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Potential Applications and Development of Cell Metabolomics in Natural Products

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Abstract

Natural products play a vital role in drug discovery. Metabolomics, the systematic analysis of small molecular metabolites in biological systems, has been increasingly applied to discovering biomarkers, identifying perturbed pathways and discovering new drugs. It enables the examination and identification of endogenous biochemical reaction products occurring within a living cell. Cell metabolomics has many potential applications and advantages to currently used methods at various aspects in the natural product era. This review covers recent developments in the use of metabonomics to characterize cellular metabolome and interpret them in terms of metabolic changes taking place in a wide range of situations, especially, highlights the key roles of the endogenous small-molecule metabolites in the natural product field.

Keywords: Metabolomics; Cell; Biotechnology; Natural Product; Metabolites

Introduction

Metabolomics is an emerging and powerful discipline that provides an accurate and dynamic picture of the phenotype of mammalian systems through the study of endogenous and exogenous metabolites in cells, and culture supernatants [1]. It enables the characterization of endogenous molecules that are the products of biochemical reactions, revealing connections between different pathways in a living cell [2]. More specifically, the ability to uncover and evaluate biochemical differences within healthy and diseased organisms provides information as to the underlying cause(s) of disease, which in turn suggests targets for pharmacological intervention [3]. Aim of metabolomics analysis is to describe qualitatively and quantitatively the final products of cellular regulatory pathways and can be seen as the ultimate response of a biologic system to genetic factors and/or environmental changes. Cell metabolome can be defined as the set of all the metabolites present in cells and considered the best indicator of an organism's phenotype [4,5].

Currently, a dramatic change in the pharmaceutical industry as many companies are downscaling their efforts to discover new drug candidates and are instead turning toward natural products that are the source of many active substances in drug discovery. This trend has been dubbed open innovation. Natural products profoundly impact many research areas, including medicine, organic chemistry, and cell biology. However, discovery of new natural products suffers from a lack of high throughput analytical techniques. Metabolomics will allow an objective assessment of the impact of candidate drugs on cells. This quantitative approach should help guide the development of new drugs reducing failure rates in clinical phase. Therefore, metabolomics was evaluated to rapidly and efficiently analyze cell-derived natural products. Metabolomics capitalizes on the small molecules in cell to construct a 'fingerprint' that can be unique to the individual. Small-molecule metabolites as primary indicators have an important role in biological systems and represent attractive candidates to understand cell phenotypes [6]. Cell applications are easier to control, less expensive and easier to interpret than analysis of both animal models and human subjects. As such, they represent an untapped resource for identification of specific metabolite biomarkers that would help distinguish the normal, and abnormal states, as well as response to drugs [7-9].

Metabolomics is a powerful tool developed to systematically analyse the metabolic fingerprint of a cell, widely used in the past to analyze cell metabolism [10]. In combination with feature selection, pattern recognition, and multivariate data analysis approaches, metabolomic profiling aims to provide a comprehensive assessment of the alterations in the metabolite levels in cells [11,12]. Broadly, the term cell metabolomics refers to the comprehensive analysis of the complete set of small weight metabolites present in and around growing cells at a given time during their growth or production cycle. Although recent efforts have provided methodologies that make cell line metabolomics much more accurate, faster and more informative [13-15]. Application of metabolomics in the area of cell culture is relatively undeveloped and thus the aim of the present review is to provide an insight into the issues pertaining to metabolome analysis as well as to explore its possible applications in cell culture.

Analytical Instrument Platforms

Metabolite concentrations represent sensitive markers of phenotypic changes. Consequently, the development of robust metabolomic platforms will greatly facilitate various applications of cell cultures. Clearly, an analytical platform capable of assaying the small molecule content of cells would be an invaluable tool for gaining insights into these areas. Variety of analytical platforms has been developed to facilitate these and other types of metabolomics

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experiments [16]. Recent technological advancements in NMR and mass spectrometry (MS) have led to wide use of these technologies for precise measurements of metabolites with improved sensitivity, resolution, and mass accuracy. For instance, NMR, gas chromatography (GC) and liquid chromatography (LC) have been coupled to MS detection methods in order to perform metabolomic profiling of biological samples [17]. LC is well-suited for single cell measurements. Using various detectors with LC has been effective for characterizing chemical content and even release from individual cells. There are a number of features of LC that make it a method of choice for such analyses. It is possible to simultaneously concentrate and separate analytes, and has been used extensively for single cell and even subcellular analyses, and it is well suited as a separation method for use in metabolomics experiments [18].

