Trading places: Peptide and small molecule alternatives to oligonucleotide-based modulation of microRNA expression

Talhat Chaudhry [a,b], **Christopher R. Coxon** [c], **Kehinde Ross** [a,b,*]

[a] School of Pharmacy and Biomolecular Science, Liverpool John Moores University, Liverpool, UK
[b] Institute for Health Research, Liverpool John Moores University, Liverpool, UK
[c] EaStChem School of Chemistry, The University of Edinburgh, Joseph Black Building, David Brewster Road, Edinburgh EH14 4AS, UK

It is well established that microRNA (miRNA) dysregulation is involved in the development and progression of various diseases, especially cancer. Emerging evidence suggests that small molecule and peptide agents can interfere with miRNA disease pathways. Despite this, very little is known about structural features that drive drug–miRNA interactions and subsequent inhibition. In this review, we highlight the advances made in the development of small molecule and peptide inhibitors of miRNA processing. Specifically, we attempt to draw attention to peptide features that may be critical for interaction with the miRNA secondary structure to regulate miRNA expression. We hope that this review will help to establish peptides as exciting miRNA expression modulators and will contribute towards the development of the first miRNA-targeting peptide therapy.

Keywords: microRNA; Peptides; Peptidomimetics; Peptide–miRNA interactions; Dicer processing

Talhat Chaudhry received her Bachelor's degree from Liverpool John Moores University in 2017. After this, she pursued a Master's in Drug Discovery and Design at the same institute, working under the supervision of Christopher R. Coxon to complete her thesis on the synthesis of small cyclic peptide analogs for CGRP receptor antagonism as potential migraine treatments. Talhat is currently completing her PhD studies, co-supervised by Kehinde Ross and Christopher R. Coxon. Her research focuses on the development of novel peptide conjugates for the delivery of bioactive peptides to cancer cells in order to inhibit the function of miRNA-21.

Chris Coxon is Senior Lecturer in Medicinal Chemistry in the EaStChem School of Chemistry, University of Edinburgh. He completed his PhD in 2010 at the Northern Institute for Cancer Research, Newcastle University. He then worked as a post-doctoral research associate at Durham University, before undertaking academic positions at Liverpool John Moores University and Heriot-Watt University. His research interests are at the biology-chemistry interface, focusing on peptide drug discovery and the development of chemical tools and medicines for diseases of the CNS. He has interests in migraine, neurodegenerative diseases and oncology. In addition to his academic role, he is director and co-founder of Pepmotec Ltd.

Kehinde Ross is a Reader (Associate Professor) in Molecular and Cellular Biology at Liverpool John Moores University. He obtained his PhD from the University of Sheffield under the supervision of Dr Francois Guesdon and Professor Steve Dower. He then undertook postdoctoral research at Newcastle University in the laboratory of Professor Nick Reynolds. In 2008, Kehinde moved to Liverpool John Moores University to pursue independent research in relation to microRNA function in the skin. More recently, his research interests have expanded to the development of miRNA-directed interventions for skin disorders and cancer, spanning nucleic acid, peptides and nanoparticles.

* *Corresponding author.Ross, K. (o.k.ross@ljmu.ac.uk)*

Introduction

Over the past two decades, work on non-protein coding RNAs has led to some of the most important progress in understanding and regulating cell biology and human health. From the transformative impact of guide RNAs in therapeutic gene editing,[1] to the first approval of short-interfering RNA (siRNA)-based drugs (Onpattro® (patisiran), Givlaari® (givosiran), Oxlumo® (lumasiran) and inclisiran (Leqvio®)) by the US Food and Drug Administration (FDA),[2–5] and the growing pipeline of approved antisense oligonucleotide (ASO) drugs,[6] it appears that we are on the cusp of a revolution in which patient outcomes will be improved by RNA-directed medicines. Concurrently, small molecules and peptides, the traditional cornerstones of drug discovery, are also undergoing a renaissance due to technological innovations. This has facilitated their exploitation as modulators of traditionally 'undruggable' macromolecular targets, including RNA and RNA–protein interactions.[7–9]

Mature microRNAs (miRNA) are evolutionarily conserved, single-stranded, small non-coding RNAs (~22 nucleotides in length) that regulate post-transcriptional gene expression.[10] The first known miRNA, lin-4, was reported in 1993 as a regulator of development in the roundworm *Caenorhabditis elegans*.[11,12] The first known human miRNA, let-7, was identified around 7 years later, with several subsequent studies showing widespread expression of small RNAs in Metazoa.[13–15] Since then, miRNA sequences have been identified in at least 271 organisms: 38,589 hairpin precursors and 48,860 mature miRNAs have been deposited in the miRbase online repository.[16]

Dysregulation of miRNA processing or expression has been implicated in the development and progression of a wide array of human diseases, including cardiovascular disease (CVD),[17] neurodegenerative diseases,[18] dermatological conditions,[19] and cancer.[20,21] Given the role of miRNAs in pathological processes, it is unsurprising that the modulation of miRNA activity has been the subject of extensive fundamental and translational research.

Conventional inhibition of miRNAs relies on chemically stabilized antisense oligonucleotides (ASOs), which have been reviewed elsewhere.[22] Indeed, miRNA-targeted ASOs such as MRG-106 (cobomarsen) and miravirsen have entered phase II clinical trials for the treatment of cutaneous T cell lymphoma and hepatitis C, respectively.[23,24] However, they have yet to gain FDA approval. It seems that this lack of approval appears is primarily due to toxicity concerns associated with the off-target effect of miRNA-targeted ASOs and the requirement for complex ASO delivery systems.[6,25]

In recent years, attention has shifted towards alternative miRNA-targeting therapies, particularly towards the small drug-like molecules and peptides employed to inhibit RNA molecules that have defined secondary structures, including human immunodeficiency virus (HIV).[26,27] As outlined below, miRNA biogenesis features hairpin structures that are amendable to ligand binding to block oncogenic miRNA maturation, or in some cases to enhance the expression of tumor-suppressing miRNAs.[28,29] Hence, peptides and small drug-like molecules have emerged as potential therapeutics that could be exploited for the development of miRNA-processing inhibitors.

Therefore, this review discusses the successes and limitations in targeting secondary miRNA structures using small-drug-like molecules, the progress in peptide-based modulation of oncogenic miRNAs, and our understanding of peptide and miRNA interactions. Finally, we consider the outlook for the future of peptides and peptidomimetics in the development of miRNA-targeting cancer therapies.

Biogenesis of miRNA

The canonical pathway of miRNA biogenesis is a multistep process that includes both nuclear and cytoplasmic cleavage events performed by the ribonuclease III enzymes, Drosha and Dicer, respectively (Fig. 1). The canonical pathway for miRNA generation begins in the nucleus with the transcription of primary miRNA (pri-miRNA) by RNA polymerase II (pol II). The transcribed pri-miRNA is polyadenylated and capped. Structurally, pri-miRNA consists of a stem (33–35 base pairs), a terminal loop, and single-stranded RNA segments at both the 5′ and 3′ sides (Fig. 1).[30,31] The pri-miRNA is cleaved by a complex consisting of the RNase III enzyme Drosha and the DGCR8 protein to yield the hairpin precursor miRNA (pre-miRNA; approximately 70 nucleotides).[32,33] Subsequently, pre-miRNA is exported from the nucleus to the cytoplasm by the exportin 5 (XPO5) and RanGTP complex.[34]

In the cytoplasm, the pre-miRNA is processed by the RNase III enzyme Dicer and RNA-binding protein TRBP to remove the terminal loop, producing a mature miRNA duplex.[35,36] The miRNA duplex consists of the 5p and 3p strands, which arise from the 5′ and 3′ end of the pre-miRNA hairpin, respectively.[37] Once the miRNA duplex unwinds, the miRNA strand that is deemed the guide strand is loaded onto the Argonaute protein (AGO), with the aid of Hsp70 and Hsp90 chaperone proteins,[38] to form the miRNA-induced silencing complex (miRISC). The guide strand is selected depending on the thermodynamic stability at the 5′ end of the miRNA duplex or the presence of a 5′ uracil.[37,39] Until recently, the passenger strand (miRNA*) was thought to be degraded, but it is now clear that miRNA* can accumulate to functionally relevant levels and can regulate target transcripts.[40–42] Non-canonical pathways of miRNA biogenesis have also been reported. These typically employ a combination of proteins that differs from those used in the canonical pathway and can be grouped into Drosha/DGCR8 and Dicer-independent pathways. These pathways have been reviewed elsewhere.[10,37]

Once the miRISC complex has assembled, the miRNA binds to mRNA at the complementary 3′ untranslated region (3′UTR); this primarily induces mRNA degradation by targeting the transcripts to processing bodies (P-bodies) and inducing mRNA decay through decapping, deadenylation and exonucleolytic degradation.[43] This accounts for the majority of miRNA-mediated target gene suppression. In some cases, miRNA may mediate translational repression instead, but this only contributes to 10–25% of all miRNA-mediated activity.[44,45]

Targeting oncogenic microRNAs in cancer

The roles of miRNA in cancer pathogenesis can be subcategorized into two groups: oncogenic and tumor-suppressing miRNAs, both of which have been targeted for the development of novel

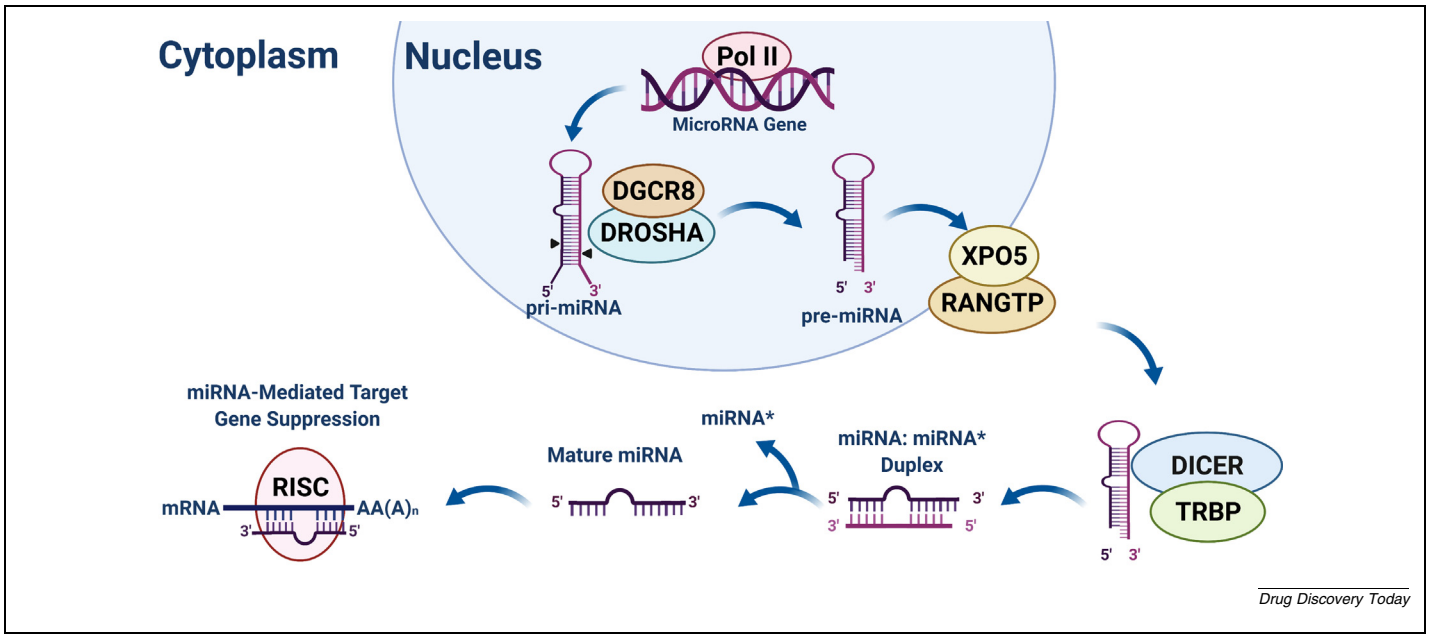


FIG. 1

Stages of microRNA biogenesis: from transcription by RNA polymerase II to miRNA maturation and mode of action. Created with BioRender.com.

