Exosomal Cargo in Ovarian Cancer Dissemination

Subjects: Oncology

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Ovarian cancer (OC) has the highest mortality rate among all gynecologic cancers and is characterized by early peritoneal spread. The growth and development of OC are associated with the formation of ascitic fluid, creating a unique tumor microenvironment. Understanding the mechanisms of tumor progression is crucial in identifying new diagnostic biomarkers and developing novel therapeutic strategies. Exosomes, lipid bilayer vesicles measuring 30–150 nm in size, are known to establish a crucial link between malignant cells and their microenvironment. Additionally, the confirmed involvement of exosomes in carcinogenesis enables them to mediate the invasion, migration, metastasis, and angiogenesis of tumor cells. Functionally active non-coding RNAs (such as microRNAs, long non-coding RNAs, circRNAs), proteins, and lipid rafts transported within exosomes can activate numerous signaling pathways and modify gene expression.

exosomes exosomal cargo microRNA proteins liquid biopsy ovarian cancer

1. Introduction

Ovarian cancer (OC) ranks among the top ten causes of cancer mortality in women. According to Global Cancer Statistics, there were 313,959 new cases and 207,252 deaths diagnosed worldwide in 2020 [1]. The incidence of OC continues to rise annually, primarily due to the challenges of early diagnosis with only 20% of cases being detectable at stages I–II [2]. The challenge of early OC diagnosis stems not only from its asymptomatic development but also from the prolonged course of nonspecific symptoms, such as asthenic syndrome, weight loss, abdominal pain, urinary symptoms, etc. The primary methods for diagnosing OC and determining the possibility of optimal cytoreduction involve preoperative biopsy, histological analysis, and molecular study of tumor tissues. In cases where obtaining tissue samples is not feasible, clinical manifestations, ascites cytology, measurement of serum tumor marker levels (CA-125, CEA, HE4), and instrumental methods (CT, ultrasound, MRI) are used for diagnosis [3][4]. However, these biochemical markers are not reliable for the early detection of OC due to their limited specificity and sensitivity. Approximately 50–60% of OC cases do not exhibit elevated CA-125 levels [2], and approximately 30% do not show elevated HE4 levels [4][5].

Instrumental methods are commonly employed for OC diagnosis, but their effectiveness in detecting tumors smaller than 10 cm is limited. Transvaginal ultrasonography is the most common diagnostic, offering a sensitivity of 86% and a specificity of 91%. Additionally, it provides accurate visualization of the peritoneum, retroperitoneum, and inguinal lymph nodes, which is crucial for planning cytoreductive surgery [6].

High-field MRI is often used to determine the spread of malignant processes with a sensitivity of 91% and specificity of 88% [7]. One of the most promising instrumental diagnostic methods worth mentioning is positron emission tomography (PET). This method relies on the ability. One of the most promising instrumental diagnostic methods worth mentioning is positron emission tomography (PET). This method relies on the ability of actively dividing tumor cells to accumulate 18F-dGlu, which can be detected by PET [7]. Therefore, PET effectively addresses the issue of visualization of small-sized primary focus, metastases, and lymph nodes [3], and plays a crucial role in planning cytoreductive surgery [8][9]. Its sensitivity and specificity for detecting primary OC tumors are 67% and 79%, respectively. However, for recurrent OC, the sensitivity and specificity increased to 94.5% and 100%, respectively [7][9]. Despite the availability and widespread use of instrumental diagnostic methods, the detection of neoplasms remains a challenging endeavor.

One of the promising methods for early OC diagnosis is the detection of tumor biomarkers in extracellular vesicles (EVs) within biological fluids such as blood plasma and ascites, referred to as liquid biopsy. Liquid biopsy offers a comprehensive view of the molecular profile of the neoplasm since all tumor cells secrete EVs into the extracellular space.

Exosomes, which are lipid bilayer vesicles measuring 30–150 nm, have been shown to carry the tetraspanins CD9, CD63, and CD81 on their surface [10]. Exosomes are produced by all cells in the body, and the content of these vesicles varies depending on the condition of the donor cells. An elevated concentration of exosomes has been observed in tissues and biological fluids of various cancer patients, including those with OC [11][12].

2. Formation and Secretion of Exosomes

The process of exosome formation includes the following steps:

(1) Invagination of the plasmalemma and formation of early endosomes.

(2) Early endosomes mature into multivesicular bodies (MVBs), which contain intraluminal vesicles (ILVs) filled with various proteins, lipids, and nucleic acids. Notably, the cargo composition of ILVs is specific to the parent cell.

