The Role of Exosomes and its Cargos in Drug Resistance of Cancer

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Abstract: Chemotherapy is one of the main therapies in cancer and plays an important role in controlling tumor progression, which can offer a longer overall survival (OS) for patients. But as the accumulation of drugs used *in vivo*, cancer cells develop drug resistance, even multi-drug resistance (MDR), that can cause failure of the whole therapy. The similar phenomenon can be observed *in vitro*. There are several mechanisms of drug resistance such as drug efflux, mediated by extracellular vesicles. Exosomes, a subset of extracellular vesicles (EVs), can be secreted by many types of cells and transfer proteins, lipids, and miRNA/mRNA/DNAs between cells *in vitro* and *in vivo*. Particularly cancer cells secrete more exosomes than healthy cells and resistance cells secrete more exosomes than sensitive cells. Exosomes have function of intercellular communication and molecular transfer, both associated with tumor growth, invasion, metastasis, angiogenesis, and drug resistance. In this paper, we will review the current knowledge regarding the emerging roles of exosomes and its cargo in drug resistance.

Keywords: Exosomes, drug resistance, drug efflux, antibody, miRNAs, lncRNA, P-glycoprotein, EMT.

INTRODUCTION

Chemotherapy is the major treatment used in cancer management. Development of resistance to drugs in cancer remains a major obstacle to the success of chemotherapy [1]. More than 90% of patients with metastatic disease relapse and become unresponsive to treatment due to the development of drug resistance [2, 3]. There are different mechanisms of developing drug resistance such as drug efflux mediated by exosome and intercellular communication. Here in this paper, we mainly focus on the relationship between tumor-derived exosomes (TD-exosomes) and drug resistance in cancer. There are multiple reasons for drug resistance, including mediating drug efflux of tumor cells [4], the inhibition of tumor suppressor proteins by miRNAs [5], the presence of a subset of cancer stem-like cells with high drug-resistance [6], and the reduction in interaction between anti-cancer drugs and cancer cells [7]. Exosomes may play a role in the above pathways of developing drug resistance.

THE STRUCTURE AND FUNCTIONS OF EXOSOMES

Exosomes were first discovered in sheep in 1983 as transferrin associated 50 nm vesicles extruding from reticulocytes and then were found to be secreted by a wide range of mammalian cell types [8]. Thirty years past, considerable amount of researches haves done in order to understand the exosomes. Exosomes are a kind of extracellular vesicles secreted from the original cell. Exosomes are 50-100 nm in diameter and 1.13-1.19 g/mL in density, with a classic “cup” or “dish” morphology. Exosomes can be released by the fusion of multivesicular bodies (MVBs) to the plasma membrane, or can be formed by the breakage of endosome-like bodies from the membrane [9]. Exosomes consist of a lipid bilayer membrane, which matches the characteristics of the original cell, surrounding a small cytosol. The structured lipids are involved in cell communication by regulating cell signaling pathways away from the origin. The lipid structures of exosomes can carry various important proteins and nucleic acids, and guide cell signaling pathways between the normal and disease states.

It is clear that exosomes are an important way for intercellular communication [10]. It has been revealed various new information of material transport across biological membranes. To a great extent, the role of exosomes in disease development has been confirmed, especially in cancer [11, 12]. Exosomes were initially considered as garbage bags for abandoned membrane parcels and molecular fragments. In the mid-1990s, exosomes were recognized as being closely related to the function of the immune system with the finding of the role of exosomes in the presentation of B lymphocyte antigens [13]. In the 2010s, researchers found that miRNA and mRNA can be loaded as “goods” in exosomes. Actually many types of protein, breakdown products of signaling pathways, viruses [14] and RNAs [15], miRNAs [16] can be transported through exosomes. In recent years,

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another function of exosomes was revealed: they can serve as "communication shuttles" and transduction signals between cells. Exosomes play important roles in many physiological and pathological processes such as immune surveillance, inflammation, tumorigenesis, and drug resistance.

Many studies confirm that exosomes can interact with recipient cells [17], but it is unclear how the exosomes interact with and regulate the function of target cells. Based on indirect evidence and studies *in vitro*, several mechanisms of interactions have been proposed: (1) binding to the surface of the recipient cell through exosomal adhesion molecules; (2) direct fusion of vesicles with recipient plasma membrane after adhesion; or (3) internalization of vesicles into endocytic compartments through receptor-mediated endocytosis or phagocytosis. The interaction between exosomes and target cells can lead to direct stimulation of target cells *via* surface-expressed growth factors (EGF) or bioactive lipids, transfer of membrane receptors, or delivery of proteins and nucleic acids to target cells. Additionally, the presence of mRNA and miRNA, termed "exosomal shuttle RNA," in exosomes suggests that genetic material exchange could be an additional level of exosome-mediated intercellular communication [18]. The diversity of export cargos by exosomes indicates that much to be learned on the effects of exosome transport. In the following paragraphs, the role of exosomes and its cargos in different aspects will be reviewed.

EXOSOME-MEDIATED DRUG ACCUMULATION AND EFFLUX

Traditionally, exosomes are in charge of waste product export and less needed molecules from cells. In cancer cell models, exosomes could also export chemotherapeutic drugs, which partly play a role in cancer cell resistance to chemotherapy.

