The Diagnostic and Therapeutic Prospects of Exosomes in Ovarian Cancer

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Abstract

Exosomes are nano-sized vesicles derived from the endosomal system and involved in multitudinous biological and pathological processes. Emerging evidence has demonstrated that exosomes are closely related to the tumorigenesis and progression of ovarian cancer. Ovarian cancer-related exosomes have cell-specific constituents that could be leveraged for diagnosis. Additionally, exosomes are promising to treat ovarian cancer through functioning as delivery systems or participating in immunotherapy or serving as direct targets. In this review, we discussed the association of exosomes in ovarian cancer progression and look forward to the clinical significance of exosomes relevant to diagnosis and treatment.

1. Introduction

Ovarian cancer, one of the most common malignancies in the female reproductive system, has the highest mortality rate among gynecological cancers [1]. Albeit the five-year relative survival rate of patients diagnosed at the early stage (FIGO stage I or II) can reach 90%, more than 70% of patients are diagnosed with advanced stage (FIGO stages III-IV) for lack of early symptoms and sensitive diagnosis [2]. Patients diagnosed at advanced stages are generally accompanied by poor prognosis, which possibly subsumes extensive peritoneal dissemination, massive ascites, acquired chemoresistance, and a five-year survival rate of less than 25%[3]. Despite the indisputable fact that novel therapies, such as targeted therapy and immunotherapy, have been continuously emerging in recent years, current treatments for ovarian cancer are still limited[4]. Hence, there is an urgent need to identify specific diagnostic biomarkers and potential therapeutic interventions for ovarian cancer.

Recently, as promising biomarkers of ovarian cancer, exosomes have been widely investigated and have exhibited their immense potential in the medical field due to their diverse functions in pathology[5,6]. After the discovery of membrane exfoliation vesicles by Eberhard G. Trams, the term "exosome" was initially used by Johnstone to describe the specific type of extracellular vesicles (EVs) in 1987 [7,8]. Nowadays, exosomes specifically refer to those membranous vesicles with a diameter of ~40 to160 nm (average ~100 nm) that have the same topology as the cells and are enriched in lipids, nucleic acids, and protein complexes[5,9]. Research has revealed that exosomes abnormally initiate or suppress various signaling pathways in cancer cells though transmitting heterogeneous cargoes, which potentially contribute to the development of cancer[10]. In ovarian cancer, exosomes play a crucial role in mediating epithelial-to-mesenchymal transition (EMT), non-mutational epigenetic reprogramming, immune modulation, thus involved in the promotion of tumorigenesis, peritoneal dissemination, and drug resistance [11]. Apart from this, exosomes are promising biomarkers in biological fluids for the multicomponent diagnosis of ovarian cancer [12]. Herein, this review is aimed to describe the characteristics of exosomes, with a particular focus on their emerging functions and mechanisms

in ovarian cancer, we hope to bring new insights into the latest advances in diagnosis and treatment of ovarian cancer.

2.Biological characteristics ofexosomes

The biogenesis of exosomes occurs within the endosomal system, covering the involution of cell membranes to form endosomes, the generation of intraluminal vesicles (ILVs) inside the multivesicular bodies (MVBs), and the fusion between the MVBs and the plasma membrane to release exosomes (Figure 1)[5]. The exosomal membranes mainly consist of lipid layers, and some specific lipids are enriched in exosomes compared to their parent cells [13]. Additionally, exosomes possesses several families of proteins unique to the endosomal pathway, such as tetraspanins (CD9, CD63, CD81), tumor-sensitive gene 101 (TSG101), heat shock proteins (Hsc70), lysosomal proteins (Lamp2b) and fusion proteins (flotillin and annexin), which are commonly used to characterize exosomes and distinguish them from other vesicles [14]. Moreover, exosomes contain multiple types of bioactive molecules such as cargos including proteins, nucleic acids, lipids, and metabolites[5]. A straightforward approach based on analyzing the variation of key contents is helpful to uncover the biological functions of exosomes coming from different cell types. For more extensive information about bio-active contents in exosomes identified in multiple organisms, ExoCarta (http://www.exocarta.org) can provide further information for researchers to conduct in-depth exploration.

