DR ALFRED GRECH, DR MARIO FARRUGIA, DR MICHAEL BALZAN

Metabolomics

ABSTRACT

Metabolomics is the process whereby metabolites are profiled in biofluids, cells and tissues. With the advance of informatics and other analytical technologies, metabolomic profile analyses are being used to understand physiological and pathophysiological mechanisms. Thus with other –omics, research into metabolomics is showing potential to be another useful tool in understanding health and disease, and help in their management.

INTRODUCTION

By definition, a metabolite is an organic molecule with a molecular weight (MW) of less than 1500 Da (Dalton).[1] Metabolites encompass many different chemical families. Examples include peptides, oligonucleotides, sugars, nucleosides, organic acids, ketones, aldehydes, amines, amino acids, lipids, steroids, alkaloids, foods, food additives, toxins, pollutants, drugs and their metabolites. The spectrum also includes human and microbial products.

Metabolomics is a field of life science research that identifies and/or characterises metabolites in many matrices[2] like saliva, serum, urine, exhaled air, cells, tissue or organism. The total profile of the metabolites is called the metabolome.

About 50,000 metabolites have been identified in humans. Slowly but surely, this number is likely to expand as other metabolites which as yet cannot be detected or unknown will make this number higher. Indeed, these undetected or unknown metabolites have been nicknamed the 'dark matter of the metabolome'.

Broadly, metabolites can be categorised into endogenous or exogenous metabolites. Endogenous metabolites are far more numerous than exogenous ones. Besides being the most abundant, the endogenous metabolites also span the greatest concentration ranges. Indeed, their concentration varies from low picomolar up to almost molar levels. They can be located in a database called the 'Human Metabolic Database'. Urea is the most abundant in terms of its concentration in the body. Endogenous metabolites are generally further divided into primary metabolites and secondary metabolites. Primary metabolites are generated in primary pathways of metabolism, like the Kreb's cycle or glycolysis. On the other hand, secondary metabolites are metabolites that are generated from primary metabolites.

Exogenous metabolites are those that are derived from an outside source. Examples include food, drugs and their metabolites, toxins, and environmental pollutants. Drugs span typically a lower range in concentration, around micro-molar levels. Toxins and environmental chemicals (pollutants) hopefully range in the nano-molar concentration or below. Higher concentration spells trouble.

Metabolism has been basically 'understood' and worked in the 1950's and 60's and one can connect many metabolites to many genes. Indeed, metabolism is exquisitely detailed far more than any other biology field. So by knowing well the metabolome, researchers end up connecting it with the proteome and the genome to bring together the concept of systems biology.

Innovation is still happening in metabolomics and it is moving more forward from the back room to the front room. Having said this, it is still falling behind the other –omics. This is because of the involved chemistry. Genomics deals with 4 bases while proteomics deals with 20 amino-acids, in different combinations, but metabolomics deals with thousands of different chemicals, which have different behaviour and different features. Even though metabolomics is the endpoint in the 'omics' flow (genomics–transcriptomics–proteomics–metabolomics), it comes with its own important advantages which are listed in table 1.

TABLE 1. ADVANTAGES ASSOCIATED WITH THE STUDY OF METABOLOMICS.

Advantage	Reference
Levels of several metabolites could be profiled and their perturbation could be connected to a pathophysiological condition.	3
Small alterations in gene expression or protein synthesis result in bigger changes of metabolite levels.	4
Compared with the genome or proteome, the metabolome is more sensitive to pathophysiological changes.	5
The metabolome is dynamic and it changes rapidly with the cell status. This allows the cell state to be evaluated temporally.	3
Metabolome profiling could decipher pathways of disease progression.	6
Metabolome perturbations are intimately linked with the phenotype.	6

Metabolomic profiling can be done through an 'untargeted' or a 'targeted' approach. In the untargeted

method the aim is to detect as many metabolites as possible.[4] In the targeted approach, specific metabolites from a particular metabolic pathway are profiled and quantified. The targeted approach can be used to confirm results obtained by the untargeted method.[4,6]

Along the years, several branches emerged from metabolomics. Amongst these are 'lipidomics' and 'volatilomics'. The latter analyses 'volatile organic compounds' (VOCs), like ketones, aldehydes, alcohols, hydrocarbons and aromatic compounds.[7] These VOCs are end products of biological processes in the human body and can be found in blood, sweat, urine, faeces, saliva or breath.[7,8] It was observed that trained sniffer dogs could 'smell' these VOCs in urine or skin samples of patients with specific cancers. This led to their investigation as possible cancer biomarkers.[9] Indeed, it was found that VOC composition was different in different types of cancer[10-12] and could be used clinically using what is called an 'electronic nose' (e-nose), which is a technological biological sensor.[7]

