



Proteomic Biomarkers for Early Detection and Patients' Stratification in Ovarian Cancer: A Brief Overview

6

Marco Petrillo, Carlo Ronsini, Davide Calandra, Margherita Dessole, and Salvatore Dessole

Abstract

Quantitative proteomic profiling is progressively emerging as a reliable strategy to achieve early diagnosis, and prognostic stratification in epithelial ovarian cancer (OC). In particular, specific proteomic profiles of tumor-derived circulating proteins involved in regulating apoptosis, epithelial-to-mesenchymal transition, and cellular motility seem to show promising performances in early disease identification and prognostic stratification. Furthermore, proteomic characterization of ascites and pleural effusions will significantly improve the accuracy of predicting outcomes and selecting OC patients to benefit from the current therapies. Cancer tissues, pleural effusions, and ascitic fluids should be considered as the best biological samples for proteomic profiling to achieve the optimal use of biomarkers. On the other hand, plasma circulating-free proteins, or tumor-derived extracellular vesicles-embedded proteins are considered as the most appropriate source of data for early disease identification in OC patients. In the next decade, proteomic profiling will certainly be introduced in the clinical algorithms of the management of OC.

M. Petrillo (✉) · S. Dessole
PhD School in Biomedical Sciences, University of Sassari, Sassari, Italy
Obstetrics and Gynecology Clinic, University of Sassari, Sassari, Italy

C. Ronsini
Department of Woman and Child Health, IRCCS Fondazione Policlinico Agostino Gemelli, Rome, Italy

D. Calandra
Obstetrics and Gynecology Clinic, University of Chieti, Chieti, Italy

M. Dessole
PhD School in Biomedical Sciences, University of Sassari, Sassari, Italy

KeywordsOvarian cancer · Biomarkers · Proteomics · Extracellular vesicles

6.1 Introduction

Epithelial ovarian cancer (OC) is a leading cause of cancer-related deaths in the female population (Sung et al. 2021). Due to the lack of early symptoms, patients with OC are often diagnosed with an advanced stage of disease. In fact, approximately 60% of women have stage III–IV disease at diagnosis, which is associated with a 5-year survival below 30% (Elstrand et al. 2012). The most relevant issue to achieve early detection of the disease is the absence of related symptoms before the occurrence of diffuse peritoneal carcinomatosis. Only two biomarkers, cancer antigen 125 (CA-125) and human epididymis 4 (HE4) are currently used in clinical practice as reliable serological tests for diagnosis and disease monitoring of OC (El Bairi et al. 2017, 2020). In this perspective, several studies have demonstrated that the serum dosage of both HE4 and CA-125 has the highest sensitivity in the detection of OC and in particular when combined (Moore et al. 2008; Leung et al. 2016; Montagnana et al. 2009). Several clinical algorithms based on the combined assessment of CA-125 serum levels, and ultrasound pelvic examination have been developed as screening approaches in women with ovarian mass. However, overall discriminating performances in terms of sensitivity and specificity appeared to be disappointing; therefore, nowadays, despite initial promising findings, there is no validated screening algorithm able to accurately detect OC earlier. Furthermore, with the advent of personalized medicine, there is a growing awareness in the scientific community that OC does not represent a unique disease, but a complex, and heterogeneous biological entity (Petrillo et al. 2016). Therefore, emphasizing the need to completely change our point of view, moving from the traditional clinical approach that one fits for all, to the evidence-based strategy that every clinical strategy should be tailored to the patients' specific disease. In this context, it is expected that the proteomic strategies support the genomic-based approach for disease profiling. As previously mentioned, the lack of effective clinical strategies in achieving early diagnosis has created an increasing interest in proteomic approaches. In particular, genomic-based profiling is certainly useful to characterize the pattern of gene expression in cancer cells, but the functional role of a specific gene product can be definitely assessed only by focusing on the proteins level. For these reasons, there is a great expectation on the potential benefits in terms of accurate disease characterization that can be achieved with the advent of the proteomic era. In this context, proteomic analysis includes several different strategies, including protein structural identification, quantification of protein levels, description of protein–protein interaction, posttranslational modifications, and functional analysis. Proteomics has greatly advanced from initial gel-based procedures (one- and two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis) to mass spectrometry-based (MS) methods. In particular, innovative

approaches such as electrospray ionization-MS and matrix-assisted laser desorption/ionization (MALDI)-MS are emerging as reliable strategies to achieve an accurate and reliable protein profiling in oncology. The availability of quantitative methods that are able to identify deregulated protein expression represents a further step toward future use of proteomic platforms for disease characterization in patients with OC. The aim of this chapter is to briefly give an overview of the current knowledge on investigated proteomic biomarkers in OC.

6.2 Proteomics and Ovarian Cancer

6.2.1 Ovarian Cancer Cell Lines and Tumor Tissues

OC cell lines traditionally represent the first step of preclinical cancer research. These experimental models enable the investigation of biological mechanisms sustaining proliferation and development of metastatic potential as well as the characterization of gene and protein expression. On the other hand, recent evidence has clearly demonstrated that several OC cell lines are characterized by a hypermutated genotype, which is frequently very different from OC tissues retrieved from tumor biopsies (Domcke et al. 2013). For these reasons, the results obtained from preclinical *in vitro* models should be always considered with great caution, and *in vivo* validation is mandatory. Focusing on proteomic profiling of OC cell lines, several interesting data have been published suggesting that specific protein panels may be involved in driving drug resistance (Agarwal and Kaye 2003; Li et al. 2010; Chappell et al. 2012; Chen et al. 2014). In particular, a study conducted by Li et al. identified a panel of 28 proteins in several cancer cell lines involved in the development of cisplatin resistance (Li et al. 2010). These potential biomarkers were classified into eight functional groups: calcium-binding proteins, chaperones, extracellular matrix, DNA damage repair complex, mitochondrial proteins, transcription factor, cytoskeletal proteins, and signaling transducing factors (Li et al. 2010). Unfortunately, these interesting preliminary data were not validated in patients' samples. The complete proteomic profiling of tumor tissues is certainly a very. However, it is well known that formalin tissue fixation produces cross-links among proteins on cancer tissues; thus, masking epitopes in proteomic characterization. Furthermore, surgical contamination and tumor disease heterogeneity are also other potential pitfalls. On the other hand, the availability of novel techniques for protein extraction, together with improvement of quantitative proteomic strategies allow a reliable proteomic characterization even on formalin-fixed embedded protein (FFPE) blocks. Few studies that investigated the differences in terms of proteomic profiles in OC tumor histotypes have been published. A specific proteomic profile has been suggested for high-grade serous histology (An et al. 2006). Notably, the most relevant findings have been reported by Wiegand et al. which identified 50 proteins differentially expressed in clear cell and endometrioid OC as compared with high-grade serous histology (Wiegand et al. 2014). In particular, this study found a specific biological mechanism at a proteomic level that is probably involved

