Messing up with the regulator: A rational AI-driven drug design strategy to disrupt miRNA-target interactions

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Functional products of the transcription and processing of the non-coding genome constitute a relevant and flexible regulatory layer in cell function. Among them, micro-RNAs (miRNAs) are a widely represented class of small regulatory non-coding RNAs (ncRNAs) that exert a negative regulatory effect over genomic output at the post-transcriptional level. This regulatory effect is based on the base pairing of miRNAs to cognate mRNA targets and, by nature, has limited specificity and a high degree of promiscuity. Consequently, miRNAs can establish and build complex regulatory networks, simultaneously targeting several mRNA transcripts that could also be under control by different miRNAs. The imbalance of miRNA-centered regulatory networks has been described and demonstrated as an important driving factor for many human diseases, being, in consequence, a potential target for therapeutic intervention.[1]

Targeting miRNAs has been classically achieved by two different strategies: antisense oligonucleotides or "antagomirs" have been employed to inhibit the mature miRNAs, whereas miRNA mimics or miRNA precursor hairpins have been used to increase the levels of specific miRNAs.[2] Both strategies are sequence specific and agnostic for the miRNA functional targets, as they are designed against the regulatory RNA. Considering the promiscuity of the miRNA regulatory action, both therapeutic strategies targeting the mature miRNA are prone to produce side effects. A recent example of the side effects of the direct targeting of miRNAs is represented by miravirsen, a competitive antagonist of miR-122, designed for the treatment of hepatitis C. Miravirsen is a locked nucleic acid short antagomir, complementary to the sequence of human liver-enriched miR-122.[3] The hepatitis C virus (HCV) has an evolutionary tropism to infect liver cells due to its requirement for miR-122, which binds to the 5′ end of the viral genome, triggering the replicative cycle of the virus. Despite the specificity of miravirsen, the abolition of miR-122 regulatory activity was responsible for the production of adverse side effects, which prevented the continuation of the drug's clinical trials.

Besides their individual nucleotide sequences, another specific feature of miRNAs is their unique biogenesis pathway (Figure 1A). The mature miRNAs are generated by successive endonuclease processing of specific RNA transcripts, first at the nucleus and later at the cytoplasm, producing intermediate RNA species with potentially druggable characteristics.[4] Stable RNA structures present in miRNA precursors can be targeted by small molecules, interfering with the miRNA biogenesis and abolishing their regulatory functions (Figure 1B). This strategy has been developed by Matthew Disney's lab with the design of the Inforna 2.0 platform, a comprehensive computational strategy aimed at identifying small molecules that can selectively bind to secondary RNA structures. Secondary RNA structures of interest are analyzed to identify their constituent motifs, such as hairpins, internal loops, or bulges. These motifs are then matched against the Inforna database, curated to include experimentally validated RNA-small-molecule interactions. By computationally screening small molecules for compatibility with the target RNA motifs, the system prioritizes candidates that are pre-dicted to bind with high affinity and specificity. The successful proof of concept of the Inforna method allowed the selective inhibition of the miR-96 biogenesis by pre-miRNA targeting with a small molecule. Interestingly, this miRNA is located within a tri-membered gene cluster that also contains miR-182 and miR-27a.[5] Recently, a quantum leap of this strategy has also been achieved by Disney's team with the development of the RIBOTAC technology, where an RNA-binding molecule is appended to a heterocycle that binds and activates RNase L, which degrades the targeted RNA. The RIBOTEC technology has proven its efficacy over the pre-miR-155, being able to induce a targeted degradation of the miRNA precursor.[6]

All the described strategies for drug design against miRNAs, tackling the mature species or its biogenesis intermediates, do not consider the miRNA functional targets. Consequently, there is a current need for the development of more specific drug design protocols that could circumvent the pleiotropic effects of miRNA action by acting over a specific miRNA-mRNA targeting event. The manuscript by Xiao and co-workers[7] describes a novel approach for drug design against miRNAs that takes into consideration both the miRNA and its cognate target. This work relies on previous evidence obtained from the same research group that demonstrated how small organic molecules could be active against specific RNA complexes between miRNAs and mRNA targets formed in the catalytic center of the AGO2 effector of the RNA-induced silencing complex (RISC).[8] Interestingly, the partial complementarity between the miRNA and its target allows the formation of structural bulges that are specific for each miRNA-mRNA pair and can be druggable (Figure 1C). This elegant concept allowed Xiao and co-workers to develop a complete