Lipidomics involves qualitative and quantitative metabolic profiling methods that analyse lipid metabolites in biological samples.[13] The interest in lipidomics has increased because cancer cells exhibit perturbations in the lipidome signatures and thus offer the possibility of using lipids in theranostic applications, like finding lipid-based biomarkers and lipid therapeutic targets.[14,15] Also, research in lipidome profiles is helping to understand carcinogenesis and cancer progression. Mammalian cells have around 10,000 lipid species[16] which can be classified into classes and subclasses, of which glycerophospholipids (GPLs), sphingolipids (SL), and lysophospholipids (LPLs) (a subclass of GPLs) are the most studied in cancer research.

METABOLMICS AND DRUGS

The field of metabolomics has its specific strengths in the landscape of drug repurposing, especially when used integrally with other–omics like genomics, epigenomics, transcriptomics, and proteomics.

Combination therapies have the potential to offer new opportunities in fighting antibiotic resistance. Using high-throughput metabolomics, Campos et al.[17] designed an approach to predict drug-drug interactions and opened insights for repurposing compounds to be used in combination therapies. Specifically, the authors predicted some novel drug combinations like sulfamethizole (a sulfonamide antibiotic) and zidovudine (a dideoxynucleoside used in the treatment of HIV infection) against *E. coli*.

Lima et al.[18] also used metabolomics to understand the mode of action of the anti-depressant sertraline when used against *Leishmania infantum*. The authors found that sertraline kills *Leishmania* by targeting essential metabolic pathways of the parasite. They propose that sertraline can be a valuable drug against visceral leishmaniasis.

Metabolomics has also been utilized to predict the efficacy of antivirals in Covid-19.[19] Indeed, metabolomics showed that antiviral efficacy could depend on the metabolic phenotype of the patient. It was found that the most important metabolomic perturbations were increased metabolites associated with *de novo* NAD (nicotinamide adenine dinucleotide) production, mitochondrial function and ATP production. This could explain the decreased effectiveness of nucleoside-based antivirals used or tried for treating SARS-CoV-2 (e.g., remdesivir, ribavirin, and favipiravir) as these need host enzymes to be activated by endogenous ATP, changing them into the active triphosphate kind. Thus one can propose clinical trials of antivirals or other drugs, to monitor the metabotypes of patients (stratified by viral load, outcome, sex, age, day/s of treatment, and so on). This could be potentially critical in optimizing their efficacy, like what drugs can be used and when.

METABOLOMICS IN CANCER

Generally, early detection of many neoplasms is very important in their management. Several approaches have been suggested and such potential candidates are metabolomic-based biomarkers. This is because metabolites are closely related to the neoplastic phenotype and also because metabolomic profiling can be easily done on different human bio-fluids, like blood, urine, and faeces. Indeed, such metabolomic biomarkers are being investigated for several neoplasms.

Erben et al.[20] did a literature search in 'PubMed' and 'Web of Science' for potential metabolomic biomarkers for the early detection of colorectal neoplasms. They analysed 41 studies and concluded that single metabolites are of limited use to discriminate people with or without colorectal neoplasm. However, metabolite panels are potential candidates; specifically, amino acid panels in blood samples and nucleosides in urine samples.

Using a multi-centred study (ClinicalTrials.gov NCT01486745), Eisner et al.[21] investigated and assessed the possibility of using urine metabolomics for the screening of colorectal cancer (CRC). They were able to create an automated diagnostic test using a urine sample to differentiate those patients who need a colonoscopy from those who do not.

Prostate-specific antigen (PSA) is an essential biomarker for prostatic cancer diagnosis. Nevertheless, when it comes to levels of 4-10ng/ml, dubbed the 'gray zone', PSA is not accurate. Xu et al.[22] used serum metabolomic profiling to differentiate between benign prostatic hyperplasia (BPH) and prostate cancer (PCa) in patients with PSA levels in the gray zone. They identified 18 lipids and lipid-related metabolites as potential biomarkers that could discriminate between patients with BPH and PCa with PSA levels in the gray zone.

