The Role of Reversible AKAP12 Lysine Myristylation in PKA signalling

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The role of the lone class IV histone deacetylase (HDAC), HDAC11, is poorly understood. It was recently revealed that HDAC11 is a 10,000-fold more efficient lysine demyristoylase than deactylase, removing 14-carbon fatty acid moieties from lysine residues. Previous work from the McKinsey lab identified the A-kinase anchoring protein, AKAP12, as a demyristoylase substrate of HDAC11 in adipocytes, publishing just the second ever described HDAC11 substrate in 2022. Upon pharmacological inhibition or genetic inactivation of HDAC11, AKAP12 myristoylation increases, promoting PKA signalling and thermogenic (UCP1) gene expression in adipocytes, in a manner that is independent of the presence of beta-adrenergic receptors. In vivo, loss of HDAC11 enhances the proportion of thermogenic, UCP1-expressing adipose tissue and protects mice from weight gain and metabolic disease in the context of high fat feeding.

We are now looking for more specific molecular approaches that will enhance AKAP12 myristoylation and PKA signalling and promote UCP1 expression in adipocytes in vitro and in vivo.

Our lab has identified a conserved 10-amino acid sequence in rat AKAP12 that is essential for HDAC11 binding. Treating 3T3L1 adipocytes with a ‘disruptor’ peptide that reflects the 10-amino acid sequence of mouse AKAP12, also promoted PKA signalling and UCP1 activation, even in the absence of beta-adrenergic stimulation.

However, this approach does not successfully enhance PKA signalling in human adipocytes. We hypothesise that the HDAC11 binding site on human AKAP12 is not identical to that in rat and mouse AKAP12. We are now using a molecular cloning approach to identify the amino acid stretch that is necessary for HDAC11 binding on human AKAP12.

The experimental plan is to clone full length human AKAP12 and a series of C-terminal truncations into the pCMV-3X-Flag vector backbone. We will subsequently co-transfect these constructs along with Myc-HDAC11 and perform co-immunoprecipitation to look at the interaction of AKAP12 and HDAC11 in human adipocytes.

Our work provides an alternative approach to enhancing thermogenesis in adipocytes to that of classic beta-adrenergic receptor agonism. This may guide future development of therapeutics for obesity and metabolic disease.