Lipidomics: New Approaches to the Studies of Cell Signaling and Prospects of Use in Medicine

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A new modern stage in the development of lipid biochemistry is presented: lipidomics, which emerged on the basic of new highly sensitive fractionation methods, primarily, mass spectroscopy. Lipidomics is defined mainly as systemic evaluation of all molecular types of lipids in an object, their cell functions, and molecules with which they react. Lipidomic approaches identifying picomole levels of individual lipids in combination with modern genome technology provide detailed information about the involvement of minor phospholipids in the cell signaling processes. Brief data on the functions of lysophospholipids as second messengers of signal transfer, their effects on cell processes, and possible involvement in the pathogenesis of some diseases are presented. It is expected that introduction of lipidomics in biomedical studies will promote the detection of targets for new drugs and development of new diagnostic tests.

Key Words: *cell signaling; lysosphingolipids; lysophosphatide acid; lipidomics*

Three major periods can be distinguished in the development of lipid biochemistry (or lipidology), determined, similarly as in other branches of biochemistry, by the levels and potentialities of analytical methods, on the one hand, and by our knowledge of cell processes in which these substances are involved, on the other. The first period is "prechromatographic", when lipids (at that time more often called just fat) were regarded only as a source of energy and were analyzed only by calorimetric methods. The appearance of column and thin layer chromatography in 1960-70s, together with the first models of the structure of biological membranes and understanding of their function in cells, brought about the first remarkable progress in lipidology: transition to the notion of separate classes of lipids, particularly individual phospholipids (PL), as the most important "construction blocks" for biomembranes, essential for their characteristics.

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However, the cause, or biological usefulness of so great a variety of membrane lipids remains unclear; considering the variability of polar heads (PL classes) and the fatty acid set, it seems that more than a hundred of lipid compounds of different structure simultaneously exist in a biological object. Even a slight difference, for example, in the length of chain or number or position of double bonds in one of the lipid molecule fatty acids, is essential for its properties, or, in other words, for its reactions with the "metabolic partners" or adjacent molecules, which cannot but influence, in turn, the pattern of a local biochemical process or characteristics of a membrane microregion (for example, its permeability). These fine effects remain beyond our attention in traditional lipid fractionation and analysis. For comparison let us note, that replacement of just one amino acid in protein structure can lead, as we know, to significant consequences, and this knowledge (classified in recent years due to proteomics) are used in medicine. For lipids only common characteristics of the object were studied for many years; at the best, it could be the content and composition

of lipids, PL classes and sums of their fatty acids (evaluated by thin-layer and gas-liquid chromatography), which was largely due to the intricate nature of lipids and lack of adequate instruments for their analysis. For this reason the informative burst in genomics and proteomics was not paralleled by corresponding achievements in the study of lipids. As a result, lipids, up to the recent decade, were regarded as a little perspective object as regards deciphering of the reactions of cell metabolism, performing just a "housekeeper" function, important, but little interesting [23], in contrast to proteins and nucleic acids.

This situation crucially changed during the last decade due to two factors. First of all, numerous "signaling" effects of individual lipid molecules were revealed, indicating their involvement in transmission of biochemical signal outside and inside the cells and determining coordinated triggering of activities of functionally important proteins. This became the starting point for "double life" of membrane lipids, active participants in many vital processes in the cell. The methodological factor played the key role here: new mass spectrometry technologies, involving "soft ionization" methods, sparing the native characteristics of lipid molecules, were created. These methods are matrix-assisted laser desorption ionization mass spectrometry (MALDI) or electrospray ionization [18]. The use of these methods, sometimes with preliminary liquid chromatography, led to identification of molecular types of individual PL and revealed minor lipid molecules, present in picomole quantities, which could not be studied by previously available analytical methods.

This resulted in the development of a new trend of research, which was called "lipidomics (by analogy with proteomics and genomics, which were in full bloom by that time).

