REVIEW

The potential role of miRNAs and exosomes in chemotherapy in ovarian cancer

Mona Alharbi¹, Felipe Zuñiga², Omar Elfeky¹, Dominic Guanzon¹, Andrew Lai¹, Gregory E Rice^{1,3,4}, Lewis Perrin^{5,6}, John Hooper^{5,6} and Carlos Salomon^{1,2,3}

¹Exosome Biology Laboratory, Centre for Clinical Diagnostics, University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital, The University of Queensland, Brisbane Queensland, Australia

²Department of Clinical Biochemistry and Immunology, Faculty of Pharmacy, University of Concepción, Concepción, Chile

³Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Ochsner Clinic Foundation, New Orleans, Louisiana, USA

⁴Perinatology Research Branch, NICHD/NIH, Wayne State University, Detroit, Michigan, USA

⁵Mater Research Institute, University of Queensland, Translational Research Institute, Woolloongabba, Queensland, Australia

⁶Mater Ovarian Cancer Research Collaborative, Mater Adult Hospital, South Brisbane, Queensland, Australia

Correspondence should be addressed to C Salomon: c.salomongallo@uq.edu.au

Abstract

Chemoresistance is one of the major obstacles in the treatment of cancer patients. It poses a fundamental challenge to the effectiveness of chemotherapy and is often linked to relapse in patients. Chemoresistant cells can be identified in different types of cancers; however, ovarian cancer has one of the highest rates of chemoresistance-related relapse (50% of patients within 5 years). Resistance in cells can either develop through prolonged cycles of treatment or through intrinsic pathways. Mechanistically, the problem of drug resistance is complex mainly because numerous factors are involved, such as overexpression of drug efflux pumps, drug inactivation, DNA repair mechanisms and alterations to and/or mutations in the drug target. Additionally, there is strong evidence that circulating miRNAs participate in the development of chemoresistance. Recently, miRNAs have been identified in exosomes, where they are encapsulated and hence protected from degradation. These miRNAs within exosomes (exo-miRNAs) can regulate the gene expression of target cells both locally and systemically. Exo-miRNAs play an important role in disease progression and can potentially facilitate chemoresistance in cancer cells. In addition, and from a diagnostic perspective, exo-miRNAs profiles may contribute to the development of predictive models to identify responder and non-responder chemotherapy. Such model may also be used for monitoring treatment response and disease progression. Exo-miRNAs may ultimately serve as both a predictive biomarker for cancer response to therapy and as a prognostic marker for the development of chemotherapy resistance. Therefore, this review examines the potential role of exo-miRNAs in chemotherapy in ovarian cancer.

Key Words

- ▶ ovarian cancer
- ▶ chemoresistance
- ▶ exosomes
- ▶ miRNA

Endocrine-Related Cancer
(2019) **25**, R663–R685

Introduction

Ovarian cancer is the leading cause of gynecological-related deaths and the fifth leading cause of cancer mortality in women worldwide (Malvezzi *et al.* 2016,

Wang *et al.* 2018). Due to the lack of overt symptoms, the majority of patients are diagnosed with advanced-stage disease (Maringe *et al.* 2012). Primary cytoreductive

surgery followed by platinum-based chemotherapy has been the cornerstone of treatment and the absence of residual tumor after cytoreduction considered the most important prognostic factor (Mandić *et al.* 2001, Gerestein *et al.* 2009, Di Donato *et al.* 2017). Recent clinical guidelines, however, recommend the use of neoadjuvant chemotherapy and interval cytoreduction among women with stage IIIC or IV epithelial ovarian cancer (Wright *et al.* 2016). Despite these advances, current treatments become ineffective after a few cycles of administration and disease recurrence is estimated to be around 80–85% (Foley *et al.* 2013, Siegel *et al.* 2013). Recurrent disease usually presents as distant metastases involving other organs (such as, the bowel, bladder, liver, etc.) (Lengyel 2010) and an overall 5-year survival rate of only 20% (Sharma *et al.* 2017). The challenge remains in the diagnosis of the disease at a stage when treatment increases survival. While longitudinal multimodal screening offers some advances in this area (Jacobs *et al.* 2016), predictive biomarkers of early stage disease may further improve survival rates (Yap *et al.* 2009).

The initial issue that results in the poor overall survival rate is that cancer cells can communicate and propagate oncogenic information in their local microenvironment and beyond, which results in cancer progression and metastasis. At the beginning, cancer cells activate the endogenous oncogenic pathways and prompt the cells to proliferate in their local niches. Nevertheless, at some point, these cells recruit endogenous secretions, such as extracellular vesicles (EVs), to expand communication beyond the local microenvironment. The alteration in the vital pathways of recipient cells can result in disease metastasis and cancer progression. Although the underlying mechanism is not yet understood, it is suggested that EVs, in particular exosomes, play an important role in cell–cell communication (Escrevente *et al.* 2011).

Exosomes have distinguishing features that differentiate them from other EVs, including their endosomal origin and nano size of approximately 100 nm (Tomasetti *et al.* 2017). More importantly, exosomes shuttle unique compositions of molecular cargo that are representative of origin cells (Tomasetti *et al.* 2017). In the context of cancer progression and metastasis, exosomes are able to transfer oncogenic molecules, including miRNAs, mRNAs and proteins, from parent cells to contiguous organs and distal sites. Since exosomes are present in biological fluids, they are of significant clinical relevance and are highlighted as a potential source of diagnostic aid and for monitoring response to treatment. Exo-miRNA molecules, in particular, are resistant to

RNase and stable in biological fluids; therefore, circulating exosomes in plasma are proposed to provide a unique snapshot of oncogenic miRNAs secreted within exosomes from resistant cells and could be an ideal source for cancer prognosis *via* minimal invasive procedures (Joyce *et al.* 2016).

This review focuses on the potential role of miRNAs, in particular, exo-miRNAs in cancer progression and chemotherapy resistance in ovarian cancer. In particular, we will summarize the current knowledge on the relationship between exo-miRNAs and the regulation of chemotherapy resistance in patients with ovarian cancer.

Ovarian cancer

Despite current advances in research, ovarian cancer remains the leading cause of gynecological mortality (Mezzanzanica 2015). Ovarian cancer is a heterogeneous disease differentiated on biological behavior of tumors and associated risk factors (Rosen *et al.* 2009) and is classified into three broad subgroups: stromal, germ and epithelial cell tumors. Stromal cell tumors develop from the connective tissue surrounding the ovary and make up approximately 7% of all cases (Lancaster 2010). Ovarian germ cell tumors arise from germ cells in the ovary and are rare, accounting for less than 3% of all ovarian cancers (Lancaster 2010). Epithelial cell tumors are the most prevalent subtype, accounting for 90% of all cases (Lancaster 2010) and can be grouped into type I and II tumors. Type I cancers include clear cell, mucinous, endometrioid and low-grade serous and are often diagnosed at early stages of the disease, whereas type II cancers, including high-grade serous, are typically diagnosed at advanced stages of the disease and are associated with poor survival rates (Lim *et al.* 2016).

The high rate of ovarian cancer mortality can be attributed to the fact that symptoms are often dismissed, resulting in an inability to detect the disease at an early stage (Rossing *et al.* 2010). Patients with early-stage ovarian cancer often experience vague symptoms, including pelvic pain, abdominal pain, early satiety and increased abdominal size, all of which may not necessarily indicate a gynecological cause (Goff *et al.* 2000). Thus, concrete indicators do not exist until the disease has metastasized throughout the abdomen, and 75% of cases are often diagnosed at advanced stages (i.e., stages III and IV) (Mandić *et al.* 2001, Jelovac & Armstrong 2011). The high mortality rate of ovarian cancer can largely be associated to late diagnosis (Su *et al.* 2013) and resistance to chemotherapy (Ling *et al.* 2005).

Chemotherapy treatment

Surgical tumor de-bulking and chemotherapy are the most common treatments for ovarian cancer. The current standard of chemotherapy choice for women with ovarian cancer is to use a platinum agent with a taxane, for example carboplatin plus paclitaxel or cisplatin and/or docetaxel (Vasey 2003). Patients initially respond well to these treatments, but the majority relapse within 18 months, often due to chemoresistance (Foster *et al.* 2013). Such resistance becomes apparent when patients are re-exposed to chemotherapy treatments after a disease relapse. Although the underlying mechanisms leading to chemoresistance remain unclear, several factors have been associated with chemotherapy failure (Liu *et al.* 2012), including heterogeneous tumor cells (Kroeger & Drapkin 2017), genetic instability or epigenetic alterations (Meinhold-Heerlein & Hauptmann 2014) and the tumor microenvironment (Castells *et al.* 2012). Only 10–20% of patients respond to re-administered chemotherapeutic agents (Liu *et al.* 2012) and have a 10-year patient survival rate of ~10–15% (Adams *et al.* 2005).

