VIEW POINTS
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A MicroRNA "Target Pools" Remains Mysterious

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To the Editor: MicroRNAs (miRNAs) are a recently discovered class of endogenous regulators of gene expression. They bind to complementary sequences in the 3′-UTR of target mRNAs and typically inhibit their translation. Studies in vitro and in vivo have shown that miRNAs can influence tumor development processes, cardiomyocyte hypertrophy, and other diseases.

To date, a variety of methods to predict and verify the relationship between miRNAs and their target genes have been developed, for example, one strategy is to use a co-immunoprecipitation assay to isolate miRNA target mRNAs based on the observation that the Ago proteins can bind both miRNAs and mRNAs. Another strategy is to search for miRNA target genes by a proteomics-related approach based on altered protein levels. However, the basal strategy that is widely used and accepted includes the following three steps: (1) bioinformatic prediction, (2) miRNAs interference, and (3) reporter gene activity assays [Baccarini and Brown, 2010]. In the present study, we hypothesized that miRNAs can regulate housekeeping genes, which would result in a false negative result in step (2) above (Supplementary Fig. 1).

To test this hypothesis, we first applied four popular algorithms, TargetScan, miRanda, Pictar, and miRGen, to predict which miRNAs can target the housekeeping genes, glyceraldehyde phosphate dehydrogenase (Gapdh), β-actin, and β-tubulin. We found many miRNAs were predicted to bind to their UTRs simultaneously (Table I). We then choose miR-138 and Gapdh, its potential target to verify this further. Unexpectedly, miR-138 did not regulate Gapdh at either the mRNA or protein level, however, the luciferase activity, which originated from a recombinant plasmid containing a wild-type 3′ UTR of Gapdh, was regulated by the miR-138 mimics and inhibitor (Supplementary Fig. 2).

Although miR-138 did not regulate the expression of Gapdh in our experiments, it is impossible to exclude that other miRNAs would not affect this housekeeping gene. At the same time, our results suggested that the luciferase activity assay might produce a false positive result.

The functions of miRNAs are quite diverse. In addition to the aforementioned mechanisms of gene regulation through 3′-UTR interactions, other "non-canonical" miRNA-mediated mechanisms of the modulation of gene expression are emerging. Some miRNAs have been shown to bind to the open reading frame or to the 5′-UTR of their target genes, some miRNAs can bind to ribonucleoproteins in a seed sequence, and some miRNAs can also regulate gene expression at the transcriptional level by binding directly to the DNA. Many factors, such as the abundance of target mRNA [Arvey et al., 2010], length of UTR [Cheng et al., 2009], spatial location of the UTR [Majoros and Ohler, 2007] and alternative polyadenylated variants of the UTR [Ghosh et al., 2008], have a potentially large impact on gene regulation by miRNAs. These findings suggest that the algorithms that depend on base complementarity now need to improve, or new prediction software based on these new principles need to be invented, such as mRNA cytoplasmic/nucleic ratios strategy [Li et al., 2010] or HOCTAR software.

Regardless, our analyses are relevant to investigators embarking on canonical strategies for miRNA target identification. We suggest that one point should be considered: before choose housekeeping gene, a prediction made by an alternative algorithm should be applied to determine whether this individual miRNA could target the housekeeping gene, as the miRNAs "target pools" in cells has not been elucidated.

Ning Ma and Ying Xiang contributed equally to this work.

Additional Supporting Information may be found in the online version of this article.

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TABLE I. miRNAs Predictions for Housekeeping Gene

Housekeeping gene (length of 3′ UTR)	Popular software			
	TargetScan	miRanda	Pictar	miRGen
Gapdh (200 nt)	miR-138, miR-18a, miR-18b[a]			
Actb (599 nt)	miR-145, miR-205			
Tubb (1019 nt)	miR-200b/c, miR-429			

Gapdh, glyceraldehyde phosphate dehydrogenase; Actb, β-actin; Tubb, Tubulin-beta.
[a]Gapdh is not present in Pictar database.

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