BBA Clinical 7 (2017) 105–114

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: www.elsevier.com/locate/bbaclin

The plasma lipidome in acute myeloid leukemia at diagnosis in relation to clinical disease features

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ARTICLE INFO

Article history:
Received 13 January 2017
Received in revised form 4 March 2017
Accepted 4 March 2017
Available online 08 March 2017

Keywords:
Acute myeloid leukemia
Lipidomics
Fatty acids
Eicosanoids
Blast cell number
Prognostic risk

ABSTRACT

Background: Early studies established that certain lipids were lower in acute myeloid leukemia (AML) cells than normal leukocytes. Because lipids are now known to play an important role in cell signaling and regulation of homeostasis, and are often perturbed in malignancies, we undertook a comprehensive lipidomic survey of plasma from AML patients at time of diagnosis and also healthy blood donors.
Methods: Plasma lipid profiles were measured using three mass spectrometry platforms in 20 AML patients and 20 healthy blood donors. Data were collected on total cholesterol and fatty acids, fatty acid amides, glycerolipids, phospholipids, sphingolipids, cholesterol esters, coenzyme Q10 and eicosanoids.
Results: We observed a depletion of plasma total fatty acids and cholesterol, but an increase in certain free fatty acids with the observed decline in sphingolipids, phosphocholines, triglycerides and cholesterol esters probably driven by enhanced fatty acid oxidation in AML cells. Arachidonic acid and precursors were elevated in AML, particularly in patients with high bone marrow (BM) or peripheral blasts and unfavorable prognostic risk. PGF2α was also elevated, in patients with low BM or peripheral blasts and with a favorable prognostic risk. A broad panoply of lipid classes is altered in AML plasma, pointing to disturbances of several lipid metabolic interconversions, in particular in relation to blast cell counts and prognostic risk.
Conclusions: These data indicate potential roles played by lipids in AML heterogeneity and disease outcome.
General significance: Enhanced catabolism of several lipid classes increases prognostic risk while plasma PGF2α may be a marker for reduced prognostic risk in AML.

Abbreviations: AML, acute myeloid leukemia; MUFA, monounsaturated fatty acid; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; FAO, fatty acid oxidation; CPT1a, carnitine palmitate transferase 1a; SCD1, stearoyl CoA desaturase 1; FAB, French-American-British classification; GCMS, gas chromatography–mass spectrometry; FAME, fatty acid methyl ester; PCA, principal components analysis; PLS-DA, projection to latent structures-discriminant analysis; OPLS-DA, orthogonal PLS-DA; UPLC-ESI-QTOFMS, ultraperformance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry; ESI+, electrospray ionization positive mode; ESI-, electrospray ionization negative mode; PUFA, polyunsaturated fatty acid; FLC-QqLIT-MS, fast liquid chromatography-quadrupole linear ion-trap mass spectrometry; MRM, multiple reactions monitoring; LPE, lysophosphatidylethanolamine; MG, monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol (triglyceride); FAA, fatty acid amide; POEA, palmitoleoyl ethanolamide; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; Cer, ceramide; CE, cholesterol ester; CoQ10, coenzyme Q10; DGLA, dihomo-γ-linoleic acid; AA, arachidonic acid; 8,9-DHET, 8,9-dihydroxy-5*Z*,11*Z*,14*Z*-eicosatrienoic acid; EPA, eicosapentaenoic acid (20:5;5*Z*,8*Z*,11*Z*,14*Z*,17*Z*); 12-HEPE, 12-hydroxy-5*Z*,8*Z*,10E,14*Z*,17*Z*-eicosapentaenoic acid; 12-LOX, 12-lipoxygenase; PGE2, prostaglandin E2; PGF2α, prostaglandin F2α; PGF1α, prostaglandin 1α; TxB2, thromboxane B2; TxA2, thromboxane A2; mPGES-1, microsomal prostaglandin E synthase-1; 2OG, 2-oxoglutarate; 2HG, (*R*)-2-hydroxyglutarate; DIC, disseminated intravascular coagulation; PGH2, prostaglandin H2.

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http://dx.doi.org/10.1016/j.bbacli.2017.03.002

1. Introduction

Acute myeloid leukemia (AML) is a fatal disease with a heterogeneous genomic and cytogenetic profile. While traditional cell signaling cascades have attracted considerable research interest in an attempt to understand the pathogenesis of AML and to optimize AML therapy [1], little is known regarding the role of lipid mediators in AML cell proliferation or in disease prognosis. However, the study of lipid profiles and lipid metabolism in myeloid leukemias such as AML has a long history. In the 1960s, the lipid content of various normal and abnormal leukocytes was reported, in which total cholesterol, for example, was significantly lower in AML cells than in normal leukocytes [2]. In a single chronic myelogenous leukemia (CML), total lymphatic cell lipid was almost five-times lower than from corresponding healthy cells. In addition, the pattern of ether-linked neutral glycerides also differed considerably between CML and healthy cells [3]. Subsequently, the lipid composition of AML myeloblasts and immature bone marrow myeloid cells from healthy persons was compared with that of normal mature human neutrophils. There was a decreased total cholesterol and cholesterol/phospholipid ratio, with an increased percentage of unsaturated fatty acids, when compared with normal mature neutrophils [4]. Recently, AML patients were reported to display lower HDL cholesterol than either healthy controls or ALL patients, with lower total cholesterol and LDL cholesterol seen only in male AML patients [5]. It thus appeared that AML cells might display an increased lipid catabolism. A later study showed that pharmacological inhibition of fatty acid oxidation (FAO) retarded proliferation of AML cells cultured on a feeder layer of mesenchymal stromal cells. The authors proposed that the shift to nonoxidative fatty acid metabolism, such as generation of ceramide, may decrease cell survival [6]. Accordingly, FAO may represent a biochemical characteristic of AML proliferation and this was borne out by a study of the proliferation of AML blasts from 23 patients that was inhibited by incubation with the non-β-oxidizable fatty acid tetradecylthioacetic acid [7]. FAO has recently been recognized as a key component of cancer cells [8].