anticancer therapies.[6,22] This review, however, focuses on progress in developing oncogenic miRNA-targeted small drug-like molecules and peptides. To date, the majority of reported peptide and small-molecule inhibitors of miRNA processing target pre-miR-21 or pre-miR-155. This is probably because the mature miRNAs miR-21-5p and miR-155-5p, which arise from those precursors, are two of the most extensively studied oncogenic miRNAs and have a well-established role in the development and progression cancer.[46]

miR-21 was one of the first miRNAs to be identified in the human genome and displays strong evolutionary conservation.[14] Extensive profiling revealed miR-21 elevation in various solid cancers and hematological malignancies.[47] Furthermore, a comprehensive microarray study across 540 samples from lung, breast, colon, gastric, pancreatic and prostate cancer found that miR-21 was upregulated in all six solid tumours.[48] The oncogenic role of miR-21 stems largely from its anti-apoptotic contributions to tumor pathogenesis and other stages of carcinogenesis.[49] Typically, miR-21 modulates the function of key tumor suppressor genes, including PTEN, PDCD4, SPRY1, SPRY2, TIMP3 and TP63, which are downregulated when miR-21 is overexpressed.[50,51]

Fundamental and translational interest in miR-155-5p stems from cancer-related and immunological roles that have been reviewed recently.[52] Depending on the cellular context, both oncogenic and tumor-suppressor roles have been defined for miR-155-5p.[53] The cancer and immunological axes of miR-155-5p are intricately linked as the roles played by miR-155 in immune cells underlie its association with hematological malignancies, such as diffuse large B-cell lymphoma (DLBCL), acute myeloid leukemia, acute and chronic lymphocytic leukemia, and mantle cell lymphoma.[53] However, miR-155-5p has also been implicated in non-hematological neoplasms, including glioma, non-small cell lung cancer, colorectal cancer, hepatocellular carcinoma and breast cancer.[54] An example of oncogenic miR-155-mediated downregulation of important tumor suppressor genes in cancer cells is the repression of WEE1, which encodes a checkpoint kinase that plays an important role in the DNA damage response and cell cycle regulation.[55] Other key mRNA targets that are downregulated by miR-155-5p include SHIP1, HDAC4, RHOA, SOX6 and TP53INP1.[56,57] Notably, miRagen Therapeutics developed a locked nucleic acid (LNA)-based miR-155 inhibitor (MRG-106; Cobomarsen) for cutaneous T-cell lymphoma, specifically mycosis fungoides.[24] However, a phase 2 clinical trial of the compound (NCT03713320) was terminated prematurely and no plans for further development are evident.

Design of molecules to bind miRNA secondary structures

When designing and developing RNA-directed small molecules and peptide therapeutics, the key structural features of RNA that can be targeted using bioactive molecules must be understood. Typically, RNA molecules such as pri-miRNA and pre-miRNAs exist as A-form RNA helical structures, which have deep and narrow major grooves and shallow and wide minor grooves. When the RNA A-form helical structure is fully base-paired, the RNA grooves are sterically inaccessible and difficult to target using small molecules or peptide-based therapeutics. Nevertheless, structural studies suggest that perturbations in the A-form RNA helix can lead to the formation of various secondary RNA structures, including hairpin loops, internal loops and bulged regions (Fig. 2). The formation of secondary RNA structures, due to mismatches and unpaired bases, widens the RNA grooves to create a surface-exposed binding pocket that can be accessed by small molecules and peptide therapeutics.[58]

In support of this, the bulge near the hairpin loop of HIV-1 *trans*-activating response (TAR) RNA is crucial for TAR interactions with the arginine-rich segment of the Tat protein. The ini-

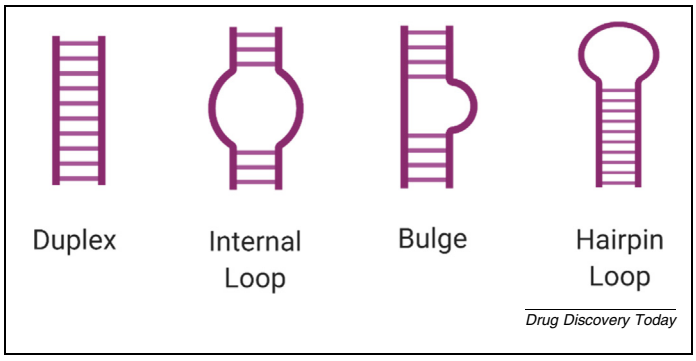


FIG. 2

Schematic representation of the different RNA structures: RNA duplex, internal loop, bulge and hairpin loop. Typically, perturbations in RNA structures—the internal loop, bulge and hairpin loop—are targeted by small molecules and peptide. Created with BioRender.com.

tial recognition of the arginine side chains prompts a conformational change in the TAR RNA major groove that promotes Tat–TAR interactions.[59,60] Following this discovery, disruption of this RNA–protein interaction was considered a potential anti-HIV strategy. Given that aminoglycosides had previously been reported to bind RNA structures, they were screened for their ability to disrupt Tat–TAR interactions. Neomycin was shown to bind TAR RNA non-competitively in the minor groove, to induce a conformational change of the RNA structure and to reposition key TAR functionalities away from the major groove, limiting Tat–TAR interactions.[61] Subsequently, the first D-Tat peptide was reported to bind in the major groove of TAR RNA and thus to inhibit Tat–TAR interactions competitively. This was one of the first peptidomimetics reported to target an RNA structure.[27] Following the initial discoveries, various groups have designed several types of peptidomimetics to target the apical loop region of HIV-1 TAR RNAs, including branched,[62] α-helical,[63] and cyclic β-hairpin peptidomimetics.[64] Together, these studies have helped to establish the validity of peptide-based targeting of RNA secondary structure for the development of novel therapeutics.

Small molecule-based inhibitors of microRNA processing

Small molecules have proven to be useful tools for the modulation of miRNA moieties.[8,65] Small molecules act by targeting miRNA transcription, miRNA biogenesis and the function of mature miRNA.[66,67] In this review, we focus on the development of pri-miRNA- and pre-miRNA-targeted small molecules.

The design and discovery of small molecules that selectively bind and inhibit miRNA processing has proven challenging due to a limited understanding of the structural motifs that promote selective recognition of and affinity for RNA structures. To compensate for this lack of understanding, the 'rules' that are regularly applied in the development of small molecules that target proteins and macromolecule have largely been ignored.[8,68] This approach has yielded several small-molecule inhibitors of miRNA processing that function by binding to the stem-loop of pre-miRNA or pri-miRNA. This list includes small molecules that target pre-miR-21,[69,70] pri-miR-96, [71,72] pre-miR-155,[73] pre-miR-372,[74] pre-miR-10b,[75] and pre-miR-210 (Fig. 3).[76] Thus

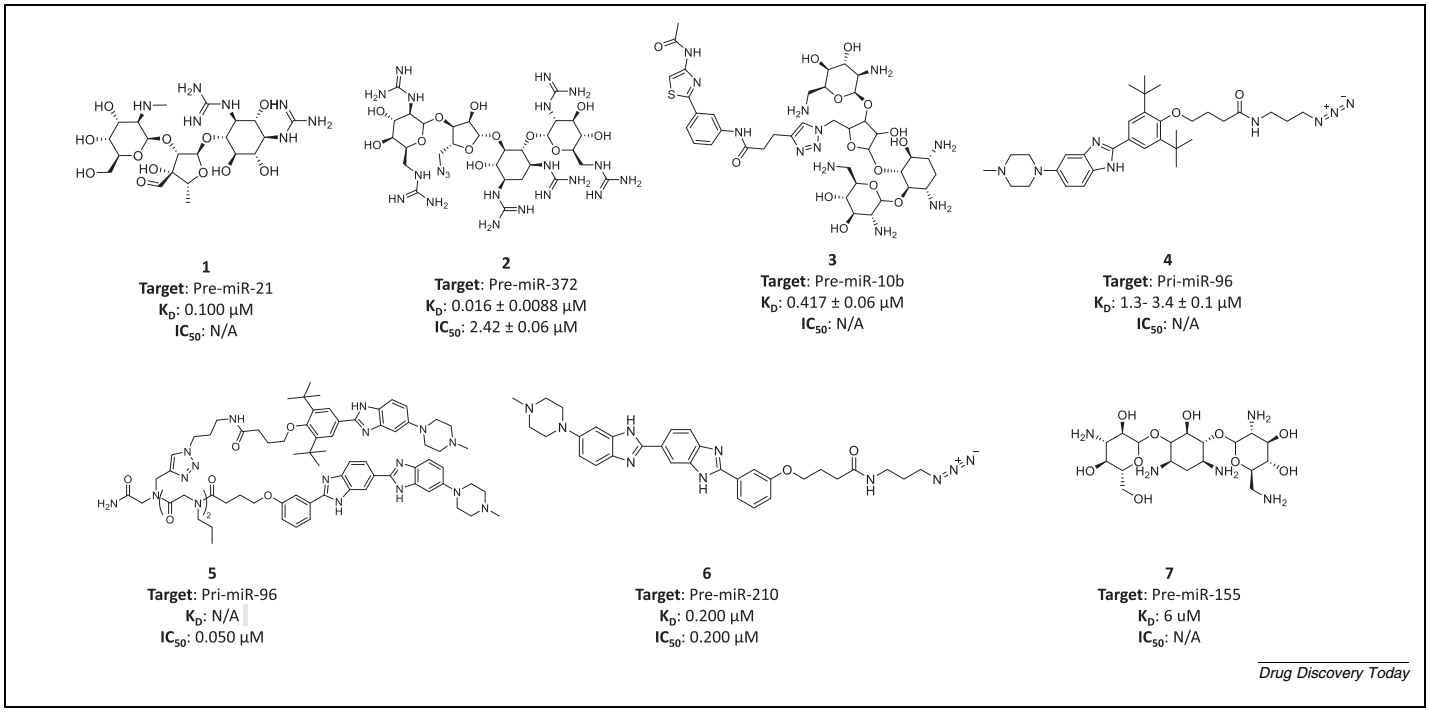


FIG. 3

Small molecules reported to bind and inhibit the function of various miRNA secondary structures. Note: there are various other small-molecule inhibitors of miRNA processing that are not included in this figure. This figure merely aims to highlight that small molecules have been reported to bind and inhibit the function of various oncogenic miRNAs.

