Chapter

Extracellular Vesicles and Ovarian Cancer

Diego Aviles, David Warshal, Lauren Krill and Olga Ostrovsky

Abstract

Extracellular vesicles (EVs) are a varied group of cell-derived, microscopic, fluid-filled pouches released from cells into neighboring microenvironments that are quickly gaining recognition as a potentially powerful tool against epithelial ovarian cancer (EOC). Recent studies show that not only do EVs play an integral part in the development of cancer through intercellular communication, cell survival, and immune modulation but also may assist with early diagnosis and improved treatments. EOC currently has few effective screening options for early detection of this disease; and, therefore, it is detected at an advanced stage where it is more likely to recur, develop chemoresistance, and ultimately become fatal. Newer research has evaluated EVs as biomarkers for early screening and diagnosis and as novel targets for treatment of EOC. Moreover, EVs are possible targets for novel immunomodulatory therapies to directly target cancer cells or make cancer cells more susceptible to other treatment modalities. Therefore, EVs present an exciting, promising approach which may improve clinical outcome for EOC patients.

Keywords: extracellular vesicles, exosomes, epithelial ovarian cancer, ovarian cancer, diagnosis, prognosis, novel therapy, gynecologic oncology

1. Introduction

Extracellular vesicles (EVs) are a varied group of cell-derived, microscopic, fluid-filled pouches that cells release into the neighboring microenvironment. Recent studies show that not only do EVs play an integral part in the development of cancer through intercellular communication, cell survival, and immune modulation but also may assist with the early diagnosis and improved treatment of diseases such as epithelial ovarian cancer (EOC) [1]. EVs are quickly gaining recognition as an important enabler of EOC propagation and may potentially serve as a powerful tool in inhibiting and even reversing the progression of this disease. Historically, EOC has been a frustrating gynecologic malignancy characterized by its furtive early course that leads to an advanced presentation at initial diagnosis with subsequent poor outcomes [1]. Public health entities are ineffective at screening for early disease, leaving patients with few warnings to herald a lurking predator that affects 1–3% of women throughout their lifetime [2]. Once an EOC has manifested, the primary treatment options are surgery in combination with chemotherapy. While initially effective, these treatments

are often fruitless at abating the malignancy due to the persistence of microscopic disease and the development of chemoresistance [3]. EOC patients desperately need new treatments, and EVs may provide an opportunity to gain an improved understanding about EOC proliferation and metastasis while hopefully providing novel, effective treatments.

1.1 Why is epithelial ovarian cancer so hard to treat, and how can EVs help?

Worldwide, ovarian cancer is the seventh most common malignancy among women; and over 280,000 cases were diagnosed in 2012 alone [1, 4]. In the United States ovarian cancer is the fifth deadliest cancer among women and is the deadliest cancer originating in the female reproductive system [2]. The most common type of ovarian cancer is EOC, making up more than 90% of cases [5]. EOC encompasses numerous histologic subtypes, including serous, mucinous, endometrioid, and clear cell types; additionally, EOC can proliferate rapidly, known as high-grade disease, or have a more insidious course, known as low-grade disease [6]. Interestingly, in the past decade researchers discovered that high-grade serous EOC originates in the fallopian tubes and then migrates to the ovary; so clinicians treat EOC and fallopian tube cancer as the same entity [7]. Additionally, high-grade serous fallopian tube, ovarian, and primary peritoneal cancer are all considered the same clinical entity based on common behaviors and treatments [8]. The chapter will primarily discuss high-grade epithelial ovarian carcinoma of the ovary, fallopian tubes, and peritoneum because it is the predominant subtype of EOC and because publications prioritize this subtype when studying EVs.

EOC is a difficult disease. When testing detects this malignancy at an early stage, 80% of these patients are free of cancer at 5 years [8]. However, the early signs of EOC are nonspecific and insidious, ranging from abdominal discomfort or pain to bloating and early satiety [1]. Unfortunately, these vague symptoms lead to a late diagnosis for most patients, with about 80% of patients diagnosed with advanced disease that is more challenging to cure [9]. While surgery and chemotherapy are initially effective in treating advanced EOC, most patients experience a relapse of the cancer that is chemoresistant, with a five-year survival under 30% [5]. Based on these grim outcomes, patients need new diagnostic and therapeutic tools to improve detection and treatment of EOC.

1.2 Why are EVs so exciting?

EVs are generating excitement within the field of gynecologic oncology because they not only help researchers to better understand how cancers grow and spread but also because they can assist with the diagnosis and management of EOC at every step of the disease course. EOC EVs carry a wide array of information including microRNA (miRNA), non-coding RNA, messenger RNA, DNA, lipids, glycans, and proteins that play a role in the proliferation and metastasis of this disease. In fact, patients with EOC are known to have an upregulation in EV secretion, transforming the microenvironment surrounding the cancer and causing normal cells to secrete tumorigenic factors [10]. Researchers will someday be able to detect EOC EVs readily in blood or urine for early detection of the cancer so that clinicians can treat it at an earlier stage, preventing metastasis and resistance to chemotherapy from ever occurring. By understanding the information carried inside of EVs, patients will have access to personalized treatment regimens specifically tailored to their cancer. By exploring

different ideas, scientists will be able to unlock the potential for EVs to provide dramatic breakthroughs in the diagnosis and treatment of EOC.