3.Roles of exosomes in ovarian cancer

Exosomes presenting in biological fluids are tightly connected with multiple pathological and physiological processes. Generally, exosomes participate in expelling excess and/or nonfunctional cellular components, recycling materials between cells for intercellular communication, remodeling the tumor microenvironment (TME), and many more [15]. Studies have shown that tumor cells secrete more exosomes than normal cells, and exosomes derived from tumor cells have a potent capacity to promote tumor progression by modifying both local and distant microenvironments [16]. Herein, we summarize the functions of exosomes in the metastasis, chemotherapy resistance, and immune regulation of ovarian cancer (Figure 2).

3.1 Role inmetastasis

Recent studies have unraveled the significant contribution of TME to ovarian cancer metastasis, of which exosomes are an important composition[17]. Exosomes are chiefly involved in the formation of pre-metastatic niches and the process of promoting metastasis. Primary ovarian tumor-derived exosomes can prepare the distant tumor microenvironment via regulating intercellular communication between tumor cells and normal stroma, cancer-associated fibroblasts, and local immune cells to promote metastatic invasion[18]. Plasma cells are capable of mediating phenotypic switch and shaping the mesenchymal identity of ovarian cancer in an exosome-dependent manner [19]. Specifically, plasma cell-derived exosomes contain miR-330-3p, thereby uplifting the expression of junctional adhesion molecule B in non-mesenchymal ovarian cancer cells in a noncanonical fashion. Moreover, exosomes in ascites promote epithelial-mesenchymal transition (EMT) of ovarian cancer cells by delivery of miR-6780b-5p and eventually facilitate ovarian cancer metastasis[20]. Other exosomal miRNAs can be synthesized and secreted by ovarian cancer cells as well. It has been found that miR-205 was up-regulated in ovarian cancer tissues and promoted ovarian cancer metastasis in an exosome-dependent manner by inducing angiogenesis[21]. Furthermore, proteomic analysis reveals that exosomes from ovarian cancer cells contain a specific set of proteins that are representative of their origin and invasive capacity[22]. Similarly, Alharbi *et al.* unveiled that 40 proteins associated with Wnt canonical pathway (β-catenin) were differentially expressed in tumor tissues between mice injected with high invasiveness capacity cell line (exo-SKOV-3) and low invasiveness capacity cell line (exo-OVCAR-3), and exosomes secreted by a high invasiveness capacity cell line boosted tumor metastasis *in vivo*[23]. To sum up, exosomes modulate TME via autocrine-paracrine signaling that reinforces the formation of pre-metastatic niches and enhances the invasion and migration of ovarian cancer cells.

3.2 Role inchemotherapyresistance

Apart from metastasis, chemotherapy resistance is the major cause of treatment failure in patients with

ovarian cancer, especially in patients with advanced ovarian cancer [24]. And exosomes released in TME are prone to bring about chemotherapy resistance. For instance, exosomal miRNA miR-1246 assists in establishing paclitaxel resistance in ovarian cancer via targeting the Cav1/p-gp/M2-type macrophage axis [25]. Au Yeung*et al.* demonstrated that exosomes transfer stroma-derived miR21 from cancer-associated adipocytes (CAAs) and fibroblasts (CAFs) to the cancer cells, therefore helping ovarian cancer cells to develop paclitaxel resistance through targeting APAF1 [26]. And Zhu *et al* . indicated that macrophages-derived exosomes deliver miR-223 to epithelial ovarian cancer (EOC) cells to elicit a chemo-resistant phenotype [27]. To put it more specifically, this study emphasized the role of exosomes in the crosstalk between macrophages and EOC cells for chemotherapy resistance through the exosomal miR-223/PTEN-PI3K/AKT signaling pathway. Lately, a hypothesis has been proposed that hypoxia-induced changes in exosomes composition and bioactivity might render carboplatin resistant to target cells [28]. Conformably, hypoxia-induced exosomes carry more STAT3 and FAS that are capable of inducing chemo-resistance[29]. Besides, plasma gelsolin (pGSN) transported by exosomes upregulates HIF1α-mediated pGSN expression in chemo-resistant ovarian cancer cells in an autocrine manner, coupled with leading to cisplatin resistance in other chemo-sensitive ovarian cancer cells in a paracrine manner[30]. Notably, exosomes are also a direct mechanism of intercellular drug transfer. What's more, exosomes promote drug efflux in a drug concentration-dependent manner and exceed the p-glycoprotein efflux when it is saturated [31].