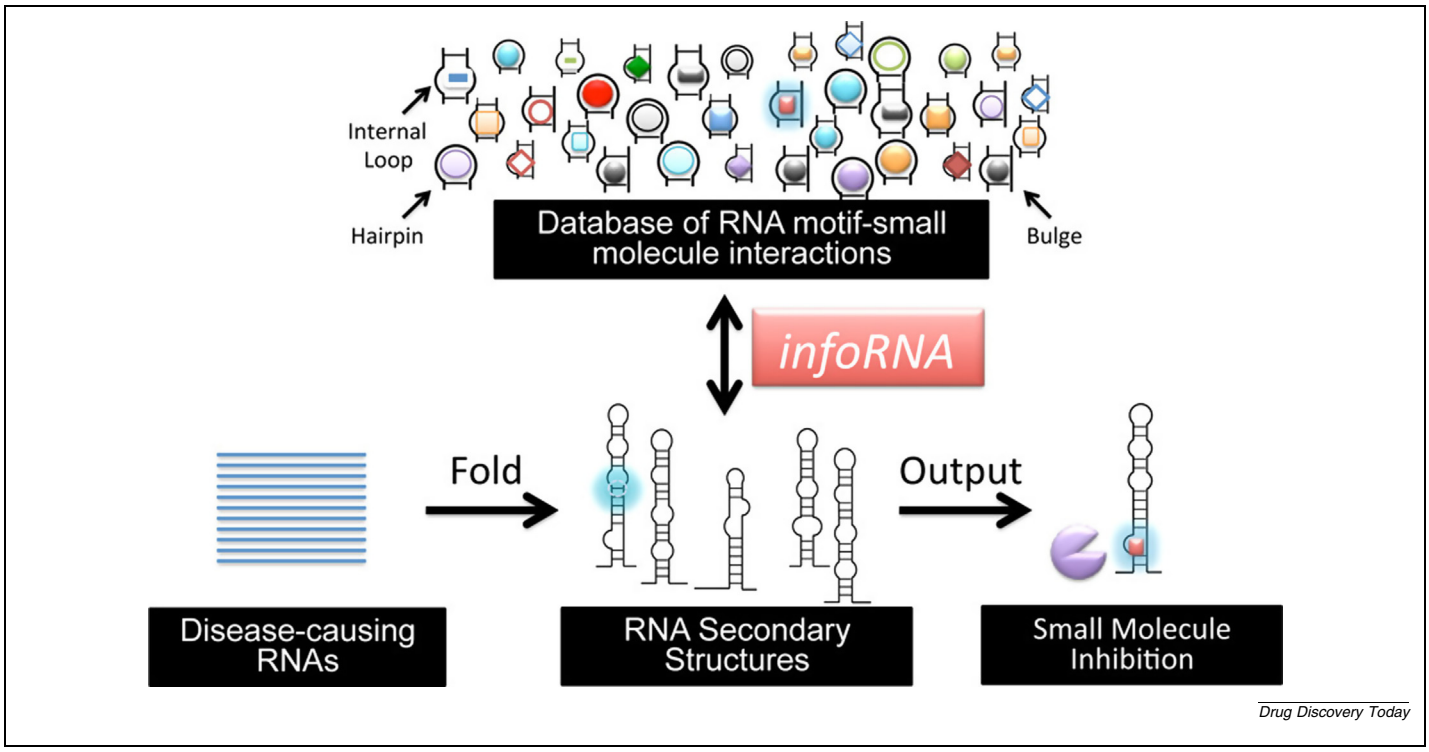


FIG. 4

Design of lead small molecules that target RNA secondary structures (such as those of pri-miRNA and pre-miRNA), using information on RNA motif–small molecule interactions stored in the Inforna database. Figured reused with permission.[79] Copyright 2021 American Chemical Society.

miRNA precursors have been established as valid therapeutic targets for small-molecule-mediated inhibition of miRNA maturation and function. These studies also helped to further our understanding of the features and characteristics of small molecules that facilitate their association with pre-miRNA. This has led to the rational structure-based design of pre-miRNA- and pri-miRNA-binding small molecules.

The interaction between streptomycin **1** and pre-miR-21 was the first reported modulation of miRNA secondary structure using a small molecule, probably mediated by the presence of a bulged region in the secondary structure of pre-miR-21 that created an ideal binding pocket.[69] Using this information, Vo *et al*. hypothesized that aminoglycosides can be adapted to regulate pre-miR-372 and pre-miR-373, which are implicated in gastric cancer.[74] Therefore, after identifying neomycin as a complete inhibitor of pre-miR-372, Vo and co-workers chemically modified neomycin with artificial bases and discovered a potent pre-miR-372 inhibitor **2** with a IC_{50} of 2.42 μM (Fig. 3).[74] This was one of the first reported examples of the rational structure-based design of a miRNA-targeted small molecule, using previous literature on RNA–small molecule interactions rather than the cellular assays typically employed in the discovery process. However, although aminoglycosides are the main class of small molecules reported to bind a variety of miRNA secondary structures,[69,73–75] they interact with RNA nucleotides in a non-specific manner.[58,77] Therefore, the therapeutic utility of aminoglycoside derivatives in the selective modulation of oncogenic miRNAs is questionable.

Employing an alternative strategy, Disney and co-workers combined the sequence information for various pri-miRNA and pre-miRNA available on miRBase with data on all previously reported interactions between small ligands and RNA secondary structural motifs to create a computational tool called Inforna (Fig. 4).[26,78,79] This tool can be used to predict the interaction of novel small molecules and the stem-loop structure of pre-miRNA and pri-miRNA structures. The data obtained can be employed to design novel modulators of miRNA expression. What makes computational tools such as Inforna particularly exciting is the impact they have had on our understanding of the use of small molecules to target pri-miRNA, pre-miRNAs and RNAs in general, given that such nucleic acids were not previously considered amenable to targeting by small molecules. As an example, the Disney group successfully designed non-aminoglycoside small-molecule inhibitors of pri-miR-96 processing (**4** and **5**) using Inforna (Fig. 3). Both of these small molecules were reported to inhibit miR-96 biogenesis selectively in MCF-7 breast cancer cells, with micro- and nanomolar affinities, respectively. Notably, **4** was reported to be ineffective against 149 other profiled miRNAs, including precursors of miR-182 and miR-183, which are transcribed from the same pri-miRNA transcript as miR-96. This indicates that the ligands functioned exclusively by targeting pri-miR-96, rather than other elements of miRNA biogenesis.[71,78,79] Furthermore, the knockdown of miR-96 mediated by **4** and **5** increased FOXO1 expression in MCF-7 cells, subsequently increasing apoptosis. Following this, Inforna was also used to develop a highly selective pre-miR-210 targeting ligand **6**, which was shown to downregulate miR-210

levels in cellular and mouse tumor models.[76] Together, these data suggest that, despite the concerns related to aminoglycosides, small molecules can be designed to be highly selective towards the targeted miRNA secondary structures.

Furthermore, on the basis of the high selectivity of the pri-miR-96 and pre-miR-210 binding small molecule (**4**, **5** and **6**), it is clear that small drug-like molecules can be designed to be highly specific for the target miRNA. Previously, this was a major concern associated with targeting miRNAs using small molecules. The large difference in the surface area of small drug-like molecules (typically < 150 Å^2) compared to that of RNA grooves (1100–1500 Å^2) also raises concerns about the activity of small molecules in comparison to larger bioactive molecules, such as peptides, proteins and oligonucleotides. These larger molecules can facilitate competition for larger macromolecular interfaces, i.e. RNA and RNA–protein interfaces, which is difficult to mimic with small molecules.[80] Therefore, although small molecules are invaluable tools for probing the function of miRNAs in various disease states, they may not be able to facilitate the level of competitive interaction with pre-miRNAs required for therapeutic use. We thus turn now to the use of peptides to inhibit miRNA production for therapeutic ends.

One further area in which small-molecule approaches to modulating miRNA expression have been explored relates to RNA-binding proteins (RBPs). Several RBPs have been shown to regulate miRNA biogenesis selectively, as reviewed elsewhere.[81] This has offered up novel paradigms for controlling the biogenesis of mature miRNAs by targeting RBP interactions. For instance, using a large-scale chemical screen, Sliz and colleagues[82] identified six compounds that inhibited Lin-28 homolog A (LIN28A) interactions with let-7 precursors, thus relieving LIN28A-dependent blockage of let-7 maturation. Likewise, Michlewski and co-workers[83] very recently reported an innovative RNA pull-down confocal nanoscanning strategy that identified quercetin as a small molecule disruptor of HuR binding to pri-miR-7, thus elevating mature miR-7 levels. Nevertheless, it remains to be seen whether these RBP-targeted strategies to elevate miRNA can compete in translational pathways with the direct enhancement of miRNA using miRNA mimics.

Peptide inhibitors of microRNA processing

Peptides occupy the chemical space between ASO and small-molecule-based miRNA inhibitors. They have greater molecular weights and typically occupy a larger area than small drug-like molecules, and so can facilitate competition for larger macromolecular interfaces such as RNA and RNA–protein interfaces.[80] In addition, peptides can be specifically designed to adopt highly