2. Extracellular vesicles and their role in epithelial ovarian cancer

2.1 What is an extracellular vesicle?

For the past decade the classification of EVs has been based on size, ranging from exosomes that are 30–100 nm, microvesicles (MVs) that are 100–1000 nm, and apoptotic bodies that are 0.1–5 μm [1]. Exosomes are the smallest EV and appear to originate within the lumen of multivesicular bodies [1]. Oncosomes, a subtype of MVs, are released by budding from malignant cells [1]. As cells undergo apoptosis, they release apoptotic bodies [1]. One factor that limits this classification system is that some EVs that function as oncosomes are larger than the typical 100–1000 nm and can be as large as 1–10 μm [1]. When trying to isolate and study EVs, it became apparent that size did not adequately capture the breadth of heterogeneity among EVs with their varied functions and content. In 2019 the International Society of Extracellular Vesicles published a recommendation for the use of the term *extracellular vesicle* to encompass all types of EVs while still including a subclassification system that incorporated size [11]. Therefore, in this chapter the term *extracellular vesicle* will be used. Eventually, the optimal method of classification for EVs will be based on the specific phenotype and content of an EV that would better describe its origin and function. However, current testing methods and understanding of this topic need to be further studied.

2.2 How do extracellular vesicles impact epithelial ovarian cancer?

EVs provide a pertinent target for research because EOC cells exploit EVs for intercellular messaging. By hijacking the EV communication system, cancer cells distort key biological processes that enhance cancer survival, including angiogenesis, immunity, apoptosis, inflammation, migration, invasion, and even activation of secretion of tumorigenic factors by stem cells [1]. Malignant cells dramatically increase EV synthesis, manipulating crucial intercellular communication, exerting control over the tumor's surrounding environment, and transforming this microenvironment into a tumorigenic niche that facilitates chemoresistance and progression of disease [10]. Since EVs affect many aspects of EOC propagation and spread, they are suitable candidates for the development of new diagnostic and therapeutic management options.

3. Role of EVs in the diagnosis of epithelial ovarian cancer

3.1 How good are we at diagnosing epithelial ovarian cancer?

With the current tools available, clinicians are unable to reliably identify EOC early in its disease course, losing a valuable opportunity at early intervention and higher rates of cure. For patients who are diagnosed with EOC at stage I, disease that is confined to the ovaries, their five-year survival approaches 90% [2]. Survival drops precipitously for women with advanced stages of the disease, which is unfortunately the most

common presentation. Attempts at establishing screening systems have certainly been investigated, with the United Kingdom famously conducting a randomized controlled study in which women were screened for EOC with a combination of serum markers and ultrasound [12]. When compared to women who underwent no screening, no impact was observed on overall survival from EOC [12]. Based on the results of this trial, no screening is currently recommended for the general population because modern diagnostic tests do not help patients that are diagnosed with EOC live longer and these same tests lead more women with benign ovarian diseases to have unnecessary procedures because the testing does not distinguish well between benign ovarian disease and cancer [12]. EVs present a promising new frontier for EOC screening because they are detectable in the serum and urine of patients, providing a potential novel method for diagnosing this cancer at an early stage when the patient can be cured more easily.

3.2 MicroRNA in EVs: how can they help to diagnose epithelial ovarian cancer?

One promising method for early cancer detection involves the analysis of EVs carrying microRNA, or miRNA, in the blood of patients. As strands of non-coding RNA that are 19–25 nucleotides in length, miRNAs are transcription products of DNA that regulate genes, a process that can activate or suppress the expression of different factors that can promote the growth and metastasis of EOC [1]. While most miRNA found in body fluids is cell-free and easily degradable, miRNA that is present in EVs is more stable, amplifying the role of this information in intercellular communication because it reaches cells more effectively [13]. When normal cells, such as stem cells, receive the miRNA from EVs, they produce tumorigenic factors that enhance the cancer's ability to survive and promote invasion and dissemination [1].

When compared to healthy individuals, patients with EOC have levels of certain circulating miRNAs carried in EVs that are often elevated [13]. Numerous specific miRNAs have already been linked to EOC. For example, miR-222-3p, which Ying et al. showed promotes the conversion of normal macrophages into tumor-supporting macrophages through the activation of the SOCS3/STAT3 pathway, is elevated in patients with EOC. Once normal macrophages are transformed, they exert immunosuppressive effects that assist EOC cells in evading identification while also secreting factors that promote migration and growth. Since EV miR-222-3p levels are increased in this cancer, its detection in serum can serve as a diagnostic biomarker for early detection [14].

