

Metabolomics Analysis of Marker Metabolites for Patients with Pancreatic Cancer

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Abstract

Metabolomics is a rapidly growing field of research used in the identification and quantification of the small molecule metabolites within an organism, thereby providing insights into processes important in clinical medicine. It offers comprehensive information about thousands of low-molecular metabolites (<1500 Da) that represent a wide range of metabolism pathways. In human studies, metabolomics has been applied to define biomarkers related to prognosis or diagnosis of a disease. Pancreatic cancer is a devastating disease and has a very low 5 years-survival rate and its diagnosis is often late and imprecise due to the lack of specificity of currently used markers for pancreatic cancer. Obviously, more sensitive biomarkers for early diagnosis and treatment are urgently needed. Fortunately, metabolomics is becoming increasingly popular, and may provide clinically useful biomarkers applied toward identifying metabolic alterations in pancreatic cancer and has introduced new insights into the pathology of pancreatic cancer. By measuring the endogenous metabolites, metabolomics can be used for delineating metabolic networks and discovering metabolic markers. Studying pancreatic cancer through metabolomics could reveal new biomarkers that could be useful for its future prognosis, diagnosis and therapy. In this review we take a closer look at the metabolomics used within the field of pancreatic cancer disease diagnosis.

Keywords: Metabolomics; Pancreatic cancer; Biomarkers; Metabolites; Early diagnosis

Introduction

Pancreatic cancer is the main leading cause of cancer mortality in the world since early diagnosis is difficult and challenging [1]. The annual death rate from the disease almost equals the annual incidence due to the aggressive nature of the cancer as well as to the lack of effective means of screening for it during its early curable stage [2]. Endoscopic brushing cytology or biopsy is still suffers from low sensitivity and specificity [3]. Classical tumor markers are reliable parameters to determine disease progression during chemotherapy or recurrence after surgery, but they are not adequate to identify suspected disease or for screening [4]. The diagnosis and management of pancreatic cancer continues to be an overwhelming challenge. Metabolomics, a dynamic portrait of the metabolic status of living systems, offers potential advantages that classically diagnose approaches do not, based on following discovery of a suite clinically relevant biomarker that are simultaneously affected by the pancreatic cancer [5,6]. Because small changes in living systems can lead to large changes in metabolite levels, the metabolome can be regarded as the amplified output of a biological system. Monitoring fluctuations of certain metabolite levels in body fluids, has become an important way to detect early stages in pancreatic cancer [7]. Moreover metabolomic approaches are likely to be used to screen for potential diagnostic and prognostic biomarkers of pancreatic cancer [8]. Experimental setup for metabolomics analysis was shown in Figure 1.

Metabolomics represents a tool to assess the biochemical activity of a living system through the analysis of substrates and products processed during the metabolism [9]. The emergence of powerful metabolomics technologies in conjunction with advanced bioinformatic tools allows the simultaneous analysis of thousands of biological molecules [10-15]. These techniques yield the discovery of new cancer signatures, which are sensitive and specific enough for early cancer detection, for monitoring disease progression and for proper treatment selection, paving the way to individualized cancer treatment [16]. Key idea of metabolomics is to determine the low-weight molecular metabolites, by a series of analytical methods, then to transform the data of metabolic pattern into useful information,

by chemometric tools and pattern recognition software, and to reveal the essence of life activities of the body [17-21]. With advantages of high-throughput, high-sensitivity and high-accuracy, metabolomics shows great potential and value in cancer individualized treatment. To improve performance, combinations of biomarkers, or combinations of biomarkers and clinical parameters or laboratory test results, might be required. In this review we describe recently discovered metabolites of pancreatic cancer and discuss challenges to their development and application, highlights the potential value of metabolomics for the noninvasive analysis of pancreatic cancer.