To analyze such a tiny quantity of metabolites in a single cell, various techniques have been tried and developed, especially in MS [19]. A high-throughput mass spectrometer will open up the possibility to perform metabolomics on large populations of cells. In fact, MS has already become the main analytical platform for cell metabolomics, and also been used to characterize the contents of individual neurons, with considerable efforts devoted to characterizing their peptide complements. Many of these studies employed direct MS profiling; however, pairing LC to MS often leads to better analyte coverage. Furthermore, ESI coupled with tandem MS allows for the identification and/or characterization of unknown or unexpected compounds, such as metabolites, via their mass-to-charge ratio (*m/z*) and MS/MS fragmentation pattern, and complements the other detection schemes used for cell measurements.

Intracellular metabolites can reflect the physiological state of cells. Hyphenated LC/MS, and NMR techniques are becoming a useful tool in the study, represent a promising microseparation platform in metabolomics and has a strong potential to contribute to cell [20]. UPLC-MS is a relatively new technique for the separation of complex samples. UPLC-MS coupled with pattern recognition show promise for metabonomics. It is a potential and very promising technology for the classification of the cell lines. Cell growth, metabolic activity and protein productivity measurements, which are currently used to monitor the cellular physiological state, suggested consistency across bioreactors and over the course of the cultivation.

Methodology of Sample Preparation and Extraction

A cell culture metabolomic experiment can be divided into five general steps: experimental design, cell culture growth and/or stimulation, quenching and metabolite extraction, metabolomic measurement, and data preprocessing and analysis. Metabolomic analysis has emerged as an important technique for studying cellular biochemistry [21]. The first necessary step termed 'quenching' is to rapidly stop any inherent enzymatic activity or any changes in the metabolite levels. Freezing in liquid nitrogen is generally considered to be the easiest way of stopping enzyme activity provided that cells or tissues are not allowed to partially thaw before extracting metabolites. Despite the power of these methods, sample preparation and extraction are critical to achieving meaningful results. Apart from the development of sensitive and comprehensive detection systems, a big challenge in cell metabolomics is to cope with the fast changes of intracellular concentrations of metabolites. This imposes stringent requirements on the sample preparation [22]. The time and method of sampling are important issues to be considered to ensure reproducibility in the analytical sample, especially since a large number of biological replicates are commonly used.

Hence integrated procedures that allow for sampling, quenching and extraction to be simplified into a single step have been devised [23,24]. Intracellular metabolite concentrations are subsequently determined by subtracting the metabolite content of the cell-free extracellular medium from the resultant mixture. A simple, fast, and reproducible sample preparation procedure has been developed for relative quantification of metabolites in adherent mammalian cells using the clonal β-cell line INS-1 as a model sample. The method was developed by evaluating the effect of different sample preparation procedures on LC-MS quantification of 27 metabolites involved in glycolysis and the tricarboxylic acid cycle on a directed basis as well as for all detectable chromatographic features on an undirected basis [25]. Separation of quenching and extraction steps provides the benefit of increased experimental convenience and sample stability while maintaining metabolite content similar to techniques that employ simultaneous quenching and extraction with cold organic solvent. Maximal recovery was achieved using a single rapid extraction step. The utility of this rapid preparation method was demonstrated through precise metabolite measurements associated with step changes in glucose concentration that evoke insulin secretion in the clonal β-cell line INS-1. Given the power of the metabolomics technology, the availability of standardized sample preparation methods for cell lines is critical toward augmenting research in this direction.

Bringing Metabolomics into the Forefront of Cell Research

It has been noted that present testing of drugs on animals is insufficient in clinical testing and that these human cell cultures may be an alternative for understanding the specific metabolism of drug candidates [26]. Currently, the metabolic analysis of cell cultures has many potential applications and advantages to currently used methods for cell line testing. Metabolite concentrations represent sensitive markers of phenotypic changes. Metabolomics represents a global quantitative assessment of metabolites within a biological system. It is the downstream of systems biology and has drawn significant interest for studying the metabolic networks from cells to organisms [27]. Cell culture has to-date used metabolomics analyses, attempting to resolve significant questions regarding cell culture performance. Cell metabolomics is an emerging field that addresses fundamental biological questions and allows one to observe metabolic phenomena in heterogeneous populations of single cells. Furthermore, some extremely difficult problems facing other metabolomic applications are not issues in cell culture applications. Consequently, the development of cell metabolomics will greatly facilitate various applications of cell cultures and provides information of biological systems. In addition, metabolomic analysis of cell lines provides information, either independently or in conjunction with other omics measurements, essential for system level analysis and modeling of biological systems. Thus, metabolomics has been used tused for optimizing cell cultures for antibody production [28], testing drugs [29], cell transfections, determinant of apoptosis and comparing lung cancer cell phenotypes, identifying novel underlying metabolic pathways [30].