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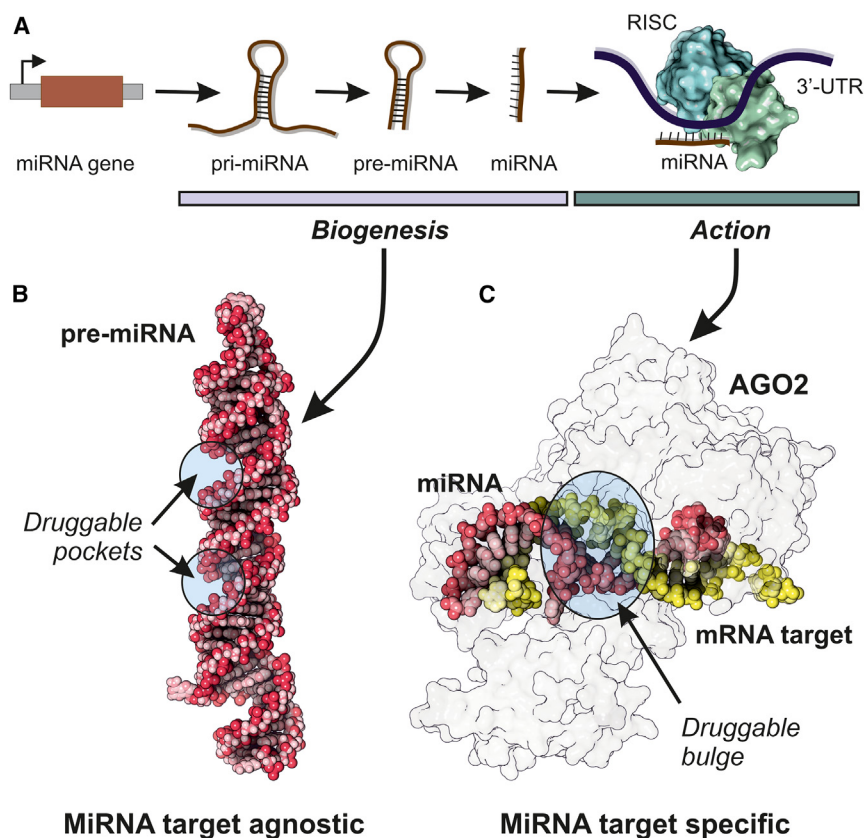**E-mail:** fenguita@medicina.ulisboa.pt

Figure 1. Strategies for structure-based drug design targeting miRNA biogenesis and action
(A) miRNAs are produced from specific RNA transcripts that undergo several steps of processing to generate biogenesis intermediates (pri-miRNAs and pre-miRNAs) with potential druggable characteristics. (B) Pockets on the surface of the characteristic hairpin loop structure present in pre-miRNAs have been used as target spots for the design of small-molecule binders that interfere with miRNA biogenesis.[5] However, this strategy is agnostic for the miRNA targets and can further generate unwanted secondary effects due to the reduction in the levels of the drugged miRNA precursor. (C) Xiao and co-workers[7] described SMTRI, a new miRNA target-specific protocol for drug design that takes advantage of the characteristic RNA bulges formed within the catalytic pocket of AGO2 protein during miRNA binding to its cognate target. This strategy has the major advantage of isolating and targeting the specific regulatory event involving an miRNA over an mRNA transcript, avoiding the pleiotropic effects caused by the inhibition of miRNA biogenesis. Structure representations were prepared with 3D Protein Imaging software (https://3dproteinimaging.com).

AI-driven pipeline for the selection of lead drug compounds that will bind to a specific miRNA-mRNA hybrid.[7] The SMTRI (small molecules targeting miRNA-mRNA interactions) software uses a convolutional neural network (CNN) model for predicting small molecules that directly target the RNA structural motifs formed by miRNA-mRNA interactions. Moreover, the SMTRI pipeline uses a simplified numerical language to translate the information extracted from the secondary structure motifs present in the miRNA-mRNA interface, which reduces the computing times. The software training set included information from experimentally validated small molecules that interact with RNA motifs, extracted from RNALigands, PDB, PubChem, and RPocket databases, and was *in silico* validated with test cases involving experimentally proven miRNA-mRNA interactions.[7] The authors also built a web-based platform that implements the whole SMTRI pipeline behind a graphical user interface (http://www.smtri.net).

Overall, the SMTRI method is based on a novel and smart approach that considers the specific structural features that characterize miRNA-mRNA interactions and a numerical representation of these RNA complexes. Driven by deep-learning algorithms, SMTRI can select lead compounds to specifically bind the RNA structural bulges formed inside the core of the RISC, opening a new avenue for the design of small molecules targeting miRNA action. Compared with other strategies used to modulate miRNA action, the SMTRI approach offers the unique feature of target specificity that could prevent secondary effects related to the general regulatory action of miRNAs. The method would need further laboratory validation with different test cases, but it harbors a strong potential to become an election protocol to tackle specific miRNA-target interactions by small compounds.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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