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Rational Design of Small Molecules Targeting Oncogenic Noncoding RNAs from Sequence

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Conspectus

The discovery of RNA catalysis in the 1980s and the dissemination of the human genome sequence at the start of this century inspired investigations of the regulatory roles of noncoding RNAs in biology. In fact, the Encyclopedia of DNA Elements (ENCODE) project has shown that only 1–2% of the human genome encodes protein, yet 75% is transcribed into RNA. Functional studies both preceding and following the ENCODE project have shown that these noncoding RNAs have important roles in regulating gene expression, developmental timing, and other critical functions. RNA's diverse roles are often a consequence of the various folds that it adopts. The single-stranded nature of the biopolymer enables it to adopt intramolecular folds with noncanonical pairings to lower its free energy. These folds can be scaffolds to bind proteins or to form frameworks to interact with other RNAs. Not surprisingly, dysregulation of certain noncoding RNAs has been shown to be causative of disease.

Given this as the background, it is easy to see why it would be useful to develop methods that target RNA and manipulate its biology in rational and predictable ways. The antisense approach has afforded strategies to target RNAs via Watson–Crick base pairing and has typically focused on targeting partially unstructured regions of RNA. Small molecule strategies to target RNA would be desirable not only because compounds could be lead optimized via medicinal chemistry but also because structured regions within an RNA of interest could be targeted to directly interfere with RNA folds that contribute to disease. Additionally, small molecules have historically been the most successful drug candidates. Until recently, the ability to design small molecules that target non-ribosomal RNAs has been elusive, creating the perception that they are "undruggable".

In this Account, approaches to demystify targeting RNA with small molecules are described. Rather than bulk screening for compounds that bind to singular targets, which is the purview of the pharmaceutical industry and academic institutions with high throughput screening facilities, we focus on methods that allow for the rational design of small molecules toward biological RNAs. One enabling and foundational technology that has been developed is two-dimensional combinatorial screening (2DCS), a library-versus-library selection approach that allows the identification of the RNA motif binding preferences of small molecules from millions of combinations. A landscape map of the 2DCS-defined and annotated RNA motif–small molecule interactions is then placed into Inforna, a computational tool that allows one to mine these

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interactions against an RNA of interest or an entire transcriptome. Indeed, this approach has been enabled by tools to annotate RNA structure from sequence, an invaluable asset to the RNA community and this work, and has allowed for the rational identification of “druggable” RNAs in a target agnostic fashion.

Introduction

In the field of small molecule probes of function or lead therapeutics, we currently live in a protein-centric world;(1) however, many believe that life on earth is a direct descendent of an RNA world. Large-scale functional genomics studies as well as the ENCODE project suggest that much more of the human genome is transcribed than is translated.(2) Thus, RNA plays essential and critical roles in cellular biology beyond translation of a disease-causing protein. An analysis of the protein targets that are “druggable” with small molecules suggested that 15% of all proteins are in “druggable” families, whereas 85% are not.(3) This analysis greatly underestimates the number of “undruggable” biological targets because RNA has not been considered, as most of the genome (~75%) is transcribed into RNA whereas only a small portion (1–2%) encodes protein.(4) Although some of these mRNAs may encode a “druggable” protein target, many encode for proteins that are not in “druggable” families. Combined with the large number of noncoding RNAs, the number of biologically important targets that have yet to be targeted with a small molecule in a cell greatly increases.

Although the antisense approach is a rules-based approach to design compounds that target unstructured regions,(5–7) RNA is a well-known target of chemical probes of function or lead therapeutics. For example, antibacterial agents targeting the bacterial ribosome are tried and true RNA-targeting small molecules.(8–10) Likewise, bacterial riboswitches, which change their shapes by binding small molecule metabolites,(11, 12) are also targets of small molecules.(13) Mimicking the structure of the endogenous ligand to identify higher affinity binders is typically used to target riboswitches, although other target sites have been recently disseminated.(13) Both the bacterial ribosome and riboswitches are generally outliers compared to RNA targets in the human transcriptome. The ribosome comprises 80% of the total RNA in a cell(14) and plays the critical function of translating cellular protein, likely contributing to its status as a good target and why many ribosome-targeting drugs lie outside of chemical compositions that would be viewed as “drug-like.” These drugs can have higher molecular weights and larger charges than what would typically be considered “drug-like”, and because of the large abundance of ribosomes relative to other off targets, ribosomes are the more likely occupied cellular target of these compounds. A typical mRNA, however, accounts for ≪1% of total cellular RNA, and thus, more precise recognition is required for one of these targets to be occupied by a small molecule in a cell. Because most RNAs do not have an endogenous small molecule ligand, structural mimicry approaches are not a general, viable design strategy. Lead compounds have been identified by high throughput screening (HTS), but this screening method is expensive, labor intensive, and often results in nonselective compounds, some of which have unclear targets that could be either protein or RNA. In cases such as the efforts of PTC Therapeutics, Roche, and Novartis to identify compounds that modulate RNAs that are causative of muscular dystrophy and spinal

muscular atrophy, however, lead optimization after screening has afforded molecules that have been or are in the clinic.(15–17) There have been other RNAs that are the targets of small molecules, including quadruplexes,(18–20) but rational approaches, as discussed in this Account, have been sparsely reported.

