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What Have Mass Spectrometry-Based Proteomics and Metabolomics (Not) Taught Us about Psychiatric Disorders?

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Abstract
Understanding the molecular causes and finding appropriate therapies for psychiatric disorders are challenging tasks for research; -omics technologies are used to elucidate the molecular mechanisms underlying brain dysfunction in a hypothesis-free manner. In this review, we will focus on mass spectrometry-based proteomics and metabolomics and address how these approaches have contributed to our understanding of psychiatric disorders. Specifically, we will discuss what we have learned from mass spectrometry-based proteomics and metabolomics studies in rodent models and human cohorts, outline current limitations and discuss the potential of these methods for future applications in psychiatry.

Introduction

Due to their phenotypic heterogeneity, the lack of accurate diagnostic classification and the absence of molecular correlates which hamper our understanding of disease pathogenesis, psychiatric disorders constitute a challenging field of research. Various hypothesis-free -omics technologies have been used to disentangle the molecular mechanisms underlying psychiatric disorders. In this review, we will discuss the current status of mass spectrometry-based proteomics and metabolomics studies in rodent models and human cohorts. We will mainly focus on anxiety, depression, bipolar disorder and schizophrenia as they represent core psychiatric conditions. We will then assess what proteomics and metabolomics studies have delivered for psychiatric research, their limitations and what we can still learn in order to get meaningful results that will help us understand psychiatric disorders.

Proteomics and Metabolomics in Psychiatric Disorders: Current Status

Proteomics and Metabolomics Toolbox

Mass spectrometry is the core technology behind most proteomics and metabolomics approaches [1]. In a disease framework, a mass spectrometry-based proteomics or metabolomics study has a quantitative character and compares the levels of proteins/metabolites between two conditions, namely disease versus control or treatment versus no treatment. In the context of psychiatric disorders, the majority of proteomics studies to date have primarily used two-dimensional (2D) gel electrophoresis

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and its variations to compare two states (reviewed by Craft et al. [2]). With this technique, equal amounts of protein homogenates from the groups to be compared are loaded on 2D gels, proteins are separated according to both their charge and molecular weight, gels are stained, and gel spots that have different signal intensities between the two groups are processed and sent to the mass spectrometer for protein identification. Stable isotope labeling or label-free approaches have recently been implemented for relative quantification of two states. Stable isotope labeling proteomics is based on introducing heavy stable isotopes (e.g. ^{2}H, ^{13}C, ^{15}N or ^{18}O) into peptides and proteins. Stable isotopes result in a predictable mass difference between a labeled peptide/protein and an unlabeled peptide/protein. Label-free proteomics is based on the assumption that the chromatographic peptide peak area corresponds to its concentration [3]. In both cases, protein extracts are digested, and the resulting peptides are analyzed with mass spectrometry. Mass spectrometry peptide signal intensities, indicative of their abundance, are compared between the two groups with quantification software.

Mass spectrometry-based metabolomics is tailored towards the analysis of small molecules. There are two main approaches: targeted metabolomics, which measures a selected set of metabolites [4], and untargeted metabolomics, which assesses metabolites in an unbiased manner. In the latter approach, not all mass spectrometry signals can be assigned to a metabolite of known structure [5]. In both targeted and untargeted metabolomics, quantification is performed by comparing signal intensities across different sample groups.

Specimens from Animal Models and Human Cohorts

The proteomics and metabolomics toolbox has been applied to animal models representing psychiatric phenotypes with a particular emphasis on rodents. This is due to their straightforward, time-efficient and highly controllable breeding and maintenance combined with the availability of a plethora of test paradigms to assess animal behavior that reflects psychiatric symptoms, relevant secondary read-outs and endophenotypes [6, 7]. Work with human brain specimens is inevitably based on postmortem tissue, which presents a number of challenges related to protein and metabolite stability [8]. For clinical translation, peripheral biofluids have received increasing attention. Cerebrospinal fluid is in close proximity to the brain and contains secreted brain-derived proteins and metabolites. Blood/plasma is easily accessible and available for different time points to carry out longitudinal and follow-up analyses. Saliva has been analyzed to compare protein expression differences between patients suffering from schizophrenia and bipolar disorder and matching controls [9]. Metabolomics analyses of urine samples have also been performed to study depression [10, 11] and effects of antidepressant treatment in rat models [12]. For further proteomics and metabolomics studies in animal models and human cohorts, the reader is referred to several review papers [3, 13–16].

