

1 Title – Detecting Ovarian Cancer Using Extracellular Vesicles: Progress and 2 Possibilities

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16 Abstract

17 Ovarian cancer (OC) is the deadliest gynecological malignancy. Most patients are diagnosed when
18 they are already in the later stages of the disease. Earlier detection of OC dramatically improves
19 the overall survival, but this is rarely achieved as there is a lack of clinically implemented
20 biomarkers of early disease. Extracellular vesicles (EVs) are small cell-derived vesicles that have
21 been extensively studied in recent years. They contribute to various aspects of cancer pathology,
22 including tumour growth, angiogenesis and metastasis. EVs are released from all cell types and the
23 macromolecular cargo they carry reflects the content of the cells from which they were derived.
24 Cancer cells release EVs with altered cargo into biofluids, and so they represent an excellent
25 potential source of novel biomarkers for the disease. In this review we describe the latest
26 developments in EVs as potential biomarkers for earlier detection of OC. The field is still relatively
27 young, but a number of studies have shown that EVs and the cargo they carry, including miRNAs
28 and proteins, can be used to detect OC. They could also give insight into the stage of the disease
29 and predict the likely therapeutic outcome. There remain a number of challenges to the use of EVs
30 as biomarkers, but through ongoing research and innovation in this exciting field there is great
31 potential for the development of diagnostic assays in the clinic that could improve patient
32 outcome.

33 34 Keywords

35 Extracellular vesicles, exosomes, ovarian cancer, miRNAs, diagnosis, liquid biopsy.

36 37 Introduction

38
39 Ovarian cancer (OC) is the 7th most commonly diagnosed cancer in women and the disease causes
40 more than 150,000 deaths around the world each year, making it the deadliest gynecological

41 malignancy^{1, 2}. The 5-year survival for OC patients is less than 50%³, and this is mostly due to the
42 frequently late presentation of the disease (when the cancer has usually already spread to the
43 peritoneal space) and the subsequent acquisition of therapeutic resistance during treatment^{3, 4}.

44
45 OC is a heterogeneous disease that is thought to originate from the epithelium of the ovaries⁵,
46 though recent studies suggest that it may originate from cells shed from the fallopian tube⁶. It can
47 be classified into two groups based on their distinct origin and histological characteristics⁵. The
48 first group, originating from epithelial cells and known as epithelial ovarian cancer (EOC), accounts
49 for almost 90% of all OC cases; this group can be further subdivided on the basis of histological
50 morphology into serous (the majority of these are high-grade serous carcinoma [HGSC] and the
51 rest are low-grade serous carcinoma [LGSC]), mucinous, endometrioid, and clear cell carcinomas⁷.
52 The second group can originate from stromal and germ cells and include non-epithelial ovarian
53 cancers that account for 10% of all OC cases⁸.

54
55 The treatment of most patients with EOC comprises surgical debulking of the tumour mass
56 followed by chemotherapy with platinum-based compounds such as carboplatin, cisplatin and
57 oxaliplatin⁹. Successful debulking is the best correlate for subsequent survival^{10, 11}. The treatment
58 regime for EOC has changed little in the past few decades, with the addition of taxanes such as
59 paclitaxel (which work by inhibition of microtubule function) being the only substantial change to
60 the way in which EOCs are treated⁹. The report that the presence of tumor infiltrating
61 lymphocytes is a good indicator of chemotherapy response and improved survival for OC implies a
62 synergistic effect of immunotherapy and chemotherapy that may benefit some
63 patients¹². Immunotherapy for OC is still limited to clinical trials and only a low proportion of
64 patients appear to benefit clinically, unlike immunotherapy treatment in melanoma which appears
65 to be particularly successful¹³. There is therefore an urgent need for improved therapeutics for OC,
66 but most importantly, an urgent need for the identification of improved high-sensitivity
67 biomarkers to facilitate earlier detection of the disease and to monitor treatment response to
68 standard and new therapy.

