Development of a Novel Method for the Determination of Acyl-CoA Compounds by Liquid Chromatography Mass Spectrometry to Probe Cell Signaling Activation

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**Purpose**

Acyl-Coenzyme As (acyl-CoAs) are a group of endogenous molecules participating in multiple cellular processes including lipid synthesis, metabolism of fatty acids, transcriptionsal regulation and protein post-translational modification. Quantification of cellular acyl-CoAs is challenging due to their instability in aqueous solutions and lack of blank samples. A high-fat diet is a provocative factor for the implication of obesity in multiple types of cancer. Endogenous fatty acids are susceptible to metabolic abnormalities during cancer pathogenesis. Both exogenous and endogenous fatty acids are activated in the form of fatty acyl-coenzyme As (acyl-CoAs). In this poster, we demonstrate an LC-MS/MS assay which allows for absolute quantitation with broad coverage of cellular acyl-CoAs. To study the remodeling of acyl-CoA profile under the stress of fatty acids, this assay was applied to determine acyl-CoAs in prostate and hepatic cell lines cultured with a variety of dietary fatty acids.

**Methods**

We established a new method, with protein precipitation for sample preparation, reversed phase chromatography for separation, multiple reaction monitoring (MRM) mass spectrometry for detection, to simultaneously quantitate a variety of acyl-CoAs in human cell lines. Absolute quantitation was achieved with calibration curves made from serially supplemented acyl-CoAs, with pentadecanoyl CoA (C15:0 CoA) as the internal standard. Hepatic and prostate cells are models for this study, because the liver is the main metabolic site for fatty acids, and there is increasing evidence of an association between fatty acids and prostate cancer initiation and progression. To study the impact of dietary fatty acids on the acyl-CoA profile, different fatty acids including decanoic acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (cis-9-C18:1), elaidic acid (trans-9-C18:1) and aracidic acid (C20:0), were individually introduced to each cell line at a concentration of 400 μM and incubated for 24 h before the determination of acyl-CoAs. The tumorigenic and nontumorigenic cell lines from the same tissue were compared. Heat maps and hierarchical clusterings were done to identify the pattern of fatty acids affecting acyl-CoA profiles, and to identify individual acyl-CoAs which responded differently in cells with different states of tumorigenicty under the stress of fatty acids.

**Results**

We developed a sensitive LC-MS/MS method to absolutely quantitate various acyl-CoAs in prostate and hepatic cell lines. For the first time, how a variety of dietary fatty acids affect the pool of cellular acyl-CoAs was determined quantitatively. Individual acyl-CoAs were identified which were altered differently by exogenous fatty acids in divergent tumorigenicity states of cells. Hierarchical clustering in the remodeling of acyl-CoA profiles revealed a fatty acid-specific pattern across all tested cell lines, which provides a valuable reference for making predictions in other cell models. Individual acyl-CoAs which corresponded differently to fatty acids in cells at divergent states of tumorigenicity were identified.

**Conclusion**

We believe this method provides a valuable tool for studying the role of fatty acids and subsequent remodeling of the acyl-CoA profile, in the progression of tumors and other diseases associated with nutrition and/or metabolic abnormalities.

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