As presented, researchers found that anti-cancer drugs may be subjected to efflux by exosomes leading to reduction of efficacy of cancer treatment [4]. The relationships between drug efflux and drug sensitivity in different tumor models strongly suggest that forming and shedding of exosomes is closely related to drug resistance in many tumors [19]. Drugs are accumulated in intracellular vesicles which can prevent drugs from killing the cancer cells. When shedding intracellular vesicles become exosomes secreted into the exterior, the intracellular concentration of the drug is also reduced, which shows the reduction of chemo-sensitivity. Based on the theory that exosomes can mediate drug efflux, much research has been conducted to confirm whether exosomes participate in the process of tumor resistance. The doxorubicin encapsulated in exosomes was captured using fluorescence microscopy, which proved the hypothesis that drugs were wrapped and physically excluded by exosomes [20]. The resistance of docetaxel is probably related to the increased secretion of exosomes, and even leads to a change in cell phenotype in prostate cancer [20, 21] and breast cancer models [22]. In cisplatin-resistant human ovarian cancer cell lines compared with cisplatin-sensitive cells, the accumulation of cisplatin in the lysosomal compartment is significantly reduced owing to the release of exosomes, and even stored cisplatin is rapidly discharged from the lysosomes with the help of enhanced cisplatin transporter proteins [23]. In addition, after treatment with cisplatin, the amount of cisplatin in exosomes released from cisplatin-resistant cells is 2.6 times higher than from cisplatin-sensitive cells, indicating that exosomes can be used as an efflux mechanism for anticancer drugs by tumor cells [23]. A similar phenomenon was also observed with melanosome [24]. The above studies support the viewpoint that the exclusion of drugs or their decomposed products by exosomes will result in a reduction of drugs in tumors, and even guide the drugs to act on nearby non-target organs. Exosome-mediated drug efflux is now an emerging concept in the area of drug resistance.

THE EXOSOME-ANTIBODY INTERACTION INHIBITED THE ANTI-TUMOR EFFECTS

As a kind of extracellular vesicles, the membrane of exosomes which matches the characteristics of the original cell, can express and carry the same antigen as the original cell. To an extent exosomes may counteract the effect of antibody drugs.

Exosomes with HER2 Expression Binding to Trastuzumab Contribute to Drug Resistance

Researchers found that Exosomes from HER2-overexpressed breast cancer cell lines also contain full-length HER2 molecules and can inhibit the drug effects [25]. Exosomes secreted by HER2-overexpressing breast carcinoma cell lines were analyzed *in vitro* and *in vivo* their potential role in interfering with the therapeutic activity of the humanized antibody Trastuzumab. The result showed that exosomes released by the HER2-overexpressing tumor cell lines express a full-length HER2 molecule that is also

activated, although to a lesser extent than in the original cells. Release of these exosomes was significantly modulated by the EGF and heregulin, two of the known HER2 receptor-activating ligands and naturally present in the surrounding tumor microenvironment. Exosomes secreted either in HER2-positive tumor cell-conditioned supernatants or in breast cancer patients' serum bound to Trastuzumab. Functional assays revealed that both xenogeneic and autologous HER2-positive exosomes inhibited the anti-proliferative effects of trastuzumab by preventing it from binding to tumor cells. These findings point to the role of HER2-positive exosomes in modulating sensitivity to Trastuzumab [25].

Specific Exosomes Binding to CD20 and other Immune Factors

Another research showed that Lymphoma exosomes carry CD20, which bind therapeutic anti-CD20 antibodies and protect target cells from antibody attack [26]. Moreover, antibodies detained in exosomes can reduce the antibody-dependent cellular cytotoxicity against tumor cells by immune effector cells. The cell-released exosomes contain abundant complement proteins and complement membrane attack complex (MAC) and can be used as a preventive mechanism to prevent membrane lysis *via* the complement system [27]. The exosome-secreted membrane attachment type TNF-α can prevent cell death induced by cytotoxic T cells [28].

THE ROLE OF EXOSOME-DERIVED NUCLEIC ACIDS IN DRUG RESISTANCE

Exosome-Derived microRNAs Regulate Drug Sensitivity

MicroRNAs (miRNA) are small noncoding RNAs that are usually 20–25 nucleotide long sequences with diverse functions [29, 30]. They can regulate many genes by binding to non-coding regions of target mRNA, causing disorders in the target genes [29]. They are recognized as an important mechanism of intercellular communication in exosomes [31, 32]. MiRNAs have also been shown to be included into exosomes and to be capable to regulate the function of distant cells entering the blood stream [33, 34] and may affect the processes of receptor cells, especially by promoting interaction between various cells in the tumor microenvironment (TME)[35].

As more and more studies have revealed the significance of exosomal miRNAs in intercellular communication [36], miRNA expression patterns differed between exosomes of drug-resistant and drug-sensitive cells. Drug-resistant breast cancer (BCa) cells are an abundant source of exosomes [37], researchers have begun to explore the relationship between exosomal miRNA and drug resistance. The ability of drug- resistant BCa cells to transmit resistance capacity is probably due to their release of exosomes. When exosomes were treated with RNase transfer of drug resistance was impaired [38]. This phenomenon may reveal that exosomes could alter chemo-susceptibility in recipient sensitive cells by modulating cell cycle distribution and drug-induced apoptosis after binding, absorption, and internalization [39, 40]. Recently Chen and colleagues reported that exosomes from drug resistant BCa cells are capable of delivering a subset of miRNAs (miR-100, miR-222 and miR-30a) to sensitive cells [40]. *MiR-34a*, detected as both intracellular and exosomal biomarker, was recently found also to influence prostate cancer cell response to docetaxel by regulating anti-apoptotic *BCL-2* gene [41]. Another study identifies exosomic miR-21 and miR-155 triggered drug resistance to chemotherapy through dendritic cells in neuroblastoma and identifies exosomes within the TME as important molecular targets to restore drug sensitivity [42].