In another study Cappellesso et al. identified elevated levels of EVs with miR-21, a known regulator of the tumor suppressor gene programmed cell death 4 (PDCD4), in patients with EOC compared to patients with benign ovarian disease [15]. The gene PDCD4 typically prevents cancer through the regulation of apoptosis. However, in EOC, the increased expression of miR-21 directly inhibits PDCD4, allowing the cancer cell to further mutate and to invade other tissues. Similarly to miR-222-3p, EVs with miR-21 can enhance diagnostic testing and clinical staging of EOC.

While looking at individual EV miRNAs can provide clues for early detection of EOC, their true value will come from evaluating the miRNAs in large groups as diagnostic panels that together will provide screening with high sensitivity and specificity. In their study Taylor and Gercel-Taylor reviewed a panel of 8 miRNAs found in EVs—miR-141, miR-214, miR-200a, miR-200b, miR-200c, miR-21, miR-205—that displayed distinct biological profiles between patients with benign ovarian disease and those with EOC [16]. By utilizing this panel of EV miRNAs and including other EV miRNAs, a simple blood sample may serve as a powerful test that can be employed by clinicians to apprehend EOC in asymptomatic populations before it lethally spreads.

Proteins are also transported in EVs and can potentially serve as biomarkers for early diagnosis of EOC. One example of these EV proteins is EpCAM, which is recognized for its role in tumorigenesis and tumor proliferation and is elevated in patients with EOC. However, the diagnostic utility of EpCAM and other proteins is limited because the proteins can be elevated in patients with benign ovarian disease, decreasing the specificity of these markers [16]. If such proteins are then implemented into screening protocols, patients may have false-positive test results and may subsequently undergo invasive procedures with their associated complications without any benefit.

However, some proteins carried by EVs appear to be specific to EOC. CD24, a known poor prognostic marker for EOC, can be detected within EVs in malignant ascites of EOC patients [17]. Additionally, about half of the blood samples from a cohort of EOC patients contained EV claudin-4, another protein that can potentially serve as a diagnostic marker [18]. With the development of new diagnostic panels that combine EV proteins and miRNAs, patients will 1 day obtain testing that identifies EOC early and gives them a better chance at a cure.

Even easier to obtain than blood, urine is another potentially rich source for EOC EVs. Studies have identified numerous EV miRNAs such as miR-92a and miR-30a-5p that are elevated in the urinary samples of patients with EOC when compared to healthy controls [19, 20]. Specifically, miR-30a-5p is elevated in EOC but decreased in other malignancies such as gastric and colon cancer, making it a potentially unique biomarker [20]. While EV miRNAs found in urine are a potentially exciting

Type of EV Content	**Content**
miRNA	miR-21
	miR-30a-5p
	miR-21
	miR-92a
	miR-141
	miR-155
	miR-181a
	miR-200a
	miR-200b
	miR-200c
	miR-205
	miR-214
	miR-222-3p
	miR-223
	miR-486
	miR-1908
Protein	CD24
	EpCam
	Claudin-4

Table 1.
Potential panel of EV biomarkers for the diagnosis of epithelial ovarian cancer [1, 16, 17, 19, 20, 22].

biomarker for diagnosing EOC, more research is required to further take advantage of this easily accessible opportunity.

While these many EV factors provide appealing options for future diagnostic applications, some barriers hinder the utilization of EVs in the clinical setting. Current methods for isolation and purification of EVs are still constrained, relying on identification of these vesicles by size, a non-specific criterion that does not distinguish EVs from large proteins and other types of vesicular structures. The purification process involves ultracentrifugation, a process that is inefficient and cumbersome, especially for serum samples [21]. Also, current methods of molecular identification are limited by the small size of EVs as well as by the difficulty in detecting the EV content [21]. Once scientists solve these issues and answer other questions regarding the viability and concentration of EVs in blood and urine samples, the detection of EOC EVs will bolster the strength of diagnostic tools (**Table 1**).

4. Role of EVs in the prognosis of epithelial ovarian cancer

EVs are positioned to provide valuable prognostic information for EOC because current prognostic tools struggle to accurately predict an individual's disease course and response to treatments. If there was a better understanding of how a patient's particular cancer would grow and which medicines would be effective against it, providers would better optimize treatment strategies that would extend a patient's life and even grant a better opportunity for cure. Currently, the prognosis for EOC is estimated based on generalized characteristics about this disease process within the context of the patient's health status and medical history [8]. Some factors include age, stage of the cancer at the time of diagnosis, and performance status [8]. In recent years genetic research has played a significant role in patient prognosis. BRCA mutations, a pathologic process that affects the repair of double-strand DNA breaks, place patients at increased lifetime risk for EOC but also confer an improved prognosis for EOC especially with new therapies that are targeted toward patients with these mutations. While these factors provide some helpful guidance regarding a patient's treatment outcomes, neither providers nor patients can accurately predict how an individual patient's EOC will respond to therapies. However, with EVs new factors are being identified that can help in better understanding which patients will respond to certain therapies and what personalized treatment regimens will best address the cancer.

