Targeting RNA structure in SMN2 reverses Spinal Muscular Atrophy molecular phenotypes

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Modification of *SMN2* exon 7 (E7) splicing to increase SMN protein production is a validated therapeutic strategy against Spinal Muscular Atrophy (SMA). Based on this, we have performed the first small molecule screening described for SMA, choosing a stem-loop RNA structure TSL2 that partially overlaps with the E7 5' splicing site (5' ss) of SMN2 as the biological target. TSL2-binding hit PK4C9 was found to also increase E7 splicing and rescued downstream molecular alterations in transfected HeLa cells, transgenic Drosophila, and SMA patient cells. High-resolution NMR combined with *in silico* modeling revealed that PK4C9 binding to TSL2 promotes a conformational shift towards a triloop conformation, which we also demonstrate that is associated with an enhanced E7 splicing efficiency. This work not only provides one of the few examples of small molecules with direct *SMN2*-spllicing modifier activity, but also opens new avenues for rational drug discovery in SMA and other splicing-mediated diseases where similar RNA structures are involved.