Experimental verification of microRNA targets is essential, prediction alone is insufficient

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Dear Sir,

We refer to recent ‘Letter-to-the-Editor’ from Ma N and Gao X regarding the use of β-actin, a predicted target of hsa-miR-145, as housekeeping control in our earlier published article (1). The authors suggested four target prediction tools, namely TargetScan, miRanda, PicTar and miRGen, could identify β-actin as a potential target of hsa-miR-145 and hence questioned its suitability as internal control in western blot analysis.

In response to the authors’ concern, we first would like to draw attention to some practical aspects of computational micro RNA (miRNA) target analysis. It is widely known that many current algorithms used for the identification of miRNA targets have limited positive predictive value (2,3). In fact, most predicted results would require careful scrutiny of target:miRNA complementarity, and experimental validation to confirm target association and influence on gene expression. We surveyed the four proposed target prediction tools for the energy score between hsa-miR-145 and β-actin. Using the developer’s criteria for classifying a ‘successful predicted target’ of each tool, we found only TargetScan and miRanda would regard β-actin as a potential target of hsa-miR-145 and hence questioned its suitability as internal control in western blot analysis.

More importantly, validity of predicted miRNA targets can only be established in validation experiments. In our earlier publication on hsa-miR-145 in hepatocellular carcinoma (1), we have taken precaution in realizing that some housekeeping genes can be influenced by miRNA re-expression. We had assessed both β-actin and glyceraldehyde 3-phosphate dehydrogenase in the presence of hsa-miR-145 mimic, and found stable protein expression of β-actin in repeated experiments. Of interest, glyceraldehyde 3-phosphate dehydrogenase, not a predicted target of hsa-miR-145, was shown to be consistently downregulated; suggesting the probable indirect effect from hsa-miR-145. The protein loading in these experiments was controlled by Bradford quantitation and results from hsa-miR-145 mimic were compared with mock control (1). In support of our finding, published articles of hsa-miR-145 in other cancer types also utilized β-actin as housekeeping control for western blot experiments (4,5). Moreover, experimentally validated hsa-miR-145 targets from miRTarBase (6) documented many genes but not β-actin. Overall, these results would suggest β-actin is unlikely a true target of hsa-miR-145.

In sum, we believe computational software can assist in predicting potential target genes of miRNA; however, they remain candidates until proven experimentally.

References

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