Exosomes can also increase chemoresistance of the recipient cancer cells. When cisplatin is added to lung cancer cells (A549), exosome secretion is strengthened, and the addition of this secreted exosomes to other A549 cells can increase the resistance of these cells to cisplatin [43]. When A549 is exposed to cisplatin, the expression levels of several miRNA and mRNA, which are reportedly associated with cisplatin sensitivity, change significantly in secreted exosomes. This phenomenon implies that the changes of potential associated miRNA and mRNA may mediate the resistance of A549 cells to cisplatin, but the precise underlying mechanisms are still being studied.

LncRNAs Transferring by Extracellular Vesicles Modulate Chemosensitivity

Long non-coding RNAs (lncRNAs) are defined as non-coding RNAs more than 200 nucleotides in length [44-47]. Like miRNA, these lncRNA can regulate the expression of associated genes at transcriptional, post-transcriptional, and epigenetic levels [48] and have an impact on many different cellular processes. Recently, Several lncRNA have been implicated in human liver diseases. In a hepatocellular cancer (HCC) model,

researchers found that lncRNA was enriched in exosomes from HCC cells can reduce chemotherapy-induced cell death in recipient cells by mediating TGFβ-dependent chemoresistance [49]. Amongst the lncRNA, the lincRNA-ROR is the most significantly upregulated lncRNA in malignant hepatocytes. This lncRNA has been recognized to contribute to epigenetic regulators involved in pluripotency and lineage commitment [50]. LincRNA-VLDLR (linc-VLDLR) ,another lncRNA found in EVs, was also significantly up-regulated in malignant hepatocytes[51]. Exposure of HCC cells to diverse anti-cancer agents such as sorafenib, camptothecin, and doxorubicin increased linc-VLDLR expression in cells as well as within EVs released from these cells. RNAi-mediated knockdown of linc-VLDLR decreased cell viability and abrogated cell cycle progression. Moreover, knockdown of VLDLR reduced expression of *ABCG2* (ATP-binding cassette, sub-family G member 2), whereas over- expression of this protein reduced the effects of VLDLR knockdown on sorafenib-induced cell death[51]. Therefore, linc-VLDLR is identified as an extracellular vesicle enriched lncRNA that contributes to cellular stress responses.

Exosomes Secreted with mRNA Related to Drug Resistance

Excluded miRNA and lncRNA, exosomes also carry nucleic acids in larger size, like mRNA. Inhibitors of apoptosis (IAP) are a kind of functional proteins which can regulate cell survival and are often deregulated in cancers. The high levels of IAP expression in cancer cells are associated with disease progression and therapy resistance [52, 53]. Exosomes secreted from human cancer cell lines contain full-length IAP mRNA transcripts and were absorbed by recipient cells. These mRNA may be translated into functional proteins in the recipient cells and may increase cell resistance to anticancer drugs [54, 55].

THE ROLE OF EXOSOME-DERIVED PROTEINS IN DRUG RESISTANCE

Exosomes Transferring Drug Resistance by Delivering P-gp

The development of MDR in cancer is clinically correlated with the overexpression of the efflux transporters P-glycoprotein (P-gp) or Multidrug Resistance-Associated Protein 1 (MRP1) in many cancers such as lung, breast, neuroblastoma and prostate cancer [56]. MDR present in cancer arising from epithelium may be associated with high P-gp expression [57, 58]. P-gp is the best characterized efflux pump mediating MDR. It is a 170 kDa membrane protein, member of the ATP-binding cassette (ABC) superfamily of transporters [59], which can prevent the absorption of drugs[60]. P-gp is encoded by the human *MDR-1* gene located at chromosome 7, being synthesized in the endoplasmic reticulum (ER) as a glycosylated intermediate. It contains 1,280 amino acids arranged in two halves, each encompassing a transmembrane domain (TMD) which spans the membrane and two intracellular nucleotide-binding domains (NBD)[61]. The glycosyl moietyin the first extracellular loop of P-gp appears to have a role in the trafficking or stability of P-gp to the cell surface, although it does not seem to be essential for drug transport [62].

Exosomes share the same pattern of P-gp expression as their original cells: Exosomes secreted from drug-resistant cells expressed high level of P-gp while exosomes from sensitive cells expressed low level of P-gp. Exosomes can also deliver P-gp from drug-resistant cells to sensitive cells [22]. Consequently, Exosomes are effective in transferring drug resistance from drug-resistant cancer cells to sensitive ones. There is a reduction in tumor cell death as a result of intracellular drug accumulation deficit [2, 63, 64]. This delivery may be a mechanism of exosome-mediated drug resistance transfer.

Moreover, the expression and exosomic transfer of P-gp regulate by miRNA in some ways. Indeed, exosomes from resistant leukemia and BCa cells were shown to incorporate and transfer both P-gp protein and transcripts, together with miRNAs, to drug-sensitive recipient cells. This transfer resulted in the acquisition of the drug resistant by the recipient cells [65-67]. In a recent study, the authors analysed the molecular basis for the acquired traits and found miR-27a and miR-451as enhancers of P-gp expression in drug resistant cancer cells [68-71]. Another significantly expressed and shed miRs, miR-455-3p, is also possibly related to P-gp levels [72]. The results from microarray analysis showed that miR-455-3p was less expressed in a P-gp overexpressing resistant leukemia cell line, when compared with the parental sensitive cell line. Moreover, following the transfer of microvesicles, another type of vesicles, from the resistant to the sensitive cell lines, it was observed that the sensitive cells acquired lower miR-455-3p and higher P-gp levels. They demonstrated that the transfer of transcripts and miRs through microvesicles plays an important role in conferring MDR by “turning” recipient