(3) Fusion of MVB with the plasmalemma results in the secretion of ILVs into the extracellular space. However, MVBs can also fuse with lysosomes or autophagosomes, leading to the degradation of ILVs [10][13].

Various published sources indicate that the regulation of exosome secretion involves proteins such as T-SNARE, SNAP23, and STX4 [13]. Additionally, the NSF and SNARE complexes play a role in the fusion of MVBs with the plasmalemma [14]. The Rab5 family GTPase is a key regulator of exosome formation and secretion. In particular, the conversion of Rab5 to Rab7, loss of Rab4, Rab11, Rab22, and the attachment of Rab9 are required during the maturation of MVBs [15]. Rab35 and Rab11, in turn, together with the GTPase activator protein TBC1D10A-C regulate the secretion of exosomes into the extracellular space [16].

The ESCRT complexes also play an important role in the formation of exosomes. Several types of these complexes have been identified: ESCRT-0, -I, -II, -III, and Vps4. All complexes closely interact with each other. ESCRT complexes are involved not only in the process of ILVs formation but also in the sorting of biologically active molecules in them. It has been observed that ubiquitinated proteins can be sorted into ILVs, a process that requires ESCRT-0. ESCRT-0, working in conjunction with clathrin-rich sites, captures ubiquitinated proteins and isolates them within the endosomal membrane. The ESCRT-I and -II complexes are involved in the invagination of the MVBs membrane and the formation of vesicles with clusters of ubiquitinated proteins inside. The ESCRT-III complex with its CHMP6 subunit, in turn, promotes the separation from the membrane and the release of vesicles within the MVBs by recruiting the CHMP4 protein. The ESCRT-0 complex requires the HRS protein, which is involved in recognizing monoubiquitinated proteins and sorting them into phosphatidylinositol-3-phosphate-rich endosomal compartments. HRS, together with Tsg101, recruits ESCRT-I [13][17].

An ESCRT-independent pathway of exosome formation has also been shown [18]. According to some studies, ceramides are an important part of the ESCRT-independent pathway due to their involvement in the invagination of the MVBs membrane and the formation of ILVs [15][19]. Ceramides are known to be formed by the action of nSMase from sphingomyelin. During this process, ceramide-rich platforms (CPRs) containing more than 20 ceramides can be formed. Some researchers propose that exosomes might transport CPRs or ‘mobile lipid rafts’ and activate specific signaling pathways in recipient cells that were previously active in donor cells. This mechanism could potentially play a pivotal role in the formation of pre-metastatic niches and carcinogenesis. Inhibition of nSMase has been found to lead to reduced exosome secretion both in vitro and in vivo [19].

Tetraspanins are also involved in exosome biogenesis within the ESCRT-independent pathway. Tetraspanins CD9, CD81, and CD63 are abundantly present in exosomes and are considered key markers of vesicles of exosomal origin [10][11]. CD63 is known to be involved in exosome biogenesis. A recent study showed a decrease in exosome secretion in HEK293 cell culture after CRISPR/Cas9 knockout of CD63. The interaction of CD9 and CD82 with ceramides leads to the enrichment of exosomes with β-catenin, which plays an important role in intercellular adhesion [13][20].

Exosomes have the ability to influence both nearby and distant cells as they spread through tissues via the blood and lymphatic system. As a result of exosomes spreading throughout the body, these vesicles can be found in saliva [21], blood plasma [22][23], ascites [24], urine [25], cerebrospinal fluid [26], breast milk [27], and tears [28]. It is known that 1 mL of blood from healthy donors contains 5–30 × 10^7 exosomes [12][23][29]. Each exosome contains metabolites, lipids, proteins, and nucleic acids (**Figure 1**) [25][30].