Metabolomics for Phytomedicine Research and Drug Development

Metabolomics is the global quantitative assessment of endogenous metabolites within a biological system, and is fast becoming the approach of choice across a broad range of sciences including systems biology, drug discovery, molecular and cell biology, and other medical and agricultural sciences. New analytical and bioinformatics technologies

and techniques are continually being created or optimized, significantly increasing the crossdisciplinary capabilities of this new biology. The metabolomes of medicinal plants are particularly a valuable natural resource for the evidence-based development of new phytotherapeutics and nutraceuticals. Natural products as pure compounds have been involved in western medicine as drugs or lead compounds for drug discovery and development. Metabolite detection, either individual or grouped as a metabolomic profile, is usually performed in cells by either NMR or MS followed by sophisticated multivariate data analysis. Consequently, the development of robust metabolomic platforms will greatly facilitate various applications of cell cultures and aid in their rapid incorporation into novel therapeutic settings.

Current Applications in Cell Culture

Drug discovery is a complex and unpredictable endeavor with a high failure rate. Current trends in the pharmaceutical industry have exasperated these challenges and are contributing to the dramatic decline in productivity observed over the last decade. Recent advances in metabolomics are responding to these self-imposed limitations and are providing opportunities to increase the success rate of drug discovery [31]. The use of metabolomics in cell cultures allows for the determination of important secondary metabolites such as taxol, which have been proved to be effective pharmaceutical ingredients [32]. Metabolomic studies on *Taxus* (yew) cell cultures have allowed for the identification new taxoids, which could lead to the further understanding of the induction of taxoid production in *Taxus* cells and the production of synthetic versions in other plant cells. NMR metabolomic profiling was used to study the effect of BEZ and MPA on three AML cell lines and shed light on the underlying mechanism of action [33]. The findings indicate that the actions of combined BEZ and MPA against AML cells are indeed mediated downstream of the generation of ROS. A study exploited a combined metabolomics and in silico modeling approach to gain a deeper insight into the cellular mechanisms of Chinese hamster ovary (CHO) fed-batch cultures [34]. Extracellular and intracellular metabolite profiling analysis reviewed that key metabolites associated with cell growth limitation within the energy, glutathione, and glycerophospholipid pathways that have distinct changes at the exponential-stationary transition phase of the cultures. Key information on growth-related mechanisms derived from the current approach can potentially guide the development of new strategies to enhance CHO culture performance.

Cancer cells have several specific metabolic features, which have been explored for targeted therapies. Agents that promote apoptosis in tumors are currently considered as a powerful tool for cancer therapeutics. Metabolomic signatures might be used in the tests of efficacy of agents causing apoptosis in cell culture. HEK 293 and in cancer cell lines HepG2, PC3, and MCF7 were searched for metabolic biomarkers of apoptosis differing from that of necrosis [35]. Already nontreated cell lines revealed distinct concentrations of metabolites. Several metabolites indicative for apoptotic processes in cell culture including aspartate, glutamate, methionine, alanine, glycine, propionyl carnitine, and malonyl carnitine were observed. These signatures could be obtained in fast high-throughput screening. Metabolic differences was to examine between a novel chronic myelogenous leukemic (CML) cell line, MyL, and a sub-clone, MyL-R, which displays enhanced resistance to the targeted Bcr-Abl tyrosine kinase inhibitor imatinib [36]. NMR was carried out on cell extracts and conditioned media from each cell type. Specific metabolite identification and quantification were used to examine metabolic differences between the cell types. MyL cells showed enhanced glucose removal from the media compared to MyL-R cells with significant differences in production rates of the glycolytic end-products, lactate and alanine. Interestingly, the total intracellular creatine pool was significantly elevated in MyL-R compared to MyL cells. It demonstrated a clear difference in the metabolite profiles of drug-resistant and sensitive cells, with the biggest difference being an elevation of creatine metabolites in the imatinib-resistant MyL-R cells.

GC/MS was employed to profile the metabolites in two different cell lines infected with influenza A virus [37]. Metabolic profiling allowed the differentiation of fatty acid biosynthesis and cholesterol metabolism during viral replication in the cell lines. It revealed the different responses between A549 and AGS cell lines to the virus infection. From the pattern recognition results, AGS cell line might be more susceptible to influenza A virus. Regarding the fact that AGS is a poorly differentiated gastric adenocarcinoma cell line whereas A549 is a relatively differentiated lung tumor one, it is speculated that viral replication might be associated with the cell differentiations. A multiplatform analytical approach combining proton nuclear magnetic resonance NMR and MS, together with pattern recognition tools in a metabolomic study was used to investigate the effects of dengue virus infection [38]. The four serotypes of dengue, DEN-1, DEN-2, DEN-3, and DEN-4, were inoculated into the EA.hy926 cell line, which was then incubated for various time intervals. Distinct effects of infection by each serotype were demonstrated, and these differences were attributed to changes in levels of metabolites, including amino acids and dicarboxylic acids related to the tricarboxylic acid cycle. These studies demonstrated that application of metabolomics to improve understanding of the effect of dengue infection on endothelial cells' metabolome. Since the interaction between metabolites and specific targets is dynamic, knowledge regarding genetics, susceptibility factors, timing, and degree of exposure to natural products are fundamental to understanding the metabolome and its potential use for predicting and preventing early phenotypic changes. Understanding the metabolome will not only provide insights into the critical sites of regulation in health promotion, but will also assist in identifying intermediate or surrogate cancer biomarkers for establishing preemptive/preventative or therapeutic approaches for health.