in tumor development for both clear-cell and endometrioid OC. In fact, the authors detected increased levels of phosphorylated AKT protein in tumor tissues, together with a reduced expression of BAF250a; this protein acts as tumor suppressor promoting apoptotic cascade. It can be hypothesized that in the process of endometrioid and clear-cell carcinogenesis, phosphorylation of AKT protein occurs as an early event, and in turn suppresses BAF250a expression at the genomic level (Wiegand et al. 2014). Awaiting further experimental confirmations, these data represent a relevant contribution of proteomic tissue characterization for early diagnosis and disease profiling of OC (Wiegand et al. 2014). Furthermore, the experimental evidence showing a relevant biological role of phosphorylated AKT protein in OC introduced another crucial point of proteomic tissue characterization which is represented by the identification of posttranscriptional modifications. In fact, it is well known that the biological processes such as glycosylation or phosphorylation may produce activation, or silencing of a protein function, and these relevant biological mechanisms can be detected only through proteomic analysis, and not using a traditional genomic approach. A plethora of studies have been published and showed the relevance of phosphorylated protein isoforms in driving tumor angiogenesis, apoptosis blockade, epithelial-to-mesenchymal transition, and chemoresistance through the activation of several pathways including NF κ B, mitogen-activated protein kinase (MAPK), Src, and PI3K (Elzek and Rodland 2015). Unfortunately, despite the important amount of literature suggesting, and clearly demonstrating the role of these phosphorylated proteins in cancer development, none of these molecules have successfully entered into clinical practice as diagnostic biomarkers. One of the potential reasons to explain this contrasting scenario is the lack of proteomic data confirming at the protein level the above-mentioned findings that have been identified only at the genomic level.

6.2.2 Proteomic Plasma Analysis

Serum derived from cancer patients certainly represents the most appropriate sample to be used for proteomic characterization. Compared with tumor tissues, serological samples can be easily achieved, and during sampling, it can avoid contamination using an appropriate protocol for collection and early processing (Fig. 6.1). Furthermore, compared with FFPE blocks, no fixation is required, and tumor tissue is not manipulated; thus, avoiding cross-links between proteins. On the other hand, the number of tumor-derived proteins released in the blood is very low. Therefore, it is not surprising that only with the availability of innovative quantitatively spectroscopic techniques such as SELDI-TOF that we were able to correctly identify tumor-derived circulating proteins.

In OC, a relevant proteomic serological profiling has been conducted by Zhang et al. that showed that a panel of circulating proteins has been found to be differentially expressed in OC patients as compared with healthy subjects, thus, allowing the earliest proteomic-based strategy for early diagnosis (Zhang et al. 2004). These results have been further evaluated to develop a five proteins algorithm, called

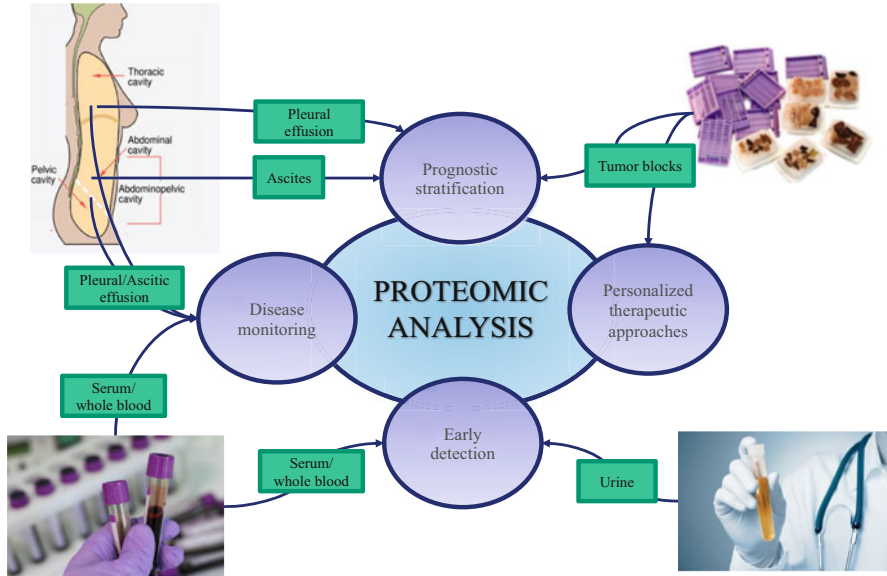


Fig. 6.1 The proteomic approach in ovarian cancer

OVA1 test, based on the combined dosage of apolipoprotein A1, prealbumin, transferrin, β -2 microglobulin, and CA-125 (Ueland et al. 2011). The OVA1 assay also received class II FDA approval to be used in combination with ultrasound evaluation for the triage of suspicious pelvic mass. Unfortunately, premarketing approval is still needed despite these interesting data. Definitive clinical data are still omitted, but these results, for the first time, opened the route for serological proteomic profiling that is able to increase the diagnostic performance of CA-125 alone in the detection and stratification of OC patients (Fig. 6.2). Based on proteomic profiling of TCGA samples, Yang et al. showed a high-throughput protein profiling which allowed the identification of an algorithm of nine proteins called PROVAR which is able to predict disease progression in OC (Yang et al. 2013). Again, also for the PROVAR test, a clinical validation has not been performed, thus not allowing a safe translation from laboratory to clinical practice. Another experimental approach for proteomic profiling of OC patients is represented by the combined evaluation of blood and tumor samples. This strategy is of great value to correctly identify tumor-derived proteins that may be involved not only in carcinogenesis, but also in the development of drug resistance. An interesting study based on this approach showed a statistically significant lower expression of APOA1 and serotransferrin in both serum and cancer tissue samples of OC patients compared with healthy subjects (Wegdum et al. 2014), thus providing a partial confirmation of the Zhang's findings (Zhang et al. 2004). Another emerging scientific field is represented by the so-called circulating *secretomes* or secretomics which analyzes the secreted extracellular proteins in the blood (Madden et al. 2020). Circulating extracellular proteins in the