Historical Approaches in Nucleic Acids as Inspiration and Indispensable Tools

Given the goal of developing rational and predictable methods to target RNA, ideally from sequence, we were inspired by work in several areas. One of the major advantages of studying RNA is that its structure can be annotated and predicted from sequence.(21–23) The nexus of this work was the study of the thermodynamic stability of RNA base pairs and of noncanonically paired regions studied by Doty, Tinoco, Turner, and others.(24–27) This data set of free energy increments of RNA pairs was used by Zuker to construct algorithms to predict the structure of RNA from sequence.(28) Subsequent work by Turner and Zuker improved the parameters for RNA secondary structure stability.(29, 30) Additionally, it became clear that prediction alone would not be sufficient to deduce the correct structure for larger RNAs and some shorter ones. Thus, free energy minimization and alignment protocols(31) were developed as well as the ability to incorporate chemical modification and other experimental parameters to restrain folding.(22) The latter modification restraints can be obtained in cells to define regions of an RNA that are not structured by using dimethyl sulfate (DMS) and other reagents originally used for Gilbert–Maxim sequencing(22) or selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE).(32) RNA folding in vivo is often not at thermodynamic equilibrium, and the use of other mapping and prediction methods can help to decipher folding landscapes in cells and living organisms.(33–35) Each of these methods go hand-in-hand with RNA secondary structure prediction by phylogenic comparison, which has been invaluable to not only define RNA structures but also to reveal new kingdoms of life by Woese.(36, 37)

Another foundational approach that inspired the field of RNA small molecule targeting is Dervan's polyamides that target DNA.(38, 39) Initially inspired by the molecular recognition of the DNA minor groove by natural products such as distamycin, Dervan and co-workers defined building blocks in small molecules that sequence-specifically target a DNA duplex. Thus, by custom assembling these compounds together, small molecules can "read out" DNA sequences. Unfortunately, there is no distamycin equivalent for RNA that can be used to decipher broad rules for recognition of RNA base pairs. Recent studies, however, have identified small molecules that target RNA AU base pairs specifically. These compounds have been used to design a modulator of cellular toxicity in spinocerebellar ataxia type 10.(40)

Given the ability to obtain structural information about an RNA from sequence, the question is how to leverage these data to enable the design of small molecules targeting RNA in a comprehensive and general way. Key strategies include (i) the development of a set of rules or at least the identification of the types of ligands that can bind to RNA motifs with high affinity and selectivity and (ii) the development of a high throughput and facile method to

define privileged RNA motifs that bind small molecules and privileged small molecules that bind RNA motifs. These types of data could be merged with RNA secondary structure prediction and annotation from sequence to identify preferred small molecule target sites and the RNAs that contain them. If one could target these RNAs in cells, functional consequences and target selectivity could be probed, the latter of which would be particularly important due to the polyanionic nature of the biopolymer and the often incorrect perception that RNA motifs are not unique to a given RNA.(41)

Development of Two-Dimensional Combinatorial Screening to Identify RNA Motif–Small Molecule Binding Partners

Given the polyanionic nature of RNA and the observation that small molecules targeting the ribosome do not display exquisite selectivities that are typically observed with protein-targeting ligands, a challenge of high importance in this area is to develop a framework in which designer chemical probes targeting RNA could be informed from a knowledge base of highly selective interactions. Systematic evolution of ligands by exponential enrichment (SELEX) and directed evolution experiments have shown that RNAs can be evolved from large pools to bind small molecule ligands with high affinity and selectivity.(42–44) Because of the complexity of the starting library, which could theoretically have trillions of members, multiple rounds of selection are completed to identify a population of binders that recognize a target. These selected RNAs, although they bind ligands with high affinity and selectivity, are quite large and would be difficult to identify within the human transcriptome.

We sought to develop a framework in which small RNA motifs that are present in the transcriptome, such as bulges, hairpins, and internal loops, could be rapidly screened for binding to small molecules with high affinity and selectivity (Figure 1). If such an approach were possible, it would define privileged RNA space that binds small molecules and chemotypes that engender affinity and selectivity for RNA. In an initial test of this method, the aminoglycosides neamine and kanamycin A were acylated at their 6′ positions with 5′-hexynoic acid.(45) These two compounds were selected as they are derivatives of known compounds that bind RNA, but acylation of the 6′ amine, a main mode of aminoglycoside resistance,(46) ablates binding to mimics of the bacterial A-site. Thus, although built on an RNA-binding scaffold, the ability of these compounds to bind RNA motifs was unknown. Indeed, these studies identified RNAs that bind the neamine and kanamycin A derivatives with nanomolar affinity and high selectivity compared to those of other RNAs in the library that were not selected for binding to the small molecules.(45)

Given the myriad of RNA structural motifs that are present in the transcriptome, we sought to develop an approach that allowed us to screen small molecule libraries for binding to RNA motifs in a single library-versus-library experiment (Figure 2). When used with nucleic acids, such an approach allows for a straightforward read-out of the binding RNAs by using standard sequencing approaches. To develop this method, we required a platform that would allow us to screen small molecules quickly for binding to targets using minimal amounts of material. Small molecule microarrays,(47) in which compounds are spatially arrayed onto a surface, provided such a platform.

The advantage of small molecule microarrays is that they allow for screening of thousands, if not millions, of interactions in a single experiment. Thus, RNA binding to small molecule microarrays was straightforward, but the following step of identifying the bound RNAs was not. To address this problem, we utilized agarose-coated microarrays, which allowed bound RNAs (derived from an RNA library) to be harvested by simple gel excision.(48, 49) Subsequent RT-PCR amplification of the harvested RNA allowed for easy identification by using standard sequencing approaches. This library-vs-library screening method was dubbed 2-dimensional combinatorial screening (2DCS). 2DCS has been used to mine the binding capacity of various small molecules to RNAs including *bis*-benzimidazoles,(50) amino-benzimidazoles,(51) aminoglycosides,(49) and others.(52) These studies identified lead compounds that bound RNA with high affinities and selectivities (over other RNAs and DNA), or a set of privileged RNA motif–small molecule partners.

Structure–Activity Relationships Through Sequencing (StARTS)

Sequencing reactions to deconvolute selections such as 2DCS and SELEX are used to identify lead binding compounds, but not to score the relative affinities of the population of binders. Thus, we sought to develop theoretical frameworks to allow for a 2DCS experiment to read out binders and to score binding interactions, thus defining the affinity and selectivity of all members of the RNA library. Our statistical method was dubbed Structure-Activity Relationships Through Sequencing, or StARTS.(51, 53)

In StARTS, the RNA motifs that are selected to bind a small molecule are identified, and the substructures within the selected motifs are analyzed by using the statistical score Z_{obs}. The premise behind StARTS is that, for a given selected RNA motif, there are multiple submotifs that can interact with the small molecule and drive apparent affinity; the greater the occurrence of a submotif, the larger the contribution to apparent affinity and the higher the value of Z_{obs}. For each RNA motif, a sum of all Z_{obs} features (ΣZ_{obs}) is computed. A plot of ΣZ_{obs} as a function of apparent affinity can be fit to define a scoring function for the entire RNA library as the apparent affinity of the complex scales with the sum of Z_{obs}. This approach can also allow one to determine the selectivity of small molecules between different RNA motifs.(51) Normalization of ΣZ_{obs} values from a given selection affords a fitness score for every RNA in the library.

