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Construction of shRNA vectors against BTB-Kelch substrate adaptor proteins

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The BTB-Kelch substrate adaptor proteins are proteins that contain an N-terminal BTB and a C-terminal Kelch domain. These proteins function as substrate adaptors for protein ubiquination. BTB-Kelch substrate adaptor proteins may influence the differentiation of C2C12 myoblasts into myotubules. To better understand the role of BTB-Kelch proteins for the differentiation of myoblasts cells, we designed short hairpin RNA (shRNA) molecules to inhibit mRNA expression of five BTB-Kelch proteins found in Mus musculus (mouse). These proteins are: klhl9, klhl24, kbtbd8, klhdc8b, and Enc1. Expression vectors for the shRNAs were constructed using pSico and pSicoR, which are lentiviral vectors that contain an internal U6 promoter for expression of a shRNA molecule and a CMV-GFP cassette. Positive clones were identified by restriction mapping. Positive clones were purified and sequenced. The plasmid DNAs were transfected into 293T cells to generate virus. The viruses were used to infect C2C12 myoblasts. Two days after infection, the cells will be examined for GFP expression, which will show a green fluorescent marker in the infected cells verifying successful uptake of the virus. Further analysis will identify any differences in the differentiation of C2C12 myoblasts into myotubules following shRNA-mediated knockdown of these BTB-Kelch proteins.