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INHIBITING MICRORNA FUNCTION GLOBALLY TO TARGET CANCER: AN EMERGING NEW SCENARIO

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Abstract – *MicroRNA (miRNA) is an important class of small non-coding RNAs that act as post-transcriptional modulators of gene expression, and whose dysregulation has been implicated in various stages of cancer development. Although promising experimental evidences point toward a potential use of miRNA as targets for cancer treatment, to date, technical challenges have hindered the translation of this information from bench to bedside. Here, we review some of the most promising approaches that have been explored to develop new anti-cancer therapies based on the regulation of miRNA function.*

The objective of this review is to draw the attention on recent observations suggesting that global inhibition of miRNA function may inhibit tumor development, and which may set the stage for new therapeutic avenues. We discuss the characteristics of some of the most promising strategies to inhibit miRNA function and describe their advantages as well as their potential drawbacks.

Although pharmacologic interventions aimed to modulate miRNA activity are still at the primordial stage, exciting new evidences highlight the need to persist in studying the relationship between miRNA function and cancer. Information gathered from these studies may have the potential to lead to new opportunities of treatment of cancer.

KEYWORDS: *microRNAs, Cancer, RNA-induced silencing complex.*

INTRODUCTION

MicroRNAs (miRNAs) are short non-coding RNAs that repress gene expression at the post-transcriptional level by binding to partially complementary sequences on target mRNAs, blocking their translation, and eventually inducing their degradation[1].

Computational analyses suggest that the majority of mammalian genes are targeted by miRNAs. Accordingly, miRNAs play a critical role in virtually every physiologic process, and their dysregulation has been linked to several pathological conditions, including cancer[2].

The majority of miRNAs are generated from longer primary transcripts via a series of successive processing steps catalyzed by two RNAses: DROSHA[3,4] (in the nucleus) and DICER[5,6] (in the cytoplasm). The mature miRNAs are then incorporated into one of four Argonaute proteins[7] (AGO1-4) (Figure 1).

A mere AGO2-bound miRNAs unit can directly induce cleavage of target mRNAs harboring perfectly complementary sites[8]. While this process is common in plants, it is exceptionally rare in animals. In fact, target mRNAs in animals generally have only partial complementarity to the cognate miRNA and AGO-bound miRNAs act as part of a large (>2MDa) multiprotein complex known as miR-Induced Silencing Complex (RISC[9]).

AGO proteins and TNRC6 proteins (TNRC6A/GW182, TNRC6B and TNRC6C) are the core components of the RISC. AGO binds directly to the miRNA and structurally promotes specific miRNA-mRNA interaction. TNRC6 binds to AGO and acts as a docking platform for the recruitment of decapping protein (DCP) and deadenylases (deA), the enzymes that ultimately induce mRNA degradation[10,11].

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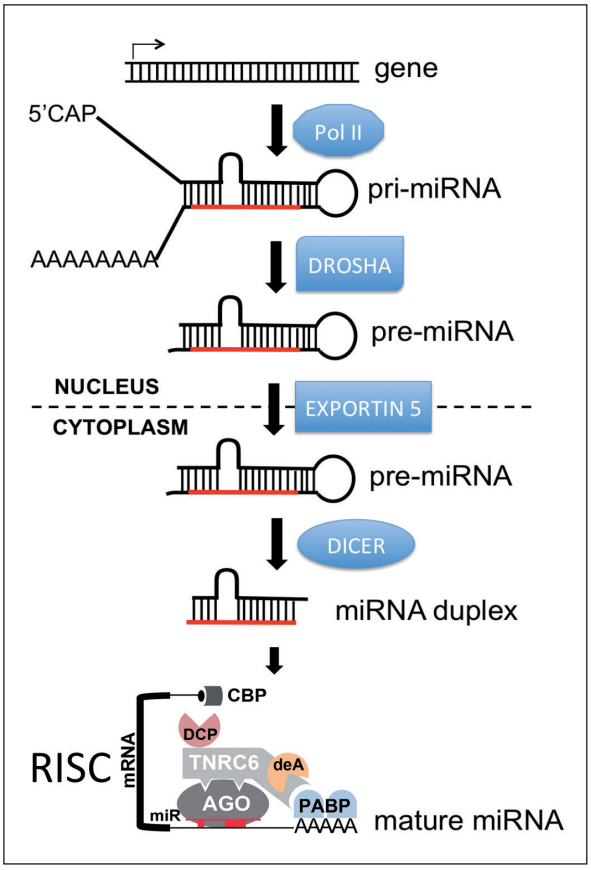

