Restoration of microRNA metabolism trigger robust antitumor responses

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In this study, Qi and Ding et al. show that RDR1, a plant RNA-dependent RNA polymerase, can rescue microRNA biogenesis defects and promote antitumoral activity in cancer cells and tumors.[1]

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression by post-transcriptional mechanisms. The biogenesis of miRNAs is a stepwise process that requires the sequential cleavage of longer precursors by DROSHA and DICER1. The resulting mature miRNAs provide specificity to AGO proteins to induce gene repression. It is well established that miRNAs play critical roles in the development of many diseases including cancer. Disruptions of miRNA biogenesis enzymes DROSHA and DICER1 are known to be involved in tumorigenic processes. Indeed, hotspot mutations in these key components of the pathway are associated with both pediatric and adult tumors.[2]

More recently, editing on the 3' end of miRNAs has been linked to tumorigenesis through multiple mechanisms. The addition of non-templated nucleotides is mediated by members of the family of terminal nucleotidyl transferases (TENTs). By contrast, miRNA trimming is mediated by RNA nucleases. For instance, uridylation of pre-miR-324 by TUT4/7 (TENT3A/B) led to a distinct DICER1 cleavage, and a subsequent switch in strand selection, relevant to glioblastoma.[3] Also, TENT4B and PARN have been involved in regulating the levels of miR-21 and other miRNAs through adenylation in colorectal and glioblastoma cells.[4] In general, trimming and tailing of mature miRNAs can also result from binding to certain target RNAs.[5] Despite these and other examples, little is known about the general impact of miRNA metabolism in tumors.

Here, Qi and Ding et al. present evidence that trimming of the last nucleotide of miRNAs is a general signature of tumors. The analysis of the datasets from The Cancer Genome Atlas (TCGA) show that all 32 tumor types present increased trimming when compared with normal tissue, with frequencies ranging between 70% and 30% of the patients. The same phenomena are present in frequently studied cancer cell lines such as A549, HepG2, or HCT116 but not in normal cells such as RPE-1, induced pluripotent stem cells (iPSCs), or umbilical vein endothelial cells (UVECs). This can be detected in northern blots, where a one nucleotide difference can be detected for multiple miRNAs when comparing cancer cell lines with RPE-1. The authors identify this one nucleotide trimming as a mark of impaired miRNA biogenesis. They show that miRNA duplexes presenting trimming of the last nucleotide present difficulties to get efficient loading onto AGO2 (Figure 1).

In the present study, the authors seek to rescue this tumor-specific defect in miRNA metabolism by expressing plant RNA-dependent RNA polymerase 1 (RDR1) from *Arabidopsis thaliana* (At) and *Oryza sativa* (Os). Upon induction with doxycycline, both *Arabidopsis* and rice RDR1 show the capability to add non-templated nucleotides at the 3' end of miRNAs. This restoration of the terminal nucleotides enables increases between 2- and 8-fold in the expression levels of nearly all mature miRNAs in cancer cells. By contrast, the levels of miRNAs in normal cells remain unchanged. This increase in global miRNA levels is accompanied with an inhibition of cell proliferation in cancer cells. Similar results are also observed in other characterization assays such as colony formation, wound healing, and cell invasion. To examine whether such antitumoral effects are exclusive from the RDR1-dependent tailing on miRNAs or not, the authors use the knockout of key factors on the miRNA biogenesis pathway (DGCR8, DROSHA, and AGO2). The knockout of any of the miRNA biogenesis genes blocks the antiproliferative effects of RDR1 expression, indicating that there are no major effects mediated by the tailing of other RNA substrates.

Next, they sought to characterize the downstream molecular targets repressed by rescuing the normal miRNA metabolism. To this end, the authors use AGO2 crosslinking immunoprecipition sequencing (CLIP-seq) to compare the targetomes of cells with and without induction of RDR1. This analysis shows increased repression of direct miRNA targets, in particular mRNAs involved in cell-cycle regulation such as CCNE1, CDK6, and CDC25A, among others. The impact of RDR1 rescue on miRNAs involved in the regulation of cell-cycle genes is further validated by western blot and miRNA reporter assays.

Lastly, to assess the impact of the RDR1 treatment on tumor growth, the authors performed *in vivo* studies in mice using models of solid and liquid tumors. Briefly, cell lines that expressed RDR1, a catalytic-dead RDR1, or no RDR1 were administered subcutaneously and systemically. The authors also evaluate the delivery of RDR1 into tumors using nanoparticles and adeno-associated virus (AAV) vectors. The analysis of cell-cycle genes in the

