Lipid Biomarkers: Lipidomics of the Macrophage and Plasma Lipidomes

Prof. Edward A. Dennis

Overview

A. LIPID MAPS Initiative in Lipidomics
B. Macrophage Eicosadomics
C. Human Plasma Lipidome

LIPID MAPS "Glue Grant" NIH U54 GM 69338

LIPID MAPS
LIPID Metabolites And Pathways Strategy

Genomics
(transcriptomics)

4 base side chains

Proteomics

20 amino acid side chains

Metabolomics

Nucleic acids, amino acids, sugars and fats: >10^5 ?

"Lipidomics"

All the fats: >10^5 ?
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LIPID MAPS cores / bridges
PI
Edward A. Dennis - UCSD

Mass Spec Imaging Bridge
Robert C. Murphy - Colorado
Nicholas Winograd - Penn State

Bioinformatics / Networks Bridge
Shankar Subramaniam - UCSD

Oxidized Lipids Bridge
Joseph Wilburn - UCSD

Glycerolipids
Robert C. Murphy - Colorado

Prenol Lipids
Christian Rezz - Duke

CLASS: Comprehensive Lipidomics Analysis using Separation Simplification
a "divide-and-conquer" strategy

Cells or tissues
Cells
Pulse
Homogenate
Sonicate
Internal standards
Extract
GC LC
Category specific
(deuterated, odd-chain carbon)
Category optimized
(GC, NP-HPLC, RP-HPLC, chiral, specialty)

Mass spectrometer (variables)
1. Mass spectrometer types
2. Ionization mode
3. Additives (for ionization)
4. Mass spectrometer monitoring modes

Kdo₂-Lipid A (KLA, LPS subspecies)
A specific agonist of TLR-4 on RAW 264.7 macrophages
Raetz et al., 2008. J. Lipid Res. 47: 1097

Nuclei – DAPI
Mitochondria – MitoTracker Red

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Macrophage lipid metabolism

- Sphingolipids
- Glycerophospholipids
- Glycerolipids

- Eicosanoids
- Fatty Acids
- Sterol esters
- Sterols
- Phospholipids

- Acetyl CoA

- TCA cycle
- Oxidative phosphorylation

Dennis et al., 2010, J. Biol. Chem., 51: 39976-85

Lipidomics components

- Pathways
- Annotations
- Proteins

See: www.lipidmaps.org

KLA ratio

Ctrl

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Phospholipase A₂ (PLA₂) function in arachidonic acid release

Arachidonic Acid

Aspirin
NSAID

Cyclooxygenase

Prostaglandins

Lipoxygenase

Leukotrienes

Eicosanoid signaling pathways in RAW264.7 macrophage

Numerous eicosanoid metabolites

Buczynski et al., 2007, JBC 282: 22034
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Basic macrophage experimental scheme

Macrophages

\[ \pm \text{KdL lipid A (TLR4)} \]

\[ \pm 2 \text{ mL ATP (P2X7)} \]

Isolate media & Solid phase extraction (Eicosanoids)

LC-MS/MS

Norris et al., 2011, J. Leukocyte Biology, 90: 563-74

Cellular eicosanoid metabolism

\[ 5-\text{LOX} \]

\[ 12-\text{LOX} \]

\[ \text{CYP(EET)} \]

\[ \text{COX} \]

\[ \text{CYP(o)} \]

\[ 15-\text{LOX} \]

\[ 12-\text{LOX} \]

\[ \text{15-LOX} \]

\[ \text{12-LOX} \]

Buczynski, Dumlao, Dennis, 2009, JLR, 50: 1015-38

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Cellular eicosanoid metabolism

Macrophage phenotypes
Resident Peritoneal (RPM) Thiglycolate-Elicited Peritoneal (TGEM)
Bone Marrow-Derived (BMDM) RAW264.7 Cell Line (RAW)

Eicosanoid changes by phenotype

Fold Decrease Fold Increase Not detected
PGI2 PGE2 PGD2 TxB2 8 Hr
COX-2 metabolites and transcripts

PGE$_2$/PGD$_2$ vs. PGES/PGDS transcripts

Proportionality of eicosanoids and transcripts
Directed proteomics on enzymes of eicosanoid biosynthesis

Protein abundance after KLA stimulation

Eicosanoid genes to proteins to metabolites

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Human plasma lipidomics

- NIST collected (pooled) fasting plasma from 100 individuals
- 50% female and 50% male; age 40-50

15% of the total taken from individuals of Hispanic origin

Quehenberger & Dennis, 2011, New Eng. J. Medicine, 365: 1812-23

Human plasma metabolites (mg/dL)

Lipids
Amino Acids
Sugars
Nucleic Acids

Human plasma lipid categories (mM)

Sterol Lipids
Sphingolipids
Glycerolipids
Glycerophospholipids
Prenols
Fatty Acyls

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SRM: Prostaglandins, Isoprostanes

SRM: Sterols

Cholesterol 3.8 mmol/ml

Human plasma lipid diversity

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