sequential stages involving guide-target recognition, conformational fitting, and catalysis [82].Target mRNAs are first recognized by base-pairing interactions between the seed region (guide nucleotides g2–g8) of the small RNA bound to AGO2. In the initial phase, base-pairing interactions occur with the 3′ supplementary region (g13–g16) after which additional interactions with the action center of the guide (g9–g12) take place while the central region remains unpaired. At the time perfect duplexes form between the seed and 3′ supplementary regions and the target, AGO2 drives a rotational movement of the 3′ supplementary duplex region [83,84]. This change enables the guide center (g9–g12) to base-pair with the target sequence to be determined if it is complementary. However, the lack of restriction on the central and 3′ supplementary segments allows for this conformation despite AGO2 stabilizing the phosphate backbone of the seed region [83]. When base-pairing is fully extended to the central region of the target RNA, the target is precisely positioned within the PIWI domain of AGO2 containing the conserved D–E–D–H catalytic tetrad [83]. These tagged residues work alongside the divalent metal ions to stimulate a water molecule for a nucleophilic attack of the phosphodiester backbone. Target Cleavage AGO2 cleaves the target RNA endo-nucleolytically between t10 (t = 1) and t11 (P = 5′ end of the guide RNA). During this process target

RNA is cleaved into two unique fragments, which are then released and degraded resulting in efficient post-transcriptional gene slicing [84].

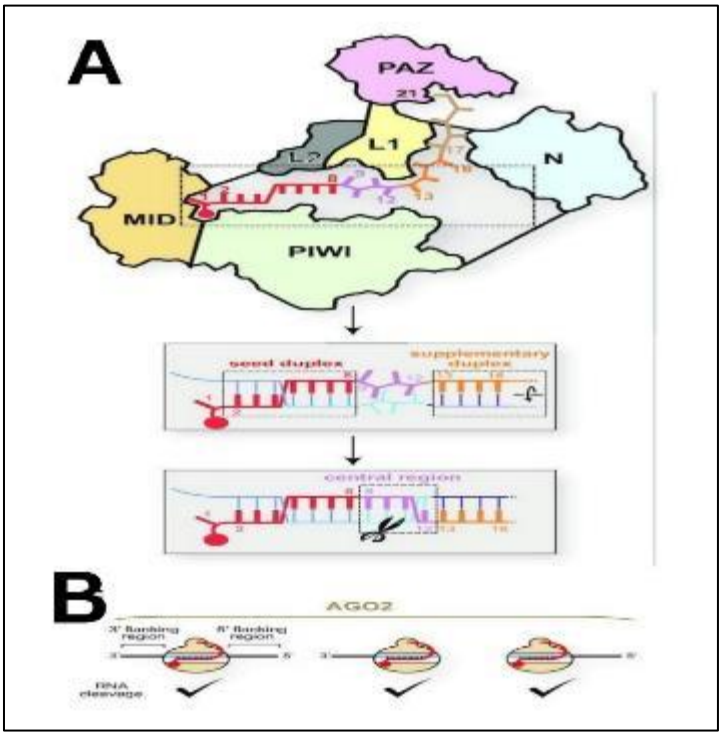

Figure 5 Ago2-mediated RNA cleavage. "Figure adapted from Nucleic Acids Res, Volume 50, Issue 12, 8 July 2022, Pages 6618-6638, https://doi.org/10.1093/nar/gkac519

4. siRNA-Mediated Silencing of Oncogenes and Downstream Pathways

Specific suppression of oncogenes with an essential role in tumor initiation and progression can be achieved using siRNA technology [85]. They have the ability to direct the degradation of its complementary oncogenic mRNAs through RNA-induced silencing complex (RISC) mediation [86], leading to no protein output. Crucially, silencing an oncogene also disrupts the downstream signaling pathways it controls, amplifying the therapeutic effect [85]. This module combines oncogenic targets KRAS, MYC, and BCL2 with their relevant pathways [14], emphasizing how siRNA-mediated knockdown re-monitors cancer signals [87].

