



## Perspective

## Opinion: miRNAs – The new wave of molecular cancer therapeutics

Hernando Lopez-Bertoni<sup>a,b,\*</sup>, John Laterra<sup>a,b,c,d</sup><sup>a</sup> Hugo W. Moser Research Institute at Kennedy Krieger, 707N. Broadway, Baltimore, MD 21205, USA<sup>b</sup> Department of Neurology, Johns Hopkins School of Medicine, 600N. Wolfe Street, Baltimore, MD 21287, USA<sup>c</sup> Department of Neuroscience, Johns Hopkins School of Medicine, 600N. Wolfe Street, Baltimore, MD 21287, USA<sup>d</sup> Department of Oncology, Johns Hopkins School of Medicine, 600N. Wolfe Street, Baltimore, MD 21287, USA

## A B S T R A C T

Cutting-edge advances in nanomedicine and the recent approval of two siRNA-based therapeutics by the Food and Drug Administration (FDA) has rekindled the interest in RNA interference (RNAi) as vehicles for the development of novel cancer therapeutics. In this perspective, we will briefly discuss how miRNAs are becoming the next-generation RNAi therapeutic, the advances in delivery vehicles for *in vivo* miRNA delivery, and where miRNA technology stands in terms of clinical translation.

Over the last two decades our molecular understanding of RNA interference (RNAi) and the technology to deliver oligonucleotides *in vivo* has grown exponentially [1]. The growing appeal of using RNAi technology as therapeutics is based, in large part, on the potential to achieve highly specific, rationally designed therapies based on the primary sequence of the targeted transcripts. The use of this technology allows for the possibility to target disease-specific *undruggable* molecular events providing new options for treatment. Since these inhibitor-target interactions are based on Watson–Crick base pairing, off-target effects can be predicted and minimized [2].

One RNAi modality that is gaining pre-clinical traction as a promising new treatment approach for cancer are microRNAs (miRNAs). These short, non-coding RNAs (ncRNAs), are a class of endogenous double-stranded RNAs 19–22 nucleotides long that repress activity of complementary messenger RNAs (mRNAs). miRNAs are highly conserved and thought to regulate ~30% of mammalian gene products. Not surprisingly, these molecules are potent determinants of cell fate and their dysregulation has causative effects in several disease conditions, including cancer [2]. Numerous miRNAs have been found to regulate tumorigenesis by targeting tumor-suppressing or tumor promoting transcripts [3]. It is now well-accepted that activation of tumorigenic cascades involves the dysregulation of multi-dimensional molecular networks with miRNAs playing key roles in the process [3].

We now understand that miRNAs participate in the regulation of vast gene-expression networks by modulating effectors of DNA methylation, histone modifications, and chromatin architecture [4]. Conversely, epigenetic events linked to oncogenesis can affect miRNA expression. Dysregulation of this carefully balanced feedback contributes to neoplastic cell growth and resistance to cancer therapeutics [4]. Growing evidence indicates that reconstituting tumor-suppressive miRNAs or inhibiting oncogenic miRNAs can normalize these dysregulated molecu-

lar networks, inhibit tumor growth, and enhance the effects of current standards-of-care [5,6]. Understanding these context-specific, multi-dimensional interactions can lead to a sophisticated, mechanism-based, rational approach to designing molecular cancer therapeutics. For instance, we recently identified a mechanism by which the Oct4 and Sox2 transcription factors repress miRNAs (*i.e.* miR-148a and miR-296–5p) that inhibit the expression of DNA methyl transferases (DNMTs) and the chromatin-associated protein HMG1A and thereby modify the DNA methylation landscape, chromatin structure and induce the tumor propagating capacity of GBM cells. Reconstituting these miRNAs in tumor-propagating GBM stem cells (GSCs) was found to potently inhibit GBM cell stemness based on multiple cell responses including the inhibition of tumor propagating capacity *in vivo* and the prolongation of animal survival [6]. These results highlight that identifying epigenetic regulators that function as GSC modifiers can inform new approaches to treat tumors, such as GBM.

One feature of miRNAs, the advantages of which are frequently overlooked, is the ability of one miRNA to target multiple mRNA transcripts [2]. This promiscuous quality of miRNAs is often perceived as a source of concern for therapeutic development. However, carefully selecting one miRNA to target multiple mRNAs dysregulated during tumorigenesis can allow the targeting of multiple parallel oncogenic pathways using a single agent, enhancing therapeutic efficacy and reducing chances of tumor recurrence. Integrating next-generation sequencing (NGS) data and bioinformatics allows for an agnostic approach to selecting miRNAs with therapeutic potential. This rational “top down” approach to using miRNAs as therapeutic tools has the potential to significantly advance the development of next generation molecular therapeutics.