Challenges in Cell Metabolomics

Metabolomics, the 'global' study of metabolite changes in a biological system, has drawn a significant amount of interest over the last few years [39]. It can be said to be an amalgam of traditional areas such as metabolite analysis, bioanalytical development and chemometrics. Most work to date has been focused on plant, microbial, as well as tissue and biofluid samples. However, the diverse potential of metabolomics in many fields, including cell engineering, has made it a universal tool for industrial, medical and research purposes. It is also a vital component of a 'systems biology' approach, as it is believed to be a good reflection of the phenotype of any cell or tissue [40]. At the heart of metabolomics' growth is the issue of method development, including sample preparation, instrument analysis, data processing and bioinformatics.

A large amount of structural and functional information is obtained by molecular cell phenotype analysis of natural products at cell level by image or flow cytometry in combination with bioinformatic knowledge extraction concerning metabolites as well as cell function parameters like intracellular pH, transmembrane potentials or ion gradients. Identification and quantification of metabolites occurring within specific cell types or single cells of natural products and other organisms is of particular interest for natural product chemistry, chemical ecology, and biochemistry in general. Metabolic

fingerprinting, although much more technically challenging because it requires metabolite extraction from cells, provides more complete information about cellular metabolic processes. The comprehensive characterization of the metabolome, however, is a daunting task, as the endogenous metabolites vary widely in their physical and chemical properties, which in turn, makes their concurrent extraction, separation, and detection a major challenge [23]. The development of cell metabolomics, however, has been impeded by several challenges specific to this application. Limited work has focused on development of sample preparation techniques for metabolomic analysis of adherent mammalian cells. The differences in optimized cell culture growth conditions present another major concern for cell metabolomics. The standard enhancement of cell culture medium can add another level of complexity in cell growth condition optimization.

Cultured cell lines are useful models in biomedical research that characterize metabolic responses to various stimuli and explore the underlying mechanisms [41]. In addition to the main challenges imposed by cell analysis, sample preparation protocols that do not alter the metabolic status of the cells analyzed must be developed. Yet, these important studies are not sufficient for generating a complete picture of the molecular components regulating cellular function. Without analytical standards and quality control rules, quantification of individual metabolites and validation of the cell metabolomic methods will not be possible. With the rapid developments in powerful analytical technologies, we can expect a plethora of metabolite data. The grand challenges are to validate and make sense of the data, put molecular components and dynamic changes into particular pathways, integrate with other types of data, and connect into molecular networks of cell functions [42]. In addition, analysis of cell cultures does not require the same level of ethics consideration as is required for applications in animal and human subjects [43]. Focusing on a specific cell type can reduce variability and provide a more constant background against which more subtle metabolic changes become apparent [44].

Conclusion and Future Trends

Metabolomics is the newest "omics" science and is a dynamic portrait of the metabolic status of living systems [45]. It has brought new insights on metabolic fluxes and a more comprehensive and holistic understanding of a cell's environment. It has also been proven to be a valuable analytical tool for the identification of metabolites from natural products. The purpose of this review is to describe the state of the art of a new emerging discipline, cell metabolomics in the understanding how the metabolites affect cell behavior and function and highlight the past successes in applications of cell metabonomics to contribute to low-molecular-weight metabolites discovery in natural products. In recent years, metabolomic technologies have moved beyond simple cataloging towards large-scale molecular quantification and network analysis. As a vital component of a 'systems biology' approach, it is believed to be a good reflection of the phenotype of any cell. A systemic knowledge of how cells work will certainly aid in our effort toward a holistic understanding of cell function. Metabolomics produce highly useful information about cell biology. In addition, future fundamental research should provide a more complete list of metabolites. The detailed experimental design, experimentation and analysis for metabolomics provide useful information and become a truly essential analysis for cells. We believe that the availability of high throughput methods for analysis of metabolites in cells will substantially enhance our abilities to gain insight into biochemical reaction networks, to mechanistically understand how the metabolites affect cell behaviors.

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Competing Financial Interests

The authors declare no competing financial interests.

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