

The application and outlook of proteomics in Ovarian Cancer, Breast Cancer, and Colon Cancer

Yiru Xie

The Ohio State University, Columbus, Ohio, United States

Abstract: Cancer, a pervasive challenge of global health, necessitates advanced technologies for diagnostics and therapies. Ovarian, breast, and colon cancers, notable for their complexity, have made significant discoveries with the help of proteomics. Ovarian cancer, diagnosed late, benefits from proteomics biomarkers like CA-125 and plasma CRP, offering early detection and insights into sub-types. Breast cancer leverages proteomics for sub-type classification, treatment prediction, and liquid biopsy development. Markers like uPA and PAI-1 guide therapies, while phosphorous proteomics unveils crucial signaling pathways. Colon cancer, marked by its prevalence, sees proteomics aiding biomarker discovery, sub-type classification, and predicting treatment responses, fostering personalized treatments. Liquid biopsy development holds promise for real-time monitoring. Propelled by advanced technologies like LC-MS/MS and iTRAQ, proteomics provides a holistic view of cancer biology. Integrating with genomics refines precision medicine. Insights from proteomics in ovarian, breast, and colon cancers mark a era of transformation, offering personalized cancer care and improved clinical outcomes.

1. Introduction

Cancer is a complex and multifaceted group of diseases which continue to pose a significant challenge on global health. Among the various forms of cancer, ovarian cancer, colon cancer, and lung cancer stand out as hazardous disease, affecting millions of individuals worldwide. More than 30% of people will develop some form of cancer during their lifetime, and cancer is the leading cause of the death worldwide. Implementation of proteomics for cancer research: past, present, and future. *Asian Pac J Cancer Prev*, 15, 2433).

For example, Ovarian cancer remains the most lethal gynecologic cancer. It ranks fifth in cancer-related deaths among women in the United States. The American Cancer Society estimates that in 2022, 19,880 women will receive a new diagnosis of ovarian cancer and about 12,810 women will die from ovarian cancer^[1]. Colorectal cancers (CRC) are the 3rd most common malignancies in the world. The global mortality of CRC is expected to be million deaths and the new emerging more than 2.2 million cases by 2030^[2]. Lung cancer is a deadly disease. It is the most common cause of cancer-related mortality among both men and women in the western countries, resulting in an overall 5-year survival rate of approximately 15%^[3]. Proteomics is very popular these days, searching for proteomics application in cancer in the last five years, we found that the number of articles appearing in ovarian cancer, Colon Cancer, and Lung Cancer is very high. The application of proteomics in cancers such as bone cancer and esophagus cancer are

relatively rare, so the cancers we chose will be good representatives of proteomics in cancer medical research. The quest for effective diagnostic tools, prognostic markers, and targeted therapies including mammography--a widely used diagnostic tool for breast cancer screening; hormone receptor status -- a marker determining the hormone receptor status of breast cancer cells, specifically estrogen receptor (ER) and progesterone receptor (PR) status, is crucial for predicting the cancer's behavior; and BRCA Gene Mutations -- markers associated with an increased risk of ovarian cancer for these malicious cancers has driven scientific research to explore sophisticated medical technologies, overcome including proteomics. Proteomics has been used previously to identify biomarkers of ovarian cancer, lung cancer, and colorectal cancer.

Despite of there are currently existing markers, tools, and therapies, they all have their shortcomings. Traditional diagnostic tools like imaging techniques (e.g., mammography or CT scans) provide structural information about tumors but offer limited molecular insights. Proteomics, on the other hand, can provide detailed information about the proteins expressed within tumors, offering a deeper understanding of their biology and potential drug targets. Many cancers, including breast, ovary, colon, and lung cancers, are known for their varieties/heterogeneity, meaning that different regions of the tumor may have distinct molecular characteristics. Traditional tools often sample a small portion of the tumor, potentially missing critical information. Proteomics can capture this heterogeneity by analyzing multiple proteins simultaneously.