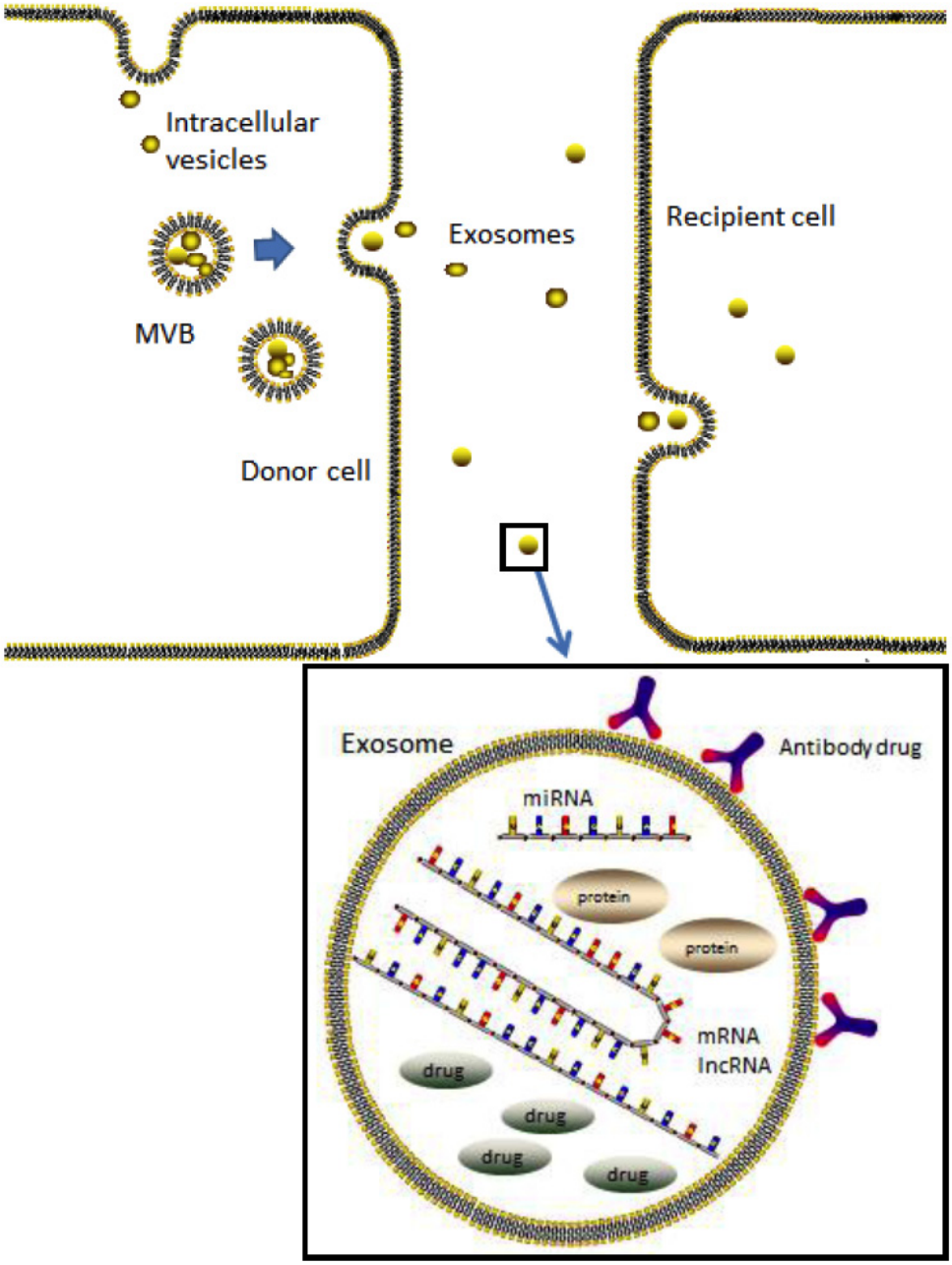

Figure 1: A simplified illustration of how exosomes tranfer its cargos and effect the chemosensitivity.
TD-exosomes secreted by different donor cells can transfer proteins including P-gp, EMT inducers, bioactive factors of CSCs, and nucleic acids, like miRNAs, lncRNAs, mRNAs to recipient cells in order to effect the chemosensitivity of anticancer drugs. Anticancer drugs can be accumulated in the intracellular vesicles and excreted to the exterior by exosomes diretly. Exosomes carry the same antigen as the original cell and counteract the effect of antibody drugs.

cells from low P-gp expressed to P-gp overexpressed as the donor cell [72] but the mechanisms involved in the regulation of P-gp by this miR are not fully understood.

In addition, it is known that miRs involved in P-gp regulation could also be transferred *via* cell-to-cell contact and drive drug resistance. Indeed, miR-21 was shed *via* contact dependent intercellular transfer, mediated by a transmembrane channel [73].

Exosomes Participated in EMT by Transferring EMT Inducers

Epithelial-mesenchymal transition (EMT) is a complex interaction network and is one of the important lables of cancer. Cancer cells that underwent EMT are usually resistant to multiple anticancer drugs [74]. EMT inducers, such as annexin A2, integrin 3, metal matrix proteinase, IL-6, TGFβ and hepatoma-derived growth factor, have been found in some TD-exosomes,

suggesting that TD-exosomes might play a role in the EMT process in cancer cells [75-79]. Among these inducers, the *WNT* signaling pathway is well-studied that it can promote gene expression program and can favor EMT [80]. Human-derived exosomes contained Wnt protein can be transferred to recipient cells and activate *WNT* signaling pathway. Therefore TD-exosomes may have a close relationship with EMT [81-83]. Recently exosomes were found to be generated from nasopharyngeal carcinoma (NPC) contain latent membrane protein1 (LMP1), a principal oncoprotein of EBV that can drive oncogenic process and tumor progression of NPC [84]. EBV-negative cell lines treated with LMP1 exosomes increases migration and invasiveness of NPC cell lines, which associated with EMT [84]. Despite that the number of studies on this topic is limited, it can be concluded preliminarily that TD-exosomes are associated with EMT, and such association might consequently influence the sensitivity of chemotherapy [85].