Chemotherapy resistance associated mechanisms in ovarian cancer

Cancer cells exposed to chemotherapeutic agents can be classified as either sensitive or resistant cells (intrinsic or extrinsic resistance). Cells with intrinsic (*de novo*) resistance have the capacity to resist chemotherapeutic agents from inception. Contrastingly, extrinsically resistant cells acquire the resistance phenotypes after exposure to several cycles of treatment (Gottesman 2002). To understand the underlying mechanisms involved in the development of resistance, it is imperative to identify the pathways through which anti-cancer drugs function. One of these pathways is the association between anti-cancer drugs and epigenetic mutation in cells. This relationship has been implicated in the development of acquired resistance phenotypes (i.e. impairing the synthesis of, or damaging, DNA and/or mutations in the mitotic spindles of cells undergoing mitosis) (Liu *et al.* 2012). Epigenetic mutations also often lead to alterations in the DNA sequence, including modification, deletion, amplification and translocation of nucleotide bases. Upon undergoing these changes, cancer cells demonstrate resistant phenotypes and aggressive behavior.

The response of cancer cells to chemotherapeutic agents depends on the activation and deactivation of a series of signaling pathways that produce: (i) increased

tolerance to DNA lesions and the development of DNA repair mechanisms, (ii) increased efflux of the anti-cancer agents leading to reduced build-up of chemotherapeutic drugs within the cell, (iii) increased detoxification of the drugs within the cellular environment and (iv) mutations in B-tubulin (Gatti & Zunino 2005, Fodale *et al.* 2011). The molecular mechanisms involved in these phenomena are presented in Figs 1 and 2 and are discussed in detail below.

DNA damage response

Chemotherapy drugs, such as platinum, cause irreversible DNA damage. As illustrated in Fig. 1, after carboplatin enters a cell, a cytosol activation cascade is initiated by mitochondrial cytochrome c release. Release of cytochrome c from the intermembrane space into the cytoplasm results in the proteolytic activation of procaspase-9 to active caspase-9. Proteolytic cleavage of procaspase-3 by active caspase-9 then activates caspase-3. The active caspase-3 cleaves several substrates, resulting in characteristic morphological changes in the nucleus and DNA fragmentation, which triggers apoptotic pathways (Wisnovsky *et al.* 2013). Carboplatin also causes DNA damage, as it interacts with DNA and forming crosslinks in the DNA, that lead to inhibition of DNA synthesis and structural disruption. The configuration of the DNA is recognized by the mismatch repair (MMR) system that induces the expression of P53 and mediates the apoptosis pathway (Sousa *et al.* 2014). Cancer cells can subvert these effects by the activation of the DNA damage response (DDR) to prevent apoptosis (Bonanno *et al.* 2014). The DDR mechanism involves tumor suppressor P53, pro-arrest gene *P21* (downstream of P53) (Abbas & Dutta 2009, Zhang *et al.* 2012) and nuclear factor kappa B (NF-KB) (McCool & Miyamoto 2012). The major regulator of the DDR mechanism, however, is P53 (Petitjean *et al.* 2007).

P53 is a tumor suppressor protein that responds to cellular stress and can induce a number of different responses including cell cycle arrest, cell death, regulation of mitochondrial respiration and the DNA repair mechanism (Liu & Xu 2011). Under normal cellular conditions, P53 expression is low, and it is negatively regulated by MDM2 (Wu *et al.* 1993). Expression increases in response to cellular stress or following DNA damage (Kastan *et al.* 1991). Under conditions of low stress, P53 activates the expression of genes that drive a temporary cell cycle arrest, that allows cells to eliminate intracellular reactive oxygen species (ROS) and repair damage (Kastan *et al.* 1991, Bensaad & Vousden 2005). In contrast,

Figure 1

Mechanisms associated with the action of carboplatin on cells. (A) *Sensitive cancer cells*: Carboplatin can cross the cell membrane by passive diffusion via lipids or active transport using ion channels (such as the copper influx transporter (CTR1)) (Sousa *et al.* 2014, Safaei & Howell 2005). (B) *Resistant cells*: Once cells become resistant to carboplatin, cross membrane transport of carboplatin becomes limited via rapid downregulation in the expression of CTR1, other transporters and cholesterol levels whereas cell membrane rigidity increases (Stewart 2007, Sousa *et al.* 2014). A resistant cell increases levels of heavy metal detoxification proteins in its cytoplasm. These proteins, such as glutathione and metallothionein, are rich in the sulfur-containing amino acids cysteine and methionine. Platinum is chelated by glutathione (GSH). This conjugation is catalyzed by glutathione S-transferases (GSTs) and effluxes via an ATP-dependent mechanism, such as the glutathione S-conjugate export (GS-X) pump (Kelland 2007, Sousa *et al.* 2014). The resistant cells also increase the sequestration of metals into lysosomes, mitigating the effects of platinum therapy (Holmes 2015, Zhitomirsky & Assaraf 2016).

when the cells are exposed to acute stress, P53 initiates programmed cell death. The transcriptional targets of P53 include a number of pro-apoptotic proteins such as the BH3-only proteins that target NOXA and PUMA (Ghosh *et al.* 2009, Elkholi *et al.* 2011). Decreased expression of PUMA or NOXA reduce protection against DNA damage and induce apoptosis (Villunger *et al.* 2003). Furthermore, P53 functions by inducing the loss of the inner mitochondrial membrane. Subsequently, this damage leads to the release of cytochrome c and other proapoptotic and apoptogenic factors, caspase cascade activation and apoptosis/cell death (Marchenko *et al.* 2000). Loss of the WT P53 or a mutation in P53 (MutP53) in cancer cells leads to an elevation in intracellular ROS thus causing an increase in the overall mutation rate, uncontrolled cellular proliferation and chemoresistance

(Bensaad & Vousden 2005, Vogiatzi *et al.* 2016). Cancer cells resist carboplatin by increasing the expression of anti-apoptotic genes and the loss of DNA MMR and decreasing pro-apoptotic factors, such as downregulation of P53 and caspase-9. Evidence shows that loss of MMR proteins is associated with drug resistance in ovarian cancer (Sousa *et al.* 2014). MutP53 is also involved in the activation of various mechanisms relating to chemoresistance including promoting drug efflux, resistance to apoptosis signaling, activation of survival signals and upregulation of the DNA repair mechanism (He *et al.* 2017).

Efflux pump proteins

Efflux pump proteins are transmembrane proteins that reduce intracellular drug accumulation by transporting

Figure 2

Mechanisms associated with the action of paclitaxel in cells. (A) *Sensitive cells*: Paclitaxel can be up-taken by cancer cells via passive diffusion and/or high binding affinity to intracellular binding sites (e.g. microtubulin) (Jang *et al.* 2001). Paclitaxel has been shown to activate MAPK through Toll-like receptor 4 (TLR4)-myeloid differentiation gene 88 (MyD88) signaling; this in turn causes mitotic arrest at G2/M and thus induces the activation of BCL-2 (apoptosis regulator protein) and cell death (Kampan *et al.* 2015). Paclitaxel can induce DNA damage and increase p53 levels; this can then activate a G1 cell-cycle arrest mediated by P21, to promote apoptosis (Kampan *et al.* 2015). (B) *Resistant cells*: Cancer cells can develop a number of molecular mechanisms that can contribute to paclitaxel resistance. Reference page 9 of text.

drug molecules out of the cell, thus decreasing their cytotoxic effects (Sauna & Ambudkar 2001). One such efflux pump protein is P-glycoprotein (PGP), also known as the ATP-binding cassette sub-family B (ABCB) or multidrug resistance (MDR) protein (Gottesman *et al.* 2002). PGP contains two main domains: (i) a nucleotide-binding domain, which has a highly conserved sequence and (ii) a transmembrane domain, which varies between cells and species. When drug molecules bind to the transmembrane domain of PGP, ATP hydrolysis at the nucleotide-binding site, inducing a conformational change that translocates the drug molecules to the cell surface. This efflux mechanism plays an important role in preventing the accumulation of drugs within the cells (Stavrovskaya & Stromskaya 2008, Liu 2009). For example, resistant cancer cells increase the expression of drug efflux transporters such as ATP7 A/B and PGP that pump drugs out to decrease drug accumulation in the cytosol (Sousa *et al.* 2014).

Detoxification

Detoxification is the process of inactivating drugs to prevent drug response. Drugs can be inactivated through several mechanisms, including (i) the partial degradation of the drugs and (ii) the use of specific proteins to modify drug structure. Glutathione (GSH) and metallothionein proteins are involved in the inactivation of platinum-based drugs. In addition, Glutathione S-transferase (GST) is primarily responsible for catalyzing the interaction between reduced GSH and electrophilic molecules (e.g. platinum) (Townsend & Tew 2003). Interestingly, overexpression of GST has been shown to contribute to the development of resistance in ovarian cancer cells to platinum-based antineoplastic drugs (Saburi *et al.* 1989, Hamada *et al.* 1994). An increased expression of GST enhances the detoxification of chemotherapeutic drugs and reduces their efficacy (Manolitsas *et al.* 1997, Cumming *et al.* 2001). Resistant cells have also been observed to display abnormal mitochondrial membrane

potential to avoid mitochondrial dysfunction through the binding of platinum drugs into mitochondrial DNA. Mitochondria also reduce intracellular distribution and act on the upregulation of cytochrome c specifically in the mitochondria instead of releasing cytochrome c to the cell cytoplasm (Stewart 2007).