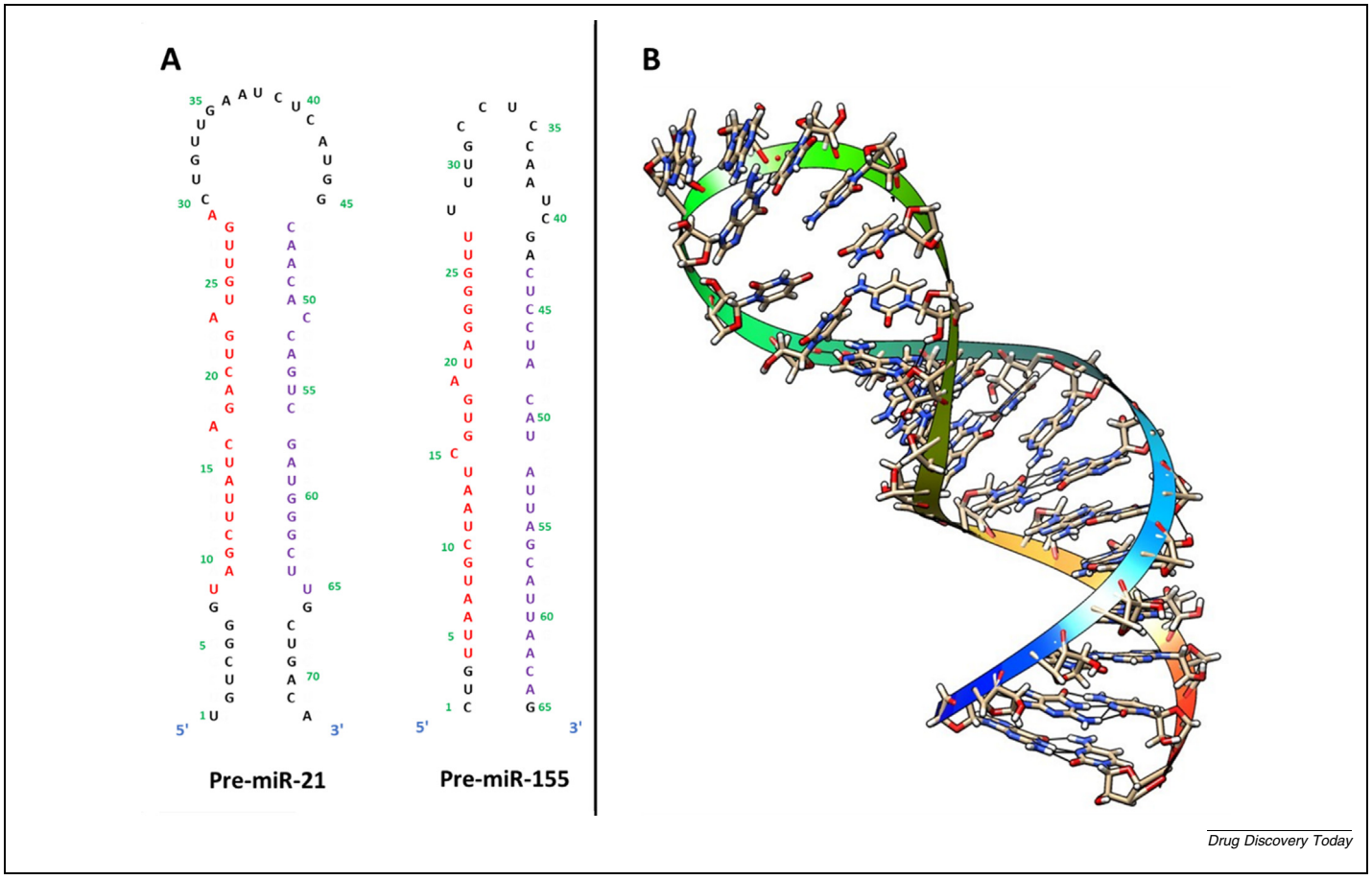
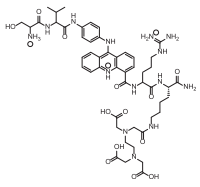
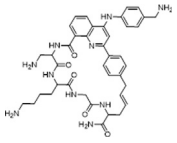
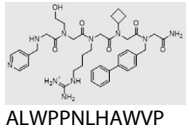
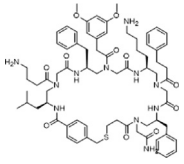
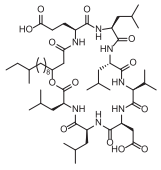


FIG. 5

Structures of precursor miRNAs (pre-miRs). A. The full-length sequences of pre-miR-21 and pre-miR-155. The red sequences represent mature miR-21-5p and miR-155-5p, whereas the purple sequence represents mature miR-21-3p and miR-155-3p, respectively. **B.** Molecular model of a truncated version of pre-miR-21, spanning G22 to C52. Reported by Shortridge *et al.*[80] using solution NMR-based molecular modelling (PDB: 5UZT modelled in Chimera).

TABLE 1

Structure and activity of peptide (and peptidomimetic) inhibitors of miRNA processing.

No	Structure	Targeted miRNA	Binding affinity K_D (μM)	Microprocessor inhibition IC_{50} (μM)	Reference
8		Pre-miR-39	16.5 ± 6.9	N/A	96
9		Pre-miR-23b	2.3 ± 0.6	N/A	98
10		Pri-miR-21	12.0	N/A*	99
11	ALWPPNLHAWVP	Pre-miR-21	0.0127	0.5	92
12	Cyclo(RVRTRGKRRIRRpP) (head to tail cyclized)	Pre-miR-21	0.2	10	80
13	pgrc*kpkrrrratc*rav (*disulfide bridge)	Pre-miR-21	N/A	0.54	93
14	LKKLLKLLKKWLKLKG	Pre-miR-155	0.047	0.77	88
15		Pre-miR-155	0.515	22.0	90
16		Pre-miR-21	0.78 ± 0.08	38–48	91

stabilized secondary structures that can interact with RNAs in a protein-like manner, which is difficult to mimic using small molecules.[64,84] These peptide characteristics have previously led to the development of therapeutic peptides that disrupt intracellular protein–protein interactions (PPIs); Imfinzi (AstraZeneca; approved in 2017) is an example that targets PD-1–PD-L1 interactions to treat non-small lung cancer.[85–87] Prior to the application of peptides, it was difficult to disrupt such macromolecular interactions using traditional methods. Thus, peptides may have similar success in the regulation of oncogenic miRNA by targeting and preventing protein–RNA interactions such as Dicer- or Drosha-mediated pre-miRNA or pri-miRNA cleavage processes.

The exploitation of peptides to regulate the expression of oncogenic miRNAs is, however, widely underexplored. To our knowledge, only nine peptides and peptidomimetics have been reported to bind to miRNA. Out of these, seven were reported to inhibit the function of the oncogenic miRNAs miR-21 and miR-155 (Fig. 5). The peptides and peptidomimetics (Table 1) functioned primarily by binding to grooves at the junction of the hairpin loop and the double-stranded RNA region of pri-miRNAs or pre-miRNAs, thereby preventing Drosha- or Dicer-mediated cleavage processes.

The exploitation of peptide inhibitors of miRNA processing has been slow, largely because of a limited understanding (and, therefore, difficult prediction) of the peptide characteristics that drive peptide–miRNA interaction at the targeted stem-loop junction of pre-miRNA and pri-miRNA. This issue prevents the development of highly selective miRNA-targeting peptides using structure-based design approaches. Instead, to date, the discovery of miRNA binding peptides has primarily been achieved through high-throughput screening methods, including the use of microarrays,[88,89] electrophoretic mobility shift assays (EMSA),[80,90] the catalytic enzyme-linked click chemistry assay (cat-ELCCA),[91] and phage display.[92,93] The peptides discovered through these screening methods were reported to bind the respective miRNA targets selectively with nano or micromolar affinities. Insight into the key peptide motifs that enable specific miRNA binding may help to facilitate the structure-based design of highly selective miRNA-binding peptidomimetics that have picomolar binding affinities.

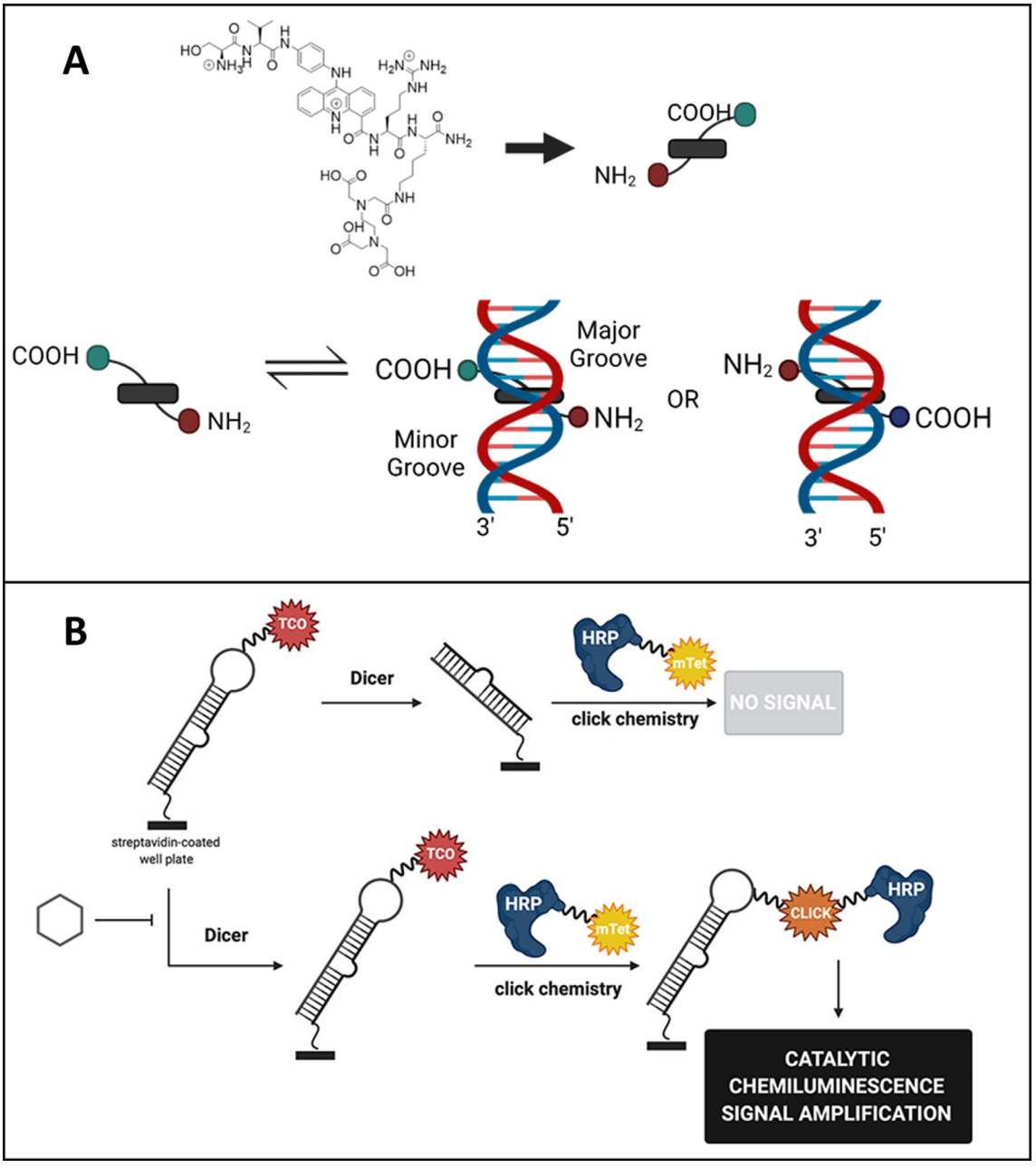


FIG. 6

MicroRNA-targeting peptides. A. Schematic representation of the helix threading peptides (HTP) bound to the RNA duplex. The N-terminus interacts in the minor groove while the C-terminus interacts in the major groove. Reprinted and adapted with permission from Gooch and Beal.[96] Created with BioRender.com. **B.** Schematic representation of how the biochemical assay, cat-ELCCA, may be used to identify peptide inhibitors of miRNA processing. HRP, horseradish peroxidase; mTet, methyltetrazine; TCO, trans-cyclooctene. Figure reused with permission.[91] Copyright 2021 American Chemical Society.

MicroRNA-targeting helix threading peptides

The helix threading peptides (HTPs; **8** and **9**; Table 1) reported by the Beal group were the first class of peptides reported to bind to miRNA.[94] This class of peptides incorporate known RNA and DNA intercalators, such as quinoline and acridine, into a peptide sequence. The heterocyclic core of the HTP directs the peptide to the RNA site that is predisposed to threading intercalation. At the binding site, the intercalator is inserted between nucleic acid base pairs while the peptide appendage passes or 'threads' through the RNA duplex in the opposite grooves of the RNA secondary structure (Fig. 6a).[94–98] Beal and co-workers[96,97] determined that, as a minimum, single-base bulges on both RNA strands towards the 3′ end of the intercalation site were necessary to maintain HTP-binding affinity.[96,97] By defining the minimal binding parameters required for HTP: RNA duplex interaction, these researchers were able to identify-two HTPs that bound pre-miR-39[96] and pre-miR-23b,[98] respectively.