4.1. KRAS and MAPK/ERK, PI3K/AKT Pathways

Mutations of KRAS are one of the most common oncogenic drivers in lung, pancreatic and colorectal cancer [88]. KRAS stimulates prominent signaling pathways, specifically MAPK/ERK and PI3K/AKT cascades that govern cell growth, survival and metastasis [89]. siRNA-induced depletion of KRAS diminishes both ERK and AKT phosphorylation and subsequent tumor cell growth and apoptosis [85]. KRAS siRNAs delivered by nanoparticles led to substantial inhibition of tumor growth and metastasis in preclinical lung cancer models [90]. Furthermore, simultaneous KRAS repression and MYC abrogation has resulted in sustained tumor regression raising perspectives that such a dual assault may have even more pronounced therapeutic effects [91].

4.2. MYC and PI3K/AKT Pathway

The transcription factor MYC that controls cell cycle, metabolism and apoptosis relevant genes has been found overexpressed in many hematological and malignancies and solid tumors [92]. MYC expression is also inhibited by siRNA, and results in markedly decreased cell proliferation mediated by KB-3-1 and K562 cells [93]. Biologically, suppression of MYC also interferes with the PI3K/AKT pathway that is pivotal in the metabolic adaptation and survival of cancer cells [94]. Inhibition of this pathway causes suppression of glycolysis, decreased biosynthesis and increased sensitivity to apoptotic signals [95]. These data indicate that MYC is an important hub through which siRNA therapy can intersect with PI3K/AKT-stimulated tumorigenic signaling mechanism.

4.3. BCL2 and Apoptotic Signaling

BCL2 is a classical oncogene whose anti-apoptotic activity are over-expressed in around 50% of all human cancers with approximately 50–70% of patients with breast cancers displaying deregulated expression [96]. Overexpression inhibits programmed cell death, promoting chemoresistance and radioresistance [97]. The nanoliposomal formulations (NL-Bcl-2 siRNA) of siRNA-based silencing of BCL2 exert potent antitumor effects on estrogen receptor-positive (MCF7) and estrogen receptor-negative (MDA-MB-231) breast cancer xenografts [87]. Mechanistically, the BCL2 inhibition included in silencing would re-enable apoptotic machinery by freeing pro-apoptotic proteins (e.g., BAX, BAK) [98], which further yields a radiation-resistant status to tumor cells upon conventional therapies [87]. This shows how siRNA can directly re-engage intrinsic death pathways that cancer cells evade.

Table 2 siRNA-Mediated Silencing of Oncogenes and Downstream Pathways in Cancer

Target (oncogene and pathway)	Cancer model	siRNA Delivery Method	Observed Effect	Ref.
KRAS / MAPK–ERK, PI3K–AKT	Lung Cancer (xenograft models)	Nanoparticle-delivered KRAS siRNA	Reduced KRAS expression, inhibited ERK and AKT signaling, suppressed tumor growth and metastasis	[99]
BCL2 / Apoptotic Pathway	Breast Cancer (ER^+ MCF7, ER^- MDA-MB-231 xenografts)	Nanoliposomal BCL2 siRNA (NL-Bcl-2 siRNA)	Suppressed tumor growth, enhanced chemosensitivity, restored apoptotic signaling	[87]
MYC / PI3K–AKT	Leukemia (K562 cells), Cervical carcinoma (KB-3-1 cells)	Synthetic siRNA against c-myc	Decreased MYC mRNA, inhibited proliferation, induced apoptosis, disrupted PI3K–AKT survival signaling	[100]
KRAS + MYC / MAPK–ERK and PI3K–AKT	Lung Cancer models	Combined siRNA silencing	More frequent and durable tumor regression compared to single gene targeting	[101]

5. Role of Argonaute 2 (Ago2) in Cancer

Among the argonaute family, the only catalytically active member Ago2 has been identified as a core component of the RNA-induced silencing complex (RISC) and a pivotal player in microRNA (miRNA)-guided gene silencing [10,72]. Ago2 has vital roles in mediating post-transcriptional regulation that affect normal cellular homeostasis and cancer [10,72]. Ago2 has been widely studied in the field of cancer for its aberrant expression and dysregulation in numerous types of cancers, highlighting its role in tumorigenesis and progression [102,103].

5.1. Expression Patterns of Ago2 in Tumors Versus Normal Tissues

Multiple studies have shown that Ago2 is often upregulated in tumor tissue compared to adjacent normal tissues. Increased expression in bladder urothelial carcinoma, hepatocellular carcinoma, glioma, hypopharyngeal carcinoma, adrenocortical carcinoma, gastric carcinoma, ovarian carcinoma, and colon cancer. In these cancers, increased Ago2 levels correlate with higher cellular proliferation, migration, metastasis, and overall aggressiveness of tumors. However, decreased or variable expression has been observed in melanoma with low Ago2 protein yet stable mRNA expression and in ER positive breast cancers where active estrogen signaling suppressed Ago2 expression. Yet these heterogeneous patterns reveal Ago2 's cancer-type-specific functions.