B-tubulin

One of the more commonly used chemotherapy drugs for the treatment of ovarian cancer is paclitaxel, which functions by binding to the microtubules during cell division (Weaver 2014). Binding inhibits the depolymerization of the microtubule, which disrupts chromosome segregation, inhibits mitotic progression and prevents cell division (Hamada *et al.* 1994). β -Microtubulin is important for the formation of the mitotic spindle during cell division and is required for the maintenance of cell structure, motility and cytoplasmic movement (Wittmann *et al.* 2001). Microtubule assembly occurs during the G2 and pro-mitosis phases. Altering the mitotic spindle function induces mitotic arrest at the G2/M phase of the cell cycle and ultimately causes apoptosis (Ganguly *et al.* 2010, Weaver 2014, Kampan *et al.* 2015). As illustrated in Fig. 2, cancer cells can develop a number of molecular mechanisms that can contribute to paclitaxel resistance. Five of them are as follows: (i) there can be alterations to membrane lipids, which would give rise to compartmentalization and prevent the passive diffusion of paclitaxel. (ii) Point mutation occurs at the tubulin gene site (Greenberger & Sampath 2006, Ganguly *et al.* 2010). (iii) TLR4 negatively regulates paclitaxel chemotherapy by increasing transcriptional factors and gene inductions (e.g. NF-KB), which can subsequently lead to the upregulation of cytokine expression (e.g. IL-6 and IL-8) (Greenberger & Sampath 2006, Vyas *et al.* 2014). This can also increase the function of significant anti-apoptotic proteins such as platelet-derived growth factor (PDGF) (Kampan *et al.* 2015). (iv) Resistant cells can amplify membrane drug-efflux pumps (Kampan *et al.* 2015). (v) Drug molecules can be inactivated, and catalysis can occur by detoxifying the enzyme family of cytochrome P450 and glutathione metabolism (Sparreboom *et al.* 1998, Jang *et al.* 2001, Kampan *et al.* 2015).

Taxol-resistant tumors induce extensive post-translational modifications, including the following: (i) point mutations at the tubulin, (ii) changes in drug-binding affinity due to selective alterations in the expression of tubulin isotypes (Class III B-tubulin) (Ferrandina *et al.* 2006), (iii) alterations in the stability of the microtubule

network (McGrail *et al.* 2015) and (iv) a reduction in total intracellular tubulin concentration (Duran *et al.* 2017). These alternations then lead to reduced drug efficacy.

Influences of the tumor microenvironment and chemoresistance on ovarian cancer

The pathogenesis of ovarian cancer is largely distinguished from other tumor types, since the tumor microenvironment plays a significant role in ovarian cancer dissemination (Sun 2016, von Strandmann *et al.* 2017). Malignant ovarian ascites is the primary contributing factor to this unique environment; at the time of diagnosis, more than a third of ovarian cancer patients exhibit ascites (i.e., the presence of fluid in the abdominal cavity) and almost all recurrent cases include ascites (Ahmed & Stenvers 2013, Smith 2017). Ascites includes shed membrane particles that are rich in proteolytic enzymes, including matrix metalloproteinase 9 (MMP9), matrix metalloproteinase 2 (MMP2) and urokinase receptor (UPA) (Graves *et al.* 2004, Reiner *et al.* 2017), and MMP9 degrades the endothelial basement membrane and other matrix components to facilitate macrophage extravasation toward tumor clusters and concomitantly liberates VEGF to promote angiogenesis (Kessenbrock *et al.* 2010). Additionally, vesicle-associated MMP2 activity had been positively linked to malignant potential of metastasis (Graves *et al.* 2004) and by degrading native collagen, cancer cell invasion is facilitated by MMP- (Kenny & Lengyel 2009).

Ginestra *et al.* (1999) found that vesicle content was higher in females with malignancies compared to those with benign diseases after analyzing vesicles derived from the ascites of 33 women with different gynecologic pathologies (i.e., 19 benign ovarian lesions, 10 ovarian carcinomas and 4 endometrial carcinomas). The zymogen pro-form and active MMP2 and MMP9 were present in vesicles from malignancies, and minimal gelatinolytic activity was present in vesicles from benign serous cysts. This study indicated that membrane vesicles in ascites may act as storage for active proteinases and assist the metastatic process (Ginestra *et al.* 1999). In other studies, shed or extracellular vesicles were identified in different biological fluids obtained from patients with ovarian cancer (Runz *et al.* 2007, Peng *et al.* 2011), who were found to have claudin-containing vesicles in their plasma (Li *et al.* 2009). Thus, the diagnosis of ovarian cancer and monitoring of the therapeutic efficacy of antitumor drugs can be assisted by analyzing the biological fluids of ovarian cancer-derived EVs.

There are four main categories of extracellular vesicles: microvesicles, nanovesicles (e.g., exosomes), shedding vesicles and ectosomes. Exosomes have received much attention in the literature as they are released from the intracellular (endosomal) compartments of a cell, which differs from other extracellular vesicles. Interestingly, exosomes are capable of evading the effects of chemotherapy drugs via the regulation of reactive oxygen species (ROS) concentrations (Patel *et al.* 2017). Defining the role of exosomes in cancer progression, screening and treatment is of increasing contemporary interest and clinical relevance.

Diagnostic and prognostic gap

The clinical management of ovarian carcinoma is not greatly affected by histological differences, even though distinct subtypes of epithelial ovarian cancer have been supported by molecular and clinicopathological data (Muinao *et al.* 2017). Serum biomarkers (e.g. CA-125), imaging (e.g. transvaginal ultrasound) and physical examination are the primary methods of monitoring ovarian cancer. However, the sensitivity and specificity of traditional examinations are poor, and none of these approaches are able to detect early recurrence or to predict the prognosis of the disease. For this reason, identifying a panel of different biomarkers is crucial for screening, diagnosis and treatment of ovarian cancer. Gene expression studies have been used to develop predictive biomarkers, including miRNAs and proteins.

miRNAs have been shown to be dysregulated when expressed in ovarian cancer, and their expression can be differentiated as normal vs cancerous. In a cancer diagnosis, monitoring patient response to chemotherapy drugs using miRNA signatures is vital. In comparison to mRNA classification, miRNA-based identification of cancers provides more appropriate diagnoses for unknown primaries. Nevertheless, the utilization of miRNA profiling in the absence of a mass to be biopsied remains difficult, and clinical translation is a challenge mainly because specific tumor-derived miRNAs must be identified (Ogata-Kawata *et al.* 2014). Identification and analysis, however, requires a technical specialist to analyze circulating miRNAs in biological fluids acquired from patients with cancer (Chen *et al.* 2008, Hunter *et al.* 2008, Liu *et al.* 2014) and circulating 'free' miRNAs isolated from plasma are easily degraded by prolonged storage (Taylor & Gercel-Taylor 2008, Cheng *et al.* 2014).

In contrast, miRNAs encapsulated in exosomes are protected from degradation by RNase regardless of storage

times by the compartmentalization of miRNAs within the exosomal lipid bilayer membrane (Joyce *et al.* 2016). For example, during a number of freeze-thaw cycles, the content of exosomes is protected, whereas concentrations of non-vesicles associated miRNA decrease after each cycle (Ge *et al.* 2014). The highly stable nature of exosomes (and their content) and the encapsulated content makes them attractive as identifying biomarkers for cancer progression and future clinical applications.

miRNA in ovarian cancer

miRNAs are short, non-coding RNAs consisting of approximately 22 nucleotides that play a vital role in the regulation of gene expression (Ha & Kim 2014). It has been commonly thought that miRNAs can only inhibit translation and messenger RNA stability; however, it has been reported that miRNAs may also upregulate the expression of their targets (Vasudevan *et al.* 2007). Given the important role of miRNAs in normal physiology, it is not a surprise that in ovarian cancer, many of these miRNAs are dysregulated, affecting cellular processes that have been associated with cancer hallmarks such as invasion, proliferation, apoptosis and cell differentiation (Melo & Esteller 2011). Of particular interest are the miRNAs that are associated with chemoresistance in ovarian cancer.

miRNAs that have been found to be associated with chemoresistance pathways in ovarian cancer can be broadly divided into two main categories (Table 1). (1) miRNAs that mediate the expression of MDR transport protein such as the ATP-Binding Cassette Subfamily B Member 1 (ABCB1) or (2) non-transporter mediated (Bush & Li 2002). Included in Table 1 are the miRNAs' mechanisms of action, listing whether the regulation is direct (binding to 3'-untranslated region) or indirect (regulation of an intermediary target). Table 2 details the expressions of particular miRNAs in ovarian cancer cell lines and their gene targets of these miRNAs in response to chemotherapy treatment. An analysis of the gene ontology of the miRNA targets revealed that a large number of the target proteins are classed as protein phosphatase, giving weight of their potential importance in chemoresistance. miRNAs can mediate the expression of the MDR transporter and induce chemoresistance. For example, the expression of miR-27a and miR-451 was evaluated in ovarian cancer cells A2780 and multidrug-resistant counterpart cells A2780 Dx5, and their expression, was significantly higher in resistant cells compared with sensitive cells. Interestingly, overexpression of miR-27a or miR-451 in

Table 1 miRNAs involved in the chemo resistance pathways in ovarian cancer.