Inforna: Mining for Druggable RNA Targets from Sequence: A Target Agnostic Approach

Each of the above studies was completed to define the ligandability of RNA motifs for small molecules. These data were incorporated into a lead identification strategy to identify druggable RNAs that we named Inforna.(54) Inforna compares the secondary elements (motifs) within a target RNA to motifs in our database of RNA motif–small molecule interactions, affording lead compounds (Figure 3). By computing the potential ligand binding, identified by 2DCS and annotated by StARTS, the approach essentially uses the output of a selection for a small molecule to identify its preferred RNA target in a cell. Thus, the approach is not focused on a specific target but rather is “target agnostic”.

We applied this data set to design chemical probes of RNA function in a target agnostic fashion for human microRNA (miRNA) precursors. Precursor miRNAs are perhaps an ideal class for a target agnostic approach for drug discovery.(55) These RNAs play diverse roles in biology, and their dysregulation causes cancer, immune diseases, and a variety of other maladies. The secondary structures of miRNA hairpin precursors are readily predictable from sequence. Further, Dicer and Drosha cleavage sites within hairpin precursors can be used to restrain secondary structure prediction;(56) Dicer and Drosha cleavage sites can be easily discerned from RNA-seq reads of miRNAs, which provide rich data on the sequences of mature miRNAs. Binding of a small molecule to the Dicer or Drosha nuclease processing sites could affect the biogenesis of the miRNA.

Prior to these studies, there were no known selective small molecule modulators of miRNA function. Given the ability to predict their secondary structures accurately, which miRNA precursors were targetable with the small molecules in our Inforna database could be easily computationally queried. Further, the output from Inforna could also be used to predict other miRNA precursors with the same targetable motif and how selectively a compound can modulate a specific miRNA. That is, it is important to know how many of these targetable RNA motifs are present in miRNA precursors and the small molecule's preference for each site, which is predicted by their fitness scores.

Inforna identified over two-dozen human miRNA precursors that are disease-associated and could be targeted with a small molecule.(54) The most avid interaction was between a benzimidazole (**1**) and the Drosha site in the microRNA(miR)-96 hairpin precursor. The benzimidazole has favorable properties to accommodate binding to RNA, and the bulky *t*-butyl groups located at the benzene ring ablate any potential binding to DNA. MiR-96 is upregulated in triple negative breast cancer and silences FOXO1 transcription factor.(57) The Drosha target site for **1** was predicted via StARTS and confirmed via experiments to be the highest affinity RNA motif that binds to the ligand from the entire 4,096-member RNA motif library.(54) Thus, this RNA motif–small molecule partner is a highly selective interaction that, in theory, could provide selective inhibition of miR-96 biogenesis in breast cancer cells.

Compound **1** was validated for binding to the Drosha site in miR-96 hairpin precursor in vitro by using a nuclease protection assay. These studies confirmed that the compound indeed recognized the predicted site.(54) Interestingly, at 10 μM, **1** inhibits the production of mature miR-96 but does not affect miR-182 and miR-183, which are produced as a single transcript (pri-miRNA) with miR-96.(57) Thus, in this particular case, a small molecule can precisely inhibit biogenesis of one mature miRNA without affecting the others in the transcript, showing a lack of cooperativity in the processing of the cluster. The compound was also found to boost the production of pri-miR-96 and inhibit production of pre-miR-96, which is consistent with the compound targeting the Drosha site.(54) Additionally, **1** de-repressed expression of FOXO1 (regulated by miR-96) but not control proteins.(54)

In breast cancer cells, **1** triggered apoptosis. To probe if the compound triggered apoptosis via the FOXO1-miR-96 circuit, siRNA was used to ablate *FOXO1* mRNA. When *FOXO1* was ablated, the effect of the compound for triggering apoptosis was reduced by ~75%.(54)

Notably, siRNA ablation of a control RNA did not affect compound potency. There are several implications of this study. First, **1** is a pathway-selective inducer of apoptosis. Second, the interaction between miR-96 and *FOXO1* plays a highly significant role in silencing apoptosis in breast cancer cells. Pleotropic effects are a hallmark of miRNA activity as multiple miRNAs can affect a single mRNA and a single miRNA can affect multiple mRNAs. In this case, however, it appears that a major driver of phenotype is the activity of a single miRNA (miR-96) against a single mRNA (*FOXO1*).

These functional studies suggest that **1** is selective, as predicted by 2DCS and StARTS. To gain further insight into the miRNA selectivity of the compound, qRT-PCR studies were completed on all miRNAs that had sufficient expression in MCF-7 breast cancer cells, nearly 200 different miRNAs. No other miRNA's expression was significantly affected at a dose of **1** that inhibited 90% of the biogenesis of miR-96.(54) The small molecule's ability to inhibit biogenesis of miR-96 was compared to antisense oligonucleotides, both full antagomirs and seed-targeting locked nucleic acids (LNAs). In both cases, the small molecule had selectivity that rivaled or exceeded that observed with these oligonucleotides.(54) Collectively, these studies have shown that small molecules can precisely target miRNA precursors and display selectivities that rival modalities that target miRNAs based on Watson–Crick base pairing.

In Vivo Activity of Designer Compounds Targeting Oncogenic Noncoding RNAs

One challenge with drugging RNA is pushing compounds beyond cellular models and into disease-relevant in vivo models. For the case of the miR-96 targeting benzimidazole, the low micromolar activity of the compound was not sufficiently potent to translate into in vivo models. Thus, we sought methods to quickly lead optimize **1** for both potency and selectivity of binding. Ideally, the binding constants and concentrations of compound that are required to affect biology should be in the nanomolar regime such that an in vivo delivered dose of the compound saturates affected tissues.

