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Review

Metabolomics in cancer biomarker discovery: Current trends and future perspectives

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ABSTRACT

Cancer is one of the most devastating human diseases that causes a vast number of mortalities worldwide each year. Cancer research is one of the largest fields in the life sciences and despite many astounding breakthroughs and contributions over the past few decades, there is still a considerable amount to unveil on the function of cancer. It is well known that cancer metabolism differs from that of normal tissue and an important hypothesis published in the 1950s by Otto Warburg proposed that cancer cells rely on anaerobic metabolism as the source for energy, even under physiological oxygen levels. Following this, cancer central carbon metabolism has been researched extensively and beyond respiration, cancer has been found to involve a wide range of metabolic processes, and many more are still to be unveiled. Studying cancer through metabolomics could reveal new biomarkers for cancer that could be useful for its future prognosis, diagnosis and therapy. Metabolomics is becoming an increasingly popular tool in the life sciences since it is a relatively fast and accurate technique that can be applied with either a particular focus or in a global manner to reveal new knowledge about biological systems. There have been many examples of its application to reveal potential biomarkers in different cancers that have employed a range of different analytical platforms. In this review, approaches in metabolomics that have been employed in cancer biomarker discovery are discussed and some of the most noteworthy research in the field is highlighted.

Contents

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1. Introduction

For decades, cancer research has involved studying the molecular features that are different between cancer cells and their healthy counterparts, with the aim of revealing biomarkers representative of the cancer phenotype as well as possible therapeutic targets. This has led to the identification of many molecular features involved in cancer that function in signal transduction [1], cell senescence [2] and other hallmarks of cancer cells [3]. Although the functional levels of a biological system include the genome, transcriptome, proteome and metabolome, the latter is considered most representative of the phenotype [4]. Exploring the cancer metabolome may be the best way to reveal the phenotypic changes relative to biological function, especially where subtle changes in metabolite concentrations can be tractable. For these reasons metabolomics is considered as one of the fastest developing disciplines in cancer research as well as many other aspects of life science. It is hoped that for a range of cancers, specific biomarkers will be revealed that could be used in screening for diagnostic and prognostic purposes. A reliable biomarker should be reproducibly detected in samples and collection of samples containing the biomarker should be performed with uniformity involving minimal invasion to the patient or subject. The application of metabolomics to cancer is increasing year by year in the search for candidate biomarkers that define a particular cancer, whose directional variation is significantly higher than all other endogenous metabolites that comprise the often complex sample for analysis.

Although metabolomics is still an emerging field, particularly in cancer research, aspects of cancer metabolism have long been a focus to understand central mechanisms in tumours. The best studied feature of cancer metabolism is central carbon metabolism and the relationship between glycolysis, the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. One key hypothesis is that cancer cells preferentially convert pyruvate to lactate rather than fuelling the TCA cycle even in aerobic conditions [5,6]. More recently it has been shown that this is not the exclusive rule for cancer metabolism, however it is a common understanding that tumours display enhanced glycolytic activity along with a down regulation of the TCA cycle and oxidative phosphorylation [7–9]. This is known as the Warburg effect. Hypothesised by Otto Warburg in 1956, the Warburg effect suggests that tumour cells originate from healthy cells in two phases: an irreversible injuring of respiration followed by a replacement of the lost respiration energy with fermentation energy [8]. Furthermore, the Warburg effect implies that cancer cells show elevated uptake of glucose. This is the main feature of the highly sensitive and accurate positron emission tomography (PET) currently employed in solid tumour diagnostics [10].

Using a metabolomics approach, it is possible to detect a range of metabolites in a single assay and therefore metabolomics can be defined as a holistic and data-driven study of the low molecular weight metabolites present in biological systems [4]. This allows further investigation into central carbon metabolism but also the revelation of other biochemical pathways that contribute to cancer function. The metabolome consists of both endogenous and exogenous components: those catabolised or anabolised by the biological system itself, or those that are extra-organism or extracellular respectively. It is inclusive of metabolites present in a biological sample that represent metabolic activity required for growth, maintenance and function, as well as metabolites consumed from the external environment [4]. Fig. 1 highlights the main steps of a metabolomics experiment that can be characterised by three main stages: data collection, processing and analysis.

Metabolomics can be performed on a range of different sample types including tissue, cells, bio-fluids such as serum, plasma, urine and saliva, and recently it has been shown using ion mobility that cancer biomarkers may be detected in breath odour from volatile organic compounds exhaled [11]. Likewise, a range of different analytical platforms and appropriate methodologies for sample preparation can be used in metabolomics, many of which are discussed in the proceeding section. Finally, data are analysed in different ways depending on the experimental design, but commonly involve univariate and/or multivariate analyses that assign statistical significance to the difference in individual metabolite concentrations between experimental groups or determine the multi-variation between groups collectively from all metabolites identified respectively. Practical applications of metabolomics software with particular reference to cancer metabolomics has been reviewed previously, providing an explanation of the different methodologies employed for data processing and analysis [12].

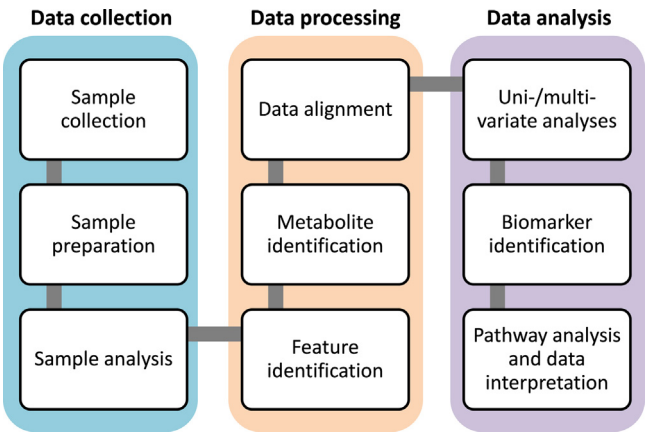

Fig. 1. The pipeline for metabolomics in biomarker discovery. The experiment is defined by three main categories: data collection, data processing and data analysis. Sample preparation is dependant both on the sample and on the analytical platform employed, and sample analysis is designed to suit the type of sample collected. Features are identified in the spectra and metabolite identifications are assigned where possible, commonly employing publically available metabolite databases. Data from different experimental groups are aligned and pre-treated for their comparison by univariate or multivariate analysis. From this possible biomarkers are suggested and can be further analysed to interpret the origin of their control through pathway analysis. If a certain subset of metabolites are exposed these can be further investigated in a more focused version of this pipeline.

2. Methodologies for metabolomics based biomarker discovery

2.1. Analytical platforms

The key pathways that behave differently between tumour and normal cells include glycolysis and the pentose phosphate pathway, nucleotide and protein biosynthesis, lipid and phospholipid turnover, the TCA cycle and redox stress pathways. No single analytical platform can detect all the compounds that can be altered in cancer. Metabolomics experiments can employ one or more different analytical platforms depending on the application; where the pipeline for a metabolomics experiment is similar for each. In general, metabolomics based biomarker discovery typically employs either nuclear magnetic resonance spectroscopy (NMR) [13–15] or mass spectrometry [16–19]; where the latter can be coupled with a separation technique such as gas chromatography (GC–MS) [20–23], liquid chromatography (LC–MS) [24–27] or capillary electrophoresis (CE-MS) [28–30]. Also, mass spectrometry can be performed using a range of different mass analysers depending on the type of experiment.

NMR benefits from being highly reproducible (>98% [31]) and offers the potential to quantify compounds in complex mixtures precisely due to the direct relationship between peak area and the