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Review

Metabolomics in cell culture—A strategy to study crucial metabolic pathways in cancer development and the response to treatment

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ABSTRACT

Metabolomics is a comprehensive tool for monitoring processes within biological systems. Thus, metabolomics may be widely applied to the determination of diagnostic biomarkers for certain diseases or treatment outcomes. There is significant potential for metabolomics to be implemented in cancer research because cancer may modify metabolic pathways in the whole organism. However, not all biological questions can be answered solely by the examination of small molecule composition in biofluids; in particular, the study of cellular processes or preclinical drug testing requires *ex vivo* models. The major objective of this review was to summarise the current achievement in the field of metabolomics in cancer cell culture—focusing on the metabolic pathways regulated in different cancer cell lines—and progress that has been made in the area of drug screening and development by the implementation of metabolomics in cell lines.

Introduction

This review is focused on the metabolomics of cancer cell lines. In the first three sections, a compendium of metabolomics-related information is provided as follows: (1) definitions, methods and tools used to study small molecules; and (2) introduction to cell culture as well as strategies applied to study metabolomics in cells. However, the primary aim of this review is to highlight the metabolic signatures of cancer cell lines from different origins, primarily how metabolomics can be applied for "cause-and-effect" studies in which, through the effect—metabolic patterns—the cause—molecular mechanisms (gene regulation)—can be revealed and how metabolomics can support drug screening and development. In the final section, conclusions and perspectives are presented regarding metabolomics and cell culture studies.

Metabolomics—an overview

The era of "omics" emerged along with our growing understanding of the complexity of diseases and a demand for their comprehensive analysis. We can study biological systems under healthy and diseased conditions on several omics levels, including genomics (genomic variance), transcriptomics (gene expression), proteomics (protein abundance) and metabolomics (metabolite concentrations), which complement each other. With respect to the strongest correlation to phenotype, metabolites (unlike genes and proteins, whose function is determined by epigenetic regulation or posttranslational modification) constitute direct readouts of biochemical events. The term metabolome describes all low-mass molecules (metabolites below 1500 Da) in a biological system, with a single cell as the smallest unit. The metabolic composition of the cell is a consequence of its genetic characteristics, regulation of gene expression, protein abundance and environmental influences [2,3]. Although the term metabolome [1], metabolomics [4] or metabonomics [3] first appeared in the literature in the late 1990s, the first technological, analytical and conceptual steps were already taken in the 1970s when Pauling applied gas–liquid partition chromatography for the quantitative analysis of urine vapour and breath [5]. The continuous technological development strongly improved the low resolution of direct analysis by the implementation of separation steps, including gas chromatography (GC[1]), multidimensional gas chromatography (GC × GC), liquid chromatography (LC), high performance liquid chromatography (HPLC)

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[1] *Abbreviations used:* GC, gas chromatography; LC, liquid chromatography; HPLC, high performance liquid chromatography; UPLC, ultra-high performance liquid chromatography; NMR, magnetic resonance; MS, mass spectrometry; 1H, proton; 1H-MRS, proton magnetic resonance spectroscopy; BMI, body mass index; ATCC, American Type Culture Collection; TCA, tricarboxylic acid; PLs, phospholipids; PI3K, phosphoinositide-3 kinase; SUMO, Small Ubiquitin-like Modifier; FDA, Food and Drug Administration; HCS, high content screening.

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or ultra-high performance liquid chromatography (UPLC). Although technology has significantly improved over the years, no single analytical approach is suitable for the accurate identification and quantification of all of the metabolites of interest. Thus, metabolomics relies on the two most frequently applied analytical approaches based on proton (1H) nuclear magnetic resonance (NMR) and mass spectrometry (MS), with their unique strengths and weaknesses [6]. There are also two different strategies to study metabolomics. Non-targeted metabolomics, used for hypothesis generation, is based on, for example, NMR, proton magnetic resonance spectroscopy (1H-MRS), GC–MS or UHPLC–MS techniques, whereas targeted metabolomics, applied to confirm the hypothesis, is implementing mostly GC–MS, LC–MS and UHPLC–MS techniques, which are also applicable for quantification [7]. Furthermore, targeted metabolomics can be specified towards particular component classes, including lipids (lipidomics) [8,9], carbohydrates (glycomics) [10,11] or steroids (steromics) [12]. Targeted metabolomics is used preliminary in diagnostics and is based on LC–MS, GC–MS, electrospray ionisation (ESI)-MS or flow injection analysis (FIA)-MS [13]. Large data sets are the main products of metabolic measurements and require advanced bioinformatical tools. Annotation of the generated peaks and NMR spectra of the metabolites, followed by their further analysis, is supported by various tools [14–17]. Recently, web-based, easy-to-use approaches have been developed for the statistical analysis of metabolomics data [18,19]. Among the available tools, the *metaP*-server provides an interactive exploration and interpretation of metabolic data within the context of multiple multiclass and metric sample phenotypes [19] and is cross-linked to KEGG [20], LipidMaps [21], PubChem [22], and HMDB databases [23], which are frequently used for data interpretation [24]. Although human metabolism and the correlation between metabolites have been extensively studied for decades, the computational research field still struggles with the organisation of metabolic patterns into metabolic networks. Recently, this issue was addressed by Krumsiek et al. [25] by applying a Gaussian graphical model for pathway reconstruction of metabolics data from their high-throughput study. The Gaussian graphical modelling approach together with metabolomics genome-wide association studies [26] has been successfully applied and reveals a link between unknown metabolites with known metabolic classes and biological processes [27]. All of the described technologies, tools, methods and concepts are actually directed towards the same two primary objectives: (1) to understand the metabolic homeostasis of an organism and mechanism of disease and (2) to support diagnosis by the identification and validation of biomarkers specific for disease prediction as well as to monitor the progression or response to treatment [28].

