Metabolomics applications in disease diagnosis, treatment, and drug discovery

Hassan Z. Ghanem, Mai O. Kadry, Rehab M. Abdel-Megeed, Abdel-Hamid Z. Abdel-Hamid

Department of Therapeutic Chemistry, National Research Centre, El Buhouth St., Dokki, Giza, Egypt

Correspondence to Rehab M. Abdel-Megeed. Tel: +20 101 007 5907; e-mail: rehabzenbaa@gmail.com

Received: 2 March 2019
Accepted: 19 November 2019

Egyptian Pharmaceutical Journal 2019, 18:290–295

Metabolomics-based discovery has an important role in disease diagnosis, discovery of drug, and treatment expansion. This tool is a novel biomarker that can provide a biochemical insight into disease stages and could estimate the activity of certain drugs; it can observe the preclinical and clinical cases, leading to discovery of more efficient methods for treatment approach. This review discussed the potential of metabolomics technology as a very important approach in disease diagnosis, therapy, as well as new drug discovery.

Keywords:
biomarkers, drug discovery, metabolomics

Egypt Pharmaceut J 18:290–295

1687-4315

Introduction

Metabolic analysis provides a biochemical snapshot of molecules that are produced during cell metabolism and can directly reflect physiological situation that improves both preclinical and clinical trials, efficiency of drug, and success of therapy [1]. Metabolomics may be subdivided into targeted or untargeted metabolomics [2], fluxomics [3], lipidomics [4], and xenometabolomics [5,6].

Metabolomics can detect a specific biomarker(s) in diseases after genetic or environmental interference, which cannot be obtained from other omics technology [7]. Metabolomics helps in diagnosing cancer at early stage[8], diabetes [9], cardiac as well as vascular diseases [10], and neonatology [11–13]. Nowadays, pharmaco-metabolomics is termed as the most novel technique to denote efficacy of the drug, in addition to classify patients into responsive or nonresponsive patients to develop clinical trials [14]. It is a new tool that includes metabolic signature and chemo-informatics to predict drug target, toxicity, and efficiency [15], in addition to providing an efficient and cheap technique [16,17]. Increasing knowledge about metabolic profiling in addition to the other omics technologies could be an important tool indetermination of safety margin for new drugs [18].

Target and nontarget metabolomics

Nontarget metabolomics can allow a hypothesis-free global overview of more important metabolite that is influenced by a disease or experimental disorder. However, targeted metabolomics provide us knowledge about metabolic pathways that help in evaluating the drug efficiency candidate on metabolite regulations [19].

Platforms technology of target metabolites depends on distinct optimizing strategy for analysis of various pathways. This technology can clarify accommodation in addition to the wide variation in stability and physiochemical makeup [20].

Metabolomic platforms

Metabolomics is the most accurate technology as compared with other omics technologies. There are three main metabolomic platforms: gas chromatography, liquid chromatography (LC-MS), and nuclear magnetic resonance (NMR) [21,22].

Advantages and disadvantages of different metabolomics technologies

Nuclear magnetic resonance spectroscopy

NMR spectroscopy has different advantages. This technique is quantitative, nondescriptive, and fast (2–3 min/sample) [20]. It does not require derivatization or separation. Furthermore, it has the ability to identify novel chemicals. It could be fully automated in addition to its ability to be compatible with liquid, sand, and solids. This instrument has long lifetime (over 20 years) [22].

On the contrary, NMR spectroscopy is not sensitive, in addition to its high startup cost (>US$1 million). It cannot detect or identify salt sand inorganic ions. It cannot detect nonprorogated compounds. NMR spectroscopy requires larger sample volumes (0.1–0.5 ml) [20].

 DOI: 10.4103/epj.epj_10_19

Gas chromatography

It can be considered as robust, mature technology with moderate startup cost (~$150 000).

It requires modest sample volume with good sensitivity. It has large body of software and databases. This technology has excellent separation reproducibility. It can be mostly automated and compatible with gases and liquids [20]. Although several advantages are detected with gas chromatography, it declares different disadvantages. It is a descriptive technology (sample Notre coverable). It requires sample derivatization and separation. It could be slow for sample analysis (20–40 min/sample). Furthermore, it cannot be used in imaging. It is not compatible with solids. Furthermore, this technique has difficulty in identifying novel compounds [20].

