Exosomes in cancer: small vesicular transporters for cancer progression and metastasis, biomarkers in cancer therapeutics

Atefe Abak Corresp., 1, Alireza Abhari 2, Sevda Rahimzadeh 2

1 Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2 Department of Biochemistry and Clinical Laboratory, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Corresponding Author: Atefe Abak
Email address: atefeh.abak@gmail.com

Cancer progression is a polygenic procedure in which the exosomes can function as substantial roles. Exosomes are tiny, phospholipid bilayer membrane nanovesicles of endocytic derivation with a diameter of 40-100 nm. These nanovesicles can transport bioactive molecules containing mRNAs, proteins, DNA fragments, and non-coding RNAs from a donor cell to recipient cells, and cause the alteration in genetic and epigenetic factors and reprogramming of the target cells. Many diverse cell types such as mesenchymal cells, immune cells, and cancer cells can induce a release of exosomes. Increasing evidence illustrated that the exosomes derived from tumor cells might trigger the tumor initiation, tumor cell growth and progression, metastasis, and drug resistance. The secreted nanovesicles of exosomes can play significant roles in cells communicate via shuttling the nucleic acid molecules and proteins to target cells and tissues. In this review, we discussed multiple mechanisms related to biogenesis, load, and shuttle of the exosomes. Also, we illustrated the diverse roles of exosomes in several types of human cancer development, tumor immunology, angiogenesis, and metastasis. The exosomes may act as the promising biomarkers for the prognosis of various types of cancers which suggested a new pathway for anti-tumor therapeutic of these nanovesicles and promoted exosome-based cancer for clinical diagnostic and remedial procedures.
Exosomes in cancer: small vesicular transporters for cancer progression and metastasis, biomarkers in cancer therapeutics

Atefe Abak¹, Alireza Abhari², Sevda Rahimzadeh²

¹Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
²Department of Biochemistry and Clinical Laboratory, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Corresponding Author:
Atefe Abak¹

Email Address: Atefeh.abak@gmail.com, abaka@tbzmed.ac.ir

ABSTRACT. Cancer progression is a polygenic procedure in which the exosomes can function as substantial roles. Exosomes are tiny, phospholipid bilayer membrane nanovesicles of endocytic derivation with a diameter of 40-100 nm. These nanovesicles can transport bioactive molecules containing mRNAs, proteins, DNA fragments, and non-coding RNAs from a donor cell to recipient cells, and cause the alteration in genetic and epigenetic factors and reprogramming of the target cells. Many diverse cell types such as mesenchymal cells, immune cells, and cancer cells can induce a release of exosomes. Increasing evidence illustrated that the exosomes derived from tumor cells might trigger the tumor initiation, tumor cell growth and progression, metastasis, and drug resistance. The secreted nanovesicles of exosomes can play significant roles in cells communicate via shuttling the nucleic acid molecules and proteins to target cells and tissues.

In this review, we discussed multiple mechanisms related to biogenesis, load, and shuttle of the exosomes. Also, we illustrated the diverse roles of exosomes in several types of human cancer development, tumor immunology, angiogenesis, and metastasis. The exosomes may act as the promising biomarkers for the prognosis of various types of cancers which suggested a new pathway for anti-tumor therapeutic of these nanovesicles and promoted exosome-based cancer for clinical diagnostic and remedial procedures.
1. INTRODUCTION