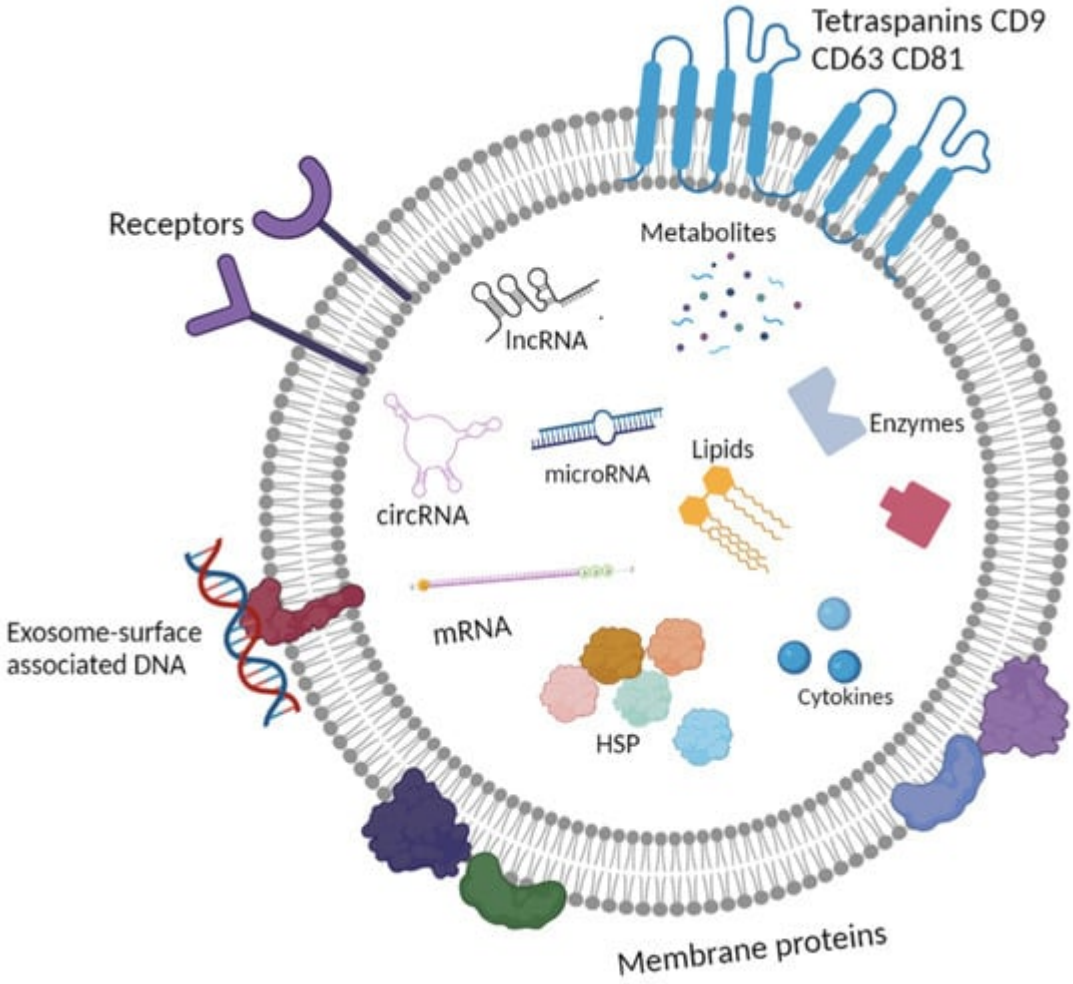

Figure 1. Scheme of the molecular content of the exosomes. Created with BioRender.com, accessed on 1 April 2023.

3. Morphology and Content of Exosomes

The extensive use of transmission electron microscopy (TEM) has confirmed that exosomes are extracellular vesicles (EVs) characterized by low electron density and a cup-shaped morphology [31][32] in both the plasma and ascites of the OC patients (**Figure 2**). Additionally, cryo-electron microscopy has identified subpopulations of exosomes with single-, double-, and multi-layer membranes [31][33][34]. Although fundamental differences in the structure of exosomes in the blood of healthy women and cancer patients have been identified, data specific to OC are currently unavailable.

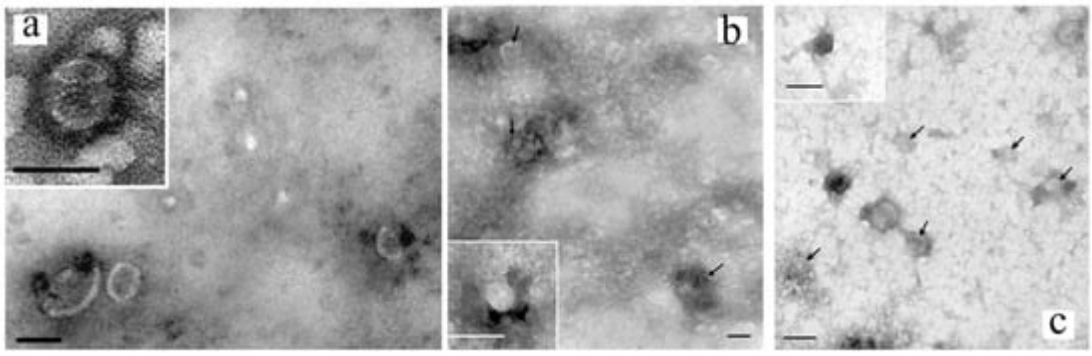

Figure 2. Total view of exosome preparation obtained from blood plasma of HFs (**a**), blood plasma of OCPs (**b**), ascitic fluid of OCPs (**c**). Inserts and arrows show exosomes. Scale bars correspond to 100 nm. Electron microscopy, negative staining using phosphotungstate acid [35].