Transporter target	miRNA expression profile in chemo resistance	miRNA mode of action	Reference
<i>ABCB1</i>	miR-27a↑	Indirect regulation of transporter target. miR-27a targets the intermediary <i>HIPK2</i> , causing an increase in <i>MDR1/PGP</i> expression	Li et al. (2010)
	miR-27a↑, miR-451↑	Treatment of cells with miR-27a and miR-451 caused an increased expression of <i>MDR1/PGP</i> mRNA	Zhu et al. (2008)
	let-7g↓	Let-7g inhibits the RNA binding protein <i>IMP-1</i> , which in turn stabilizes <i>MDR1</i> mRNA	Boyerinas et al. (2012)
	miR-186↓	Direct binding target (3'-untranslated region) of <i>ABCB1</i>	Sun et al. (2015)
Target genes	miRNA expression in chemo resistance	Biological effects	Reference
<i>TUBB3</i>	miR-200 family	Inverse correlation between the levels of miR-200 family and B-tubulin protein levels	Leskelä et al. (2011)
EMT related	miR-20a↑	Increased levels of miR-20a increased the cell migration of cancer cells via activation of EMT pathways	Liu et al. (2017b)
<i>ERCC2</i>	miR-770-5p↓	miR-770-5p negatively regulates <i>ERCC2</i> gene, which caused an inhibition to apoptosis due to impaired nucleotide damage repair and DNA repair capacity	Zhao et al. (2016)

sensitive cells increased the expression of MDR and PGP proteins, which suggested that miR-27a and miR-451 regulated the expression of MDR protein by inhibiting the transcriptional factors involved in the suppression of *MDR* gene expression ([Zhu et al. 2008](#)). Consistent with these data, [Li et al. \(2010\)](#) demonstrated that the inhibition of miR-27a in A2780/taxol cells decreased the expression of MDR and PGP leading to increased cell apoptosis, involving the upregulation of homeodomain-interacting protein kinase 2 (*HIPK2*), suggesting that miR-27a targets *HIPK2*. Therefore, miR-27 affects the development of chemoresistance by targeting *HIPK2* expression and regulates the expression of *MDR* and *PGP* ([Li et al. 2010](#)). miR-130a has been associated with chemotherapy resistance via inhibition of *MDR1* gene expression and upregulation of *PTEN* protein expression ([Li et al. 2015](#)). In contrast, overexpression of miR-186-induced apoptosis and cell sensitivity to chemotherapeutic agents (cisplatin and paclitaxel) in resistant cells A2780/DDP and A2780/taxol cells ([Sun et al. 2015](#)). miR-186 downregulated the expression of *MDR* via direct binding to *ABCB1* transporter mRNA, suggesting that restoring the expression of miR-186 might increase cell sensitivity to chemo drugs.

miR-376c targeting the activin receptor-like kinase 7 (ALK7) has also been associated with tumor growth and chemotherapy resistance. Higher levels of miR-376c is associated with an increase in cell proliferation, survival and chemo resistance using ovarian cancer cell lines cultured in both monolayer and as a 3D spheroid, and these effects were partially mediated by ALK7. Interestingly, a decrease in the expression of

miR-376c and higher levels of ALK7 were found in tumor tissue samples obtained from patients who responded to the treatment with chemotherapy drugs compared with patients that did not respond ([Ye et al. 2011](#)).

Real-time PCR, Western blotting and transfection assays were used to investigate the role of miR-31 in ovarian cancer cells and paclitaxel resistance ([Mitamura et al. 2013](#)). The expression of miR-31 is lower in resistant compared to sensitive cells in response to paclitaxel. Over expression of miR-31 induces sensitivity to paclitaxel, involving a direct targeting to hepatocyte growth factor receptor (MET).

miRNA can also mediate the expression of the non-transporter via direct binding to the 3'-untranslated region or via indirect binding by regulation of an intermediary target. In a study of ovarian carcinoma tissue, miR-200 family expression was upregulated and negatively correlated to B-tubulin 3 expression via direct binding to the 3'-untranslated region. In the miR-200 family, only the low expression of miR-200c was associated with patients who did not achieve complete clinical treatment response and had disease recurrence. Hence, miR-200c is suggested to target B-tubulin 3 and increase cell resistance to paclitaxel-based therapy ([Leskelä et al. 2011](#)). In addition, miRNA-mediated chemoresistance via indirect regulation of an intermediate target, such as the mitochondrial inner membrane protease subunit 1 (*IMP-1*). Let-7d was downregulated in patients who had recurrent post-primary treatment with the chemotherapy drugs carboplatin and paclitaxel. Boyerinas *et al.* reported that let-7d was downregulated in recurrent patients and showed a shorter disease-free survival rate than those who have a

Table 2 The miRNAs expression profile in OvCa cells upon treatment with platinum or taxol-based drugs.

miRNAs	miRNA's sources	Response to treatment	Targets	PANTHER protein class of target	References
Drug: Platinum					
miR-93	OVCAR3/CDDP and SKOV3/CDDP	↑	<i>PTEN/AKT</i>	Protein phosphatase	Fu et al. (2012)
miR-214	HIOSE cells	↑	<i>PTEN/AKT</i>	Protein phosphatase	Yang et al. (2008)
miR-27a		↑	<i>RKIP</i>	Protease inhibitor	Li et al. (2014)
miR-216b	SKOV3/CDDP	↓	poly(ADP-ribose) polymerase (<i>PARP</i>)-1	–	Liu et al. (2017c)
miR-376c	OV2008 cells	↑	Nodal/activating receptor-like kinase 7 (<i>ALK7</i>)	TGF-B receptor serine/threonine protein kinase receptor	Ye et al. (2011)
miR-130a	A2780/DDP	↑	<i>PGP</i> levels and <i>MDR1</i> mRNA and <i>PTEN</i>	cysteine protease serine protease	Li et al. (2015)
miR-489	SKOV3/CDDP and OVCAR3/CDDP	↓	<i>AKT3</i>	Annexin calmodulin non-receptor serine/threonine protein kinase transfer/carrier protein	Wu et al. (2014)
mir-770-5p	A2780S cells	↓	<i>ERCC2</i>	Helicase	Zhao et al. (2016)
mir-643	A2780 DDP cells	↓	<i>CCND1</i> , and Ras-MAPK pathway components (<i>GRB2</i> , <i>ERK2</i> and <i>RSK2</i>)	Kinase activator	van Jaarsveld et al. (2015)
miR-let-7i	Chemo-resistant epithelial ovarian cancer (EOC) tissue	↓	<i>BRCA1</i> and <i>RAD51</i>	Non-receptor serine/threonine protein kinase	Xiao et al. (2017)
miR-125B	C13	↑	Pro-apoptotic <i>BCL2</i> antagonist killer 1.	Ubiquitin-protein ligase	Kong et al. (2011)
miR-29 a/c	CP70, HeyC2 and SKOV3	↓	Collagen type I alpha 1 (<i>COL1A1</i>), signal-regulated kinase 1/2 and glycogen synthase kinase 3 beta	Signaling molecule	Yu et al. (2014)
miR-130a	A2780/DDP	↓	<i>XIAP</i>	Non-receptor serine/threonine protein kinase	Zhang et al. (2013)
miR-21-3p	CP70	↑	<i>NAV3</i>	Protease inhibitor	Pink et al. (2015)
Resistance drugs: Taxol					
miR-27a	EC9706	↑	<i>HIPK2</i>	–	Li et al. (2010)
miR-182	13 ovarian cancer tissue samples and Eight ovarian carcinoma cell lines, OVCAR3, SKOV3, OV2008, HEY, 3AO, A2780, HO8910, and C13	↑	<i>MAD2</i> or <i>PDCD4</i>	Non-receptor serine/threonine protein kinase non-receptor tyrosine protein kinase	Wang et al. (2013)
miR-663	OvCa tissues (patients) and sixteen human ovarian cancer cell lines	↑	<i>P53</i> , TUOS-3 and TUOS-4	Translation elongation factor	Kim et al. (2014)
miR-31	PTX-resistant KFr13Tx cells	↓	Increase receptor tyrosine kinase MET activity	<i>P53</i> -like transcription factor	Mitamura et al. (2013)
mir-106a	Six different PTX-resistant sublines were generated from the parent cell line (SKOV3) and tissue samples from 39 ovarian serous tumours	↑	<i>BCL10</i> and <i>Caspase-7</i>	–	Huh et al. (2013)
				Cysteine protease protease inhibitor	