An important part of caring for patients with EOC is selecting the best treatment for their specific tumor. When determining a patient's clinical management, the available prognostic information offers limited value in guiding clinicians about how to best care for their patients. However, novel therapeutic agents are demonstrating the need for refined prognostic tools that can identify a particular tumor's sensitivity or resistance to certain treatments. For example, the breakthrough use of PARP inhibitors for the treatment of EOC over the past decade served as an important demonstration of the necessity to discover new patient factors that facilitate targeted treatments [23]. In cancer cells with impaired repair of double-stranded DNA breaks, also known as homologous recombination deficiency (HRD), PARP inhibitors promote double-stranded breaks through the inhibition of secondary single-stranded DNA repair that triggers apoptosis [23]. When EOC with HRD is treated with a PARP inhibitor, these patients experience a significant improvement in the management of their cancer. Therefore, patients with EOC are now tested for homologous recombination

deficiency [23]. While PARP inhibitors are clearly a success, patients need new biomarkers for individualized treatments; and EVs can be these new targets.

EOC quickly becomes resistant to front-line chemotherapy regimens, but it is currently not possible to predict which patients will develop chemoresistance [16]. Evidence from multiple studies suggest that EVs can predict which patients will have a tumor that is sensitive to chemotherapy [1]. For example, Yan studied a cohort of 50 patients and demonstrated an increase in serum EV annexin A3 levels, a protein involved in exocytosis and vesicle trafficking, among patients with resistance against primary chemotherapy drugs when compared to patients that are still sensitive to those chemotherapies [24]. In a second study protein RAB7A functioned as a potential mediator of chemoresistance [25]. Functioning as a key regulator of the influx of chemotherapy agents into cells, RAB7A is downregulated in chemoresistant cells, potentially affecting drug sequestration [25]. Finally, some groups are studying serum panels of EV miRNAs that are associated with chemoresistance and include miR-181a, miR-1908, miR-21, miR-486, and miR-223 [22]. Together, these different EV molecular targets may serve as prognostic biomarkers to identify chemoresistance in patients with EOC and help tailor the appropriate medication combination for each patient.

5. Can extracellular vesicles inspire novel cancer therapies?

Based on its role in tumor invasion, chemoresistance, angiogenesis, cancer metastasis, and immunologic suppression, EVs present a promising opportunity to target important regulators of EOC progression and to mobilize the immunologic response to combat the malignancy. EVs and associated miRNAs are generating excitement as novel therapeutic targets for drug development.

5.1 EVs as a drug delivery system

EVs can be employed as a drug delivery system that targets cancer cells directly. Harboring packages of chemotherapeutic agents, the EVs can be manufactured to express cell surface antigens and receptors that target it toward cancer cells and spare normal cells, maximizing cytotoxic effect while sparing healthy tissue. In recent work Tang developed a model in which they incubated tumor cells with chemotherapy; and the tumor cells subsequently packaged the chemotherapy into EVs [26]. Tang's group then took these EVs and demonstrated tumor-killing effect in mice with minimal side effects [26]. Therefore, if researchers translate this murine model into a therapy for EOC patients, EVs can be customized to carry the antitumor agents that are effective for a particular tumor. While chemotherapy affects tumor cells, it is also a treatment that damages healthy tissue, causing patients to experience a wide range of side effects. By having a treatment that can precisely target cancer cells while sparing normal tissues, clinicians could safely administer treatments to patients that could control or even cure EOC while avoiding harm.

5.2 Why are EVs so important in establishing the tumor microenvironment? Can the microenvironment become a target for new treatments?

In developing new therapeutic targets, one key area of focus is the immediate, small-scale environment surrounding cancer cells known as the microenvironment. The tumor microenvironment includes the extracellular matrix, neighboring blood