Lipidomics denotes for the majority of authors a total systems analysis of lipids and their reactive partners [3] or "systemic deciphering of lipid-related information on lipid identification and their transmitters" [19]. The notion of "oxidative lipidomics" was introduced, including the description of mechanisms of successive biochemical processes, related to free radical oxidation of mitochondrial PL and its consequences for the cell [14]. Recently a wider definition of lipidomics was offered: "systemic study of all lipids, molecules with which they react, and their functions in the cells" [22].

Actually, it denotes not just a sort of molecular "inventory" or list of all lipid molecules present in the studied object (called a lipidome), but also the study of reactions in which they are involved, and these are primarily various processes associated with cell signaling. The lipid metabolic pathways are dissociated in many human diseases, such as atherosclerosis, diabetes, obesity, cancer, and neurodegenerative diseases, and detailed lipidomic studies can offer new promising potentialities for their diagnosis and treatment [23]. This stimulated intense lipidomic studies in many laboratories of the world.

Minor "signaling" PL analyzed by lipidomic methods

Due to extremely high sensitivity of lipidomic, particularly mass spectrometry methods, it is now possible to isolate and analyze minor PL present in biological fluids in picomole quantities (by several orders of magnitudes lower than common PL classes). Their level in biological membranes is also extremely low (less than 1% of all PL), and they are undetectable by the traditional methods. These are mainly lysoglycero- and lysosphingo-PL: lysophosphatide acid (lyso-PA), sphingosine-1-phosphate (S1P), and sphingosylphosphorylcholine (SPC); in some objects ceramides (sphyngomyelin cleavage product) are present. These lipids, no less than previously known phosphoinositides and lysophosphatidylcholine (lyso-PC) [2], function as mediators, causing numerous cell responses [9], including mitogenesis, differentiation and migration of cells, are essential for cell viability (apoptosis resistance or susceptibility). Cell effects induced by the mediators causes, in turn, respective physiological events, such as vasoconstrictor activity, wound healing, immunomodulation, angiogenesis, platelet aggregation. Working largely in the same direction as polypeptide growth factors, lyso-PL have a wider spectrum for manifestation of their biological activity.

Lyso-PA, a product of phospholipase cleavage of PL, belongs to a new family of lipid mediators, endogenous growth factors causing various biological effects, primarily, cell proliferation [5].

A significant elevation of lyso-PA level was detected in the sera of patients with ovarian carcinoma [8]. At a concentration corresponding to that in ascitic fluid lyso-PA indirectly stimulates cell growth by increasing the expression of vascular endothelial growth factor. Another mechanism of lyso-PA action was shown: through elevation of D1 cyclin (stimulating proliferation) level in tumor cells. It is hypothesized that lyso-PA is produced and released by tumor cells. It was shown on DLD1 human carcinoma cells that lyso-PA in physiological concentrations stimulated cell migration, secretion of endothelial growth factor and IL-8, adhesion and secretion of angiogenic factors, thus increasing the metastatic potential of the cells [20].

The presence of lyso-PA was shown in normal human follicular fluid, which suggests that this biological mediator is an element of ovarian physiology and is locally generated by the reproductive tissue. It is hypothesized to play numerous roles in male and female reproductive physiology and diseases [5].

Lysosphingo-PL (S1P and SPC) are the key signaling molecules involved in stimulation of cell growth, are potential calcium-mobilizing agonists, induce constriction of capillaries and coronary arteries [1,24].

Sphingosine-1-phosphate plays a regulatory role in angiogenesis, is involved in cerebral vasospasm, stimulates the proliferation and chemotaxis of endothelial cells [2,24]. Accumulation of S1P leads to an increase in the level of adhesion molecules (E-selectin and VCAM-1) and is associated with the effects of some factors, for example, is induced under the effect of TNF-α or in platelet aggregation. Sphingosylphosphorylcholine promotes an increase in Ca^{2+} sensitivity of cells, causing its intracellular accumulation, induces endothelium-dependent relaxation in the coronary artery, which is attributed to its activation of NO-synthase [6].