Metabolomic Features

Pancreatic cancer is a devastating disease with incidence increasing at an alarming rate and survival not improved substantially during the past three decades [22]. Although enormous efforts have been made in early detection and comprehensive treatment for this disease, little or no survival improvement was obtained, which necessitates the development of novel strategies. Early diagnosis of pancreatic cancer is difficult owing to late presentation of symptoms [23-25]. Hence, finding a marker to identify cancer stage early would be useful to improve survival. Emerging metabolomics provides a means for noninvasive screening of tumor-associated perturbations in metabolism. In the last decade, metabolomics has contributed substantially to our understanding of different diseases [26]. Metabolomics aims to comprehensively identify metabolites to gain insight into the

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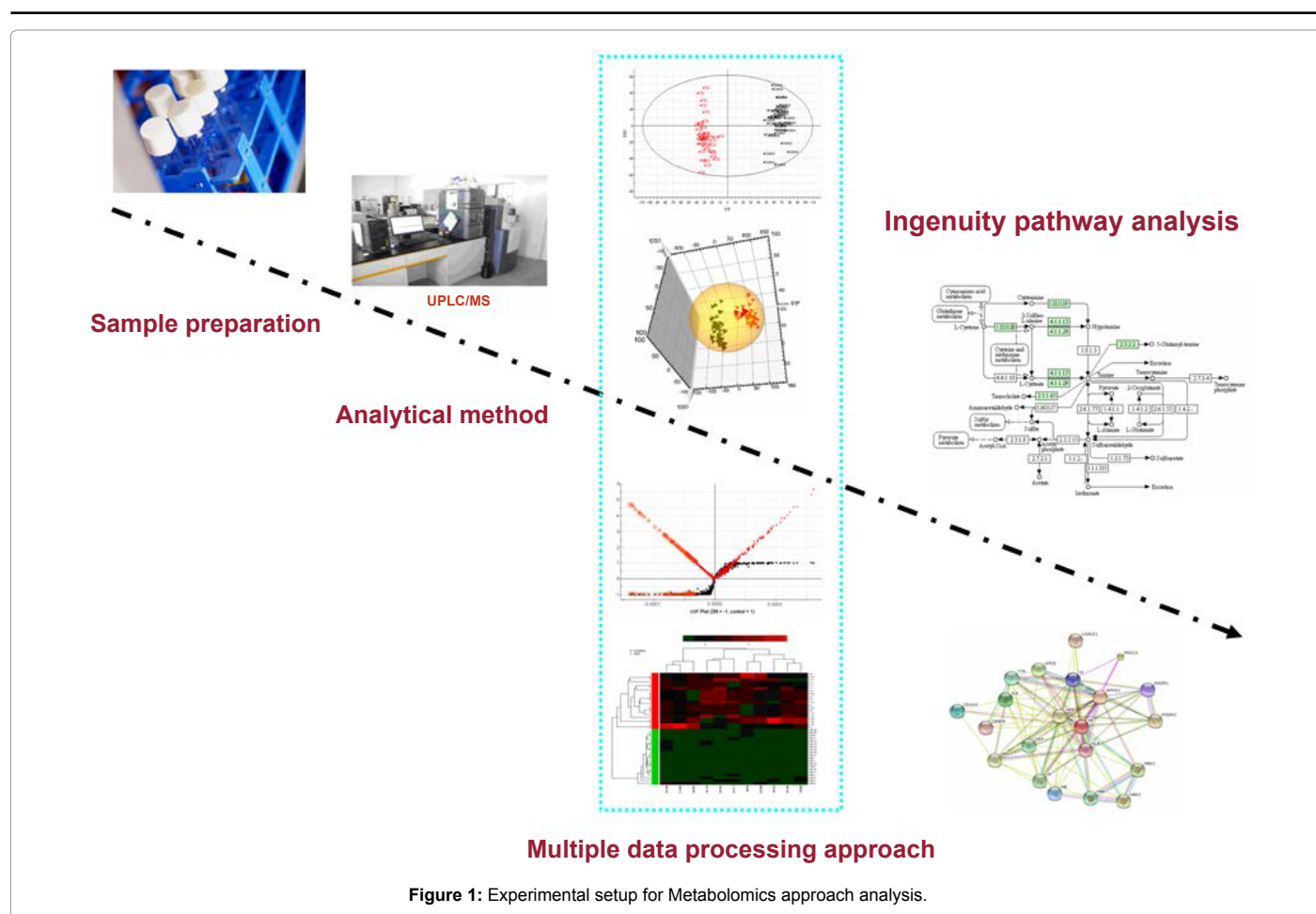


