

# MicroRNAs in Brain Cancer: Look at the Forest, Not at the Tree

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**ABSTRACT:** Much is known about microRNA biology and their involvement in essentially any biological processes in eukaryotic cells, including cancer. Now, to take advantage of them in clinics, a change in perspective is needed and a reappraisal of their features is warranted to re-ignite interest and translational hype. As we recently reported, their strength is in numbers, size and simplicity.

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Much is known about microRNA biology and their involvement in essentially every biological process in eukaryotic cells, including cancer. To take advantage of microRNAs in clinics, a change in perspective is needed and a reappraisal of their features is warranted to re-ignite interest and translational hype. As we recently reported, microRNA strength is in numbers, size, and simplicity.

Two decades have passed since the revolutionary discovery of microRNAs, tiny non-coding RNAs naturally produced by cells to regulate the protein output of their coding genome.<sup>1</sup> Within a very short time it also became evident that they have an important role in cancer biology, some acting as oncogenes, others as tumor suppressors.<sup>2,3</sup>

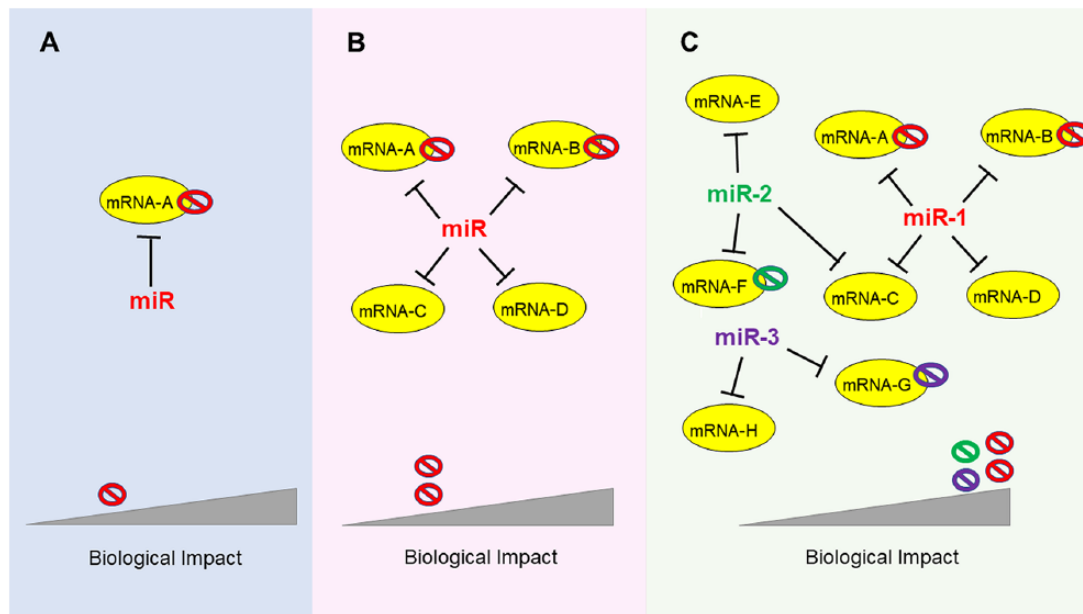
This has been followed by an impressive research effort detailing the functions and targets of many microRNAs in an ever-growing number of tumors, including glioblastoma, the deadliest of brain cancers.<sup>3</sup> It is undeniable that this has led to a deep understanding of their multifaceted roles. However, out of this immense mound of basic science and preclinical work, only two microRNAs have so far made it to clinical trials and the results have not been particularly encouraging, showing minimal clinical response.<sup>4,5</sup> Even worse, no new clinical trials investigating microRNAs as anticancer therapeutics are currently on the horizon ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). The prevailing feeling is that microRNA research in oncology has hit a stalling point, not able to go beyond a descriptive characterization of microRNA targeting, and for the most part, devoid of real translational relevance. Consequently, the initial hype has progressively declined over time to little more than lukewarm interest. Yet, the reason for this still unkept promise might have more to do with a failure on our part to see the whole picture rather than on microRNAs' biological shortcomings.

To begin with, microRNAs are "peculiar" genes: Different from protein-coding genes, they have no direct role as final

effectors of the cell's functions. Instead, they work by negatively modulating the abundance of their targets and this modulation is usually partial, rarely dramatic. It is thus not surprising that no microRNA has been shown to be necessary, nor sufficient for tumorigenesis. It is becoming obvious that the existence of a "silver bullet" microRNA whose modulation can cure cancer is an unlikely possibility. In fact, since the very first studies in the early 2000s, it is evident that tumors are characterized by not just one, but a multitude of abnormally expressed microRNAs, suggesting that many are involved simultaneously in the oncologic process.<sup>6</sup> Our tendency to "cherry pick" and investigate a selected microRNA among these ever-growing lists of deregulated microRNAs in cancer has on one side, positively contributed to the granular understanding of the function of certain microRNAs, but on the other side, has prevented the development of a more comprehensive and likely more biologically relevant vision of their broader impact, and, more importantly, how to take advantage of them in therapy.

