Review Article

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Metabolomics: a systems approach in studying of cancer biology

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Abstract

Metabolomics is a systems approach in medical research that aims to quantitatively analyze small molecules (less than 1500 Da) in a given biospecimen. Improvement in analytical technologies enabled metabolic research in various fields of medicine including oncology. Metabolic reprogramming is a firmly established hallmark of cancer. It includes changes in tumor cells and in the stromal cells as well. In this review, we focus on metabolomics in the field of cancer research. In the first part, we discuss the main technical approaches. In the second part, we address altered cancer metabolism and additional roles of some metabolites besides energetic and structural roles.

Keywords

Metabolism, metabolomics, cancer, MS, NMR

Introduction

Metabolomics is a new „omics" field in biomedicine that provides comprehensive information on cellular metabolism [1]. It aims to identify and measure hundreds of small molecules (less than 1500 Da) in a given sample (biofluids, tissue, cell culture) and finally, give them biological significance [2]. Metabolome is considered as a downstream point and reflection of genome, transcriptome, proteome, epigenome and also environmental influences [1]. Metabolome is sensitive, highly-dynamic, fast-responding and levels of metabolites are changing in a real-time fashion, being directly connected to the physiological and pathological process. Relation to genomics, transcriptomics and proteomics is not simple, one to one. It is more similar to „net" with multiple bidirectional connections. On the other hand, genome is less susceptible to change and not directly associated with the function [3]. In comparison to other omics, which usually require one platform to extract or explore molecule of interest,

metabolomics requires a whole mixture of different techniques to uncover a broad spectrum of metabolites [4]. Consequently, metabolomics could be more error-prone and reproducibility between investigators is still a problem [5]. Therefore, great effort is invested in the development of standardized procedures [1,4]. Metabolomics is especially evolving in the field of cancer research [1,6], motivated by the well-known fact that metabolism is highly altered in cancer. From historical Warburg's observations about the phenomenon of aerobic glycolysis [7] and recognizing altered metabolism as one of the cancer hallmarks [8], metabolism started to be an interesting field for developing new cancer therapies and biomarkers, potentially leaving classical biomarkers behind [9].

This review is mainly focused on the application of metabolomics in the field of cancer research. First part will address technical perspective and challenges in planning experiment and making decisions. The aim is to point out importance of carefully designing metabolomics experiment, to obtain reliable results. In the second part, emphasis will be on altered cancer metabolism and the additional roles of several enumerated metabolites, besides being a building block or source of energy. These metabolites play a role in various signaling and modulating pathways that may have tumor-promoting or tumor-suppressive effects. Therefore, metabolic changes within a tumor are still going to be an exciting area of research with a big potential for future breakthroughs.

Methods

In metabolomics, two approaches are considered: untargeted and targeted. Untargeted approach is used to assess a broad spectrum of metabolites and reveal their identity and role in metabolic pathways. The untargeted approach provides hypothesis, whereas targeted approach aims to test it. Targeted approach is used to measure exact levels of metabolites or to measure the conversion rate of one metabolite to another [10].

Sampling and preparation

Different samples are appropriate for metabolomics studies: cell cultures, lab animals and human biospecimens such as blood plasma, serum, urine, breath, tissue or a single cell for compartment focused studies [1,3]. The first step is to choose the right sample according to the question of the research. When exploring tumor biology and potential therapeutic targets, tissues and cell cultures are appropriate. Intact tissue can be analyzed during surgery to assess resection margins via NMR [11]. MS-based techniques in combination with surgical methods can also be used for intraoperative tissue assessment. Such techniques include intelligent knife (iKnife) and Mass Spec and application of picosecond infrared laser (PIRL) and desorption electrospray ionization (DESI) [12]. Biological fluids such as blood serum, plasma and urine are more applicable to general population metabolomics research because of relatively easy handling and non or low invasiveness. Hence, fluids are ideal biospecimens for discovering new potential biomarkers for screening, diagnosis, surveillance and evaluation of therapeutic efficacy. There is a growing interest in metabolome studies because it is an endpoint of genome, epigenome and exposome. Studying metabolome may provide more specific and sensitive insight into dysregulated metabolic pathways in comparison with classical biomarkers [13–16]. Standardized protocols in sampling and handling are highly significant for providing reliable information. For appropriate sampling few parameters should be considered: a) time of sampling b) number of samples c) amount of each sample d) sampling and storage consistency between population of cells, animals or humans [15]. Firstly, to eliminate effect of different exposures (such as dietary compounds and medications), diurnal variations, physical activity and dietary variations should be considered and therefore, their impact should be minimized. To differentiate whether is metabolite result of exposure or not, sampling at different exposure levels and types can be obtained. Number of samples vary depending on: a) complexity (cell culture <<< humans) b) variability (cell culture << syngenic rodents << different genders, races and age of humans) c) subtility of phenotype or effect that is researched d) number of sites of collection e) storage conditions and quality for epidemiological studies with already taken samples [15,16]. Amount of each sample is growing with complexity of organism and sample type, liquids << tissues [15]. However, each type of sample requires an appropriate protocol for metabolites extraction. It is very important to choose the right method which is characterized by solvent type, temperature, and pH value. For example, when analyzing a tissue or cells as a solid sample, disruption step is required. Also, for these samples, so-called quenching (stopping enzymatic activity) is crucial for stable extraction because of

high metabolic activity and high rate metabolite turnover [17,18]. Features of blood and urine metabolic analysis are summarized in Table 1.

Table 1. Comparison of blood and urine features

Biospecimen type	BLOOD (whole blood, plasma, serum, dried blood spot)	URINE (spot urine, 24h urine)	References
Number of metabolites in HMDB*	220945 metabolites	8610 metabolites	[84]
Interfering factors	Proteins, fasting state, circadian rhythm, diet, drugs, stress, exercise, body mass index, gender, age, race, hemolysis, prolonged clotting time (>60min)	food and drug derivates and bacterial contamination, diet, exercise, gender, age, circadian rhythm, gut microflora, genetics	[14,19,85,86–88]
Sampling recommendations	12h fasting	24 h collection	[85,87,89]
Time of processing and storage	centrifugation at 4°C (separation of cells) within 4h, stepwise freezing at -80°C in liquid nitrogen separated in multiple aliquots	within 2 hours of sampling storage at 4°C while collectin samples or 24 h urine; -80°C in liquid nitrogen separated in multiple aliquots	[23,85,87,90]
Endogenous/exogenous metabolic ratio	reflects metabolome mostly endogenous	both, endo- and exogenous metabolic products, environmental exposures	[23,86]
Scope of variation	relatively stable, tightly regulated	high variability, inter- and intraindividual	
Sample and analysis preparation complexity	higher	lower	
Error analysis susceptibility	higher	lower	
Invasiveness	minimal	non-invasive	