69 70 **Current biomarkers for ovarian cancer detection**

71
72 Almost 70% of OC cases are diagnosed at an advanced stage (III or IV) of the disease^{7,14}. Early
73 symptoms of OC, including back pain and digestive problems such as dyspepsia, are general and
74 non-specific and are often related to benign conditions. It tends to be only later, once the less
75 serious causes of symptoms have been eliminated and/or symptoms become more intense or
76 severe, that the cancer is diagnosed¹⁵. Late diagnosis has a significant impact on prognosis;
77 patients that are diagnosed in earlier stages have a five-year survival rate of >70% but this drops
78 to <40% for those diagnosed in later stages^{4, 16}. Transvaginal ultrasonography is an imaging-based
79 approach, which could be used to identify earlier-stage disease, but it is invasive and trials have
80 shown that as a screening tool it only has limited ability to reduce overall survival¹⁷⁻¹⁹. The inability
81 to detect OC at an earlier stage reveals one of the biggest challenges in biomarker research: the

82 need of finding a highly sensitive, non-invasive screening method applicable to an asymptomatic
83 population subject to develop OC during their life.

84

85

86 Due to the high incidence and low survival rates, much research effort has been put into finding
87 potential biomarkers for screening the disease. One of the first OC biomarkers identified was
88 Cancer Antigen 125 (CA125), also known as MUC16²⁰. CA125 is a high molecular weight trans-
89 membrane glycoprotein produced by coelomic epithelium, and thus far it is the most clinically
90 utilised biomarker for monitoring the response to treatment and detecting disease recurrence²¹.
91 When the treatment is successful the level of circulating CA125 can decrease, while increased
92 levels are correlated with drug resistance and disease progression²². Although CA125 is used in the
93 clinic, it has some limitations for the purpose of early detection, as it can be associated with both
94 false positive and false negative results²¹. Non-epithelial ovarian tumours are not associated with
95 increased levels of this mucin, and the various EOC subtypes produce different levels of CA125
96 (levels are higher in HGSC compared to mucinous carcinoma, for example) ²²⁻²⁴. Some studies
97 reported a higher correlation between the FIGO (International Federation of Gynecology and
98 Obstetrics) staging and preoperative CA125 levels, whilst other studies suggest the correlation is
99 less clear-cut^{23, 24}. CA125 can also be detected in other physiological or pathological conditions
100 leading to false positives; for example, it can be raised in the presence of other cancers including
101 cervix, breast, colon and lung cancer²⁵, and it can be altered in inflammatory pathologies²⁶, during
102 the menstrual cycle and in pregnancy^{27, 28}. Moreover, in some cases of EOC the level of CA125 is
103 not raised, and this is particularly true in the early stages of the disease when the presence of a
104 biomarker would be most clinically useful²⁹. CA125, therefore, does not represent the ideal
105 biomarker for robust and diagnostic detection of early OC, and this is confirmed by clinical trials
106 that reveal CA125 as a screening tool lead to only modest (if any) improvements in patient
107 survival^{17, 18}.

108

109 Another biomarker that has been investigated is the Human Epididymis protein 4 (HE4)³⁰. This is a
110 small protein encoded by the *WFDC2* (WAP Four-Disulfide Core Domain 2) gene and secreted by
111 epithelial cells. HE4 is secreted by pulmonary epithelial cells, appears to be involved in sperm
112 maturation, but is also highly expressed in the serum of OC patients when compared with healthy
113 individuals^{30, 31} and it could be used for distinguishing OC from benign disease and to monitor
114 treatment and recurrence³². Unfortunately, like CA125, HE4 has limitations as a biomarker; its
115 expression has been associated with other conditions (especially with endometrial and lung cancer
116 and cystic fibrosis) and different factors (including menstrual cycle, hormone treatment, age and
117 smoking) can modify its expression, leading to false negatives and false positives^{28, 33}.

118

119 Greater predictive power could be achieved by combining different biomarkers. For example, the
120 Risk for Ovarian Malignancy Algorithm combines CA125 and HE4 levels, leading to increases in
121 both sensitivity and specificity and thus diagnostic accuracy³⁵. The Risk of Malignancy Index
122 combines CA125 levels, ultrasound results and menopausal status³⁶. Another multivariate index
123 assay known as OVA1 combines the results of measuring several different proteins alongside

124 CA125³⁷. However, even these multiparametric tests suffer from false positives and negatives and
125 the only definitive way to diagnose patients is during surgery, making these tests unsuitable for
126 routinely screening OC in the female population. Further research is therefore needed to identify
127 other potential biomarkers for earlier detection of OC.

