Review Article
Exosome mediated multidrug resistance in cancer

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Received August 26, 2018; Accepted October 8, 2018; Epub November 1, 2018; Published November 15, 2018

Abstract: Extracellular vesicles (EVs), named as exosomes, were recently found to play important roles in cell-cell communication by transducing various biochemical and genetic information. Exosomes, secreted from either tumor cells or stromal cells including immune cells, can eventually remodel tumor environment to promote tumor progression such as metastasis and multidrug resistance (MDR). Therefore, the detection or targeting of biochemical and genetic cargos like proteins, lipids, metabolites and various types of RNAs or DNAs are believed to be valuable for the diagnosis and treatment of human cancer. In this review, we will summarize recent progresses in the research of exosomes especially its biological and clinical relevance to MDR. By doing so, we hope it could be valuable for the prevention, detection and intervention of MDR which is one of the major challenges for the clinical management of human cancers.

Keywords: Exosomes, multidrug resistance, chemotherapy, immune suppression, signal transduction

Introduction
Cancer is a group of diseases that are characterized by uncontrolled cell growth, morphological and cellular transformation, angiogenesis, deregulation of apoptosis and metastasis [1]. With its incidence rates still rising, cancer is the second leading cause of death after cardiovascular diseases worldwide. Chemotherapy is one of the most important treatments for various cancer entities. However, cancer cells often have intrinsic resistance or develop acquired multidrug resistance to chemotherapeutic drugs, thus limiting its clinical efficacy. The development of chemotherapeutic drug resistance during the course of treatment for primary and metastatic tumors is a common phenomenon. Molecular mechanism for the development of chemotherapeutics resistance in cancer treatment is a point of common interest across the globe. Many cellular and genetic factors associated with chemotherapeutics drug resistance have been disclosed. However, the exact molecular mechanism underlying the phenomenon of multidrug resistance of tumors remains to be validated.

Exosomes are small nano-molecules secreted by extracellular vesicle bodies (EVBs) which carries various biochemical or genetic information. It plays a vital role in the maintenance of stable physiological and morphological functions. The dynamic studies elucidate the contribution of exosomes to the process of tumor chemo-resistance by facilitating the drug efflux. The drug and its metabolites can be associated with the production and movement of encapsulated exosomes in the cell microenvironment [2]. Recent studies suggested that the multidrug resistance (MDR) proteins MRP, LRP, and several tumor-derived exosomes miRNAs are involved in chemotherapy-associated resistance [3]. Production of exosomes and other components may be influenced by molecular signaling, depending on the origin of the cells and types of cells. Consequently, exosomes have specific roles to play in developing MDR and transferring genetic signals to control metabolism, tumorigenesis, intercellular signaling, and the immune system [4]. Circular DNA, miRNAs and lncRNAs act as either tumor suppressor genes or oncogenes which participate in cancer progression and resistance to therapy [5].
Biogenesis of exosome

Exosomes are small lipid bilayer extracellular vesicles (EVs) secreted by the luminal membranes of the multivesicular bodies (MVBs) and released from mammalian cells by exocytosis [6, 7]. Exosomes were first discovered by Trams and his colleagues in sheep reticulocytes early in 1980 [8] and later found in other mammalian including human cells [9]. Exosomes are one of the most heterogeneous groups of MVBs distinguished by their specific size of 30-100 nm and show a cup or dish-like morphology under transmission electron microscope (TEM) in numerous cells like stem cells, immune cells, neurons, cancer cells and some other body fluids like saliva, blood plasma/serum, semen, breast milk, and urine [10]. Before 1990’s, exosomes were considered as garbage bags between membranes and in cytoplasm [11], and later they were found to have a significant role in physiological as well as pathological processes [12]. They intercede cell-to-cell communication by transferring DNA, RNA, proteins and lipids among the cells [13, 14]. As a biological messenger in cancer cells, exosomes can transfer both intercellular and intracellular signals. O’Brien et al. found that exosomes level in the serum of breast cancer patients is normally higher when compared to normal samples [15]. However, functions of exosomes are versatile and largely depend upon the origin of cells. For example, exosomes, originating from cancer cells, serve as vehicles for immune system regulation and other pro-cancer properties like tumor growth and propagation [16].

