Synthetic Small RNA Delivery for *In Situ* Muscle Tissue Regeneration

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Statement of Purpose: Selectively controlling the expression of the target genes through small RNAs has significant potential for treatment of numerous injuries and diseases. Taking advantages of this process, we utilized small RNAs for muscle tissue regeneration. In addition, we introduced combinations of multiple small RNAs to direct the production of various cellular factors, which could promote *in situ* muscle tissue regeneration in a targeted site. Using small interfering RNA (siRNA) targeting myostatin (siGDF-8), a major inhibitory factor in development and postnatal regeneration of skeletal muscle, we expect to decrease myostatin expression hence leading to muscle development. In addition to siGDF-8, muscle-specific microRNAs (miRNAs), well-known regulators of muscle development, were utilized to further elevate muscle regeneration. We expect this novel combination of small RNAs would enhance natural mechanism of muscle recovery and regeneration.

Methods: To evaluate the myogenic potentials of small RNAs in vitro, we used three different small RNAs, siGDF-8, miR-1, and miR-206. Murine myoblasts were transfected with individual or combinations of the RNAs, and incubated in differentiation media until used for gene expression, proliferation, and differentiation analyses. For *in vivo* evaluation, chemically injured Lewis rat tibialis anterior (TA) muscles were treated with RNAs and analyzed for functional and structural recovery.

Results: All of the individual small RNAs increased gene expression of myogenic regulatory factors (MRFs), including MyoD, myogenin, Pax7, and myosin heavy chain 1 (MyHC1). Combining two miRNAs, miR-1 and miR-206, did not have significant differences on the gene expression of MRFs. However, adding siGDF-8 to these miRNAs significantly increased gene expressions of all the myogenic regulatory factors tested. This demonstrates synergistic effects of this particular combination of small RNAs on myogenic development. This combination of small RNAs also enhanced myosin protein expression by miR-1 and miR-206 suggesting improved differentiation of myoblasts into myotubes. Moreover, it also resulted in acceleration of myoblast proliferation by the action of siGDF-8. The differentiation and proliferation attests that miRs and siGDF-8 improved myoblast development without compromising the mechanisms of each other when used together. As a result of this dual enhancement on differentiation and proliferation, the combination delivery significantly increased overall myofibers development showing improved fusion index.

This synergistic effect of small RNAs was reflected well on the recovery of chemically injured rat tibialis anterior (TA) muscles. Structural and functional recovery of hind leg was significantly accelerated by this combination delivery of siGDF-8, miR-1, and miR-206.

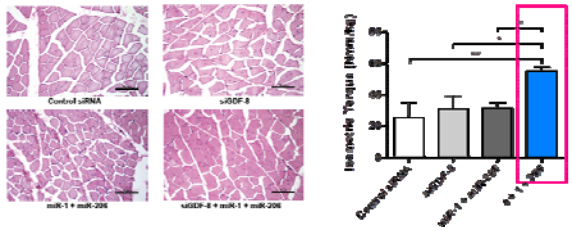

Figure 1. Improved skeletal muscle recovery by combination delivery of small RNAs (n = 5, **P*<0.05).

Conclusions: Combinations of small RNAs enhanced myogenic activation by overexpressing MRFs such as MyoD, myogenin, Pax7, and MyHC1. This improved gene expression boosted the overall development capacity of myoblasts. The combination delivery also accelerated *in vivo* regenerative efficiency compared with the effects of any single- or two-factor mixture. This novel combination of siGDF-8, and miR-1 and miR-206 is expected to have a great therapeutic potential to fine-tune skeletal muscle recovery from traumatic injury.

References:

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

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