128

129 **Extracellular Vesicles as cancer biomarkers**

130

131 Extracellular vesicles (EVs) are a heterogeneous group of cell-derived submicron vesicles
132 surrounded by a lipid bilayer³⁸. EVs can be broadly classified into three main types depending on
133 the mechanisms of their biogenesis (Fig 1). Apoptotic bodies are considered to be larger EVs
134 (>1000 nm in diameter, though smaller apoptotic vesicles can also be released) produced by
135 apoptotic cells. Microvesicles (MVs) are a class of EVs produced by outward membrane budding of
136 a cell with sizes ranging between 50 nm and 1 μ m. Exosomes are small (30-150 nm in diameter)
137 EVs produced when multivesicular bodies (MVBs) fuse with the plasma membrane leading to the
138 release of the intraluminal vesicles (which, upon release, are then redefined as exosomes).
139 Another class of EVs that is gaining attention in the tumour microenvironment is large oncosomes
140 (LOs). LOs are a big class of EVs (3-4 μ m diameter), mostly originated from highly aggressive tumor
141 cells and characterized by carrying a variety of oncogenic signals^{39, 40}. More recently, the group of
142 David Lyden (Weill Cornell Medicine, New York, US) reported the identification of a new class of
143 EVs, dubbed exomeres⁴¹. These particles, whose origin and mechanism of formation are still
144 unknown, were found using asymmetric flow field-flow fractionation, are smaller than exosomes
145 (<50nm) and were described as the most predominant particle secreted by cancer cells⁴¹.

146

147 EVs were, until recently, thought to primarily perform the role of facilitating the release of
148 unwanted cellular material⁴². While this may be one of their roles, we now know that they can
149 serve a variety of functions⁴³. They play significant roles in many biological processes, including
150 angiogenesis and the immune response⁴³. EVs are released from all cell types and they carry
151 various types of cargo such as long nucleic acids (including mRNAs, lncRNAs and DNA), short
152 nucleic acids (including miRNAs, vault RNAs, tRNAs and YRNAs), proteins, glycoproteins,
153 carbohydrates, metabolites and lipids^{44, 45}. Their biologically active cargo can be transferred into
154 and used by recipient cells, leading to changes in the function of these cells^{46, 47}. EVs are therefore
155 an important part of the signaling dialogue that occurs between cells. Many of the EVs produced
156 by cells can make their way into biofluids, including saliva, urine, cerebrospinal fluid, blood,
157 semen, sweat and tears⁴³. The molecular composition of EVs partially reflects the molecular
158 landscape of the parental cell; given that this landscape can be altered in cancer, and that EVs can
159 reach biofluids, these vesicles could serve as an easily accessible window into the state of a
160 tumour⁴⁸. Indeed, the very presence in biofluids of vesicles carrying cancer-related cargo could be
161 a diagnostic indicator that a tumour is present.

162

163 One approach that is often taken is to identify potential vesicular biomarkers in cancer patients is
164 to initially use cultured cancer cell lines as a proxy. The cargo (miRNAs in particular) of EVs

165 released by cancer cell lines (compared to non-cancer cell lines) can be profiled using relatively
166 unbiased techniques (such as microarrays or RNA-seq); transcripts that are identified as altered in
167 cancer cell-derived EVs can then be measured in biofluids to see if they are also de-regulated in
168 patients⁴⁸. However, one potential issue is that, even though the foetal bovine serum that the
169 cultured cells grow in has been pre-cleared of vesicles, it still contains bovine-derived EVs carrying
170 bovine miRNAs⁴⁹. Directly testing biofluids using unbiased techniques may, therefore, be
171 preferable as a method for identifying suitable EV biomarkers; however, working with EVs
172 presents several technical challenges and there are a number of pre-analytical variables that affect
173 the interpretation of the results of such experiments^{50, 51}. Further work is also needed to establish
174 the most reliable EV isolation strategy for the analysis of vesicular cargo in biofluids⁵².

175

176 Despite these barriers, there is great excitement for the potential use of EVs as biomarkers to
177 inform clinicians not just on the *presence* of tumours but also on the *state* of the tumour and its
178 microenvironment^{48, 53}. EVs can play an active role in the pathology of cancer, contributing to an
179 increase in various undesirable phenotypes such as metastasis⁵⁴, angiogenesis⁵⁵ and drug
180 resistance⁵⁶. EVs released by stressed cells (including stress induced by the chemotherapeutic
181 agent cisplatin) are able to induce a range of effects in neighbouring tumour cells, including
182 increased invasion, bystander DNA damage and an adaptive response⁵⁷⁻⁵⁹. miRNAs are short non-
183 coding RNAs that can repress the expression of multiple genes, meaning that changes in their
184 levels can lead to substantial phenotypic effects in a cell⁶⁰. They are known to be involved in stress
185 response⁶¹ and in mediating drug resistance in ovarian cancer⁶²⁻⁶⁴. miRNAs can also contribute to
186 other cancer phenotypes, including migration and angiogenesis⁶⁵. For these reasons, the miRNA
187 content of EVs is of particular interest as a potential diagnostic in cancer⁴⁸. A growing body of
188 literature describes the early attempts to capitalize on EV cargo as a biomarker in a variety of
189 cancer types. In the following section we will review current work investigating EVs as biomarkers
190 in OC (Table 1).