Malignant cells discharge specific sets of EVs that are not secreted by normal eukaryotic cells [17, 18]. Further studies may lead to help the detection of specific pathways associated with biosynthesis of distinctive cancer cell-derived EVs. Some tumor cells are resistant to chemotherapy due to MDR-associated proteins MDR1 and ATP-binding cassette (ABC) transporter efflux system. It was reported by Jones that these proteins were carried by exosomes [19]. For example, docetaxel resistant prostate cancer released more exosomes than sensitive cells [20] and so did cisplatin resistant ovarian cancer [21]. Exosomes of colon cancer showed negative effects on proton irradiation and positive influence on the proliferation and metastasis of tumor cells [22]. Emerging evidence supported that exosomes from stromal cells as well as cancer cells might be potentially affected by therapeutic response via transfer of micro-RNAs (miRNAs) and proteins [23, 24].

Exosomes in multidrug resistance (MDR)

The key role of exosomes in cancer cells to act as mediators of cell-cell communication with the micro-vesicles has received considerable attention. It can transfer a variety of DNA, RNA and proteins in both paracrine and autocrine manners [13, 14]. For example, mesenchymal stem cells (MSC) derived exosomes significantly induce chemotherapy resistance of gastric cancer and also modulate the immune system in a suppressive mechanism [25]. Reported findings proposed that MSC exosomes have deep effects on the development of MDR-associated proteins like LRP, MRP and kinase in gastric cancer cells against 5-fluorouracil and cisplatin [20]. Recent studies have shown that chemotherapy enhances the secretion of exosomes in multiple tumor cells, which contain the chemo-resistance related mi-RNAs and mRNAs of the cells that alter their sensitivity to chemotherapeutic drugs like doxorubicin [26]. MDR genes like BCRP, MDR1 and MRP1 induce chemo-resistance and phenotypic change in breast and prostate cancer cells. They are also associated with the enhanced secretion of exosomes [20, 27]. Similarly, Lv et al. found exosomes function as mediators of MDR to transfer drug resistance from drug resistant cells to sensitive cells in MCF-7 breast cancer cell lines [28]. Exosomes might function to facilitate drug efflux like cisplatin through various miRNAs and proteins [29]. The lysosomes, where cisplatin accumulates, release significant exosomes with the help of transporter proteins [21]. Ciravolo and his colleagues found that exosomes are constitutively secreted by HER2-over-expressing breast cancer cell lines both in vitro and in vivo. HER2-positive exosomes bind with trastuzumab and inhibit its anti-proliferative effect on tumor cells. In addition, exosomes released by SKBR3 and BT474, HER2-over-expressing cell lines were significantly regulated by EGF and heregulin growth factors, HER2 receptor-activating ligands present in the surrounding tumor microenvironment [30]. Zhang et al. have found emerging functions of circulating exosome-mediated miRNAs in bortezomib resistance of multiple myeloma, which provided...
Exosome and MDR in cancer

Table 1. Function of exosome mediated microRNA (miR) in cancer

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Exosome miR</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>miR-100, miR-222, miR-125a-3p</td>
<td>Pathogenesis, diagnosis, chemo-resistance</td>
<td>[35, 125, 126]</td>
</tr>
<tr>
<td>Leukemia</td>
<td>miR-146, miR-138, miR-203, miR-30a, 135-b, 196-b, 181-c</td>
<td>Autophagy, chemo-resistance</td>
<td>[3, 127, 128]</td>
</tr>
<tr>
<td>Prostate and endometrial carcinoma</td>
<td>miR-204, miR-551b, 96, 183, 182, 153, 625, miR-141, 193b, 200c, 193a-3p, 205, 708, 365, 34a</td>
<td>Tumor development and progression, diagnosis</td>
<td>[56, 129]</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>miR-21, miR-24, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-205, and miR-214</td>
<td>Diagnostic marker</td>
<td>[54, 130]</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>miR-203</td>
<td>IL-12, TNF-α suppression</td>
<td>[98]</td>
</tr>
<tr>
<td>Lung cancer, leukemia, head and neck cancer</td>
<td>miR-17-3p, 21, 106a, 146, 155, 191, 192, 203, 205, 210, 12, 214, 451</td>
<td>Diagnostic marker, chemo-resistance</td>
<td>[131-134]</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>miR-21, miR221</td>
<td>Tumor progression, chemo-resistance</td>
<td>[135, 136]</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>miR-21</td>
<td>Chemo-resistance</td>
<td>[137]</td>
</tr>
<tr>
<td>Glioblastoma multiform</td>
<td>miR-9</td>
<td>Temozolomide resistance</td>
<td>[46]</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>miR-25</td>
<td>Cisplatin resistance</td>
<td>[134]</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>miR-20, miR-19b-3p, miR-106a-5p</td>
<td>Cisplatin resistance</td>
<td>[138]</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>miR-9-3p</td>
<td>Diagnosis biomarker</td>
<td>[65]</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>miR-21, miR-100</td>
<td>Cell signaling</td>
<td>[139, 140]</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>miR940, miR-23a</td>
<td>Tumorigenesis, intercellular communication</td>
<td>[141, 142]</td>
</tr>
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</table>