We argue that the real strength of microRNAs lies in their synergistic action and that there are a critical number of functionally related microRNAs, which if coordinately modulated, can perturb the biology of cancer significantly enough to reach the threshold required for its destruction (Figure 1). Indeed, this tendency of microRNAs to function in groups is also apparent in nature: the most striking example is the miR-17-92 cluster, a group of 6 microRNAs encoded within a short 1 kb genomic sequence, each targeting different tumor suppressor genes.<sup>7</sup> Another hint at this fundamental synergism is suggested by the expression of otherwise genetically independent microRNAs is mainly regulated at the epigenetic level, so that transcription modules of specific microRNAs are activated or deactivated simultaneously in response to specific cellular programs, including oncogenic ones.<sup>8</sup> We have recently shown that among the most downregulated microRNAs in glioblastoma, there are





**Figure 1.** Widening the concept of microRNA-mediated interference. From a translational perspective, expanding the granularity of one microRNA targetome does not change the net biological impact of that specific microRNA (A and B), also in consideration that many targets are in common among multiple microRNAs. On the contrary, the impact of multiple microRNAs is synergistic by affecting a growing number of targets that are functionally related (C). Barred circles represent targets which are components of complex and redundant cellular pathways and are color-matched with the corresponding microRNAs.

three (miR-124, miR-128, and miR-137) which, although genetically discreet and transcriptionally independent, are always co-expressed during neuronal differentiation. The relevant part of this finding is that each one of these three microRNAs contributes to the regulation of specific chromatin repressor oncoproteins, which unless simultaneously targeted are able to rescue each other's impaired function.<sup>9</sup> For cancers like glioblastoma, characterized by the simultaneous involvement of multiple oncogenic pathways, and for which targeted therapies have shown no effect, this multi-microRNA approach allows to effectively interfere with a large number of intertwined oncogenes, curbing the tumor's ability to overcome selective knockdowns.

Recognizing that microRNAs work in synergy is not just an academic exercise. On the contrary, the simple biogenesis of microRNAs and their small size makes it easy to engineer artificial transgenes that are able to simultaneously encode multiple microRNAs of choice within DNA segments well below the capacity limits of any common vector used for gene therapy.<sup>9</sup> This strategy allows the simultaneous expression of crucial microRNAs and thus regulates a broad spectrum of otherwise difficult-to-target oncogenic pathways. To prove the feasibility, as well as the potential therapeutic advantage of such an approach, we have devised a very facile and highly reproducible method for engineering these artificial microRNA clusters in a way that they can also be easily delivered to cells *in vitro* and *in vivo*.<sup>9</sup>

Yet, realism is important when investigating the translational potential of new experimental therapies: despite combining the action of three (or even five, as demonstrated in unpublished

material) among the most relevant tumor suppressive microRNAs in glioblastoma, we still were not able to kill the most recalcitrant cancer cells. Instead, we found that the broad interference provided by these microRNA clusters could be used much more effectively by preventing cancer cells to adapt and survive the additional toxicity of standard chemoradiation, a process that normally occurs in cancer and is responsible for its recurrence after therapy. We demonstrated that this phenomenon, not surprisingly, depends on the concerted action of multiple proteins. The simultaneous downregulation of these proteins by a combinatorial microRNA approach resulted in a lethal hit to the tumor by hampering its defense against standard therapies that normally would have not been particularly effective.<sup>9</sup>

Efficient delivery of microRNAs continues to be a major limitation to microRNA-based therapeutics. A major effort is currently underway to optimize delivery of therapeutic oligonucleotides by chemical modifications or with the use of nanomolecular carriers.<sup>10</sup> It is evident, however, that this approach might be difficult to employ for the simultaneous delivery of multiple microRNAs. Due to their small size, microRNAs are known to be transferred from cell to cell within the tumor microenvironment through extracellular vesicles.<sup>11</sup> We have taken advantage of this feature and shown that while the transfer of single microRNAs from one cell to another has negligible effects on receiving cells, the transfer of a group of microRNAs is able to significantly have an impact, almost to the same extent observed in the cells of origin.<sup>9</sup> This observation is important because, in the realistic scenario where there

is only a fraction of tumor cells transduced by a multi-microRNA transgene delivered locoregionally, this microvesicle-mediated trafficking appears to be a promising strategy to “flood” the tumor with therapeutic microRNAs before a second and lethal hit to the tumor is provided with chemotherapy or radiation.

The criticism that microRNA combinations would exponentially increase the already poorly defined targeting landscape characteristic of each microRNA is probably more an academic problem than a real drawback: if normal tissues display an array of specific microRNAs, it probably makes sense to use them together therapeutically, even if we are not aware (and probably will never be) of all the possible targets that such a combination could impact.

In conclusion, translating microRNAs into therapeutics requires the acceptance of their positive qualities (such as simple biogenesis, small size, ease of genetic modification, and tendency to diffuse through the tumor microenvironment) to offset their shortcomings (ie, low biological potency). With a bit of creativity and some pragmatism, microRNAs have all the features to be an important tool for the treatment of cancers and many other diseases.

#### Author Contributions

PP conceived and wrote the manuscript. VB made the figure and helped with writing of the article.

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