Proteomics and Metabolomics Studies in Psychiatric Disorders: What Have We Learned So Far?

Disease-Relevant Molecular Mechanisms

The hypothesis-free nature of quantitative proteomics and metabolomics methods has revealed molecular mechanisms beyond the neurotransmitter systems that are commonly associated with psychiatric disorders.

Energy Metabolism and Oxidative Stress. An imbalance of major energy metabolism pathways has been demonstrated in brain and peripheral material by proteomics and metabolomics for all major psychiatric disorders, including schizophrenia [17–20], anxiety [21, 22], depression [23–25] and bipolar disorder [25, 26], as well as for pharmacological studies with antidepressants [27–29]. In these studies, glycolysis appears to be one of the major pathways affected, in line with the well-established aberrant glucose metabolism in schizophrenia patients [30] and in patients receiving antipsychotic drug treatment [31]. Converging lines of evidence have linked oxidative stress to psychiatric disorders [32]. In anxiety this is supported by -omics findings in mouse models [21, 22, 33]. Proteomics and metabolomics studies in schizophrenia [19, 34–37] and depression [38] have also reported alterations for protein and metabolite oxidative stress markers.

Other Mitochondrial Functions. In brain tissue, mitochondrial oxidative phosphorylation has been the predominant mechanism implicated in energy metabolism. Protein expression alterations have been reported for electron transport chain complexes in rodent models of trait anxiety and antidepressant treatment [21, 39]. Besides energy metabolism and oxidative stress, alterations in other mitochondrion-related mechanisms and functions have been reported [21, 40–42]. The majority of these studies have used proteomics approaches, as mitochondrion-specific metabolomics in psychiatry is just beginning to be applied [43]. These studies have revealed alterations in mitochondrial import, transport and structural elements in high versus low trait anxiety mice [21,

40], in mitochondrion-orchestrated apoptosis in a mouse model of schizophrenia-like symptoms [41] and in structural mitochondrial components in a methamphetamine-induced behavioral sensitization mouse model of schizophrenia-related symptoms [42]. These data point towards a causal role of mitochondria in psychiatric disorders extending beyond their role in energy production and oxidative stress regulation, in line with data acquired from other methodologies [44].

Immune System. Proteomics and metabolomics studies have implicated a role for the immune system in brain [45], serum [46], blood [47] and peripheral blood T cells [48] of schizophrenia patients, and in brains of a rat model of depression [49].

Posttranslational Modifications

Apart from alterations in protein expression levels, the tight regulation of disease-related pathways and signaling cascades is orchestrated by protein posttranslational modifications (PTMs) such as phosphorylation and acetylation. Advances in the global analysis of PTMs by mass spectrometry-based proteomics have been reported both for protein characterization and relative quantification [50]. In the field of psychiatry, high-throughput quantitative analysis of PTMs is still in its infancy. Label-free proteomics was used to compare phosphorylation levels in postmortem dorsolateral prefrontal cortices of depressed patients versus control donors [51]. An example of the potential that the study of PTMs holds for elucidating the regulation of molecular events involved in psychiatric phenotypes includes a global quantitative phosphoproteomics analysis based on stable isotope labeling in cells which revealed differences in serotonin receptor phosphorylation when exposed to hallucinogenic versus non-hallucinogenic agonists. These results were also confirmed in vivo in mice [52]. Since hallucinations are a core symptom of schizophrenia, this work may pave the way for more effective treatments of positive symptoms of this disorder.