3.3 Role inimmune regulation

The double-edged role of exosomes originating from TME in immune regulation has gained continuously increasing attention. Most tumor-associated exosomes have immunosuppressive effects, which collectively inhibit the antitumor response of tumor-specific T cells and induce the functional arrest of adoptively transferred tumor-specific T cells or chimeric antigen receptor T cells[32]. Consistent with this, Zhou *et al.* found that exosomes released from tumor-associated macrophages deliver miRNAs, including miR-29a-3p and miR-21-5p, into $CD4^+$ T cells, which could induce a Treg/Th17 cell imbalance in EOC [33]. Additionally, exosomes carry PD-L1 on their surface, thus contributing to immunosuppression by anti-PD-1 response [34]. For immunosuppressive exosomes, targeting exosomal phosphatidylserine by ExoBlock, a novel PS-binding molecule, represents a promising strategy to enhance antitumor T-cell responses in TME [35]. However, some exosomes have antipodal effects. In other words, these exosomes can activate dendritic cells (DCs), natural killer cells, and T cells to exert an antitumorigenic effect through stimulating both the innate and adaptive immune systems [36]. Since the immune microenvironment of ovarian cancer is of considerable complexity, supererogatory studies are required to help thoroughly understand the role of exosomes in immune regulation to provide the possibility for effectively designing immunotherapeutic regimens.

4.Exosomes serve as biomarkers of ovarian cancer

To date, in actual clinical application, the dominating diagnostic and prognostic biomarkers of ovarian cancer have been carbohydrate antigen 125 (CA125) and human epididymis protein 4 (HE4)[37,38]. Nevertheless, these markers usually present superior sensitivity and poor specificity or vice versa and are linked with several other pathological conditions. Recent studies have revealed that exosomes turn out to be ideally noninvasive or less-invasive biomarkers for ovarian cancer diagnosis and monitoring[39]. Because exosomes represent mobile information regarding the molecular makeup of parental tumors and can be conveniently detected in many physical fluids such as blood, urine, saliva, and malignant effusions of ascites. In this section, we mainly summarize exosomal proteins and miRNAs as diagnostic and prognostic biomarkers of ovarian cancer (Figure 3). Remarkably, in addition to exosomal proteins and miRNAs, long noncoding RNAs (lncRNAs) and phosphatidylserine (PS) carried by exosomes can also be potential and attractive biomarkers. For example, exosome-delivered lncRNA SOX2-OT contributed to motility and proliferation of ovarian cancer cells and was highly expressed in ovarian cancer. PS-expressing exosomes in patient blood and ascites can be a surrogate biomarker for ovarian cancer, since exosome membranes derived from the plasma membrane of parental tumor cells commonly expose PS [40].

4.1Exosomal proteins asbiomarkers

Compared with exosomes derived from normal tissue, exosomes from ovarian cancer contain some types of protein with significantly increased levels, such as CD24 and Claudin-4, suggesting that exosomal protein can serve as biomarkers probably connected with FIGO classification [41,42]. Other potential biomarkers, small heat shock proteins have remarkably high expression levels in exosomes from serum and peritoneal fluid of ovarian cancer patients, while more studies are needed to exploit their diagnostic value[43]. In addition, the exosomal proteome of the ovarian cancer ascites may provide monitoring for the therapeutic response to different therapy strategies. Meshach*et al.* have reported that elevated expression of plasma gelsolin (pGSN) transported by exosomes (Ex-pGSN), correlated with poorer overall survival and relapse-free survival in patients with ovarian cancer[30]. Consistently, exosome-mediated pGSN confers cisplatin resistance in chemosensitive ovarian cancer cells in an autocrine manner. Indeed, in drug-resistant cancer cells derived exosomes, chemoresistance-related proteins, such as annexin A3, MRP2, ATP7A, and ATP7B, are highly expressed, indicating the potential value to predict the effectiveness of chemotherapy methods in patients with ovarian cancer[44]. Surprisingly, a microfluidic device enabled the isolation of exosomes and the establishment of their protein profiles for the early detection of high-grade serous ovarian cancer (HGSOC)[45].