Table 3 Expression Patterns of Ago2 in Tumors Versus Normal Tissues

Cancer Type	Expression Pattern	Comparison with Normal	Ref.
Urothelial Carcinoma	Upregulated	Tumor > normal bladder tissue	[104]
Hepatocellular Carcinoma (HCC)	Upregulated	Tumor > adjacent normal liver tissue	[105,106]

Gliomas	Upregulated (higher in high-grade)	High-grade > low-grade tumors	[107]
Hypopharyngeal Cancer	Upregulated	Tumor > adjacent epithelial tissue	[108]
Adrenocortical Carcinoma (ACC)	Upregulated	Tumor > normal adrenal/adenoma	[103,109]
Gastric Carcinoma	Upregulated	Tumor > normal gastric tissue	[110]
Ovarian Carcinoma	Upregulated along tumor progression	Advanced stage > early stage	[111]
Colorectal Cancer (CRC)	Upregulated / variable	Tumor > paired normal tissues	[112]
Breast Cancer	Variable: ↑ in ERα- tumors; ↓ in ERα+	ERα- > ERα+ cell lines and tumors	[113]
Melanoma	Downregulated (protein level)	Melanoma < melanocytes/other cancers	[114]
Multiple Myeloma (MM)	Upregulated	Tumor > normal controls	[115]

5.2. Ago2-Related Pathways in Carcinomas

Ago2 plays oncogenic functions can be consolidated by its involvement in several signaling pathways that regulate cell proliferation, metastasis, angiogenesis, and survival [72]. Such interactions underscore its function as a miRNA function mediator and its function as a direct oncogenic signaling regulator.

5.2.1. Ago2 and Focal adhesion kinase (FAK)

Ago2 directly associates with the promoter of FAK, a major gene in the regulation of cell migration and invasion in hepatocellular carcinoma [105]. This interaction activates FAK/PI3K/AKT pathway which promotes growth and metastasis of the tumor. Knockdown of Ago2 stably inhibits this pathway in hypopharyngeal and colorectal cancers deactivating their metastatic potential [108].

5.2.2. Ago2 and EGFR/MAPK Signaling

Ago2 is indispensable for oncogenesis driven by KRAS in pancreatic ductal adenocarcinoma [109]. Removal of it by means of genetic ablation causes oncogene-induced senescence, and blocks the transition of early precursor lesions to invasive cancer. The impact is largely accomplished by disrupting the EGFR–RAS–MAPK signaling axis[116], thus consolidating Ago2 as the main mediator of KRAS-dependent tumorigenesis [117].

5.2.3. Ago2 and AKT3

Phosphorylation of Ago2 at Ser387 by AKT3 alters the functional state of Ago2 in gliomas. The phospho-form of Ago2 is more involved in gene silencing, whereas the non-phosphorylated one has an increased capacity for mRNA cleavage [118]. This phosphorylation-dependent switch modifies the ratio of direct mRNA degradation to miRNA-mediated silencing, which affects the growth and progression of tumor cells [119]. This phosphorylation-facilitated switch changes the balance between direct mRNA degradation and miRNA-mediated silencing that influence the tumor cell growth and metastasis capacity.

5.2.4. Ago2 and Prolyl-4-Hydroxylase (P4H)

Ago2 associates with P4H, an enzyme controlling hypoxia-inducible factor (HIF) stability, in a hypoxic condition [120]. This interplay leads to hypoxia-induced angiogenesis by supporting survival pathways that promote tumor vascularization and adaptation to low-oxygen environments [121].

