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Metabolomics in lupus; opportunities and challenges

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Abstract

Systemic lupus erythematosus (SLE) is characterized by an inappropriate autoimmune response to self-antigens. This disease is a heterogeneous autoimmune disease that shows variable clinical course. Metabolomics employs advanced analytical chemistry techniques to comprehensively measure many small molecule metabolites in biological cells and tissues. Metabolites are downstream of translation processes and are thought to be associated with disease phenotypes. This technology is recognized as a powerful tool with excellent potential for detecting prognostic and diagnostic biomarkers in rheumatic diseases. In this review; we summarized the recent available results of studies on metabolomics in lupus and the importance of metabolomics in the finding of diagnostic and prognostic biomarkers was investigated.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease that may involve several organs of the body and show different clinical courses (1).

The incidence of SLE in females is approximately nine times that of males. It is estimated that SLE costs approximately $13 billion annually for US health care systems, indicating that SLE is a big diagnostic and therapeutic challenge for nations.

The disease is notably more frequent among African, Asian, Hispanic and local American populations and these experience the highest mortality rate, too (2,3).

SLE is characterized by an irrelevant autoimmune reaction to self-antigens. Environmental factors in genetically susceptible individuals administrate to break down self-tolerance and activate innate immunity of cells and autoreactive lymphocytes against body cells (4, 5).

Due to the heterogeneous character of lupus, a broad spectrum of clinical manifestations exists. The first clinical manifestations of SLE may appear as (fatigue), myalgia, fever, anorexia and weight loss. SLE usually involves several organs and systems, such as skin, joints, kidneys, lungs, central nervous system and hematopoietic system. Actually, no two cases of lupus are exactly alike (6-8).

Key point

SLE is an autoimmune disease with various clinical presentation. Due to heterogeneous characteristics of this disease, its diagnosis is challenging. Metabolites are produced by cell metabolisms. Metabolite profiles can be used as a possible biomarkers for SLE detection and prognosis.

The diagnosis of SLE is based on clinical features within the skin, joints, kidneys and central nervous system and serological parameters like anti-nuclear antibodies (ANA), specifically, anti-double stranded DNA (anti-dsDNA) antibodies. Currently, patients are usually diagnosed based on the American College of Rheumatology (ACR) or Systemic Lupus International Collaborating Clinics (SLICC) criteria and still therapy is limited to the general immunosuppressants which have many unwanted side effects (8,9).

Metabolomics

On the molecular level, the human body is an exceptionally dynamic system, with lots of chemical and molecular responses taking place within millions of cells at any given moment.
These biochemical reactions are liable for preserving cell activity and maintaining cell shape and cell contact with one another (10).

Metabolomics utilizes advanced analytical

chemistry techniques to degree a large number of small molecule metabolites in biological cells and tissues and refers back to the systematic analysis of metabolites (low molecular weight biochemical substances such as carbohydrates, amino acids, organic acids, nucleotides and lipids) in an organic sample (10,11).

Small molecule metabolite profiles are capable to be used as potential biomarkers for the detection, prognosis of disease, response to treatment and disease status

In addition, some genetic disturbances are associated with many diseases but they account for a little portion of the disease risk. Due to the multifactorial nature of disease mechanisms, the excessive influence of the environment and the intricacy of gene-environment interactions and also the effect of post-translational changes (10-12).

Approaches to the metabolomics study

The strategies for conducting metabolic tests are classified in two directions: 1) Non-targeted analysis, and 2) Targeted analysis.

Non-targeted metabolomic investigations are used as a hypothesis-generating strategy and are defined by qualitative and quantitative measurement of enormous numbers of metabolites in the samples (12). In this way, it is possible to create metabolic profiles, detect and analyze metabolites or panels like lipids, including phospholipids, amino compounds and carbohydrates without focusing on a particular compound.

Targeted metabolism concerns hypothesis-driven experiments and is defined by gathering quantitative data on a predetermined set of metabolites with high profile of precision. In fact, targeted methods include multiplexed analysis of known metabolites. For example, targeted metabolism, the measurement of analyzes based on common biochemistry and/or previously well-established non-targeted studies, is selected (13, 14).

Employing efficient statistical analysis, shifts in metabolites can be traced to definitive pathways, allowing information to be obtained throughout the pathophysiological process of a specific disease (12,14,15).

Techniques for studying metabolomics

Metabolism is usually utilized in two different ways:
1. Proton (1 H) nuclear magnetic resonance (NMR) spectroscopy
2. Mass spectrometry (MS) coupled with gas or liquid phase chromatography (GC and LC, respectively).

The samples are usually separated into gas (GC) or liquid chromatography (LC) before performing MR and the compounds are characterized by mass and fragmentation patterns (16,17). In contrast, NMR spectroscopy measures all metabolites at once without the need for separation, but this method is less sensitive (18).

Mass spectrometry is often used for semi-targeted or untargeted metabolomics because it is more sensitive, more powerful and also can determine more molecules in a biological specimen (19).

NMR detects molecular properties by measuring the intrinsic magnetic substances of atomic nuclei. NMR is selective and non-destructive but of relatively low sensitivity (20).

Metabolomics as a possible tool for biomarker studies in SLE

Initial diagnosis and differentiation between early SLE and other rheumatic diseases are incredibly difficult within the early stages and sometimes the disease has not been diagnosed for many years.

Common methods of diagnosing and evaluating the disease include the employ of acute phase markers, such as erythrocyte sedimentation rate and C-reactive protein and anti-dsDNA antibodies have inadequate sensitivity and specification (21).