Abbreviations: *HMDB – human metabolome database

Less-frequently used samples are saliva, tears, exhaled breath condensate (EBC) and cerebrospinal fluid (CSF). These samples are simpler and containing fewer compounds than blood and urine [19] and more organ related, as well. Integrating information obtained from these biospecimens with well-known blood and urine, result in comprehensive insight into disease. Additionally, analysis of these specimens can be used for identification of potential metabolic biomarkers. Some of the advantages (except CSF) are non-invasiveness and easy access [20–22]. CSF is difficult to obtain but it provides some essential information about central nervous system diseases whereas other samples seem to be insufficient [23]. The main disadvantages of EBC, saliva and tears are lack of standardization and many parameters which should be considered for convenient analysis. For EBC saliva cleanness, temperature and sampler device efficacy are important parameters [20]. Confounding factors in saliva can easily be avoided if following several simple rules. Avoiding cigarettes, alcohol and coffee 12 hours before sampling and avoiding collection within 24 hours of dental work [21]. Examples of application of these types of samples are shown in Table 2. Many studies tried to choose the optimal analytical method depending on the type of sample.

Table 2. Invasiveness and clinical application of less-frequently used samples

Biospecimen	*CSF	Saliva	Tears	Breath
Invasive	Yes	No	No	No
Clinical application	#CNS diseases -schizophrenia, Alzheimer's disease, gliomas, lymphomas, traumatic brain injury etc.	Oral cavity cancer, periodontal diseases, breast cancer, hepatocellular carcinoma, colon and pancreatic cancer, Alzheimer's disease etc.	Ocular diseases - glaucoma, dry eye disease, keratoconus, diabetic retinopathy, trachoma etc. Systemic diseases, breast, colon and pancreatic cancer	Lung, esophageal, gastric cancer; asthma, chronic obstructive pulmonary disease, infections etc.
References	[91–93]	[21,94–96]	[22]	[20,21,97]

Abbreviations: *CSF – cerebrospinal fluid, #CNS – central nervous system

Data acquisition techniques

The two main acquisition techniques in metabolomics are mass spectrometry (MS) and nuclear magnetic resonance (NMR). Separation techniques such as liquid (LC) or gas (GC) chromatography, typically precede MS as a detection technique [15]. Optimization of separation in GC-MS is achieved by temperature gradient changes and for LC by changing gradient and mobile phase. When using GC-MS, metabolites are derivatized to become volatile and electron impact (EI) for ionization is performed. Ion is dissociated into multiple fragments which are passing through m/z quadrupole filter to signal detector. After selecting parent ion by the first quadrupole, the second quadrupole performs fragmentation and the third quadrupole provides a selection of obtained fragments [3]. That means that final detected signal depends on the specific fragment pattern. Because of many overlapping sig-

nals, aligning and deconvolution are done via software. The signal peaks are compared with metabolites in databanks to reveal identity [15]. However, GC-MS is convenient for small molecules (<400 Da) [15] short-chain fatty acids and sugar alcohols [17], sterols, sugars and monophosphorylated sugars and hydroxy acids. It is not suitable for thermally fragile and nonvolatile molecules [3]. On the other hand, for LC-MS electrospray ionization (ESI) is used to ionize dissolved metabolites. The process is followed by high-resolution MS, either time-of-flight (TOF) or OrbitrapTM, or by tandem MS/MS to achieve specificity. A process can be accompanied with collision-induced dissociation (CID), fragmentation of ions by inert gas bombing. Therefore, CID is useful to provide information about structure based on different fragmentation patterns, when metabolite of interest is part of a complex solution [24]. LC-MS is appropriate for temperature sensitive and large weight molecules, whereas compounds that do not ionize cannot be measured [3,17]. LC-MS may disclose several thousand of ions within a sample but some of them are the result of contamination. Besides minimal sample preparation, advantages of LC-MS are high throughput and high sensitivity.

Signal intensity, which depends on mass-to-charge (m/z) ratio, and retention time are compared with standards to provide metabolite identity. These processes are followed by database matching measured ion and the standard in the database. Another approach is chromatography-free, direct-injection MS and it is suitable for single cell metabolomics and certain type of extractions which are not too complex [17].

Targeted metabolomics is favorable when the identity of metabolite is a priori known and it normally follows untargeted approach, as well. Process is usually done using triple quadrupole MS or Qtrap [15]. Quantification can be relative or absolute. Relative quantity can be obtained comparing the curve of signal intensity of the analyzed sample with the standard curve of signal intensity. Absolute quantity can be obtained by comparasion with standards: internal (using stable isotope labeled internal standards) or external [17,25].

Promising and broadly used stable isotopes enable to ascertain the role of metabolite in metabolic pathway and for exact quantification, as well. Classical metabolomics analysis does not provide information about relative activities in metabolic networks. Some metabolites participate in hundreds of metabolic reactions (for example glutamine) [10,26]. Consequently, there is a lack of information about how and how much each pathway contributes to the metabolite level of interest [26].

Stable isotopes may be used for exact quantification: stable isotope-labeled internal standards (IS) with a priori known quantity are used to minimize loss of signal. How to use stable isotopes in metabolic pathway analysis? Stable isotopes can be used for both, in vivo and in vitro studies. Labeled nutrients are brought into a living tissue of interest and then metabolites are extracted and analyzed using MS and/or NMR [27]. This method is low-invasive and requires an injection. Big advantages of this method are: safe for introducing into a living organism and information about dynamics (showing the process, not just a result as in the classic metabolomics approach). Therefore, stable isotope pathway enrichment provides more and precise information about pathway utilization. How to pick appropriate isotope? It depends on the question of a survey. For example, when tracing amino acid utilization in further synthesis carbon (13C) may be chosen, whereas nitrogen (14N) is useful when observing transamination [28]. Studies based on stable isotopes may provide novel information on metabolic pathway's targets and response to anti-cancer agents [29]. The main issues are the lack of all IS for all metabolites and the fact that stable isotope IS are pricey. Another role of stable-isotope labeling is determination of the flux through the pathways. Finally, stable-isotope labeling can be used to detect distinct pathways when some metabolites cannot be measured [30].

