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Review

Mass spectrometry as a tool for biomarkers searching in gynecological oncology

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ABSTRACT

Tumors of the female reproductive tract are an important target for the development of diagnostic, prognostic and therapeutic strategies. Recent research has turned to proteomics based on mass spectrometry techniques, to achieve more effective diagnostic results. Mass spectrometry (MS) enables identification and quantification of multiple molecules simultaneously in a single experiment according to mass to charge ratio (m/z). Several proteomic strategies may be applied to establish the function of a particular protein/peptide or to identify a novel disease and specific biomarkers related to it. Therefore, MS could facilitate treatment in patients with tumors by helping researchers discover new biomarkers and narrowly targeted drugs. This review presents a comprehensive discussion of mass spectrometry as a tool for biomarkers searching that may lead to the discovery of easily available diagnostic tests in gynecological oncology with emphasis on clinical proteomics over the past decade. The article provides an insight into different MS based proteomic approaches.

Contents

1. Introduction

The field of genomics dates back to 2001, when an initial sequence of human genome was decoded. This new branch of science deals with the complete genome as opposed to focusing on individual genes [1]. Therefore, it enables a better understanding of the mechanisms controlling the human body [2,3]. It was expected that further studies on the human genome would result in discovering innovative biomarkers, developing novel diagnostic tests and gene therapies that would cure many diseases such as neoplasms and other chronic conditions. However, it turned out that the knowledge on our genome alone was not sufficient to elucidate the interactions at molecular level occurring in the course of a disease. As a result, a rapid progress in interdisciplinary

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systeomics (systems biology), combining the fields of genomics, transcriptomics, proteomics and metabolomics has been observed. Recently various omics areas of science provide relatively new tools for cancer research, including searching for diagnostic, prognostic and predictive biomarkers. These technologies make it possible to study the human body's response to physiological and/or pathological conditions on DNA, RNA, protein, peptide, and metabolite levels. Although standard bioanalytic methods such as enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence immunoassay (ECLIA) are still commonly used in medicine, numerous studies focus on mass spectrometry (MS) based technologies. MS is an analytical technique that enables identification and quantification of multiple molecules in a single experiment according to their mass to charge ratio (m/z). Mass spectrometers equipped with matrix assisted laser desorption ionization (MALDI), surface enhanced laser desorption ionization (SELDI) and electrospray ionization (ESI) ion sources have become widely used in the field of proteomics and metabolomics [4]. The use of MS is not limited to the studies on previously identified compounds but when combined with chemometric tools and open access databases it may be helpful in identification of previously unknown biopolymers with possible biological roles. Several strategies may be applied to establish the function of a particular gene at the protein level or to identify a novel disease and specific biomarkers related to it. MS could facilitate treatment in patients with tumors by helping clinicians discover new biomarkers and narrowly targeted drugs [5–7].

Detailed explanation and understanding of processes and interactions on the proteomic level have to be supported by chemometric and bioinformatic tools [8]. This is due to the fact that the current studies involving MS techniques generate huge amount of data. Using dedicated software to deal with the findings allows for gaining more information on diagnostic potential of physiological and/or pathological conditions of the human body. Furthermore, chemometric tools are essential to compile clinical and multi-omic data (genomic, proteomic, metabolic), which result in modern medicine being more individualized and patient-oriented.

Gynecological neoplastic diseases include ovarian cancer, endometrial cancer, cervical cancer, vulvar cancer, breast cancer, and gestational trophoblastic disease. Gynecological oncology already uses various helpful protein biomarkers, such as carbohydrate antigen 125 (CA 125), human epididymis protein 4 (HE4), chorionic gonadotropin β (βhCG), tumor-associated glycoprotein 72 (CA72-4), soluble Fas (sFas), and serum levels of immunosuppressive acidic protein. Currently used diagnostic tools are not effective enough and their sensitivity and specificity are insufficient. Hence, clinical proteomics seems to be a very promising way to gain broad knowledge on the underlying mechanisms of neoplastic processes [9], which in turn will contribute to discovery and depiction of novel specific and sensitive markers.