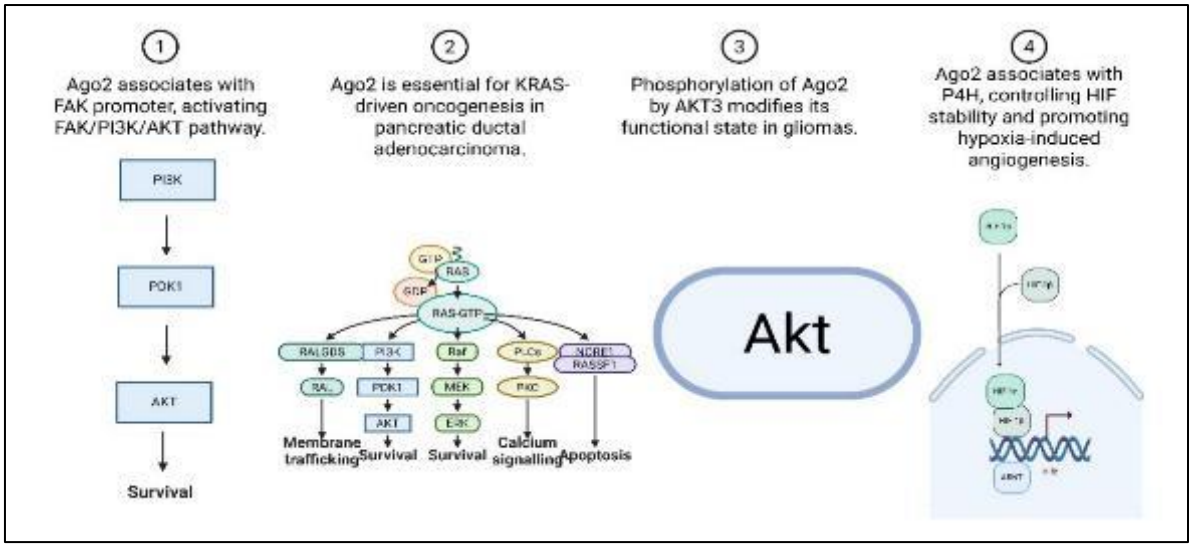

Figure 6 Ago2 associated signaling pathways in carcinomas. Ago2 promotes tumor growth, survival, and angiogenesis through its roles in the FAK/PI3K/AKT, KRAS/MAPK, AKT3, and P4H/HIF pathways

6. Drug Design and Delivery Strategies for siRNA–Ago2-Based Cancer Therapy

6.1. Challenges in Clinical Translation

Although RNA interference (RNAi) has tremendous potential as an anticancer treatment [122], the development of siRNA–Ago2-based therapies still faces several biological and physicochemical obstacles [123]. Bare siRNAs are inherently unstable in the systemic circulation, where they are rapidly degraded by serum nucleases targeting the double-stranded RNA, leading to low bioavailability and an extremely short therapeutic half-life. In addition, the negatively charged phosphate backbone also impedes cellular uptake limiting applications for vectors or chemical modifications. Even after internalization, a large portion of siRNA molecules get trapped in endocytic vesicles and eventually degraded in lysosomes, thereby greatly inhibiting the cytoplasm release, Ago2 uptake and downstream gene silencing effect. Furthermore, heterologous effects and stimulation of the innate immune sensors like Toll-like receptors expose additional safety and efficacy concerns. In combination, these obstacles highlight the critical roles of rational siRNA design and innovative delivery systems for exploiting RNAi for therapeutic uses. A concise overview of these challenges, their implications, and possible mitigation strategies is provided in Table 4 [124–127].

Table 4 Key biological and physicochemical barriers hindering the clinical translation of siRNA–Ago2-based cancer therapy, along with their therapeutic implications and potential strategies to overcome them

Challenge Area	Underlying Issue	Impact on Therapy	Potential Mitigation Strategies
Stability and Circulation	Rapid nuclease degradation and renal clearance	siRNA degraded or cleared before reaching tumour; low bioavailability	Chemical modifications (2′-O-Me, 2′-F, phosphorothioate), nanoparticle encapsulation, PEGylation, controlled-release systems
Biodistribution and Tumor Targeting	Non-specific organ uptake (liver, spleen) and poor tumor accumulation	Reduced delivery to tumor cells; off-target exposure	Active targeting ligands (antibodies, peptides, aptamers), tumor-penetrating peptides, local/regional delivery
Cellular Uptake and Endosomal Escape	Endocytosis traps siRNA in endosomes	Very little siRNA reaches cytosol for Ago2 loading	Ionizable lipids, fusogenic peptides, pH-responsive polymers, photochemical internalization

