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Role of Acetyl-CoA Synthetase 2 and Acetyl-CoA Precursors, Acetate and Ethanol, on Hepatocyte Gene Expression

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Role of acetyl-CoA synthetase 2 and acetyl-CoA precursors, acetate and ethanol, on hepatocyte gene expression

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Introduction

- Abnormally high blood and tissue lipid levels observed in the setting of metabolic disorders and cardiovascular diseases are associated with extensive gene expression changes. Gene expression is modulated by epigenetic mechanisms, which include modifications of DNA-bound protein histones without alteration of DNA sequence. Histone hyperacetylation is observed in the setting of metabolic disorders but the causes have not been described in any detail. We know that histone acetylation is positively correlated with the abundance of acetyl-coenzyme A.
- This study tests the hypothesis that acetyl-coenzyme A metabolizing enzyme acetyl-coenzyme A synthetase 2 (ACSS2, cytoplasmic, aka AceCS1) impacts liver function through histone acetylation, and that dietary precursors of acetyl-CoA (acetate and ethanol) further contribute to these effects. Our long-term goal is to understand mechanistic relationships between diet and histone modification particularly where they present opportunities for the prevention and treatment of cardiovascular disease risk factors.

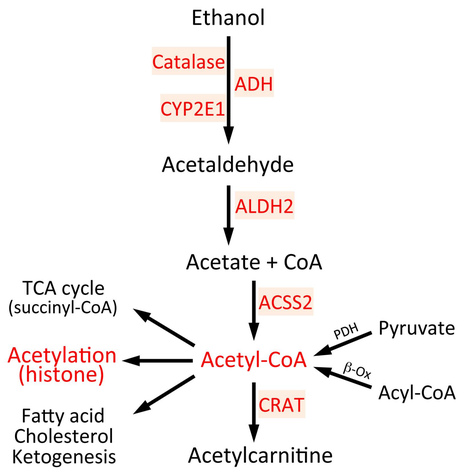


Figure 1. **Metabolism of acetyl-CoA.**
Abbreviations: ADH, alcohol dehydrogenase; CYP2E1, cytochrome P450 2E1; ALDH2, aldehyde dehydrogenase 2; CRAT, carnitine O-acetyltransferase

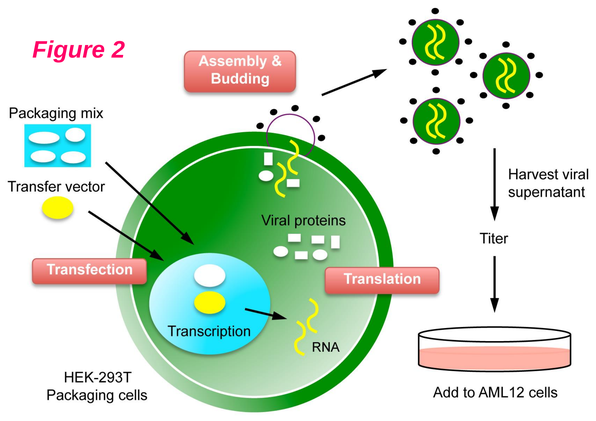
Objectives

- To understand the regulation of gene expression by acetyl-coenzyme A.
- To characterize the role of ACSS2 in liver function in response to acetyl-CoA precursors.
- To assess whether ACSS2 is potential therapeutic target.

Methods

- Lentiviral-based stable overexpression of ACSS2 in mouse AML12 hepatocytes (***Figure 2***).
- AML12 cells treated with Na acetate (5 mM) or ethanol (86 mM, eqv. to 0.5%) for 24 hr.
- qRT-PCR, Western blotting, ACSS activity, and subcellular fractionation according to standard protocols.

Figure 2



Conclusions

- ACSS2 plays a determining role in the gene expression of key liver proteins; albumin and fatty acid synthase (FASN).
- Providing acetate (5 mM) to AML12 cells overexpressing ACSS2 further induced albumin gene expression and albumin secretion. These effects correlated with an increased in histone H3 acetylation.
- Ethanol treatment (86 mM, eqv. to 0.5%) was not as effective as acetate treatment at inducing albumin and FASN gene expression even in ACSS2 overexpressing AML12 cells. However, providing ethanol to AML12 cells overexpressing ACSS2 markedly induced albumin secretion.