Liquid chromatography

LC-MS is supersensitive (LOD=0.5 nmol/l). It is a very flexible technology. It can detect most organic and some inorganic molecules. LC-MS requires small sample volumes (10–100 µl). It can be done without separation (direct injection). This technology has the potential to detect the largest portion of metabolomics. It can be mostly automated and is compatible with solid, sand, and liquids [21].

On the contrary, LC-MS has a few disadvantages, as it is destructive (sample not recoverable). Furthermore, it is not very quantitative. It has high cost (>$300 000). This technique is slow (15–40 min/sample). It usually requires separation, with poor separation resolution. It is not compatible with gases. Most spectral features are not yet identifiable. In addition, novel compound identification is difficult. The instrument lifetime is short (< 9 years) [20,21].

Metabolomics applications in human disease diagnosis

Gene expression analysis is the first step in molecular information flow from the genome to the proteome then to metabolomics. Proteomics may asses the discovery of functional proteins that regulate metabolic pathways, 'their synthesis and degradation, and their modification of both physiological and pathological conditions' [3]. So, it could provide valuable, complementary information about the pathways that may be affected by both early prognosis and diagnosis through different samples (saliva, sputum, serum, plasma, blood, urine, tissue, fecal water, or feces) [1].

Metabolomics workflow and parameters influencing research into human diseases

Metabolomics is often applied in the assessment of biological system disturbances resulting from diseases where the disease stage can be indicated according to changing of metabolite concentrations [1].

Cancer

Metabolomics showed that the metabolite profiles of various tumors are correlated with the type of the tumor [7]. Metabolites biomarkers in cancer have been established as follows.

Central nervous system and brain

The most abundant metabolites were valine, proline, alanine, total choline compounds, myoinositol, creatine, taurine, glutamate, glutamine, γ-aminobutyric acid, malate, N-acetyl-aspartate, and acetate [23].

Cervical carcinoma

Cervical carcinoma was linked to phosphocholine, choline, glycerophosphocholine, creatine, taurine, glucose, and lactate [24].

Breast cancer

Methylated nucleosides, like 5-hydroxymethyl-2-deoxyuridine, and 8-hydroxy-2-deoxyguanosine, are potential tumor markers for early diagnosis of breast cancer. 3-N-acetyl-glycine, hydroxybutyrate, 3-hydroxy-2-methyl-butanoic acid, and nonanedioic acid have been identified in this disease also [25].

Lung cancer

HPLC allowed the identification of 11 biomarkers (eight amino acids and their metabolites, one bile acid, one dipeptide, and one organic acid), which underwent a significant modification ($P<0.05$, t test) of their urinary concentration in patients with lung cancer relative to healthy volunteers [26,27].

Hepatocellular carcinoma

In benign hepatic disease, creatine increased and glutamine was the only metabolite in common with hepatocellular carcinoma [28,29].

Hypertension

Hypertension is associated with increasing in alpha-1-acid glycoproteins and choline. In urine, the discriminatory metabolites include alpha-1-acid glycoproteins and choline format [30].

Alzheimer's

Alterations of the lecithin, amino acid, and phospholipids metabolism in Alzheimer's disease may be very relevant for clinical diagnosis and

treatment. Besides, bile acid and lipid components may play an important role in Alzheimer's disease through cell signaling mechanisms, cholesterol homeostasis, and metabolism of amyloid [31].

Drug discovery

It includes discovery of new compounds or determination of a lead compound that can lead to a real drug. It is well-known that innovating or designing a new drug from herbal or synthetic origin to lunch a finished product is a very difficult pathway. The total cost of drug discovery or development ranges from US$ 897 million to US$ 19 billion and the typical development time taken for this process is 10–15 years [12]. Searching for a target can arise from a diversity of sources. It can take number of years to make a strong proof before assigning a target. Once a target has been known, the pharmaceutical companies have streamlined a number of early processes to identify molecules that possess suitable characteristics to make acceptable drugs [22,32].

