Identifying novel miRNAs targeting NDRG2 regulation in skeletal muscle

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate genes involved in skeletal muscle biological processes. Ndrg2 is a stress-responsive gene highly expressed in differentiated muscle and plays a role in muscle cell growth and metabolism. In this study, we investigated whether miRNAs contributed to the control of Ndrg2 expression in skeletal muscle. MiRNA prediction softwares, miRWalk2.0 and microRNA.org, and literature searches were used to identify possible miRNAs that target the mouse 3′-untranslated region (UTR) of the Ndrg2 gene in the context of skeletal muscle. As a result, the miRNAs, miR-23a, -23b, and -28, were identified. To confirm an interaction with Ndrg2 3′UTR, luciferase reporter assays were performed in HEK293 cells and found that all 3 mimics significantly decreased luciferase activity by 33%, 35% and 44% for miR-23a (P<0.05), miR-23b (P<0.01) and miR-28 (P<0.0001), respectively. Their sites of interaction were further confirmed through luciferase assays containing only the Ndrg2-specific seed sequences. No binding was observed, however, when the seed sites were mutated. These findings suggest that these miRNAs could potentially target and regulate the endogenous Ndrg2 gene in muscle, which was next investigated. Individually, miR-23a, -23b, and -28 mimic overexpression had no influence on NDRG2 mRNA or protein levels up to 72 h post treatment in mouse myotubes. Next, we speculated whether the overexpression of a miRNA could impact the expression of another due to a compensatory effect. Indeed, the overexpression of each mimic caused a subsequent decrease in the endogenous expression of the other miRNA. As an example, miR-23a overexpression caused a 35% decrease in endogenous miR-23b levels (P<0.05). Therefore, myotubes were transfected with a cocktail of all three mimics to determine if the combined treatment could suppress endogenous NDRG2 levels. This approach, however, still did not result in any significant change in NDRG2 expression. In conclusion, these findings established that specific miRNAs were able to bind the 3’UTR of Ndrg2 gene and inhibit luciferase activity *in vitro*; however, we were not able to observe any overt impact on endogenous NDRG2 regulation in skeletal muscle.