Lipidomic Profiling of Arachidonic Acid-Containing Phospholipids in Phagocytic Cells

Jesús Balsinde

*Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas (CSIC)*
*Universidad de Valladolid, 47003 Valladolid, Spain*

September 13, 2011

(Transcription of the lecture presented on Tuesday September 13, 2011 at the International Workshop on Metabolomics; Technology and Applications, held in Derio, Bilbao, Spain)

I will present our most recent data on the regulation of free arachidonic acid (AA) in cells of phagocytic origin, in particular the identification of markers of activation as revealed by lipidomic profiling of arachidonate-containing phospholipids. I will focus on two particular examples, namely the use of lipidomics to identify novel lipid markers of activation, and the use of lipidomics to unveil novel protein effectors regulating arachidonic acid availability.

I will begin the presentation of results from our lab with the work of David Bálgoma concerning the identification of lipid markers of activation in human monocytes. Nowadays, lipidomic approaches utilizing mass spectrometry provide a new frame for the analysis of unique phospholipid species involved in fatty acid release from and incorporation into cellular phospholipids. Utilizing high performance liquid chromatography coupled to electrospray ionization mass spectrometry, we have characterized changes in the levels of AA-containing phospholipid species in human monocytes. In resting cells, AA was found esterified into various molecular species of phosphatidylinositol (PI), choline glycerophospholipids (PC) and ethanolamine glycerophospholipids (PE). All major AA-containing PC and PI molecular species decreased in zymosan-stimulated cells; however, no PE molecular species was found to decrease. On the other hand, the levels of three AA-containing species increased in zymosan-activated cells compared to resting cells. These were 1,2-diarachidonyl-glycero-3-phosphoinositol (PI(20:4/20:4)), 1,2-diarachidonyl-glycero-3-phosphocholine (PC(20:4/20:4)), and 1-palmitoleoyl-2-arachidonyl-glycero-3-phosphoethanolamine (PE(16:1/20:4)). PI(20:4/20:4) and PC(20:4/20:4), but not PE(16:1/20:4), also significantly increased when platelet activating factor or phorbol myristate acetate were used instead of zymosan as stimulants of the monocytes. Analysis of the pathways involved in the synthesis of these three lipids suggest that PI(20:4/20:4) and PC(20:4/20:4) were produced in a deacylation/reacylation pathway via acyl-CoA synthetase-dependent reactions, while PE(16:1/20:4) was generated via a CoA-independent transacylation reaction. Collectively, our results define the rises of PI(20:4/20:4) and PC(20:4/20:4) as lipid metabolic markers of human monocyte activation, and validate lipidomics as a powerful tool for the identification of specific molecular markers under various experimental conditions [1-20].

In the second part of my talk I will discuss the work of Alma Astudillo on the use of lipidomics to identify new proteins involved in activation, in particular the effect of caveolin-1 deficiency on the mechanisms that regulate free arachidonic acid availability and its impact on inflammation. Caveolin-1-deficient mice exhibit elevated fatty acid incorporation and remodeling due to a constitutively increased CoA-independent transacylase activity. Mass spectrometry-based lipidomic analyses reveal stable alterations in the profile of AA distribution among phospholipids, manifested by reduced levels of AA in choline...
glycerophospholipids but elevated levels in ethanolamine glycerophospholipids and phosphatidylinositol. Furthermore, macrophages from caveolin-1 null mice show decreased AA mobilization and PGE₂ and LTB₄ production upon cell stimulation, and a defective inflammatory response in an in vivo model of mouse peritonitis. Collectively, these results provide insight into the role of caveolin-1 in AA homeostasis and suggest an important role for this protein in regulating innate immune responses [21-36].

Slide List.

Slide 1 – Title
Slide 2 – What Is Lipidomics?
Slide 3 – Biological Applications
Slide 4 – Lipidomics – Mass Spectrometry
Slide 5 – Lipidomics in Valladolid
Slide 6 – Role of Phospholipase A₂ in AA Release
Slide 7 – Free AA levels depend on a deacylation/reacylation cycle
Slide 8 – AA Into and Remodeling Within Phospholipids
Slide 9 – Markers of Activation
Slide 10 – Lipidomic Analyses of AA-Containing Phospholipids
Slide 11 – AA-Containing Phospholipids in Unstimulated Human Monocytes
Slide 12 – Time-dependent Changes of Major AA-containing PC Species After Zymosan Stimulation
Slide 13 – Time-dependent Changes of Major AA-containing PI Species After Zymosan Stimulation
Slide 14 – Time-dependent Changes of Major AA-containing PE Species After Zymosan Stimulation
Slide 15 – The Role of CoA-dependent Acyl Transferases
Slide 16 – What About Other Stimuli?
Slide 17 – Conclusions
Slide 18 – Lipidomics as a Useful Approach...
Slide 19 – Caveolae and Caveolin-1
Slide 20 – Caveolin-1 KO Mice
Slide 21 – [³H]AA Incorporation Into Phospholipids
Slide 22 – Incorporation of [²H]AA Into Phospholipids
Slide 23 – Analysis of Endogenous Species
Slide 24 – Phospholipid Fatty Acid Content (GC/MS)
Slide 25 – AA-containing Phospholipids (LC/MS/MS)
Slide 26 – Assay of CoA-IT activity
Slide 27 – CoA-IT Activity Is Augmented in Homogenates from Cav-1-deficient Cells
Slide 28 – Free AA Levels Depend on a Deacylation/Reacylation Cycle
Slide 29 – AA Mobilization in Control and Cav-1-deficient Mice
Slide 30 – Eicosanoid Production in Control and Cav-1-deficient Mice
Slide 31 – Caveolin-1
Slide 32 – Acknowledgments.

REFERENCES