e-mail: yiruxie025@gmail.com

Proteomic analysis is defined as a group of the analytical methodology that used to characterize qualitatively and quantitatively protein groups^[4]. Proteomics is the study of the entire complement of proteins expressed by an organism or a specific tissue, has emerged as a new yet promising discipline within the field of molecular biology. Proteomics is introduced by Wilkins and Williams in 1995, and it refers to a way of all protein in organism and its activities^[5]. The products of gene---protein---is the main part of the organism to perform a variety of complex physiological functions^[6]. This marked a pivotal moment in the history of biology, as it signaled a shift from the study of individual proteins to a holistic approach that aimed to comprehensively analyze the entire proteome of an organism, tissue, or cell. With the advent of advanced mass spectrometry techniques, gel electrophoresis, and bioinformatics tools, proteomics began to evolve rapidly. In recent years, proteomics has matured into a robust discipline, with advancements in high-throughput technologies. Liquid chromatography-mass spectrometry (LC-MS/MS) and other proteomic techniques have enabled the identification and quantification of thousands of proteins in a single experiment. Furthermore, the integration of proteomics with genomics, transcriptomics, and metabolomics has provided a more comprehensive view of cellular processes and their malfunction in diseases like cancer.

Proteomics offers a comprehensive view of the complex molecular mechanism controlling cellular processes and has been instrumental in explaining the molecular aspects of various diseases, including cancer. In recent years, human beings have higher needs in terms of life expectancy and health, the application of proteomics for curing cancer has a huge impact, so we should pursue advanced new proteomics technologies. It is urgent for researchers to unravel the intricate molecular landscapes of breast, ovary, and colon cancers. This study reviews the application of proteomics to these cancers and the prospective.

2. The application of Proteomics in the Ovarian Cancer

Ovarian cancer, a challenging malignancy often diagnosed in late stages, which has been a focal point for proteomics research due to its clinical significance and complexity. In 2021, a research found out that plasma CRP levels help monitor the progression of ovarian tumor and combination of novel protein biomarkers have a good distinguish between cancer and non-cancer patients. In 2022, the result of a research identified makers unique to the VTE group that could contribute to development of thrombosis and the associated morbidity and mortality in ovarian cancer patients^[7].

Proteomics has played a pivotal role in biomarker discovery for ovarian cancer. By scrutinizing the protein profiles of ovarian cancer tissues and comparing them to healthy counterparts, researchers have identified candidate biomarkers. Consequently, the research the application of proteomics in ovarian cancer is utmost

importance.

Cancer Antigen 125 (CA-125) is a high molecular weight mucinous glycoprotein found on the surface of ovarian cancer cells CA-125, also referred to as Carbohydrate Antigen 125, has played the most significant role in screening, detecting, and managing ovarian cancer for decades^[8]. Notably, the well-established ovarian cancer biomarker CA-125 was discovered through proteomics approaches. Bast and colleagues first described CA125 in 1981 by developing a monoclonal antibody (OC125) against this antigen. The biomarkers such as CA-125 hold the promise of enabling earlier detection and diagnosis, which is essentially given the disease's propensity for late-stage presentation. Moreover, proteomics techniques have unveiled the diverse sub-types and multiple targets within ovarian cancer. Ovarian cancer is not a singular entity but comprises distinct sub-types with varying molecular profiles. At least 5 different histological sub-types of EOC exist and may reflect the clinical heterogeneity of this disease in terms of chemotherapeutic response and outcome^[9]. Proteomics has been instrumental in characterizing these sub-types by discerning differences in protein expression patterns. Such knowledge has significant implications for tailoring personalized treatment strategies, as different sub-types may respond differently to therapies. For example, in 2019, Hongyu Xie, Huan Xu, et al conducted an experiment where iCluster method was utilized to integrate mRNA and protein data of ovarian cancer from TCGA and Clinical Proteomics Tumor Analysis Consortium (CPTAC) database, iCluster method is a data-integration clustering method based on a Gaussian latent variable model with lasso.¹⁴ We found that HGSC patients were falling into six groups after iCluster analysis. Combining groups with similar survival time, two sub-types of HGSC were identified, and the survival profile was different obviously between these two sub-types.