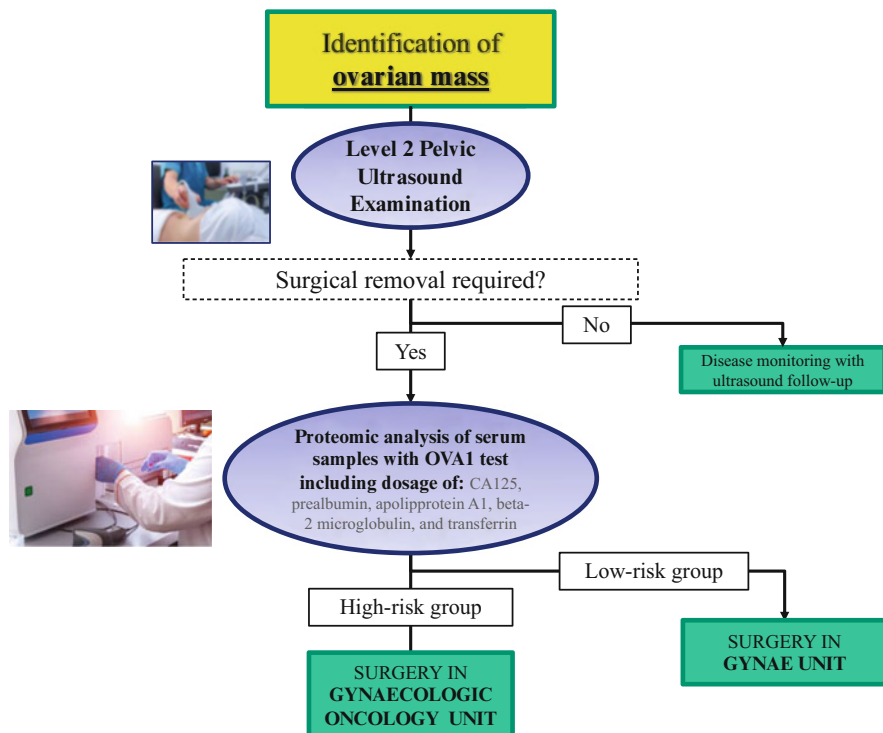


Fig. 6.2 Integration of proteomics in the diagnostic algorithms for early identification of epithelial ovarian cancer

blood are glycosylated which makes them suitable for proteomic biomarker discovery. Interestingly, several previous studies used this approach (Tian et al. 2011; Pan et al. 2011; Faca et al. 2008; Gunawardana et al. 2009). In conclusion, the profiling of circulating proteins appears as a promising field for the identification of biomarkers for the diagnosis and stratification of OC patients.

6.2.3 Proteomic Analysis of Ascitic and Pleural Effusions

The vast majority of OC patients develop ascites along with their disease natural history. Unfortunately, this event is related to peritoneal cancer spread, and it is obviously associated with late FIGO stages. Therefore, ascitic fluids are certainly a relevant source for biomarkers development and their proteomic profiling may be of great value to study the mechanisms of disease spread, and patients' prognostic stratification. However, ascitic samples cannot be used for early disease detection. Interestingly, a complete proteomic profiling of ascites from OC patients revealed a panel of 50 differentially expressed proteins (Gortzak-Uzan et al. 2008; Kuk et al. 2009). However, as described in Table 6.1, these studies do not have a potential

Table 6.1 Proteomic analysis in ovarian cancer patients: Comparison of different biological sources

| | Biological source | | |
|--------------------------------|---|--|---|
| | Tumor tissue | Plasma | Ascitic and pleural effusions |
| Samples collection | <ul style="list-style-type: none"> • Need of invasive procedure • High risks of contamination | <ul style="list-style-type: none"> • Easy • Low risks of contamination | <ul style="list-style-type: none"> • Need of invasive procedure • Low risks of contamination |
| Technical aspects | <ul style="list-style-type: none"> • High amount of tumor-derived proteins • Need of accurate microdissection to reduce contaminations bias • Epitopes masking: potential concerns in detecting specific protein profiles due to formalin fixation | <ul style="list-style-type: none"> • Low amount of tumor-derived proteins: need of high sensitivity proteomic strategies | <ul style="list-style-type: none"> • High amount of tumor-derived proteins |
| Clinical role and implications | <ul style="list-style-type: none"> <i>Early disease diagnosis</i> • Limited value <i>Disease stratification</i> • Potentially relevant value | <ul style="list-style-type: none"> <i>Early disease diagnosis</i> • Great value <i>Disease stratification</i> • Great value, particularly when combined with tumor, and ascitic fluids evaluation | <ul style="list-style-type: none"> <i>Early disease diagnosis</i> • No value <i>Disease stratification</i> • Potentially relevant value |
| Scientific evidence | <ul style="list-style-type: none"> <i>Early disease diagnosis</i> • Limited evidences for clinical translation <i>Disease stratification</i> • Limited evidences for clinical translation | <ul style="list-style-type: none"> <i>Early disease diagnosis</i> • FDA approved panel to be further validated in a clinical scenario • Relevant evidences on circulating extracellular vesicles ready to be validated in clinical scenarios <i>Disease stratification</i> • Limited evidences for clinical translation | <ul style="list-style-type: none"> <i>Early disease diagnosis</i> • Limited evidences for clinical translation <i>Disease stratification</i> • Limited evidences for clinical translation |

clinical horizon as this approach has no clinical value for performing proteomic profiling of ascitic fluids to achieve early disease detection. On the other hand, the role of proteomic profiling of pleural effusion in the prognostic stratification of OC patients seems to be promising (Davidson et al. 2006; reviewed elsewhere: El Bairi et al. 2017; Carvalho et al. 2019). Reduced survival was seen in patients with increased levels of AKT, and JNK proteins; thus, another opportunity for further clinical validation of these biomarkers for prognostic disease stratification (Davidson et al. 2006).