Metabolite profiles of small molecules can be used as possible biomarkers for the detection and prognosis of the disease. Metabolomic studies in SLE have examined metabolic changes in SLE pathology over the past decade.

Metabolomics studies in lupus have been developed to screen for fewer invasive bio-fluids, such as serum or urine, for separating metabolomics related to disease activity and response to therapy. These will assist in diagnosing, evaluation of disease activity and treatment choices (22,23).

Serum and plasma metabolomes

Liang et al in 2018 described the coagulation cascade relationship to the complement system in SLE. Using LC-MS (liquid chromatography coupled to MS), the serum metabolome of 24 SLE patients was compared to 24 healthy controls. In addition, the levels of ten coagulation factors, seven complements and three cytokines were measured in 112 SLE patients. In this study, clinical data were obtained from 2025 SLE patients.

An total of 195 significantly changes in metabolites were seen in SLE patients. The main metabolites that are differentially displayed in SLE patients contain tyramine, L-tryptophan valproic acid, 1-alpha-25-dihydroxyvitamin, linoleic, L-leucine, L- 2-phenylethylamine. In this study the metabolic pathways that were changed in SLE patients have been determined, which include steroid hormone biosynthesis, tryptophan metabolism and interestingly coagulation cascade pathway and complement system.

The findings indicated that the coagulation cascade and complement system had cooperation effects on the severity of SLE. This analysis shows relationships between the coagulation cascade components and the complement system (24).

Another mass spectroscopy-based metabolomics study (LC/MS and GC/MS) referred to SLE that compared the metabolites in SLE patients and healthy controls, which showed100 metabolites were outstandingly different in SLE. Much of the difference observed is related to

energy metabolism. The most important of these energy production pathways include glycolysis, the Krebs cycle and beta-oxidation of lipids. These findings mention a severe reduction in ATP production. The study also found an increase in oxidants and a decline in most free long-chain fatty acids in SLE patients (25).

Recent studies showed some members of the acylcarnitine family have undergone major changes in SLE. This indicates the dysregulation of β oxidation processes in this disease. This study also suggested a moderate-to-strong relation between elected individual metabolic peaks and bacterial genera in the bowel (26).

Urine SLE metabolome

In a study, the larger sample size was used to analyze urine metabolome profiling of six of the most common immune-mediated inflammatory diseases (IMIDs) (rheumatoid arthritis, SLE, psoriatic arthritis, Crohn's disease, psoriasis and ulcerative colitis) by the use of NMR. Compared to healthy controls, patients with immune-mediated inflammatory diseases (especially SLE) had 28 important associations between urinary metabolite level and diagnosis of disease. Moreover, significant relationships of three significant metabolites with disease activity were found [PFDR (false-discovery rate) <0.05]. Some of the metabolite variations were prevalent across all or almost all diseases, thereby, they were considered as hub metabolites. Citrate had the strongest hub properties, which in most of IMIDs showed a definitely lower concentration in the urine. Compared to controls, there were another five hub metabolites, which were significantly associated with several IMIDs. Compared to healthy controls, alanine, N-acetyl amino acids (N-acetyl AAs), methyl succinate and trigonelline displayed lower accumulation in the urine of some different IMIDs. Additionally, in the urine of SLE patients, lower level of citrate had been determined compared to controls (27).

Network analysis revealed lots of similarity between the three major metabolic pathways. The citric acid cycle was the main pathway identified and citrate had a common relationship with IMID. The phenylalanine metabolism pathway was the second major metabolic pathway. An important role of glycine and serine metabolism pathway was determined in IMIDs analyses.

Further comparison between patients with SLE and others revealed 11 metabolites were very different. It can use for differential diagnosis in IMIDs (27, 28).

Fecal SLE metabolome

This is a new metabolic model for better SLE diagnosis. Feces is also an excellent biological fluid for new biomarkers because of its uncomplicated and noninvasive collection.

Ultra-high-performance liquid chromatography equipped with mass spectrometry studies conducted by Zhang et al in 2019 compared fecal metabolic profiles of SLE patients with healthy controls for detecting a potential biomarker for SLE. Around 23 important metabolites that include the amino acids, purine, lipids and vitamin B metabolisms were changed in the feces of SLE group, compared with healthy group. The most important pathways that changed were aminoacyl-tRNA (aa-tRNA) biosynthesis, nitrogen metabolism, thiamine metabolism, tryptophan metabolism and cyanoamino acid metabolism. Interestingly glucogenic amino acids, such as L-methionine, proline, and L-asparagine increased in the stool of SLE patients. Furthermore, the glucogenic and ketogenic amino acids, L-tyrosine, increased in SLE patients' stool. This finding suggests that there may be impairments in metabolism of glucose energy. Metabolism of these amino acids can emerge as possible energy sources. These changes are also seen in studies of serum samples from patients (25).

Besides, the metabolite profiles of fecal specimen make it possible to distinguish SLE patients from healthy normal controls. The mixed diagnosis of phosphatidylglycerol 27:2 and proline was of the biggest importance to identify SLE patients from normal and healthy controls (29).

Conclusion

Lupus heterogeneity precludes the creation of an objective tool that can be used for a wide range of patients. Identifying biomarkers for diagnosis, prognosis determining treatment response has always been one of the major challenges in this disease. In the past decades, significant developments in technology aimed to a better understanding of the risk factors and pathophysiology of SLE. Further studies can improve and increase biomarker accuracy too.

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