Early work by Gooch *et al.*[96] identified the ability of HTPs to bind pre-miRNAs, such as pre-miR-39, due to the invariable presence of bulges, mismatches, and loops in the pre-miRNA duplex. Following this proof-of-concept study, the same group demonstrated that macrocyclic peptides, in comparison to their linear peptide counterparts, had high binding affinities for target RNAs. This is probably because peptide cyclization restricts the conformational flexibility, which in turn reduces the entropic penalty paid upon RNA binding and increases HTP selectivity for the RNA binding site. This is reflected by the 7-fold lower disassociation constant (K_D) of a macrocyclic peptide **9** (K_D = 2.3 μM) compared to that of a linear peptide **8** (K_D = 16.5 μM).[96,97] This work did not investigate the activities of **8** or **9** in inhibiting microprocessing, but it was the first example of selective peptide–miRNA interactions and kick-started the exploitation of peptides as therapeutically active inhibitors of miRNA processing.

Peptoids that inhibit pri-miR-21

The first peptidomimetic discovered to bind and inhibit miRNA function was reported by Diaz *et al.* (Table 1).[89] The peptoid (N-substituted oligoglycine) was identified through a microarray screen of a library of 14,024 peptoids. The design of this library of compounds was informed by the structure–activity relationships reported for a previously identified 'hit' compound (structure not shown).[99] In the previous work, the central arginine residue was identified as being vital for binding to pri-miR-21. The presence of two C-terminal monomers, in this instance two glycine residues, also contributed to target affinity. Thus, the library of peptoids that was synthesized retained the central arginine residue while two variable peptoid monomers were substituted on either side of the arginine. This led to the discovery of 11 lead compounds, and out of these, peptoid **10** had the lowest disassociation constant (K_D = 12 μM; Table 1). Thus, peptoid **10** was further analyzed for microprocessing inhibiting activity. The peptoid was found to abrogate Drosha's microprocessor capabilities completely at 250 μM concentration, preventing the formation of pre-miR-21 and the maturation of miR-21.

The selectivity of **10** was further investigated to confirm that it could bind to pri-miR-21 in a sequence-specific manner. At 250 μM concentration, the peptoid was found to decrease pre-miR-16 expression by less than 10%, whereas pre-miR-21 expression was completely abrogated. These findings indicate that **10** acted in a sequence-specific manner and did not act by inactivating the Drosha microprocessor.[89] This inhibitory activity is lower than desired, and thus it is evident that there is potential for further ligand optimization. Following on from the work by the Beal group, however, this study provided proof-of-concept that peptidomimetics could be designed to target and inhibit miRNA function selectively.

Peptidomimetics that inhibit pre-miR-21 and pre-miR-155

Subsequent to the discovery of miR-21-targeted peptoids, a range of other peptides and peptidomimetics have been reported to inhibit the maturation of miR-21[80,91–93] and miR-155. [88,90] Noteworthy examples include a type II β-hairpin mimetic (**12**) and an α-helical peptide (**7**), which were reported, by Shortridge *et al.*[80] and by Pai *et al.*,[88] respectively, to inhibit pre-miRNA maturation. Another group reported an interesting head-to-tail cyclized γ-substituted-N-acylated-N-aminoethylamino acid (γ-AA) peptidomimetic (**15**), which inhibited pre-miR-155 maturation and which showed enhanced stability towards proteolysis, enhanced cell permeability properties and increased selectivity in comparison to linear peptides.[90] Interestingly, the Garner group employed their in-house cat-ELCCA to identify a cyclic lipodepsipeptide that could bind and inhibit Dicer cleavage of pre-miR-21.[91,100].

The structural constraint of peptides and peptidomimetics, such as **12**, **15** and **17**, reportedly enhance their binding selectivity for the targeted macromolecule surface when compared to linear unstructured peptides.[8,90] Nevertheless, a comparison of peptide–pre-miRNA binding affinities suggested that the linear peptide **11** (K_D = 0.0127 μM), which reportedly did not form a defined secondary structure, was more selective towards its respective pre-miRNA target than more structured peptide counterparts.[92] This may be explained by the propensity of **11** to adopt relatively stable turn motifs due to prolyl bond isomerism. This is particularly prevalent where a proline side chain can interact with an adjacent aromatic residue through CH–π interactions, thereby stabilizing the *cis*-prolyl conformer.[101] Thus, the 'Trp-Pro-Pro' motif of peptide **11** may give rise to peptidylprolyl *cis* or *trans* conformers, leading to the formation of a favorable stable secondary structure; for example, a turn motif centered around the Pro-Pro sequence may be essential to engage the pre-miR-21 target.[101] The ability of the peptide to form a turn motif would explain why peptide **11** had the lowest miRNA binding affinity and IC_{50} seen in this study. This needs to be confirmed experimentally and is a possible new consideration in inhibitor design.

Understanding what drives peptide–pre-miRNA interactions

Molecular modelling studies, employing solution NMR analysis and docking, have been reported for three out of five of the pre-miRNA binding peptidomimetics.[80,88,92] These studies are crucial in identifying peptide–pre-miRNA interactions that inhibit Dicer cleavage, as this information can inform the rational design of future miRNA-targeted peptide therapies. Current data from the limited number of molecular modelling studies that have been performed indicate that the peptide-binding pocket is situated near the stem-loop junction of pre-miR-21 and pre-miR-155, near the Dicer binding site (Fig. 5A).

More specifically, NMR analysis indicated that peptide **12** bound to the major groove of pre-miR-21 by straddling the A29 bulge, with specific contact in the lower helix and secondary contact with loop residues. Furthermore, amino acid residues that are positively charged at physiological pH, Arg and Lys, interacted with the negatively charged phosphate backbone of the pre-miR-21.[80] Meanwhile, Bose *et al.*[92] used a docking model to probe pre-miR-21–peptide **11** interactions, and concluded that the 'ALWPPN', 'W10' and 'P12' residues interacted with the pre-miR-21 residues G32 to G35 and U43 to C46. Further enzymatic 'footprinting' confirmed this mode of interaction. In addition, the A29 bulge also proved crucial for pre-miR-21–peptide **11**

interactions; peptide **11** was shown to have very low binding affinity towards a pre-miR-21 mutant without the A29 bulge.[92] This was consistent with a previous binding study on small molecules that indicated that the A29 bulge in pre-miR-21 was important for ligand-binding activity.[69] Specific peptide–miR-155 interactions were only reported for peptide **14**. Solution NMR studies indicated that upon peptide **14** binding, there were large chemical perturbations ($\Delta\delta_{max} > 0.01$ ppm) in pre-miR-155 bases U28-G31, C36, A38 and G41. This suggested that peptide **14** bound to pre-miR-155 at the stem-loop junction, where the peptide–pre-miR-21 interactions described above also occurred. Further molecular modelling studies suggested that both hydrophobic and hydrophilic amino acid side chains of amphiphilic peptide **14** interacted with pre-miR-155 bases or with phosphate groups through electrostatic, hydrogen bonding and van der Waals interactions.[88]

Finally, at least two of the reported peptidomimetics (**12** and **14**) functioned by binding to the major groove of the targeted pre-miRNA.[8,88] This may explain why the Dicer-inhibiting activity of the peptides was significantly lower than the binding affinity of the peptides, where these were reported (Table 1). Given that the Dicer enzyme reportedly binds to the minor groove of pre-miRNA, the low inhibitory activity of the peptides could be due to a lack of direct competition between the ligands and the Dicer complex.[102,103]

Evidently, the data available to tell us what drives peptide–pre-miRNA interactions and the subsequent inhibition of miRNA maturation is limited. A greater pool of molecular modelling data for various types of peptides that inhibit miRNA processing is required to reveal fully the peptide features that drive miRNA interaction and inhibition. Such data will improve our understanding of peptide–miRNA interactions and will allow the structure-based design of future miRNA-targeted peptide therapeutics. Nevertheless, the limited number of molecular docking and solution NMR studies conducted to date suggest that, as might be predicted, perturbations in the secondary miRNA structure near the hairpin region are critical for peptide–miRNA interactions and to inhibit miRNA maturation. This is a step in the right direction towards increased understanding of peptide–miRNA interactions and brings us closer to the exploitation of peptides in the development of miRNA-directed drugs.

Tools that facilitate the discovery of miRNA-targeted peptides

To accelerate the discovery of pre-miRNA-targeted peptide inhibitors of miRNA processing, Garner *et al.*[77] developed cat-ELCCA. This is a high-throughput screening (HTS) biochemical assay that depends on a click chemistry reaction with modified horseradish peroxidase (HRP) to detect biomolecular events through the generation of an amplified chemiluminescence signal. To identify peptides that bind and inhibit pre-miRNA, the group designed Dicer cat-ELCCA, which monitors the integrity of the terminal loop of immobilized pre-miRNA. In the absence of a peptide inhibitor, the Dicer enzyme cleaves the pre-miRNA terminal loop, leading to no chemiluminescent signal. By contrast, the presence of a peptide inhibitor prevents cleavage of the pre-miRNA terminal loop, yielding a chemiluminescent signal (Fig. 6b).[91]

Previously, cat-ELCCA has been employed to identify small-molecule inhibitors of miRNA processing.[100,104] To discover a peptide inhibitor of miRNA maturation, a library of approximately 30,000 pre-fractionated marine natural product extracts (NPEs) were screened, leading to the discovery of a family of RNA-binding lipodepsipeptides, known as surfactins, which had previously been studied for their antibacterial, antifungal and antiviral properties. The fraction with the highest inhibitory activity was further purified and the structures of the peptides were determined by MS, MS/MS and 1D and 2D NMR experiments. This identified peptide **16**, which was shown to bind pre-miR-21 (K_D 0.78 ± 0.08 μM) and to inhibit their subsequent maturation (IC_{50} 13–48 μM).[91] The ability of the surfactins to bind pre-miR and to inhibit miRNA activity is surprising, given that surfactins are largely hydrophobic and do not contain aromatic or positively charged residues, two of the most commonly reported characteristics of miRNA-binding peptides (Table 1).

Toxicity concerns related to peptide **16** limited the further application of this peptide. Furthermore, peptide **16** was shown to be only moderately selective for pre-miR-21 when compared to pre-let-7d. More broadly, concerns related to the toxicity and selectivity of RNA-targeted peptides have been a key bottleneck in the progression of this field of research. Thus, these concerns will need to be addressed before miRNA-targeted peptides may be considered as potential therapeutics. Nonetheless, the development and application of cat-ELCCA in the discovery of a peptide that inhibits miRNA processing is ground-breaking. It eliminates the need to isolate completely and purify compounds prior to testing, which is often a laborious process. In the future, it would allow the rapid screening and identification of miRNA-targeting peptides. To date, the largest cat-ELCCA screen involved a library of approximately 130,000 compounds, but the group predicts that this technology could be used for the HTS of 1,000,000 compounds.[104] This could significantly accelerate the identification of classes of peptides that bind and inhibit miRNA function, could further our understanding of the peptide features that drive peptide–miRNA interaction, could eliminate toxicity and selectivity concerns and could lead to the development of novel miRNA-targeted therapies.