The distinct relevance of metabolomics has been recognised in the field of oncology. Metabolic signatures for several cancer types, including colorectal [29–31], breast [32–34], pancreatic [35–37], liver [38,39], ovarian [40–42], prostate [43,44] and lung [45,46] cancer, have been already reported. The potential clinical applications of metabolomics in oncology have already been reviewed [47]. However, not all biological questions can be solved by analysing human bio-fluids or tissue samples because factors such as gender, body mass index (BMI), age and diet increase noise rather than improve the metabolic signature of interest. In particular, the metabolism of cellular processes or preclinical drug testing requires *ex vivo* models.

Cell culture system and metabolomics

The history of cell culture experiments dates back to 1907, when Harrison cultivated frog nerve fibres *ex vivo* [48]. Subsequently, the first animal cell line [49] and first human cancer cell line (HeLa) [50] was established. Recently, different cell lines from diverse organs have been collected in cell culture banks (see Table 1). For example, American Type Culture Collection (ATCC), established in 1962, is one of the largest bio-resources offering a selection of cell lines from over 150 different species, including nearly 4000 human cell lines (http://www.atcc.org). Cell culture systems were already defined and divided [51] into primary cell culture, cell lines and cell strains, which can grow adherently or in suspension (see Definition box 1). Our knowledge of fundamental processes that occur in human cells—whether physiological or pathological—is dependent to a large extent on using HeLa and other cell lines as a model system [52]. The questions arise: what about metabolomics and cell culture? Recently, Cuperlovic-Culf et al. [53] and Leon et al. [54] presented a detailed overview on the experimental design and sample preparation for mammalian cell metabolomics. Briefly, the main goal of cell metabolomics is to analyse as many metabolites as possible in a defined cell state or condition in which all enzymatic and chemical processes are stopped to gain a metabolic snapshot of a targeted metabolic status of the culture from which they were drawn. Fig. 1 illustrates the cell culture workflow reflecting both adherently growing and suspension-growing cells. The cells growing in suspension are separated from the medium by filtration [55] or centrifugation [56], which are suitable for metabolomics study. Quenching is required to gain the metabolic snapshot, ideally by stopping all cellular activities that have an influence on metabolic alterations. Several quenching and extraction protocols were recently examined by Dietmair et al. [57] for cells growing in suspension. Among all of the evaluated quenching strategies, cold isotonic saline (0.9% [w/v] NaCl, 0.5 °C) was highlighted as the most convenient because it did not damage the cells and effectively inhibited the conversion of ATP to ADP and AMP, a hallmark of metabolic arrest. Furthermore, extraction in cold 50% aqueous acetonitrile resulted in a superior standard recovery and a higher metabolite concentration than that with the other 12 tested solutions. Protocols applied for adherently growing cells require additional concern, namely the detachment of cells from the cell culture flask. Two strategies are frequently applied—trypsinisation and cell scraping. It has been reported that trypsinisation is generally less suitable for metabolomics studies in cells because it results in lower metabolite concentration in almost all metabolic classes except lipids [58] and may contribute to cell membrane damage and metabolite leakage [59]. Recently, Dettmer et al. [60] reported cell scraping in extraction solvent, which causes simultaneous cell quenching, as the most suitable method for the harvesting of adherently growing cells. Moreover, among 7 examined extraction solvents, pure acetone yielded the lowest efficiency in contrast to 80% methanol in water, which resulted in the best and most reproducible extraction efficiency overall [60]. Because cell counting is a common choice for the normalisation of metabolomics data, direct scraping, which impairs counting, requires the implementation of equivalent

Table 1
Biological resource centres offering a broad coverage of cell lines.

Cell culture collection	Web site
American Type Culture Collection (ATCC)	http://www.atcc.org
Coriell Cell Repository	http://ccr.coriell.org/
Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)	http://www.dsmz.de/
European Collection of Animal Cell Cultures (ECACC)	http://www.phe-culturecollections.org.uk/
National Institute of Biomedical Innovation JCRB Cell Bank	http://cellbank.nibio.go.jp
RIKEN BioResource Center	http://dna.brc.riken.jp/index.html