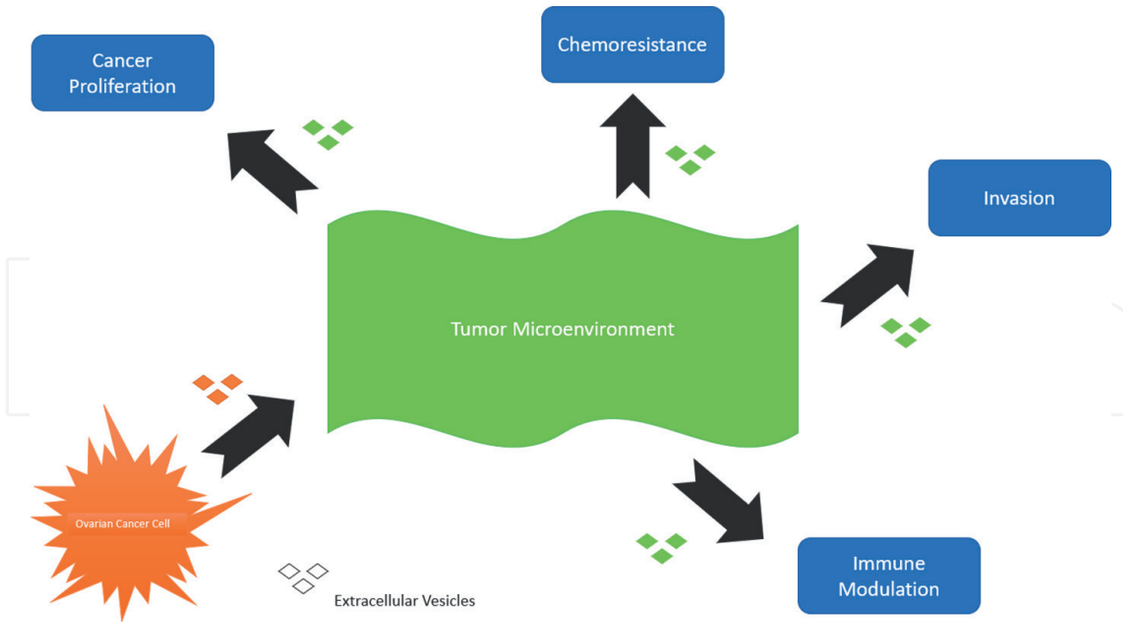

Figure 1.
The interaction between EOC cells and their microenvironment. Cancer cells release EVs that instruct normal cells to produce EVs that support cancer growth and invasion while also facilitating the development of chemoresistance and protection from the immune system [1, 2].

vessels, stromal cells such as macrophages and fibroblasts, stem cells, signaling molecules, and immune cells [22]. For EOC the microenvironment becomes an integral component for drug targeting because of the role that it plays in protecting the tumor from chemotherapy and the immune system as well as in promoting proliferation, invasion, and metastasis. By targeting EOC EVs that transform healthy tissue into this tumorigenic niche, researchers will enhance the effectiveness of current treatments by reversing chemoresistance as well as limit the deadly growth and spread of this disease (**Figure 1**).

EVs derived from EOC cells promote the transformation from a normal microenvironment into a tumorigenic one through intercellular communication that stimulates angiogenesis, immune suppression, and stromal invasion [27]. Specifically, altered expression of miRNA such as miR-214, miR-31, and miR-155 has been linked to the conversion of fibroblasts, a support cell within the connective tissue, into cancer-associated fibroblasts (CAFs)— cells that participate in cancer propagation, support of the tumorigenic microenvironment, alteration of the extracellular matrix, and metastasis [1]. The CAFs then produce EVs enriched with TGFβ1 that then trigger the invasive properties of the tumor. With almost deliberate malintent, the cancer cells drive their own invasive potential by directing the formation of CAFs that provide the necessary growth factors that allow the malignancy to spread. Following treatment with cisplatin, a frontline chemotherapy agent, EOC cells release EVs that promote tumorigenic activity of mesenchymal stem cells that eventually stimulate cancer progression [28]. By developing therapies targeted at these factors that transform the healthy tissue surrounding cancer into the tumorigenic microenvironment, scientists can inhibit the cancer's ability create its own protective environment that fuels its ability to grow and invade.

5.3 EVs and chemoresistance

Among the many challenges limiting the treatment of EOC, resistance to standard chemotherapy regimens exists as a frustrating inevitability in most patients

with advanced disease; and EVs seem to play an integral role in this process. As the first-line regimen for EOC, platinum-based chemotherapy is the most effective treatment for EOC; yet 80% of patients with advanced EOC relapse, most within 2 years [3]. Following recurrence of the cancer, most people develop chemoresistance and succumb to the disease. An EOC that is *platinum-resistant* is defined as disease that recurs or progresses within 6 months of completion of the last treatment with a platinum-based regimen. Once a patient's cancer reaches this state, expectations for disease control change, with low response rates to subsequent chemotherapies and a median survival falling below 12 months [3].

While platinum resistance is a complicated, multifactorial process that still needs further elucidation, EVs may help to better understanding this transformation. EOC EVs function as an intercellular communication system. Interestingly and frighteningly, EVs excreted by platinum-resistant tumor cells are capable of inducing resistance in other tumor cells [29]. While this mechanism is not well understood, once the EVs that mediate this process are better defined, they can become targets for possible therapeutic intervention. Furthermore, by understanding the EV content that conveys chemoresistance between cancer cells, scientists can alter the EVs to send information directly to tumor cells that reverses this resistance, allowing first-line treatments to again become effective.

The cytotoxic effect of platinum-based drugs such as cisplatin relies on the uptake of the chemotherapy into cells followed by DNA binding, leading to the formation of DNA crosslinks and breaks that result in apoptosis [3]. In patients that develop platinum resistance, some of their cancer cells exhibit reduced uptake or increased efflux of platinum agents, a process that EVs may facilitate [1, 3]. Transport proteins that have been implicated in this mechanism of drug resistance such as the lysosomal proteins ATPase copper-transporting alpha and beta have been found in EOC EVs, allowing cancer cells to survive against chemotherapy [22]. Is it possible to negate the effect of these EVs through targeted therapies? By disrupting this EV communication system with antibodies or other novel therapies, researchers can provide hope to these patients by overcoming chemoresistance and making their chemotherapy more effective.