Figure 1: Experimental setup for Metabolomics approach analysis.

cellular signaling pathways underlying disease and to discover novel biomarkers for screening, early detection and diagnosis, as well as for determining prognoses and predicting responses to specific treatments [27,28]. For comprehensive analysis of metabolites, analytical methods of wider dynamic range higher resolution and good sensitivity are required. Technological advances have made and will continue to make possible earlier, more accurate, less invasive diagnoses, all while enhancing our understanding of the root causes of disease [29]. Liquid chromatography-mass spectrometry (LC-MS) is currently one of the most versatile techniques [30]. Integration of metabolomics into the pancreatic cancer would make it possible to embrace the arrival of 'Pancreatic Cancer-OMICS' era, and might be the direction to enable a revolution for future health care.

Bringing Metabolomics into Pancreatic Cancer Research

Pancreatic cancer is one of the most commonly diagnosed cancers and cause of cancer-related deaths worldwide. Studies have demonstrated that patient outcome is substantially influenced by cancer stage at the time of diagnosis [31-33]. Pancreatic cancer is a malignant tumor with the worst prognosis among all cancers. At the time of diagnosis, surgical cure is no longer a feasible option for most patients, thus early detection of pancreatic cancer is crucial for its treatment. It is important to develop effective methods for early diagnosis as well as for precise staging of this disease process. Metabolomics has recently emerged as a novel method of pancreatic cancer detection owing to its ability to monitor changes in the metabolic signature that reflect changes in phenotype and function [34]. Metabolomics promises to

be a valuable tool in the early detection of pancreatic cancer that may enable earlier treatment and improved clinical outcomes.

Potential roles for metabolomics in the clinical trials include biomarker discovery and validation, molecular target discovery, therapy decisions, and patient monitoring [35-38]. 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 cancers that have employed a range of different analytical platforms. This novel metabolomic approach can potentially lead to pancreatic cancer screening or precancer diagnosis and may provide useful information on the cancer type and the disease's stage of progression. We keep the hope that a combination of some of these novel biomarkers can be a useful tool for early pancreatic cancer diagnosis before image techniques and/or patient's symptoms reveal disease in an incurable state.

Metabolomics Applications in Pancreatic Cancer Research

Pancreatic cancer is the main leading cause of cancer mortality with a high mortality rate in the world. Effective early detection methods are needed since this is the best way to cure this disease. Currently, although CA19-9 is one test used, the sensitivity and specificity for the disease are less than optimal. The emerging field of metabolomics attempts to profile all metabolites within a biological system, painting a broad picture of the altered pathways [39]. While a large fraction of cancer metabolomics research is focused on finding

diagnostic biomarkers, metabolomics is also being used to obtain more fundamental mechanistic insight into cancer. It has potential power to improve our understanding of the underlying mechanisms of cancer, has been shown to provide a detailed snapshot of the body's processes at any particular point in time, opening up the possibility of monitoring health and disease, prevention and treatment. Metabolomics in cancer research is most often used for case-control comparisons. Secondary applications include translational areas, such as patient prognosis, therapy control and tumor classification, or grading [40].