Cellular activity of peptide inhibitors of miRNA processing

In addition to assessing the ability of peptide inhibitors to target Dicer processing, the cellular activity of the peptides was also examined. Peptides **11**, **12**, **14**, **15** and **16** were shown to downregulate targeted mature miRNAs at a cellular level. It was expected that the cellular knockdown of oncogenic miR-21 and miR-155 expression would increase the expression of the mRNA target genes and promote apoptotic cell death. To this end, peptide **11** was demonstrated to increase moderately the expression of PCDD4 and PTEN, both tumor-suppressing genes that are known targets of miR-21, in MCF-7 cells.[105] Peptide **11** also increased the extent of apoptosis by 30% in MCF-7 cells and reduced cell invasion and migration by up to 50% and 30%, respectively. Meanwhile the control peptide had no effect on cel-

lular apoptosis, invasion, or migration.[92] The overexpression of miR-155 is associated with the resistance of cancer cells to apoptosis.[106] Amphiphilic peptide **14** was reported to stimulate cellular apoptosis through the generation of caspare-3, indicating that peptide **14** induced apoptosis in MCF-7 cells through caspase-dependent pathways.[88] Similarly, the ability of peptide **15** to mediate miR-155 knockdown and to upregulate FOXO3a expression was determined. The miR-155 target, FOXO3a, is a key tumor-suppressing gene. Loss of FOXO3A function is associated with increased resistance to apoptosis in cancer cells.[107] In MCF-7 cells, treatment with peptide **15** led to an increase in FOXO3A expression and increased cell apoptosis by 12%.[90] This showed that, by targeting pri-miRNA and pre-miRNA processing, peptides are able to modulate the expression of mature miRNAs at a cellular level. Thus, they can regulate the expression of target genes whose dysregulation may play a key role disease.

Conclusions and future perspectives

Traditionally, miRNA secondary structures were considered undruggable and unsuitable for drug discovery efforts because complex 3D structures with protein-like folds were thought to be pre-requisites for the ideal drug target. Fortunately, these early views were largely disregarded, and as evidenced by this review, strides have been made in establishing RNA secondary structures as valid drug targets. Peptidic drugs in particular have shown great potential for the future development of miRNA-directed therapies. A plethora of future work is required, however, to provide an understanding of peptide–miRNA interactions before the first peptide inhibitor of miRNA processing can be approved for clinical use.

To further advance the understanding of peptide–miRNA interactions, with the goal of bringing miRNA-directed peptide therapies to the market, more work is required from a medicinal chemistry perspective. Specifically, now that peptide-based inhibitors of miRNA processing have been identified, further studies are needed to identify which amino acid moieties in the inhibitor facilitate interactions with the nucleobases or backbone of the miRNA. For example, techniques, such as alanine-scanning mutagenesis of known miRNA inhibitors could help to identify the amino acids and motifs that are crucial for peptide affinity, and could help to reveal which features drive peptide–miRNA interaction. In addition, out of the eight peptide inhibitors of miRNA processing that have been reported, three are likely to form some type of secondary structure. Incidentally these three peptides also have the lowest dissociation constant. Thus, it is possible that the secondary structure of these peptides, when compared to linear unstructured counterparts, may help to facilitate preferential binding to targeted miRNA secondary structures. However, further solution molecular docking and solution NMR studies of a larger library of peptides that are reported to bind secondary miRNA structures are required to confirm this, and to identify which peptide secondary structures facilitate the most favorable peptide–miRNA interactions. An enhanced understanding of the peptide secondary structures and amino acids that drive peptide–miRNA interactions would help to facilitate the structure-based design of peptide inhibitors of miRNA processing with picomolar binding affinities.

Once a lead peptide inhibitor of miRNA processing has been identified, potential poor pharmacokinetic properties will have to be addressed and an *in vivo* peptide delivery system may have to be developed. In the past, this has been the major bottleneck in bringing peptide pharmaceuticals onto the market. On the basis of the rapidly growing number of peptide therapeutics approved by regulatory bodies, however, it is clear that these concerns can easily be addressed. Tools such as PepTherDia,[108] which curates the key information available on approved peptide pharmaceutics, can help to support the rational design and development of future peptide drugs.

To conclude, peptide inhibitors of miRNA processing are still in the very early stages of development and much work remains to optimize their design, delivery and pharmacokinetic properties. Nevertheless, we anticipate that we will begin to see miRNA-targeted peptide drugs enter clinical trials for treatment of various cancers within the next 10 years. Therefore, such peptides represent an exciting area for continuing research spanning fundamental discovery and translational medicine.

Declaration of interest

There are no conflicts of interest or necessary disclosures associated with this manuscript.

Acknowledgements

TC is supported by a Liverpool John Moores University Institute for Health Research PhD scholarship.

References

1 E.A. Stadtmauer, J.A. Fraietta, M.M. Davis, A.D. Cohen, K.L. Weber, E. Lancaster, et al., CRISPR-engineered T cells in patients with refractory cancer, Science 367 (2020) eaba7365, https://doi.org/10.1126/science.aba7365.

2 D. Adams, A. Gonzalez-Duarte, W.D. O'Riordan, C.C. Yang, M. Ueda, V. Kristen, et al., Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis, N Engl J Med 379 (2018) 11–21, https://doi.org/10.1056/NEJMoa1716153.

3 M. Balwani, E. Sardh, P. Ventura, P.A. Peiró, D.C. Rees, U. Stölzel, et al., Phase 3 trial of RNAi therapeutic givosiran for acute intermittent porphyria, N Engl J Med 382 (2020) 2289–2301, https://doi.org/10.1056/NEJMoa1913147.

4 S.F. Garrelfs, Y. Frishberg, S.A. Hulton, M.J. Koren, W.D. O'Riordan, P. Cochat, et al., Lumasiran, an RNAi therapeutic for primary hyperoxaluria type 1, N Engl J Med 384 (2021) 1216–1226, https://doi.org/10.1056/NEJMoa2021712.

5 F.J. Raal, D. Kallend, K.K. Ray, T. Turner, W. Koenig, R.S. Wright, et al., Inclisiran for the treatment of heterozygous familial hypercholesterolemia, N Engl J Med 382 (2020) 1520–1530, https://doi.org/10.1056/NEJMoa1913805.

6 S. Bajan, G. Hutvagner, RNA-based therapeutics: from antisense oligonucleotides to miRNAs, Cells 9 (2020) 137, https://doi.org/10.3390/cells9010137.

7 X. Jing, K. Jin, A gold mine for drug discovery: strategies to develop cyclic peptides into therapies, Med Res Rev 40 (2020) 753–810, https://doi.org/10.1002/med.21639.

8 M.D. Shortridge, G. Varani, Structure based approaches for targeting non-coding RNAs with small molecules, Curr Opin Struct Biol 30 (2015) 79–88, https://doi.org/10.1016/j.sbi.2015.01.008.

9 P. Wu, Inhibition of RNA-binding proteins with small molecules, Nat Rev Chem 4 (2020) 1–18, https://doi.org/10.1038/s41570-020-0201-4.

10 D.P. Bartel, Metazoan microRNAs, Cell 173 (2018) 20–51, https://doi.org/10.1016/j.cell.2018.03.006.

11 R.C. Lee, R.L. Feinbaum, V. Ambros, The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14, Cell 75 (1993) 843–854, https://doi.org/10.1016/0092-8674(93)90529-y.

12 B. Wightman, I. Ha, G. Ruvkun, Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*, Cell 75 (1993) 855–862, https://doi.org/10.1016/0092-8674(93)90530-4.

13 A.E. Pasquinelli, B.J. Reinhart, F. Slack, M.Q. Martindale, M.I. Kuroda, B. Maller, et al., Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA, Nature 408 (2000) 86–89, https://doi.org/10.1038/35040556.

14 M. Lagos-Quintana, R. Rauhut, W. Lendeckel, T. Tuschl, Identification of novel genes coding for small expressed RNAs, Science 294 (2001) 853–858, https://doi.org/10.1126/science.1064921.

15 R.C. Lee, V. Ambros, An extensive class of small RNAs in *Caenorhabditis elegans*, Science 294 (2001) 862–864, https://doi.org/10.1126/science.1065329.

16 A. Kozomara, M. Birgaoanu, S. Griffiths-Jones, miRBase: from microRNA sequences to function, Nucleic Acids Res 47 (2019) D155–D162, https://doi.org/10.1093/nar/gky1141.

17 E.S. Ovchinnikova, D. Schmitter, E.L. Vegter, J.M. Ter Maaten, M.A.E. Valente, L. C.Y. Liu, et al., Signature of circulating microRNAs in patients with acute heart failure, Eur J Heart Fail 18 (2016) 414–423, https://doi.org/10.1002/ejhf.332.

18 M. Baghi, M. Rostamian Delavar, E. Yadegari, M. Peymani, D. Pozo, M.H. Nasr-Esfahani, K. Ghaedi, Modified level of miR-376a is associated with Parkinson's disease, J Cell Mol Med 24 (2020) 2622–2634, https://doi.org/10.1111/jcmm.14979.

19 R.-Y. Huang, L. Li, M.-J. Wang, X.-M. Chen, Q.-C. Huang, C.-J. Lu, An exploration of the role of microRNAs in psoriasis: a systematic review of the literature, Medicine (Baltimore) 94 (2015) e2030.

20 G.A. Calin, C. Sevignani, C.D. Dumitru, T. Hyslop, E. Noch, S. Yendamuri, et al., Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers, Proc Natl Acad Sci U S A 101 (2004) 2999–3004, https://doi.org/10.1073/pnas.0307323101.

21 M. Cui, H. Wang, X. Yao, D. Zhang, Y. Xie, R. Cui, X. Zhang, Circulating microRNAs in cancer: potential and challenge, Front Genet 10 (2019) 626, https://doi.org/10.3389/fgene.2019.00626.

22 R. Rupaimoole, F.J. Slack, MicroRNA therapeutics: towards a new era for the management of cancer and other diseases, Nat Rev Drug Discov 16 (2017) 203–222, https://doi.org/10.1038/nrd.2016.246.

23 M. Lindow, S. Kauppinen, Discovering the first microRNA-targeted drug, J Cell Biol 199 (2012) 407–412, https://doi.org/10.1083/jcb.201208082.

24 C. Querfeld, F.M. Foss, Y.H. Kim, L. Pinter-Brown, B.M. William, P. Porcu, et al., Phase 1 trial of cobomarsen, an inhibitor of Mir-155, in cutaneous T cell lymphoma, Blood 132 (2018) 2903, https://doi.org/10.1182/blood-2018-99-119861.

25 C. Chakraborty, A.R. Sharma, G. Sharma, S.-S. Lee, Therapeutic advances of miRNAs: a preclinical and clinical update, J Adv Res 28 (2021) 127–138, https://doi.org/10.1016/j.jare.2020.08.012.

26 M.D. Disney, Targeting RNA with small molecules to capture opportunities at the intersection of chemistry, biology, and medicine, J Am Chem Soc 141 (2019) 6776–6790, https://doi.org/10.1021/jacs.8b13419.