(Continued)

Table 2 Continued.

miRNAs	miRNA's sources	Response to treatment	Targets	PANTHER protein class of target	References
miR-591	Six different PTX-resistant sublines were generated from the parent cell line (SKOV3) and tissue samples from 39 ovarian serous tumours	↓	<i>ZEB1</i>	KRAB box transcription factor	Huh <i>et al.</i> (2013)
miR-187	Eight human ovarian cancer cell lines (SKOV3, OVCAR-3, TOV-21G, TOV112D, OV-90, BR, MDAH2774, CAO3)	↓	Disabled homolog-2	Signaling molecule	Chao <i>et al.</i> (2012) , Sulaiman <i>et al.</i> (2016)
miR-145	KOV3/PTX and A2780/PTX cells	↓	<i>SP1</i> and <i>CDK6</i>	DNA binding protein transcription cofactor zinc finger transcription factor Non-receptor serine/threonine protein kinase non-receptor tyrosine protein kinase	Zhu <i>et al.</i> (2014)
miR-134	Chemoresistant ovarian cancer tissues and paclitaxel-resistant SKOV3-TR30	↓	Reduction in <i>TAB1</i>	Kinase inhibitor protein phosphatase	Shuang <i>et al.</i> (2017)

higher expression. Patient samples showed upregulation of IMP-1 and MDR protein expression, suggesting that downregulation of let-7d caused an increase in the expression of its target IMP-1 and allowed it to stabilize MDR expression and induce chemoresistance ([Boyerinas *et al.* 2012](#)). Additionally, Kong *et al.* demonstrated that miR-125b directly targeted the BCL2 antagonist/killer 1 gene (*BAK1*), which is a member of BCL2 family that acts as a pro-apoptotic protein. The results from this study demonstrated that miR-125b directly targeted the *BAK1* gene, which is the gene that regulates a cell's resistance to cisplatin. Upregulation of miR-125b caused suppression of *BAK1*, which resulted in ovarian cancer cells' increased resistance to cisplatin. This miR-125b-*BAK1* axis appears to be critical for cisplatin resistance and could potentially be exploited for further therapeutic strategies ([Kong *et al.* 2011](#)).

Moreover, miRNAs are able to regulate the expression of apoptotic proteins, such as caspase 7 (*CASP7*) and B cell CLL/lymphoma 10 (*BCL10*). Huh *et al.* investigated the function and mechanisms of miR-106a and miR-591 in paclitaxel-resistant ovarian cancer cells ([Huh *et al.* 2013](#)). They found that upregulation of miR-106a and downregulation of miR-591 was discovered in ovarian cancer cells that were resistant to paclitaxel compared to sensitive cells. In addition, inhibition of miR-106a and upregulation of miR-591 in paclitaxel-resistant ovarian

cancer cells enhanced apoptosis, suppressed migration and reduced proliferation, thus re-sensitizing these cells. Furthermore, these results revealed that miR-106a can directly target *CASP7* and *BCL10*, while miR-591 can directly target zinc finger E-box-binding homeobox 1 (*ZEB1*). These genes have been demonstrated in other studies to be important proteins involved in chemoresistance. The results of this study are interesting, and the authors should further investigate through their assays whether *CASP7*, *ZEB1* and *BCL10* are responsible for paclitaxel resistance in ovarian cancer cells ([Huh *et al.* 2013](#)). The expression and function of miR-130a in ovarian cancer cells and their response to cisplatin has also been discussed ([Zhang *et al.* 2013](#)). miR-130a is reduced in cisplatin resistance ovarian cancer cells, while overexpression of this miRNA sensitizes these cells to cisplatin. Furthermore, luciferase assays revealed that miR-130a directly targets the gene x-linked inhibitor of apoptosis (*XIAP*), and this gene could induce cisplatin sensitivity in ovarian cancer cells ([Zhang *et al.* 2013](#)). The data, however, do not establish whether or not the regulation of cisplatin sensitivity in ovarian cancer cells by miR-130a is the result of its interaction with *XIAP*.

miRNA is involved in the inhibition of tumor suppressor genes, such as *PTEN* and programmed cell death 4 (*PDCD4*), and in the activation generation of oncogenic genes, such as mutant *P53*, mutant *BRCA1* and

poly (ADP-ribose) polymerase 1 (*PARP1*). Fu *et al.* showed that miR-93 was upregulated in resistant cancer cell lines. Over-expression of miR-93 and knock-down showed association with cell survival, through the actions of its binding target *PTEN* (Fu *et al.* 2012). In line with this study, Yang *et al.* using microarray, real-time PCR, *in vitro* reporter assays, cell viability and apoptosis experiments reported the potential role of miR-214 in cisplatin resistance. miR-214 was found to be deregulated in drug-resistant cells and the target of miR-214 was found to be *PTEN* (Yang *et al.* 2008). The expression and function of miR-182 in ovarian cancer tissues and cells, as well as their response to taxol have been suggested (Wang *et al.* 2013). Luciferase transfection assays demonstrated that miR-182 was able to directly target and suppress *PDCD4* expression. The miRNA miR-182 also enhanced colony formation and viability, while reduced ovarian cancer cell sensitivity to Taxol, through the regulation of *PDCD4*.

The absence of WT *P53* and the presence of mutant *P53* has been linked to aggressive cancer cells and chemoresistance. Immunoblotting, real-time PCR and microarrays were used to investigate the role of miRNAs and Taxol resistance in (Kim *et al.* 2014). Microarray analysis in ovarian cancer cells in taxol-sensitive compared with taxol-resistant cells identified a total of 17 miRNAs differentially expressed, including miR-663. This study also identified *P53* pathways and proteins that interact with the *P53* pathway to be differentially expressed between Taxol-sensitive and -resistant ovarian cancer cells. The relationship between *P53* and miR-663, and whether these biomolecules have a role in taxol resistance in ovarian cancer remains to be established.

Mutation in *BRCA1* contributes to resistance to chemotherapy. *In situ* hybridization, immunoblotting and transfection assays were used to investigate the expression and function of let-7e in epithelial ovarian cancer tissues and cells and their response to cisplatin (Xiao *et al.* 2017). The results from this study demonstrated that let-7e was reduced in chemoresistant ovarian cancer tissues compared to chemo-sensitive tissues. In addition, transfection assays showed that let-7e sensitized epithelial ovarian cancer cells to cisplatin and downregulated *RAD51* and *BRCA1* gene expression. These results suggest that low expression of let-7e in epithelial ovarian cancer may cause the activation of *RAD51* and *BRCA1* that results in cisplatin resistance in epithelial ovarian cancer cells. Further studies are required to understand the mechanisms which let-7e regulates *RAD51* and *BRCA1*, whether it is through direct targeting or a downstream effect. *PARP1* is a DNA repair enzyme that causes a change in chromatin structure and promotes

DNA repair. *PARP1* has been shown to be implicated in chemoresistance (Ganesan 2011). Microarrays, real-time PCR, *in vitro* assays and an animal model were used to identify and investigate the role of miR-216b in cisplatin sensitivity in ovarian cancer cells. Through a differential microarray study, miR-216b was chosen and was found to be downregulated in drug-resistant cells. Cell-based assays revealed that this miRNA can induce apoptosis. Furthermore, *PARP1* expression was found to be directly inhibited by miR-216b. This was a comprehensive study using both *in vitro* and *in vivo* models (Liu *et al.* 2017b). On the other hand, inhibition of miRNA by transcriptional factors can cause an upregulation of oncogenic genes and alteration in cancer cell response to chemo drugs. For example, the role of miR-134 in paclitaxel resistance within ovarian cancer cells has been studied (Shuang *et al.* 2017). The results of this study revealed that NF-KB, C-REL and ELK1 transcription factors can bind to the promoter region of miR-134 and suppress its expression. In addition, these genes were upregulated in paclitaxel-resistant ovarian cancer cells. Through luciferase assays, it was demonstrated that miR-134 was able to bind to TGF-B activated kinase 1-(MAP3K7) binding protein 1 (*TAB1*) and suppress its translation. The gene *TAB1* was also found to be elevated in ovarian cancer tissue and was further shown to promote chemoresistance in ovarian cancer cells. Overall, in ovarian cancer, paclitaxel-resistant cells, NF-KB, C-REL and ELK1 are elevated, which suppresses miR-134 expression, resulting in elevated levels of *TAB1* and promoting chemoresistance.