191

192 **EVs as biomarkers in ovarian cancer**

193

194 Early forays into EV biomarker research in OC focused on simply counting the number of vesicles
195 in circulation. Cancer cells produce more EVs when compared with normal cells, and this could be
196 related to specific conditions in the tumour microenvironment⁶⁶. The levels of circulating EVs were
197 seen to be elevated in patients with EOC and these may correlate with disease stage^{67, 68}.

198

199 For the reasons described in the previous section, the detection of circulating vesicular RNA,
200 particularly miRNA, could be used as a biomarker for OC. In addition, the RNA is protected from
201 degradation whilst encapsulated in vesicles, and sensitive techniques can be used to amplify
202 specific targets from relatively few copies of the nucleic acid, allowing a global view of the
203 complex RNA landscape in these organelles^{69, 70}. Many studies have analysed changes of miRNA
204 levels in biofluids such as plasma or serum, but it is not always clear whether these changes
205 represent free circulating miRNAs or those encapsulated in EVs; a more comprehensive review of

206 non-vesicular biomarkers in OC has been previously published⁷¹. Here we will focus on studies
207 where EVs have been specifically investigated.

208

209 In one early study it was shown that certain miRNAs (including miR-21, miR-141, miR-200a/b/c
210 and miR-214) were more highly expressed in circulating EVs from OC compared to patients with
211 benign disease⁶⁸. In another study the level of miR-200a/b/c and miR-373 were shown to be
212 elevated in circulating EVs of EOC patients⁶⁷. Correlations with stage and lymph node involvement
213 were observed for miR-200a and miR-373, while lower overall survival correlated with levels of
214 miR-200b/c⁶⁷. EV miRNAs were measured in peritoneal or pleural effusions and the level of a
215 combination of miRNAs was correlated with stage, progression free survival and overall survival⁷².
216 An expression signature of eight miRNAs circulating in serum was also shown to distinguish
217 healthy control from patients with OC⁷³. Most of these miRNAs were shown to be vesicular when
218 released by cell lines and xenografts⁷³. In another study the levels of miR-21 were elevated in
219 ovarian carcinoma EVs⁷⁴. Pan *et al* showed that the levels of a number of miRNAs are altered in
220 the plasma EVs of ovarian cancer patients. Interestingly, the levels of miR-200b correlated with
221 CA125 and overall survival⁷⁵. Urine could also be used as a source of diagnostic EVs. In one study
222 the levels of miR-30a-5p were elevated in the urine of ovarian cancer patients, and this miRNA
223 was shown to be found in EVs⁷⁶.

224

225 Proteins in EVs have also been investigated as potential OC biomarkers. Interestingly, CA125 has
226 been identified in EVs and its vesicular levels were higher than freely circulating CA125 plasma
227 levels at an earlier stage, suggesting that studying CA125 vesicular levels instead of freely
228 circulating serum CA125 could be used to detect OC earlier⁷³. Another study demonstrated that
229 the expression of Claudin 4 protein in EVs obtained from plasma of OC patients was positively
230 correlated with tumor stage, with a sensitivity of 51% and specificity of 98%⁷⁸. Furthermore, the
231 dual measurement of CA125 and Claudin4 inside EVs could be used as a new combination
232 biomarker, although further validation experiments need to be performed⁷⁸. Patients with stage I
233 EOC had a low level of circulating CA125 but high levels of Claudin 4, suggesting that relative levels
234 of the two could be informative⁷⁸. It has been shown that EVs derived from OC patients' plasma
235 contain increased levels of TGF β 1 and melanoma associated antigen 3 (MAGE3) compared with
236 patients with benign disease, suggesting they could serve as potential biomarkers to distinguish
237 between malignant and benign patients⁷⁹. The levels of EpCAM, ADAM10 and EMMPRIN have also
238 been shown to increase in EOC^{68, 80}. Zhang *et al* developed a microfluidic device combined with
239 ELISA to detect EVs; with this device they show that in a small cohort of patients the levels of
240 EpCAM in plasma EVs from OC patients was higher compared to controls⁸¹. CD24 has been
241 associated with poor prognosis in OC and it is highly enriched in EVs from ascitic fluid⁸², with most
242 of the CD24 positive exosomes being secreted by tumour cells⁸². Combined measurement of
243 vesicular EpCAM and CD24 can distinguish between patients that are responsive or non-
244 responsive to therapy⁸³. Zhao *et al* developed a microfluidic device named 'ExoSearch Chip' to
245 isolate serum exosomes that contain CA125, EpCAM and CD24. When EVs were isolated using
246 antibodies against CA125 it was noted that patient samples contained a greater amount of EVs
247 compared to healthy controls⁸⁴. Similarly, another microfluidic-based platform was used to show