Beyond biomarker discovery and sub-type classification, proteomics has significantly contributed to identifying potential drug targets within ovarian cancer cells. Utilizing advanced mass spectrometry techniques, such as liquid chromatography-mass spectrometry (LC-MS/MS), researchers delve into the intricate protein landscapes associated with ovarian cancer progression. LC-MS is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Linking MS techniques with liquid chromatography (LC) or capillary electrophoresis allows for obtaining high resolution spectral proteomic patterns from numerous sample types. "The method has already been reported as an accurate tool for discovering multi-component classifiers, which significantly discriminate cancer samples from control biofluids or tissues^[10]. By studying the quantitative and qualitative aspects of these proteins, scientists have pinpointed molecules that could serve as targets for innovative therapeutic agents. This includes the identification of over-expressed or abnormally regulated proteins that play pivotal roles in cancer development. For instance, the application of quantitative proteomics

using isobaric tags for relative and absolute quantitation (iTRAQ) has allowed for a comprehensive understanding of the proteomics alterations in ovarian cancer cells. iTRAQ is a technology that utilizes isobaric reagents to label the primary amines of peptides and proteins and is used in proteomics to study quantitative changes in the proteome by tandem mass spectrometry. “These techniques allow for simultaneous identification as well as relative quantification of proteins in multiple samples. ITRAQ labeling combined with tandem MS techniques enabled a selection of a few potential OC biomarkers. Moreover, iTRAQ analysis was proved to be a useful method to track changes in proteins during a transition from benign to malignant tumor-like PI3K/Akt signaling pathway^[10].

In summary, proteomics has profoundly advanced our comprehension of ovarian cancer. It has been pivotal in biomarker discovery, sub-typing, identification of drug targets, elucidation of drug resistance mechanisms, and the development of prognostic tools. These findings hold substantial promise for enhancing early detection, treatment strategies, and overall outcomes in ovarian cancer patients.

3. The application of Proteomics in the Breast Cancer

Breast cancer is the most commonly diagnosed malignancy and the second commonest cause of cancer related mortality in women^[11]. It is a prevalent and heterogeneous malignancy that has seen substantial researches in the realm of proteomics. The application of proteomics in breast cancer research has not only deepened our understanding of this disease but has also yielded valuable clinical insights and therapeutic advancements. Proteomics plays a pivotal role in identifying biomarkers for breast cancer. Proteomics information is essential to classify the functional sub-types and stages of the breast cancer, to decipher its tumorigenesis mechanisms, cancer behaviour and aggressiveness, to predict recurrence, to assess and reduce the cancer cell resistance, to choose and monitor the most appropriate breast cancer treatment^[12]. By analyzing the protein profiles of breast cancer tissues and comparing them to healthy counterparts, researchers have uncovered potential biomarkers, which can aid in early detection and diagnosis. For instance, both urokinase- type plasminogen activator (uPA) and its inhibitor plasminogen-activator type-1(PAI-1) are validated as prognostic biomarkers available for lymph node-negative breast cancer. Cysteine-rich intestinal protein 1 (CRIP1) expression was proposed as a biomarker in HER2+ breast cancer patients, has been identified through proteomics approaches. HER2 status is now a critical factor in guiding treatment decisions and has led to the development of HER2-targeted therapies like trastuzumab (Herceptin). In terms of sub-type classification, breast cancer, like ovarian cancer, encompasses distinct molecular sub-types with varying clinical behaviors. Proteomics has been instrumental in classifying these sub-types based on their unique protein

expression patterns. Notably, compared to healthy control plasma donors, researchers identified 191, 166, 182, and 186 differentially expressed proteins in the Luminal, Lumina-HER2, HER2, and TN sub-types through proteomics analyses^[13]. This classification has significant importance in determining appropriate treatment strategies, because each sub-type may respond differently to therapies. For example, hormone receptor status (estrogen receptor and progesterone receptor) is determined through proteomics trials and influences the choice of hormone therapy.