The drug discovery program is the cornerstone of the pharmaceutical research in industry. It is the process that encompasses early stages of research, from target discovery to validation of lead compound or drug candidate. During lead discovery, a thorough searched is performed to find a small, drug-like molecule or biological drug candidate, the likes of which can be progressed into later stages starting by preclinical and finally clinical trials. Well thought-out and thorough analysis at this early stage will reduce risks and increase the chances of success throughout the drug development pipeline (http://www.combichemistry.com/drug-discovery.html, http://chiesipakistan.com/index.php?page=R%26D+Pharmaceutical+Industry).

Stages of drug discovery

Target identification and validation

The target is the naturally existing cellular or modular structure that appears to have an important role in a particular disease pathway and will be targeted by the drugs that will subsequently be developed.

A significant target needs to be safe and efficacious, be accessible by the drug module. Some drug molecules elicit a biomedical response upon binding to the target, such as antibodies binding with proteins to block and prevent protein–-protein interactions [19]. Good validation is essential in increasing confidence in the relationship between the target and disease and allows exploring whether the target will be responsive to development of novel medicines designed to elicit a specific therapeutic result [19].

Target validation provides a deep understand if and how your lead candidates are performing. It ranges from in-vitro study to in-vivo methods and continue into drug development where phase III clinical trials are the ultimate test [19].

High-throughput screening

High-throughput screening is define as screening a compound or candidate library directly against the target which is the most popular method as it allows the assaying of a large number of potential molecules against the chosen targets [33].

A knowledge-based approach

When enough knowledge on the identified target is available, it must be analyzed and assessed. Alternatively, we can use any existing compounds to focus a screen, potentially reducing cost or aid the development of a virtual screen [34].

Virtual screening

It provides coverage across a wide chemical space using computer-assisted analysis to reduce the number of compounds screened, and potentially reduce costs. Such virtual screening can provide the starting chemical structures for a focused screen, without the need to physically screen a large, expensive compound library screen [35].

Hit identification and validation

Compound screening assays are developed following target phases of the drug discovery process. Hit molecules can mean slightly different things according to different researches. Hits can be identified through several techniques, which depend on the target and what previous knowledge exists [33].

Absorption, disposition metabolism, excretion and toxicity screening

Absorption, disposition metabolism, excretion and toxicity screening acts as a screening tool and utilization approach in drug discovery and development [34,35].

Metabolomics and drug discovery

Applications in targeted metabolite identification

Targeted determination is an important step in drug discovery and metabolites are considered definitive targets of drug discovery such as receptors, enzymes, ion channels, and transporters except for few cases, such as uric acid [36].

The efficacy of metabolomics in the identification of protein and gene targets is well illustrated by the following.

Improving an aberrant metabolism is a common target for several drug discovery trials for controlling diseases caused by metabolic problems.

Regulators of deranged metabolism in many diseases are perfect goals for phamacotherapies. Bidirectional interaction commonly occur among the alterations in gene, protein expression, and metabolites clarifying early and specific metabolic perturbations caused by the breakdown of biosystems; this allows identification of targets, which are functionally necessary in pathogenesis.

However, the metabolic system is complex and unpredictable. Common targeted metabolite analyses are limited to detect different changes in case no reliable hypothesis is available.

It is greatly probable that the usage of metabolomics approach in the research of uncommon medical status and rare diseases will enable the development of new drug targets.

These biomarkers may be applied for detecting diseases, toxicity, and efficiency besides the different targets of drug discovery [37].

Applications in command development

A new chemical structure determined by this approach may be defined as a novel chemical entity. Efforts based on these misanalyses could result in the promising discovery leads for further progress [38]. Promising compounds include synthesized and natural compounds. Metabolomics has been previously applied for discovering novel synthesized and other active compounds in plant, marines, and microbial extracts [38,39].