NMR spectroscopy is the second main tool with two traits: the obtained signal is proportional to metabolite concentrations and the signal provides more information about the structure. It doesn't require destruction of tissue and it is highly reproducible [15]. Furthermore, it can be coupled with isotope-labeling [29] and therefore used to measure metabolite fluxes. However, the main issues are lack of sensitivity [1] and overlapping signal peaks. Detection threshold is significantly higher (μM) in comparison to MS (pM) [14]. NMR is a valuable method for non-invasive in vivo assessment of metabolism and currently deemed to be the best method for assessing urine metabolome [19]. The problem of low sensitivity is even higher when NMR is used to measure isotope-labeled metabolites, especially when using 13C for labeling. To overcome

this problem, different hyperpolarization techniques (dynamic nuclear polarization technique) can be used [17,27].

In the era of bioinformatics, computational tools enable to acquire more precise information about identity, validity and the role of diverse metabolites. Interpretation of data collected by MS and other techniques is much faster and more accurate because these tools pick peaks, deconvolute, realign, match and remove artifacts, as well. Moreover, different computational tools and Internet platforms (XCMS Online and MetaboAnalyst) can reveal the role of the distinct molecule in metabolic networks. In the end, the main goal is to establish the connection between pathological process, ensuing metabolic change and measured metabolite. With this knowledge, metabolites are becoming more than biomarkers because they are placed in the context of metabolic pathways which means that we have insight into causality. Changes in distinct pathway are followed by predictable changes in metabolite type or level. That opens up opportunities to target different key-points in metabolism, assess the response of therapeutic interventions and follow the development of disease.

Metabolism of tumors

Metabolic changes in tumors became its well-established hallmark [8] as a result of various factors: predominance of oncogenic signaling over tumor-suppressors, a tissue of origin, interaction with microenvironment, availability of nutrients and oxygen, and therapy exposure [31]. The most characteristic change is aerobic glycolysis, known as Warburg effect [7]. This core change is characterized by low-effective adenosine triphosphate (ATP) production and redirection of intermediates to anabolic synthesis of macromolecules, which enables sustainable proliferation and growth [32]. The first presumption of Otto Warburg was that the cancer cells have impaired mitochondria [33]. However, it turned out to be incorrect – mitochondria have a role in biosynthesis and also energy production in some cancer cells. Lactate can be used as a fuel in TCA. Many highly glycolytic cells live in symbiosis with oxidative phosphorylation (OXPHOS)-driven cells and it is seen between cancer cells and also between cancer and stromal cells. So-called „lactate shuttle" is facilitated by high expression of monocarboxylate transporters (MCTs) MCT1 and MCT4 on a cell surface [34–38]. Thus, MCT1 inhibition may have anticancer effect (Figure 1). For example, cancer stem cells (CSCs) rely on oxidative metabolism and use lactate as a source of energy, produced by adjoining cancer-associated fibroblasts (CAFs). This phenomenon is called „reverse Warburg effect" [34,39]. Oxidative metabolism turned out to be significantly important in metastatic cells. These cells have high energy demands, whereas proliferation and growth are less accentuated in that phase [40]. Besides glucose, glutamine is highly consumed by cancer cells, and some tumors show „glutamine addiction" [41,42]. Glutamine serves as a carbon and nitrogen source for biosynthesis of non-essential amino acids and nucleotides. It can participate in TCA to fulfill energy demands or get out of the cycle and continue in amino acid synthesis. Moreover, glutamine transport can be coupled with uptake of essential amino acids. Glutathione enables evasion of reactive oxygen species (ROS)-induced cell death and its generation relies on glutamine [41]. The metabolism of glycine (which is also a part of glutathione) and serine appeared to be upregulated in tumor cells. Serine participates in so-called „one-carbon" metabolism which is important for the generation of S-adenosylmethionine (SAM) and methylation reactions, nucleotide synthesis and has the biggest share in reduced nicotinamide adenine dinucleotide phosphate (NADPH) generation [41]. The glycine-creatine pathway is an alternative energy source for metastatic cells. Increased glycine demands cause increased glutaminolysis due to conversion of glutamate to glycine [40]. Some tumors, such as melanoma and hepatocellular carcinoma show arginine auxotrophy, although arginine is a non-essential amino acid [43]. The explanation might be in generating precursors for polyamine and nucleotide biosynthesis via silencing enzymes involved in de novo arginine synthesis [41,44]. Various lipid species have a special role in membrane biogenesis, modulation of apoptosis, protein modification, cell differentiation, signaling within a cell and tumor microenvironment, and serve as a fuel for beta-oxidation. Normally, cells use exogenous fatty acids (FAs), whereas cancer cells (especially aggressive [45]) increase the biosynthesis of lipids [45,46] or at least increase uptake [47]. Cells that synthesize lipids de novo have been shown to have a higher share of saturated lipids in their membranes which is possible protection from oxidative damage [48]. Moreover, this change leads to decreased fluidity of membrane and it turned out to be characteristic of aggressive cancer [49]. Acetyl-CoA is a

starting point for both, FA and cholesterol biosynthesis, and it is mostly coupled with high glycolytic rate and TCA. However, glutamine and exogenous acetate may also serve for generating acetyl-CoA [41]. Some tumors prefer fatty acid oxidation (FAO) for ATP generation: prostatic cancer [50], diffuse large B cell lymphoma [51], lung cancer [45] and metastasis-initiating cells [52]. Under hypoxic conditions, cancer cells can accumulate lipid droplets and produce lipid-enriched extracellular vesicles (EV) and use stored lipids when conditions become favorable. EVs have increased content of arachidonic acid which can be used in distant, normoxic cells for production of bioactive lipids – eicosanoids and therefore, contribute to invasive traits [53]. Besides CAFs, which can fuel cancer cells with lactate, other types of stromal cells can serve as an energy source. Thus, cancer cells can induce lipolysis in surrounding cancer-associated adipocytes (CAAs) due to lipase activation [54] and directly bind FAs to fatty acid binding proteins (FABs) or can uptake different lipid species from extracellular vesicles originating from tumorous and non-tumorous cells [55]. Released glycerol can also be utilised, through the glycolysis and thus provide energy when conditions are not favorable for mitochondrial oxidation [56]. All enumerated metabolic alterations in tumors and surrounding stromal cells show high metabolic complexity, plasticity and flexibility of tumors, and remarkably contribute to growth, invasiveness and therapy failure [57].