Thus, Inforna was used to lead optimize **1** by mining the miR-96 hairpin precursor for compounds that bind motifs adjacent to the Drosha site. A lead compound that binds the adjacent site could then be linked to **1** to afford a dimeric small molecule (Figure 4). Indeed, a *bis*-benzimidazole was identified that bound to a 1 × 1 nucleotide GG internal loop that is adjacent to the Drosha site in the miR-96 hairpin precursor. By using rules for spanning the distance of two base pairs,(58) the compound Targaprimir-96 was developed (Figure 4).(59) This compound bound with a low nanomolar dissociation constant to the target miRNA hairpin precursor. Mutational analysis in which either the 1 × 1 GG internal loop was mutated to a GC base pair or in which the 1 × 1 UU internal loop in the Drosha site was mutated to the AU base pair showed that the binding of Targaprimir-96 was ablated.(59) Additionally, the compound is RNA-selective as there is no saturable binding of the compound to AT-rich DNA despite the fact that the *bis*-benzimidazole that targets the 1 × 1 GG internal loop is a known DNA binder.

Cell culture studies showed that Targaprimir-96 decreased mature and pre-miR-96 levels and boosted pri-miR-96 levels at a concentration as low as 30 nM with an IC_{50} of ~50 nM.

Targaprimir-96 also inhibited Drosha processing of pri-miR-96 at low nanomolar concentrations in vitro, and the binding site was further confirmed by protection from cleavage by S1 nuclease in vitro. The compound also derepressed the effect of miR-96 on FOXO1 expression, enhancing protein amounts by 2-fold when 50 nM of Targaprimir-96 was used.(59) These studies were completed in both MDA-MB-231 (a cellular model of triple negative breast cancer) and 4175 (a breast cancer cell line that metastasizes to lung).(60) Additionally, the compound triggers apoptosis at 50 nM. This effect is ablated upon exogenous overexpression of miR-96.(59)

For the compound's properties to be studied in vivo, drug metabolism and pharmacokinetics studies were completed. These studies showed that Targaprimir-96 has a favorable pharmacokinetic and metabolism profile as the concentration in serum is greater than 1 μM at 48 h post-IP injection of either 2 or 7 mg/kg, 20-fold higher than the amount of compound needed to trigger apoptosis in cell culture.(59) Thus, the effect of Targaprimir-96 on tumor growth in vivo was assessed. IP injection of 10 mg/kg compound was well tolerated in animals and ablated tumor growth over the course of 21 days. Resected tumors were analyzed for protein and miRNA content and showed that Targaprimir-96 boosted FOXO1 expression and silenced miR-96 in vivo (Figure 4).(59)

Conclusions and Future Perspectives

As we have described in this Account, it is possible to design small molecules targeting RNA from sequence by using entire segments of the transcriptome. Although the focus of this Account was on the application of technologies to target miR-96, this approach can also provide small molecules that affect other cancer-associated miRNAs.(61, 62) As more is learned about noncoding RNAs and their roles in disease biology, it is provocative to think that Inforna could quickly deliver lead therapeutics and chemical probes of function. Although the foundation for this idea has been set, there will be a need for further advancement of the database of RNA motif–small molecule binding partners. Additional strategies to modulate the function of RNA, including small molecule cleavage and reaction, would also be particularly useful.(63, 64) Both of these approaches importantly can be leveraged to answer fundamental questions about the RNA targets of small molecules in cells and to identify on- and off-targets. Such studies may help fulfill the promise of using the vast data from genome sequencing to directly inform the design of small molecule lead therapeutics, advancing a disease-to-gene-to-drug paradigm.

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References

1. Arrowsmith CH, Audia JE, Austin C, Baell J, Bennett J, Blagg J, Bountra C, Brennan PE, Brown PJ, Bunnage ME, Buser-Doepner C, Campbell RM, Carter AJ, Cohen P, Copeland RA, Cravatt B, Dahlin JL, Dhanak D, Edwards AM, Frederiksen M, Frye SV, Gray N, Grimshaw CE, Hepworth D, Howe T, Huber KV, Jin J, Knapp S, Kotz JD, Kruger RG, Lowe D, Mader MM, Marsden B,

Mueller-Fahrnow A, Muller S, O'Hagan RC, Overington JP, Owen DR, Rosenberg SH, Roth B, Ross R, Schapira M, Schreiber SL, Shoichet B, Sundstrom M, Superti-Furga G, Taunton J, Toledo-Sherman L, Walpole C, Walters MA, Willson TM, Workman P, Young RN, Zuercher WJ. The promise and peril of chemical probes. Nat Chem Biol. 2015; 11:536–541. DOI: 10.1038/nchembio.1867 [PubMed: 26196764]