One of the biggest challenges for RNAi-based therapeutic strategies is their stability, delivery and bioavailability [7]. Two main approaches have been at the forefront of tackling the problem of RNA instability:

\* Corresponding author at: Department of Neurology, Johns Hopkins School of Medicine, 600N. Wolfe Street, Baltimore, MD 21287, USA.  
E-mail addresses: [lopezbertoni@kennedykrieger.org](mailto:lopezbertoni@kennedykrieger.org) (H. Lopez-Bertoni), [laterra@kennedykrieger.org](mailto:laterra@kennedykrieger.org) (J. Laterra).

stabilization by chemically modifying the RNA oligonucleotides or the use of delivery vehicles to protect the RNA molecules until it reaches the desired site of action. Both strategies have yielded success, however the use of delivery vehicles such as viral vectors and nanocarriers seem to be gaining momentum. The use of viral vectors as a mode of delivery for RNAi-mediated therapies has been broadly explored and despite initial enthusiasm, concerns about potential side-effects of integrating vectors and efficiency of non-integrating viral vectors have dampened the initial momentum of this technology. Recent advances in nanomedicine are tilting the balance towards the use of nanocarriers to deliver the RNAi cargo as a more flexible and advantageous mode of delivery [8]. A wide range of nanocarriers have been developed to date, including polyamidoamine (PAMAM) dendrimers, lipopolyamines, and polyethylenimine (PEI), each with their own unique characteristics. A powerful advantage of these next-generation nanocarriers is that they readily accommodate payloads consisting of multiple miRNAs and/or miRNA inhibitors (*i.e.* antagomirs), and thus offer an ideal vehicle for implementing multi-miRNA normalization strategies as proposed above. These types of carriers can be optimized for siRNA/miRNA delivery to specific cell types, providing an extra level of control to minimize off-target effects. We recently developed and characterized novel bioreducible poly( $\beta$ -amino ester) (PBAE) polymers that can effectively deliver miRNA mimics and/or antagomirs to inhibit the GBM stem cell phenotype *in vitro* and *in vivo* [6]. These polymers have a high loading capacity allowing for codelivery of nanoparticles containing multiple miRNA types to cells of interest [6]. We also show that this technology can be combined with other treatment modalities (*e.g.* radiation) [5] to create a window of opportunity that sensitizes tumor cells to chemo/radiation for long-term disease management.

These observations highlight how novel RNAi-mediated mechanisms of cell fate regulation can combine with nanomedicine to provide new avenues to develop innovative molecular therapeutics. Thus far, two siRNA-based therapeutics have been approved by the Food and Drug Administration (FDA) to treat hereditary transthyretin amyloidosis (Patisiran) and acute hepatic porphyria (Givosiran) [9,10] with several others currently in clinical trials [1]. The positive momentum from the clinical success of siRNA-based drugs are paving the way for clinical trials to test miRNA-based therapeutics. To date no miRNA-based therapeutic has entered phase 3 clinical trial, however, there are several candidates in phase 1 and 2. miRagen, one of the leaders in the development of miRNA-based therapeutics ([www.miragen.com](http://www.miragen.com)), has active phase 1 trials for miR-29 (MRG-201) to treat keloid and scar tissue formation in addition to a phase 2 trial for miR-155 (MRG-106) to treat T-cell lymphoma. Regulus Therapeutics (<http://regulusrx.com>) is recruiting patients for phase 1 testing of a miR-10b inhibitor to treat patients with GBM and a phase 2 trial for a drug candidate to inhibit miR-21 in patients with Alport syndrome.

As our knowledge regarding how miRNA *targetomes* contribute to disease increases and technological advances to deliver miRNAs *in vivo* advance, we will undoubtedly see an increase in the rational mechanism-based design of miRNA-based molecular therapeutics and their promise of durable clinical impact for cancer patients.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgement

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Institute of Health (NIH) [1R01NS096754-01A1 (Laterra) and 1R01NS110087-01A1 (Laterra)].

### Author's contribution statement

John Laterra, MD, PhD and Hernando Lopez-Bertoni, PhD contributed equally to the manuscript.

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