6.3 Proteomics and Extracellular Vesicles: A Promising Approach in Ovarian Cancer

In the last decade, the role of extracellular vesicles and their cargoes as diagnostic, prognostic, and predictive biomarkers have been widely studied in cancer (Srivastava et al. 2021; Amintas et al. 2021). Extracellular vesicles (EVs) are divided into three types based on their size: exosomes (30–100 nm), microvesicles (100 nm–1 μ m), and apoptotic bodies (500 nm–3 μ m). Regarding their functional features, exosomes seem to play a crucial role in regulating several biological mechanisms involved in cancer growth, and metastatic development, acting as mediators of cellular crosstalk in cancer tissue (Elewaily and Elsergany 2021). Exosomes can contain a complex cargo of materials, including microRNAs and occasionally genomic DNA. The vast majority of miRNAs circulates in body fluids of patients as cell-free RNAs, and for these reasons, they have been considered for several years as potential biomarkers to be used in liquid biopsy approaches. However, circulating miRNAs are quickly removed by enzymatic RNase activity. Therefore, these biomarkers do not appear as easily manageable diagnostic tools to be used in screening diagnostic tools. On the other hand, circulating miRNAs embedded in tumor-derived EVs are certainly more stable, and easier to be used in diagnostic algorithms, particularly considering that some specific miRNAs panels are differentially expressed in OC patients compared to healthy women (Mahdian-Shakib et al. 2016; Montagnana et al. 2017). Finally, EVs are easily identifiable in various body fluids, such as blood, serum, and urine, making them reliable markers that are easy to find and potentially very useful in clinical practice. Recently, Barnabas et al. conducted a proteomic analysis of EVs-related proteins in utero-tubal lavage from healthy women, and OC patients and showed a panel of nine proteins (SERPINB5, S100A14, MYH11, CLCA4, S100A2, IVL, CD109, NNMT, ENPP3) that were differentially expressed in the two groups, and involved in regulating kinase activity, cellular motility, and apoptosis modulating p53 pathway (Barnabas et al. 2019). Unfortunately, the diagnostic performance of these proteomic biomarkers in the early detection of OC was around 75%, being therefore promising, but still not adequate for clinical use (Barnabas et al. 2019). Furthermore, as previously mentioned, proteomic profiling of ascites and pleural effusion may be certainly regarded as a potentially useful tool to achieve final diagnosis. In particular, the evaluation of EVs embedded miRNAs, and proteins may be certainly regarded as a very interesting approach with a panel of proteins (NANOG, SPINT2, and ZEB2), and miRNAs (miR-29a, miR-30d, and miR-205) differentially expressed in OC patients and healthy women (Yamamoto et al. 2018). However, this experimental approach appears very questionable, since ascitic fluids, which appear in women with late-stage disease, do not represent a useful biological sample to be used for early diagnosis. For, these reasons, the studies comparing the proteomic profile of ascitic fluids in OC patients and healthy controls do not have the appropriate design to provide clinically useful insights. Interestingly, a previous report failed to identify differences in terms of proteomic profile between OC patients and healthy subjects (Zhao et al. 2014). However, when focusing only on women with an advanced stage

of disease, a higher level of circulating HSP27-related EVs in patients with peritoneal carcinomatosis was noticed (Zhao et al. 2014). Thus, again highlighting the need to focus scientific efforts on specific subgroups of OC patients in future biomarker research. Another crucial point is represented by the potential role of EVs proteomic profiling for early identification of chemoresistance. A recently published study by Guerra et al. showed a correlation between reduced circulating levels of EVs-embedded RAB7A protein and the development of cisplatin resistance (Guerra et al. 2019). Furthermore, poor drug response is related to several complex biological mechanisms involving also epithelial-to-mesenchymal transition which is principally based on cytoskeletal and extracellular matrix modifications. Therefore, it is not surprising that the recently published data showed increased levels of EVs-embedded matrix metalloproteinase 1 (MMP1) in peritoneal lesions with intrinsic chemoresistant features. Furthermore, the overexpression of circulating EVs-derived MMP1 was found to be associated with reduced overall and progression-free survival in women with OC. In conclusion, the proteomic profile of circulating EVs appears as a promising field for future developments for early diagnosis and prognostic stratification of OC patients.

6.4 Future Perspectives: A Focus on microRNAs

Quantitative proteomic profiling techniques extended the horizon of proteomics by assessing several other biomarkers beyond proteins such as miRNAs. Deregulation of mi-RNAs expression has been shown to be associated with malignant development of OC. Therefore, quantitative proteomic assessment of miRNAs expression patterns represents a further approach to improve early detection of OC. Previously, Taylor et al. reported that eight circulating exosomal miRNAs (miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-205, and miR-214) are overexpressed in OC patients compared to benign controls (Taylor and Gercel-Taylor 2008). Similarly, another report showed that the expression levels of four serum miRNAs (miR-182, miR-200a, miR-200b, and miR-200c) were significantly elevated in women with high-grade serous OC as compared with healthy controls (Kan et al. 2012). Moreover, serum levels of miR-25 and miR-93 were found downregulated, while miR-7 and miR-429 were found upregulated in OC patients compared with healthy women (Meng et al. 2015). This suggests that the differential expression of some selected miRNAs can be used as biomarkers.