Fig. 1. Representation of the miRNA pathway. The majority of miRNAs are transcribed as primary transcripts (pri-miRNAs) by RNA polymerase II (Pol II). The pri-miRNA contains one or more hairpins, and miRNAs are localized in their double-stranded stem. Processing by the nuclear microprocessor complex, which contains the RNase III enzyme Drosha, releases the hairpin: this intermediate is referred to as the precursor miRNA (pre-miRNA). After export to the cytoplasm by exportin 5, a second RNase III, Dicer, chops off the loop of the hairpin and generates a double-stranded RNA. The mature miRNA strand is subsequently incorporated into the RISC, binding directly to a member of the AGO protein family. The other strand of the miRNA duplex is referred to as miRNA* and is normally degraded.

MIRNAS AND CANCER

Calin et al[12] published the first evidence of association between miRNAs with cancer, showing that miRNA-15 and miRNA-16 are located at chromosome 13q14, a region deleted in more than 50% of B cell chronic lymphocytic leukemias. Shortly after, the same group demonstrated that the majority of miRNAs reside in cancer-associated genomic region or fragile sites[13].

Since then, the hunt for new miRNAs rushed in, and the analysis of miRNAs expression became a vast enterprise across many research fields. As a result, the list of miRNAs has been growing ever since, and currently about 2600 human miRNAs have been identified[14]. Many of these show patterns of expression that reflect developmental lineage and differential state of tumors[15], proving the extraordinary potential of miRNA profiling in cancer diagnosis. However, due to inconsistencies in miRNA expression even among cancers of the same type, there is not consensus on whether miRNAs are ready to be used as biomarkers in the clinical routine[16].

Early mechanistic studies led to the classification of miRNAs between oncomiRs (miRNAs that promote cancer), such as the miR-17–92 cluster, miR-155, and miR-21[17-21] and tumor suppressor miRNAs, such as let-7, miR-15 and miR-16[22,23]. However, soon the literature became populated with conflicting reports as to whether specific miRNAs were oncogenic or tumor suppressive. Indeed, the function of specific miRNAs turned out to be context-specific, as the same miRNA would behave as oncogenic in one scenario, but tumor suppressive in another. Therefore, it soon became clear that the classification of a miRNA as oncogenic or tumor suppressive represented a naïve oversimplification[24].

Therapeutic approaches targeting miRNA function started to be developed. They are based on the delivery of miRNA mimics or antisense agents, and they are meant to adjust the activity of individual miRNAs whose misregulation has been linked to cancer[25,26]. However, the ambiguous role of miRNAs in affecting cancer biology makes the identification of safe and specific miRNA therapeutics a significant challenge[27].

The reason why it has been difficult to predict the effects of miRNA-targeting therapies may reside on the fact that a single miRNA can repress hundreds of different mRNAs simultaneously. As a result, its manipulation will result in different phenotypes depending on cell type and state. For example, a single miRNA can simultaneously repress the expression of tumor suppressors and oncogenes in a cell. Which leads us to the question: what would it be the resulting outcome of its inhibition or overexpression? Conceivably, perturbation of the expression of a miRNA will either support or inhibit tumor development depending on the balance and roles that its target genes have in that specific cancer type.

In conclusion, although promising observations have pointed out a role of miRNA as biomarkers for cancer diagnosis and targets for the development of anti-cancer therapies, technical barriers have hindered the translation from bench to bedside.

GLOBAL INHIBITION OF MIRNA FUNCTION TO IMPAIR CANCER DEVELOPMENT

A seminal observation published in 2009 by Kumar et al[28], raised the possibility that global loss of miRNA function may impair tumor development. In

particular, this report showed that, although several human tumors present inactivating mutations of one *DICER* allele – suggesting that global downregulation of miRNAs promotes cancer– the other allele is never lost during tumorigenesis, suggesting that miRNA function is indeed required for tumors to develop. This phenomenon has been referred as the "the paradox of DICER in cancer"[29].

