The potential of ^{15}N metabolic labeling for schizophrenia research

O potencial da rotulação metabólica de ^{15}N para a pesquisa de esquizofrenia

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Abstract

Psychiatric research is in need of non-hypothesis driven approaches to unravel the neurobiological underpinnings and identify molecular biomarkers for psychiatric disorders. Proteomics methodologies constitute a state-of-the-art toolbox for biomarker discovery in psychiatric research. Here we present the principle of *in vivo* ^{15}N metabolic labeling for quantitative proteomics experiments and applications of this method in animal models of psychiatric phenotypes, with a particular focus on schizophrenia. Additionally we explore the potential of ^{15}N metabolic labeling in different experimental set-ups as well as methodological considerations of ^{15}N metabolic labeling-based quantification studies.

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Keywords: Schizophrenia, quantitative proteomics, ^{15}N metabolic labeling, biomarker, G72.

Resumo

Pesquisas em psiquiatria ainda necessitam de estudos não dirigidos por hipóteses para revelar fundamentos neurobiológicos e biomarcadores moleculares para distúrbios psiquiátricos. Metodologias proteômicas disponibilizam uma série de ferramentas para esses fins. Apresentamos o princípio de rotulação metabólica utilizando ^{15}N para proteômica quantitativa e suas aplicações em modelos animais de fenótipos psiquiátricos com um foco particular em esquizofrenia. Exploramos o potencial de rotulação metabólica por ^{15}N em diferentes tipos de experimentos, bem como suas considerações metodológicas.

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Palavras-chave: Esquizofrenia, proteômica quantitativa, rotulação metabólica de ^{15}N, biomarcadores, G72.

The need for quantitative proteomics in schizophrenia research

In the last years, the development of holistic approaches such as genomics, transcriptomics, proteomics and metabolomics has given rise to quantitative, non-hypothesis driven research applications. In this regard, populations of genes, proteins or metabolites of two states (*e.g.* disease *vs.* control) can be compared to identify expression level differences relevant to the observed phenotypic alterations. As the proteome of an organism can reflect phenotypic changes at the molecular level, proteomics constitutes a valuable tool to investigate the underlying mechanisms involved in psychiatric disorders.

Research in schizophrenia suffers from a lack of molecular correlates for the observed behavioral alterations and disease symptoms. Molecular biomarkers for schizophrenia can aid the assessment of predisposition risk, the accurate subcategorization of patients, the monitoring of disease progression and the discovery of novel therapeutic targets. To this end, quantitative proteomics has the potential to provide sensitive molecular biomarker information and therefore offer valuable insights for schizophrenia prognosis, diagnosis and treatment.

^{15}N metabolic labeling

A number of quantitative proteomics approaches are available and applicable to schizophrenia research[1]. Among them, *in vivo* ^{15}N metabolic labeling holds great potential for studies in animal models as well as in patient cohorts. The principle of ^{15}N metabolic labeling is based on the introduction of the stable nitrogen isotope ^{15}N in an organism through either a ^{15}N-labeled diet or ^{15}N-labeled growth media. The ^{15}N-labeled protein population (labeled specimen) is then compared to a protein sample that contains only natural-abundance isotopes (unlabeled specimen). The introduction of the ^{15}N isotope label results in a predictable mass difference between a labeled peptide and its unlabeled counterpart. Relative protein quantification is enabled by comparing the signal intensities of the unlabeled/labeled peptide pairs of a given protein. *In vivo* metabolic labeling methods provide high accuracy compared to other existing quantitative proteomics approaches because the labeled and unlabeled specimens are combined prior to sample preparation, thus avoiding biased errors during experimental handling. Apart from protein quantification, ^{15}N metabolic labeling allows the study of protein turnover *in vivo* through assessing ^{15}N incorporation levels at different time points[2].