Ago2/RISC Engagement	Inefficient guide strand selection and loading	Reduced potency and specificity of gene silencing	Optimized siRNA thermodynamic design, selective strand modification, 5′-phosphate mimetics
Immunogenicity and Safety	siRNA or carriers activate innate immunity or cause toxicity	Cytokine storms, infusion reactions, hepatotoxicity	Sequence screening, 2′-O modifications, safer ionizable lipids/polymers, dose optimization
Off-Target Effects	Partial complementarity and unintended gene silencing	Unwanted toxicity and altered gene networks	Bioinformatic sequence design, chemical modifications, targeted delivery to reduce intracellular siRNA dose
Tumor Microenvironment Barriers	Dense stroma, high interstitial pressure, hypoxia	Hinders penetration and uniform tumor distribution	Matrix-degrading enzymes, tumor-penetrating peptides, TME-responsive carriers
Translational and Regulatory Hurdles	Complex nanocarrier manufacturing and quality control	Limits scalability and clinical reproducibility	Scalable microfluidic LNP production, standardized assays, early regulatory alignment

6.2. Chemical Modifications for Stability and Specificity

Extensive research has been devoted on chemical modifications of siRNA molecules to overcome fast nuclease degradation and also to have better target selectivity [128]. Ribose sugar modifications, including 2′-O-methyl and 2′-fluoro modifications, are among the most commonly used strategies [128,129]. These changes help resist nucleolytic cleavage and bind better to the RNA-induced silencing complex (RISC) [130]. An additional, highly active class of modifications are locked nucleic acids (LNAs), within which the ribose ring is conformationally restrained, leading to increased degree of stabilization inside duplexes and level of thermal resistance, stability, and specificity in binding [131].Modifications of sugar and also of the siRNA backbone itself have been critical. Phosphorothioate (PS) linkages, where a non-bridging oxygen atom is replaced with sulfur, provide increased resistance to nucleases and improve binding to plasma proteins to prolong circulation time [132]. For example, the 3′ and 5′ ends of siRNA strands can be modified with PSs, and relatively high levels of metabolic stability without loss of gene-silencing activity have been observed when these modifications are strategically positioned [133]. In addition to improving their stability, chemical modifications have also been applied to reduce potential off-target effects and minimize immune stimulation [134]. For example, by incorporating 2′-O-methyl nucleotides, which selectively reduce off-target transcript binding, in the seed region of the guide strand the potential of innate immune activation can be reduced selectively [135].Collectively, these chemical engineering strategies not only stabilize siRNA in vivo but also refine its pharmacological profile, laying the foundation for clinically viable siRNA–Ago2-based therapeutics.

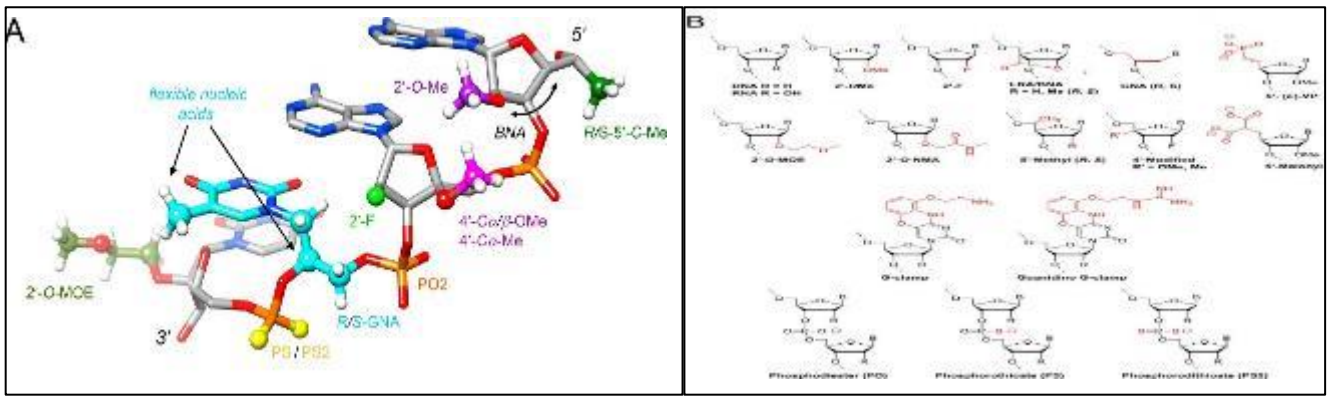

Figure 7 Representative examples of first-, second-, and third-generation chemical modifications of nucleic acids applied in therapeutic oligonucleotides. These structural analogues, including 2′-O-methyl (2′-OMe), 2′-fluoro (2′-F), phosphorothioate (PS), locked nucleic acids (LNA), and others, enhance nuclease resistance, improve binding affinity, and refine pharmacological properties of siRNA. Adapted with permission from Egli M, Manoharan M. Re-Engineering RNA Molecules into Therapeutic Agents. Acc. Chem. Res. 2019; 52:1036–1047. Copyright © 2019 American Chemical Society