Network-Based Etiology and Biomarker Panels

Along with the realization that a better understanding of human disease mechanisms requires an investigation of networks [53] in order to gain a holistic view of brain and central nervous system dysfunction [54], modeling tools for pathway changes are used to integrate information acquired from proteomics and metabolomics experiments [55]. The complex molecular underpinnings of psychiatric disorders are reflected by a combination of mild quantitative molecular changes rather than by a few prominent biomarkers. Indeed, alterations identified by proteomics and metabolomics studies include small alterations in protein/metabolite levels that are part of distinct molecular pathways. This pathway-based view may aid the transition to clinical translation, as many protein sequences are not fully conserved between rodents and humans, whereas major neurobiological circuits regulating behavior (i.e. anxiety, reward and fear) are well preserved across vertebrates [56].

Proteomics and Metabolomics in Psychiatric Disorders: What We Have Not Yet Learned

Pathway Activity

Quantitative proteomics and metabolomics approaches provide information on differences in relative protein/metabolite levels in a given experimental setup. Once appropriately analyzed, these data may reveal dysregulated protein/metabolite networks. However, such experiments do not measure the pathway activity or reaction rates of enzymes with altered expression levels. Increased protein/metabolite levels do not necessarily imply an increased pathway activity or vice versa, and this should be taken into account when interpreting proteomics and metabolomics findings. To get insights on pathway activity levels, activity assays for the pathway/process under investigation should be performed.

Causality

The information acquired via proteomics and metabolomics analyses does not specify whether the identified changes in protein and metabolite abundance are a cause or a consequence of disease pathology. At the level of mechanisms/pathways, it is therefore unknown whether affected pathways across different psychiatric conditions represent common causal mechanisms or nonspecific effects of disease manifestation. As -omics methods do not rely on previous molecular knowledge of the condition studied, no conclusions on the cause-effect relationship of the observed changes can be drawn. Moreover, these data cannot always shed light on the underlying biological mechanisms. As an example, increased levels of mitochondrial electron transport chain proteins in a tissue may either indicate a higher number of mitochondria or an increased concentration of electron transport chain molecules in existing mitochondria that may or may not result in higher electron transport activity. To answer such questions, complementary, targeted methodological approaches are required.

Disease-Relevant Alterations in Expression Levels

Technical and biological variability needs to be taken into account in mass spectrometry measurements. The contribution of technical variability to overall variability can be estimated a priori by testing the repeatability of the workflow employed (i.e. sample preparation and accuracy of instrumentation) [57–59]. It is important that technical variability is addressed for each workflow employed [60] as well as for the sample type studied [61], as sample complexity has been shown to affect relative variability in quantification [57]. Biological variability is more difficult to estimate a priori. Each proteomics or metabolomics experiment should be able to answer the question, 'Which of the observed changes in protein/metabolite levels are disease relevant, and which are within the range of physiological variability across different subjects?' This can be achieved by assessing the method to be employed in combination with the material to be studied [62]. Disease-relevant changes in expression levels can be reported with confidence provided they are higher than the method variability together with the normal biological variation of the study sample. Numerous studies have reported fold changes that lie within the technical variability range, and these results are not reliable. It should also be noted that metabolite levels are highly sensitive to external stimuli, and therefore small fold changes may be of no relevance to disease pathology. Taken together, all of these parameters should be taken into account to establish clear-cut fold change thresholds for reporting meaningful results.

Implementation

Perhaps the most serious issue that needs to be addressed in the -omics field as a whole is the implementation of the acquired results. The enthusiasm for the advances in mass spectrometry instrumentation and application was followed by a disillusionment concerning the disproportionately small number of mass spectrometry-based clinical applications. Especially in the field of psychiatric disorders, there is admittedly a discontinuity between technical advances in the instrumentation and the implementation of the acquired knowledge with regard to disease understanding and development of effective treatments [63]. Although an enormous amount of raw data is generated, validation and follow-up studies for proteomics and metabolomics findings are rare, which may be due to a lack of highly accurate alternative methods to validate mass spectrometry data. What is also needed for clinical translation are consortia and initiatives to approach psychiatric disorders from a multidisciplinary point of view.