Applications of ^{15}N metabolic labeling in animal models of psychiatric phenotypes and patient specimens

^{15}N metabolic labeling has been applied to a plethora of model organisms ranging from bacteria to rodents and was recently used to label mouse models of disease[3]. In the context of psychiatry, a ^{15}N metabolic labeling protocol *via* a bacteria-based diet was established and applied to study the mouse model of high (HAB), normal (NAB) and low (LAB) anxiety-related behavior[4]. ^{15}N-labeled NAB mice were used as internal standards and quantitative proteomics studies in cingulate cortex, hippocampus and plasma were performed to compare HAB and LAB mice. These proteomics analyses revealed an involvement of mitochondrial and immune system-related pathways in the modulation of anxiety-related behavior[5,6].

^{15}N metabolic labeling has also been applied to a mouse model of schizophrenia. The primate-specific G72/G30 locus is one of the

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most replicated findings in schizophrenia genetic studies. However, the function of the corresponding G72 protein remains to a large extend unknown. To investigate the function of the G72 protein *in vivo*, transgenic mice that carry the G72/G30 gene locus and express the G72 protein were generated[7]. The G72/G30 transgenic mice exhibited schizophrenia-like symptoms including increased compulsive behaviors, impaired locomotor coordination, increased sensitivity to phencyclidine, impaired odorant discrimination and learning deficits[7,8]. To compare the cerebellar proteomes of G72/G30 transgenic mice and wild type controls, ^{15}N-labeled CD1 mice were used as internal standards and several proteins related to affected molecular pathways in schizophrenia were found differentially expressed[9].

Besides animal models, ^{15}N metabolic labeling can be employed to examine human specimen by labeling cell lines of human origin and using them for quantitative comparisons with patient material. Human cell lines can be grown in ^{15}N-labeled media and the deriving labeled protein populations can then be utilized as internal labeled standards for pair wise comparisons of patient and control groups. This way the *in vivo* metabolic labeling at the whole organism level is circumvented while the comparative proteomics analysis still benefits from the high quantification accuracy achieved by this method.

Methodological considerations

When applying ^{15}N metabolic labeling to schizophrenia research, several considerations need to be taken into account. The cost of the ^{15}N-labeled diet is high and long labeling periods are required for complex organisms (*e.g.* rodents) to achieve high ^{15}N incorporation rates for quantitative proteomics comparisons. Importantly, the introduction of ^{15}N may have an effect on the behavioral phenotype[4] or on protein expression levels[10]. As a consequence, labeling controls (either using internal labeled standards or reciprocal labeling) should be implemented in the experimental design to avoid artifacts caused by the ^{15}N isotope affecting relative protein quantification results. Although the cost of metabolically labeling whole organisms with ^{15}N is not negligible, the method eventually results in ^{15}N-labeled material (*e.g.* organs, blood, brain tissue etc.) that can be used for a great number of different quantitative proteomics experiments. Given the high accuracy, fewer biological replicates compared to other less sensitive quantitative methods may be required to achieve accurate quantification results. Notably, employing ^{15}N-labeled cell lines as internal proteome standards drastically reduces costs and labeling time to achieve high ^{15}N incorporation, enabling a routine application of this methodology. It should be also noted that the computational challenges concerning the assessment of fully or partially ^{15}N-labeled spectra have to a large extend been addressed by the development of appropriate software and optimized data processing workflows, which have made high-throughput data analysis possible[11,12]. A detailed methodological evaluation of ^{15}N metabolic labeling in comparison to other quantitative proteomics methods is discussed elsewhere[13].

Conclusion

Taken together, *in vivo* ^{15}N metabolic labeling provides a powerful and versatile tool for schizophrenia research that can be used both for animal model and patient-based studies. The high quantification accuracy achieved by this method may shed light on new molecular entities relevant for schizophrenia etiology and contribute to the discovery of protein biomarkers. Additionally, the study of protein turnover by ^{15}N metabolic labeling may pinpoint protein metabolism mechanisms pertinent to the pathophysiology of schizophrenia.

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