The first report in 2002 demonstrated the possibility of using SELDI in ovarian cancer diagnostics [10]. Since then, multiple papers confirming the usefulness of MALDI and SELDI ionization techniques in the study of various diseases have been published. However, these methods were also criticized for insufficient interlaboratory reproducibility. Therefore, this review presents a comprehensive discussion concerning mass spectrometry as a tool for biomarkers searching in gynecological oncology with special emphasis on clinical proteomics. The article provides an insight into different MS based proteomic approaches that may lead to the discovery of easily available diagnostic tools.

2. Mass spectrometry strategies in medicine

Proteomics is a useful omics tool in the field of biomarkers characterization and clinical evaluation of diseases. The two main strategies that are currently used in research laboratories are: top-down and bottom-up. Among them, the bottom-up strategy is more common and comprises data-dependent, data-independent and targeting methods, that can be applied for different purposes [11,12]. Classic data-dependent bottom-up strategy deals with the complexity of biological matrices by involving the analytical procedures beginning with the separation of proteins using a two-dimensional gel electrophoresis (2D-GE), followed by proteolytic digestion of the separated proteins and MS-based identification of proteins [13].

Present proteomic studies frequently take advantage of the shotgun technique which is in fact a kind of bottom-up MS approach. It involves proteolytic digestion of the entire sample as the first step. The resulting mixture of proteins and peptides is separated by means of liquid chromatography (LC) and further identified by applying tandem mass spectrometry (MS/MS). The aforementioned shotgun proteomics strategy, in which the complex mixture of digested peptides is analyzed in data dependent mode, allows to detect even more than 10000 proteins in a single run. In order to obtain the quantitative data from such experiments, stable isotopes or label-free methods are used for estimation of relative protein abundance. Although the isotope labeling techniques are characterized by better precision and accuracy comparing to label-free strategies, the latter ones become recently more common due to being less tedious, time-consuming and cheaper.

One of the emerging methods which allows to perform label-free quantification is sequential window acquisition of all theoretical mass spectra (SWATH) [14]. It is a data-independent acquisition technique based on MRM-like (multiple reaction monitoring) mode. In data-independent mass spectrometry acquisition, the instrument produces all the MS/MS spectra from *all* parent ions within a predefined m/z range, thus it provides much more information than in the case of data-dependent strategies. Therefore, data-independent approach is becoming a method of choice for biomarkers searching. Applying this approach it is possible to retrospectively process the data in order to search for markers or other compounds that were unknown at the time of data acquisition.

In contrast to data-dependent and data-independent approaches, where no prior information of analyzed compounds is required, the targeted strategy is focused only on previously defined analytes and is not suitable for global assessment of the matrix composition [15,16] For a protein of interest, its specific fragment ions in the predefined list are selected in the first (Q1) quadrupole in a triple quadrupole mass spectrometer (QqQ). They are fragmented in the second (Q2) quadrupole that serves as a collision cell. Next product ions are analyzed by the third (Q3) quadrupole. Each MRM transition is monitored over the LC–MS run.

Disadvantages of the bottom-up strategies include low repeatability and the fact that 2D-GE separation is time-consuming and often troublesome, especially when repeated multiple times. Advances in compound fragmentation has led to the implementation of the top-down strategy. This strategy involves an analysis of native compounds without prior separation and digestion of proteins. Its main drawbacks include complicated MS/MS spectra of large proteins that are difficult to interpret and require the mass spectrometer's operator to have vast experience.

Proteomic strategies are used for the analysis of very broad spectrum of biological samples: tissues, organs, body fluids and cell cultures. From a clinical perspective, body fluids are undoubtedly the best source of material for searching for biomarkers. This is particularly true for easily available fluids such as serum/plasma, urine and saliva, as sampling may take place at the comfort and convenience of the patient's home. With