miRNAs can enhance DNA repair efficacy and develop chemoresistance. miRNA expression was evaluated in ovarian cancer patients who showed complete or incomplete response to platinum-based therapy. The expression of miR-770-5p was significantly lower in incomplete response cases compared with complete response cases, and the reduced expression was negatively correlated with the expression of ERCC excision repair 2 (*ERCC2*) and associated with a shorter survival rate. *ERCC2* participates in nucleotide excision repair, which has been reported to play a role in chemoresistance. The *in vitro* overexpression of miR-770-5p in ovarian cancer cells OV2008 and A2780 and their cisplatin resistance counterpart cells C13 and A2780 CP caused a reduction in cell viability and induced cell apoptosis. Silencing of *ERCC2* mRNA in cells transfected with miR-770-5p inhibitor caused the cells to become more resistant to a platinum-based drug. A low expression of miR-770-5p is suggested to result in a high expression of *ERCC2*, which caused impaired nucleotide damage and DNA

repair mechanism (Zhao *et al.* 2016). Taken together, these studies emphasize the role of miRNAs in mediating chemoresistance event in ovarian cancer cells.

miRNAs play an important role in activating or inhibiting essential proteins in upstream and downstream MEK/MAPK and PI-3K/AKT. For example, Ras is a switcher that activates intracellular signaling according to the extracellular stimulus. The downstream pathway of Ras is known as the MAPK pathway. miRNA microarrays, transfection studies using mimics and pathway analysis with luciferase reporter constructs were used to study the miR-634 candidate and its role in drug sensitivity in resistant ovarian cancer cells. It was found that the miR-634 downregulation was associated with cisplatin resistance. When miR-634 was overexpressed in a drug-resistant cell line, their sensitivity was restored. Additionally, an overexpression of miR-634 was found to interact and repress cell cycle regulators associated with the Ras-MAPK pathways. Taken together, miR-634 should be investigated as a potential target of therapeutics (van Jaarsveld *et al.* 2015). The role of miR-489 and its protein target AKT serine/threonine kinase 3 (AKT3) in cisplatin-resistant ovarian cancer cells has been proposed. The expression of miR-489 in cisplatin-resistant ovarian cancer cell lines SKOV-3 and OVCAR-3 were downregulated, and overexpression of miR-489 restored their chemotherapy sensitivity. In addition, this study through bioinformatics and transfection with miR-489 mimics that AKT3 is a direct target of miR-489, suppressing AKT3 expression. The role of AKT3 in cisplatin resistance was further explored with the use of an AKT3 inhibitor, MK-2206. Taken together, miR-489 and Akt3 have been demonstrated to be associated with cisplatin-resistant ovarian cell lines, additional experiments using animal models will still need to be conducted (Wu *et al.* 2014). miR-29 has been linked to chemoresistance in ovarian cancer cells (Yu *et al.* 2014). The findings from this study revealed that miR-29 directly targeted collagen I, and knockdown of miR-29 caused upregulation of collagen I, activation of ERK1/2 and inactivation of glycogen synthase kinase 3 beta (GSK3B). In addition, overexpression of miR-29 in ovarian cancer cells increases cisplatin sensitivity and suppresses tumor formation in mice models. The *in vivo* results of this study are exciting, and further studies should investigate the miR-29 regulatory mechanisms *in vivo* and its potential as a therapeutic target.

The expression of miR-145 is decreased in paclitaxel-resistance ovarian cancer cells (Zhu *et al.* 2014). Interestingly, the low expression of miR-145 was associated with high levels of Cell division protein

kinase 6 (CDK6) and transcription factor (SP1), suggesting that miR-145 is targeting CDK6 and SP1. Moreover, miR-145 reduced abdominal size and tumor burden *in vivo*. These findings are interesting, and further studies could investigate whether altering expression of miR-145 is a suitable therapeutic target.

The inhibition of miRNA by transcriptional factors can cause an upregulation of oncogenic genes and alteration in cancer cell response to chemo drugs. Shuang *et al.*, established that NF-KB, C-REL and ELK1 transcription factors can bind to the promoter region of miR-134 and suppress its expression, and these genes were upregulated in paclitaxel-resistant ovarian cancer cells (Shuang *et al.* 2017). Using luciferase assays, it was demonstrated that miR-134 was able to bind to TAB1 and suppresses its translation. The gene TAB1 was also found to be elevated in ovarian cancer tissue and was further shown to promote chemoresistance in ovarian cancer cells. A summary of the signaling pathways regulated by miRNAs in ovarian cancer is presented in the Fig. 3.

Exosomes as a potential marker for cancer prognostics

Exosomes are nanovesicles (30–120nm) that consist of a lipid bilayer membrane and contain multiple molecular components. Exosomal content includes molecules from the cell of origin, extracellular stimuli (i.e. infectious agents) and molecules reflective of pathological conditions (state of cell). They are released after the fusion of multivesicular bodies with the cell membrane (Colombo *et al.* 2014). Exosomes are spherical vesicles enriched with membrane lipids such as sphingolipids, ceramide and cholesterol (Zhang *et al.* 2015). The Rab family proteins, including Rab27a and Rab27b, regulate the release of exosomes (Ostrowski *et al.* 2010, Colombo *et al.* 2014). Exosomes are found in almost all biological fluids, including blood, urine, ascites and amniotic fluid (Kobayashi *et al.* 2014). These vesicles carry a specific set of molecules including nucleic acids (e.g. DNAs and miRNAs) and proteins that are delivered to the target cells (Alvarez *et al.* 2012, Kobayashi *et al.* 2014). Exosomes function to modify the biological function of target cells under both normal and pathological conditions.

Exosome mediated ovarian cancer progression and metastasis

Several studies have shown that cancer cell-derived exosomes reprogram or educate other cells to aid tumor

Figure 3

Summarizes how alteration of miRNA expression may regulate the cell's production of key proteins involved in chemoresistance. Red (sharp or dashed) arrows indicate downregulation or inhibition and green (sharp or dashed) arrows indicate upregulation or activation of miRNA expression or targets. miRNA may regulate cell chemoresistance at many levels, including: (i) mediating the expression of the MDR transporter; (ii) inhibition of tumor suppressor genes (e.g. *PTEN* and *PDCD4*); (iii) enhancing DNA repair mechanism (e.g. *ERCC2*, *SP1*, *CDK6*, and *PARP1*); (iv) inhibiting the secretion of proapoptotic proteins (e.g. *BAK1*) and apoptotic proteins (e.g. *CASP7*); (v) alteration of drug targets (e.g. B-tubulin); (vi) enhancing the expression of growth factor (e.g. insulin-like growth factor 2); (vii) targeting the activin receptor-like kinase 7 (*ALK7*); or (viii) MET receptor induction.

survival and promote metastasis and cancer progression (Saleem & Abdel-Mageed 2015, Lu *et al.* 2017), however, to date, only a limited number of reports have investigated the ovarian cancer-promoting effect of a particular protein or miRNA in cancer-derived exosomes. Exosome content is highly regulated by proteins that function together to form the endosomal sorting complex that is required for the transport of exosomes (Soekmadji *et al.* 2013, Oskarsson *et al.* 2014). The protein content of exosomes released from cancer cells is different from that released by normal cells and may modify target cell phenotype and promote cancer progression (Saleem & Abdel-Mageed 2015, Lu *et al.* 2017).

Ovarian cancer-derived exosome proteins were found to be highly enriched in cell signal pathways correlated with tumorigenesis; a group of proteins were upregulated in ovarian cancer tissue and were also present in exosome. These proteins were as follows: the epithelial cell surface antigen (EPCAM), tubulin B-3 chain (TUBB3), proliferation cell nuclear antigen (PCNA), epidermal growth

factor receptor (EGFR), apolipoprotein E (APOE), fatty acid synthase (FASN), ERBB2, L1CAM (CD171), and claudin 3 (CLDN3) (Liang *et al.* 2013).

Hu *et al.*, showed that epithelial ovarian cancer (EOC)-derived exosomes promoted ovarian cancer invasion by transferring exosomes containing CD 44 to human peritoneal mesothelial cells (HPMCs), which reprograms them to more of an epithelial–mesenchymal transition (EMT) phenotype, thereby promoting ovarian cancer cell invasion and metastasis (Hu *et al.* 2017). Ovarian cancer invasion and metastasis is promoted by EOC-derived exosomes, as they transfer exosomes containing CD44 to HPMC which resulted in reprogramming of these cells to a more mesenchymal phenotype (Nakamura *et al.* 2016, Hu *et al.* 2017). A separate study has discovered that the gap-junction protein Claudin 4 was overexpressed in serum exosomes derived from ovarian cancer patients compared with the control group (Li *et al.* 2009). Interestingly, Claudin 4 is highly expressed in the majority of ovarian cancer tissue, and it controls paracellular barrier

permeability and induces cancer metastasis (Litkouhi *et al.* 2007). In addition, ovarian cancer cells derived from exosomes have an overexpression of LIN28A, which promotes EMT in non-metastatic recipient cells (Litkouhi *et al.* 2007).