Recently, several molecules were found to be specifically expressed in pancreatic cancer, and these novel molecular markers are reported to improve the sensitivity of cytology or biopsy. Urinary metabolomics detected distinct differences in the metabolic profiles of pancreatic cancer compared with healthy controls and benign pancreatic disease [41]. Significant differences in metabolite concentrations between cancers and controls were noted. Sensitivity and specificity of the multivariate OPLS-DA model were summarized using a ROC (0.988), indicating strong predictive power. It suggested that metabolomic approaches may facilitate discovery of novel pancreatic cancer biomarkers. Tesiram et al. had determined nuclear magnetic resonance spectroscopic characteristics and metabolite profiles of serum samples from patients with pancreatic cancer compared with control samples and to ascertain if the accuracy of metabolite identification can be improved upon by confirmation of spin-system assignment using more sophisticated experiments [42]. Result show that total choline, taurine, and glucose plus triglycerides are significantly higher in cancer versus control samples. To develop a noninvasive and accessible diagnostic method for pancreatic cancer, Zhang et al. presented a metabolomic method to investigate the plasma metabolites obtained from 20 patients with pancreatic cancer and 20 healthy individuals [43]. Metabolic changes associated with pancreatic cancer included abnormal amino acid and lipid metabolism. Pancreatic cancer elevated plasma levels of N-acetyl glycoprotein, dimethylamine, very low density lipoprotein, and acetone, and reduced levels of 3-hydroxybutyrate, lactate, high density lipoprotein, low density lipoprotein, citrate, alanine, glutamate, glutamine, histidine, isoleucine, lysine, and valine. These metabolites could be a biomarker group for pancreatic cancer that distinguishes between pancreatic cancer patients and healthy individuals. Metabolomic strategy appears as a promising approach for distinguishing pancreatic cancer and identifying new strategies for prevention or therapy in the clinical practice.

NMR spectroscopy was applied to investigate the urine metabolome of pancreatic cancer patients and to detect altered metabolic profiles in comparison with healthy matched controls [44]. Group discrimination was possible due to average concentration differences of several metabolite signals, pointing to a multimolecular signature of the disease. The robustness of the determined statistical model is confirmed by its predictive performance. Application of NMR combined with principal components analysis was to discriminate pancreatic cancer patients from healthy controls based on metabolomic profiling of the serum [45]. The metabolic analysis revealed significant lower of 3-hydroxybutyrate, 3-hydroxyisovalerate, lactate, and trimethylamine-N-oxide as well as significant higher level of isoleucine, triglyceride, leucine, and creatinine in the serum from pancreatic cancer patients compared to that of healthy controls. It demonstrated that the subtle differences in metabolite profiles in serum of pancreatic cancer patients and that of healthy subjects as a result of physiological and pathological variations could be identified by metabolomics and exploited as metabolic markers for the early detection of pancreatic cancer. In future work, these potential biomarkers should be further validated with a large enough patient cohort to achieve earlier diagnosis of pancreatic

cancer. In a study by Urayama et al., metabolic profiling of plasma samples from selected cancer patients and noncancerous controls was performed to seek novel metabolic biomarkers of pancreatic cancer [46]. It was found that selective amino acids, bile acids, and polar lipids were detected with increased or decreased levels in pancreatic cancer samples compared to controls. These findings on blood plasma levels of the relevant metabolites might be very useful clinical parameters for early diagnosis of pancreatic cancer.

Metabolomics, a high-throughput global metabolite analysis, is a burgeoning field, and in recent times has shown substantial evidence to support its emerging role in pancreatic cancer diagnosis, recurrence, prognosis, and identifying novel cancer biomarkers as well as developing cancer therapeutics [47]. Recent developments in the area of metabolic analysis may help to close the gap between clinical metabolomics research and the development of cancer metabolome [48-50]. Any findings associated with relevance to pancreatic cancer, once passed to the clinical level, will be eventually combined with other diagnosis approaches to hopefully reach the 100% detection level for high-risk patients. Recent advances in metabolomics along with the novel strategies to analyze, understand, and construct the metabolic pathways opens this window of opportunity in a very effective manner. We predict an intensified use of metabolomic screens in clinical and preclinical studies focusing on the onset and progression of pancreatic cancer development.

Conclusions and Future Perspectives

Pancreatic cancer is the most common cancer in worldwide and the high prevalence of pancreatic cancer has caused a huge burden on the society. Metabolomics has the potential to impact our understanding of molecular mechanisms of pancreatic cancer. Changes in metabolite concentrations reveal the range of biochemical effects induced by a disease or its therapeutic intervention. With the recent emergence of novel technologies, the field of biomarker discovery has been the subject of intense research and activity. It has the potential to generate novel noninvasive diagnostic tests, which are the high sensitivity and specificity characteristics. Therefore, this literature review presents an overview of the status of current advances in metabolomics diagnostics for pancreatic cancer, which in turn leads to improvement in cancer screening, prognosis and management of therapeutic response in cancer patients.

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