Proteomics has enabled the identification of predictive markers for treatment response in breast cancer. By analyzing the proteins associated with drug sensitivity or resistance, researchers can guide treatment decisions. For instance, studies have identified proteins associated with resistance to chemotherapy drugs, allowing for the exploration of alternative treatment options. The discovery of proteins like Ki-67, a marker of cell proliferation, has been instrumental in assessing the aggressiveness of breast tumors and predicting response to specific therapies^[14]. Moreover, phospho-proteomics is a specialized branch of proteomics, has been applied to breast cancer research to study protein phosphorylation patterns. Phosphorylation is a critical regulatory mechanism in cell signaling pathways, and abnormal phosphorylation events can foster cancer development. Phospho-proteomics allows for the identification of phosphorylated proteins, which can serve as potential therapeutic targets^[15]. This approach has unveiled novel insights into the signaling networks involved in breast cancer and the potential for targeted interventions.

Proteomics plays a role in the development of liquid biopsies for breast cancer. Liquid biopsies, which involve the analysis of circulating proteins and nucleic acids in the blood, have the potential to provide real-time information about disease progression and treatment response. Proteomics techniques are pivotal in the identification of circulating protein markers that can be used for non-invasive monitoring and early detection of breast cancer^[16].

4. The application of Proteomics in the Colon Cancer

Colon cancer is one of the most common malignant tumors, accompanied with high morbidity and mortality. The incidence is even higher in the most developed countries. With continuing progress in developing countries, the cases of colorectal cancer worldwide are predicted to increase to 2.5 million in 2035^[17]. As one of the leading causes of cancer-related mortality, colon cancer has been a focal point for proteomics research. The application of proteomics in colon cancer has significantly contributed to our understanding of this disease, leading to valuable clinical insights and potential therapeutic advancements.

Proteomics has played a pivotal role in identifying potential biomarkers for colon cancer. By analyzing the

protein profiles of colon cancer tissues and comparing them to healthy counterparts, researchers have identified candidate biomarkers. For instance, carcinoembryonic antigen (CEA), an established biomarker in colon cancer, was initially discovered through proteomics approaches. Although CEA lacks specificity, its identification through proteomics marked a critical early step in understanding potential markers for colon cancer, leading to further biomarker exploration. Colon cancer exhibits significant heterogeneity, prompting the need for sub-typing and personalized treatment strategies. Proteomics, aided by mass spectrometry, has been instrumental in characterizing these sub-types based on their unique protein expression patterns. Various sub-types such as micro-satellite stable (MSS), micro-satellite instable (MSI), and different molecular subgroups within these categories have been characterized through proteomics analyses. This classification has critical implications for tailoring treatment strategies, as different sub-types may respond differently to therapies. For example, MSI status, determined through proteomics assays, influences the response to therapies of immunity, highlighting the importance of proteomics classification for personalized treatments. Proteomics has enabled the identification of potential markers for treatment response in colon cancer. By analyzing the proteins associated with drug sensitivity or resistance, researchers can guide treatment decisions. For example, studies have identified specific proteins associated with resistance to chemotherapy drugs, facilitating the exploration of alternative treatment options. Additionally, proteins linked to the activation of certain pathways, such as the PI3K-AKT- mTOR pathway, have been identified through proteomics studies and hold promise as potential targets for therapeutic intervention in resistant cases^[18]. The integration of proteomics with genomics data has led to a more comprehensive understanding of the molecular landscape of colon cancer. This multiple genomics approach has provided insights into the genetic alterations, protein expression profiles, and signaling pathways involved in colon cancer development and progression. By combining proteomics and genomics information, researchers aim to uncover novel therapeutic targets and refine precision medicine approaches for colon cancer treatment. Proteomics has also contributed to the development of liquid biopsies for colon cancer. Liquid biopsies, which involve the analysis of circulating proteins and nucleic acids, hold the potential for non-invasive monitoring and early detection. Proteomics techniques have identified circulating protein markers that can be utilized in liquid biopsies, providing a promising avenue for real-time monitoring of disease progression and treatment response in colon cancer patients.