The role of miRNAs isolated from serum, tissue, and ascites was analyzed by Chung et al. and identified five miRNAs (miR-132, miR-26a, let-7b, miR-145, and miR-143) as the most significantly downregulated miRNAs in the sera of OC patients (Chung et al. 2013). Moreover, Zhou et al. investigated the diagnostic value of urinary miRNAs in OC patients and identified a significant upregulation of miR-30a-5p in the urine samples of women with OC when compared to healthy controls (Zhou et al. 2015). The miRNA signatures from exosomes were concordant to those from the originating tumor cells, indicating that circulating miRNAs profiles accurately reflect the tumor profile. Furthermore, Zheng et al. evaluated plasma

samples of 360 OC patients and 200 healthy controls, and they found a higher expression of plasma miR-205 and lower expression of let 7-f in OC patients (Zheng et al. 2013). The authors were able to propose a combination of mir-205 and let-7f to provide high diagnostic accuracy (Nakamura et al. 2016; Zheng et al. 2013). Similarly, Zuberi et al. showed that miR-200a was significantly upregulated in mucinous adenocarcinoma when compared with histotypes in 70 OC patients (Zuberi et al. 2015). Another interesting experience has been recently published evaluating the differences in terms of circulating EVs derived miRNAs between OC patients and healthy controls (Chi Pan et al. 2018). A specific panel of miRNAs (miR-23a, miR-92a, miR-21, miR-100, and miR-200b, miR-320, miR-16, miR-93, miR-126, and miR-223) was identified as potentially useful diagnostic biomarkers, but the overall discriminating performance was indecisive being below 85%, thus not allowing a further clinical validation. Very interesting results have been reported in 2017 by Yokoi et al., which demonstrated that a combination of eight circulating serum miRNAs (miR-142-3p, miR-26a-5p, let7d-5p, miR-374a-5p, miR-766-3p, miR-200a-3p, miR-328-3p, and miR-130b-3p) was able to successfully discriminate OC patients from healthy controls with remarkable diagnostic performances at ROC analysis (AUC 0.97; sensitivity 0.92 and specificity 0.91) (Yokoi et al. 2017a, b). The eight miRNAs classification model had a different AUC, sensitivity, and specificity for the different histological types of OC, thus emphasizing the need to identify histology-based diagnostic models (Yokoi et al. 2017a, b). In addition, in the same study, the authors developed a predictive algorithm able to differentiate early-stage OC from benign tumor using seven mi-RNAs (miR-200a-3p, miR-766-3p, miR-26a-5p, miR-142-3p, let-7d-5p, miR-130b-3p, and miR-328-3p) (Yokoi et al. 2017a, b). In this model, the diagnostic performance appeared promising with an AUC of 0.92, but the sensitivity and specificity were lower being 0.861, and 0.833, respectively (Yokoi et al. 2017a, b). Similarly, Yoshimura et al. identified circulating EVs embedded miR-99a-5p as a potentially useful diagnostic tool for early detection of OC patients (Yoshimura et al. 2018). Furthermore, a quantitative proteomic approach detected a relevant reduction of circulating miR-99a-5p after cytoreductive surgery, thus suggesting that this biomarker may be used for disease monitoring. Unfortunately, the diagnostic performances were always below 85% with relevant differences according to tumor histotypes, and specificity for detecting clear cell and mucinous OC above 90%. It should be acknowledged that the results of this study do not support the use of this miRNA in clinical setting; however, this is the first well-conducted experimental approach that stratified prognostic and diagnostic performances of specific proteomic profiles according to tumor histology (Yoshimura et al. 2018), which certainly support this approach to be furtherly developed. In case of endometriosis-associated OC, Suryawanshi et al. found that three plasma miRNAs (miR-16, miR-191, and miR-195) are overexpressed in peritoneal endometriotic lesions and discriminated between healthy subjects and patients with deep infiltrating endometriosis (sensitivity and specificity of 88% and 60%, respectively) (Suryawanshi et al. 2013). Kobayashi et al. showed that serum miR-1290 is significantly increased in patients with high-grade serous OC, and it can be used to early identify these patients (Kobayashi et al. 2018). In particular, this

study demonstrated that CA-125 retains a better performance to early identify the OC patients as compared with miR-1290 serum levels. However, the assessment of miR-1290 serum levels showed better performance as compared to CA-125 in discriminating high-grade serous OC patients from women with non-serous ovarian malignancies. Furthermore, the authors compared the levels of miR-1290 before and after the primary debulking surgery and suggested that serum miR-1290 reflects tumor burden, which may help disease monitoring (Kobayashi et al. 2018). Similarly, in a cohort of 56 high-grade serous OC patients, Shah et al. showed that the combination of miR-375 and CA-125 was the strongest discriminator of healthy versus high-grade serous OC patients, and that the combination of miR-34a-5p and CA-125 was the strongest predictor of complete surgical debulking (Shah et al. 2018). In addition, the role of the EVs derived miRNAs have been studied also in terms of prognosis because of their implication in the development of drug resistance in OC patients. In particular, increased circulating levels of annexin A3 (Yin et al. 2012) together with a panel of miRNAs including miR-181a, miR-1908, miR-21, miR-486, and miR-223 were identified as markers of platinum-resistance in women with OC, thus suggesting a potential clinically relevant role for these biomarkers (Kuhlmann et al. 2019). To date, this approach using microRNAs and other liquid biopsy components is under investigation in several human studies but the current evidence is not mature yet for clinical use.

6.5 Conclusion

In the last decade, quantitative proteomic approaches have been used as a promising tool to be used in clinical practice. In particular, compelling evidence seems to support the role of a panel of proteins and circulating microRNAs as reliable biomarkers to achieve early diagnosis and accurate prognostic stratification of OC patients. On the other hand, despite a plethora of experimental data suggesting potential diagnostic and prognostic proteomic profiles, only a few reports have entered clinical evaluation, with contrasting results, thus producing an impressive gap between preclinical evidences, and clinical findings. Therefore, there is an urgent need to design clinically focused studies with an immediate reliable translation into clinical practice. The combination of proteomic profiles, serum CA-125 levels, *BRCA* gene status, and ultrasound examination appears as the most promising strategy. For further reading, see Box 6.1.

