Splicing misregulation leads to metabolic vulnerability: vitamin B12 as a candidate therapy for PUF60-related Verheij syndrome

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Background: mRNA splicing shapes proteome diversity and cellular state, and its disruption underlies a growing family of spliceosomopathies whose pathomechanisms remain poorly defined. Verheij syndrome, a rare multisystem disorder of developmental retardation and short stature, arises from mutations in the core splicing factor PUF60. How mis-splicing produces this disease, and whether it can be mitigated, has remained unknown. Method: We modeled PUF60 deficiency in Caenorhabditis elegans by introducing a hypomorphic mutation into the sole ortholog rnp-6 or inducibly depleting the protein. Mechanistic studies included unbiased bacterial and mutagenesis screens, transcriptome-wide splicing analysis, metabolomics, lipidomics, and validation in PUF60-depleted human cells and patient plasma. Results: In C. elegans, RNP-6/PUF60 deficiency resulted in mis-spliced genes governing one-carbon and phospholipid metabolism, lowering methylation potential (SAM/SAH ratio) and depleting phosphatidylcholine. These perturbations activated the integrated stress response and suppressed mTORC1, delaying growth. Two independent screens converged on a single corrective input: vitamin B12. Supplementation of vitamin B12 restored SAM-dependent phospholipid remodeling and mTORC1 activity and rescued Verheij-like phenotypes. Mechanistically, intron retention in the transcription factor nhr114/HNF4 emerged as a proximal disease driver. Forcing retention reproduced the disease state, intron deletion relieved the defects. Depleting another splicing factor, PRP-19, recapitulated the same metabolic disruption, and PUF60-deficient human cells and patient plasma showed parallel dysregulation of one-carbon and lipid metabolism. Conclusion: Mis-splicing of a metabolic transcription factor links spliceosomal dysfunction to disrupted methylation and phospholipid metabolism. Vitamin B12 supplementation reverses metabolic phenotypes to restore growth. Our findings suggest one-carbon metabolism as a conserved vulnerability across spliceosomopathies, and warrant further investigation of vitamin B12 as a therapeutic strategy for spliceosomopathies.