A proteomic analysis of IGROV-1 and OVCAR-3-derived exosomes detected that 2230 proteins are related to multiple cellular pathways, and both cells share approximately 50% of the total protein content (Liang *et al.* 2013). This protein content carried by exosomes is involved in multiple biological pathways, including adhesion, signal transduction, membrane transport and fusion, and immune response modulation. Exosomes derived from SKOV-3 and OVCAR-3 cells cause adipose-derived stem cells to obtain myofibroblast properties, activating the transforming growth factor beta (TGF- β) pathway (Cho *et al.* 2011). Exosomal-TGF- β 1 and the melanoma-associated antigen family (MAGE3 and MAGE6) could be used as biomarkers to distinguish ovarian cancer cases from those with benign tumors (Szajnik *et al.* 2013).

Furthermore, CD24 can be found in the cytoplasm within MVBs and is released into the surrounding environment via exosomes. CD24 contributes to the poor prognosis of ovarian carcinomas and a short patient survival period (Li *et al.* 2017).

Exosomes in drug resistance

Tumor-derived exosomes regulate a wide range of biological processes associated with cancer progression and resistance to chemotherapy, including proliferation (Richards *et al.* 2017), immune regulation (Greening *et al.* 2015), and metastasis (Costa-Silva *et al.* 2015, Soung *et al.* 2016). Exosomes also regulate the cellular accumulation of anti-cancer drugs. It was previously reported that exosomes flush chemotherapeutic agents from cells through the accumulation of drugs into shed vesicles (Shedden *et al.* 2003). There could also be a correlation between exosome content and clinical outcome; a recent study that examined ovarian cancer patients' exosomal proteins before and after chemotherapy found that exosome levels were relatively unchanged in patients who were unresponsive to chemotherapy, while significantly altered levels were observed in responders, suggesting that the protein content of exosomes may be useful for predicting treatment response (Szajnik *et al.* 2013).

Exosomes have been found to transfer a resistance phenotype to other cells through the activation of multiple pathways, both *in vitro* and *in vivo* (Corcoran

et al. 2012, Challagundla *et al.* 2015, Yu *et al.* 2015, Au Yeung *et al.* 2016, Zheng *et al.* 2017), and recent research has identified efflux PGP proteins in exosomes (Lv *et al.* 2014). Drug molecules and proteins resulting from drug metabolism (i.e. PGP) are released into the extracellular environment by cancer cells through exosomes. Resistant cells in comparison to parental sensitive cells, it is that 2.6-fold more exosomes were released by cancer cells following treatment with cisplatin (Safaei *et al.* 2005). Accordingly, as exosomes serve as an efflux mechanism and remove chemotherapeutic drugs from cancer cells, the accumulation of the drug in the intracellular space is reduced (Shedden *et al.* 2003). It has been demonstrated that a high level of cisplatin export transporters (e.g. MRP2, ATP7A, and ATP7B) is present in exosomes isolated from a cisplatin-resistant human ovarian cancer cell line (Safaei *et al.* 2005).

In addition to transferring bioactive proteins, exosomes are enriched in non-coding RNAs (i.e. miRNAs), and specific loading of miRNAs into exosomes has been associated with cell invasiveness (Kobayashi *et al.* 2014) and chemoresistance (Chen *et al.* 2014). Notably, cells exposed to chemotherapeutic agents selectively package miRNAs into exosomes that repress mRNA targets in recipient cells and activate chemoresistant pathways. Figure 4 illustrates the involvement of exosomal cargo in transferring chemoresistant properties from resistant cells to sensitive cells, which reveals new opportunities for the development of exosome-based diagnosis and therapies.

Exosomal-miRNA in cancer

Recent studies provided evidence that miRNAs can be transported within the body via extracellular vesicles, specifically via exosomes (Valadi *et al.* 2007, Crescitelli *et al.* 2013). Exosomes carry a wide range of bioactive molecules, including nucleic acids, proteins, and lipids, which are delivered to target cells (Rak 2013, Hornick *et al.* 2015, Soung *et al.* 2017). Interestingly, exosomes and their content (e.g. miRNAs) in the cancer microenvironment can regulate communication between tumor cells and neighboring and/or distant cells to promote cancer progression, leading to metastasis, angiogenesis, and ultimately chemotherapy resistance (Shapira *et al.* 2014, De Toro *et al.* 2015, Hannafon *et al.* 2016, Liu *et al.* 2017a, Richards *et al.* 2017).

Exo-miRNA-21s and miRNA-29 as have been reported to promote tumor growth and metastasis by binding to toll-like receptors (TRLs) on immune cells, resulting in the activation of the TLR-mediated NF- κ B (Fabbri *et al.* 2012).

Figure 4

Potential role of exosomes in chemotherapy resistance. The heterogeneity and diversity of ovarian tumor cells in the tumor micro-environment is presented and some of the cells are resistance and other are sensitive to anti-cancer drugs. When cancer cells are treated with chemo drugs, the resistant cells survive because of their inherent chemoresistance which may lead to a relapse of the disease. The transmission of resistant phenotype derived from resistant cells via exosomes may cause acquired chemoresistance that transforms sensitive cells in resistance. These exosomes contain molecular factors including RNAs (e.g. miRNAs and mRNAs), proteins (e.g. transmembrane proteins, and cytosolic proteins) and cytosol from the originating cells. These factors may modulate the response of sensitive cancer cells to chemo drugs, which leads to the activation of several pathways involving in expression of chemoresistance proteins, controlling the cellular uptake of chemodrugs, increasing the progression of cancer, EMT, and metastasis.

Also, exo-miRNA-21 has been shown to target *PDCD4*, activating its downstream c-Jun N-terminal kinase (JNK) signaling pathway and resulting in the enhancement of the migration and the invasion of recipient esophageal cancer cells (Liao et al. 2016). Exo-miRNA-122 reportedly enhances metastasis via reprogramming the glucose metabolism in the pre-metastatic niche. The inhibition of miR-122 reduces the incidence of metastasis by restoring the glucose uptake in distant organs (Fong et al. 2015).

Angiogenesis is the formation of blood vessels to increase nutrition and blood supply to tumor cells (Nishida et al. 2006). Exo-miRNA has been shown to contribute to the angiogenesis process. Exosomes derived miR-21 in cancer cells promote the vascular endothelial growth factor (VEGF) near normal bronchial cells, through a STAT3-dependent mechanism (Liu et al. 2016). Notably, under hypoxia, tumor cells can promote angiogenesis by the secretion of exosomes to their surrounding microenvironment. Exosomes derived from hypoxic A549 lung cancer cells contain miR-494 genes that promote the angiogenesis of cancer cells by suppressing of *PTEN*

AKT/eNOS in their neighboring endothelial cells (Mao et al. 2015).

Exosomes-mediated chemoresistance via delivery of bioactive miRNAs to other cells

The use of miRNA profiles has been shown to be highly informative for the diagnosis of cancer. Using miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-205, and miR-214 as diagnostic miRNAs, exo-miRNA (i.e. miRNAs within exosomes) profiles highly reflect tumor miRNA profiles. More importantly, the levels of these miRNAs in exosomes from the plasma of ovarian cancer patients have been found to be significantly higher compared to benign diseases and could not be detected in normal controls (Taylor & Gercel-Taylor 2008). Differences in miR-200c and miR-214 expression during tumor stages suggests that exo-miRNA profiles may not be limited for use as biomarker for early detection but might also be helpful for prognosis of ovarian carcinomas (Taylor & Gercel-Taylor 2008). In fact, a strong correlation was found between

exo-miRNA-21 and overall survival among ovarian cancer patients (Vaksman *et al.* 2014).

It has been shown that exo-miRNA can induce chemoresistance by blocking the apoptotic pathways and altering cell cycles. For example, exosomes derived from neuroblastoma chemotherapy-resistant cells enhanced the expression of exo-miRNA-21 in chemotherapy-sensitive cells (Challagundla *et al.* 2015). Consistent with this finding, in ovarian cancer, miR-21 induced chemoresistance in cancer cells by targeting apoptotic protease-activating factor-1 (APAF1) (Yeung *et al.* 2016), which normally has a diminished ability to recruit and activate caspase-9 and activate the cell apoptosis pathway (Tan *et al.* 2011). In prostate cancer, resistant cells had decreased expressions of miR-34a, which regulates B-cell lymphoma 2 (BCL2) (Corcoran *et al.* 2014), which is a key protein that regulates cell death and has been recently reported to regulate proteins involved in the induction of platinum resistance in ovarian cancer (Dai *et al.* 2017). In addition, ovarian cancer-derived exosomes enriched with miR-222-3p can be transferred to macrophages to induce a tumor-associated macrophage (TAM)-like phenotype with SOCS3/STAT3 pathway involvement, that facilitates the progression of cancer (Ying *et al.* 2016).