27 Z. Wang, X. Wang, T.M. Rana, Protein orientation in the Tat–TAR complex determined by psoralen photocross-linking, J Biol Chem 271 (1996) 16995–16998, https://doi.org/10.1074/jbc.271.29.16995.

28 S. Kim, J.H. Lee, I. Kang, S. Hyun, J. Yu, C. Shin, An amphiphilic peptide induces apoptosis through the miR29b-p53 pathway in cancer cells, Mol Ther Nucleic Acids 5 (2016) e330.

29 Y.-T. Sun, M.D. Shortridge, G. Varani, A small cyclic β-hairpin peptide mimics the Rbfox2 RNA recognition motif and binds to the precursor miRNA 20b, Chembiochem 20 (2019) 931–939, https://doi.org/10.1002/cbic.201800645.

30 M. Ha, V.N. Kim, Regulation of microRNA biogenesis, Nat Rev Mol Cell Biol 15 (2014) 509–524, https://doi.org/10.1038/nrm3838.

31 T.M. Rana, Illuminating the silence: understanding the structure and function of small RNAs, Nat Rev Mol Cell Biol 8 (2007) 23–36, https://doi.org/10.1038/nrm2085.

32 S.R. Feldman, R. Garton, W. Averett, R. Balkrishnan, J. Vallee, Strategy to manage the treatment of severe psoriasis: considerations of efficacy, safety and cost, Expert Opin Pharmacother 4 (2003) 1525–1533.

33 J. Han, Y. Lee, K.H. Yeom, Y.K. Kim, H. Jin, V.N. Kim, The Drosha-DGCR8 complex in primary microRNA processing, Genes Dev 18 (2004) 3016–3027, https://doi.org/10.1101/gad.1262504.

34 R. Yi, Y. Qin, I.G. Macara, B.R. Cullen, Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs, Genes Dev 17 (2003) 3011–3016, https://doi.org/10.1101/gad.1158803.

35 R.C. Wilson, A. Tambe, M.A. Kidwell, C.L. Noland, C.P. Schneider, J.A. Doudna, Dicer-TRBP complex formation ensures accurate mammalian microRNA biogenesis, Mol Cell 57 (2015) 397–407, https://doi.org/10.1016/j.molcel.2014.11.030.

36 Y. Peng, C.M. Croce, The role of MicroRNAs in human cancer, Signal Transduct Target Ther 1 (2016) 15004, https://doi.org/10.1038/sigtrans.2015.4.

37 J. O'Brien, H. Hayder, Y. Zayed, C. Peng, Overview of microRNA biogenesis, mechanisms of actions, and circulation, Front Endocrinol 9 (2018) 402, https://doi.org/10.3389/fendo.2018.00402.

38 K. Tsuboyama, H. Tadakuma, Y. Tomari, Conformational activation of Argonaute by distinct yet coordinated actions of the Hsp70 and Hsp90 chaperone systems, Mol Cell 70 (2018) 722–729.e4, https://doi.org/10.1016/j.molcel.2018.04.010.

39 A. Khvorova, A. Reynolds, S.D. Jayasena, Functional siRNAs and miRNAs exhibit strand bias, Cell 115 (2003) 209–216, https://doi.org/10.1016/s0092-8674(03)00801-8.

40 J.S. Yang, M.D. Phillips, D. Betel, M.u. Ping, A. Ventura, A.C. Siepel, et al., Widespread regulatory activity of vertebrate microRNA* species, RNA 17 (2011) 312–326, https://doi.org/10.1261/rna.2537911.

41 Y. Ge, L. Zhang, M. Nikolova, B. Reva, E. Fuchs, Strand-specific in vivo screen of cancer-associated miRNAs unveils a role for miR-21(*) in SCC progression, Nat Cell Biol 18 (2016) 111–121, https://doi.org/10.1038/ncb3275.

42 B. Tang, B. Lei, G. Qi, X. Liang, F. Tang, S. Yuan, et al., MicroRNA-155-3p promotes hepatocellular carcinoma formation by suppressing FBXW7 expression, J Exp Clin Cancer Res 35 (2016) 93, https://doi.org/10.1186/s13046-016-0371-6.

43 H.O. Iwakawa, Y. Tomari, The functions of microRNAs: mRNA decay and translational repression, Trends Cell Biol 25 (2015) 651–655, https://doi.org/10.1016/j.tcb.2015.07.011.

44 E. Elkayam, C.R. Faehnle, M. Morales, J. Sun, H. Li, L. Joshua-Tor, Multivalent recruitment of human Argonaute by GW182, Mol Cell 67 (2017) 646–658.e3, https://doi.org/10.1016/j.molcel.2017.07.007.

45 S.W. Eichhorn, H. Guo, S.E. McGeary, R.A. Rodriguez-Mias, C. Shin, D. Baek, et al., mRNA destabilization is the dominant effect of mammalian microRNAs by the time substantial repression ensues, Mol Cell 56 (2014) 104–115, https://doi.org/10.1016/j.molcel.2014.08.028.

46 C. Li, T. Zhou, J. Chen, R. Li, H. Chen, S. Luo, et al., The role of exosomal miRNAs in cancer, J Transl Med 20 (2022) 6, https://doi.org/10.1186/s12967-021-03215-4.

47 S.D. Selcuklu, M.T. Donoghue, C. Spillane, miR-21 as a key regulator of oncogenic processes, Biochem Soc Trans 37 (2009) 918–925, https://doi.org/10.1042/bst0370918.

48 S. Volinia, G.A. Calin, C.G. Liu, S. Ambs, A. Cimmino, F. Petrocca, et al., A microRNA expression signature of human solid tumors defines cancer gene targets, Proc Natl Acad Sci U S A 103 (2006) 2257–2261, https://doi.org/10.1073/pnas.0510565103.

49 S.R. Pfeffer, C.H. Yang, L.M. Pfeffer, The role of miR-21 in cancer, Drug Dev Res 76 (2015) 270–277, https://doi.org/10.1002/ddr.21257.

50 Y.H. Feng, C.J. Tsao, Emerging role of microRNA-21 in cancer, Biomed Rep 5 (2016) 395–402, https://doi.org/10.3892/br.2016.747.

51 D. Bautista-Sánchez, C. Arriaga-Canon, A. Pedroza-Torres, I.A. De La Rosa-Velázquez, R. González-Barrios, L. Contreras-Espinosa, et al., The promising role of miR-21 as a cancer biomarker and its importance in RNA-based therapeutics, Mol Ther Nucleic Acids 20 (2020) 409–420, https://doi.org/10.1016/j.omtn.2020.03.003.

52 L. Witten, F.J. Slack, miR-155 as a novel clinical target for hematological malignancies, Carcinogenesis 41 (2020) 2–7, https://doi.org/10.1093/carcin/bgz183.

53 P. Ranganath, MicroRNA-155 and its role in malignant hematopoiesis, Biomark Insights 10 (2015) 95–102, https://doi.org/10.4137/bmi.S27676.

54 R. Bayraktar, K. Van Roosbroeck, miR-155 in cancer drug resistance and as target for miRNA-based therapeutics, Cancer Metastasis Rev 37 (2018) 33–44, https://doi.org/10.1007/s10555-017-9724-7.

55 E. Tili, J.J. Michaille, D. Wernicke, H. Alder, S. Costinean, S. Volinia, C.M. Croce, Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer, Proc Natl Acad Sci U S A 108 (2011) 4908–4913, https://doi.org/10.1073/pnas.1101795108.

56 I. Faraoni, F.R. Antonetti, J. Cardone, E. Bonmassar, miR-155 gene: a typical multifunctional microRNA, Biochim Biophys Acta 1792 (2009) 497–505, https://doi.org/10.1016/j.bbadis.2009.02.013.

57 E. Tili, C.M. Croce, J.J. Michaille, miR-155: on the crosstalk between inflammation and cancer, Int Rev Immunol 28 (2009) 264–284, https://doi.org/10.1080/08830180903093796.

58 J.R. Thomas, P.J. Hergenrother, Targeting RNA with small molecules, Chem Rev 108 (2008) 1171–1224, https://doi.org/10.1021/cr0681546.

59 S. Roy, U. Delling, C.H. Chen, C.A. Rosen, N. Sonenberg, A bulge structure in HIV-1 TAR RNA is required for Tat binding and Tat-mediated trans-activation, Genes Dev 4 (1990) 1365–1373, https://doi.org/10.1101/gad.4.8.1365.

60 K.M. Weeks, D.M. Crothers, RNA recognition by Tat-derived peptides: interaction in the major groove?, Cell 66 (1991) 577–588, https://doiorg/10.1016/0092-8674(81)90020-9.

61 C. Faber, H. Sticht, K. Schweimer, P. Rösch, Structural rearrangements of HIV-1 Tat-responsive RNA upon binding of neomycin B, J Biol Chem 275 (2000) 20660–20666, https://doi.org/10.1074/jbc.M000920200.

62 D.I. Bryson, W. Zhang, P.M. McLendon, T.M. Reineke, W.L. Santos, Toward targeting RNA structure: branched peptides as cell-permeable ligands to TAR RNA, ACS Chem Biol 7 (2012) 210–217, https://doi.org/10.1021/cb200181v.

63 S. Jang, S. Hyun, S. Kim, S. Lee, I.S. Lee, M. Baba, et al., Cell-penetrating, dimeric alpha-helical peptides: nanomolar inhibitors of HIV-1 transcription, Angew Chemie Int Ed Engl 53 (2014) 10086–10089, https://doi.org/10.1002/anie.201404684.

64 T.C. Leeper, Z. Athanassiou, R.L. Dias, J.A. Robinson, G. Varani, TAR RNA recognition by a cyclic peptidomimetic of Tat protein, Biochemistry 44 (2005) 12362–12372, https://doi.org/10.1021/bi0510532.

65 R. Fan, C. Xiao, X. Wan, W. Cha, Y. Miao, Y. Zhou, et al., Small molecules with big roles in microRNA chemical biology and microRNA-targeted therapeutics, RNA Biol 16 (2019) 707–718, https://doi.org/10.1080/15476286.2019.1593094.

66 N. Ankenbruck, R. Kumbhare, Y. Naro, M. Thomas, L. Gardner, C. Emanuelson, A. Deiters, Small molecule inhibition of microRNA-21 expression reduces cell viability and microtumor formation, Bioorganic Med Chem 27 (2019) 3735–3743, https://doi.org/10.1016/j.bmc.2019.05.044.

67 Y. Naro, N. Ankenbruck, M. Thomas, Y. Tivon, C.M. Connelly, L. Gardner, A. Deiters, et al., Small molecule inhibition of microRNA miR-21 rescues chemosensitivity of renal-cell carcinoma to topotecan, J Med Chem 61 (2018) 5900–5909, https://doi.org/10.1021/acs.jmedchem.7b01891.

68 S.P. Velagapudi, B.R. Vummidi, M.D. Disney, Small molecule chemical probes of microRNA function, Curr Opin Chem Biol 24 (2015) 97–103, https://doi.org/10.1016/j.cbpa.2014.10.024.