EXOSOMES EXPAND DRUG RESISTANCE BY REGULATING CANCER STEM-LIKE CELLS (CSCs)

Mesenchymal stem cells (MSCs) play an important role in chemoresistance. In a recent study, it was found that MSC-exosomes significantly induced the resistance of gastric cancer cells to 5-fluorouracil both *in vivo* and *ex vivo*. MSC-exosomes antagonized 5-fluorouracil-induced apoptosis and enhanced the expression of multi-drug resistance associated proteins, including MRP and lung resistance protein (LRP). MSC-exosomes could induce drug resistance in gastric cancer cells by activating CaM-Ks/Raf/MEK/ERK pathway [86]. Thus, MSC-exosomes have profound effects on modifying gastric cancer cells in the development of drug resistance. Moreover, exosomes secreted by bone marrow mesenchymal stem cell induce multiple myeloma cells resistant to bortezomib through the activation of several survival relevant pathways [87].

Stromal communication with cancer cells can influence treatment response. Stromal cells, which are primarily fibroblasts but can also be other cell types, can promote survival after genotoxic and targeted therapy through the secretion of paracrine factors [25]. Many of these interactions between stromal cells and tumor cells may support the maintenance of CSCs analogously to how normal stem cells depend on a niche [88]. It has been reported that tumor and stromal exosomes induce signal transducer and activator of transcription1 (STAT1) through the *retinoic acid-inducible gene-I*, an RNA sensor, in CSCs[89]. Stromal and BCa cells utilize paracrine and juxtacrine signaling to drive chemotherapy and radiation resistance. Upon heterotypic interaction, exosomes are transferred from stromal to BCa cells. The paracrine antiviral and juxtacrine NOTCH3 pathways converge as STAT1 facilitates transcriptional responses to NOTCH3 and expands therapy-resistant tumor-initiating cells. Stromal cells orchestrate an intricate crosstalk with BCa cells by utilizing exosomes to instigate antiviral signaling. This expands BCa adept at resisting therapy and promoting tumor growth [89]. Therefore, exosomes secreted by both CSCs and stromal cells can contribute to tumor drug resistance by regulating the bioactive factors of CSCs.

SUMMARY

In conclusion, Exosomes contribute to chemoresistance to cancer cells in multiple ways. Anticancer drugs can be excreted to the exterior by exosomes and the exosomes can drive overexpression of P-gp which can prevent the absorption of drugs. More and more kinds of miRNA, lncRNA, mRNA and chemoresistance-related proteins are found in exosomes secreted by cancer cells. Exosomes secreted by CSCs and stromal cells can expand drug resistance by regulating CSCs. There may be other ways that exosomes work on drug resistance still not be found out. It is clear that exosome is an obstacle to the success of chemotherapy. More researches are needed to focus on how exosome developing drug resistance and how to reverse this type of drug resistance in order to expand the drug effects and extend the OS of cancer patient ultimately

REFERENCES

[1] Bebawy M, *et al*. Membrane microparticles mediate transfer of P-glycoprotein to drug sensitive cancer cells. Leukemia 2009; 23(9): 1643-9. http://dx.doi.org/10.1038/leu.2009.76

[2] Longley DB, Johnston PG. Molecular mechanisms of drug resistance. The Journal of Pathology 2005; 205(2): 275-292. http://dx.doi.org/10.1002/path.1706

[3] Morgan G, Ward R, Barton M. The contribution of cytotoxic chemotherapy to 5-year survival in adult malignancies. Clin Oncol (R Coll Radiol) 2004; 16(8): 549-60. http://dx.doi.org/10.1016/j.clon.2004.06.007

[4] Azmi AS, Bao B, Sarkar FH. Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. Cancer Metastasis Rev 2013; 32(3-4): 623-42. http://dx.doi.org/10.1007/s10555-013-9441-9

[5] Li H, Yang BB. Friend or foe: the role of microRNA in chemotherapy resistance. Acta Pharmacol Sin 2013; 34(7): 870-9. http://dx.doi.org/10.1038/aps.2013.35

[6] Holzel M, Bovier A, Tuting T. Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance? Nat Rev Cancer 2013; 13(5): 365-76. http://dx.doi.org/10.1038/nrc3498

[7] McMillin DW, Negri JM, Mitsiades CS. The role of tumour-stromal interactions in modifying drug response: challenges and opportunities. Nat Rev Drug Discov 2013; 12(3): 217-28. http://dx.doi.org/10.1038/nrd3870

[8] Harding CV, Heuser JE, Stahl PD. Exosomes: looking back three decades and into the future. J Cell Biol 2013; 200(4): 367-71. http://dx.doi.org/10.1083/jcb.201212113

[9] Dai S, *et al.* Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. Mol Ther 2008; 16(4): 782-90. http://dx.doi.org/10.1038/mt.2008.1

[10] Staals RH, Pruijn GJ. The human exosome and disease. Adv Exp Med Biol 2011; 702: 132-42. http://dx.doi.org/10.1007/978-1-4419-7841-7_11