5.4 EVs and immunosuppression

One important technique that allows EOC to proliferate and spread is the ability to suppress the immune system. By further understanding the elaborate underlying mechanisms through which EOC EVs dampen immunity, researchers will be able to block immune escape by the cancer cells, producing new treatments. By preventing immune suppression within the tumorigenic microenvironment, ovarian cells that were previously protected within this nurturing space would be freshly susceptible to immune cells that could find and eliminate the cancer cells [1].

Given the significant promise for novel treatments for EOC that reactivate the suppressed immune system, studies are already underway that target EOC EVs. One therapy utilizes dendritic cells as a map that directs the immune system toward the cancer. In one study these dendritic cells, known for presenting specific foreign antigens to the immune system for identification and targeting, were exposed to EVs isolated from the ascites of EOC patients [1]. The dendritic cells then presented tumor-specific antigens from the cancer EVs to resting T cells that subsequently differentiated and then killed EOC cells [30]. Dendritic cells may be harvested from a patient with EOC, cultured with isolated EOC EVs, and then reintroduced to the patient as an autologous injection that then directs the patient's own T cells to

eradicate the cancer. This concept elegantly demonstrates the potential for unleashing the immune system on cancer cells using EVs.

Another interesting avenue for treating EOC is through the utilization of immunoglobulins that directly target EVs. The serum of patients with EOC is more immunologically reactive when compared to the serum of healthy patients and patients with benign ovarian disease, indicating a robust immune response against the malignancy. As many studies have proven before, the natural immunoreactivity that the human body mounts against EOC is insufficient because the cancer employs tactics to evade the immune system, a process in which EVs play a significant role [22]. While immune evasion is a hallmark characteristic of EOC, the immune system may be mobilized against the cancer by findings ways to target EVs with immunoglobulins. Researchers can develop antibodies that specifically target EOC EVs, tagging them for the immune system so that they can be destroyed, effectively dismantling the vital EV communication system for the cancer cells and limiting the cancer's ability to grow and spread. While this novel use of EVs is exciting, more research is needed to use this method. Mainly, scientists need to better characterize EVs to develop targets for immunoglobulins. Also, it is difficult for antibodies to target the content within EVs because it is protected by the vesicular walls, so proteins on the vesicle wall may provide a unique target for the antibodies. As scientists better understand the unique protein signatures of EOC EVs, immunity-based therapeutics may provide promising new avenues for treating these patients.

5.5 EVs and angiogenesis

Angiogenesis, a vital component of cancer proliferation and progression, has become an important focus in the care of patients with EOC. Ovarian cancers have previously been recognized for their role in promoting angiogenesis; so, by targeting these specific EVs in combination with other antitumor treatments, more effective regimens may be developed for combating this cancer. In the study GOG 218, Burger et al. conducted a clinical trial in which they incorporated a vascular endothelial growth factor (VEGF) inhibitor into the standard primary chemotherapy regimen for advanced EOC [31]. While patients on the VEGF inhibitor experienced a longer period of progression-free survival, they did not live any longer when compared to those who did not receive the treatment. While the inhibition of VEGF, a family of proteins recognized for stimulating the formation of blood vessels, clearly has some effect on tumor growth, other factors appear to be at play that limit the effectiveness of this therapy. One explanation is that EVs play a role in angiogenesis that circumvents the use of VEGF. Ovarian cancer-derived EVs that contain proteins such as CD147, metastasis-associated protein 1, and activating transcription factor 2 appear to have a key effect on angiogenesis that promotes cancer proliferation [32, 33]. A treatment for EOC could include antibodies or some other novel therapy that targets cancer EVs that carry these proteins that stimulate angiogenesis and then eliminate the ability for the cancer to develop its own blood supply.

Another appealing area of active research is the study of common dietary supplements that may have antiangiogenic properties through the production of antiangiogenic EVs. A promising supplement, Amla extract, derived from the Indian Gooseberry tree, has long been suspected to have cancer preventative properties [34]. One recent study tested the supplement on EOC cells and noted increased expression of EV miR-375 which appears to block the proangiogenic proteins SNAIL1 and IGF1R [34]. With a better understanding of the mechanism of this supplement and many

others, scientists may 1 day provide dietary recommendations that can enhance a patient's standard chemotherapy regimen or even derive a novel pharmacologic treatment that blocks blood vessel formation, helping to better destroy EOC cells [4].