Results

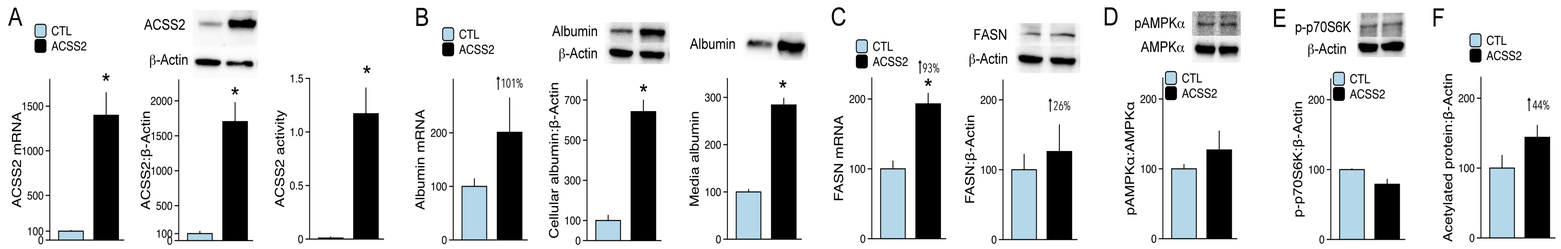


Figure 3. **Characterization & effects of ACSS2 overexpression on albumin secretion, fatty acid synthase expression, AMPK & mTORC1 activities, and protein acetylation.**

A) Characterization of mouse AML12 hepatocytes stably overexpressing ACSS2.
B) ACSS2 overexpression induced the transcription, translation and secretion of albumin.
C) ACSS2 overexpression induced significantly (+97%) the gene expression fatty acid synthase (FASN) and only slightly FASN protein abundance (+26%).
D) AMPK phosphorylation state was unaffected by ACSS2 overexpression.
E) mTORC1 activity, measured by the phosphorylation of its downstream target p70S6 kinase, was unaffected by ACSS2 overexpression.
F) Overall cellular protein acetylation was increased (+44%) although not significantly by ACSS2 overexpression.
*Indicates statistical significance of Student *t*-test. P < 0.05. n = 3-4.

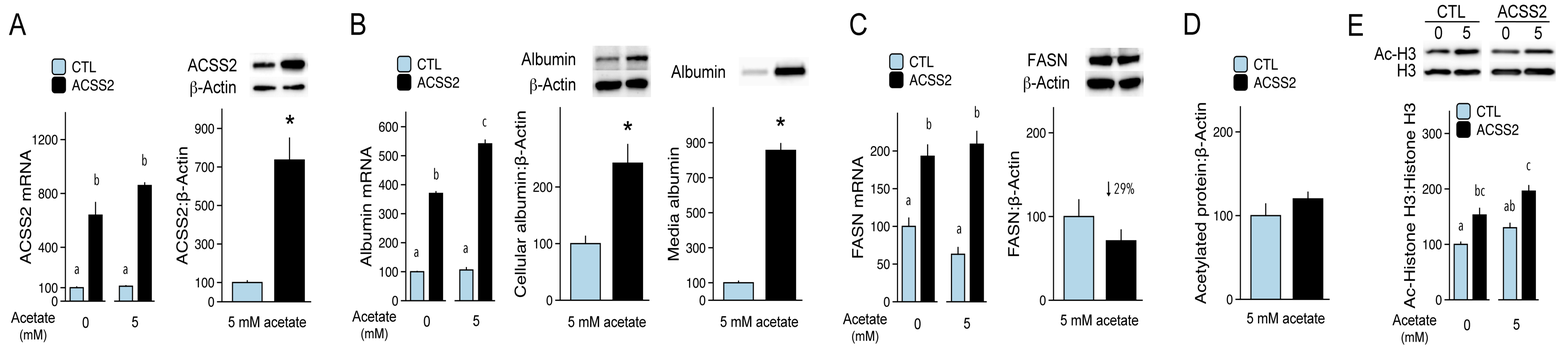


Figure 4. **Sodium acetate enhanced the effects of ACSS2 overexpression on albumin expression, histone acetylation, but did not impact FASN expression.**