2. The ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012; 489:54–74.
3. Hopkins AL, Groom CR. The druggable genome. Nat Rev Drug Discov. 2002; 1:727–730. DOI: 10.1038/nrd892 [PubMed: 12209152]
4. Clamp M, Fry B, Kamal M, Xie X, Cuff J, Lin MF, Kellis M, Lindblad-Toh K, Lander ES. Distinguishing protein-coding and noncoding genes in the human genome. Proc Natl Acad Sci U S A. 2007; 104:19428–19433. DOI: 10.1073/pnas.0709013104 [PubMed: 18040051]
5. Stephenson ML, Zamecnik PC. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. Proc Natl Acad Sci U S A. 1978; 75:285–288. DOI: 10.1073/pnas.75.1.285 [PubMed: 75546]
6. Zamecnik PC, Stephenson ML. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. Proc Natl Acad Sci U S A. 1978; 75:280–284. DOI: 10.1073/pnas.75.1.280 [PubMed: 75545]
7. Gude L, Berkovitch SS, Santos WL, Kutchukian PS, Pawloski AR, Kuimelis R, McGall G, Verdine GL. Mapping targetable sites on human telomerase RNA pseudoknot/template domain using 2′-OMe RNA-interacting polynucleotide (RIPtide) microarrays. J Biol Chem. 2012; 287:18843–18853. DOI: 10.1074/jbc.M111.316596 [PubMed: 22451672]
8. Poehlsgaard J, Douthwaite S. The bacterial ribosome as a target for antibiotics. Nat Rev Microbiol. 2005; 3:870–881. DOI: 10.1038/nrmicro1265 [PubMed: 16261170]
9. Schlunzen F, Zarivach R, Harms J, Bashan A, Tocilj A, Albrecht R, Yonath A, Franceschi F. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature. 2001; 413:814–821. DOI: 10.1038/35101544 [PubMed: 11677599]
10. Borovinskaya MA, Pai RD, Zhang W, Schuwirth BS, Holton JM, Hirokawa G, Kaji H, Kaji A, Cate JH. Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. Nat Struct Mol Biol. 2007; 14:727–732. DOI: 10.1038/nsmb1271 [PubMed: 17660832]
11. Nahvi A, Sudarsan N, Ebert MS, Zou X, Brown KL, Breaker RR. Genetic control by a metabolite binding mRNA Chem. Biol. 2002; 9:1043–1049. DOI: 10.1016/S1074-5521(02)00224-7
12. Mandal M, Breaker RR. Gene regulation by riboswitches. Nat Rev Mol Cell Biol. 2004; 5:451–463. DOI: 10.1038/nrm1403 [PubMed: 15173824]
13. Blount KF, Breaker RR. Riboswitches as antibacterial drug targets Nat. Biotechnol. 2006; 24:1558–1564. DOI: 10.1038/nbt1268
14. Johnson LF, Abelson HT, Penman S, Green H. The relative amounts of the cytoplasmic RNA species in normal, transformed and senescent cultured cell lines. J Cell Physiol. 1977; 90:465–470. DOI: 10.1002/jcp.1040900310 [PubMed: 192738]
15. Naryshkin NA, Weetall M, Dakka A, Narasimhan J, Zhao X, Feng Z, Ling KK, Karp GM, Qi H, Woll MG, Chen G, Zhang N, Gabbeta V, Vazirani P, Bhattacharyya A, Furia B, Risher N, Sheedy J, Kong R, Ma J, Turpoff A, Lee CS, Zhang X, Moon YC, Trifillis P, Welch EM, Colacino JM, Babiak J, Almstead NG, Peltz SW, Eng LA, Chen KS, Mull JL, Lynes MS, Rubin LL, Fontoura P, Santarelli L, Haehnke D, McCarthy KD, Schmucki R, Ebeling M, Sivaramakrishnan M, Ko CP, Paushkin SV, Ratni H, Gerlach I, Ghosh A, Metzger F. Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. Science. 2014; 345:688–693. DOI: 10.1126/science.1250127 [PubMed: 25104390]
16. Palacino J, Swalley SE, Song C, Cheung AK, Shu L, Zhang X, Van Hoosear M, Shin Y, Chin DN, Keller CG, Beibel M, Renaud NA, Smith TM, Salcius M, Shi X, Hild M, Servais R, Jain M, Deng L, Bullock C, McLellan M, Schuierer S, Murphy L, Blommers MJ, Blaustein C, Berenshteyn F, Lacoste A, Thomas JR, Roma G, Michaud GA, Tseng BS, Porter JA, Myer VE, Tallarico JA, Hamann LG, Curtis D, Fishman MC, Dietrich WF, Dales NA, Sivasankaran R. SMN2 splice modulators enhance U1-pre-mRNA association and rescue SMA mice. Nat Chem Biol. 2015; 11:511–517. DOI: 10.1038/nchembio.1837 [PubMed: 26030728]

17. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, Paushkin S, Patel M, Trotta CR, Hwang S, Wilde RG, Karp G, Takasugi J, Chen G, Jones S, Ren H, Moon YC, Corson D, Turpoff AA, Campbell JA, Conn MM, Khan A, Almstead NG, Hedrick J, Mollin A, Risher N, Weetall M, Yeh S, Branstrom AA, Colacino JM, Babiak J, Ju WD, Hirawat S, Northcutt VJ, Miller LL, Spatrick P, He F, Kawana M, Feng H, Jacobson A, Peltz SW, Sweeney HL. PTC124 targets genetic disorders caused by nonsense mutations. Nature.
18. Bugaut A, Balasubramanian S. 5′-UTR RNA G-quadruplexes: translation regulation and targeting. Nucleic Acids Res. 2012; 40:4727–4741. DOI: 10.1093/nar/gks068 [PubMed: 22351747]
19. Ecker DJ, Griffey RH. RNA as a small-molecule drug target: doubling the value of genomics Drug Discov. Today. 1999; 4:420–429. DOI: 10.1016/S1359-6446(99)01389-6
20. Tor Y. Targeting RNA with small molecules. ChemBioChem. 2003; 4:998–1007. DOI: 10.1002/cbic.200300680 [PubMed: 14523917]
21. Zuker M. M fold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 2003; 31:3406–3415. DOI: 10.1093/nar/gkg595 [PubMed: 12824337]
22. Mathews DH, Disney MD, Childs JL, Schroeder SJ, Zuker M, Turner DH. Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. Proc Natl Acad Sci U S A. 2004; 101:7287–7292. DOI: 10.1073/pnas.0401799101 [PubMed: 15123812]
23. Tinoco I Jr, Borer PN, Dengler B, Levine MD, Uhlenbeck OC, Crothers DM, Gralla J. Improved estimation of secondary structure in ribonucleic acids. Nat New Biol. 1973; 246:40–41. DOI: 10.1038/newbio246040a0 [PubMed: 4519026]
24. Tinoco I Jr, Uhlenbeck OC, Levine MD. Estimation of secondary structure in ribonucleic acids. Nature. 1971; 230:362–367. DOI: 10.1038/230362a0 [PubMed: 4927725]
25. Uhlenbeck OC, Baller J, Doty P. Complementary oligonucleotide binding to the anticodon loop of fMet-transfer RNA. Nature. 1970; 225:508–510. DOI: 10.1038/225508a0 [PubMed: 5411856]
26. Freier SM, Kierzek R, Jaeger JA, Sugimoto N, Caruthers MH, Neilson T, Turner DH. Improved free-energy parameters for predictions of RNA duplex stability. Proc Natl Acad Sci U S A. 1986; 83:9373–9377. DOI: 10.1073/pnas.83.24.9373 [PubMed: 2432595]
27. Xia T, SantaLucia J Jr, Burkard ME, Kierzek R, Schroeder SJ, Jiao X, Cox C, Turner DH. Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick base pairs. Biochemistry. 1998; 37:14719–14735. DOI: 10.1021/bi9809425 [PubMed: 9778347]
28. Zuker M. On finding all suboptimal foldings of an RNA molecule. Science. 1989; 244:48–52. DOI: 10.1126/science.2468181 [PubMed: 2468181]
29. Jaeger JA, Turner DH, Zuker M. Predicting optimal and suboptimal secondary structure for RNA. Methods Enzymol. 1990; 183:281–306. DOI: 10.1016/0076-6879(90)83019-6 [PubMed: 1690335]
30. Jaeger JA, Turner DH, Zuker M. Improved predictions of secondary structures for RNA. Proc Natl Acad Sci U S A. 1989; 86:7706–7710. DOI: 10.1073/pnas.86.20.7706 [PubMed: 2479010]
31. Mathews DH. Using the RNAstructure software package to predict conserved RNA structures. Curr Protoc Bioinformatics. 2014; 46:12.4.1–12.4.22. DOI: 10.1002/0471250953.bi1204s46
32. Deigan KE, Li TW, Mathews DH, Weeks KM. Accurate SHAPE-directed RNA structure determination. Proc Natl Acad Sci U S A. 2009; 106:97–102. DOI: 10.1073/pnas.0806929106 [PubMed: 19109441]
33. Ding Y, Tang Y, Kwok CK, Zhang Y, Bevilacqua PC, Assmann SM. In vivo genome-wide profiling of RNA secondary structure reveals novel regulatory features. Nature. 2014; 505:696–700. DOI: 10.1038/nature12756 [PubMed: 24270811]
34. Rouskin S, Zubradt M, Washietl S, Kellis M, Weissman JS. Genome-wide probing of RNA structure reveals active unfolding of mRNA structures in vivo. Nature. 2014; 505:701–705. DOI: 10.1038/nature12894 [PubMed: 24336214]
35. Wan Y, Qu K, Zhang QC, Flynn RA, Manor O, Ouyang Z, Zhang J, Spitale RC, Snyder MP, Segal E, Chang HY. Landscape and variation of RNA secondary structure across the human transcriptome. Nature. 2014; 505:706–709. DOI: 10.1038/nature12946 [PubMed: 24476892]

36. Woese, C., Pace, N. Probing RNA structure, function, and history by comparative analysis. In: Gesteland, RF.Cech, TR., Atkins, JF., editors. The RNA World. 2. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 1993. p. 91-117.
37. Woese CR, Magrum LJ, Gupta R, Siegel RB, Stahl DA, Kop J, Crawford N, Brosius J, Gutell R, Hogan JJ, Noller HF. Secondary structure model for bacterial 16S ribosomal RNA: phylogenetic, enzymatic and chemical evidence. Nucleic Acids Res. 1980; 8:2275–2293. DOI: 10.1093/nar/8.10.2275 [PubMed: 6159576]
38. Dervan PB, Burli RW. Sequence-specific DNA recognition by polyamides. Curr Opin Chem Biol. 1999; 3:688–693. DOI: 10.1016/S1367-5931(99)00027-7 [PubMed: 10600731]
39. Dervan PB, Edelson BS. Recognition of the DNA minor groove by pyrrole-imidazole polyamides. Curr Opin Struct Biol. 2003; 13:284–299. DOI: 10.1016/S0959-440X(03)00081-2 [PubMed: 12831879]
40. Yang WY, Gao R, Southern M, Sarkar PS, Disney MD. Design of a bioactive small molecule that targets r(AUUCU) repeats in spinocerebellar ataxia 10 Nat. Commun. 2016; 7:11647.doi: 10.1038/ncomms11647
41. Liu B, Childs-Disney JL, Znosko BM, Wang D, Fallahi M, Gallo SM, Disney MD. Analysis of secondary structural elements in human microRNA hairpin precursors. BMC Bioinformatics. 2016; 17:1–11. DOI: 10.1186/s12859-016-0960-6 [PubMed: 26817711]
42. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990; 249:505–510. DOI: 10.1126/science.2200121 [PubMed: 2200121]
43. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990; 346:818–822. DOI: 10.1038/346818a0 [PubMed: 1697402]
44. Robertson DL, Joyce GF. Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. Nature. 1990; 344:467–468. DOI: 10.1038/344467a0 [PubMed: 1690861]
45. Disney MD, Childs-Disney JL. Using selection to identify and chemical microarray to study the RNA internal loops recognized by 6′-N-acylated kanamycin A. ChemBioChem. 2007; 8:649–656. DOI: 10.1002/cbic.200600569 [PubMed: 17394189]
46. Llano-Sotelo B, Azucena EF Jr, Kotra LP, Mobashery S, Chow CS. Aminoglycosides modified by resistance enzymes display diminished binding to the bacterial ribosomal aminoacyl-tRNA site. Chem Biol. 2002; 9:455–463. DOI: 10.1016/S1074-5521(02)00125-4 [PubMed: 11983334]
47. MacBeath G, Koehler AN, Schreiber SL. Printing small molecules as microarrays and detecting protein- ligand interactions en masse. J Am Chem Soc. 1999; 121:7967–7968. DOI: 10.1021/ja991083q
48. Childs-Disney JL, Wu M, Pushechnikov A, Aminova O, Disney MD. A small molecule microarray platform to select RNA internal loop-ligand interactions ACS Chem. Biol. 2007; 2:745–754. DOI: 10.1021/cb700174r
49. Disney MD, Labuda LP, Paul DJ, Poplawski SG, Pushechnikov A, Tran T, Velagapudi SP, Wu M, Childs-Disney JL. Two-dimensional combinatorial screening identifies specific aminoglycoside-RNA internal loop partners. J Am Chem Soc. 2008; 130:11185–11194. DOI: 10.1021/ja803234t [PubMed: 18652457]
50. Velagapudi SP, Seedhouse SJ, French J, Disney MD. Defining the RNA internal loops preferred by benzimidazole derivatives via 2D combinatorial screening and computational analysis. J Am Chem Soc. 2011; 133:10111–10118. DOI: 10.1021/ja200212b [PubMed: 21604752]
51. Velagapudi SP, Pushechnikov A, Labuda LP, French JM, Disney MD. Probing a 2-aminobenzimidazole library for binding to RNA internal loops via two-dimensional combinatorial screening ACS Chem. Biol. 2012; 7:1902–1909. DOI: 10.1021/cb300213g
52. Tran T, Disney MD. Identifying the preferred RNA motifs and chemotypes that interact by probing millions of combinations Nat. Commun. 2012; 3:1125.doi: 10.1038/ncomms2119
53. Velagapudi SP, Seedhouse SJ, Disney MD. Structure-activity relationships through sequencing (StARTS) defines optimal and suboptimal RNA motif targets for small molecules. Angew Chem, Int Ed. 2010; 49:3816–3818. DOI: 10.1002/anie.200907257