[11] Schorey JS, Bhatnagar S. Exosome function: from tumor immunology to pathogen biology. Traffic 2008; 9(6): 871-81. http://dx.doi.org/10.1111/j.1600-0854.2008.00734.x

[12] Vlassov AV, *et al.* Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim Biophys Acta 2012; 1820(7): 940-8. http://dx.doi.org/10.1016/j.bbagen.2012.03.017

[13] Raposo G, *et al.* B lymphocytes secrete antigen-presenting vesicles. J Exp Med 1996; 183(3): 1161-72. http://dx.doi.org/10.1084/jem.183.3.1161

[14] Fevrier B, *et al.* Exosomes: a bubble ride for prions? Traffic 2005; 6(1): 10-7. http://dx.doi.org/10.1111/j.1600-0854.2004.00247.x

[15] Vinciguerra P, Stutz F. mRNA export: an assembly line from genes to nuclear pores. Curr Opin Cell Biol 2004; 16(3): 285-92. http://dx.doi.org/10.1016/j.ceb.2004.03.013

[16] Gibbings DJ, *et al.* Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. Nat Cell Biol 2009; 11(9): 1143-9. http://dx.doi.org/10.1038/ncb1929

[17] Corrado C, *et al.* Exosome-mediated crosstalk between chronic myelogenous leukemia cells and human bone marrow stromal cells triggers an interleukin 8-dependent survival of leukemia cells. Cancer Lett 2014; 348(1-2): 71-6. http://dx.doi.org/10.1016/j.canlet.2014.03.009

[18] Valadi H, *et al.* Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9(6): 654-9. http://dx.doi.org/10.1038/ncb1596

[19] Shedden K, *et al.* Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles. Cancer Res 2003; 63(15): 4331-7.

[20] Corcoran C, *et al.* Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer *via* exosomes. PLoS One 2012; 7(12): e50999. http://dx.doi.org/10.1371/journal.pone.0050999

[21] Corcoran C, *et al.* Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer *via* exosomes. PLoS One 2012; 7(12): e50999. http://dx.doi.org/10.1371/journal.pone.0050999

[22] Lv MM, *et al.* Exosomes mediate drug resistance transfer in MCF-7 breast cancer cells and a probable mechanism is delivery of P-glycoprotein. Tumour Biol 2014; 35(11): 10773-9. http://dx.doi.org/10.1007/s13277-014-2377-z

[23] Safaei R, *et al.* Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. Mol Cancer Ther 2005; 4(10): 1595-604. http://dx.doi.org/10.1158/1535-7163.MCT-05-0102

[24] Chen KG, *et al.* Melanosomal sequestration of cytotoxic drugs contributes to the intractability of malignant melanomas. Proc Natl Acad Sci U S A 2006; 103(26): 9903-7. http://dx.doi.org/10.1073/pnas.0600213103

[25] McMillin DW, Negri JM, Mitsiades CS. The role of tumour-stromal interactions in modifying drug response: challenges and opportunities. Nat Rev Drug Discov 2013; 12(3): 217-28. http://dx.doi.org/10.1038/nrd3870

[26] Aung T, *et al.* Exosomal evasion of humoral immunotherapy in aggressive B-cell lymphoma modulated by ATP-binding cassette transporter A3. Proc Natl Acad Sci U S A 2011; 108(37): 15336-41. http://dx.doi.org/10.1073/pnas.1102855108

[27] Pilzer D, Fishelson Z. Mortalin/GRP75 promotes release of membrane vesicles from immune attacked cells and protection from complement-mediated lysis. Int Immunol 2005; 17(9): 1239-48. http://dx.doi.org/10.1093/intimm/dxh300

[28] Zhang HG, *et al.* A membrane form of TNF-alpha presented by exosomes delays T cell activation-induced cell death. J Immunol 2006; 176(12): 7385-93. http://dx.doi.org/10.4049/jimmunol.176.12.7385

[29] He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5(7): 522-31. http://dx.doi.org/10.1038/nrg1379

[30] Mitchell PS, *et al.* Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008; 105(30): 10513-8. http://dx.doi.org/10.1073/pnas.0804549105

[31] Jung T, *et al.* CD44v6 dependence of premetastatic niche preparation by exosomes. Neoplasia 2009; 11(10): 1093-105. http://dx.doi.org/10.1593/neo.09822

[32] Skog J, *et al.* Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008; 10(12): 1470-6. http://dx.doi.org/10.1038/ncb1800

[33] Ogawa R, *et al.* Adipocyte-derived microvesicles contain RNA that is transported into macrophages and might be secreted into blood circulation. Biochem Biophys Res Commun 2010; 398(4): 723-9. http://dx.doi.org/10.1016/j.bbrc.2010.07.008

[34] Zhang Y, *et al.* Secreted monocytic miR-150 enhances targeted endothelial cell migration. Mol Cell 2010; 39(1): 133-44. http://dx.doi.org/10.1016/j.molcel.2010.06.010

[35] Lasser C. Exosomal RNA as biomarkers and the therapeutic potential of exosome vectors. Expert Opin Biol Ther 2012; 12 Suppl 1: S189-97. http://dx.doi.org/10.1517/14712598.2012.680018

[36] Simons M, Raposo G. Exosomes--vesicular carriers for intercellular communication. Curr Opin Cell Biol 2009; 21(4): 575-81. http://dx.doi.org/10.1016/j.ceb.2009.03.007

[37] O'Brien K, *et al.* Exosomes from triple-negative breast cancer cells can transfer phenotypic traits representing their cells of origin to secondary cells. Eur J Cancer 2013; 49(8): 1845-59. http://dx.doi.org/10.1016/j.ejca.2013.01.017