6.3. Delivery Platforms for siRNA–Ago2 Therapeutics

Chemical modifications help with the stability and specificity of siRNAs, however, the most critical step for clinical translation remains an efficient delivery [136]. Moreover, delivery platforms are intended to localize siRNA against enzymatic degradation, enhance cellular uptake, facilitate the escape of siRNA from endosome and achieve a tumor-selective distribution [137]. Of these, lipid and polymer nanoparticle-based technologies are the more prevalent and promising technologies [138].

Lipid nanoparticles (LNPs): Lipid nanoparticle (LNP) platform remains the most clinically-advanced technology among siRNA delivery methods that have been miRNA-therapeutics technologies developing (e.g., Onpattro the first FDA-approved siRNA drug is delivered with an LNP formulation) [139]. Effective LNPs are usually characterized by small particles (<100 nm), high encapsulation efficiency, and the use of ionizable lipids, which are neutral at physiological pH but become positively charged in the acidic endosomal environment allowing the endosomal escape [140]. The main materials in these nanoparticles are ionizable lipids, cholesterol, PEGylated lipids, and helper phospholipids [141]. A recent study has shown that structural changes in the linker region of ionizable lipids can lead differences in delivery potency and biodistribution [142]. In addition, the improvement of lipid chemistry has been accompanied with higher gene silencing mRNA levels and lower immune activation, thus turning LNP into the gold standard for the systemic delivery of siRNA [143].

Polymeric nanoparticles: Biodegradable polymers constitute a versatile alternative approach to the delivery of siRNA. Several candidates in this category have gone beyond the preclinical stage to testing [144]. The main three substances have been the chitosan (CS), the poly(lactic-co-glycolic acid) (PLGA), and the polyethyleneimine (PEI) [145]. Cationic polymers are capable of forming stable complexes with the negatively charged siRNA via electrostatic interactions. Thus, they eliminate the need for direct chemical modification of RNA [146]. Intracellular delivery is being improved by pH-responsive systems [147]. For instance, one can consider guanidine chitosan derivatives which, when exposed to an acid environment such as that of endosomes, liberate CO_2. This, in turn, leads to a local "proton-sponge" effect causing endosomal escape and the siRNA enters the cytoplasm [148]. Meanwhile, tumor-targeted polymeric nanocarriers with a tumor cell membrane-coated surface have been prepared to elevate tumor infiltration, thus decreasing systemic distribution [149]. By and large, these interventions considerably enhance silencing efficiency and show a conspicuous anti-tumor effect in vivo [150].

7. Future Perspectives and Emerging Trends

The proof-of-concept therapeutic potency of siRNA via Ago2 continues to grow [4]. The scientific progress in molecular design, delivery strategies, and integrative approaches with emerging technologies should boost the further clinical translation of siRNA-based cancer therapeutics [18].

7.1. Next-generation RNAi therapeutics

This work has recently led to some exciting innovations on siRNA design tackling the fundamental challenges of stability, delivery and specificity [4]. In contrast, circular siRNAs (circ-siRNAs), formed by covalently linking the 5′ and 3′ ends, exhibit a robust resistance to exonuclease degradation, while retaining Ago2 compatibility. Circ-siRNAs appear to be suitable candidates for oncology due to improved durability and reduced dosing frequency based on preclinical evidence [151,152].

Another very interesting strategy is self-delivering siRNAs (sd-siRNAs) [153]. These molecules circumvent the requirement of carrier systems and enhance the tumor penetration by chemical conjugation with a lipophilic group, GalNAc moieties, or peptide ligands [154]. The carrier-free design minimizes systemic toxicity and enables higher tissue selectivity [153]. Conversely, the use of AI to guide sequence optimization is gaining wider acceptance [154]. We developed computational pipelines that allow predicting strand asymmetry, scanning transcriptome-wide seed matches to minimize off-target interactions, and modeling Ago2–mRNA binding dynamics [155]. These platforms allow researchers to quickly design effective, patient-specific siRNAs against oncogenes to facilitate the development of RNAi therapeutics that are targeted to individual patients.