248 that the number of EpCAM+ EVs is correlated with disease progression in OC⁸⁵. In another study,
249 soluble E-cadherin was found to be released with EVs into ascitic fluid and the levels were able to
250 distinguish between OC and benign disease⁸⁶. Taken together, these results suggest that vesicular
251 proteins have promising potential as biomarkers and that they could be potentially used as point
252 of care testing (POCT) as quick, cheap and sensitive technique that could help to overcome the
253 challenges related with early diagnosis.

254
255 Other EV-associated molecules can also be used as biomarkers. A recent study published by Lea *et*
256 *al* developed an ELISA assay that can detect and bind picogram-levels of phosphatidylserine (PS)-
257 containing EVs from the plasma of OC patients, and, based on the difference in the number of PS-
258 positive EVs, it can differentiate between malignant and benign disease⁸⁷. The use of single EV-
259 methods that can distinguish between subpopulations of vesicles may also be useful in identifying
260 cancer-specific EVs⁸⁸. In another study, microvesicle-associated tissue factor procoagulant activity
261 was able to distinguish between plasma from OC patient and healthy controls⁸⁹.

262

263 **Future perspective**

264 EVs are not the only potential source of non-invasive circulating biomarkers for detecting and
265 monitoring cancer. In particular, the use of cell-free DNA, circulating tumour DNA and circulating
266 tumour cells have shown both diagnostic and prognostic utility in the diagnosis and monitoring of
267 OC^{90, 91}.

268

269 Although EVs have gained a lot of attention in recent years as a potential biomarker for OC and
270 other cancer types, there is still much work required before their potential can be realised.

271

272 There remain many challenges associated with working with EVs^{48, 50, 52}. There are several
273 methods for extracting EVs but no universal agreement on which technique is most appropriate
274 for diagnostic purposes. The most commonly used techniques are ultracentrifugation (UC) and size
275 exclusion chromatography (SEC), but both of these approaches lead to the enrichment of EVs with
276 different biofluid impurities including soluble proteins and lipoproteins. Combining
277 methodologies, such as SEC and density cushion centrifugation can help to remove lipoproteins
278 and therefore improve EV purity^{52, 92}. Antibody-bound magnetic beads that are specific to EV
279 markers such as CD63 can be used to isolate vesicles and enhance purity⁹². Methods that rely on
280 polyethylene glycol-based precipitation result in much lower EV purity but give higher yields⁹³;
281 miRNA/protein biomarkers discovered using such methods cannot be definitively attributed as
282 vesicular (unless further validation is performed) but if the biomarker is reproducibly and robustly
283 altered in OC then it has value. Microfluidic approaches for EV isolation are being developed that
284 can be combined with novel sensor technologies^{81, 84, 85}; these approaches may be less useful for
285 biomarker discovery (due to the smaller scale of material that they produce) but could be used
286 effectively to detect differences in specific EV cargo in ovarian cancer patients. Further research is
287 needed to develop isolation and detection technology, and to assess the effect of isolation
288 methodology on EV purity.

289

290 Purifying EVs from different biofluids presents various challenges⁹⁴, and there are many pre-
291 analytical variables that can affect the measurement of biomarkers such as vesicular miRNA and
292 proteins⁵¹. These include the method of biofluid collection, time of day, whether the patient has
293 fasted and the presence of other conditions. These factors could affect biomarker discovery and
294 the testing of biomarkers in a clinical setting. More work is therefore required to establish the
295 effect of pre-analytical variables on the detection of EV cargo and robust procedures must be put
296 in place for diagnostic applications.