Fig. 1 Cancer cell symbiosis; therapeutic targe

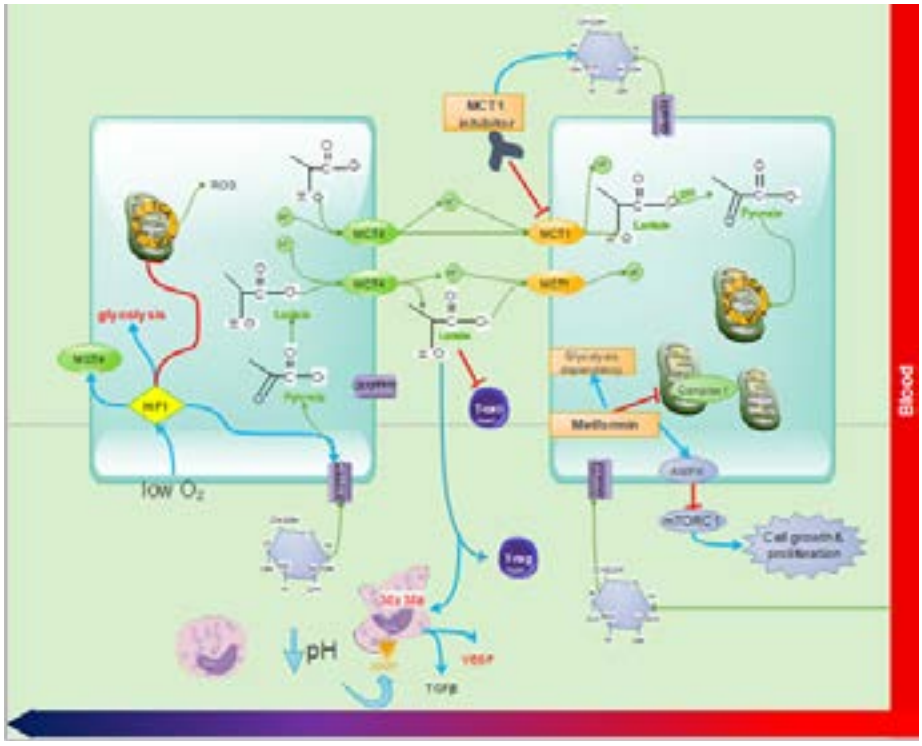

Figure Caption:
MCT1 and MCT4 play major roles in Ca-cell-Ca-cell symbiosis. Highly oxygenated Ca-cells render MCT1 on their surface which enables lactate utilization originating from low-oxygenated cells. Therefore, more glucose is left for anaerobic metabolism of low-oxygenated Ca-cells. Inhibiting MCT1 disables lactate utilization and therefore, highly oxygenated cells are also becoming dependent on glucose. Consequently, less glucose is left for low-oxygenated cells and they die due to nutrient deficiency. Metformin, the "new-old" drug, amplifies effects of MCT1-inhibitor due to inhibiting OXPHOS (complex 1) and leading to higher glycolysis dependency. MCT1-inhibitor activities: the red arrow is direct and the blue arrow is indirect. Lactate is metabolite expressing immunosuppressive (attenuation of dendritic cells, inhibition of monocyte migration, attracting M2M and T-reg) and pro-angiogenic activity.

An acidic environment is boosting MMP activity and Ca-cell mobility, improving invasive traits.
Abbreviations: MCT – monocarboxylate transporter, M2M – tumor-associated macrophages with pro-tumoral activity, often seen in hypoxic conditions, MMP – matrix metalloproteinases, TGFbeta - tumor growth factor beta, VEGF - vascular endothelial growth factor, TCA – tricarboxylic acid cycle

Additional roles of metabolites

Many small molecules are considered as nutrients and/or building units or waste products. However, most of them have some additional roles that might be underestimated. These additional roles may have an impact on carcinogenesis. Inclusively, that is confirmation of assertion that metabolic changes are the hallmark of cancer. Herein we analyze some typical examples of metabolites, from every group of nutrients, with additional roles along with some factors related to tumor microenvironment.

Microenvironment is already recognized as an active participant in tumorigenesis [8,25,34,36,41,58]. However, physical and chemical properties of tumor microenvironment (TME) also govern which types of cells are going to be in TME, as well as signals originating from cancer cells. Hypoxia has many tumor-promoting effects, such as metabolic reprogramming, sustained angiogenesis, stem cell phenotype and immune response repression [58]. The last effect is caused by several hypoxia-related factors: acidic environment (pH ≤ 6,8), vascular endothelial growth factor (VEGF) overexpression, sphingosine-1-phosphate [59], adenosine and lactate accumulation [58]. Acidosis is not just a consequence of hypoxia and aerobic glycolysis, it is also seen in excessive oxidative metabolism due to CO2 production [37,58].

Lactate, abundant metabolite in TME, often considered as a waste product has two groups of roles, nutritional and non-nutritional [8,27,35]. Besides nutritional role, lactate enables evasion of immune response, thereby connecting two emerging hallmarks defined by Hanahan and Weinberg in 2011 [8]. Lactate accumulation is the main cause of tumor acidity in most cases, which is an immune-suppressive condition by itself [58]. Lactate has acidity independent effects due to binding to cell surface lactate receptor G-protein receptor 81 (GPR81) and GPR81-independent mechanisms [38,58,60,61]. It blocks cytotoxic activities of CD8+, natural killer (NK) and natural killer-like T (NKT) cells [62], cytokine release and antigen presentation by dendritic cells (DC). Lactate impairs DC and monocyte differentiation and also lipopolysaccharide-driven cell activation [63]. Moreover, lactate upregulate expression of programmed death-ligand 1 (PDL1 or CD274) which is a major inhibitory pathway in immune cells [60]. Indirect immune-suppressive actions are due to promoting myeloid-derived suppressor cells (MDSCs), regulatory T cells and pro-tumorigenic M2 macrophages [64]. M2 polarization is achieved by hypoxia-inducible factor1 alpha (HIF-1α) stabilization which favors transcription and release of VEGF and transforming growth factor beta (TGF-β) which are responsible for angiogenesis and epithelial-mesenchymal transition (EMT) [35]. VEGF independent angiogenesis promotion might be achieved due to nuclear factor kappaB (NF-kB) activation and thereby IL-8 production in endothelial cells [61]. Lactate-enriched TME increases CD44, TGF-β and hyaluronan production [65]. It enhances cell motility and EMT, metastasis favoring properties [37]. Moreover, lactate is acting as an antioxidant, leading (partly) to radiotherapy resistance [66]. Moreover, in some tumors, lactate is considered as a prognostic biomarker and associated with risk of metastasis and poor outcome in various types of tumors such as breast cancer [67], prostatic cancer [68], head and neck tumors [69], etc.