new understanding of the intercellular cross-talk mechanism in myeloma in vivo study [31]. Lopes et al. demonstrated that drug sensitive cells have less extracellular micro-vesicles like exosomes and macroparticles than MDR cancer cells, and also proposed the potential of exosomal proteins to be used as biomarker in cancer diagnosis [32].

Docetaxel is the first-line chemotherapy in castration-resistant prostate cancer (CRPC). However, it can induce resistance through either P-glycoprotein (P-gp) dependent drug efflux or exosome. Moreover, it has been investigated that exosomes could be used as diagnostic biomarker because they possess specific mRNAs and proteins of the cells from which they are released [33]. Kharaziha et al. reported that exosomes derived from DU145 Tax-Res cells may be a valuable diagnostic biomarker of CRPC [34]. Exosomal miRNA and proteins that mediate MDR in cancer cells are shown in the Tables 1, 2. The delivery of drug-resistant mRNA and P-gp via exosomes is an effective mechanism of exosome-mediated drug resistance transfer [28]. Previous studies have been reported that drug-resistant cancer cells are the main source of exosomes that transfer genetic signal to mediate intracellular communication, either in breast cancer or in prostate cancer [15, 20].

MSCs have been shown to interact with many factors in mediating drug resistance and enhancing the proliferation of tumor cells [36-38]. Specifically, exosomes from mesenchymal cells transfer signals to the tumor to promote metastasis as well as chemo-resistance. Exosomes and pattern recognition receptors (PRRs) orchestrate heterotypic cellular communications to assist cancer progression, and involve in crosslink connection between cancer and the tumor microenvironment [39]. Balaj et al. have shown that non-coding RNAs that may persuade antiviral responses to influence therapy resistance were found in exosomes [40]. The whole mechanism of exosomes associated multidrug resistance in cancer cells are summarized in the Figure 1.

In addition, exosomal RNA transcripts can stimulate RIG-I activation, which is consistent with the known properties of RIG-I stimulatory viral RNA [41]. MDR cells produced more micro-vesicles and fewer exosomes than drug-sensitive cells because MDR cells contain P-gp, which is associated with the biogenesis of extracellular vesicles [42]. Richards et al. suggested that cancer-associated fibroblasts (CAFs) exosomes enhance chemo-resistance inducing factor Snai1, promote angiogenesis and resistance to gemcitabine in pancreatic ductal adenocarcinomas (PDACs), therefore, using exosome in-
Table 2. Exosomes associated proteins composition and functions