Ovarian cancer (OC) is another cancer where metabolomic profiling is being investigated and proposed as a potential biomarker of this silent killer. As other cancers, ovarian cancer causes metabolic perturbations to maintain its growth and metabolomic analyses can unveil these perturbations and detect cancer in its early stages. Yang et al.[23] studied these metabolic alterations of OC, aiming to find potential metabolomic-based biomarkers and potential targets for treatment. As biomarkers they proposed two metabolites that showed a sensitivity of 96.7% and a specificity of 66.7%. The two metabolites were 2-piperidinone and 1-heptadecanoylglycerophosphoethanolamine. They also found metabolic perturbations in fatty acid β-oxidation, phospholipid metabolism, and bile acid metabolism, which are associated with proliferation and metastasis of OC cells.

METABOLOMICS IN AUTISM SPECTRUM DISORDER (ASD)

ASD is a complex heterogeneous disorder. In order to reduce this complexity and heterogeneity, the 'Children's Autism Metabolome Project' (CAMP) (ClinicalTrials.gov Identifier: NCT02548442) was embarked on using a quantitative metabolomic approach. Metabolomic perturbations in ASD patients were identified and these subjects were classed into subpopulations with similar 'metabotypes' (metabolic phenotypes). Smith et al.[24] with their metabolomic screening method detected more than 50% of the autistic subjects. They propose that if their approach can be developed further to increase its sensitivity and specificity, it could help in earlier referral and diagnosis of ASD. They also claim that their method gives insight to possible targets for treatment.

METABOLOMICS IN PSYCHIATRIC DISORDERS

The molecular pathophysiological pathways of psychiatric disorders are still incomplete. Due to this fact, the treatment and diagnosis of each disorder relies on a uniform approach, despite the fact that within each disorder there is heterogeneity. This limits the ultimate goal of 'precision psychiatry' based on phenotype stratification of patients. Metabolomics is being used towards this end by detecting and investigating perturbations in metabolomic signatures which give insight of underlying mechanisms.

For example, an untargeted serum metabolomic profiling of a cohort of subjects carried out by Tasic et al.[25] could differentiate schizophrenic patients from the healthy subjects. Besides, the investigation pointed to the glycolysis pathway and confirmed the 'hyperglutamate hyothesis'.[26] The results of Yang et al.[27] implicated perturbations in fatty acid metabolism, carbohydrate metabolism, and amino acid metabolism.

Metabolomics can also be used to identify patients with mental illness who are at risk of metabolic co-morbidities related to their treatment. For example, mentally ill patients often gain weight during their treatment. Metabolomic and lipidomic profiling show that certain perturbations in lipid signatures are associated with this weight gain.[28]

Metabolomic profiling can also be used to tackle the inconsistency of patient's response to treatment.[29] It can also be used to predict recovery from depression,[30] or as a biomarker in anxiety disorders.[31]

All this shows that metabolomic biomarkers have the potential in theranostics, helping in diagnosis, patient stratification in a heterogeneous population, monitoring treatment response and disease progression, and lastly in finding targets, like eicosanoids, for novel treatments.[32]

METABOLOMICS IN CARDIOVASCULAR DISEASE

Jiang et al.[33] used serum metabolomics to try and find biomarkers in acute coronary syndrome (ACS). Their results fished four metabolites, namely betaine, acetylcarnitine, isoundecylic acid and 1-heptadecanoyl-sn-glycero-3-phosphocholine. They proposed that these differential metabolites could be used in the management of ACS.

From the ARIC study (Atherosclerosis Risk in Communities), Wang et al.[34] fished 19 differential metabolites and proposed a 'metabolite risk score' that can be used to better assess the risk of Coronary Heart Disease (CHD). They also highlight the fact that their findings point to novel causative pathways of CHD.

MICROBIOME-METABOLOMICS

Ya-Long Fenget al.[35] used a rat model for chronic kidney disease (CKD) to study the dysbiosis of the gut microbiome and CKD. A microbiome-untargeted metabolomic analyses showed decreased microbe diversity and richness and a substantial change in 291 serum metabolites. The altered metabolite profile pointed to dysregulation in the metabolism of amino acids, polyamines, bile acids and lipids. Giving the CKD rats poricoic acid A and poriacocos bettered the microbial dysbiosis and reduced renal fibrosis and hypertension. This study confirmed the association between CKD and dysbiosis of the gut microbiome, and importantly that through dietary manipulation one can improve the dysbiosis and the disarrayed metabolome, and also delay kidney disease.

Kanbay et al.[36] also suggest that gut microbiome modification can be used in CKD as prevention and treatment.

SINGLE CELL METABOLOMICS

High-throughput metabolomics is now even possible at the cellular level using 'single cell metabolomics' (SCM). Specifically, SCM is used to profile metabolites in individual cells. This makes it a very powerful tool to study cell heterogeneity. Thus, it can be used to identify cell types and their differentiation in cell colonies. Indeed, it is being applied in various fields like neurology and oncology.