297

298 Another challenge to quantifying EV cargo is choosing the most appropriate 'reference gene'. It is
299 not clear, at present, which proteins or RNAs are most appropriate as a reference for
300 normalisation. The normalisation of expression of RNAs or proteins of interest can therefore be
301 problematic, with outcomes depending on the choice of reference. More studies are needed to
302 identify EV content whose levels are the most stable in different conditions and between different
303 individuals.

304

305 EVs in any given biofluid are released from a variety of cells. A tumour may contribute EVs to this
306 heterogeneous population, and the proportion of tumour-derived vesicles will increase as the
307 disease progresses. The ability to detect a smaller number of cancer EVs in this sea of normal EVs
308 depends on several factors, including the nature of extraction methodology, the sensitivity of the
309 detection assay and the 'normal levels' of the EV-cargo being measured. In the ideal test, the EV-
310 cargo being detected would be absent in normal biofluid but present in high levels after the
311 appearance of a tumour. The test could be run on a small amount of biofluid at the point-of-care
312 and be sufficiently sensitive to pick up very small numbers of cancer-derived EVs. As the sensitivity
313 of EV-based detection methods increase it may be possible to move beyond the use of a test to
314 monitor treatment/relapse towards a true diagnostic test for early-stage OC in asymptomatic
315 individuals. Ideally, the collection of this biofluid would be minimally invasive for the patients and
316 part of their routine disease follow-up (such as blood collection, for example). Whilst
317 improvements are being made in all these areas, this hypothetical test does not currently exist.
318 Further work is necessary to identify novel potential biomarkers in EVs and develop the
319 technology required to isolate and detect them. Exciting progress is being made in this area which
320 we hope will allow us to unlock the potential of EVs for earlier diagnosis of ovarian cancer in the
321 near future.

322

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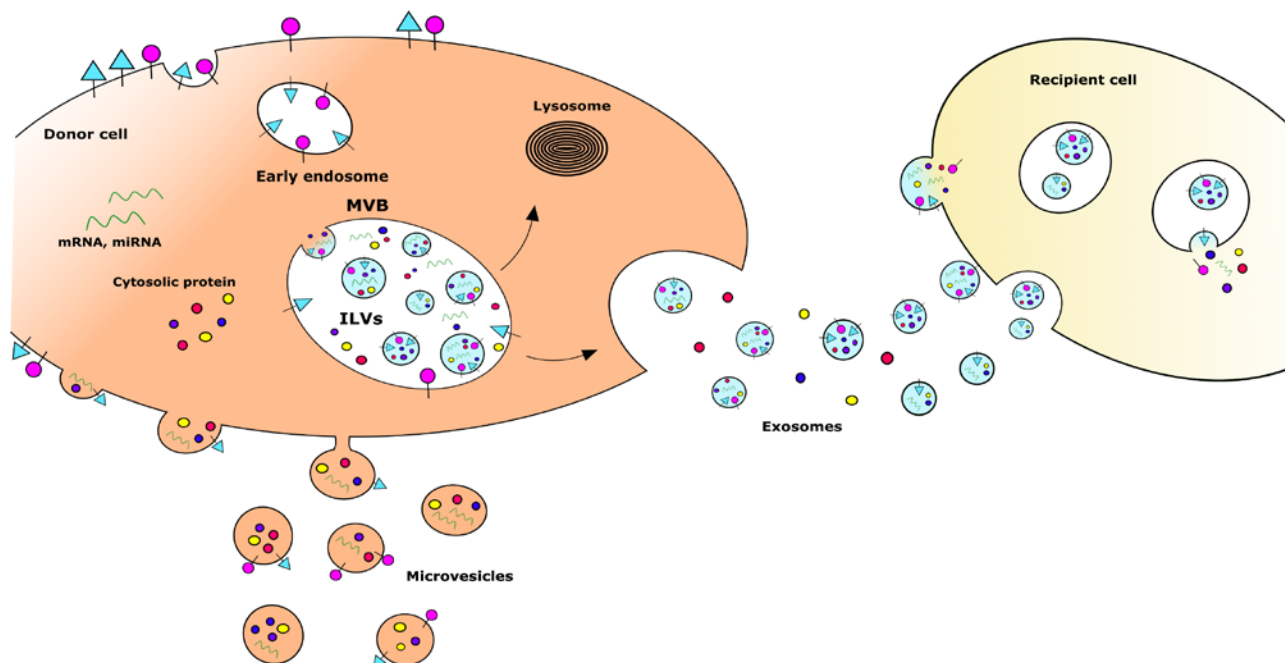
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643 Figures/Tables