54. Velagapudi SP, Gallo SM, Disney MD. Sequence-based design of bioactive small molecules that target precursor microRNAs. Nat Chem Biol. 2014; 10:291–297. DOI: 10.1038/nchembio.1452 [PubMed: 24509821]

55. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009; 136:215–233. DOI: 10.1016/j.cell.2009.01.002 [PubMed: 19167326]

56. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright A. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 2006; 34:D140–144. DOI: 10.1093/nar/gkj112 [PubMed: 16381832]

57. Guttilla IK, White BA. Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. J Biol Chem. 2009; 284:23204–23216. DOI: 10.1074/jbc.M109.031427 [PubMed: 19574223]

58. Childs-Disney JL, Tsitovich PB, Disney MD. Using modularly assembled ligands to bind RNA internal loops separated by different distances. ChemBioChem. 2011; 12:2143–2146. DOI: 10.1002/cbic.201100298 [PubMed: 21830289]

59. Velagapudi SP, Cameron MD, Haga CL, Rosenberg LH, Lafitte M, Duckett DR, Phinney DG, Disney MD. Design of a small molecule against an oncogenic noncoding RNA. Proc Natl Acad Sci U S A. 2016; 113:5898.doi: 10.1073/pnas.1523975113 [PubMed: 27170187]

60. Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J. Genes that mediate breast cancer metastasis to lung. Nature. 2005; 436:518–524. DOI: 10.1038/nature03799 [PubMed: 16049480]

61. Velagapudi SP, Disney MD. Two-dimensional combinatorial screening enables the bottom-up design of a microRNA-10b inhibitor. Chem Commun (Cambridge, U K). 2014; 50:3027–3029. DOI: 10.1039/c3cc00173c

62. Haga CL, Velagapudi SP, Strivelli JR, Yang WY, Disney MD, Phinney DG. Small Molecule Inhibition of miR-544 Biogenesis Disrupts Adaptive Responses to Hypoxia by Modulating ATM-mTOR Signaling ACS Chem. Biol. 2015; 10:2267–2276. DOI: 10.1021/acschembio.5b00265

63. Guan L, Disney MD. Covalent small-molecule-RNA complex formation enables cellular profiling of small-molecule-RNA interactions. Angew Chem, Int Ed. 2013; 52:10010–10013. DOI: 10.1002/anie.201301639

64. Guan L, Disney MD. Small Molecule-Mediated Cleavage of RNA in Living Cells. Angew Chem, Int Ed. 2013; 52:1462–1465. DOI: 10.1002/anie.201206888

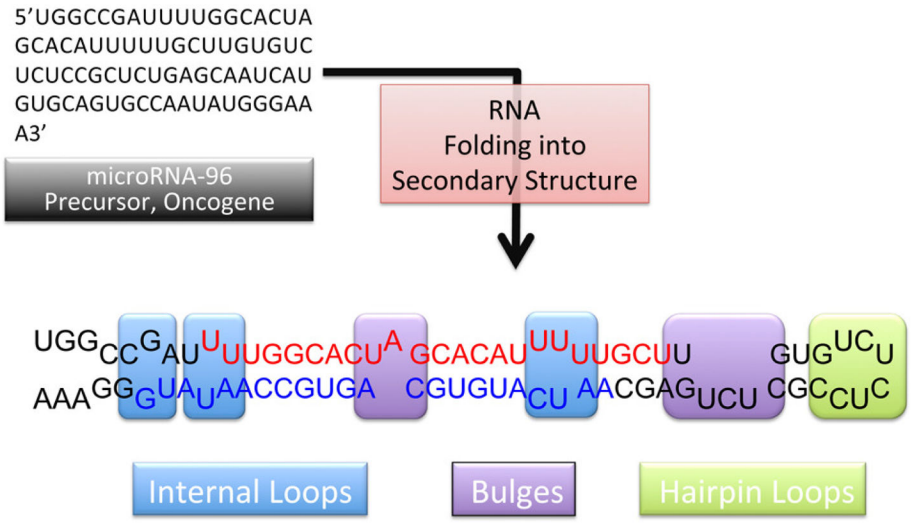

Figure 1.

RNA is a single-stranded biopolymer that adopts various conformations beyond those solely described by Watson–Crick base pairing. For example, RNA can form noncanonically paired internal loops, bulges, and hairpin loops. One major advantage that RNA has over other biopolymers is that information about its structure can be deduced from its sequence by using computational approaches with or without experimental constraints. Targeting these noncanonical structures can be accomplished with small molecules, which is the focus of this Account.