In neuroscience research,[37] SCM is being used to analyse differences between similar cells in the brain (like oligodendrocytes) and also to study differences between different cell types (like neurons and astrocytes). The studies are also being applied to unveil changes, which occur in such cells, with diet, drugs and disease. As in other fields this will surely be helpful in the theranostics of brain diseases.

In any one type of tumour, the cells could have different phenotypes, a condition called cancer heterogeneity. In oncology research, SCM is being used to identify such cancer heterogeneity and this will in the future be translated into more precise cancer diagnosis and personalized treatment. For example, Tian et al.[38] propose a SCM platform to monitor drug resistance of non-muscle invasive bladder cancer (NMIBC) treated with intravesical chemotherapy (post transurethral resection). Specifically, they used SCM of urinary exfoliated tumour cells (UETCs).

CONCLUSION

Metabolomic profiling is showing great promise in theranostics. Indeed, it can help in disease diagnosis when used as biomarkers, in the stratification of patients (and hence in more personalized treatment) and in monitoring patient's response to treatment. It can also be applied to follow disease progression; and lastly, metabolomics can unveil targets for new treatment.

REFERENCES

1. Bujak R, Struck-Lewicka W, Markuszewski MJ et al. Metabolomics for laboratory diagnostics. *Journal of Pharmaceutical and Biomedical Analysis* 2015;113:108–120.
2. Armitage EG, Barbas C. Metabolomics in cancer biomarker discovery: current trends and future perspectives. *Journal of Pharmaceutical and Biomedical Analysis* 2014;87:1–11.
3. Marchand CR, Farshidfar F, Rattner J, et al. A Framework for Development of Useful Metabolomic Biomarkers and Their Effective Knowledge Translation. *Metabolites* 2018;*8*(4):59.
4. López-López Á, López-Gonzálvez Á, Barker-Tejeda TC, et al. A review of validated biomarkers obtained through metabolomics. *Expert Review of Molecular Diagnostics* 2018;18(6):557–575.
5. Nagrath D, Caneba C, Karedath T, et al. Metabolomics for mitochondrial and cancer studies. *Biochimica et Biophysica Acta* 2011;1807(6):650–663.
6. Nagana Gowda G, Raftery D. Biomarker Discovery and Translation inMetabolomics. *Current Metabolomics* 2013;1(3):227–240.
7. Janfaza S, Khorsand B, Nikkhah M, et al. Digging deeper into volatile organic compounds associated with cancer. *Biology Methods & Protocols* 2019;4(1):bpz014.
8. Gao Q, Lee WY. Urinary metabolites for urological cancer detection: a review on the application of volatile organic compounds for cancers. *American Journal of Clinical and Experimental Urology* 2019;7(4):232-248.
9. Lubes G, Goodarzi M. GC-MS based metabolomics used for the identification of cancer volatile organic compounds as biomarkers. In *Journal of Pharmaceutical and Biomedical Analysis* 2018;147:313-322.
10. Cornu JN, Cancel-Tassin G, Ondet V, et al. Olfactory detection of prostate cancer by dogs sniffing urine: A step forward in early diagnosis. *European Urology 2011;59*(2):197-201.
11. Taverna G, Tidu L, Grizzi F et al. Olfactory system of highly trained dogs detects prostate cancer in urine samples. *Journal of Urology 2015; 193*(4): 1382-7.
12. Pirrone F, Albertini M. Olfactory detection of cancer by trained sniffer dogs: A systematic review of the literature. In *Journal of Veterinary Behavior: Clinical Applications and Research* 2017;19:105-117.
13. Perrotti F, Rosa C, Cicalini I, et al. Advances in lipidomics for cancer biomarkers discovery. In *International Journal of Molecular Sciences* 2016;17(12):1992.
14. Park JK, Coffey NJ, Limoges A, et al. The Heterogeneity of Lipid Metabolism in Cancer. In *Advances in Experimental Medicine and Biology* 2018;1063:33-55.
15. Islam SR, Manna SK Lipidomic analysis of cancer cell and tumor tissues. In *Methods in Molecular Biology* 2019;1928:175-204.
