In Vitro Validation and Characterization of Muscle-Specific Antibody-siRNA Conjugates for Targeted Delivery of siRNA

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The use of therapeutic siRNA to silence genes in a sequence-specific manner has been limited by its need for delivery to target cells. However, while antibody-mediated siRNA delivery shows promise as a delivery mechanism, discovery of extrahepatic ligand-receptor systems remains an ongoing challenge. In this study, we evaluated the effectiveness of AbA, a muscle-specific antibody, for siRNA delivery by conjugating to a muscle-targeting siRNA (siRNA1) and analyzing the knockdown and binding of AbA-siRNA1 conjugates in vitro. Furthermore, we investigated the use of differentiated C2C12 cells as a myotube model by evaluating the expression of an antigen typically only found on myotubes (AgA). Using RT-qPCR to determine mRNA levels, knockdown activity of AbA-siRNA1 was compared to that of siRNA1 alone and isotype-siRNA1 to evaluate the impact of conjugation on RNAi. Flow cytometry was then used to assess cell binding and quantify AgA expression of differentiated C2C12 cells. AbA-siRNA1 was found to efficaciously knock down the target gene in a dose-dependent manner with similar potency to the controls, suggesting that conjugation did not affect knockdown. Additionally, conjugates showed specific binding with high affinity to AgA. However, binding was slightly less potent compared to AbA alone; this was speculated to be caused by the relatively high drug-antibody ratio. Finally, differentiated C2C12s were found to express AgA to a higher extent than undifferentiated cells, indicating endogenous expression. In future studies, AbA could be a compelling candidate for muscle-targeted siRNA delivery, while differentiated C2C12s could be used to better represent in vivo skeletal muscle conditions.

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