The solid tumors are complicated structures that including the surrounding tumor stroma and cancer cells, which composed of endothelial cells, fibroblasts, and immune cells (Sund & Kalluri 2009). The surrounding cells are permanently extracting factors that alter the tumor microenvironment (TME) directly or indirectly (Dvorak et al. 2011). A persistent cross-talk among tumor cells and the distant tumor microenvironment have applied as the pivotal tumor growth, and significant targets in antitumoral intervention, and systemic diffusion (Kalluri & Zeisberg 2006; Swartz et al. 2012). Extracellular vesicles (EVs) have appeared as long-distance communicators; their outcomes in primary tumors can display as systemic effects and contribute to procedures within the circulation by many various kinds of cells. The exosomes pretense a special class of EVs, which released via various kinds of cells (Desrochers et al. 2016; Kowal et al. 2014; Mathivanan et al. 2010). Newly evidence represents that the release of exosomes has been detected to play a considerable role between human tumor cells and systemic cell-to-cell relevance in cancers. The exosomes, initially defined through several common traits in reticulocytes three decades ago, containing morphology (a classic “dish” or “cup” formed in transfer electron microscopy (SEM)), density (1.13-1.19 g/ml), size (30-100 nm in diameter), and determined increased protein markers (TSG101, HSP70, and tetraspanins) (Harding et al. 2013). Recent publications illustrated that exosomes are small membrane nanovesicles shaped in multivesicular bodies of endocytic derivation with a diameter of 40-100 nm. Exosomes were primarily considered as the trash bags for elimination of abandoned membrane segments and unwanted molecular fragments from cells, besides the critical task of exosomes in stimulation of immune response is identified as their effect on antigen presentation in the mid-1990s (Raposo et al. 1996). Interestingly, the scholars detected that noncoding RNAs (miRNAs), messenger RNAs (mRNAs), proteins and DNA fragments could be burdened as “goods” in extracellular vesicles (EVs) (Balaj et al. 2011). Likewise the exosomes as a nanovesicles were detected to function as “communication shuttles” from a donator cells to recipient cells, that could able to re-encode genes of receiver cells, reprogramming of the tumor microenvironment and recruitment to shape a pro-tumorigenic soil, and play a considerable act in the progression, invasion, metastasis, and
become insensitive to a drug of cancer (Azmi et al. 2013; Balaj et al. 2011; Valadi et al. 2007).

Here we reviewed a new science concerning the function of exosomes as a shuttle in tumorigenesis, emphasizing their biogenesis, component, significant affection, and then considering the potential of exosomes as a novel biomarkers for clinical remedial target diagnosis and prognosis.

2. SURVEY METHODOLOGY

PubMed was mostly utilized to search for relevant articles published utilizing the keyword “exosome”, “cancer” and “therapy.” Afterward, screened articles were utilized as references for this review. Additional keywords, such as “microenvironment,” “nanovesicles” and “tumor,” were also utilized.

3. Exosomes Biogenesis, Release, and Uptake

Contrary to the larger microvesicles (MVs), that straightly shed from the cell membrane, the exosomes forming is a specific process that contains four steps: beginning, endocytosis, multivesicular bodies (MVBs) creation, and finally the exosome secretion (Théry et al. 2002). Exosomes primarily can shape through the ceramide-induced procedures of inside budding from the late endosome restricted membranes (Trajkovic et al. 2008). The encapsulation of RNA molecules and functional proteins occur through this process. The Multivesicular bodies (MVBs) within the endocytic systems shaped via the budding of an endosome limited membrane into the extracellular milieu of the section by the junction and merge of the MVBs with the cell membrane. The MVBs are either classified as the destroying of cargo in the lysosome or leading to secretion within the extracellular space as exosomes after vesicular cumulation (Février & Raposo 2004; Trams et al. 1981). The procedures based on the classified of exosomal cargo within the intraluminal vesicles (ILVs) is still not completely understood. Although it has been offered to characterize the exosomes formation and releasing by both endosomal tethering complexes necessitated for
transport (ESCRT)-dependent and independent symptoms, however, alternative ways may also exist (Trajkovic et al. 2008). The ESCRT pathway discerns ubiquitination of membrane proteins and promotes their internalization within the multivesicular endosome (Wollert & Hurley 2010). The mechanism for the microvesicles formation has been illustrated to regulate through the syndecan heparan sulfate proteoglycans and their cytoplasmic adaptor syntenin (Baietti et al. 2012). The MVB trafficking and the secretion procedure of exosomes may be performed through the outside of exosomes and the microvesicles budding procedure or through multiple compositions of the endocytic machinery, containing the members of the Rab guanosine triphosphatase (GTPase) family (Rab11, Rab 27a, Rab 27b, Rab 35), elevated expression of heparanase, SNARES (soluble NSF attachment receptor), and cytoskeleton regulatory proteins (Azmi et al. 2013; Beach et al. 2014; Ostrowski et al. 2010; Pant et al. 2012). A promoted dissemination of exosomes is critically was detected to be triggered via multiple kinds of stress, including alters in PH membrane, oxidative stress, shear stress, hypoxia, thermal alters, and radiation, besides through formation of ceramide, stimulation of sphingomyelinase and following the p53-adjusted protein tumor-suppressor-activated pathway 6 (TSAP6) (Andaloussi et al. 2013; Azmi et al. 2013; Hannafon & Ding 2013; Joanne et al. 2005; Kucharzewska & Belting 2013; Lespagnol et al. 2008; Parolini et al. 2009; Yu et al. 2006). Exosomes shuttle information to the recipient cells via three major pathways: (1) interaction between receptor-ligand; (2) straight merge with cell membrane; (3) endocytosis through phagocytosis (Figure 1). Also, there are multiple proteins that can function as specific receptors to activate the uptake of the exosome, containing ICAM-1 for APCs, and Tim 1/4 for B-cells (Miyanishi et al. 2007; Segura et al. 2005).

