Theme: Lipid Metabolism

Abstract 2048

Loss of ATP-dependent Citrate Lyase Abrogates Phosphatidate Phosphatase Activity in the oleaginous yeast Yarrowia lipolytica

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Keywords: exogenous fatty acids, heterodimeric, triacylglycerol (TAG), lipogenic, mutant

The PAH1-encoded phosphatidate phosphatase (PAP) catalyzes the conversion of phosphatidate to diacylglycerol (DAG), which is esterified with a fatty acid molecule to form triacylglycerol (TAG). In Yarrowia lipolytica, TAG accumulates in the cells during the lipogenic phase, which is triggered by elevated fatty acid synthesis. The enzymatic activity of Pah1 is stimulated during lipogenesis, while the loss of Pah1 (i.e., pah1Δ) results in diminished TAG levels and elevated fatty acid levels. Also, our previous studies in Saccharomyces cerevisiae showed that exogenous fatty acids stimulate the enzymatic activity of Pah1. These findings suggest a correlation between intracellular fatty acid levels and Pah1 activity. ATP-dependent citrate lyase (ACL) catalyzes citrate cleavage to oxaloacetate and acetyl-CoA. In Y. lipolytica, the ACL reaction provides the bulk of acetyl-CoA for fatty acid synthesis. The Y. lipolytica ACL is a heterodimeric protein comprising catalytic Acl1 and regulatory Acl2 subunits. Loss of the Acl1 catalytic subunit (i.e., acl1Δ) abolishes the enzymatic activity of ACL and results in a 50% decrease in fatty acid levels and a 75% decrease in TAG levels. In this work, we examined the effect of the ACL mutation on the regulation of Pah1. We grew the wild type, acl1Δ, and pah1Δ strains in lipogenic media and harvested cells in the growth and lipogenic phases. We then analyzed the PAP activity in these cells. The results showed that in the lipogenic phase, the loss of Acl1 reduced the PAP activity to levels similar to those of the pah1Δ mutant, which lacks the PAH1 gene. In contrast, the loss of Acl1 did not significantly affect PAP activity in the growth phase. These results suggest that ACL regulates the Pah1 enzymatic activity, possibly by controlling the intracellular fatty acid levels.

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Abstract 2073

KNOCKDOWN OF CES1 IN THP-1 MACROPHAGES PROMOTES A PROINFLAMMATORY PHENOTYPE

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Keywords: Carboxylesterase 1, inflammation., macrophages, Citrate, RNA-SEq

Macrophages are innate immune cells with the capacity to differentiate into classically activated (M1) or alternatively activated (M2) cells that promote or attenuate inflammation, respectively. Inflammation caused in response to pathogen- or damage-associated molecular patterns is normally resolved during healthy physiology. However, dysregulated resolution can lead to chronic inflammation, contributing to disease development. Carboxylesterase 1 (CES1) is a serine hydrolase that can metabolize neutral lipids. Our previous results indicated that THP-1 macrophages with deficient CES1 expression (CES1KD cells) have a distinctly foamy phenotype compared to CES1-expressing THP-1 macrophages (control cells), which is due to greater quantities of TAG-containing lipid droplets. Further, CES1KD cells produce more IL-1b and PGE2 than control cells following lipopolysaccharide (LPS) stimulation. It is known that classically-activated macrophages exhibit a broken TCA cycle, causing a buildup of proinflammatory citrate and succinate metabolites. Here, we report that CES1KD cells have higher levels of several polar organic acid metabolites than control cells under both baseline and LPS-stimulated conditions. These included increased lactate (glycolytic waste product) and citrate, cis-aconitate, and a-ketoglutarate (TCA metabolites), although succinate was depressed. There was also a striking reduction in UDP-GlcNAc levels in CES1KD cells, which is a metabolite that affords O-GlcNAc PTMs on proteins during M2 macrophage polarization. In line with this, M2 gene markers ALOX15, FABP4, and CD206 in CES1KD cells were downregulated compared to those in control cells. Based on our RNA-seq data, we observed a high degree of similarity in the differentially expressed genes in baseline CES1KD cells with those of control cells treated with LPS/IFNg (r=0.728), which is concordant with the altered metabolite profiles of CES1KD cells and their more proinflammatory nature. Finally, Gene Ontology, KEGG, and Reactome database analyses of unstimulated CES1KD cells revealed an enrichment of upregulated pathways involved in antibacterial and antiviral defense that is comparable to that seen in control cells stimulated with LPS and IFN-γ. Thus, we conclude that CES1KD