16. Tumanov S, Kamphorst JJ. Recent advances in expanding the coverage of the lipidome. In *Current Opinion in Biotechnology* 2017; 43:127-133.
17. Campos AI, Zampieri M. Metabolomics-Driven Exploration of the Chemical Drug Space to Predict Combination Antimicrobial Therapies. *Molecular Cell* 2019;74(6):1291-1303.
18. Lima ML, Abengózar MA, Nácher-Vázquez M, et al. Molecular basis of the leishmanicidal activity of the antidepressant sertraline as a drug repurposing candidate. *Antimicrobial Agents and Chemotherapy* 2018;62(12): e01928-18.
19. Migaud M, Gandotra S, Chand HS, et al. Metabolomics to Predict Antiviral Drug Efficacy in COVID-19. *American Journal of Respiratory Cell and Molecular Biology* 2020;63(3): 396-398.
20. Erben V, Bhardwaj M, Schrotz-King P, et al. Metabolomics Biomarkers for Detection of Colorectal Neoplasms: A Systematic Review. *Cancers (Basel)* 2018;10(8):246.
21. Eisner R, Greiner R, Tso V, et al. A machine-learned predictor of colonic polyps based on urinary metabolomics. *Biomed Res Int* 2013;2013:303982.
22. . Xu B, Chen Y, Chen X, et al. Metabolomics Profiling Discriminates Prostate Cancer From Benign Prostatic Hyperplasia Within the Prostate-Specific Antigen Gray Zone. *Front Oncol* 2021;11:730638.
23. Yang W, Mu T, Jiang J, et al. Identification of Potential Biomarkers and Metabolic Profiling of Serum in Ovarian Cancer Patients Using UPLC/Q-TOF MS. *Cell Physiol Biochem* 2018;51(3):1134-1148.
24. Smith AM, Natowicz MR, Braas D, et al. A Metabolomics Approach to Screening for Autism Risk in the Children's Autism Metabolome Project. *Autism Res* 2020;13(8):1270-1285.
25. Tasic L, Pontes JGM, Carvalho MS, et al. Metabolomics and lipidomics analyses by ^{1}H nuclear magnetic resonance of schizophrenia patient serum reveal potential peripheral biomarkers for diagnosis. *Schizophr Res* 2017;185:182-189.
26. Meltzer HY, Rajagopal L, Huang M, et al. Translating the N-methyl-D-aspartate receptor antagonist model of schizophrenia to treatments for cognitive impairment in schizophrenia. *Int J Neuropsychopharmacol* 2013;16(10):2181-94.
27. Yang J, Chen T, Sun L, et al. Potential metabolite markers of schizophrenia. *Mol Psychiatry* 2013;18(1):67-78.
28. McEvoy J, Baillie RA, Zhu H, et al. Lipidomics reveals early metabolic changes in subjects with schizophrenia: effects of atypical antipsychotics. *PLoS One* 2013;8(7):e68717.
29. Xuan J, Pan G, Qiu Y, et al. Metabolomic profiling to identify potential serum biomarkers for schizophrenia and risperidone action. *J Proteome Res* 2011;10(12):5433-43.
30. Czysz AH, South C, Gadad BS, et al. Can targeted metabolomics predict depression recovery? Results from the CO-MED trial. *Transl Psychiatry* 2019;9(1):11.
31. Humer E, Pieh C, Probst T. Metabolomic Biomarkers in Anxiety Disorders. *Int J Mol Sci* 2020;21(13):4784.
32. Wang D, Sun X, Yan J, et al. Alterations of eicosanoids and related mediators in patients with schizophrenia. *J Psychiatr Res* 2018;102:168-178.
33. Jiang H, Jiang H, Zhang J, et al. The Serum Metabolic Biomarkers in Early Diagnosis and Risk Stratification of Acute Coronary Syndrome. Front Physiol 2020;11:776.
34. Wang Z, Zhu C, Nambi V, et al. Metabolomic Pattern Predicts Incident Coronary Heart Disease. Arterioscler Thromb Vasc Biol 2019;39(7):1475-1482.
35. Feng YL, Cao G, Chen DQ, et al. Microbiome-metabolomics reveals gut microbiota associated with glycine-conjugated metabolites and polyamine metabolism in chronic kidney disease. Cell Mol Life Sci 2019;76(24):4961-4978.
36. Kanbay M, Onal EM, Afsar B, et al. The crosstalk of gut microbiota and chronic kidney disease: role of inflammation, proteinuria, hypertension, and diabetes mellitus. *Int Urol Nephrol* 2018;50(8):1453-1466.
37. Qi M, Philip MC, Yang N, et al. Single Cell Neurometabolomics. *ACS Chem Neurosci* 2018;9(1):40-50.
38. Tian J, Fu G, Xu Z, et al. Urinary exfoliated tumor single-cell metabolomics technology for establishing a drug resistance monitoring system for bladder cancer with intravesical chemotherapy. *Med Hypotheses* 2020;143:110100.