Other active signaling lipids, the potentialities for studies of which also increased appreciably with the appearance of lipidomics, are ceramides, realizing their participation in the signaling processes via a peculiar specific mechanism. It is based on the location of ceramides (similarly as the main part of their precursor, sphingomyelin) on the membrane surface in special lateral microdomains (rafts). These "islets" with maximum orderliness of PL are conjugated with many functional proteins participating in the signaling processes, and they are therefore called membrane "signaling rafts" [17]. The intracellular signal transfer is realized in these rafts at the expense of sphingomyelinase activation with subsequent formation of ceramides. The molecular physical organization of rafts is changing during this process (it is not yet known in what way), which is essential, in turn, for the signal protein conjugated with them. This picture is observed, for example, during apoptosis. It is also hypothesized that ceramides activate nuclear κ-B factor, the key element of many biological processes [15].

It is noteworthy that biochemical reactions of biosynthesis, cleavage, and signaling of lysoglycero- and lysosphingo-PL are closely related and strictly coordinated with each other [9].

Cell receptors for lyso-PL

Despite great variety of lyso-PL effects, or presumably partially due to it, the mechanism of their realization remained unclear for a long time. Some authors hypothesized the participation of specific receptors in these processes. However, they were discovered quite recently, using the genome approaches.

The receptor sensitive to S1P was first identified; it was detected as an inducible gene product in endothelial cells. It was called EDG receptor, by the gene coding for it (endothelium differentiation gene). Other similar receptors were found later. These receptors function together with G protein (highly active regulatory heterotrimer plasma membrane protein, regulating many transcription and membrane permeability processes) [7,9]. A family of S1P-sensitive receptors was characterized; they were called EDG-1, -3, -5, -6, and -8. According to another nomenclature they are sometimes called by the ligand: S1P-1, S1P-2, S1P-3, *etc.*, respectively. They belong to GPC receptors (G-protein coupled receptors) [7].

Eight genes of EDG receptors were identified in human genome. Five of them encode S1P receptors, three others lyso-PA receptors (EDG-2, -4, and -7, or LPA-1, LPA-2, and LPA-3 by the ligand) [7]. High homology of EDG receptor proteins was noted. Sites with identical sequences were detected for some of them. The EDG receptors vary in different tissues, but all of them are similarly coupled with multiple types of G proteins [7,9].

The main S1P receptors (EDG-1, -3, and -5) are activated not only by their own ligand S1P, but also by SPC, though at much higher concentrations (micromolar), in contrast to the nanomolar for S1P [26]. On the other hand, SPC exhibits its own biological effects, not related to S1P. For example, it inhibits Ca^{2+} flow in GH_4C_1 cells, induces the formation of superoxide anions in HL-60 granulocytes, and stimulates the production of inosite phosphate in epithelial cells. These facts, together with the presence of SPC in normal plasma [16], indicate possible existence of other specific receptors to SPC [26]. And really, a specific highly affine GPC receptor to SPC, not activated by S1P, was recently identified in ovarian cancer cells [25]. This ovarian cancer GPC receptor 1 (ORG1) is an analogous orphan receptor, expressed in the placenta, lungs, liver, kidneys, brain, heart, and some immune cells. It is activated by nanomolar concentrations of SPC and does not bind other lyso-PL (S1P, lyso-PA, lyso-PC). Other receptors with less pronounced specificity for SPC, but reacting also with lyso-PC were found: lymphocyte G2A receptor and G PR4 receptor expressed in many tissues [13].

Transduction of lyso-PL signals by receptors conjugated with G protein

Despite great variety of cell receptors to lyso-PL, all of them transmit cell signals through G proteins.

The G protein is characterized by the heterotrimer structure, consisting of α-, β-, and γ-subunits (G chains), each of them in reactions of certain type regulating one or several important biochemical signaling reactions. Binding of the ligand to the corresponding receptor can cause its conformation changes, inducing, in turn, a response in some of the chains in the G protein molecule [10]. Binding to more than one receptor, lyso-PL can cause a great number of cell responses [7,9,26].