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Name</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraspanin</td>
<td>CD9, CD37, CD63, CD53, CD81, CD82, CD83</td>
<td>Biomarker, multidrug resistance</td>
<td>[143]</td>
</tr>
<tr>
<td>Heat-shock proteins</td>
<td>HSP70, Hsp84, Hsp90 αBC, HSP20, HSP27</td>
<td>Multidrug resistance, immune suppression</td>
<td>[144-146, 67]</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>G1a2, G1a3, Gsa, FRL, Erk2, Fn, Sh2, phosphatase, Rhöa, C, Gdi, syntenin, CBL, Catenin, LCK</td>
<td>Signal transformation</td>
<td>[71]</td>
</tr>
<tr>
<td>MVB formation</td>
<td>Alix, TSG 101, Gag</td>
<td>Cell trafficking, biomarker</td>
<td>[67]</td>
</tr>
<tr>
<td>Antigen presentation</td>
<td>MHC I, MHC II, CD86</td>
<td>Immune system regulation</td>
<td>[72]</td>
</tr>
<tr>
<td>Adhesion molecules/targeting proteins</td>
<td>CD146, CD166, ICAM-1, ALCAM, MAC-1, Integrin α chain, Integrin β, LFA-3, CD53, CD326, CD11a, 11b, 11c, MFG-E8/lactadherin, CD171, CLDN3</td>
<td>Intercellular communication</td>
<td>[73, 146]</td>
</tr>
<tr>
<td>Cytoskeletal proteins</td>
<td>Talin, CAP1, ezrin, tubulin, coflin, actin, moesin, rodixin, adhavin, vimentin</td>
<td>Tumorigenesis, immune system regulation</td>
<td>[147]</td>
</tr>
<tr>
<td>Membrane transport and fusion</td>
<td>AP-1, Arp2/3, SNAP, syntaxin, dynamin, Rab5, 7, Rap1B, RabGDI, annexins (I, II, IV, V, VI), epidermal growth factor receptor (EGFR)</td>
<td>Biogenesis, cell transportation</td>
<td>[148]</td>
</tr>
<tr>
<td>Enzymes</td>
<td>ATP citrate lyase, ATPase, glucose 6-phosphate isomerase, peroxiredoxin 1, Asp, amino-transferase, aldehyde reductase, fatty acid synthase, pyruvate kinase, glucose 6-phosphate isomerase, ATP citrate, lyase, ATPase, fatty acid synthase (FASN)</td>
<td>Metabolism, cell signaling</td>
<td>[149, 71]</td>
</tr>
<tr>
<td>Anti-apoptosis proteins</td>
<td>ATG7, P53, caspase 3, Bcl-2</td>
<td>Cell progression and angiogenesis, multidrug resistance</td>
<td>[69]</td>
</tr>
<tr>
<td>Domain proteins</td>
<td>Proliferation cell nuclear antigen (PCNA)</td>
<td>Proliferation, angiogenesis</td>
<td>[73]</td>
</tr>
</tbody>
</table>

Interestingly, a recent study adopted another method which validates that exosomes may participate in multi-drug resistance. It was found that exosomes released from cancer cells might resist the effect of antibody and chemotherapies by expressing tumor-derived cell surface antigens that confiscate the compound away from the target cell [44]. In addition, exosomes have been identified to reduce antibody dependent cell cytotoxicity (ADCC) by binding to cancer reactive antibodies. It is demonstrated that exosomes interfere with T lymphocytes to inhibit ADCC [45]. In glioblastoma multiforme (GBM) cells, miR-9 promoted resistance against temozolomide (TMZ), increased MDR1 gene expression, activated Sonic Hedgehog (SHH) pathway and suppressed PTCH1 level [46]. The development of de novo resistance acquired by radiation, chemotherapy, and other targeted therapies is still a stumbling issue in the cancer treatment and an emerging field of research [47]. The development of resistance is multi-faceted, as tumor cells may switch to retrieve secondary pathways to survive when the primary hallmark is blocked [48]. Further studies are needed to expand our understanding of molecular mechanism of multidrug resistance and role of exosomes in cancer cells.

Cargos of exosome nucleic acids

Comprehensive researches have been carried out on EVs over the period. The non-spherical membrane-bounded exosomes contain several families of proteins, lipids and nucleic acids originated from the parent cells when observed under high-resolution electron microscope. According to the database, more than 1639 mRNAs, 764 microRNAs, 4653 proteins and 194 lipids could be included in exosomes from various eukaryotic cells [49, 50]. For past few years emerging evidence suggests that exosomes are involved in tumor progression and development via intercellular nucleic acid communication [51]. Cancer cells derived exosomes have different kinds of genetic materials including mRNA, microRNAs, circular DNA, small nucleolar RNAs (snoRNAs), transfer RNAs (tRNAs), long noncoding RNAs (IncRNAs), ribosomal RNAs (rRNAs) and small nuclear RNAs (snRNAs), all of these are functionally active [52]. RNA in Exosomes was first detected by Valadi from mice and human derived mast cells. Exosomal RNAs transfer signal from cell to cell; therefore they are termed as “exosomal shuttle RNA” [13]. Adriamycin and docetaxel-
resistant breast cancer cells release exosomes that transfer genetic cargo and specific miRNAs between tumor cells and promote the proliferation, angiogenesis, MDR and metastasis [35]. Exosomes may also transfer circular DNA and proteins from one cell to another target cells [53]. Tumor-derived exosomes have distinct mRNA profiles across all type of cancers including ovarian [54], breast [55], prostate [56] and lung cancer [57]. Exosomes contain DNA fragments and mutated mRNA transcripts which may be involved in enhancing the growth and angiogenesis of primary and metastatic cancers [58, 59]. According to Xiao et al., miR-21 and 133b are detected in exosomes and highly expressed in DDP-resistant A549 lung cancer cells compared to those in wild-type cells [60]. Leukemia cell-derived exosomes mediate miRNAs transfer to endothelial cells and play substantial roles in enhancing interactions among specific cell populations [61]. A recent research has investigated that mRNAs and miRNAs are present in EVs and they are also transferrable to recipient cells where they can be translated into functional proteins [13].