Preclinical trials

Preclinical trials, which employ in-vivo and in-vitro studies, have to be undertaken to elucidate biosafety before being used clinically [18]. These trials are needed to elucidate risk factors and to determine safety and efficacy of lead compounds. Both drug and endogenous metabolism are critical for pharmacokinetics. Before treatment, metabolites are not detectable, and this on the contrary regarding enzootics. Exogenous metabolites are considered the main factors in the differentiation between treatment and control samples [40,41].

Applications in clinical testing

Metabolomic-based techniques can aid in many fields of clinical trials on an investigational new drug (IND), similar to their usage in targeted determination and preclinical trials; a direct sage of this technique in human research is to detect the biomarkers of efficacy and toxicity besides the exogenous metabolites. In this respect, a new metabolite of ethambutol, a medication for tuberculosis, and other metabolites from herbal sources were detected through a metabolomics assay [42,43].

In addition to determination of the metabolic end product of drugs, metabolic profile could be used to forecast pharmacokinetics and adverse effects of treatment, shown by the proof-of-concept research on tacrolimus [44].Comparing preclinical and clinical trials, the latter has greater uncertainty and is thus more expensive and time-consuming in drug discovery. This is owing to uncertainty in the genetic and epigenetic variations of human patients, opposite to the uniformity of animal and in-vitro studies used in preclinical trials.

Hence, choosing the appropriate study patients who could benefit from therapeutic applications of IND is vital for the success of a clinical trial. Pharmaco-metabolomics assay has been effective for patient stratification [45]. The metabolomics assay of serum samples from healthy participants and those with arthritis was capable of classifying four main forms of this disease [45]. Using this technique, certain biomarkers of different diseases could be used as a guideline for patient screening. As the metabo-phenotypes identified could act as the link between the genotypes and the responses to therapy, it may help in the development of personalized therapy [46]. Metabolic analysis was previously used to define the treatment biomarkers of escitalopram, a selective serotonin reuptake inhibitor for treating depression.

By comparing plasma samples from responders and non-responders to escitalopram, glycine was negatively correlated with treatment. Subsequent pharmaco-genomic analysis indicated that a single nucleotide polymorphism in glycine dehydrogenase gene is related to outcome of this drug [47,48]. Metabolomics sets up a basis for personalized usage of drugs in practice, which will likely improve the success rate of IND in achieving new therapeutics [48].

Conclusion

Metabolomics has unique properties to elucidate how we need to target drug discovery and development. It is through its applications originally developed for the drug industry that we can reach the benefits that this

technique can bring to precision medicine. Metabolic technology is thus of prime importance for early diagnosis of different diseases and hence for attainment of the different biomarkers targeted for suitable treatment intervention. Metabolomics will give a new insight for drug discovery, which will help in drug industry and improving the economic situation.

Acknowledgements

The authors are grateful to the National Research Centre for their support to complete this research.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Nicholson JK, Lindon JC, Holmes E. Metabonomics: understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 1999; 29:1181–1189.

2 Lommen A, van der Weg G, van Engelen MC, Bor G, Hoogenboom LA, Nielen MW. An untargeted metabolomics approach to contaminant analysis: pinpointing potential unknown compounds. Anal Chim Acta 2007; 584:43–49.

3 Sanford K, Soucaille P, Whited G, Chotani G. Genomics to fluxomics and physiomics-pathway engineering. Curr Opin Microbiol 2002; 5: 318–322.

4 Man X, Gross RW. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. J Lipid Res 2003; 44:1071–1079.

5 Holmes E, Loo RL, Cloarec O, Coen M, Tang H, Maibaum E, *et al.* Detection of urinary drug metabolite (Xeno-metabolome) signatures in molecular epidemiology studies via statistical total correlation (NMR) spectroscopy. Anal Chem 2007; 79:2629–2640.

6 Everett JR. Pharmaco-metabonomics in humans: a new tool for personalized medicine. Pharmacogenomics 2015; 16:737–754.

7 Corona G, Rizzolio F, Giordano A, Taffoli G. Pharmaco-metabolomics: an emerging "omics" tool for the personalization of anticancer treatments and identification of new valuable therapeutic targets. J Cell Physiol 2011; 227:2827–2831.