7.2. CRISPR–RNAi hybrid approaches

CRISPR–Cas genome editing is not a replacement for RNAi, but a new opportunity for synergy [156]. siRNA is associated with reversible and tunable oncogene suppression, whereas CRISPR can impart durable genomic edits [157,158]. Examples of these hybrid applications are starting to appear, such as: Complementary targeting, in which siRNAs enable instantaneous knockdown at the same gene locus simultaneously being inactivated by long-term CRISPR targeting

[159]. Layered regulation can be achieved by combining CRISPR interference at the transcriptional level with siRNA-mediated repression at the post-transcriptional stage [160]. Therapeutic integration to sensitize tumors using siRNAs or to mitigate compensatory signaling that occurs during CRISPR-based interventions thereby improving therapeutic efficacy [159].

7.3. Expanding therapeutic horizons

In addition to siRNA therapeutics being used for the direct knockdown of oncogenes, they are also being considered for a wider range of oncologic applications. Immune checkpoint regulators such as PD-1, PD-L1, or CTLA-4 are the most probable therapeutic targets in the immuno-oncology domain. Therefore, the delivery of the respective siRNAs could mutually enhance antitumor immunity and checkpoint blockade therapies [161]. In the tumor microenvironment, siRNAs targeting angiogenic or stromal mediators (e.g., VEGF, TGF-β) could theoretically be used to rewire or remove immune suppression cells among the staff cells of the immune system [162]. The advances in tumor sequencing technologies are also the main reason for the personalized siRNA therapy [163], in which individual patient oncogenic mutations such as KRAS G12C can be swiftly targeted. Combinatorial RNA platforms for precision oncology will also be attainable by integrating with additional RNA modalities [4], including miRNA mimics, antisense oligonucleotides, and mRNA vaccines.

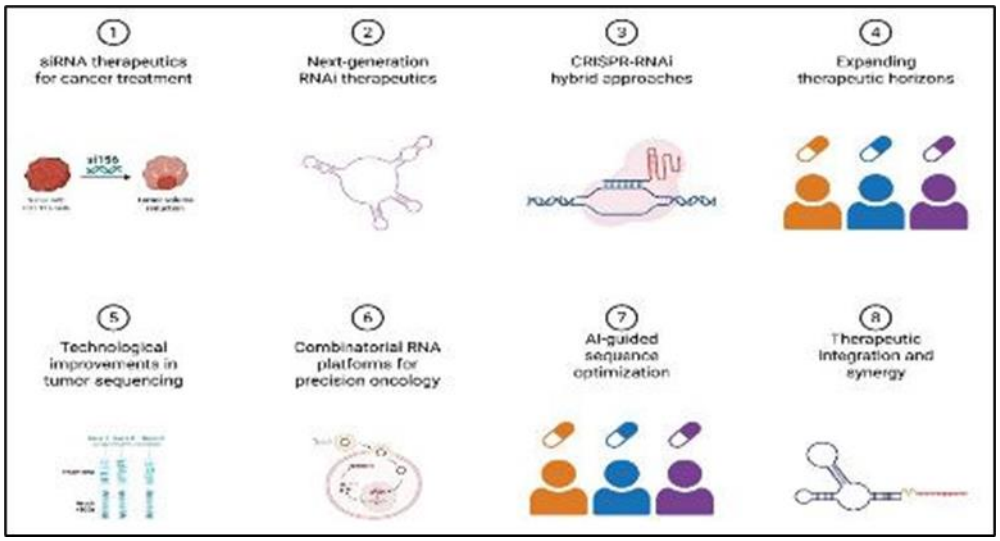

Figure 8 Emerging trends and future perspectives in RNAi-based cancer therapeutics

Advances in siRNA and RNAi technologies—including next-generation RNAi therapeutics, CRISPR–RNAi hybrid strategies, AI-guided optimization, and combinatorial RNA platforms—are expanding therapeutic horizons toward precision and personalized oncology.

7.4. Challenges and Outlook

Despite significant progress in RNAi-based therapeutics, multiple barriers continue to limit the widespread clinical application of siRNA–Ago2 platforms in oncology. These challenges span molecular, biological, and translational domains.