Outstandingly, miRNAs have been shown to be shuttled between cells by EVs, leading to the repression of mRNA expression in recipient cells [62], although current evidence suggests that miRNAs can also be transported and delivered through other mechanisms [63]. Through exchange of genetic information, EVs are thought to show pleiotropic biological functions and significances in a variety of fundamental physiological and pathological processes [64]. Wang et al. have identified novel exosomal miR-19b-3p and miR-106a-5p in gastric cancer cells and suggest that these miRNAs can be used as biomarkers for diagnosis of gastric
Exosome and MDR in cancer

As more miRNAs in exosomes released by human tumor cells and cancer cell lines are characterized [66], it is shown that all these genetic cargos are associated to MDR mechanism in tumor cells. miRNAs as genetic cargo in exosomes and their functions in cancer cells are shown in the Table 1.

**Cargos of exosomal proteins and lipids**

Exosome proteins are quite different from intercellular proteins secreted by apoptotic cells. They contain specific sets of protein families arising from endocytic pathways [67]. Numerous reports have already shown that exosomes carry membrane transport and fission proteins like Rab, GTPases, Tsg101 and annexin. Furthermore, they included tetraspanin proteins that are associated with lipid microdomains, such as CD9, CD63, CD81, CD82, CD8 [68] and heat shock proteins (HSPs) [51]. Tetraspanin proteins (CD9 and CD63) and HSPs are specifically the most prominent conserved proteins in exosomes that highlight potential biomarkers [69, 70]. Mathivanan and his co-workers have reported 11,000 proteins associated with exosomes according to ExoCarta [50]. Some of these proteins are metabolic enzymes like pyruvate dehydrogenases, peroxidases, enolases and lipid kinases [71], while others include major histocompatibility complex (MHC) and signal transduction proteins [72]. Several exosomal proteins overexpressed in plasma of ovarian cancer patients compared to patients with initial stages of tumors and healthy individual controls, including proliferation cell nuclear antigen (PCNA), epithelial cell surface antigen (EpCAM), epidermal growth factor receptor (EGFR), tubulin beta-3 chain (TUBB3), ERBB2, and L1CAM (CD171), apolipoprotein E (APOE), claudin 3 (CLDN3) and fatty acid synthase (FASN). Cellular apoptosis susceptibility (CSE1L/CAS) gene is highly expressed in several tumors that are associated with cell signal transformation and angiogenesis [73, 74]. Exosomal proteins have fundamental physiological as well as pathological functions in cell communications, immune suppression, tumor cell prolongation and multidrug resistance to cancer therapy. Exosome cargos with high incidence in cancer cells have been commonly used as biomarkers for the detection and confirmation of exosomes [75]. The composition and functions of exosome-associated proteins are listed in the Table 2.

Moreover, the composition of exosomes from the parental cells can be different due to the distinctive categories of the cargo elements. They are made up of lipid bilayer membranes surrounding part of cytosol in the cells. The structural lipids not only give specific shape to exosomes, but also have significant roles in signal transduction. Lipid is also a major component of exosomes, such as cholesterol, diglycerides, sphingolipids, phospholipids, glycerol phospholipids, phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and poly glycerol-phospholipids (i.e. bisphosphate) [49]. A previous research has found evidence that the incidence of phosphoinositides in the inner leaflet of a cell membrane is involved in multiple intracellular signaling pathways, which could be used as biomarker of lipid diseases [76].

Exosomes with bioactive components of lipids such as leukotrienes and prostaglandins can insulate from the parent cells, but they also have some active enzymes of lipid metabolism which are able to produce these bioactive compounds [77, 78]. Therefore, exosomes may function as lipid transporters, allowing the passage of the bioactive lipid molecules they carry to recipient cells. This progression of exosome trafficking, predominantly in the context of the tumor microenvironment, may lead to the enhancement of certain tumor progressive/immunosuppressive lipids, such as prostaglandins [79]. However, it may also guide to a supplementary of beneficial exosomal lipid contents, such as docosahexaenoic acid, an omega-3 polyunsaturated fatty acid with many healthy and anticancer benefits, which can affect cell-to-cell signaling, increase sensitivity to therapeutic interventions and reduce tumor cell growth [6, 80].