[38] Chen WX, *et al.* Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of microRNAs. PLoS One 2014; 9(4): e95240. http://dx.doi.org/10.1371/journal.pone.0095240

[39] Chen WX, *et al.* MicroRNAs delivered by extracellular vesicles: an emerging resistance mechanism for breast cancer. Tumour Biol 2014; 35(4): 2883-92. http://dx.doi.org/10.1007/s13277-013-1417-4

[40] Chen WX, *et al.* Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of microRNAs. PLoS One 2014; 9(4): e95240. http://dx.doi.org/10.1371/journal.pone.0095240

[41] Corcoran C, Rani S, O'Driscoll L. miR-34a is an intracellular and exosomal predictive biomarker for response to docetaxel with clinical relevance to prostate cancer progression. Prostate 2014; 74(13): 1320-34. http://dx.doi.org/10.1002/pros.22848

[42] Challagundla KB, *et al.* Exosome-mediated transfer of microRNAs within the tumor microenvironment and neuroblastoma resistance to chemotherapy. J Natl Cancer Inst 2015; 107(7). http://dx.doi.org/10.1093/jnci/djv135

[43] Ariel I, *et al.* Imprinted H19 oncofetal RNA is a candidate tumour marker for hepatocellular carcinoma. Mol Pathol 1998; 51(1): 21-5. http://dx.doi.org/10.1136/mp.51.1.21

[44] Lin R, *et al.* A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. Oncogene 2007; 26(6): 851-8. http://dx.doi.org/10.1038/sj.onc.1209846

[45] Panzitt K, *et al.* Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. Gastroenterology 2007; 132(1): 330-42. http://dx.doi.org/10.1053/j.gastro.2006.08.026

[46] Yang F, *et al.* Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology 2011; 54(5): 1679-89. http://dx.doi.org/10.1002/hep.24563

[47] Xiao X, *et al.* Exosomes: decreased sensitivity of lung cancer A549 cells to cisplatin. PLoS One 2014; 9(2): e89534. http://dx.doi.org/10.1371/journal.pone.0089534

[48] Qi P, Du X. The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. Mod Pathol 2013; 26(2): 155-65. http://dx.doi.org/10.1038/modpathol.2012.160

[49] Takahashi K, *et al.* Extracellular vesicle-mediated transfer of long non-coding RNA ROR modulates chemosensitivity in human hepatocellular cancer. FEBS Open Bio 2014; 4: 458-67. http://dx.doi.org/10.1016/j.fob.2014.04.007

[50] Boyer LA, *et al.* Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 2006; 441(7091): 349-53. http://dx.doi.org/10.1038/nature04733

[51] Takahashi K, *et al.* Involvement of extracellular vesicle long noncoding RNA (linc-VLDLR) in tumor cell responses to chemotherapy. Mol Cancer Res 2014; 12(10): 1377-87. http://dx.doi.org/10.1158/1541-7786.MCR-13-0636

[52] Altieri DC. Validating survivin as a cancer therapeutic target. Nat Rev Cancer 2003; 3(1): 46-54. http://dx.doi.org/10.1038/nrc968

[53] Mita AC, *et al.* Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. Clin Cancer Res 2008; 14(16): 5000-5. http://dx.doi.org/10.1158/1078-0432.CCR-08-0746

[54] Skog J, *et al.* Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008; 10(12): 1470-6. http://dx.doi.org/10.1038/ncb1800

[55] Valenzuela MM, *et al.* Exosomes Secreted from Human Cancer Cell Lines Contain Inhibitors of Apoptosis (IAP). Cancer Microenviron 2015; 8(2): 65-73. http://dx.doi.org/10.1007/s12307-015-0167-9

[56] Munoz M, *et al.* Role of the MRP1/ABCC1 multidrug transporter protein in cancer. IUBMB Life 2007; 59(12): 752-7. http://dx.doi.org/10.1080/15216540701736285

[57] Konieczna A, *et al.* Differential expression of ABC transporters (MDR1, MRP1, BCRP) in developing human embryos. J Mol Histol 2011; 42(6): 567-74. http://dx.doi.org/10.1007/s10735-011-9363-1

[58] Ziemann C, *et al.* Reactive oxygen species participate in mdr1b mRNA and P-glycoprotein overexpression in primary rat hepatocyte cultures. Carcinogenesis 1999; 20(3): 407-14. http://dx.doi.org/10.1093/carcin/20.3.407

[59] Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 1993; 62: 385-427. http://dx.doi.org/10.1146/annurev.bi.62.070193.002125

[60] Szakacs G, *et al.* Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006; 5(3): 219-34. http://dx.doi.org/10.1038/nrd1984

[61] Gros P, Croop J, Housman D. Mammalian multidrug resistance gene: complete cDNA sequence indicates strong homology to bacterial transport proteins. Cell 1986; 47(3): 371-80. http://dx.doi.org/10.1016/0092-8674(86)90594-5

[62] Schinkel AH, *et al.* N-glycosylation and deletion mutants of the human MDR1 P-glycoprotein. J Biol Chem 1993; 268(10): 7474-81.