In order to attempt to understand better the extent of perturbation of the plasma lipidome in AML, we have conducted a mass spectrometry-based lipidomic investigation of AML patients and healthy blood donor controls. Additionally, we have analyzed certain clinical disease features at the time of AML diagnosis in relation to eicosanoid lipid mediators and their metabolic precursors, to comprehend what role these may play in the heterogeneity of this disease.

2. Materials and methods

2.1. Patients and samples

All patients gave their written informed consent to the study, which was conducted according to the World Medical Association Declaration of Helsinki. The study was approved by the ethics committee of Canton Bern. Peripheral blood was taken from 20 patients in Bern with a diagnosis of AML that had been established from a bone marrow aspiration. Plasma was prepared by centrifugation and stored at i.e. −20 °C until analyzed. The demographic and clinical details of the AML patients are given in Table 1 and histogram distributions (with normal values) for hematology and blood chemistry are presented in Supplemental Fig. 1. Patients had a mean age of 64.0 ± 9.2 (±S.D.) and comprised 16 males and 4 females. The French-American-British (FAB) classification [9,10] of the AML patients was four M0, six M1, three M2, three M4, two M5, and two M6. The AML patients were also characterized by cytogenetics and genetic mutation analysis, which permitted prognostic risk stratification into favorable, intermediate, and unfavorable risk [11]. Healthy control patient EDTA plasmas were taken from the local blood bank from 20 blood donors from all over Switzerland (10 males and 10 females, aged 22–68 years). All control samples tested negative for antibodies and/or antigens for HIV, HCV and HBV and also tested negative for syphilis. All plasma aliquots were stored at i.e. −20 °C prior to analysis. It should be noted that, although the AML patients comprised more males and had a greater mean age than the healthy controls, this was not expected to confound the lipidomic determinations, since it had long been established (see above) that AML patients have lower cholesterol and fatty acids than expected for their age.

Table 1
Characteristics of AML patients.

Patient	Sex	Age at diagnosis	FAB	Molecular diagnosis	Cytogenetics	Prognostic risk stratification
001	f	81	M1	*NPM1*mut; *FLT3*-ITD	Normal	Intermediate
002	m	75	M5	normal	Complex abnormalities	Unfavorable
003	f	70	M1	*NPM1*mut	Normal	Favorable
005	m	72	M6	normal	Normal	Intermediate
007	m	42	M4	normal	Monosomy 7	Unfavorable
009	m	70	M0	normal	Complex abnormalities	Unfavorable
011	m	54	M1	normal	Trisomies 8, 19	Unfavorable
015	m	59	M5	*NPM1*mut	Normal	Favorable
017	m	55	M0	normal	Normal	Intermediate
022	m	68	M0	*NPM1*mut; *FLT3*-ITD	Normal	Intermediate
027	m	59	M6	normal	Normal	Intermediate
033	m	67	M4	normal	Normal	Intermediate
040	m	67	M4	normal	Normal	Intermediate
045	f	65	M1	normal	Complex abnormalities	Unfavorable
046	f	62	M2	*NPM1*mut	Normal	Favorable
050	m	49	M0	normal	Trisomy 11	Intermediate
051	m	72	M2	*CEPBA*	Normal	Favorable
052	m	64	M2	*CEPBA*	Normal	Favorable
056	m	62	M1	*NPM1*mut	Normal	Favorable
058	m	66	M1	*FLT3*-ITD	Complex abnormalities	Unfavorable

2.2. Gas chromatography–mass spectrometry fatty acid profiling

Profiles of plasma total (free and esterified) fatty acids were determined for 20 AML patients and 20 controls by gas chromatography–mass spectrometry (GCMS) after conversion to their corresponding fatty acid methyl esters (FAMEs) as we have previously described [12].

2.3. Ultraperformance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry untargeted lipidomics

An untargeted lipidomic investigation of plasma from 20 AML patients and 20 controls was conducted using ultraperformance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOFMS) by a modification of our published method [13]. Data were collected in continuum mode and the raw chromatographic data were imported into Progenesis QI 2.1 software (Nonlinear Dynamics, Newcastle-upon-Tyne, UK) for visualization of chromatograms as ion intensity maps, chromatogram alignment, peak picking, deconvolution and normalization. In ESI+ mode, the following adducts were considered when solving empirical formulae from accurate mass determinations: [M + H]+, [M + Na]+, [M + NH4]+, [2M + H]+, and [M + H-H2O]+. In ESI- mode, the following adducts were considered: [M-H]- and [M + Cl]-. When two or more different adducts were aligned, a neutral mass could be determined. Both up- and down-regulated AML plasma lipids were identified in Progenesis QI by searching various databases, including HMDB, ChemSpider, and Lipid Maps and reported on the basis of their fold change from control plasma and their statistical significance on the basis of ANOVA. Matching to database entries was made on the basis of accurate mass, isotope similarity and retention time.