6. Conclusion and clinical relevance

EOC remains a disease with a generally poor prognosis due to its asymptomatic early stages, ineffective screening mechanisms, and its predilection to develop chemoresistance with recurrence of disease. EVs are exciting within the field of EOC research because they provide the potential for many interventions that can save the lives of patients, ranging from diagnosing the cancer at earlier stages, identifying the optimal treatment for each individual patient, and even developing novel therapeutics that are more effective than the current regimens. With the ineffectiveness of screening tests, panels of EVs that can be detected in blood or urine provide hope for highly sensitive and specific tools that can give an accurate diagnosis of cancer in asymptomatic patients. Alternatively, by exploiting EVs to overcome chemoresistance, clinicians can redeploy existing treatments that typically become obsolete during a patient's disease course. The demand for new diagnostic tools and therapies for patients with EOC is high, and EVs can be the next frontier for seemingly miraculous advancements in cancer care.

Author details

Diego Aviles[1], David Warshal[1], Lauren Krill[1] and Olga Ostrovsky[2*]

1 Department of Gynecologic Oncology, MD Anderson Cancer Center at Cooper, Cooper University Health Care, Camden, NJ, USA

2 Department of Surgery, Division of Surgical Research, Cooper University Health Care, Camden, NJ, USA

*Address all correspondence to: ostrovsky-olga@cooperhealth.edu

References

[1] Lucidi A, Buca D, Ronsini C, Tinari S, Bologna G, Buca D, et al. Role of extracellular vesicles in epithelial ovarian cancer: A systematic review. International Journal of Molecular Sciences. 2020;**21**(22):8762. DOI: 10.3390/ijms21228762

[2] Islami F, Ward EM, Sung H, et al. Annual Report to the Nation on the Status of Cancer, Part 1: National Cancer Statistics. J Natl Cancer Inst. 2021;**113**(12):1648-1669. DOI: 10.1093/jnci/djab131. [Published online ahead of print, 2021 Jul 8]

[3] Pokhriyal R, Hariprasad R, Kumar L, Hariprasad G. Chemotherapy resistance in advanced ovarian cancer patients. Biomark Cancer. 2019;**11**: 1179299X19860815. Published 2019 July 5. DOI: 10.1177/1179299X19860815

[4] Zhang Y, Luo G, Li M, et al. Global patterns and trends in ovarian cancer incidence: age, period and birth cohort analysis. BMC Cancer. 2019;**19**(1):984. Published 2019 October 22. DOI: 10.1186/s12885-019-6139-6

[5] Li SS, Ma J, Wong AST. Chemo-resistance in ovarian cancer: Exploiting cancer stem cell metabolism. Journal of Gynecologic Oncology. 2018;**29**(2):e32. DOI: 10.3802/jgo.2018.29.e32

[6] Mahmood RD, Morgan RD, Edmondson RJ, Clamp AR, Jayson GC. First-line management of advanced high-grade serous ovarian cancer. Current Oncology Reports. 2020;**22**(6):64. Published 2020 Jun 4. DOI: 10.1007/s11912-020-00933-8

[7] Perets R, Wyant GA, Muto KW, et al. Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca;Tp53;Pten models. Cancer Cell. 2013;**24**(6): 751-765. DOI: 10.1016/j.ccr.2013.10.013

[8] Stewart C, Ralyea C, Lockwood S. Ovarian Cancer: An Integrated Review. Seminars in Oncology Nursing. 2019;**35**(2):151-156. DOI: 10.1016/j.soncn.2019.02.001. Epub 2019 March 11

[9] Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. CA: a Cancer Journal for Clinicians. 2018; **68**(4):284-296

[10] Nakamura K, Sawada K, Kobayashi M, et al. Role of the exosome in ovarian cancer progression and its potential as a therapeutic target. Cancers (Basel). 2019;**11**(8):1147. Published 2019 August 10. DOI: 10.3390/cancers11081147

[11] Thery et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. Journal of Extracellular Vesicles. 2018;**7**:1. DOI: 10.1080/20013078.2018.1535750

[12] Jacobs IJ, Menon U, Ryan A, Gentry-Maharaj A, Burnell M, Kalsi JK, et al. Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): A randomised controlled trial. Lancet. 2016;**387**(10022):945-956. DOI: 10.1016/S0140-6736(15)01224-6. Epub 2015 December 17. Erratum in: Lancet. 2016 March 5;387(10022):944. Erratum in: Lancet. 2016 March 5;387(10022):944

[13] Wang W, Yin Y, Shan X, Zhou X, Liu P, Cao Q, et al. The value of plasma based MicroRNAs as diagnostic

biomarkers for ovarian cancer. The American Journal of the Medical Sciences. 2019;**358**:256-267

[14] Xiang Y, Quanfeng W, Xiaoli W, Qinyi Z, Xinjing W, Lu J, et al. Epithelial ovarian cancer-secreted exosomal miR-222-3p induces polarization of tumor-associated macrophages. Oncotarget. 2016;**7**(28):43076-43087

[15] Cappellesso R, Tinazzi A, Giurici T, Simonato F, Guzzardo V, Ventura L, et al. Programmed cell death 4 and microRNA 21 inverse expression is maintained in cells and exosomes from ovarian serous carcinoma effusions. Cancer Cytopathology. 2014;**122**(9):685-693

[16] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic Oncology. 2008;**110**(1):13-14