This can result in modification of processes regulated by this protein (activities of specific mitogen-activated protein kinases or transcription nuclear factors, or opening of ionic channels), in other words, in processes involving in fact the key positions of cell life. This explains the multiplicity of lyso-PL effects and variety of cell responses induced by them.

The main effects of lyso-PL on the cell are stimulation of proliferation and functions associated with survival, suppression of apoptosis, modification of differentiation, and a wide spectrum of other effector responses, manifesting by changes in the content or activity of functional cellular proteins [2,9]. Many regulatory processes are governed by complex effects of various lyso-PL and sphingolipids, which manifests by effects on the movement of ions, processes associated with apoptosis, *etc.*

On the whole, numerous cellular effects of lyso-PL by their direction and mechanism of manifestation are arbitrarily divided into two main groups [9,26]:

- "growth-stimulating" effects, implying not only cell growth, but also a variety of processes linked with cell survival under different conditions;
- effects on the cytoskeleton proteins and thus on cell processes and reactions determined by it.

Lipidomics and modern medicine: hypotheses and prospects

Due to active involvement in various cell metabolism processes, lipids directly or indirectly participate in the pathogenesis or progress of many diseases. Today cholesterol and triglyceride concentrations in the plasma are the only lipid characteristics used in practical medicine. However, the progress in the lipidomics, attained in recent years provided new information on the involvement of minor signaling lipids in many pathological processes, which with time will lead to development of new diagnostic tests. In addition, new data were obtained, extended our notions on the pathogenesis of many diseases, for example, on the participation of signaling PL in it.

The data on the effects of lysosphingolipid on cells proliferation suggests their participation in processes associated with atherosclerosis (via smooth-muscle cell proliferation). It was found that LDL, particularly their oxidized forms, similarly as cytokines, growth factors, and ionizing radiation, activate the formation of ceramides in vascular smooth-muscle and endothelial cells, which can lead to cell proliferation, differentiation, and apoptosis [10]. Vascular smooth-muscle cells are activated under the effect of S1P [4]; S1P is generated in the cells after entry of oxidized LDL [15]. Generation of S1P and ceramides precedes proliferation of smooth-muscle cells induced by oxidized LDL [4], that is, at least some mitogenic effects of LDL are due to the influence of S1P and SPC. Participating in vascular wall cell proliferation, S1P, generated in cells under the effect of oxidized LDL and released by platelets, causes its thickening and stabilization of the atherosclerotic plaque [10].

Hence, "atherogenicity" of LDL is due to not only transport of excessive cholesterol or active oxygen forms to the cell, but also to induction of mitogenic signals by activating the formation of S1P. On the other hand, LDL inhibit the formation of S1P in endothelial cells (by inhibiting sphingosine kinase) [10], which can be one more aspect of the known antiatherogenic effect of LDL.

Measurement of lyso-PA in patients with cancer of the reproductive organs proved to be the most informative practical method. Its blood level is elevated in some types of gynecological cancer (for example, ovarian tumor), and therefore it was suggested to use lyso-PA as a new marker of these diseases [8,21]. In addition, lyso-PA induces proliferation and mitogenic signaling in prostatic cancer cells [5].

Use of lipidomic approaches (multi-dimensional mass spectroscopy) in studies on diabetic mice showed reduced levels of cardiolipin and phosphatidylglycerol in the myocardial mitochondrial internal membrane, indicating mitochondrial dysfunction [12]. The use of these methods is suggested for search for changes in the lipids in diseases of the brain and nervous system ("neurolipidomics") [3,11].

Stage 3 of clinical trials of FTY720 drug, an S1P analog, is now in progress at the Novartis Pharmaceutical Firm; this drug is planned to be used as

an immunosuppressant, replacing cyclosporin in organ transplantations. A possible target for the new drug is sphingosine kinase responsible for S1P production in the cell [23].