4. Exosomes Structure and Composition

Exosomes are commonly cup-shaped extracellular small nanovesicles ranging in size from 30 to 100 nm diameter, consist of a phospholipid bilayer comprising membrane proteins that encircles a lumen containing an extensive range of biomolecules including carbohydrates, lipids, small fragments of DNA, mRNAs, proteins, and miRNAs inward to
keep them from destruction (De Veirman et al. 2016; Hwang 2013; Raimondo et al. 2011; Vlassov et al. 2012; Wang et al. 2016).

4.1. Protein Composition of Exosomes. Exosomes from several kinds of cells include a core set of similar proteins upon 4600 various proteins have been related to these microvesicles, containing proteins from the phospholipid bilayer, endoplasmic reticulum, cytosol, and Golgi apparatus such as the heat shock proteins (HSP60, HSP70, and HSP90), the tetraspanin family (CD63, CD81, CD9, and CD82), cytoskeletal proteins (tubulin, moesin, actin, and syntenin), proteins involved in ESCRT complex (Alix, TSG101), phospholipases and lipid-related proteins (Bang & Thum 2012; Hannafon & Ding 2013; Harshman et al. 2013; Mathivanan et al. 2010; Taylor & Gercel-Taylor 2011; Vlassov et al. 2012).

4.2. Lipid Composition of Exosomes. Exosomes are containing a lipid bilayer including polyglycerophospholipids, sphingolipids (ceramide, sphingomyelin), raft-associated lipids (cholesterol), glycerophospholipids (phosphatidylethanolamine, phosphatidylerine, phosphatidylinositol, phosphatidylcholine), and phospholipids. Also, the lipid composition in exosomes varies significantly from that of the original cells (Record et al. 2014).

4.3. Nucleic Acid Composition of Exosomes. The double-stranded deoxyribonucleic acids exist in these microvesicles derived from tumoral cells and cause the aberrant regulation of the derived cells (Silva et al. 2012). The other nucleic acids that carry with exosomes contain of mitochondrial DNA (mtDNA), messenger RNAs (mRNAs), microRNAs (miRNAs), long noncoding RNAs (Inc RNA), small-nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), piwi-RNAs (pi-RNAs), transfer RNAs (tRNAs), and ribosomal RNAs (rRNAs). Exosomal-containing RNA can transfer among a variety of cells and therefore is termed “exosomal shuttle RNA” (esRNA). Recently 764 miRNAs and 1639 mRNAs have been recognized in these nanovesicles from tissues of various species via a broad range of researches (Gajos-Michniewicz et al. 2014).

