The Role of Reversible AKAP12 Lysine Myristoylation in PKA Signaling in Adipocytes

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Abstract

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. 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 (**Gravi-D**) 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 (Figure 4). 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.

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.

Background: HDAC11 Demyristoylates Two Conserved Lysines on AKAP12. (Figure 1)

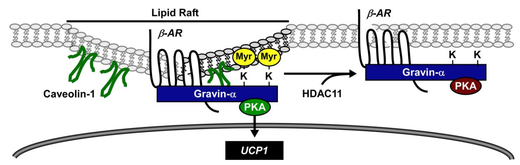

Figure 1: HDAC11 Inhibition Promotes AKAP12 Myristoylation and PKA Signaling (Bagchi et al., 2022)

Background: The HDAC11 Binding Domain on Rodent AKAP12 has been Identified (Figure 2)

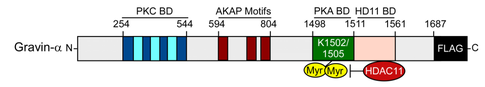

Figure 2: Schematic of Rat AKAP12, with the sites of lysine myristoylation, PKA and HDAC11 binding indicated. (Bagchi et al., 2022)

Background: Interaction of disruptor peptide 'GraviD' with HDAC11, inhibiting HDAC11's interaction with full length endogenous Gravin (Figure 3)

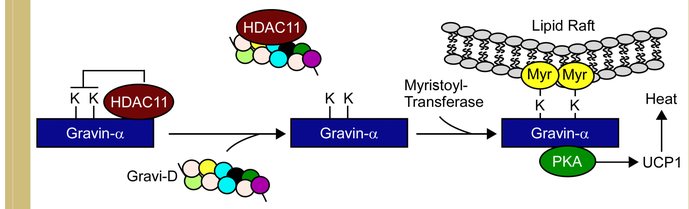

Figure 3: Schematic depicting HDCA11 interaction with GraviD, inhibiting the interaction of HDAC11 with endogenous Gravin.

Pharmacological HDAC11 inhibition or is Associated with Increased UCP1 Expression and PKA Gene Expression in Mouse Adipocytes (Figure 4)

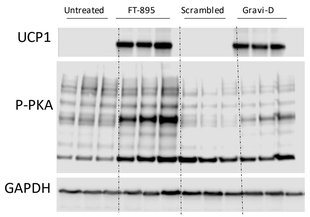

Figure 4: Immunoblot of Ucp1, p-PKA and reference gene a-tubulin in differentiated 3T3L1 murine adipocytes treated with 10 uM FT895 and 1uM GraviD peptide.

Schematic prediction of HDAC11 binding domain on human AKAP12 (Figure 5a)

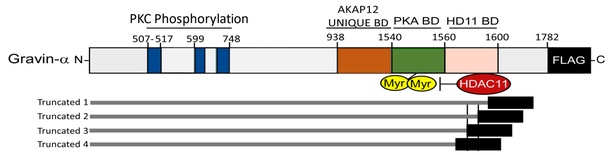

Figure 5a: Schematic of hAKAP12 with HDAC11 binding domain predicted

PCR agarose gel of Full length and truncated human AKAP12 constructs (Figure 5b)

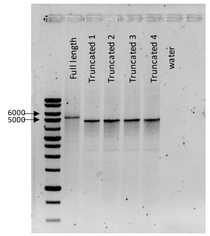

Figure 5b: PCR agarose gel full length and truncated human AKAP12

Ectopic Expression of FL human AKAP12 in HEK293 cells (Figure 6)

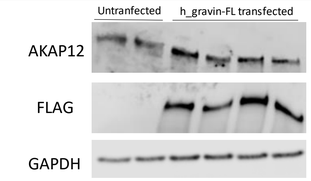

Figure 6: Immunoblot of AKAP12, Flag and reference gene GAPDH in HEK293A cells transfected with h-AKAp12/gravin-FL constructs

Conclusions

- **Pharmacological HDAC11 inhibition with FT895 and GraviD peptide in rodent 3T3L1 in culture induces thermogenic (UCP1) gene expression and PKA activation.**
- **Cloning full length h-AKAP12 into p-CMV-3x-FLAG vector was successful and we will continue the same for the C-terminal truncation constructs.**
- **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.**

Future Directions

- **Clone all the full length and truncated constructs and perform co-immunoprecipitation experiments with Myc-tagged HDAC11 to narrow down the region of human AKAP12 to which HDAC11 binds.**
- **Design a 'disruptor' peptide according to the region of AKAP12 to which HDAC11 binds and assess its ability to promote thermogenic gene expression and PKA signaling in vitro.**

Acknowledgements, Ethics and Disclosures

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NIH National Institutes of Health
American Heart Association.

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3. Qasim et al., JAHA, 2020, Jul 7;9(13):e016615.