Organization of a special scientific consortium "Lipid MAPS" (Metabolites and Pathway Strategy) at the University of San Diego (USA) uniting 30 research groups at 18 universities and aimed at the analysis of lipid structure and functions with the use of lipidomics, for which a special grant of 35 million dollars is assigned, is one more evidence of great attention paid to lipidomics and great expectations of its fruitful development. The macrophage was chosen as the first object of these studies; a complete systemic analysis of lipids in these cells with detailed investigation of the role of each metabolic reaction, each type of lipid molecules in the vital activity of this cell is planned with the aim of searching the targets for creation of new drugs [23]. The nearest tasks are lipidomic study of blood cell lipoproteins with detection of informative markers, characterizing, for example, oxidative stress and possible metabolic disorders.

REFERENCES

1. E. V. Dyatlovitskaya and A. G. Kandyba, *Biokhimiya*, **71**, 17-25 (2006).
2. N. V. Prokazova, N. D. Zvezdina, and A. A. Korotaeva, *Ibid.*, **63**, 38-46 (1998).
3. Adibhatla, J. F. Hatcher, and R. J. Dempsey, *AAPS J.*, **8**, No. 2, E314-E321 (2006).
4. N. Auge, M. Nikolova-Karakashian, S. Carpentier, *et al.*, *J. Biol. Chem.*, **274**, 21,533-21,538 (1999).
5. L. T. Budnik and A. K. Mukhopadhyay, *Biol. Reprod.*, **66**, 859-865 (2002).
6. X. L. Chen, J. Y. Grey, S. Thomas, *et al.*, *Am. J. Physiol. Heart Circ. Physiol.*, **287**, H1452-H1458 (2004).
7. J. Chun, E. J. Goetzl, T. Hla, *et al.*, *Pharmacol. Rev.*, **54**, 265-269 (2002).
8. X. Fang, D. Gaudette, T. Furui, *et al.*, *Ann. N.Y. Acad. Sci.*, **905**, 188-208 (2000).
9. E. J. Goetzl and S. An, *FASEB J.*, **12**, 1589-1598 (1998).
10. I. Gouni-Berthold and A. Sachinidis, *Ibid.*, **16**, 1477-1488 (2002).
11. X. Han, *Front. Sci.*, **12**, 2601-2615 (2007).
12. X. Han, J. Yang, H. Cheng, *et al.*, *Biochemistry*, **44**, No. 50, 16,684-16,694 (2005).
13. J. H. Kabarowski, K. Zhu, L. Q. Le, *et al.*, *Science*, **293**, 702-705 (2001).
14. V. E. Kagan, Y. Y. Tyurina, H. Bayir, *et al.*, *Biol. Interact.*, **163**, No. 1-2, 15-28 (2006).
15. T. Levade, N. Auge, and R. J. Veldman, *Circ. Res.*, **89**, 857-968 (2001).
16. K. Liliom, G. Sun, M. Bunemann, *et al.*, *Biochem. J.*, **355**, 189-197 (2001).
17. L. J. Pike, *J. Lipid Res.*, **44**, 655-667 (2003).
18. M. Pulfer and R. C. Murphy, *Mass Spectrometry Reviews*, **22**, 332-364 (2003).
19. C. N. Serhan, S. Hong, and Y. Lu, *AAPS J.*, **8**, No. 2, E284-E297 (2006).
20. S. Spiegel and S. Milstein, *J. Biol. Chem.*, **277**, 25,851-25,854 (2002).
21. M. Umezu-Goto, J. Tanyi, J. Lahad, *et al.*, *J. Cell. Biochem.*, **92**, No. 6, 1115-1140 (2004).
22. A. D. Watson, *J. Lipid Res.*, **47**, No. 10, 2101-2111 (2006).
23. M. R. Wenk, *Nat. Rev. Drug Discov.*, **4**, No. 7, 594-610 (2005).
24. Y. Yatomi, *Curr. Pharm. Res.*, **12**, 575-587 (2006).
25. K. Zhu, L. M. Baudhuin, and G. Hong, *J. Biol. Chem.*, **276**, 41 325-41 335 (2001).
26. D. M. zu Heringdorf, H. M. Himmel, and K. H. Jakobs, *Biochim. Biophys. Acta*, **1582**, 178-189 (2002).