Combination Delivery of Small RNAs Enhances Muscle Regeneration

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Statement of Purpose: Selectively controlling the expression of the target genes through synthetic small RNAs has significant potential for treatment of numerous injuries and diseases. Taking advantages of this process, we utilized small RNAs related to muscle tissue regeneration. We hypothesized that delivery of multiple small RNAs to direct the production of various cellular factors could control host microenvironment and promote natural healing ability for *in situ* muscle tissue regeneration in a targeted site. Myostatin (GDF-8) is a major inhibitory factor in development and postnatal regeneration of skeletal muscle tissue. Using small interfering RNA (siRNA) targeting myostatin (siGDF-8), we expect to decrease myostatin expression hence leading to muscle regeneration. In addition to siGDF-8, muscle-specific microRNAs (miRNAs), well-known regulators of muscle development, were utilized to further elevate muscle regeneration. These small RNAs control post-transcriptional gene expression of myogenic regulatory factors. Therefore, the objective of this study was to investigate whether combination delivery of small RNAs could synergically enhance myogenic regulatory factors for muscle regeneration.

Methods: To evaluate the myogenic potentials of small RNAs, we used three different small RNAs, siGDF-8 (Santa-Cruz), miR-1, and miR-206 (IDT). Mouse myoblast cells (C2C12) were transfected with each or in combination of the RNAs, and incubated in differentiation media with 2% horse serum for pre-determined period. For gene expression analysis, Total RNA was extracted on day 2 followed by cDNA synthesis and real-time PCR. The data was reported as mean ± standard deviation from triplicate RT-qPCR reactions of each triplicate sample. To evaluate cell proliferation and differentiation potential, the cells were cultured up to 4 days after transfection and stained for myosin using antibody against MF 20 (DSHB). Nuclei were stained with DAPI. The images were analyzed for number of cells and fusion index.

Results: Small RNAs individually increased gene expression of myogenic regulatory factors (Fig. 1). siGDF-8 increased gene expression of MyoD, myogenin, Pax7, and myosin heavy chain 1 (MyHC1) by 2-3 times more than control siRNA transfection. miR-1 also increased gene expressions of MyoD, myogenin, Pax7, and significantly increased MyHC1 gene expression. miR-206 increased gene expressions of MyoD, Pax7, and significantly increased the expressions of myogenin and MyHC1. Combining miRNAs (miR-1 and miR-206) did not have significant difference on the gene expression. However, adding siGDF-8 to these miRNAs significantly increased gene expressions of all the myogenic regulatory factors tested, which demonstrates synergistic effect of this particular combination of small RNAs on myogenic development.

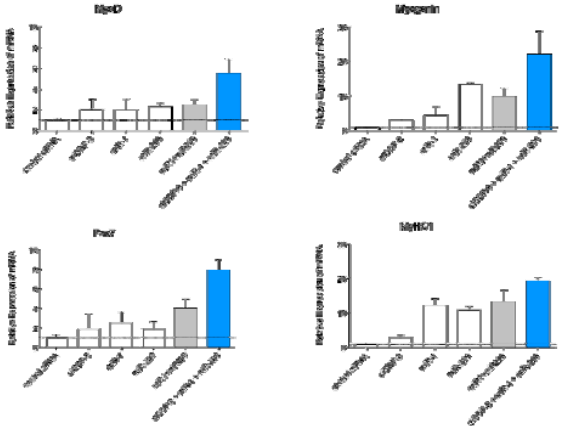

Figure 1. Increased gene expression of myogenic regulatory factors by combination delivery of small RNAs

This combination of small RNAs also enhanced myosin protein expression suggesting improved differentiation of myoblasts into myotubes. It also resulted in acceleration of myoblast proliferation. As a result of this dual enhancement on differentiation and proliferation, the combination delivery significantly increased myofibers development showing improved fusion index (Fig. 2).

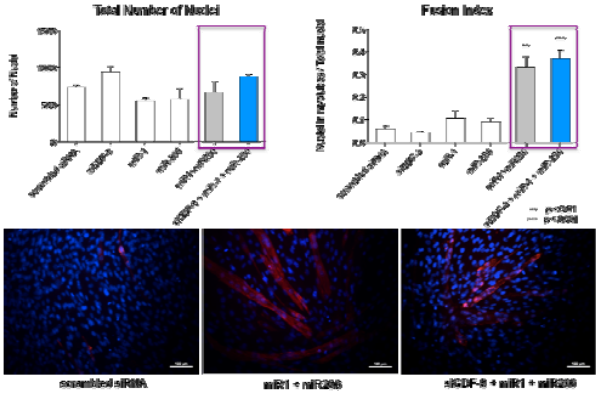

Figure 2. Enhanced myotubes differentiation and myoblast proliferation by combination delivery of small RNAs

Conclusions: Combination of small RNAs boosted myogenic activation by improving the gene expression of myogenic regulatory factors such as MyoD, myogenin, Pax7, and MyHC1. They improved the differentiating and proliferating potential of myoblasts. The myogenic impact triggered by multifactorial small RNAs was superior when compared with the effects of any single- or two-factor mixture. This novel combination of siGDF-8 and miRNAs is expected to have great therapeutic potential to fine-tune functional muscle recovery.

References:

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[2] Townley-Tilson, W.H. et al. Int J Biochem Cell Biol. 2010;42:1252-1255.