8 Suhre K, Meisinger C, Döring A, Altmaier E, Belcredi P, Gieger C, *et al.* Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. PLoS One 2010; 5:e13953.

9 Trock BJ. Application of metabolomics to prostate cancer. Urol Oncol 2011; 29:572–581.

10 Lewis GD, Asnani A, Gerszten RE. Application of metabolomics to cardiovascular biomarker and pathway discovery. J Am Coll Cardiol 2008; 52:117–123.

11 Fanos V, Barberini L, Antonucci R, Atzori L. Pharma-metabolomics in neonatology: is it a dream or a fact? Curr Pharm Des 2012; 18:2996–3006.

12 Khatib A, Choi YH, Verpoorte R. Metabolomics for bioactivity assessment of natural products. Phytother Res 2011; 25:157–169.

13 Gibney MJ, Walsh M, Brennan L, Roche HM, German B, van Ommen B. Metabolomics in human nutrition: opportunities and challenges. Am J ClinNutr 2005; 82:497–503.

14 Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, *et al.* Pharmaco-metabonomicphenotyping and personalized drug treatment. Nature 2006; 440:1073–1077.

15 Lindon JC, Holmes E, Nicholson JK. Metabonomics techniques and applications to pharmaceutical research & development. Pharm Res 2006; 23:1075–1088.

16 Malik NN. Biologics: personalized drugs should cut care costs. Nature 2012; 485:582.

17 Xu EY, Schaefer WH, Xu Q. Metabolomics in pharmaceutical research and development: metabolites, mechanisms and pathways. Curr Opin Drug Discov Dev 2009; 12:40–52.

18 Jacobs A. An FDA perspective on the nonclinical use of the X-Omics technologies and the safety of new drugs. Toxicol Lett 2009; 186:32–35.

19 Griffiths WJ, Koal T, Wang Y, Kohl M, Enot DP, Koal MN, *et al.* Targeted metabolomics for biomarker discovery. Angew Chem Int Ed Engl 2010; 49:5426–5445.

20 Wishart DS. Advances in metabolite identification. Bioanalysis 2011; 3:1769–1782.

21 Wishart DS. Quantitative metabolomics using NMR. TrAC Trends Anal Chem 2008; 27:228–237.

22 Zhang A, Sun H., Wang P., Han Y, Wang X. Modern analytical techniques in metabolomics analysis. Analyst 2012; 137:293–300.

23 Vasilopoulou CG, Margarity M, Klapa MI. Metabolomic analysis in brain research: opportunities and challenges. Front Physiol 2016; 7:183.

24 Yang K, Xia B, Wang W, Cheng J, Yin M, Xie H, *et al.* A comprehensive analysis of metabolomics and transcriptomics in cervical cancer. Sci Rep 2017; 7:433–453.

25 Yamashita Y, Nishiumi S, Kono S, Takao S, Azuma T, Yoshida M. Differences in elongation of very long chain fatty acids and fatty acid metabolism between triple-negative and hormone receptor-positive breast cancer. BMC Cancer 2017; 17:589.

26 Zhang X, Zhu X, Wang C, Zhang H, Cai Z. Non-targeted and targeted metabolomics approaches to diagnosing lung cancer and predicting patient prognosis. Oncotarget 2016; 7:63437–63448.

27 Cai Z, Zhao JS, Li JJ, Peng DN, Wang XY, Chen TL, *et al.* A combined proteomics and metabolomics profiling of gastric cardia cancer reveals characteristic dysregulations in glucose metabolism. Mol Cell Proteomics 2010; 9:2617–2628.

28 Safaei A, ArefiOskouie A, Mohebbi SR, Rezaei-Tavirani M, Mahboubi M, Peyvandi M, *et al.* Metabolomic analysis of human cirrhosis, hepatocellular carcinoma, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis diseases. Gastroenterol Hepatol Bed Bench 2016; 9:158–173.

29 Mehta KY., Wu HJ., Menon SS., Fallah Y, Zhong X, Rizk N, *et al.* Metabolomic biomarkers of pancreatic cancer: a meta-analysis study. Oncotarget 2017; 8:68899–68915.