Another glycolytic product, intermediate metabolite fructose-1,6-biphosphate (F1,6BP) directly binds to an intracellular domain of epidermal growth factor receptor (EGFR) and causes increase its phosphorylation. It results in increased glycolysis and lactate secretion, which consequently leads to immune-suppression. This interesting effect of F1,6BP is perceived in triple-negative breast cancer (TNBC) [70].

Increase in methylglyoxal (MG) is one of the consequences of high glycolytic flux. This glycating agent is highly reactive and causes „carbonyl stress". Normally, enzymes glyoxalases deal with this degradation product of glycolysis and convert it to D-lactate. Disbalance between the production of MG and its conversion leads to accumulation and carbonyl stress. MG targets observed in cancer are heat shock proteins (Hsps). Hsp90 is glycated in breast cancer, and therefore, its protein-stabilization activity is decreased. Finally, MG causes decreased large tumor suppressor 1 (LATS1), major kinase in Hippo pathway, antitumor activity. The

final impact on tumorigenesis is via sustained localization of Yes-associated protein (YAP) transcriptional factor in the cell nucleus. Consequently, it impairs Hippo pathway function [71], which normally operates in a process of contact inhibition [72].

Metabolites in lipid group also have „side-effects". In HCC, free fatty acids (FFAs) are reported to have a role in EMT. FFAs cause loss of desmoplakin which builds desmosomes and activation of both TGF-β and Wnt/β -catenin pathways [73]. FFAs are imported via CD36. This receptor is also involved in calcium signaling modulation via FFAs in prostatic cancer [74]. Omega-6 polyunsaturated fatty acids (ω-6 PUFA, for example, arachidonic acid) converts to PGE2 and promote prostatic cancer cells invasion and migration to lipid-rich bone marrow, whereas ω-3 PUFAs are opposing that effect [75]. ω-3 PUFAs (poly-unsaturated FAs) showed as a promising supplement in patients with breast cancer, treated with mammalian target of rapamycin (mTOR) inhibitor Rapamycin (Rp). Rp frequently leads to metabolic disorders which can be reduced by ω-3 PUFAs. The main beneficial effect of ω-3 PUFAs is enhanced ROS production leading to lipotoxicity and prevention of Rp caused autophagy, the main cause of therapy resistance [76].

Monounsaturated fatty acid, oleic acid (OA) seems to have a disparate impact on tumors. OA is reported to be protective in breast cancer [77]. On the other side, a recent study showed that oleic acid elevates cervical cancer tumorigenesis in vivo and in vitro. The underlying mechanism is induction of CD36 expression which further result in phosphorylation and activation of Src/ERK1/2 signaling pathway. Consequently, OA increases tumor proliferation, migration and invasion via CD36 cell surface fatty acid receptor [78]. That implies that an olive oil-rich Mediterranean diet is considered to be beneficial in many pathological conditions, but cervical cancer seems to be one of the exceptions.

Short chain fatty acids (SCFAs) are products of fiber fermentation in gut. They are well known because of their various non-nutritional effects. SCFAs have a role in maintaining intestine homeostasis, integrity and antitumor properties, which are well established in colon cancer [79]. However, their role in other types of cancer is less clear. The recent study showed role of SCFAs in reduction of invasive phenotype of breast cancer. That effect is mediated by two receptors free fatty acid receptors (FFARs) FFAR2 and FFAR3. Both receptors increase YAP1 phosphorylation and therefore decrease Hippo/Yap pathway activity. FFAR2 also increases E-cadherin levels. As a result, cancer cells are directed to epithelial-like phenotype and invasive properties of breast cancer are significantly reduced. Diminished expression of these receptors in aggressive types of breast cancer prevents the beneficial effects of SCFAs [80].

Amino acids build proteins and take part in TCA. Glutamine is major anaplerotic amino acid, and participate in biosynthesis of many important metabolites [40]. Besides being a nutrient and building unit, glutamine has anti-inflammatory properties due to induction of phosphatase MKP-1 which leads to cytosolic phospholipase A2 (cPLA2) inactivation [81].

Proline is another non-essential amino acid and can be synthesized from glutamate. It is also stored in collagen and serves as an alternative source of energy in nutrient-deprived conditions. However, proline has some regulatory roles. Proline dehydrogenase/proline oxidase (PRODH/POX) is the enzyme that catalyzes proline oxidation and therefore produces ROS. Consequently, ROS production is linked to many effects such as epigenetic changes and modulation of insulin/insulin growth factor (I/IGF) signaling axis [82]. Proline is shown to have an impact on differentiation of embryonic stem cells, as well [83].

Conclusion

In conclusion, metabolic reprogramming contributes to various aspects of tumor growth. Besides energy supply metabolic changes have additional roles including immunomodulation, alterations in signaling pathways and enhancement of metastasis-related processes. Multiple interactions between metabolome and genome, transcriptome, proteome and epigenome define cancer phenotype. Studies on cancer metabolism contribute to the complex knowledge of tumor biology and enable discovery of novel biomarkers and therapeutic targets.

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References

1. Mathé E, Hays JL, Stover DG, et al. The omics revolution continues: the maturation

of high-throughput biological data sources. Yearb Med Inform 2018; 27(1):211-222. doi:10.1055/s-0038-1667085

2. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 2016; 17(7):451-459. doi:10.1038/nrm.2016.25

3. Liu X, Locasale JW. Metabolomics: a primer. Trends Biochem Sci 2017; 42(4):274-284. doi:10.1016/j.tibs.2017.01.004

4. Clish CB. Metabolomics: an emerging but powerful tool for precision medicine. Mol Case Stud 2015; 1(1):a000588. doi:10.1101/mcs.a000588

5. Siskos AP, Jain P, Römisch-Margl W, Bennett M, et al. Interlaboratory reproducibility of a targeted metabolomics platform for analysis of human serum and plasma. Anal Chem 2017; 89(1):656-665. doi:10.1021/acs.analchem.6b02930

6. Wishart D. Metabolomics and the Multi-Omics View of Cancer. Metabolites 2022;12(2):154. doi: 10.3390/metabo12020154

7. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol 1927; 8(6):519-530.

8. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5):646-674. doi:10.1016/j.cell.2011.02.013

9. Veenstra TD. Metabolomics: the final frontier? Genome Med 2012; 4(4):40. doi:10.1186/gm339

10. Creek DJ. Stable isotope labeled metabolomics improves identification of novel metabolites and pathways. Bioanalysis 2013; 5(15):1807-1810. doi:10.4155/bio.13.131

11. Bathen TF, Geurts B, Sitter B, et al. Feasibility of MR metabolomics for immediate analysis of resection margins during breast cancer surgery. PLoS One 2013; 8(4):e61578. doi:10.1371/journal.pone.0061578

12. Zhang J, Rector J, Lin JQ, et al. Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. Sci Transl Med 2017; 9(406):eaan3968. doi:10.1126/scitranslmed.aan3968

13. Zhang A, Sun H, Yan G, et al. Metabolomics for biomarker discovery: moving to the clinic. Biomed Res Int 2015:354671. doi:10.1155/2015/354671

14. Günther UL. Metabolomics biomarkers for breast cancer. Pathobiology 2015; 82(3-4):153-165. doi:10.1159/000430844

15. Barnes S, Benton HP, Casazza K, et al. Training in metabolomics research. I. Designing the experiment, collecting and extracting samples and generating metabolomics data. J Mass Spectrom 2016; 51(7):461-475. doi:10.1002/jms.3782.

16. The Five key elements of a successful metabolomics study. https://www.metabolon.com/application/files/2415/0388/1050/The-5-Key-Elements-of-a-Successful-Metabolomics-Study.pdf. Accessed November 15, 2021.

17. Lu W, Su X, Klein MS, Lewis IA, et al. Metabolite Measurement: Pitfalls to avoid and practices to follow. Annu Rev Biochem 2017; 86(1):277-304. doi:10.1146/annurev-biochem-061516-044952

18. Wawrzyniak R, Kosnowska A, Macioszek S, et al. New plasma preparation approach to enrich metabolome coverage in untargeted metabolomics: Plasma protein bound hydrophobic metabolite release with proteinase K. Sci Rep 2018; 8(1):9541. doi:10.1038/s41598-018-27983-0

19. Bouatra S, Aziat F, Mandal R, Guo AC, et al. The human urine metabolome. PLoS One 2013; 8(9):e73076. doi:10.1371/journal.pone.0073076

20. Zamuruyev KO, Aksenov AA, Pasamontes A, et al. Human breath metabolomics using an optimized non-invasive exhaled breath condensate sampler. J Breath Res 2017; 11(1):016001. doi:10.1088/1752-7163/11/1/016001

21. Beale DJ, Jones OA, Karpe AV, et al. A review of analytical techniques and their application in disease diagnosis in breathomics and salivaomics research. Int J Mol Sci 2017; 18(1):24. doi:10.3390/ijms18010024

22. Hagan S, Martin E, Enríquez-de-Salamanca A. Tear fluid biomarkers in ocular and systemic disease: potential use for predictive, preventive and personalised medicine. EPMA J 2016; 7(1):15. doi:10.1186/s13167-016-0065-3

23. Kohler I, Hankemeier T, van der Graaf PH, et al. Integrating clinical metabolomics-based biomarker discovery and clinical pharmacology to enable precision medicine. Eur J Pharm Sci 2017; 109:S15-S21. doi:10.1016/j.ejps.2017.05.018

24. Matišić D [Proteomics in diagnosis of solid tumors] In: Vrbanec D, Kulić A, eds. [New approaches in diagnosis and tratment of solid tumors] Medicinska naklada, Zagreb, 2012: pp 13-22. Croatian

25. Muir A, Danai LV, Vander Heiden MG. Microenvironmental regulation of cancer cell metabolism: implications for experimental design and translational studies. Dis Model Mech 2018; 11(8): dmm035758. doi:10.1242/dmm.035758

26. Buescher JM, Antoniewicz MR, Boros LG, et al. A roadmap for interpreting 13 C metabolite labeling patterns from cells. Curr Opin Biotechnol 2015; 34:189-201. doi:10.1016/j.copbio.2015.02.003

27. Faubert B, DeBerardinis RJ. Analyzing tumor metabolism in vivo. Annu Rev Cancer Biol 2017; 1(1):99-117. doi:10.1146/annurev-cancerbio-050216-121954

28. Gravel S-P, Avizonis D, St-Pierre J. Metabolomics analyses of cancer cells in controlled microenvironments. In: Methods in Molecular Biology. Vol 1458. Humana Press, New York, NY, 2016: pp 273-290. doi:10.1007/978-1-4939-3801-8_20

29. Fan TW, El-Amouri SS, Macedo JKA, et al. Stable isotope-resolved metabolomics shows metabolic resistance to anti-cancer selenite in 3D spheroids versus 2D cell cultures. Metabolites 2018; 8(3):40. doi:10.3390/metabo8030040

30. Kang YP, Ward NP, DeNicola GM. Recent advances in cancer metabolism: a technological perspective. Exp Mol Med 2018; 50(4):31. doi:10.1038/s12276-018-0027-z

31. Vander Heiden MG, DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. Cell 2017; 168(4):657-669. doi:10.1016/j.cell.2016.12.039

32. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324(5930):1029-1033. doi:10.1126/science.1160809

33. Warburg O. On the origin of cancer cells. Science 1956; 123(3191):309-314. doi:10.1126/science.123.3191.309

34. Avagliano A, Granato G, Ruocco MR, et al. Metabolic reprogramming of cancer associated fibroblasts: the slavery of stromal fibroblasts. Biomed Res Int 2018. doi:10.1155/2018/6075403

35. Morrot A, da Fonseca LM, Salustiano EJ, et al. Metabolic symbiosis and immunomodulation: how tumor cell-derived lactate may disturb innate and adaptive immune responses. Front Oncol 2018; 8:81. doi:10.3389/fonc.2018.00081

36. Curry JM, Tuluc M, Whitaker-Menezes D, et al. Cancer metabolism, stemness and tumor recurrence: MCT1 and MCT4 are functional biomarkers of metabolic symbiosis in head and neck cancer. Cell Cycle 2013; 12(9):1371-1384.