Exo-miRNA may also promote changes in ROS enzyme homeostasis, which leads to tumor progression and increased oxidative stress in cancer cells, resulting in chemoresistance (Panieri & Santoro 2016). For example, pancreatic cancer exosomes have been found to cause a direct transfer of superoxide dismutase 2 (SOD2) and catalase (CAT) transcripts that encode for ROS detoxifying enzymes (Patel *et al.* 2017). This is likely to be caused by miR-155, which downregulates the gemcitabine-metabolizing enzyme and deoxycytidine kinase (DCK) (Patel *et al.* 2017). These studies all support the hypothesis that cancer cells release their tumor oncogenic or suppressor miRNAs via exosomes into the extracellular environment to maintain and promote chemoresistance (Kanlikilicer *et al.* 2016).

Do cancer-derived exosomes package selectively?

For clinical applications, it is important to identify miRNA profiles specific to tumor cells. Unique exo-miRNA profiles are sorted into exosomes, that may reflect disease conditions (Frederick *et al.* 2013, Vaksman *et al.* 2014). A comparative analysis of miRNAs in plasma or exosomes from lung cancer patients showed that miR-181b-5p and miR-21-5p were significantly enriched in exosomes

(Liu *et al.* 2017b). Other studies have reported a positive correlation between specific miRNAs and cancer tissue and exosomes isolated from the same ovarian cancer patients. These exo-miRNAs could not be detected in healthy samples (Taylor & Gercel-Taylor 2008). For other cancer types, exo-miRNA profiles have been compared, primarily between colon cancer patients and patients with metastasized (i.e. to the liver) colon cancer. This study showed that suppressor miRNAs were enriched in the patient exosomes, whereas oncogenic miRNAs were downregulated compared to donor cells. Regardless of increased oncogenic miRNA expression in tumor cells, cancer cells can selectively sort tumor-suppressing miRNAs into exosomes, thus promoting cancer progression (Teng *et al.* 2017). (Table 3 shows the comparison between the miRNA profiles of tissues and circulating exosomes in different cancer types).

Available data suggest that packaging of miRNAs is highly regulated, although the machinery that regulates this sorting into exosomes remains to be elucidated. While the underlying mechanism is unclear, it has been suggested that both, the process of exosomes biogenesis and miRNA-sequence-specific determinants may be involved in the process of packaging miRNAs into exosomes. *In silico* gene expression analysis of over-represented motifs and directed mutagenesis experiments found a potential pathway for loading miRNAs into exosomes (Villarroya-Beltri *et al.* 2013). These motifs regulate the packaging of miRNAs into exosomes and their directed mutagenesis enables the modulation of miRNA cargo in these vesicles. EXOmotifs, are a specific short sequence over-represented in miRNAs, controls the packaging of miRNAs into exosomes. These EXOmotifs mediate the binding of exosomes to a heterogeneous nuclear ribonucleoprotein, hnRNPA2B1, which directly regulates the loading of miRNAs into exosomes by binding of miRNAs to *HNRNPA2B1* (Villarroya-Beltri *et al.* 2013). HNRNPA2BI is a ubiquitously expressed RNA-binding protein that controls the transport of miRNAs into exosomes (Villarroya-Beltri *et al.* 2013). Previously, HNRNPA2B1 has been shown to regulate the intracellular trafficking and subcellular localization of specific mRNAs in neurons (Munro *et al.* 1999). Therefore, RNAs are not randomly encapsulated into exosomes. Ubiquitin proteins such as hnRNPA2B1 also mediate sorting of miRNAs into exosomes (Villarroya-Beltri *et al.* 2013). This shows that exosomes released by cancer cells are packaged with selective miRNAs to enhance the development of disease. Exo-miRNAs may potentially be used as surrogate diagnostic markers for

Table 3 Comparison between miRNA profiles of tissues and circulating exosomes in different diseases to demonstrate the selective encapsulation of miRNA in exosomes.

Cancer type	Sources	Biomarkers/outcomes	Isolation methods	References
Ovarian cancer	1. miRNAs in circulating exosomes 2. miRNAs in tissue from ovarian tumor	8 miRNAs were similar between cellular exosomal microRNA and tumor miRNA profiles (miR-200a, miR-200b, miR-200c, miR-203, miR-21, miR-141, miR-205 and miR-214)	Magnetic beads (Immunobeads; EpCAM)	Taylor & Gercel-Taylor (2008)
Lung cancer	1. miRNAs in circulating exosomes 2. miRNAs in tissue from lung tumor	20 miRNAs were similar between the circulating exo-miRNA and the tumor-derived miRNA (miR-17-3p, hsa-miR-21, hsa-miR-106a, hsa-miR-146, hsa-miR-155, hsa-miR-191, hsa-miR-192, hsa-miR-203, hsa-miR-205, hsa-miR-210, hsa-miR-212 and hsa-miR-214)	Size exclusion chromatography and magnetic activated cell sorting (EpCAM)	Rabinowits <i>et al.</i> (2009)
Hepatocyte caused by alcohol	1. miRNAs in circulating exosomes 2. Circulating miRNA in peripheral blood	1. Increases in serum miR-122 and miR-155 correlate with liver injury and inflammation 2. miR-122 and miR-155 were enriched with exosomes 3. Exosomes showed precisely the type of protein that caused liver damage, namely APAP, which induced liver necrosis	ExoQuick	Bala <i>et al.</i> (2012)
Human esophageal cancer cells	1. Human esophageal cancer cell-derived exosomes (conditioned media) 2. miRNA from an esophageal cancer cell line	1. 7727 of miRNAs in exosomes and cells were matched 2. 32 novel miRNAs were predicted in exosomes	Ultracentrifugation	Liao <i>et al.</i> (2014)
Melanoma	1. Exosomal miRNAs were purified from two normal human epidermal and two human malignant melanoma cell lines, A375 and SK-MEL-28 (cell culture supernatant) 2. miRNA from the cells was used for exosome isolation	1. miRNA in normal and melanocyte-derived exosomes were correlated with their originating cells 2. Weak correlation between miRNA profile in melanoma exosomes compared with normal melanocyte-derived exosomes	Combination of ultrafiltration and ultracentrifugation	Xiao <i>et al.</i> (2012)

tissue biopsy profiling ([Taylor and Gercel-Taylor 2008](#), [Kanlikilicer *et al.* 2016](#)).

Summary and perspectives

In summary, ovarian cancer is often diagnosed at advanced stages, when available treatment options are limited. Chemotherapy is usually effective at the start of the treatment; however, after repetitive cycles of treatment, some patients begin to acquire resistance to chemotherapeutic agents. Acquired chemoresistance is a persistent challenge in the treatment of ovarian cancer patients. Currently, there are no screening tests available to predict whether ovarian cancer patients will respond

to treatment or acquire chemotherapy resistance. miRNAs are key regulators involved in human pathologies, such as cancer, and in mechanisms related to these pathologies (i.e. chemoresistance). While miRNA profiling has shown promise in cancer diagnosis, it is currently limited to tissue biopsies. Nevertheless, it has been shown that miRNAs can be selectively packaged into exosomes and that the exo-miRNA signature parallels that of tissue miRNA. Exo-miRNA profiling has the potential to be used as a prognosis tool for cancer and may provide a reliable and noninvasive alternative to biopsies for monitoring disease recurrence and individual responses to therapies. Exosomes are considered novel biomarkers for cancer diagnosis and prognosis due to three main factors:

(i) exosomes are accessible and can be obtained from many biological fluids; (ii) exosome content (miRNAs) is significantly protected by a lipid bilayer in the exosomal membrane and (iii) exo-miRNA profiling reflects the miRNA of the original cells, which can be examined by a minimally invasive biopsy. Exo-miRNAs can provide information about tumor stage, tumor progression and therapeutic response (Simpson et al. 2009, Pant et al. 2012). In this review, we suggest that the isolation and analysis of specific tumor-derived exosomes and their miRNA content may have the potential to be used as biomedical tools in ovarian cancer for monitoring the response to chemotherapy as well as the possible progression or relapse of the disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

Lions Medical Research Foundation (LMRF), Ovarian Cancer Research Foundation (OCRF), The University of Queensland, Faculty of Medicine M+BS Emerging Leaders Medical Research Grant and Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT 1170809).

Acknowledgements

The authors acknowledge the editorial assistance of Debbie Bullock (UQ Centre for Clinical Research, The University of Queensland). M A holds a Scholarship from Saudi Arabian Ministry of Higher Education. They would like to thank the anonymous reviewers whose comments and suggestions helped improve and clarify this manuscript.

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Received in final form 20 July 2018

Accepted 26 July 2018