Box 6.1 Overview of recommended articles providing relevant scientific insights on this specific issue

| Recommended reading of particular interest | DOI |
|--|---|
| Macklin A, et al. Recent advances in mass spectrometry based clinical proteomics: applications to cancer research. <i>Clin Proteomics.</i> 2020;17:17. | https://doi.org/10.1186/s12014-020-09283-w |
| Sobsey CA, et al. Targeted and Untargeted Proteomics Approaches in Biomarker Development. <i>Proteomics.</i> 2020;20(9):e1900029. | https://doi.org/10.1002/pmic.201900029 |
| Bonifácio VDB. Ovarian Cancer Biomarkers: Moving Forward in Early Detection. <i>Adv Exp Med Biol.</i> 2020;1219:355–363. | https://doi.org/10.1007/978-3-030-34025-4_18 |
| He Y, et al. Oncoproteomics: Current status and future opportunities. <i>Clin Chim Acta.</i> 2019;495:611–624. | https://doi.org/10.1016/j.cca.2019.06.006 |
| Srivastava A, Creek DJ. Discovery and Validation of Clinical Biomarkers of Cancer: A Review Combining Metabolomics and Proteomics. <i>Proteomics.</i> 2019;19(10):e1700448. | https://doi.org/10.1002/pmic.201700448 |
| Carvalho VP, et al. The contribution and perspectives of proteomics to uncover ovarian cancer tumor markers. <i>Transl Res.</i> 2019;206:71–90. | https://doi.org/10.1016/j.trsl.2018.11.001 |
| Forshed J. Experimental Design in Clinical 'Omics Biomarker Discovery. <i>J Proteome Res.</i> 2017;16(11):3954–3960. | https://doi.org/10.1021/acs.jproteome.7b00418 |
| Huang Y, Zhu H. Protein Array-based Approaches for Biomarker Discovery in Cancer. <i>Genomics Proteomics Bioinformatics.</i> 2017;15(2):73–81. | https://doi.org/10.1016/j.gpb.2017.03.001 |
| Bonifácio VDB. Ovarian Cancer Biomarkers: Moving Forward in Early Detection. <i>Adv Exp Med Biol.</i> 2020;1219:355–363. | https://doi.org/10.1007/978-3-030-34025-4_18 |
| Labrie M, et al. Proteomics advances for precision therapy in ovarian cancer. <i>Expert Rev Proteomics.</i> 2019;16(10):841–850. | https://doi.org/10.1080/14789450.2019.1666004 |
| El Bairi K, et al. Prediction of therapy response in ovarian cancer: Where are we now? <i>Crit Rev Clin Lab Sci.</i> 2017;54(4):233–266. | https://doi.org/10.1080/10408363.2017.1313190 |

Acknowledgment and Conflicts of Interest The authors declare they have no conflict of interest. The final draft was reviewed and approved by all the authors. The contents of the chapter reflect the authors' perspectives and not of their institutions of affiliation.

Authors' Contributions MP, CR, DC, MD wrote the chapter. SD supervised the writing process.

References

- Agarwal R, Kaye SB (2003) Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer* 3(7):502–516. <https://doi.org/10.1038/nrc1123>
- Amintas S, Vendrey V, Dupin C, Buscail L, Laurent C, Bournet B, Merlio JP, Bedel A, Moreau-Gaudry F, Boutin J, Dabernat S, Buscail E (2021) Next-generation cancer biomarkers: extracellular vesicle DNA as a circulating surrogate of tumor DNA. *Front Cell Dev Biol* 8:622048. <https://doi.org/10.3389/fcell.2020.622048>
- An HJ, Kim DS, Park YK et al (2006) Comparative proteomics of ovarian epithelial tumors. *J Proteome Res* 5(5):1082–1090. <https://doi.org/10.1021/pr050461p>
- Barnabas G, Bahar-Shany K, Sapoznik S et al (2019) Microvesicle proteomic profiling of uterine liquid biopsy for ovarian cancer early detection. *Mol Cell Proteomics* 18(5):865–875. <https://doi.org/10.1074/mcp.RA119.001362>
- Carvalho VP, Grassi ML, Palma CS, Carrara HHA, Faça VM, Candido Dos Reis FJ (2019) Poersch a. the contribution and perspectives of proteomics to uncover ovarian cancer tumor markers. *Transl Res* 206:71–90. <https://doi.org/10.1016/j.trsl.2018.11.001>
- Chappell NP, Teng P, Hood BL et al (2012) Mitochondrial proteomic analysis of cisplatin resistance in ovarian cancer. *J Proteome Res* 11(9):4605–4614. <https://doi.org/10.1021/pr300403d>
- Chen X, Wei S, Ma Y et al (2014) Quantitative proteomics analysis identifies mitochondria as therapeutic targets of multidrug-resistance in ovarian cancer. *Theranostic* 4(12):1164–1175. <https://doi.org/10.7150/thno.8502.eCollection.2014>
- Chung YW, Bae HS, Song JY et al (2013) Detection of microRNA as novel biomarkers of epithelial ovarian cancer from the serum of ovarian cancer patients. *Int J Gynecol Cancer* 23(4):673–679. <https://doi.org/10.1097/IGC.0b013e31828c166d>
- Davidson B, Espina V, Steinberg SM et al (2006) Proteomic analysis of malignant ovarian cancer effusions as a tool for biologic and prognostic profiling. *Clin Cancer Res* 12(3 Pt 1):791–799. <https://doi.org/10.1158/1078-0432.CCR-05-2516>
- Domcke S, Sinha R, Levine DA, Sander C, Schultz N (2013) Evaluating cell lines as tumor models by comparison of genomic profiles. *Nat Commun* 4:2126. <https://doi.org/10.1038/ncomms3126>
- El Bairi K, Kandhro AH, Gouri A, Mahfoud W, Louanjli N, Saadani B, Afqir S, Amrani M (2017) Emerging diagnostic, prognostic and therapeutic biomarkers for ovarian cancer. *Cell Oncol (Dordr)* 40(2):105–118. <https://doi.org/10.1007/s13402-016-0309-1>
- El Bairi K, Afqir S, Amrani M (2020) Is HE4 superior over CA-125 in the follow-up of patients with epithelial ovarian cancer? *Curr Drug Targets* 21(10):1026–1033. <https://doi.org/10.2174/1389450121666200425211732>
- Elwailly MI, Elsergany AR (2021) Emerging role of exosomes and exosomal microRNA in cancer: pathophysiology and clinical potential. *J Cancer Res Clin Oncol* 147(3):637–648. <https://doi.org/10.1007/s00432-021-03534-5>
- Elstrand MB, Sandstad B, Oksefjell H, Davidson B, Tropé CG (2012) Prognostic significance of residual tumor in patients with epithelial ovarian cancer stage IV in a 20 year perspective. *Acta Obstet Gynecol Scand* 91(3):308–317. <https://doi.org/10.1111/j.1600-0412.2011.01316.x>
- Elzek MA, Rodland KD (2015) Proteomics of ovarian cancer: functional insights and clinical applications. *Cancer Metastasis Rev* 34(1):83–96. <https://doi.org/10.1007/s10555-014-9547-8>
- Faca VM, Ventura AP, Fitzgibbon MP, Pereira-Faça SR, Pitteri SJ, Green AE et al (2008) Proteomic analysis of ovarian cancer cells reveals dynamic processes of protein secretion and shedding of extra-cellular domains. *PLoS One* 3(6):e2425. <https://doi.org/10.1371/journal.pone.0002425>
- Gortzak-Uzan L, Ignatchenko A, Evangelou AI, Agochiya M, Brown KA, St Onge P et al (2008) A proteome resource of ovarian cancer ascites; integrated proteomic and bioinformatic analyses to identify putative biomarkers. *J Proteome Res* 7(1):339–351. <https://doi.org/10.1021/pr0703223>
- Guerra F, Paiano A, Migoni D, Girolimetti G, Perrone AM, De Iaco P et al (2019) Protein expression determines resistance to cisplatin through late endocytic pathway impairment and