Proteomics and Metabolomics in Psychiatric Disorders: What We Can Still Learn

Molecular Signatures

Proteomics and metabolomics are powerful systemic and hypothesis-free approaches able to identify state-specific molecular signatures. These molecular fingerprints can be characteristic of disease presence, disease progression, response to treatment, remission or relapse. Of increasing importance is the identification of these signatures in peripheral biofluids for translational applications. Indeed, distinct metabolic signatures have been found by mass spectrometry -omics in plasma [64–66], serum [46, 67, 68] and peripheral blood mononuclear cells [19, 69] of patients suffering from major psychiatric disorders (schizophrenia, depression and bipolar disorder) compared to control subjects. Furthermore, distinct metabolic signatures before and after antipsychotic treatment have been identified in plasma of patients with schizophrenia [67]. Intriguingly, it has also been suggested that disease-specific protein profiles can be detected in serum before disease onset for schizophrenia and bipolar disorder [70], whereas different metabolic profiles have been observed between patients suffering from depression with and without early-life stress [66]. Proteome profile comparison has also revealed differences in protein expression in fibroblasts from schizophrenia patients and controls [71].

Towards this goal, proteomics and metabolomics molecular signatures will be of great value to define subcategories not only for the presence or absence of a disease but also for predisposing factors, environmental effects and response to treatment. Markers with altered levels both in the brain and in the periphery are fundamental for clinical implementation. A protein that has been found to be consistently downregulated in brain, cerebrospinal fluid and red blood cells in schizophrenia is apolipoprotein A [72]. However, confirming mitochondrial energy metabolism as an underlying molecular mechanism for psychiatric disorders in peripheral tissue may be challenging, since mitochondria are not present in plasma and mitochondrial proteins can only be found in a secreted form. The combination of both proteomics- and metabolomics-derived signatures will be extremely powerful as they relate to the end point of the molecular expression of a disease. As different psychiatric disorders are hard to distinguish with existing diagnostic criteria [73], these molecular signatures will also pave the way for molecular diagnostics in peripheral material as a part of a coordinated effort between clinicians, psychologists and laboratory scientists.

Protein Turnover

Comparative proteomics and metabolomics provide a highly accurate quantitative snapshot of a steady state. Protein expression at a given time point is the result of the equilibrium between protein synthesis and degradation referred to as protein turnover. Dynamic information from the crosstalk between these two processes will provide crucial insights into protein fate in psychiatric disorders and may to some extent explain why protein differences between disease and control states exist. Proteomics-based workflows have been devised to study protein turnover by partial in vivo labeling of mice with ^{15}N and calculating the ratios of the ^{15}N-labeled, newly synthesized fraction to the unlabeled protein fraction. A workflow to investigate protein turnover in a high-throughput manner has recently been described [74]. Efforts towards implementing this technology at the levels of both sample preparation and data analysis algorithms are ongoing [75]. Since protein aggregates resulting from aberrant protein turnover are a hallmark of major psychiatric disorders [76] and alterations in proteostatic mechanisms that regulate protein turnover such as the ubiquitin proteasome system have been reported in anxiety and schizophrenia [33, 77], protein turnover studies hold great promise for unraveling pathogenesis-related mechanisms.

Pharmacological Targeting

The sensitive changes in abundance that these methods are able to detect at the pathway level in a quantifiable manner provide valuable insights into the perturbation the system undergoes. Quantitative proteomics and metabolomics have revealed alterations in protein and metabolite levels relevant for psychiatric disorders. Pharmacological manipulation may be possible in vivo by administering substances that selectively target these molecular alterations in rodent models and by assessing whether a reversal of the disease behavioral phenotype can be achieved. Monitoring the effects of selectively manipulating molecular pathways will enable mechanism-based, data-driven, targeted pharmacological studies that will reveal new molecular targets which can eventually be used for clinical translation.

Conclusions

Regardless of the amount and significance of data acquired with individual methodologies, interdisciplinary approaches that provide different types of information are imperative. Genomics, transcriptomics, microRNAs and imaging techniques including mass spectrometry-based imaging [78, 79] are complementary tools to understand how the brain (mal)functions and may eventually succeed in delivering accurate disease diagnoses and an informed choice of treatment strategies.

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Disclosure Statement

The authors declare no conflict of interest.

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