3.1. Lipidome of Exosomes

The lipid composition of exosomes is very similar to those in the plasma membrane of the parent cell. The curvature of exosomal membranes, which defines their size and biological functions, depends on their lipid content. The most commonly found lipids in exosomes are phosphoglycerolipids, sphingolipids, and sterols [10]. Exosomes exhibit a lipid composition that is notably enriched with phosphatidylserine, disaturated phosphatidylethanolamine, disaturated phosphatidylcholine, sphingomyelin, ganglioside GM3, and cholesterol when compared to their parent cells [36]. It's important to note that the lipid subset of exosomes can vary among different subpopulations. For instance, the lipid composition of the CD61-positive exosomes significantly differs from that of other subtypes [37]. Mass spectrometry analysis of exosomes from 12 healthy donors and blood plasma revealed a higher lipid content in exosomes in comparison to plasma (244 and 191, respectively).

The role of exosomal lipids in carcinogenesis is an area of active research. Lipidomic analysis of exosomes derived from the SKOV3 and HOSEPiC cell culture identified 1227 lipid species and 30 lipid classes. Notably, a significant difference in the lipid content of exosomes from the SKOV3 and HOSEPiC cell cultures was observed. The SKOV3-derived exosomes contained higher levels of GM3, zymosterol ester, lysophosphatidylinositol, lysophosphatidylserine, and cholesterol ester [38]. Exosomal lipids are also considered prospective biomarkers for tumorigenesis.

3.2. Proteome of Exosomes

The tetraspanins CD9, CD81, and CD63 are the most surface proteins on exosomes, making them markers for the exosomal nature of EVs. It is also known that the high heterogeneity of the exosomal proteins is due to the protein composition of parental cells as well as the conditions of their cultivation (hypoxia, acidic environment, etc.). According to the ExoCarta database (www.exocarta.org, accessed on 1 April 2023), which contains data from independent studies on the composition of exosomal proteins, lipids, microRNAs, and mRNAs, 9769 exosomal proteins have been identified as of 1 April 2023. The most common exosomal proteins and their functions are summarized in **Table 1**.

Table 1. Most frequently identified exosomal proteins due to ExoCarta data (as of April 2023).

No	Gene Symbol	Uniprot ID	Function
1	CD9	P21926	Membrane protein. Identified on membranes of oocytes and extracellular exosomes
2	HSPA8	P11142	Chaperone protein
3	PDCD6IP	Q8WUM4	Involved in sorting of cargo proteins of the MVBs for incorporation into ILVs
4	GAPDH	P04406	Modulates the organization and assembly of the cytoskeleton
5	ACTB	P60709	Protein that polymerizes to produce filaments
6	ANXA2	P07355	Calcium-regulated membrane-binding protein
7	CD63	P08962	Cell surface receptor for TIMP1 and plays a role in the activation of cellular signaling cascades AKT, FAK/PTK2 and MAPK
8	SDCBP	O00560	Involved in the trafficking of transmembrane proteins, exosome biogenesis, and tumorigenesis
9	ENO1	P06733	Involved in glycolysis, growth control, hypoxia tolerance, and allergic responses
10	HSP90AA1	P07900	Chaperone protein
11	TSG101	Q99816	The component of the ESCRT-I complex mediates the association between the ESCRT-0 and ESCRT-I complex
12	PKM	P14618	Catalyzes the final rate-limiting step of glycolysis generating ATP
13	LDHA	P00338	Interconverts simultaneously and stereospecifically pyruvate and lactate with concomitant interconversion of NADH and NAD+.
14	EEF1A1	P68104	Translation elongation factor that catalyzes the GTP-dependent binding of aminoacyl-tRNA (aa-tRNA) to the A-site of ribosomes
15	YWHAZ	P63104	Adapter protein implicated in the regulation of a large spectrum of signaling pathways
16	PGK1	P00558	Catalyzes one of the two ATP-producing reactions; acts as a polymerase alpha cofactor protein
17	EEF2	P13639	Catalyzes the GTP-dependent ribosomal translocation step during translation elongation
18	ALDOA	P04075	Plays a key role in glycolysis and gluconeogenesis; scaffolding protein
19	HSP90AB1	P08238	Chaperone protein

No	Gene Symbol	Uniprot ID	Function
20	ANXA5	P08758	Acts as an indirect inhibitor of the thromboplastin-specific complex

Proteomic analysis of exosomes obtained from 10 HGSOC cell cultures revealed 659 universal proteins out of all 1433 identified exosomal proteins [38]. COX2 is one of the most abundant exosomal proteins whose increased expression is associated with hypoxia. Probably, the formation of tumor spheroids and metastasis process are caused by overexpression of this protein [39].