**Immune modulation by exosome and MDR**

Previous studies elucidating the biological functions of exosomes have revealed important roles of exosomes in modulating immune responses owning to their biochemical and genetical cargos. The immune modulatory function of exosomes was first found in dendritic cells (DCs) through dynamic studies of major histocompatibility complex (MHC) class II. MHC II released from DCs-derived exosomes was capable of stimulating antigen-specific responses both in vitro and in vivo via different path-
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ways, such as cytokine modulation and chemokine transport [81]. Exosomes have several integral, peripheral cytosolic associated proteins induced as antitumor immune responses in vivo and also identify low exosomes production in mature DCs [82], while high amount of exosomes only released by immature DCs. DCs-derived exosomes as key regulators of immune system have several functions. They transport MHCs between dendritic cells, promote the initiation of adaptive immunity, enhance the tumorigenesis and promote resistance to chemotherapy [83]. Mast cell-derived exosomes indirectly activate B and T cells through cell differentiation and specific immune responses [44, 84]. Kim et al. reported IL-10 treated DCs derived exosomes reduced inflammation in autoimmune diseases like arthritis [85]. Salivary glands epithelial cells (SGECs) derived exosomes may be associated with autoimmune suppression since they have auto-antigens Ro/SSA, La/SSB, and Sm [86]. Tumor cells commonly cause autoimmune suppression, angiogenesis and progression of tumorigenesis. Tumor cells mediated exosomes have a specific mechanism that controls the activation of immune systems and enhances the tumor cell growth. Synovial fibroblast derived exosomal proteins and TNF-α may involve in the development of apoptosis resistance and suppress T-cells activation induced cell death [87]. OVA protein has stimulatory effects on CD8+ T-cell progression [88]. It induces and controls humoral immunity of specific antigen response to DCs in vivo [89], natural and autoimmune against bacterial infection in vivo [90]. Exosomes are mobile elements with cargos of proteins and miRNA that suppress the immune system and promote tumor metastasis, cell proliferation, and therapeutic resistance. Cancer-derived exosomes may inhibit natural killer cells proliferation and natural killer group 2 and member D (NKG2D), CD8 (+), and gamma delta (+) T cells [92].

Interestingly, exosomes mediate regulation to immune tolerance or immune activation and suppress hypersensitivity type-IV antigen-specific response. CD80 and CD86, which are co-stimulatory molecules via exosomes through direct interactions with lymphocytes, control natural killer cell activation [93, 94]. HCC cells exosomes contain rich amount of HSP60, 70 and 90, which act as a negative signal to inhibit NK cells mediated antitumor responses and activate MDR proteins to enhance resistance to therapy [95]. Recent studies showed that macrophages induce tumor invasion after stimulation by exosomes. Macrophages reduce levels of IL-16, TIMP1, IFNg, and induce a high level of CCL2, IL-8, MIP2 and IL-1Ra, all of which are associated with tumor cell progression [96]. Exosomes based vaccines have been used to induce antitumor immunity [97]. Zhou et al. reported interleukin IL-12 and tumor necrosis factor-α (TNF-α) expression are inhibited by exosomes and cytokines are down-regulated through pancreatic cancer derived exosomes containing miR-203 [98]. Tumor-derived exosomes have cargo elements that are capable of inducing immune suppression through various pathways. In vivo immune system suppression modulated by exosomes drives tumor progression and metastasis development [99]. The exosomes derived from dendritic cells and other immune cells have been implicated in the regulation of immune response [100], while immunological activities of exosomes in tumors are dynamic and complex [101].

Previous studies found that exosomal miRNAs are not only diagnostic biomarkers but also associated with cell signaling pathways between microvesicles. miR-24 enriched in breast cancer-mediated exosomes are involved in T-cell inhibition and tumor angiogenesis. Tumor-mediated exosomal miR-24 regulates tumor angiogenesis by inducing T-cell suppression through FGF11 cell signaling and also acts as a prognostic biomarker in nasopharyngeal malignant cells [102]. Whiteside reported in 2016 that tumor-derived exosomes have potential ability of immune suppression since they carry immunosuppressive factors like DNA, mRNA, and micro RNAs. Exosomes associated signaling pathways contribute to the down-regulation of anticancer immunity and increase MDR resistance to tumor therapy [103].