30 Cheng S, Shah SH, Corwin EJ, Fiehn O, Fitzgerald RL, Gerszten RE, *et al.* Potential impact and study considerations of metabolomics in cardiovascular health and disease: a scientific statement from the American Heart Association. Circ Cardiovasc Genet 2017; 10:e000032.

31 Varma VR, Oommen AM, Varma S, Casanova R, An Y, *et al.* Andrews RMBrain and blood metabolite signatures of pathology and progression in Alzheimer disease: a targeted metabolomics study. PLoS Med 2018; 15: e1002482.

32 Denery JR, Nunes AA, Hixon MS, Dickerson TJ, Janda KD. Metabolomics-based discovery of diagnostic biomarkers for onchocerciasis. PloS Negl Trop Dis 2010; 4:e834.

33 Mugge I, Enyedy I. Virtual screening.In Burger's medicinal chemistry and drug discovery. Wiley Intersci 2003; 1:243–279.

34 Bleicher KH, Bohm HJ, Muller K, Alanine AI. Hit and lead generation: beyond high throughput screening. Nat Rev Drug Discov 2003; 2: 369–78.

35 Shoichet BK. Virtual screening of chemical libraries. Nature 2004; 432:862–865.

36 Verdonk JC, Ric de Vos CH, Verhoeven HA, Haring MA, van Tunen AJ, Schuurink RC. Regulation of floral scent production in petunia revealed by targeted metabolomics. Phytochem 2003; 62:997–1008.

37 Joice R, Yasuda K, Shafquat A, Morgan XC, Huttenhower C. Determining microbial products and identifying molecular targets in the human microbiome. Cell Metab 2014; 20:731–741.

38 Schnackenberg LK. Global metabolic profiling and its role in systems biology to advance personalized medicine in the twenty-first century. Expert Rev Mol Diagn 2007; 7:247–259.

39 Lipinski CA, Lombardo E, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Delivery Rev 1997; 23:3–25.

40 Wishart DS. Applications of metabolomics in drug discovery and development. Drugs Res Dev 2008; 9:307–722.

41 Lenz EM, Bright J, Knight R, Wilson ID, Majoret H. A metabonomic investigation of the biochemical effects of mercuric chloride in the rat using 1H NMR and HPLC-TOF/MS: time dependent changes in the urinary profile of endogenous metabolites as a result of nephrotoxicity. Analyst 2004; 129:535–541.

42 Rochfort S. Metabolomics reviewed: a new "omics" platform technology for systems biology and implications for natural products research. J Nat Prod 2005; 68:1813–1820.

43 Yuliana ND, Khatib A, Verpoorte R, Choi YH. Comprehensive extraction method integrated with NMR metabolomics: a new bio-activity screening method for plants, adenosine A1 receptor binding compounds inOrthosiphonstamineusBenth. Anal Chem 2011; 83:6902–6906.

44 Phapale PB, Kirn SD, Lee HW, Lim M, Kale DD, Kirn YL, *et al.* An integrative approach for identifying a metabolic phenotype predictive of individualized pharmacokinetics of tacrolimus. Clin Pharmacol Ther 2010; 87:426–436.

45 Jiang M, Chen T, Feng H, Zhang Y, Li L, Zhao A, *et al.* Serum metabolic signatures of four types of human arthritis. J Proteome Res 2013; 12:3769–3779.

46 Everett JR. From metabolomics to pharmaco-metabonomics: the role of metabolic profiling in personalized medicine. Front Pharmacol 2016; 7:297.

47 Ji Y, Hebbring S, Zhu H, Jenkins GD, Biernacka J, Snyder K, *et al.* Glycine and a glycine dehydrogenase (GLDC) SNP as citalopram/escitalopram response biomarkers in depression: pharmacometabolomics-informed pharmacogenomics. Clin Pharmacol Ther 2011; 89:97–104.

48 Wishart SD. Emerging applications of metabolomics in drug discovery and precision medicine. Nat Rev Drug Disc 2016; 15: 473–484.