37. Marchiq I, Pouysségur J. Hypoxia, cancer metabolism and the therapeutic benefit of targeting lactate/H(+) symporters. J Mol Med (Berl) 2016; 94(2):155-171. doi:10.1007/s00109-015-1307-x

38. Romero-Garcia S, Moreno-Altamirano MM, Prado-Garcia H, et al. Lactate contribution to the tumor microenvironment: mechanisms, effects on immune cells and therapeutic relevance. Front Immunol 2016; 7:52. doi:10.3389/fimmu.2016.00052

39. Penkert J, Ripperger T, Schieck M, et al. On metabolic reprogramming and tumor biology: A comprehensive survey of metabolism in breast cancer. Oncotarget 2016; 7(41):67626-67649. doi:10.18632/oncotarget.11759

40. Weber GF. Time and circumstances: Cancer cell metabolism at various stages of disease progression. Front Oncol 2016; 6:1-9. doi:10.3389/fonc.2016.00257

41. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metab 2016; 23(1):27-47. doi:10.1016/j.cmet.2015.12.006

42. Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. Trends Biochem Sci 2010; 35(8):427-433. doi:10.1016/j.tibs.2010.05.003

43. Ensor CM, Holtsberg FW, Bomalaski JS, et al. Pegylated arginine deiminase (ADI-SS PEG 20,000 mw) inhibits human melanomas and hepatocellular carcinomas in vitro and in vivo. Cancer Res 2002; 62(19):5443-5450.

44. Casero Jr RA, Marton LJ. Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. Nat Rev Drug Discov 2007; 6:373.

45. Merino Salvador M, Gómez de Cedrón M, Moreno Rubio J, et al. Lipid metabolism and lung cancer. Crit Rev Oncol Hematol 2017;112:31-40. doi:10.1016/j.critrevonc.2017.02.001

46. Baumann J, Sevinsky C, Conklin DS. Lipid biology of breast cancer. Biochim Biophys Acta 2013: 1831(10):1509-1517. doi:10.1016/j.bbalip.2013.03.011

47. Ogrodzinski MP, Bernard JJ, Lunt SY. Deciphering metabolic rewiring in breast cancer subtypes. Transl Res 2017; 189:105-122. doi:10.1016/j.trsl.2017.07.004

48. Rysman E, Brusselmans K, Scheys K, et al. De novo lipogenesis protects cancer cells from free radicals and chemotherapeutics by promoting membrane lipid saturation. Cancer Res 2010; 70(20):8117-8126. doi:10.1158/0008-5472.CAN-09-3871

49. Hilvo M, Denkert C, Lehtinen L, et al. Novel theranostic opportunities offered by characterization of altered membrane lipid metabolism in breast cancer progression. Cancer Res 2011; 71(9):3236-3245. doi:10.1158/0008-5472.CAN-10-3894

50. Liu Y. Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer. Prostate Cancer Prostatic Dis 2006; 9(3):230-234. doi:10.1038/sj.pcan.4500879

51. Caro P, Kishan AU, Norberg E, et al. Metabolic signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma. Cancer Cell 2012; 22(4):547-560. doi:10.1016/j.ccr.2012.08.014

52. Aznar Benitah S. Metastatic-initiating cells and lipid metabolism. Cell Stress 2017 1(3):110-114. doi:10.15698/cst2017.12.113

53. Schlaepfer IR, Nambiar DK, Ramteke A, et al. Hypoxia induces triglycerides accumulation in prostate cancer cells and extracellular vesicles supporting growth and invasiveness following reoxygenation. Oncotarget 2015; 6(26): 22836-22856. doi:10.18632/oncotarget.4479

54. Nieman KM, Kenny HA, Penicka CV, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth.

Nat Med 2011; 17(11):1498-1503. doi:10.1038/nm.2492

55. Lazar I, Clement E, Attane C, et al. A new role for extracellular vesicles: how small vesicles can feed tumors' big appetite. J Lipid Res 2018; 59(10):1793-1804. doi:10.1194/jlr.R083725

56. Jones MG. Does fat provide energy for breast tumour cell invasion and metastasis? University of Otago. 2015. Retrieved from http://hdl.handle.net/10523/6058

57. Amoedo ND, Obre E, Rossignol R. Drug discovery strategies in the field of tumor energy metabolism: Limitations by metabolic flexibility and metabolic resistance to chemotherapy. Biochim Biophys Acta Bioenerg 2017; 1858(8):674-685. doi:10.1016/j.bbabio.2017.02.005

58. Vaupel P, Multhoff G. Accomplices of the hypoxic tumor microenvironment compromising antitumor immunity: Adenosine, lactate, acidosis, vascular endothelial growth factor, potassium ions, and phosphatidylserine. Front Immunol 2017; 8:1887. doi:10.3389/fimmu.2017.01887

59. Rodriguez YI, Campos LE, Castro MG, et al. Sphingosine-1 Phosphate: A new modulator of immune plasticity in the tumor microenvironment. Front Oncol 2016; 6:218. doi:10.3389/fonc.2016.00218

60. Feng J, Yang H, ZhangY, et al. Tumor cell-derived lactate induces TAZ-dependent upregulation of PD-L1 through GPR81 in human lung cancer cells. Oncogene 2017; 36(42):5829-5839. doi:10.1038/onc.2017.188

61. Sun S, Li H, Chen J, Qian Q. Lactic acid: no longer an inert and end-product of glycolysis. Physiology 2017; 32(6):453-463. doi:10.1152/physiol.00016.2017

62. Brand A, Singer K, Koehl GE, et al. LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. Cell Metab 2016; 24(5):657-671. doi:10.1016/j.cmet.2016.08.011

63. Gottfried E, Kunz-Schughart LA, Ebner S, et al. Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. Blood 2006; 107(5):2013-2021. doi:10.1182/blood-2005-05-1795

64. Sica A, Larghi P, Mancino A, Rubino L, et al. Macrophage polarization in tumour progression. Semin Cancer Biol 2008; 18(5):349-355. doi:10.1016/J.SEMCANCER.2008.03.004

65. Stern R, Shuster S, Neudecker BA, et al. Lactate Stimulates Fibroblast Expression of Hyaluronan and CD44: The Warburg effect revisited. Exp Cell Res 2002; 276(1):24-31. doi:10.1006/excr.2002.5508

66. Sattler UG, Meyer SS, Quennet V, et al. Glycolytic metabolism and tumour response to fractionated irradiation. Radiother Oncol 2010; 94(1):102-109. doi:10.1016/j.radonc.2009.11.007

67. Cheung SM, Husain E, Masannat Y, et al. Lactate concentration in breast cancer using advanced magnetic resonance spectroscopy. Br J Cancer 2020; 123(2):261-267. doi: 10.1038/s41416-020-0886-7. Epub 2020 May 19.

68. Yaligar J, Thakur SB, Bokacheva L, et al. Lactate MRSI and DCE MRI as surrogate markers of prostate tumor aggressiveness. NMR Biomed 2011; 25(1):113-122. doi:10.1002/nbm.1723

69. Walenta S, Wetterling M, Lehrke M, et al. High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. Cancer Res 2000; 60(4):916-921.