A) ACSS2 expression as a function of acetate availability. Acetate did not further increase ACSS2 mRNA or protein abundance.
B) Acetate treatment exacerbated albumin gene expression in ACSS2 overexpressing AML12 cells. Acetate treatment induced albumin protein abundance and secreted albumin.
C) Acetate treatment did not further enhanced FASN expression beyond ACSS2 overexpression and rather decreased FASN protein abundance (–29%).
D) Overall cellular protein acetylation was marginally increased by acetate treatment in AML12 cells overexpressing ACSS2.
E) Histone H3 acetylation was increased by acetate treatment and even more so in ACCS2 overexpressing AML12 cells.
*Indicates statistical significance of Student *t*-test, P < 0.05. For ANOVA (followed by Tukey's test), means not sharing a common letter are significantly different. P < 0.05, n = 3-4.

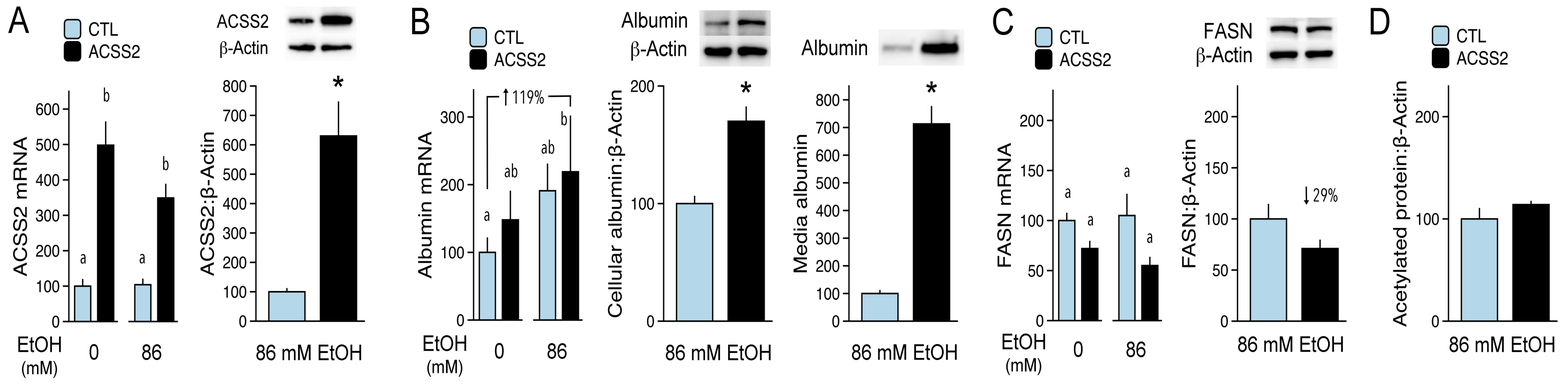


Figure 5. **Ethanol enhanced the effects of ACSS2 overexpression on albumin expression but slightly decreased FASN expression.**

A) ACSS2 expression as a function of ethanol concentration. Ethanol did not further increase ACSS2 mRNA or protein abundance.
B) Ethanol treatment induced albumin gene expression even more so in ACSS2 overexpressing AML12 cells (+119%). Ethanol treatment further increased albumin secretion.
C) Ethanol treatment slightly decreased FASN expression (–29%) in AML12 cells overexpressing ACSS2.
D) Overall cellular protein acetylation was marginally increased by ethanol treatment in AML12 cells overexpressing ACSS2.
*Indicates statistical significance of Student *t*-test. For ANOVA (followed by Tukey's test), means not sharing a common letter are significantly different. P < 0.05, n = 3-4.

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