The paradox that homozygous inactivating mutations of *DICER* impair tumor growth, while in heterozygosity these mutations are tumorigenic, can be explained hypothesizing that the amount of enzyme produced by only one *DICER* allele in cancer cells is sufficient to produce adequate levels of mature miRNAs to sustain gene regulation, but insufficient to properly catalyze the processing of other transcripts whose dysregulation may be the ultimate reason of the observed oncogenic effect.

In support of this scenario, it has been shown that DICER is involved in epigenetic regulation in the nucleus in a miR-independent manner and it is essential for the biogenesis of endogenous siRNAs[30,31]. DICER is also involved in ribosomal RNA biogenesis[32]. As a consequence, the phenotypes observed in absence of DICER cannot be unequivocally attributed to a reduction in miRNA activity.

These considerations may be a valid reason to reopen the case, and design new experimental models to test the effect of global inhibition of miRNA function in cancer development.

What we learned, though, from deleting the enzymes that mediate miRNA biogenesis, is that potential therapeutic approaches targeting these enzymes would not likely be specific, and result in unintended physiological effects in the organism, mostly due the fact that these enzymes mediate the processing of transcripts not related to the miRNA pathway.

Incidentally, studies have shown that individual deletions of either *DICER*, *DROSHA* or *EXPO5*, in human cancer cell lines, induced different proliferation-related phenotypes, consistent with the fact that another biological pathway, other than the miRNA pathway, are affected by those deletions[33].

TARGETING THE RISC

To inhibit the miRNA pathway, an alternative to interrupting miRNA biosynthesis may be the targeting the effector step, i.e. the RISC.

In a screening of small molecules that inhibit miRNA-mediated gene repression, a compound, trypaflavine (TPF), was identified with relatively non-cytotoxic profiles that suppress the loading of miRNAs into the RISC. In a cell-based model of miRNA-dependent tumorigenesis, treatment of cells with TPF effectively neutralized tumor growth[34].

In a different study, authors used structure-based molecular design techniques to identify a compound named BCI-137 with marked pharmacophore mimicking structural features of miRNAs. The authors showed that treatment of NB4 cells inhibited the binding of specific miRNAs with AGO2[35]. Although the effects of this drug on tumor growth have not been explored, the authors made the proof of concept that this molecule can be used to inhibit RISC function.

However, blocking miRNA loading may present a significant drawback: indeed, inhibiting the loading of miRNA on AGO results in structural instability of AGO, with subsequent increase in its degradation rate[36]. Because, similarly to the enzymes that mediate miRNA processing, AGO carries functions outside of the miRNA pathway, its degradation may result in the perturbation of other biological functions outside of the miRNA pathway. For example, AGO2 is found in the nucleus of mammalian cells where it can regulate transcription by RNA interference mechanisms[37] or by interaction with chromatin modifiers[38], and it can participate to DNA repair[39]. Therefore, targeting the ability of AGO to load small RNAs may affect these processes, likely resulting in unintended biological effects on cells.

Recently, new properties of the RISC have been described, and may be harnessed to design new anti-cancer therapies. Olejniczak et al[40] have shown that exponentially growing cancer cell lines present a composition in RISC strikingly different from post-mitotic cells. In particular, proliferating cancer cells in culture consistently present a fully assembled high molecular weight RISC (HMWR), while post-mitotic cells, such those forming most differentiated adult tissues, present a variable portion of AGO proteins associated with low molecular weight RISCs (LMWR). The LMWR lacks TNRC6 proteins and are not stably assembled on target mRNAs, implying that this sub-population of AGO-miRNA units is not engaged in target repression[40-42] (Figure 2). Of note, mitogenic cues can drive changes in the relative abundance of HMWR and LMWR in a given cell, with consequential changes in how effectively miRNAs can repress their targets[41].

A tempting interpretation of these observations is that the active HMWR is particularly required to supports cell proliferation and may therefore increases upon oncogenic signals. Moreover, the enrichment of LMWR in post-mitotic cells suggests the possibility that miRNA-mediated gene regulation may be dispensable in differentiated adult tissues. In support of this, *in vivo* studies conducted in animal models have shown that ablation of individual miRNAs in the heart during homeostasis results in lack of overt phenotypes[43].