70. Lim SO, Li CW, Xia W, et al. EGFR signaling enhances aerobic glycolysis in triple-negative breast cancer cells to promote tumor growth and immune escape. Cancer Res 2016; 76(5):1284-1296. doi:10.1158/0008-5472.CAN-15-2478

71. Nokin MJ, Durieux F, Peixoto P, et al. Methylglyoxal, a glycolysis side-product, induces Hsp90 glycation and YAP- mediated tumor growth and metastasis. Elife 2016; Oct 19:5. doi:10.7554/eLife.19375

72. Gumbiner BM, Kim N-G. The Hippo-YAP signaling pathway and contact inhibition of growth. J Cell Sci 2014; 127(Pt 4):709-717. doi:10.1242/jcs.140103

73. Nath A, Li I, Roberts LR, et al. Elevated free fatty acid uptake via CD36 promotes epithelial-mesenchymal transition in hepatocellular carcinoma. Sci Rep 2015; 5:14752. doi:10.1038/srep14752

74. Maly IV, Hofmann WA. Fatty acids and calcium regulation in prostate cancer. Nutrients 2018; 10(6):788. doi:10.3390/nu10060788

75. Brown MD, Hart CA, Gazi E, et al. Promotion of prostatic metastatic migration towards human bone marrow stoma by Omega 6 and its inhibition by Omega 3 PUFAs. Br J Cancer 2006; 94(6):842-853. doi:10.1038/sj.bjc.6603030

76. Zhu S, Feng N, Lin G, et al. Metabolic shift induced by ω-3 PUFAs and rapamycin lead to cancer cell death. Cell Physiol Biochem 2018; 48(6):2318-2336. doi:10.1159/000492648

77. Escrich E, Solanas M, Moral R, et al. Modulatory effects and molecular mechanisms of olive oil and other dietary lipids in breast cancer. Curr Pharm Des 2011; 17(8):813-830. doi:10.2174/138161211795428902

78. Yang P, Su C, Luo X, et al. Dietary oleic acid-induced CD36 promotes cervical cancer cell growth and metastasis via up-regulation Src/ERK pathway. Cancer Lett 2018; 438:76-85.doi:http://dx.doi.org/10.1016/j.canlet.2018.09.006

79. McNabney SM, Henagan TM. Short chain fatty acids in the colon and peripheral tissues: A focus on butyrate, colon cancer, obesity and insulin resistance. Nutrients 2017; 9(12):1348. doi:10.3390/nu9121348

80. Thirunavukkarasan M, Wang C, Rao A, et al. Short-chain fatty acid receptors inhibit invasive phenotypes in breast cancer cells. PLoS One 2017; 12(10):1-16. doi:10.1371/journal.pone.0186334

81. Lee CH, Kim HK, Jeong JS, et al. Mechanism of glutamine inhibition of cytosolic phospholipase a2 (cPLA2): Evidence of physical interaction between glutamine-Induced mitogen-activated protein kinase phosphatase-1 and cPLA2. Clin Exp Immunol 2015; 180(3):571-580. doi:10.1111/cei.12585

82. Zarse K, Schmeisser S, Groth M, et al. Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. Cell Metab 2012; 15(4):451-465. doi:10.1016/j.cmet.2012.02.013

83. Washington JM, Rathjen J, Felquer F. l-Proline induces differentiation of ES cells: a novel role for an amino acid in the regulation of pluripotent cells in culture. Am J Physiol Physiol 2010; 298(5):C982-C992. doi:10.1152/ajpcell.00498.2009

84. Human Metabolome Database. http://www.hmdb.ca/. Accessed February 21, 2022.

85. Yin P, Lehmann R, Xu G. Effects of pre-analytical processes on blood samples used in metabolomics studies. Anal Bioanal Chem 2015; 407(17):4879-4892. doi:10.1007/s00216-015-8565-x

86. Teo CC, Chong WPK, Tan E, et al. Advances in sample preparation and analytical techniques for lipidomics study of clinical samples. TrAC - Trends Anal Chem 2015; 66:1-18. doi:10.1016/j.trac.2014.10.010

87. Beger R. A review of applications of metabolomics in cancer. Metabolites 2013; 3(3):552-574. doi:10.3390/metabo3030552

88. Shen J, Yan L, Liu S, et al. Plasma metabolo-

mic profiles in breast cancer patients and healthy controls: by race and tumor receptor subtypes. Transl Oncol 2013; 6(6):757-765. doi:10.1593/tlo.13619

89. Tzoulaki I, Ebbels TMD, Valdes A, et al. Design and analysis of metabolomics studies in epidemiologic research: a primer on -Omic technologies. Am J Epidemiol 2014; 180(2):129-139. doi:10.1093/aje/kwu143

90. Dunn WB, Broadhurst D, Begley P, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nat Protoc 2011; 6(7):1060-1083. doi:10.1038/nprot.2011.335

91. Orešič M, Tang J, Seppänen-Laakso T, et al. Metabolome in schizophrenia and other psychotic disorders: A general population-based study. Genome Med 2011; 3(3):19. doi:10.1186/gm233

92. Nakamizo S, Sasayama T, Shinohara M, et al. GC/MS-based metabolomic analysis of cerebrospinal fluid (CSF) from glioma patients. J Neurooncol 2013; 113(1):65-74. doi:10.1007/s11060-013-1090-x

93. Bos I, Vos S, Vandenberghe R, et al. The EMIF-AD multimodal biomarker discovery study: design, methods and cohort characteristics. Alzheimer's Res Ther 2018; 10(1):64. doi:10.1186/s13195-018-0396-5

94. Ishikawa S, Sugimoto M, Kitabatake K, et al. Identification of salivary metabolomic biomarkers for oral cancer screening. Sci Rep 2016; 6:31520. doi:10.1038/srep31520

95. Sugimoto M, Wong DT, Hirayama A, et al. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. Metabolomics 2010; 6(1):78-95. doi:10.1007/s11306-009-0178-y

96. Kaczor-Urbanowicz KE, Martin Carras-Presas C, Aro K, et al. Saliva diagnostics - Current views and directions. Exp Biol Med (Maywood) 2017; 242(5):459-472. doi:10.1177/1535370216681550

97. Capuano R, Santonico M, Pennazza G, et al. The lung cancer breath signature: A comparative analysis of exhaled breath and air sampled from inside the lungs. Sci Rep 2015; 5:16491. doi:10.1038/srep16491