To better understand mechanisms of cancer development, most of the experimental evidences investigate the immunosuppressive role of tumor-derived exosomes in cancer progression and therapy resistance. For example, overexpression of immunoglobulin B-cell receptor in mice serum and 5T33MM cells by exosomes and identified the relationship between B-cells malignancy and MDR in multiple myelo-
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Immune suppression and prolonged angiogenesis induced by melanoma mediated exosomes are regulated by vascular endothelial mediated TNF-α [105]. As for the whole cancer microenvironment, the interaction between the cellular immune system and exosomes can promote tumorigenesis and pathological states by induction of CD4+CD25− T-cells to CD4+CD25+Foxp3+ and T-reg through phosphorylation of Stat3 and Smad2/3 [106, 107]. Exosomal tetraspanin family has the ability to induce immune suppression, metastasis and resistance to chemotherapy by systemic induction of angiogenesis signals in cancer and non-cancer cells [108]. In summary, exosomes have the ability to stop anti-tumor immune response and promote tumor development and progression (Figure 2).

**Signal transduction by exosome**

One of the most outstanding features of cancer-related exosomes is co-development of phenotypic and genotypic changes in tumor microenvironment through signal transduction between cancer cells and tumor associated stroma. For instance, the well-known mechanism of exosomes-mediated intercellular communication is via signaling molecules such as proteins and mi-RNAs to interact with the surface receptors on target cells, thereby activating intercellular pathways [109]. For example,
there are several exosomal mRNAs and miRNAs reported to be shuttled between cells that can lead to direct stimulation of target cells through bioactive lipids as potential mediators of signaling pathway [13]. Inclusive studies in the past 30 years have revealed a few signaling pathways activated by transcriptional regulators during cell development and progression such as Notch, Hedgehog (HH) family of secreted proteins, Wingless/WNT, epidermal growth factor (EGF), and fibroblast growth factor (FGF) [110]. Pikkarainen et al. have identified that WNT signaling and Mac-2BP expressions are upregulated in HEK293 cells by induction of exosomes [111]. Further, they suggested that the four-domains of Mac-2BP play a vital role in WNT binding with the C-terminal domain [112]. In vitro experiments reported that WNT signaling pathway was upregulated after treatment by exosomes in human mesenchymal stem cell (MSC) and breast cancer MCF7 cell line [113]. Bretz et al. have shown ex-vivo exosomes induce the cell signaling effects in THP-1 cells by producing IL-1β, TNF-α, and IL-6 to suppress the immune system and enhance resistance to cancer therapy [113].

Signal transduction is essential to existence and maintenance of homeostasis in all multicellular organisms through membrane adhesion molecules, gap junctions and nanotubes, or via soluble signal transduction such as cytokines, growth factors, and hormones secreted by both cancer and non-cancer cells [114]. Tumor-derived exosomes (TEX) carry cargos of several stimulatory and inhibitory biomolecules, serving as a signal network in cancer micro-environment in vivo and in vitro. The TEX-induced Ca2+ influx in cancer cells and subsequent signaling are important for T-reg to function. Thus, modulation of T-reg suppressor by TEX is through cell signaling-dependent mechanisms and does not need TEX internalization by recipient cells [115]. A recent research has investigated that chemotherapy-induced exosomes in myeloma cancer cells lead to the stimulation of ERK signaling. Moreover, an increase in flaking of the syndecan-1 proteoglycan and anti-myeloma therapy triggers a release of exosomes containing a huge amount of heparanase that remodels extracellular matrix and modifies tumor-host cell interaction, probably contributing to chemo-resistance and ultimate disease recurrence [116]. In addition, the key mediators in intercellular communications are small circular molecules like EVs secreted by host cells. They mediate the exchange of genetic materials and proteins associated with tumor angiogenesis. Exosomes derived from tumor-associated macrophages (TAMs) mediate cisplatin resistance in gastric cancer via signal molecule miR-21. Furthermore, miR-21 suppresses cell apoptosis and enhances upregulation of PI3K/AKT signaling pathway by down-regulation of PTEN in gastric cancer [117]. Transforming growth factor-beta (TGFβ) signaling mediated by exosomes in squamous cell carcinoma (SCC) is also reported. Exosomes transferring components of the TGFβ signaling pathway between tumor cells and stromal fibroblasts provide possible mechanism for MDR [118].