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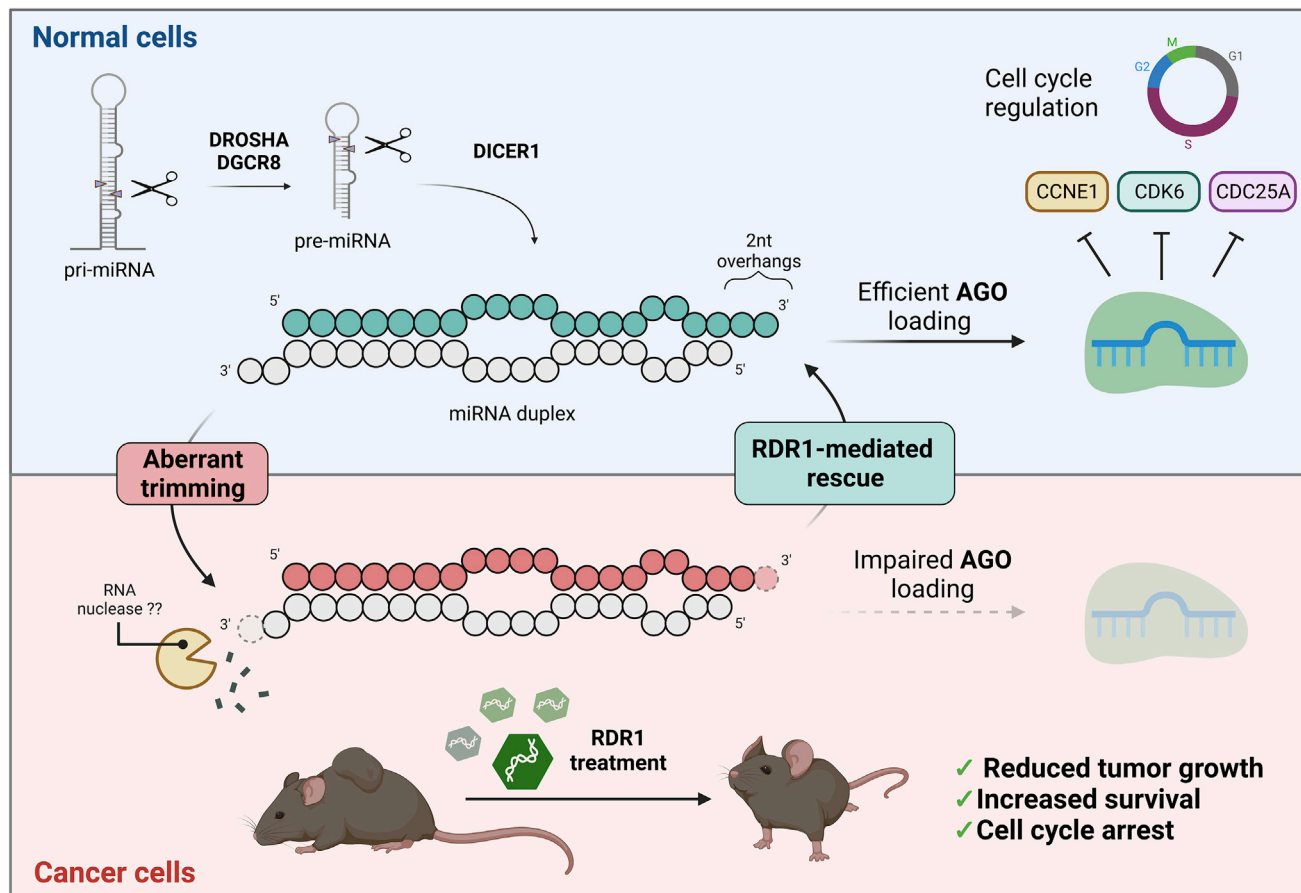
Figure 1. RDR1 can rescue AGO loading and trigger antitumoral activity

The study by Qi and Ding et al. contributes to our understanding of how defects in miRNA processing can play a role in tumorigenesis. The authors show that RDR1, a plant RNA-dependent RNA polymerase, can be used to repair miRNA 3' ends and restore normal miRNA biogenesis. The authors also show that RDR1 rescue of miRNA activity contributes to slow down cell proliferation through the modulation of cell cycle.

in vivo models confirmed that the expression of RDR1 has a profound impact on cell cycle through the restoration of normal miRNA levels. More specifically, the authors report that populations of cancer cells expressing RDR1 are more prone to remain in the G0/G1 phase and refrain from entering the G2/M phase. Consistent with the previous results in cell lines, tumors expressing functional RDR1 showed reduced growth. As a consequence, mice receiving cells treated with RDR1 had increased overall survival rates.

The study also unveils other important questions for the field. The broad 1-nt shortening in nearly all miRNAs suggests the dysregulation of an exonuclease or nibbling activity in tumors. In addition to the reduction in AGO loading described, aberrantly trimmed miRNAs could also present other defects in strand selection or target recognition. These defects combined could lead to a robust dysregulation of miRNA function. Also, it is intriguing to speculate whether any of the endogenous TENTs previously involved in miRNA tailing could play a role in repairing aberrant miRNAs or if they could be exploited therapeutically.

Overall, these results from Qi and Ding et al. provide compelling evidence of the efficacy of RDR1 to restore normal miRNA biogenesis and promote antitumor responses in cancer cells through the modula-

tion of the cell cycle. The mechanistic insights indicate the activity of RDR1 in mammalian cells has major effects in miRNAs. However, it remains to be studied whether the expression of RDR1 can tail other RNA substrates and its effects on mammalian cells. Still, the authors presented a compelling case supporting the bioengineering of cross-species enzymes in translational medicine.

AUTHOR CONTRIBUTIONS
X.B.-D.R. has conceived and written this commentary.

DECLARATION OF INTERESTS
The author has no conflicts of interest to declare.

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