4.2Exosomal miRNAs as biomarkers

While previous studies have reported that the aberrant expression of miRNAs could represent diagnostic and prognostic markers for ovarian cancer, most of these studies regarding miRNAs expression were based on findings from tumor tissue specimens. MiRNA profiling of circulating tumor exosomes in the patient's plasma has shown the clinical relevance of exosomal miRNAs as circulating biomarkers for cancer[46]. Taylor*et al.*have observed that 8 miRNAs of tumor-derived exosomes had specific expression in ovarian cancer[47]. Kanlikilicer *et al.* have reported that exosomal miR-6126 is ubiquitously released in high abundance from both chemosensitive and chemoresistant ovarian cancer cells [48]. Furthermore, Pan *et al.* have found that miR-21, miR-100, miR-200b, and miR-320 were significantly enriched in exosomes from plasma of EOC patients compared with those of healthy women, whereas miR-16, miR-93, miR-126, and miR-223 were down-regulated[49]. In particular, the levels of exosomal miR-200b and miR-200c correlate with the tumor marker CA125 and patient overall survival in advanced EOC[50]. Additionally, ovarian cancer cell-secreted exosomal miR-205 was markedly enriched in the serum of patients, and further research found that the high level of exosomal miR-205 promoted ovarian cancer metastasis via inducing angiogenesis [21]. Conclusively, despite the existing findings, we are still in sore need of more studies to elucidate the feasibility of exosomal miRNAs in ovarian cancer diagnosis and prognosis, further enabling exosomal miRNAs to be used in ovarian cancer patients soon.

5. Therapeutic Potential of Exosomes

In this section, the therapeutic potential of exosomes will be elucidated in the following three aspects, (1) exosome-mediated delivery system, (2) exosome-based immunotherapy, and (3) exosomes as therapeutic targets. Beyond that, we discuss future perspectives that exosomes can serve as therapeutic modalities as well as therapeutic targets for ovarian cancer treatment (Figure 3).

5.1Exosome-mediated delivery system

Compared to non-host vehicles, exosomes are startlingly stable in circulation and do not elicit immune rejection. Consequently, their clinical applications as agents delivery nanoplatforms can be leveraged for treating ovarian cancer[51,52]. Specifically, not only can the advances of in bio-engineering nanotechnology enable the encapsulation of therapeutic agents such as RNA, peptides, and chemotherapeutic drugs into exosomes, but also modify the exosomes with diverse ligands for tumor-targeting strategies[53]. For instance, Zhao*et al.* found that injection of RGD-modified exosomes loaded with miR-484 in xenograft model induced vessel normalization and in turn sensitized the ovarian cancer cells to chemotherapy[54]. Bioinspired hybrid nanoparticles, which were formed by CD47-expressing exosomes and the target peptide cRGD-modified liposomes with miRNA-497 and triptolide, induced ovarian cancer cells apoptosis by inhibiting the PI3K/AKT/mTOR signaling pathway and overcame drug resistance in ovarian cancer via regulating macrophage polarization

[55]. Besides, Pisano *et al.* proposed that development immune derived exosome mimetics (IDEM) as a scalable biomimetic drug-delivery system can target and treat ovarian cancer[56]. This monocyte-derived exosome mimetic can efficiently encapsulate doxorubicin with reduced immunogenicity. Prominently, natural products, such as curcumin and triptolide, show promising therapeutic effects on ovarian cancer, yet the low bioavailability and non-specific selectivity often hinder their clinical applications[57,58]. Combining exosomes with these natural anticancer products based on nanotechnology has exhibited its high efficiency for the treatment of ovarian cancer [59,60]. Accordingly, a phase I clinical trial is currently ongoing to investigate the feasibility and ability of plant exosomes to deliver curcumin (NCT01294072).