[63] Wilkens S. Structure and mechanism of ABC transporters. F1000Prime Rep 2015; 7: 14. http://dx.doi.org/10.12703/P7-14

[64] van Helvoort A, *et al.* MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 1996; 87(3): 507-17. http://dx.doi.org/10.1016/S0092-8674(00)81370-7

[65] Bebawy M, *et al.* Membrane microparticles mediate transfer of P-glycoprotein to drug sensitive cancer cells. Leukemia 2009; 23(9): 1643-9. http://dx.doi.org/10.1038/leu.2009.76

[66] Jaiswal R, *et al.* Microparticle-associated nucleic acids mediate trait dominance in cancer. FASEB J 2012; 26(1): 420-9. http://dx.doi.org/10.1096/fj.11-186817

[67] Pasquier J, *et al.* Different modalities of intercellular membrane exchanges mediate cell-to-cell p-glycoprotein transfers in MCF-7 breast cancer cells. J Biol Chem 2012; 287(10): 7374-87. http://dx.doi.org/10.1074/jbc.M111.312157

[68] Zhu H, *et al.* Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. Biochem Pharmacol 2008; 76(5): 582-8. http://dx.doi.org/10.1016/j.bcp.2008.06.007

[69] Zhang H, *et al.* Down-regulation of miR-27a might reverse multidrug resistance of esophageal squamous cell carcinoma. Dig Dis Sci 2010; 55(9): 2545-51. http://dx.doi.org/10.1007/s10620-009-1051-6

[70] Kovalchuk O, *et al.* Involvement of microRNA-451 in resistance of the MCF-7 breast cancer cells to chemotherapeutic drug doxorubicin. Mol Cancer Ther 2008; 7(7): 2152-9. http://dx.doi.org/10.1158/1535-7163.MCT-08-0021

[71] Zhao X, Yang L, Hu J. Down-regulation of miR-27a might inhibit proliferation and drug resistance of gastric cancer cells. J Exp Clin Cancer Res 2011; 30: 55.

[72] Jaiswal R, *et al.* Microparticle conferred microRNA profiles--implications in the transfer and dominance of cancer traits. Mol Cancer 2012; 11: 37.

[73] Elhassan MO, Christie J, Duxbury MS. Homo sapiens systemic RNA interference-defective-1 transmembrane family member 1 (SIDT1) protein mediates contact-dependent small RNA transfer and microRNA-21-driven chemoresistance. J Biol Chem 2012; 287(8): 5267-77. http://dx.doi.org/10.1074/jbc.M111.318865

[74] Ahmed N, *et al*. Epithelial mesenchymal transition and cancer stem cell-like phenotypes facilitate chemoresistance in recurrent ovarian cancer. Curr Cancer Drug Targets 2010; 10(3): 268-78. http://dx.doi.org/10.2174/156800910791190175

[75] Vella LJ. The emerging role of exosomes in epithelial-mesenchymal-transition in cancer. Front Oncol 2014; 4: 361.

[76] Hakulinen J, *et al*. Secretion of active membrane type 1 matrix metalloproteinase (MMP-14) into extracellular space in microvesicular exosomes. J Cell Biochem 2008; 105(5): 1211-8. http://dx.doi.org/10.1002/jcb.21923

[77] Mathias RA, *et al*. Extracellular remodelling during oncogenic Ras-induced epithelial-mesenchymal transition facilitates MDCK cell migration. J Proteome Res 2010; 9(2): 1007-19. http://dx.doi.org/10.1021/pr900907g

[78] Chairoungdua A, *et al*. Exosome release of beta-catenin: a novel mechanism that antagonizes Wnt signaling. J Cell Biol 2010; 190(6): 1079-91. http://dx.doi.org/10.1083/jcb.201002049

[79] Jeppesen DK, *et al*. Quantitative proteomics of fractionated membrane and lumen exosome proteins from isogenic metastatic and nonmetastatic bladder cancer cells reveal differential expression of EMT factors. Proteomics 2014; 14(6): 699-712. http://dx.doi.org/10.1002/pmic.201300452

[80] Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014; 15(3): 178-96. http://dx.doi.org/10.1038/nrm3758

[81] Gross JC, *et al*. Active Wnt proteins are secreted on exosomes. Nat Cell Biol 2012; 14(10): 1036-45. http://dx.doi.org/10.1038/ncb2574

[82] Luga V, *et al*. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell 2012; 151(7): 1542-56. http://dx.doi.org/10.1016/j.cell.2012.11.024

[83] Menck K, *et al*. Induction and transport of Wnt 5a during macrophage-induced malignant invasion is mediated by two types of extracellular vesicles. Oncotarget 2013; 4(11): 2057-66. http://dx.doi.org/10.18632/oncotarget.1336

[84] Aga M, *et al*. Exosomal HIF1alpha supports invasive potential of nasopharyngeal carcinoma-associated LMP1-positive exosomes. Oncogene 2014; 33(37): 4613-22. http://dx.doi.org/10.1038/onc.2014.66

[85] Greening DW, *et al*. Emerging roles of exosomes during epithelial-mesenchymal transition and cancer progression. Semin Cell Dev Biol 2015; 40: 60-71. http://dx.doi.org/10.1016/j.semcdb.2015.02.008

[86] Ji R, *et al*. Exosomes derived from human mesenchymal stem cells confer drug resistance in gastric cancer. Cell Cycle 2015; 14(15): 2473-83. http://dx.doi.org/10.1080/15384101.2015.1005530

[87] Battke C, *et al*. Tumour exosomes inhibit binding of tumour-reactive antibodies to tumour cells and reduce ADCC. Cancer Immunol Immunother 2011; 60(5): 639-48. http://dx.doi.org/10.1007/s00262-011-0979-5

[88] Korkaya H, Liu S, Wicha MS. Breast cancer stem cells, cytokine networks, and the tumor microenvironment. J Clin Invest 2011; 121(10): 3804-9. http://dx.doi.org/10.1172/JCI57099

[89] Boelens MC, *et al*. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. Cell 2014; 159(3): 499-513. http://dx.doi.org/10.1016/j.cell.2014.09.051

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