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645 Figure1 – Illustration of biogenesis pathways for EVs. Microvesicles (MVs) are directly released by
 646 outward budding of the plasma membrane. The precursors for exosomes are formed inside
 647 multivesicular bodies (MVBs) as intraluminal vesicles (ILVs). MVBs can fuse with the lysosomes
 648 leading to the degradation of their content or fuse with the PM leading to the release of exosomes
 649 into the extracellular space. Exosomes can then be taken up by recipient cells through different
 650 pathways leading to the transfer of their cargo and potentially modifying the behavior of recipient
 651 cells.

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655 Table1 – EV components identified in EOCs patients that could be used as potential biomarkers for
 656 diagnosis or prognosis of ovarian cancer patients.

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Biomarker type	Candidate	Sample source	Comment	Reference
Proteins	CA125	Ascites	Vesicular CA125 higher than circulating CA125 in early stage	Peng P <i>et al</i> 2011 ⁷⁷
	Claudin 4	Plasma and cell lines	Abundant in EVs from plasma of OC patients in late stages (potential use in combination with CA125)	Li J <i>et al</i> 2009 ⁷⁸
	TGFβ1 and MAGE3	Plasma	Higher in plasma from OCs patients. Total EV-protein concentration decreased in responsive patients after treatment	Szajnik M <i>et al</i> 2013 ⁷⁹
	ADAM10, EpCAM and EMMPRIN	Ascites and serum	Ascites EVs correlates with serum EV levels and carry late stage disease markers	Keller S <i>et al</i> 2009 ⁸⁰ , Taylor D and Gercel-Taylor 2008 ⁶⁸
	CD24	Ascites and cell lines	High CD24 is associated with poor prognosis in OC and is released in tumour-derived EVs	Runz S <i>et al</i> 2007 ⁸²
	CD24 and EpCAM	Ascites	Expression is associated with chemotherapy response	Im H <i>et al</i> 2014 ⁸³
	EpCAM	Serum	EpCAM positive EVs increase in plasma of stage IV patients	Hisey CL <i>et al</i> 2018 ⁸⁵
	E-cadherin	Ascites and non-cancer ovarian cell line	Can help distinguish between healthy and benign disease	Tang MKS <i>et al</i> 2018 ⁸⁶
miRNA	miR-21, miR-141, miR-200, miR-214	Serum	Levels of these miRNAs were similar in the EVs and tumour cells and were predictive of disease stage	Taylor D and Gercel-Taylor 2008 ⁶⁸

	miR-200 a/b/c and miR-373	Serum	Higher levels in EOC patients. miR200 specific for malignant disease. miR200b/c higher in patients with stage III-IV disease and are associated with CA125 level	Meng X <i>et al</i> 2016 ⁶⁷
	miR- 21, miR- 23b and miR- 29a	Ascites and pleural effusion	Associated with poor survival	Vaksman O <i>et al</i> 2014 ⁷²
	miR-142-3p, miR-26a-5p, let-7d-5p, miR-374a-5p, miR-766-3p, miR-200a-3p, miR-328-3p, miR-130b-3p	Serum	Specific for early-stage. Most of these found in EVs	Yokoi A <i>et al</i> 2017 ⁷³
	miR-21	Ascites	Up-regulated in malignant cells and tumour-derived EVs	Cappellesso R <i>et al</i> 2014 ⁷⁴
	miR-200b and miR-320	Plasma	EV levels higher in stage IV patients. Both miRNA are higher in patients compared with healthy controls and they are positively correlated with CA125	Pan C <i>et al</i> 2018 ⁷⁵
	miR-30a-5p	Urine	Higher in urine of ovarian cancer patients, particularly in stage I and II	Zhou J <i>et al</i> 2015 ⁷⁶
Other	Phosphatidylserine (PS)	Plasma and cell lines	Higher in cancer-derived EVs	Lea J <i>et al</i> 2017 ⁸⁷
	Microvesicle-associated TF procoagulant activity	Plasma	Improved diagnostic benefit when combined with CA-125 levels	Claussen C <i>et al</i> 2016 ⁸⁹