Translational values of exosome

In the uprisingle research of cancer, diagnosis and treatment of MDR is a big challenge. Analysis of exosomes may present potential biomarkers to monitor the progression or emergence of MDR during cancer treatment. Exosomal RNAs stimulate the receptor RIG1 to activate STAT1 signaling pathway in breast cancer cells (BrCa). In parallel, stromal cells also activate NOTCH3 in BrCa cells as STAT1 facilitates transcriptional responses to NOTCH3 and increases chemotherapy-resistant tumorigenesis [23]. Ristorcelli et al. have reported the first evidence that exosomes are involved in apoptosis pathway, where it can up-regulate the expression of Bax but down-regulate the expression of Bcl-2 in human pancreatic cancer cells. Furthermore, exosomes can induce the increased activity of phosphatase, tensin homologue (PTEN) and glycogen synthase kinase (GSK)-3β and decrease the expression of keto acid dehydrogenase [119]. Recent studies anticipated that exosomal miR-222 directs target MDR gene expression and could regulate resistance to breast cancer therapy in MCF-7 cultured cells [35], and GLT1 (astrological glutamate transporter) expression is also regulated by neuronal exosomes miRNAs [120]. Melo et al. have reported that cancer mediated exosomes contain RISC-Loading complex miRNAs along with AGO2, TRBP, and Dicer proteins, which are capable of rapidly silencing of miRNAs and reprogramming the target cell transcriptome. These findings provide significant opportunities
for the development of exosomes-based diagnostic biomarkers and cancer therapy [121]. However, an additional mechanism has recently emerged, based on the exosomes released by breast cancer cells. They revealed that psoralen can affect the exosomes and it persuades the reduction of MDR resistance proteins transmission via exosomes probably through p53 and PPAR signaling pathways [122]. This study might provide a novel approach to deal with resistance to chemotherapy in breast cancer. Further advances in our understanding with regard to translational values of exosomes and other EVs on target cells highlight the significance of establishing essential knowledge in this field.

MSC-exosomes play significant roles in post-transcriptional and translational level of genes in various carcinoma. Ji et al. demonstrated that exosomes triggered the activation of Raf/MEK/ERK kinase cascade and calcium/calmodulin-dependent protein kinases (CaMKs) in gastric cancer cells, while blocking these kinases could be achieved by inhibiting the promoting role of MSC-exosomes in chemo-resistance gastric cancer cells [25]. Exosomal proteins AnxA2 contributes to metastatic cell transformation and its overexpression and phosphorylation are related to cancer progression and tumorigenesis. However, regulation of AnxA2 function is an essential feature of cellular stability, and the prospective regulations of exosomal AnxA2 by phosphorylation or other PTMs are the most interesting studies in future [123]. Further evidence found that ubiquitinated proteins in exosomes are shed by myeloid-derived suppressor cells (MDSC). These five proteins associated with endosomal trafficking, i.e., keratin, histone, heat shock proteins, ezrin and pyruvate kinase isozyme, were detected as ubiquitinated proteins as a post-translational modification of histones in cancer cells [124].

Conclusions and perspectives

The predictive value of exosomes in cancer has expanded rapidly over the past few decades. This has led to the understanding of the fundamental role of exosomes in tumor growth and multidrug resistance. Exosomes are small but skilled mediators of immune responses, intercellular communications and tumorigenesis. The precise understanding of physiological functions of exosomes may allow us to develop novel diagnostics, therapeutics, and targeted drug delivery approaches to overcome multiple drug resistance. However, many opening questions still exist, such as the heterogeneity of cancer exosomes, exosome cargos effects on donor and recipient cells, the biogenesis of exosomes and molecular mechanisms of de novo and adopted resistance to therapy. Future studies will focus on several unknown functions of exosomes in cancer biology and need to further explore the co-contribution of nucleic acids, proteins, and lipids to the genotype as well as the phenotype of the cancer. We can further elucidate novel and useful therapeutic approaches for MDR, based on the inhibition of exosomes-facilitated intercellular communication in cancer cells.

Acknowledgements

This work was supported by Natural Science Foundation of China (81572715; 81772944), Zhejiang Natural Science Foundation (LZ18-H160001) and Department of Healthcare in Zhejiang. The authors acknowledge the input of Dr. Qurat-ul-ain Arif, Oxford University, U.K. for proofreading this review.

Disclosure of conflict of interest

None.

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