5.2 Exosomes-basedimmunotherapy

Cancer immunotherapy is a novel type of treatment based on manipulating the host immune system to reactivate the antitumor immune response and/or overcome the pathway leading to tumor escape[61]. Evidence has revealed shown exosomes generally contribute to immunosuppression and tumor immunity escapes via a variety of biological mechanisms[62]. Explicitly, Li *et al.* reported that ovarian cancer cells secrete exosomes in TME to suppress antitumor immunity based on lymphocyte-cancer cell cross-talk[63]. And Peng *et al.* concluded that exosomes existing in ascites of ovarian cancer patients induce apoptosis of cells in the immune system, such as dendritic cells (DCs) and peripheral blood mononuclear cells (PBMCs)[64]. Therefore, eliminating immunosuppressive exosomes in TME of ovarian cancer is expected to enhance antitumor immune responses for immunotherapy. Unexpectedly, some exosomes with immunogenicity can induce cytotoxic T lymphocyte-dependent antitumor response[65]. Previous studies demonstrated that exosomes containing tumor antigens for APC activation, which consequently manipulate the immune system to recognize and attack cancer cells[66]. In agreement, according to Li *et al* , ovarian cancer-derived exosomes with tumor-specific antigens can be presented by dendritic cells (DCs) derived from unrelated umbilical cord blood to induce tumor-specific cytotoxicity[67]. These provided another perspective that exosomes may employ their immunogenicity to open new avenues for immunization against ovarian cancer.

5.3 Exosomes astherapeutic targets

We considering that exosomes play pivotal roles in ovarian cancer progression, and targeting exosomes have potential clinical implications for therapy[68]. Removing exosomes from peripheral circulation and blocking exosome production and secretion from cancer cells are the mainstay of exosome-targeting strategies. Studies*in vitro* have demonstrated the selective removal of exosomes from patient blood by using Hemopurifier, an extracorporeal hemofiltration device containing fibers with a high affinity for exosomes[69]. Apart from this, emerging evidence has suggested that proteins of the Rab family of GTPases play an important part in the biogenesis and secretion of exosomes[70]. Indeed, Ostrowski *et al.* found that Rab27a or Rab27b silencing reduces MVE docking to the plasma membrane, hence indicating that targeted inhibition of the Rab27 subfamily led to reduced exosome secretion [71]. Moreover, the sphingomyelinase inhibitor GW4869 blocks the production of exosomes through depleting ceramide, which is a part of exosomes production [72]. In ovarian cancer, GW4869 inhibited CD47 overexpressed-exosome secretion in TME and consequently promoted phagocytosis by macrophages[73]. In conclusion, targeted elimination of exosomes as well as reducing exosome production and secretion are useful for inhibiting the communication between cancer cells and other cells, thus making them alternative therapeutic strategies.

6. Conclusions and Perspectives

Over the past two decades, the spectacularly rapid development of molecular biological techniques has magnificently contributed to the breakthrough of discoveries in exosomes. As a part of TME, exosomes exert a pivotal role in tumorigenesis, metastasis, drug resistance, and immune regulation of ovarian cancer. Furthermore, due to their terrific stability in body fluids and pathological features, exosomes have a promising value for both ovarian cancer diagnosis and therapy. Leveraging exosomes as a double-edged sword, novel strategies in ovarian cancer therapy, such as stimulating exosomes secretion or eliminating specific exosomes, are attractive. Nonetheless, on account of the inestimable complexity of exosomes, more studies regarding the involvement of exosomes in ovarian cancer are desperately needed to be met prior to large-scale clinical

utilization.

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Conflicts of Interest

The authors declare no conflict of interest.

Contribution to Authorship

Conceptualization, C.B. and Q.C.; investigation, C.B. and Q.C.; writing—original draft preparation, Q.C. and J.S.; writing—review and editing, Q.C. and D.R.; visualization, J.S. and D.R.; supervision, C.B. All authors have read and agreed to the published version of the manuscript.

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