Role of AIG1 and ADTRP in Endogenous FAHFA Regulation

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Abstract
Androgen-induced gene 1 (AIG1) and Androgen-dependent TFR1-regulating protein (ADTRP) are aromatic transmembrane hydrolases that rely on a catalytic threonine for enzymatic activity. Characterization of AIG1 and ADTRP in vitro identified fatty acid ester of hydroxy fatty acids (FAHFAs) as their putative substrates. Here, we generate ADTRP knockout (Adtrp-KO), AIG1 knockout (Aig1-KO), and ADTRP/AIG1 double deficient (DKO) mice using CRISPR/Cas9 to test whether these enzymes regulate FAHFAs in vivo. Deficiency of AIG1, ADTRP, or both enzymes leads to decreased FAHFA hydrolytic activity in tissue lysates. Quantitative measurement of FAHFA levels in several tissues revealed increased FAHFA levels in brown adipose tissue (BAT), subcutaneous adipose tissue (SCWAT), and perigonadal WAT (PGWAT) of Adtrp-KO mice consistent with the loss of FAHFA degrading activity. Furthermore, contribution by AIG1 was modest and only observed in kidney and BAT of DKO mice. Lipidomics analysis of tissues from mutant mice detected no significant changes in other lipid classes to indicate that these enzymes are specific for FAHFA substrates. Furthermore, we developed a potent, AIG1/ADTRP inhibitor to enable pharmacological interrogation of these enzymes in vivo. Chemical inhibition of AIG1 and ADTRP raised FAHFA levels demonstrating acute regulation of FAHFAs. In aggregate, these results establish AIG1 and ADTRP as the only endogenous FAHFA hydrolases known, and describe tools (mice, inhibitor) needed to elucidate the biochemical and physiological role of these enzymes.

Introduction
FAHFAs are a novel class of lipids with anti-inflammatory and gluoregulatory functions. They are named based on their constituent fatty acid and hydroxy fatty acid chains (e.g., Palmitic acid and hydroxystearic acid; PAHSA). Regiosomers are specified based on the carbon number where branching occurs (e.g., 9-PHSA). PAHSA levels are positively correlated with insulin sensitivity in humans. Pharmacological treatment of 5- and 9-PHSA in mice leads to improved insulin sensitivity and glucose metabolism, and decreased inflammation in adipose tissue of high fat diet induced obese mice, a colitis model, and a mouse model of Type I diabetes[1]. Given the novelty and beneficial bioactivity attributed to FAHFAs, it is important to identify the associated pathways in vivo that regulate endogenous FAHFA levels.

Figure 1: FAHFA hydrolysis by AIG1 and ADTRP. (A) Palmitic acid, 9-HS: 9-hydroxystearic acid). AIG1 and ADTRP have been identified as putative FAHFA hydrolases in vitro[2]. Here we demonstrate that both AIG1 and ADTRP act as FAHFA hydrolases endogenously using a combination of genetic and chemical approaches. We show that AIG1 and ADTRP deficient mice have elevated levels of FAHFAs in adipose depots and they can be targeted via small molecule inhibitors.

Figure 2: (A, C) CRISPR/Cas9 targeting strategy for generation of Aig1- and Adtrp-KO mice. (B, D) Confirmation of Aig1- and Adtrp-KO mice via Western blot.

AIG1 and ADTRP contribute to FAHFA hydrolysis in tissue lysates. Tissue lysates from Aig1-KO, Adtrp-KO, DKO and WT controls were incubated with excess 9-PHSA to assess contribution of AIG1 and ADTRP to FAHFA hydrolysis. End product, 9-HSA was measured to calculate hydrolytic activity. The contribution of AIG1 and ADTRP were additive in brown adipose tissue (BAT), kidney, and liver (Fig. 3).

Figure 3: Hydrolytic activity represented as percent of WT in (A) BAT, (B) kidney, and (C) liver of Aig1-KO, Adtrp-KO, DKO mice.

Figure 4: Fold change of FAHFA levels compared to WT in (A) BAT, (B) SCWAT, (C) PGWAT, (D) kidney, (E) liver, and (F) plasma of mutant mice.

AIG1 and ADTRP are specific FAHFA hydrolases. Lipidomics analysis was performed in mutant mice compared to WT controls to test whether AIG1 and ADTRP regulate other lipid classes. No differences were observed in lipids that were identified (Fig 5).

Figure 5: Lipidomics analysis of representative lipid classes in BAT, liver and brain of mutant and WT mice. PE: phosphatidylethanolamine; DG: glycerides; TG: glycerides.

Development of an in vivo-active AIG1/ADTRP dual inhibitor. A potent inhibitor was identified using a screen directed library using ABPP. Among candidates, compound ABD-110207 showed low IC50 for AIG1 and ADTRP (Fig 6A). Mice were administered orally with ABD-110207, and BAT was analyzed for FAHFA hydrolytic activity and FAHFA levels. Within 4 hours of treatment, hydrolytic activity was abolished and 9-FAHFA levels were upregulated ~2 fold (Fig 6B-C).

Figure 6: (A) Inhibition of activity by ABD-110207 on AIG1, ADTRP, FAH, and MGLL and its values. Hydrolytic activity (B), and FAHFA levels (C) in BAT from vehicle or ABD-110207 treated mice.

Conclusions
AIG1 and ADTRP are the first identified endogenous FAHFA hydrolases, though the enzymes seem to have hydrolytic activity in liver or kidney, regulation of FAHFA by these enzymes are mostly limited to adipose tissue depots under experimental conditions tested. This discrepancy could be due to presence of other FAHFA specific enzymes or low basal levels of FAHFA in liver and kidney.

9-FAHFA seems to be subject to higher regulation by AIG1 and ADTRP compared to 12/13-FAHFA suggesting presence of multiple pathways for isomer specific regulation. AIG1 and ADTRP are druggable targets where a chemical inhibitor can be used to block their activity in vivo. Use of AIG1 and ADTRP deficient mice as well as the chemical inhibitor can help elucidate the physiological role of FAHFA in a variety of contexts such as metabolic regulation, and immune response.

References

Acknowledgements
This research was supported by the NIH (DK106210, DK14785, DA033760), The Leone M. and Harry B. Helmsley Charitable Trust (grant #2012-PG-MED002 to A.J.), NCI Cancer Center Support Grant P30 (CA014196 MSSC core, A.J.S.), Dr. Frederick Pauson Chair/Farming Pharmaceuticals (A.S.), a NIH F32 postdoctoral fellowship, DK111159 (M.E.E), a Hewitt Foundation for Medical Research Fellowship (W.H.P.), Mass Spectrometry Core of the Salk Institute with funding from NIH-NCI CCSG: P30 014196, NIH 15100D021815-01 and the Heimskern Center for Genomic Medicine, and Transcend Core Facility of the Salk Institute with funding from NIH-NCI CCSG: P30 014195.

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