- extracellular vesicular secretion. *Cancers (Basel)* 11(1):52. <https://doi.org/10.3390/cancers11010052>
- Gunawardana CG, Kuk C, Smith CR, Batruch I, Soosaipillai A, Diamandis EP (2009) Comprehensive analysis of conditioned media from ovarian cancer cell lines identifies novel candidate markers of epithelial ovarian cancer. *J Proteome Res* 8(10):4705–4713. <https://doi.org/10.1021/pr900411g>
- Kan CW, Hahn MA, Gard GB et al (2012) Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer. *BMC Cancer* 12:627. <https://doi.org/10.1186/1471-2407-12-627>
- Kobayashi M, Sawada K, Nakamura K et al (2018) Exosomal miR-1290 is a potential biomarker of high-grade serous ovarian carcinoma and can discriminate patients from those with malignancies of other histological types. *J Ovarian Res* 11(1):81. <https://doi.org/10.1186/s13048-018-0458-0>
- Kuhlmann JD, Chebouti I, Kimmig R et al (2019) Extracellular vesicle-associated miRNAs in ovarian cancer-design of an integrated NGS- based workflow for the identification of blood-based biomarkers for platinum-resistance. *Clin Chem Lab Med* 57(7):1053–1062. <https://doi.org/10.1515/cclm-2018-1048>
- Kuk C, Kulasingam V, Gunawardana CG, Smith CR, Batruch I, Diamandis EP (2009) Mining the ovarian cancer ascites proteome for potential ovarian cancer biomarkers. *Mol Cell Proteomics* 8(4):661–669. <https://doi.org/10.1074/mcp.M800313-MCP200>
- Leung F, Bernardini MQ, Brown MD et al (2016) Validation of a novel biomarker panel for the detection of ovarian cancer. *Cancer Epidemiol Biomark Prev* 25(9):1333–1340. <https://doi.org/10.1158/1055-9965.EPI-15-1299>
- Li S-L, Ye F, Cai WJ et al (2010) Quantitative proteome analysis of multidrug resistance in human ovarian cancer cell line. *J Cell Biochem* 109(4):625–633. <https://doi.org/10.1002/jcb.22413>
- Madden EC, Gorman AM, Logue SE, Samali A (2020) Tumour cell secretome in chemoresistance and tumour recurrence. *Trends Cancer* 6(6):489–505. <https://doi.org/10.1016/j.trecan.2020.02.020>
- Mahdian-Shakib A, Dorostkar R, Tat M, Hashemzadeh MS, Saidi N (2016) Differential role of microRNAs in prognosis, diagnosis, and therapy of ovarian cancer. *Biomed Pharmacother* 84:592–600. <https://doi.org/10.1016/j.biopha.2016.09.087>
- Meng X, Joosse SA, Müller V et al (2015) Diagnostic and prognostic potential of serum miR-7, miR-16, miR-25, miR-93, miR-182, miR-376a and miR-429 in ovarian cancer patients. *Br J Cancer* 113(9):1358–1366. <https://doi.org/10.1038/bjc.2015.340>
- Montagnana M, Lippi G, Ruzzenente O et al (2009) The utility of serum human epididymis protein 4 (HE4) in patients with a pelvic mass. *J Clin Lab Anal* 23(5):331–335. <https://doi.org/10.1002/jcla.20340>
- Montagnana M, Benati M, Danese E (2017) Circulating biomarkers in epithelial ovarian cancer diagnosis: from present to future perspective. *Ann Transl Med* 5(13):276. <https://doi.org/10.21037/atm.2017.05.13>
- Moore RG, Brown AK, Miller MC et al (2008) The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol Oncol* 108(2):402–408. <https://doi.org/10.1016/j.ygyno.2007.10.017>
- Nakamura K, Sawada K, Yoshimura A, Kinose Y, Nakatsuka E, Kimura T (2016) Clinical relevance of circulating cell-free microRNAs in ovarian cancer. *Mol Cancer* 15(1):48. <https://doi.org/10.1186/s12943-016-0536-0>
- Pan S, Chen R, Aebersold R, Brentnall TA (2011) Mass spectrometry based glycoproteomics- from a proteomics perspective. *Mol Cell Proteomics* 10(1):R110.003251. <https://doi.org/10.1074/mcp.R110.003251>
- Pan C, Stevic I, Muller V et al (2018) Exosomal microRNAs as tumor markers in epithelial ovarian cancer. *Mol Oncol* 12(11):1935–1948. <https://doi.org/10.1002/1878-0261.12371>