The application of proteomics in colon cancer research has significantly advanced our understanding of the disease, particularly in biomarker discovery, sub-type classification, treatment response prediction, multiple genomics integration, and the development of emerging liquid biopsy approaches. These advancements have laid the groundwork for personalized treatment strategies and precision medicine in colon cancer, ultimately offering the

potential for improved clinical outcomes for patients. Continued advancements in proteomics techniques and their integration with other bio-technologies promise further breakthroughs in colon cancer research and treatment.

5. Conclusion

Faced with the challenges of late-stage diagnosis in ovarian cancer, proteomics has been a game-changer. Proteomics make contributions to biomarkers like CA-125, usher in a paradigm shift in early detection. Plasma CRP monitoring and the identification of unique protein biomarkers signify the ongoing strides in ovarian cancer proteomics. Sub-type classification, crucial for personalized treatment, finds its ally in proteomics, unraveling molecular nuances and guiding tailored therapeutic strategies. Proteomics generate in the development of prognostic tools, surpassing traditional parameters and providing clinicians with more accurate predictions of patient outcomes.

In the landscape of breast cancer, characterized by its heterogeneity, proteomics unfolds a nuanced understanding. Prognostic biomarkers like uPA and PAI-1, sub-type categorization, and assessments of drug responses mark significant contributions from proteomics. The integration of phosphorous proteomics illuminates signaling pathways, offering avenues for targeted interventions. Liquid biopsies, promising for non-invasive monitoring, leverage proteomics to identify circulating protein markers, presenting real-time insights into disease progression. Colon cancer, with its prevalence and fatality rate, confronts challenges head-on with the aid of proteomics. The discovery of CEA, a pivotal biomarker, underscores the initial triumphs of proteomics in this arena. Sub-type classification, pivotal for treatment responses, gains precision through proteomics analyses, guiding therapeutic decisions. The identification of predictive markers for drug sensitivity or resistance enhances treatment decision-making. Integrating with genomics unravels molecular intricacies, opening doors to novel therapeutic targets.

Proteomics, in synergy with advanced technologies like LC-MS/MS and iTRAQ, propels cancer research into uncharted territories. The integration with genomics, transcriptomics, and metabolomics paints a holistic picture of the molecular intricacies steering cancer development. As we stand on the cutting-edge of a new frontier in cancer research, proteomics emerges not just as an insightful tool but a tangible force propelling personalized cancer care.

As proteomics technologies continues to develop, the current exploration into the application of proteomics in ovarian, breast, and colon cancers has illuminated critical facets of these cancers, yet the journey does not stop here. Future developments in cancer proteomics are set to explore into new frontiers, driven by technological advancements and a deeper understanding of the molecular understandings of cancer. Beyond the current technologies, the horizon of cancer proteomics is expanding with the emergence of novel platforms. Next-

generation proteomics technologies, such as proximity extension assays (PEA) and data-independent acquisition (DIA) mass spectrometry, show promise in providing enhanced sensitivity and coverage. These advancements are crucial for uncovering low-abundance proteins and rare variants that may play pivotal roles in cancer initiation and progression.

In summary, the application of proteomics to ovarian, breast, and colon cancers have made strides in cancer research. Beyond biomarker discovery, proteomics resonates in sub-type classification, drug target identification, elucidation of drug resistance, and the development of prognostic tools. The promise of enhanced early detection, personalized treatment strategies, and improved clinical outcomes underscores the profound impact of proteomics in the relentless battle against these formidable diseases. The integration of proteomics into cancer research paves the way for a future where precision and individuality define the approach to conquer cancer.

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