69 D. Bose, G. Jayaraj, H. Suryawanshi, P. Agarwala, S.K. Pore, R. Banerjee, S. Maiti, The tuberculosis drug streptomycin as a potential cancer therapeutic: inhibition of miR-21 function by directly targeting its precursor, Angew Chem Int Ed Engl 51 (2012) 1019–1023, https://doi.org/10.1002/anie.201106455.

70 H. Yan, U. Bhattarai, Z.-F. Guo, F.-S. Liang, Regulating miRNA-21 biogenesis by bifunctional small molecules, J Am Chem Soc 139 (2017) 4987–4990, https://doi.org/10.1021/jacs.7b00610.

71 S.P. Velagapudi, M.D. Cameron, C.L. Haga, L.H. Rosenberg, M. Lafitte, D.R. Duckett, et al., Design of a small molecule against an oncogenic noncoding RNA, Proc Natl Acad Sci U S A 113 (2016) 5898–5903, https://doi.org/10.1073/pnas.1523975113.

72 S.P. Velagapudi, S.M. Gallo, M.D. Disney, Sequence-based design of bioactive small molecules that target precursor microRNAs, Nat Chem Biol 10 (2014) 291–297, https://doi.org/10.1038/nchembio.1452.

73 M. Maiti, K. Nauwelaerts, P. Herdewijn, Pre-microRNA binding aminoglycosides and antitumor drugs as inhibitors of Dicer catalyzed microRNA processing, Bioorg Med Chem Lett 22 (2012) 1709–1711, https://doi.org/10.1016/j.bmcl.2011.12.103.

74 D.D. Vo, C. Staedel, L. Zehnacker, R. Benhida, F. Darfeuille, M. Duca, Targeting the production of oncogenic microRNAs with multimodal synthetic small molecules, ACS Chem Biol 9 (2014) 711–721, https://doi.org/10.1021/cb400668h.

75 S.P. Velagapudi, M.D. Disney, Two-dimensional combinatorial screening enables the bottom-up design of a microRNA-10b inhibitor, Chem Commun (Camb) 50 (2014) 3027–3029, https://doi.org/10.1039/c3cc00173c.

76 M.G. Costales, C.L. Haga, S.P. Velagapudi, J.L. Childs-Disney, D.G. Phinney, M. D. Disney, Small molecule inhibition of microRNA-210 reprograms an oncogenic hypoxic circuit, J Am Chem Soc 139 (2017) 3446–3455, https://doi.org/10.1021/jacs.6b11273.

77 A.L. Garner, RNA-targeted drug discovery: moving beyond promiscuous small-molecule scaffolds, Future Med Chem 11 (2019) 2487–2490, https://doi.org/10.4155/fmc-2019-0200.

78 M.D. Disney, I. Yildirim, J.L. Childs-Disney, Methods to enable the design of bioactive small molecules targeting RNA, Org Biomol Chem 12 (2014) 1029–1039, https://doi.org/10.1039/C3OB42023J.

79 M.D. Disney, A.M. Winkelsas, S.P. Velagapudi, M. Southern, M. Fallahi, J.L. Childs-Disney, Inforna 2.0: a platform for the sequence-based design of small molecules targeting structured RNAs, ACS Chem Biol 11 (2016) 1720–1728, https://doi.org/10.1021/acschembio.6b00001.

80 M.D. Shortridge, M.J. Walker, T. Pavelitz, Y. Chen, W. Yang, G. Varani, A macrocyclic peptide ligand binds the oncogenic microRNA-21 precursor and suppresses Dicer processing, ACS Chem Biol 12 (2017) 1611–1620, https://doi.org/10.1021/acschembio.7b00180.

81 G. Michlewski, J.F. Cáceres, Post-transcriptional control of miRNA biogenesis, RNA 25 (2019) 1–16, https://doi.org/10.1261/rna.068692.118.

82 L. Wang, R.G. Rowe, A. Jaimes, C. Yu, Y. Nam, D.S. Pearson, et al., Small-molecule inhibitors disrupt let-7 oligouridylation and release the selective blockade of let-7 processing by LIN28, Cell Rep 23 (2018) 3091–3101, https://doi.org/10.1016/j.celrep.2018.04.116.

83 S. Zhu, N.R. Choudhury, S. Rooney, N.T. Pham, J. Koszela, D. Kelly, et al., RNA pull-down confocal nanoscanning (RP-CONA) detects quercetin as pri-miR-7/HuR interaction inhibitor that decreases α-synuclein levels, Nucleic Acids Res 49 (2021) 6456–6473, https://doi.org/10.1093/nar/gkab484.

84 M.A. Gelman, S. Richter, H. Cao, N. Umezawa, S.H. Gellman, T.M. Rana, Selective binding of TAR RNA by a Tat-derived β-peptide, Org Lett 5 (2003) 3563–3565, https://doi.org/10.1021/ol034977v.

85 A.C.-L. Lee, J.L. Harris, K.K. Khanna, J.-H. Hong, A comprehensive review on current advances in peptide drug development and design, Int J Mol Sci 20 (2019) 2383, https://doi.org/10.3390/ijms20102383.

86 H. Lu, Q. Zhou, J. He, Z. Jiang, C. Peng, R. Tong, J. Shi, Recent advances in the development of protein–protein interactions modulators: mechanisms and clinical trials, Signal Transduct Target Ther 5 (2020) 213, https://doi.org/10.1038/s41392-020-00315-3.

87 L. Mabonga, A.P. Kappo, Peptidomimetics: a synthetic tool for inhibiting protein–protein interactions in cancer, Int J Pept Res Ther 26 (2020) 225–241, https://doi.org/10.1007/s10989-019-09831-5.

88 J. Pai, S. Hyun, J.Y. Hyun, S.H. Park, W.J. Kim, S.H. Bae, et al., Screening of pre-miRNA-155 binding peptides for apoptosis inducing activity using peptide microarrays, J Am Chem Soc 138 (2016) 857–867, https://doi.org/10.1021/jacs.5b09216.

89 J.P. Diaz, R. Chirayil, S. Chirayil, M. Tom, K.J. Head, K.J. Luebke, Association of a peptoid ligand with the apical loop of pri-miR-21 inhibits cleavage by Drosha, RNA 20 (2014) 528–539, https://doi.org/10.1261/rna.042911.113.

90 H. Yan, M. Zhou, U. Bhattarai, Y. Song, M. Zheng, J. Cai, F.S. Liang, Cyclic peptidomimetics as inhibitor for miR-155 biogenesis, Mol Pharm 16 (2019) 914–920, https://doi.org/10.1021/acs.molpharmaceut.8b01247.

91 A.W. Robertson, J. Sandoval, O.G. Mohamed, Y. Zhuang, E.E. Gallagher, J. Schmidt, et al., Discovery of Surfactins as inhibitors of microRNA processing using Cat-ELCCA, ACS Med Chem Lett 12 (2021) 878–886, https://doi.org/10.1021/acsmedchemlett.1c00046.

92 D. Bose, S. Nahar, M.K. Rai, A. Ray, K. Chakraborty, S. Maiti, Selective inhibition of miR-21 by phage display screened peptide, Nucleic Acids Res 43 (2015) 4342–4352, https://doi.org/10.1093/nar/gkv185.

93 K. Sakamoto, K. Otake, T. Umemoto, Discovery of peptidic miR-21 processing inhibitor by mirror image phage display: a novel method to generate RNA binding D-peptides, Bioorg Med Chem Lett 27 (2017) 826–828, https://doi.org/10.1016/j.bmcl.2017.01.023.

94 C.B. Carlson, P.A. Beal, Solid-phase synthesis of acridine-based threading intercalator peptides, Bioorg Med Chem Lett 10 (2000) 1979–1982, https://doi.org/10.1016/S0960-894X(00)00388-7.

95 C.B. Carlson, M. Vuyisich, B.D. Gooch, P.A. Beal, Preferred RNA binding sites for a threading intercalator revealed by in vitro evolution, Chem Biol 10 (2003) 663–672, https://doi.org/10.1016/S1074-5521(03)00147-9.

96 B.D. Gooch, P.A. Beal, Recognition of duplex RNA by helix-threading peptides, J Am Chem Soc 126 (2004) 10603–10610, https://doi.org/10.1021/ja047818v.

97 M. Krishnamurthy, B.D. Gooch, P.A. Beal, RNA binding and thiolytic stability of a quinoline-containing helix-threading peptide, Org Biomol Chem 4 (2006) 639–645, https://doi.org/10.1039/B513591E.

98 M. Krishnamurthy, K. Simon, A.M. Orendt, P.A. Beal, Macrocyclic helix-threading peptides for targeting RNA, Angew Chem Int Ed Engl 46 (2007) 7044–7047, https://doi.org/10.1002/anie.200702247.

99 S. Chirayil, R. Chirayil, K.J. Luebke, Discovering ligands for a microRNA precursor with peptoid microarrays, Nucleic Acids Res 37 (2009) 5486–5497, https://doi.org/10.1093/nar/gkp549.

100 D.A. Lorenz, J.M. Song, A.L. Garner, High-throughput platform assay technology for the discovery of pre-microRNA-selective small molecule probes, Bioconjug Chem 26 (2015) 19–23, https://doi.org/10.1021/bc500544v.

101 N.J. Zondlo, Aromatic–proline interactions: electronically tunable CH/π interactions, Acc Chem Res 46 (2013) 1039–1049, https://doi.org/10.1021/ar300087y.

102 C. Hoehener, I. Hug, M. Nowacki, Dicer-like enzymes with sequence cleavage preferences, Cell 173 (2018) 234–247.e7, https://doi.org/10.1016/j.cell.2018.02.029.

103 G. Masliah, P. Barraud, F.H.T. Allain, RNA recognition by double-stranded RNA binding domains: a matter of shape and sequence, Cell Mol Life Sci 70 (2013) 1875–1895, https://doi.org/10.1007/s00018-012-1119-x.

104 D.A. Lorenz, T. Kaur, S.A. Kerk, E.E. Gallagher, J. Sandoval, A.L. Garner, Expansion of cat-ELCCA for the discovery of small molecule inhibitors of the Pre-let-7–Lin28 RNA–protein interaction, ACS Med Chem Lett 9 (2018) 517–521, https://doi.org/10.1021/acsmedchemlett.8b00126.

105 L.E. Buscaglia, Y. Li, Apoptosis and the target genes of microRNA-21, Chin J Cancer 30 (2011) 371–380, https://doi.org/10.5732/cjc.011.10132.

106 A. Esquela-Kerscher, F.J. Slack, Oncomirs — microRNAs with a role in cancer, Nat Rev Cancer 6 (2006) 259–269, https://doi.org/10.1038/nrc1840.

107 M. Yamamoto, E. Kondo, M. Takeuchi, A. Harashima, T. Otani, K. Tsuji-Takayama, et al., miR-155, a modulator of FOXO3a protein expression, is underexpressed and cannot be upregulated by stimulation of HOZOT, a line of multifunctional Treg, PLoS One 6 (2011) e16841.

108 V. D'Aloisio, P. Dognini, G.A. Hutcheon, C.R. Coxon, PepTherDia: database and structural composition analysis of approved peptide therapeutics and diagnostics, Drug Discov Today 26 (2021) 1409–1419, https://doi.org/10.1016/j.drudis.2021.02.019.