In silico prediction and validation of Gfap as an miR-3099 target in mouse brain

ABSTRACT

MicroRNAs are small non-coding RNAs that play crucial roles in the regulation of gene expression and protein synthesis during brain development. MiR-3099 is highly expressed throughout embryogenesis, especially in the developing central nervous system. Moreover, miR-3099 is also expressed at a higher level in differentiating neurons in vitro, suggesting that it is a potential regulator during neuronal cell development. This study aimed to predict the target genes of miR-3099 via in-silico analysis using four independent prediction algorithms (miRDB, miRanda, TargetScan, and DIANA-micro-T-CDS) with emphasis on target genes related to brain development and function. Based on the analysis, a total of 3,174 miR-3099target genes were predicted. Those predicted by at least three algorithms (324 genes) were subjected to DAVID bioinformatics analysis to understand their overall functional themes and representation. The analysis revealed that nearly 70% of the target genes were expressed in the nervous system and a significant proportion were associated with transcriptional regulation and protein ubiquitination mechanisms. Comparison of in situ hybridization (ISH) expression patterns of miR-3099 in both published and in-house-generated ISH sections with the ISH sections of target genes from the Allen Brain Atlas identified 7 target genes (Dnmt3a, Gabpa, Gfap, Itga4, Lxn, Smad7, and Tbx18) having expression patterns complementary to miR-3099in the developing and adult mouse brain samples. Of these, we validated Gfap as a direct downstream target of miR-3099 using the luciferase reporter gene system. In conclusion, we report the successful prediction and validation of Gfap as an miR-3099 target gene using a combination of bioinformatics resources with enrichment of annotations based on functional ontologies and a spatio-temporal expression dataset.

Keyword: Target gene; Neurogenesis; In silico*;* Astrogliogenesis; Bioinformatics