SignaGen® Laboratories

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Application Note

Suppression of microRNA via Tough Decoy rAAV

INTRODUCTTION:

MicroRNAs (miRNAs) are small non-coding RNAs found in plants, animals, and some viruses, which function in RNA silencing and post-transcriptional regulation of gene expression. Analysis of miRNA function as well as therapeutic manipulation of miRNA levels rely on cellular administration of miRNA inhibitors which may be achieved by the use of adeno-associated virus (AAV) vehicle. In the present application note, we compared several methodologies to inhibit miRNA expression via recombinant AAV (rAAV) vector and identified "Touch Decoy" inhibitor is a reliable and the most potent approach to inhibit miRNA expression.

MOTHEDS & RESULTS:

1. Construction of rAAV Vector-encoded miRNA Inhibitors.

To identify potency of DNA-encoded miRNA inhibitors, we made rAAV cis constructs based on three different approaches that are antagomiR, shRNA against the mature miRNA and touch decoy (TD) (Figure 1A). Briefly the antagomiR, shRNA and TD were designed, synthesized and cloned respectively into a rAAV cis vector under U6 promoter with co-expression of GFP under CMV promoter (Figure 1B). Then the antagomiR, shRNA and TD rAAVs were then packaged via helper-free system followed by purification and qPCR titration. The miRNAs including miR-221, miRNA-18a and miR-19a which are endogenous expressed in HEK293T, 293A and Lenti-X 293 cells (Figure 1C) were chosen to test the potency of the three miRNA inhibitors. To quantify the miRNA expression, the three mature miRNAs were cloned respectively in an expression vector in fusion with Renilla luciferase (rLuc) as a single transcript so that measurement of rLuc activity can reflect the degradation of miRNA. (Figure 2A).

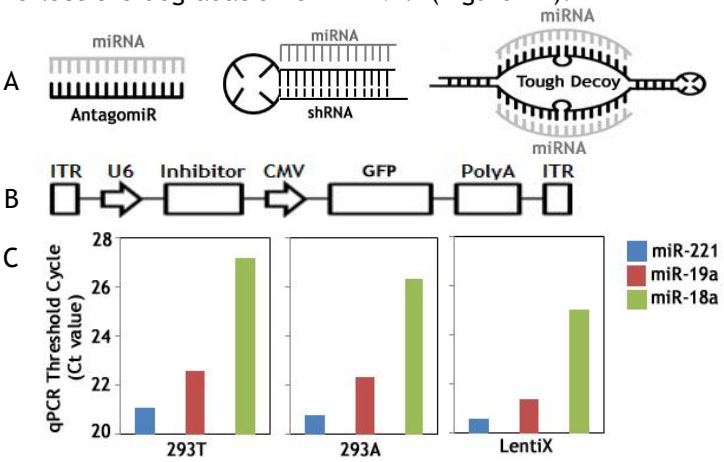

Figure 1. Strategy for miRNA Suppression. (A) A cartoon showing the three types of miRNA inhibitors used in the current study. (B) Schematic representation of the AAV cis viral construct used for expression of miRNA inhibitors under U6 promoter. (C) Endogenous miRNA expression in HEK-293T, Lenti-X and HEK293A cells. qPCR threshold cycles (Ct) were determined for miR-221, miR-19a and miR-18a.

2. Comparison of rAAV Vector-encoded miRNA Inhibitors Demonstrates Superior Suppression via Tough Decoy Approach.

Co-transfection of rAAV cis constructs that carry TD inhibitor with miRNA validation constructs to HEK293T cells significantly (average >80%) inhibited miRNAs expression 48 hours post transfection (Figure 2B). To compare the potency of the three types of miRNA inhibitors delivered via rAAV vector, we transfected HEK293 cell with miRNA validation construct followed by infection with anatagomiR, shRNA and TD rAAVs in serotype 2. To enhance rAAV infectivity, we co-infected HEK293T cell with an empty adenovirus. 4 Days after infection, the expression of all the three miRNAs was significantly deceased (average >70%) after treatment with TD rAAV (Figure 2C) in comparison of scrambled rAAV.

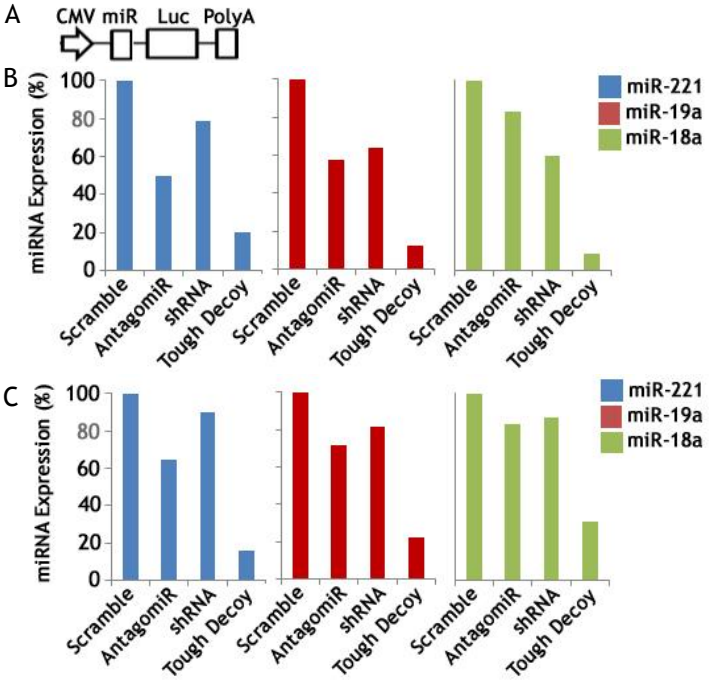

Figure 2. Superior Suppression of miRNA Expression via Touch Decoy Approach. (A) A cartoon showing miRNA validation construct. (B) Degradation of the three miRNAs via co-transfection of the miRNA validation construct and rAAV cis viral constructs carrying the three miRNA inhibitors. (C) Degradation of the three miRNAs via transfection of the miRNA validation construct followed by infection with rAAV vectors carrying the three miRNA inhibitors.

CONSCLUSION:

The present comparison study shows touch decoy rAAV vector is a reliable and the most potent approach to inhibit miRNA expression.

REFERENCES:

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