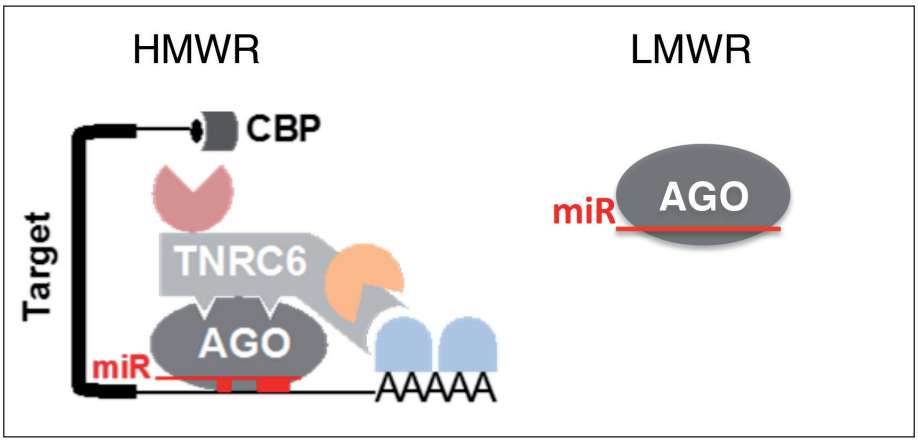

Fig. 2. Representation of HMWR and LMWR, which are enriched in exponentially proliferating cancer cell lines, and post-mitotic tissues, respectively. miR (miRNA). CBP (Cap-Binding Protein).

If these hypotheses turn out to be factual, one can envision the possibility to induce the disassembly of the HMWR to impair tumor growth *in vivo*, without affecting the physiology of normal tissues that naturally present the LMWR form.

Within the RISC, AGO proteins directly interact with TNRC6[44-47]. TNRC6 proteins are characterized by an N domain, which contains arrays of glycine–tryptophan (GW) repeats, and directly interacts with AGO proteins. In particular two tryptophan of TNRC6 bind to two hydrophobic binding pockets on the surface of the AGO[48]. The association between AGO and TNRC6 is necessary for translational repression and for the recruitment of the exonuclease XRN1, which ultimately degrades the target mRNA. Therefore, one can envision small molecules that interfere with the interaction between AGO and TNRC6 resulting in a blockage of the miRNA pathway at the effector step. This approach would have the advantage to inhibit miRNAs function without altering miRNAs and AGO levels, therefore minimizing unintended perturbations of cellular functions.

In conclusion, targeting the unique properties of the HMWR associated with proliferating cells may represent a valuable approach to specifically target cancer, with potential minimal effects on normal tissues, which in contrast are enriched in LMWR and may not depend on miRNA function.

CONCLUSIONS

Recent biochemical analyses of the RISC have suggested that miRNA activity may be required to support proliferation of cancer cells, while dispensable in post-mitotic tissues. But what are the possible mechanisms that would make cancer heavily reliant on miRNA activity in order to grow and colonize an organism?

One explanation may reside on the fact that miRNA activity may be especially required in conditions that demand prompt adjustments to the gene expression program, such as during tumorigenesis. For example, in tumors miRNA function may be essential to ensure timely clearance of cell cycle transcripts as cells proliferate, or to adapt their metabolism to facilitate growth under changing microenvironments. Conceivably, these processes demand quick adjustments of the gene program, and miRNAs may facilitate this process by decreasing the half-life and translation rate of mRNAs, thereby preventing the accumulation of undesired proteins. Although the increased turnover of transcripts, as a consequence of high RISC activity, will be energetically wasteful, it would provide an advantage in terms of transcriptome responsiveness to oncogenic stimuli. On the other end, in normal tissue the inactivation of the RISC may result in decreased target turnover and increased translation rates, allowing maximal mRNA and protein production when changes of gene expression programs are not desired, for example during homeostasis. Therefore, in absence of RISC activity optimal gene expression levels may be achieved with minimal energetic waste. If the striking difference in RISC utilization between cancer and normal tissues, which so far has been only demonstrated biochemically, will be confirmed functionally *in vivo*, we may have found a unique characteristic of tumors that can be targeted pharmacologically.

Conflict of Interest:
Authors declare no conflict of interest.

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