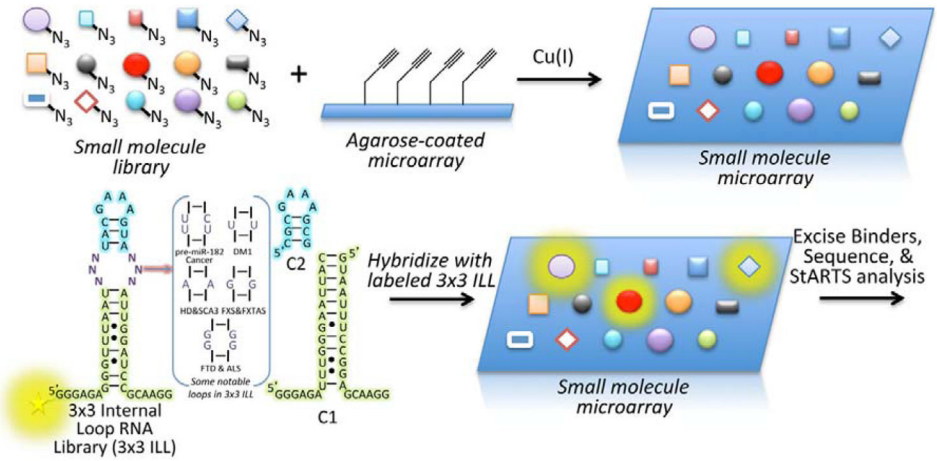

Figure 2.

Developing methods to identify the RNA motifs that bind small molecules avidly, namely 2-dimensional combinatorial screening (2DCS). 2DCS is a library-vs-library method that probes both small molecules and RNA motif space simultaneously, and the resulting data comprise a database of annotated RNA motif–small molecule binding partners. Briefly, a small molecule library is spatially arrayed onto microarray surfaces and probed for binding to a library of RNA motifs embedded in a unimolecular hairpin cassette. Small molecules on the array capture members of the RNA motif library that they bind. Binders are excised from the microarray surface and sequenced.

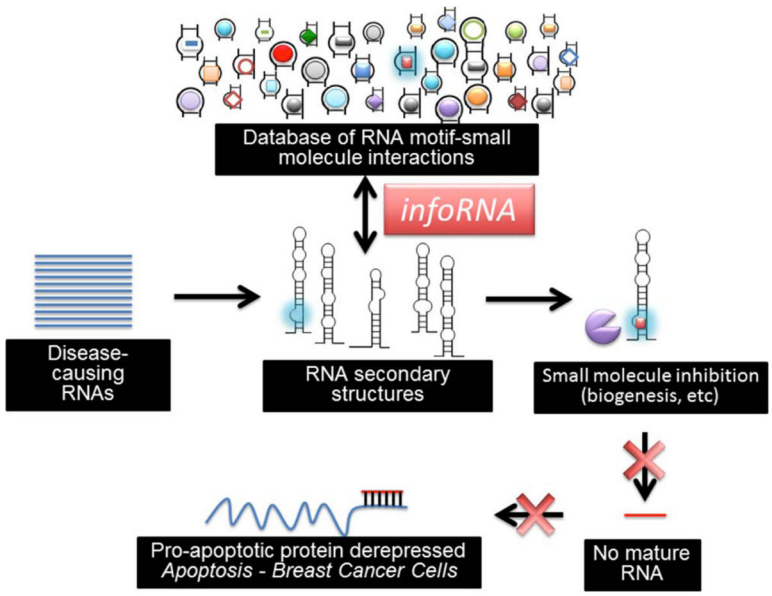

Figure 3.

Informa approach to design small molecules targeting RNA from sequence. This approach is target agnostic as it computes the most optimal binders to RNA noncanonical motifs from all human miRNA hairpin precursors. Lead compounds are then tested for bioactivity and for specific modulation of the biological functions of an RNA.

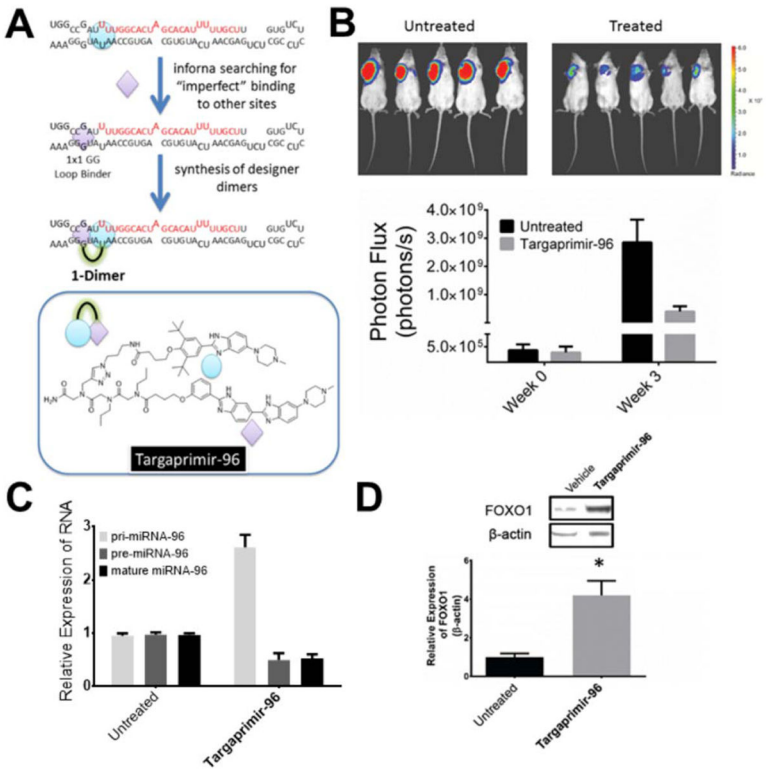

Figure 4.

Small molecules that target the miR-96 hairpin precursor affect downstream cellular processes. (A) Lead optimization of the initial compound afforded a dimeric small molecule that targets both miR-96's Drosha site and an adjacent 1 × 1 nucleotide GG internal loop, affording Targaprimir-96. (B) Targaprimir-96 inhibits biogenesis of miR-96 in vivo and impedes tumor growth as measured by photon flux (the number of photons per second per unit area). (C) Targaprimir-96 inhibits miR-96 biogenesis and (D) derepresses FOXO1 in vivo.