Proteins implicated in carcinogenesis have been identified in the cargo of exosomes obtained from blood plasma and ascitic fluid of OC patients. Specifically, proteins like AFP2, MTA1, ROCK1/2, and CD147 are involved in tumor angiogenesis, while GNA12, EPHA2, and COIA1 promote migration and metastasis. The Nanog protein plays a role in mediating the proliferation and invasion of tumor cells. Exosomes also contain Hsp90 and Hsc70, MHCI, and MHCII. Additionally, various enzymes have been detected in exosomes, such as phosphate isomerase, peroxiredoxins, aldehyde reductase, fatty acid synthase, and Dicer, which is involved in microRNA maturation, among others [40][41][42].

The important role of the PKR1 protein in the initiation of angiogenesis in vitro was demonstrated by the study of tumor-associated exosomes derived from A2780 and HO-8910 cell cultures. The PKR1 signaling pathway can be mediated by phosphorylation of STAT3. When the HUVECs cells were treated with the PKR1-positive exosomes, migration and tube formation were increased compared to controls [42].

It is known that ascitic fluid can be detected in small amounts even in the early stages of OC and then grows, ascites become a key component of the tumor microenvironment. Thus, proteomic analysis of exosomes derived from ascites may improve our understanding of OC dissemination as exosomes provide communication between cells and the tumor microenvironment. It has also been shown that up to 40% of the unique proteins of ascitic fluid are part of exosomes [43].

The formation of premetastatic niches is one of the key mechanisms of OC progression. Several studies have shown that exosomes secreted by the tumor play an important role in this process. Exosomes carry bioactive molecules such as TGFβ, TNFα, interleukins, MMPs, and many others that mediate EMT. Exosomes of cancer-associated fibroblasts are enriched with TGFβ and thus can activate the SMAD pathway in OC cells. This signaling pathway leads to enhanced cell migration and invasion. It's well-documented that OC, like many other solid tumors, often occurs under hypoxic conditions. Hypoxia impacts various stages of carcinogenesis, including the formation and secretion of tumor-associated exosomes. For instance, hypoxia enhances exosome secretion by promoting the fusion of MVBs with the cell membrane [44].

Immunosuppression is crucial for tumor proliferation and metastasis. The ability of exosomes to influence this process in tumor cells has also been shown. For example, exosomes derived from ascitic fluid express the ganglioside GD3 on their surface. This molecule can interact with the T-cell receptor, resulting in the arrest of T

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3.3. Nucleic Acids Transported by Exosomes

In addition to proteins and lipids, exosomes carry functionally active nucleic acids. It has been shown that exosomes have DNA in their crown, but the proportion of such DNA does not exceed 0.025% of blood plasma DNA in healthy women [39]. It is also known that exosomes contain various types of RNA: mRNA, microRNA, long noncoding RNA, rRNA, tRNA, circRNA, etc. [40]. According to the Exocarta database (www.exocarta.org, accessed on 1 April 2023), exosomes are involved in the transport of more than 2838 microRNAs and 3408 mRNAs. Some researchers also consider lncRNAs and circRNAs as promising diagnostic biomarkers for liquid biopsy due to their ability to influence the carcinogenesis of tumors, including OC.

4. Conclusions

Despite the effectiveness of instrumental methods in diagnosing OC, there are several limitations when it comes to detecting stage I disease, identifying cancer in situ, distinguishing between benign and malignant neoplasms, and conducting screening studies of both healthy women and cancer patients following courses of therapy. The high expectations associated with CA-125, CA 19-9, CEA, HE4, and αFP markers for OC detection have also given way to disappointment. To successfully detect neoplasms, there is now a recognized need to discover more sensitive and specific tumor markers for liquid biopsy and explore their combination with instrumental analysis methods. Furthermore, in the realm of modern personalized medicine, there is a pressing need for markers that can effectively predict aggressive tumor behavior and assess the efficacy of anti-cancer therapies. As tumor-secreted exosomes transport biopolymers critical for tumor growth and dissemination, analyzing the cargo of exosomes can provide insights into the molecular mechanisms that stimulate cancer cell proliferation, migration, invasion, angiogenesis, and immunosuppression. Research into the carcinogenic pathways involving proteins and non-coding RNAs (such as lncRNA, circRNA, and microRNA) present in exosomes circulating in the blood of OC patients holds the promise, in the near future, to not only enable the identification of effective markers for disease diagnosis and prognosis but also enhance the assessment of anti-tumor therapy.

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