- Petrillo M, Nero C, Amadio G, Gallo D, Fagotti A, Scambia G (2016) Targeting the hallmarks of ovarian cancer: the big picture. *Gynecol Oncol* 142(1):176–183. <https://doi.org/10.1016/j.ygyno.2016.03.037>
- Shah JS, Gard GB, Yang J et al (2018) Combining serum microRNA and ca-125 as prognostic indicators of preoperative surgical outcome in women with high-grade serous ovarian cancer. *Gynecol Oncol* 148(1):181–188. <https://doi.org/10.1016/j.ygyno.2017.11.005>
- Srivastava A, Rathore S, Munshi A, Ramesh R (2021) Extracellular vesicles in oncology: from immune suppression to immunotherapy. *AAPS J* 23(2):30. <https://doi.org/10.1208/s12248-021-00554-4>
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71(3):209–249. <https://doi.org/10.3322/caac.21660>
- Suryawanshi S, Huang X, Elishaev E et al (2013) Plasma microRNAs as novel biomarkers for endometriosis and endometriosis-associated ovarian cancer. *Clin Cancer Res* 19(5):1213–1224. <https://doi.org/10.1158/1078-0432.CCR-12-2726>
- Taylor DD, Gercel-Taylor C (2008) MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol* 110(1):13–21. <https://doi.org/10.1016/j.ygyno.2008.04.033>
- Tian Y, Yao Z, Roden RB, Zhang H (2011) Identification of glycoproteins associated with different histological subtypes of ovarian tumors using quantitative glycoproteomics. *Proteomics* 11(24):4677–4687. <https://doi.org/10.1002/pmic.201000811>
- Ueland FR, Desimone CP, Seamon LG et al (2011) Effectiveness of a multivariate index assay in the preoperative assessment of ovarian tumors. *Obstet Gynecol* 117(6):1289–1297. <https://doi.org/10.1097/AOG.0b013e31821b5118>
- Wegdam W, Argmann CA, Kramer G, Vissers JP, Buist MR, Kenter GG, Aerts JM, Meijer D, Moerland PD (2014) Label-free LC-MSE in tissue and serum reveals protein networks underlying differences between benign and malignant serous ovarian tumors. *PLoS One* 9(9):e108046. <https://doi.org/10.1371/journal.pone.0108046>
- Wiegand KC, Hennessy BT, Leung S et al (2014) A functional proteogenomic analysis of endometrioid and clear cell carcinomas using reserve phase protein array and mutation analysis: protein expression is histotype-specific and loss of ARID1A/BAF250a is associated with AKT phosphorylation. *BMC Cancer* 14:120. <https://doi.org/10.1186/1471-2407-14-120>
- Yamamoto CM, Oakes ML, Murakami T, Muto MG, Berkowitz RS, Ng SW (2018) Comparison of benign peritoneal fluid-and ovarian cancer ascites-derived extracellular vesicle RNA biomarkers. *J Ovarian Res* 11(1):20. <https://doi.org/10.1186/s13048-018-0391-2>
- Yang JY, Yoshihara K, Tanaka K et al (2013) Predicting time to ovarian carcinoma recurrence using protein markers. *J Clin Invest* 123(9):3740–3750. <https://doi.org/10.1172/JCI68509>
- Yin J, Yan X, Yao X et al (2012) Secretion of annexin A3 from ovarian cancer cells and its association with platinum resistance in ovarian cancer patients. *J Cell Mol Med* 16(2):337–348. <https://doi.org/10.1111/j.1582-4934.2011.01316.x>
- Yokoi A, Yoshioka Y, Hirakawa A et al (2017a) A combination of circulating miRNAs for early detection of ovarian cancer. *Oncotarget* 8(52):89811–89823. <https://doi.org/10.18632/oncotarget.20688>
- Yokoi A, Yoshioka Y, Yamamoto Y et al (2017b) Malignant extracellular vesicles carrying MMP1 mRNA facilitate peritoneal dissemination in ovarian cancer. *Nat Commun* 8:14470. <https://doi.org/10.1038/ncomms14470>
- Yoshimura A, Sawada K, Nakamura K et al (2018) Exosomal miR-99a-5p is elevated in sera of ovarian cancer patients and promotes cancer cell invasion by increasing fibronectin and vitronectin expression in neighboring peritoneal mesothelial cells. *BMC Cancer* 18(1):1065. <https://doi.org/10.1186/s12885-018-4974-5>

- Zhang Z, Bast RC Jr, Yu Y et al (2004) Three biomarkers identified from serum proteomic analysis for detection of early stage ovarian cancer. *Cancer Res* 64(16):5882–5890. <https://doi.org/10.1158/0008-5472.CAN-04-0746>
- Zhao M, Ding JX, Zeng K et al (2014) Heat shock protein 27: a potential biomarker of peritoneal metastasis in epithelial ovarian cancer? *Tumour Biol* 35(2):1051–1056. <https://doi.org/10.1007/s13277-013-1139-7>
- Zheng H, Zhang L, Zhao Y et al (2013) Plasma miRNAs as diagnostic and prognostic biomarkers for ovarian cancer. *PLoS One* 8(11):e77853. <https://doi.org/10.1371/journal.pone.0077853>
- Zhou J, Gong G, Tan H et al (2015) Urinary microRNA-30a5p is a potential biomarker for ovarian serous adenocarcinoma. *Oncol Rep* 33(6):2915–2923. <https://doi.org/10.3892/or.2015.3937>
- Zuberi M, Mir R, Das J et al (2015) Expression of serum miR-200a, miR-200b, and miR-200c as candidate biomarkers in epithelial ovarian cancer and their association with clinicopathological features. *Clin Transl Oncol* 17(10):779–787. <https://doi.org/10.1007/s12094-015-1303-1>