[17] Runz S, Keller S, Rupp C, Stoeck A, Issa Y, Koensgen D, et al. Malignant ascites-derived exosomes of ovarian carcinoma patients contain CD24 and EpCAM. Gynecologic Oncology. 2007;**107**(3):563-571

[18] Li J, Sherman-Baust CA, Tsai-Turton M, Bristow RE, Roden RB, Morin PJ. Claudin-containing exosomes in the peripheral circulation of women with ovarian cancer. BMC Cancer. 2009;**9**:244

[19] Zavesky L, Jandakova E, Turyna R, Langmeierova L, Weinberger V, Minar L. Supernatant versus exosomal urinary microRNAs. Two fractions with different outcomes in gynaecological cancers. Neoplasma. 2016;**63**(1):121-132

[20] Zhou J, Gong G, Tan H, Dai F, Zhu X, Chen Y, et al. Urinary microRNA30a-5p is a potential biomarker for ovarian serous adenocarcinoma. Oncology Reports. 2015;**33**(6):2915-2923

[21] Zhao Z, Yang Y, Zeng Y, He M. A microfluidic ExoSearch chip for multiplexed exosome detection towards blood-based ovarian cancer diagnosis. Lab on a Chip. 2016;**16**(3):489-496

[22] Li X, Wang X. The emerging roles and therapeutic potential of exosomes in epithelial ovarian cancer. Molecular Cancer. 2017;**16**:92. DOI: 10.1186/s12943-017-0659-y

[23] Mirza MR, Coleman RL, González-Martín A, Moore KN, Colombo N, Ray-Coquard I, et al. The forefront of ovarian cancer therapy: update on PARP inhibitors. Annals of Oncology. 2020;**31**(9):1148-1159. DOI: 10.1016/j.annonc.2020.06.004. Epub 2020 Jun 20. Erratum in: Ann Oncol. 2021 Aug;32(8):1066-1067

[24] Yan XD, Yin J, Yao H, et al. Increased expression of annexin A3 is a mechanism of platinum resistance in ovarian cancer. Cancer Research. 2010;**70**:1616-1624

[25] Guerra F, Paiano A, Migoni D, Girolimetti G, Perrone AM, De Iaco P, et al. Modulation of RAB7A protein expression determines resistance to cisplatin through late endocytic pathway impairment and extracellular vesicular secretion. Cancers (Basel). 2019;**11**(1):52. DOI: 10.3390/cancers11010052

[26] Tang K, Zhang Y, Zhang H, Xu P, Liu J, Ma J, et al. Delivery of chemotherapeutic drugs in tumour cell-derived microparticles. Nature Communications. 2012;**3**:1282

[27] Tang MK, Wong AS. Exosomes: Emerging biomarkers and targets for ovarian cancer. Cancer Letters. 2015;**367**(1):26-33

[28] Vera N, Acuña-Gallardo S, Grünenwald F, Caceres-Verschae A, Realini O, Acuña R, et al. Small

extracellular vesicles released from ovarian cancer spheroids in response to cisplatin promote the pro-tumorigenic activity of mesenchymal stem cells. International Journal of Molecular Sciences. 2019;**20**(20):4972. DOI: 10.3390/ijms20204972

[29] Pink RC, Samuel P, Massa D, Caley DP, Brooks SA, Carter DR. The passenger strand, miR-21-3p, plays a role in mediating cisplatin resistance in ovarian cancer cells. Gynecologic Oncology. 2015;**137**(1):143-151

[30] Li QL, Bu N, Yu YC, Hua W, Xin XY. Exvivo experiments of human ovarian cancer ascites-derived exosomes presented by dendritic cells derived from umbilical cord blood for immunotherapy treatment. Clin Med Oncol. 2008;**2**: 461-467. DOI: 10.4137/cmo.s776

[31] Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. The New England Journal of Medicine. 2011;**365**(26):2473-2483. DOI: 10.1056/NEJMoa1104390

[32] Millimaggi D, Mari M, D'Ascenzo S, Carosa E, Jannini EA, Zucker S, et al. Tumor vesicle-associated CD147 modulates the angiogenic capability of endothelial cells. Neoplasia. 2007;**9**(4): 349-357

[33] Yi H, Ye J, Yang XM, Zhang LW, Zhang ZG, Chen YP. High-grade ovarian cancer secreting effective exosomes in tumor angiogenesis. Int J Clin Exp Pathol. 2015;8:5062-70 Shender VO, Pavlyukov MS, Ziganshin RH, Arapidi GP, Kovalchuk SI, Anikanov NA, Altukhov IA. Proteome-metabolome profiling of ovarian cancer ascites reveals novel components involved in intercellular communication. Molecular & Cellular Proteomics. 2014;**13**(12):3558-3571

[34] De A, Powers B, De A, et al. Emblica officinalis extract downregulates pro-angiogenic molecules via upregulation of cellular and exosomal miR-375 in human ovarian cancer cells. Oncotarget. 2016;**7**(21):31484-31500. DOI: 10.18632/oncotarget.8966