7.4.1. Delivery efficiency and tumor specificity

Efficient delivery of siRNAs to solid tumors, Biodistribution and cellular uptake is limited by rapid renal clearance and physiological barriers including the dense extracellular matrix and aberrant vasculature [18]. While we have seen potential in lipid nanoparticles (LNPs) and polymeric carriers [164], they are frequently confronted with their limited penetration in tumors and varies in organ distributions. To overcome these obstacles, tumor-specific ligands, exosome-based carriers and self-delivering siRNAs are being explored.

7.4.2. Off-target effects and transcriptome complexity

Even with the sequence-optimized seeds, siRNAs may partially match non-target mRNAs causing off-target silencing. Such effects can block crucial pathways and result in toxicity [165]. Furthermore, alternative splicing and tumor

heterogeneity further complicate the prediction of successful siRNA targets [166]. While improvements in AI-guided sequence design and high-throughput validation pipelines could ease this, precise control is a critical requirement.

7.4.3. Immunogenicity and safety

Unmodified siRNAs can directly activate the innate immune receptors such as TLR3 and RIG-I to lead to the inflammation process [167]. Although (2′-O-methyl, 2′-fluoro and phosphorothioate linkages) have improved tolerability, the fine-tuning of immune activation versus maintaining Ago2 activity is quite challenging [168].

7.4.4. Resistance and adaptive mechanisms

Resistance to siRNA treatment cancer cells may develop by means of mutations at the target-binding site, activation of a compensatory pathway, or change in expression of RNAi machinery (for instance, downregulation of Ago2 or Dicer)[4,169]. Such resistance is similar to the acquired resistances those targeted agents. Combination tactics which combine siRNA with chemotherapy [170], immunotherapy, or genome editing may be needed in order to maintain therapeutic effect.

7.4.5. Manufacturing, scalability, and regulatory hurdles

The large-scale, economically feasible and reproducible manufacture of clinical-grade siRNA formulations has been a challenge. Variability from batch-to-batch, storage stability and QC are important considerations [18]. Regulatory models for RNAi therapeutics continue to mature, particularly in the oncology space employing advanced nanoparticle work and combinatorial approaches. Efficient production processes and uniform evaluation standards will be critical for clinical translation [18].

8. Conclusion

Small interfering RNAs (siRNAs) along with argonaute 2 (Ago2) represent the molecular core of RNA interference and are a potential hopeful path in targeted cancer therapies. Enhancements in structural biology have revealed that Ago2 serves as the catalytic center of the RNA-induced silencing complex (RISC), thus enabling the logical creation of siRNAs with improved strand selection, specificity, and stability. The decrease of oncogenic targets like KRAS, MYC, and BCL-2 is just one of the ways siRNA–Ago2 systems hold the solution for cancer to stop tumor growth, induce apoptosis, and increase the effect of current chemotherapy methods. On the other hand, the clinical application of those systems is still limited by the significant obstacles posed by nuclease degradation, immune activation, off-target effects, and the lack of tumor-targeted delivery. The stability and bioavailability of the siRNAs have been greatly improved due to the use of chemical modifications (like 2′-O-methyl, 2′-fluoro, and phosphorothioate linkages) and advanced delivery technology with lipid or polymer nanoparticles. The introduction of self-delivering siRNAs, AI-aided sequence design, and individualized RNA platforms further accelerates the progress of safe and efficient RNA interference-based therapeutics. Hypothetically, the RNAi integration with genome-editing, exosome-mediated transport, and immunomodulatory strategies might be the future of precision oncology. The implementation hybrid RNAi–CRISPR, creation tumor transcriptomics-guided personalized siRNA libraries, and using combinatorial regimens along with conventional therapies will be the subsequent stage of translation. Such a translation of siRNA–Ago2 platforms from mere tools to therapeutics that are clinically robust and capable of delivering durable, programmable, and patient-specific cancer treatment, will undoubtedly require sustained collaborative efforts across disciplines of molecular biology, bioengineering, and clinical sciences.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Md Shorif Uddin*, Md Roknuzzaman Faisal, Afif Abyad Hossain, Adib Azwad Hossain and Nusrat Jahan Bristy played pivotal roles in shaping this review paper. Their contributions encompassed the comprehensive literature review, conceptual framework development, data collection, original draft preparation, figure illustration, and the overall generation of ideas that guided the direction of the manuscript.

Mst Umma Fatama, Md Arafat Rahman Koko, Tauhedul Islam contributed significantly through formal data analysis, meticulous data curation, figure illustration, table construction, grammatical refinement, software-related adjustments, and thorough proofreading to enhance the clarity and quality of the final manuscript.

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