The composition of exosomes differs between various pathological and physiological status and originated cells. Also, the contents of these nanovesicles can discern from the derived cells because of the optional categorized of the cargo within exosomes.
5. Exosomes Function

As a communicator, exosomes can directly shuttle the bioactive molecules among multiple kinds of recipient cells, with results in targeted cellular phenotyping, contained messenger-RNA (mRNA) and microRNA (miRNA) dependent on the shuttle of genetics, and also epigenetic information and lipid trafficking among cells (Azmi et al. 2013; Xiang et al. 2009). The existence of exosomes in the circulating body fluids reveals their role in various pathological situations, the instance of infection disease, cardiovascular disease, and progression of neurodegenerative disease (Bang & Thum 2012; Fleming et al. 2014). The more substantial role of exosomes has been figured out in cancer which leads to tumor growth, angiogenesis, escaping from the immune response, causing tumor cell migration, stimulating normal cells to an invasion, and leading to metastatic colonization into distal tissues (Azmi et al. 2013).

6. Methods for the Isolation and Analysis of Biomarkers from Exosomes in Cancer Cells and Body Fluids

Recently, different methods are available for the isolation and discern of exosomes from the distinguished cells under normal and stressed situations, containing nucleic acid (DNA) sequencing, qRT-PCR analysis, western blotting assay, or Enzyme-linked immunosorbent assay (ELISA), which can identify RNA and protein of exosomes, also ultracentrifugation, source gradient ultracentrifugation combined with ultrafiltration centrifugation (SGUUC), commercial kits, magnetic activated cell sorting (MACS) can utilize as another method. To date, the international society for extracellular vesicles (ISEV) can apply for the detection of extracellular vesicles and their functions. Also, western blot and flow cytometry (FCM) commonly utilizes for recognizing of exosomes through discovering particular tetraspanins (for example CD9, CD63, and HSP70). Further, the transmission electron microscopy (TEM) can use for size and shape analysis (Lötvall et al. 2014). The common technique for the isolation of exosomes contains ultracentrifugation, which is often in combination with sucrose density gradients or sucrose cushions to float the relatively low-density exosomes. The ultracentrifugation procedure has multiple disadvantages: The manner is extremely
labor-intensive and time-consuming; due to the restrictions of the design of ultracentrifuge rotors one cannot evaluate more than six specimens at a time; the procedure needs a major amount of raw materials; exosome productions are usually low; and vast training of staff is required (Théry et al. 2006; Zeringer et al. 2015). Isolation of exosomes based on size, by the prosperous isolation of exosomes through applying the ultrafiltration methods which are less time-consuming than ultracentrifugation and do not need the usage of the particular tool (Cheruvanky et al. 2007). HPLC (high-performance liquid chromatography)-based protocols could effectively permit the preparation of extremely pure exosomes. however, these methods need appropriative material and are not negligible to scale-up (Lai et al. 2010). Besides, the intricacy is that both body fluids and cell-culture media include an extensive amount of nanoparticles in the identical size range as exosomes. For instance, many miRNAs are included within extracellular protein complexes rather than exosomes (Wang et al. 2010). In addition, volume-excluding polymers such as polyethylene glycols (PEGs) could be used to precipitate exosomes from empirical specimens. The precipitate can be separated applying either low-speed centrifugation or filtration. System Biosciences presents an appropriative reagent called ExoQuick, which can be added to conditioned cell media, urine or serum, which precipitates these nanovesicles (Adams 1973; Lewis & Metcalf 1988; Yamamoto et al. 1970). In principle, a preferable resource for special purification of exosomes should be affinity isolation with antibodies to Alix, annexin, CD63, CD81, CD82, CD9, EpcAM, and Rab5. These antibodies could be collected on multiple media, containing microfluidic devices, plates, magnetic beads and chromatography matrices(Chen et al. 2010; Théry et al. 2006).

7. Exosomes and Cancer

7.1. Role of Exosomes in Cancerogenesis. As mentioned earlier, the exosomes revealed important roles in cancer progression. The exosomes released by human cancer cells are known as tumor-derived (TD) exosomes. The TD exosomes through autocrine signals can modulate the local growth progression of human cancer cells. The exosomal autocrine signaling pathway is related to kinds of cells and cellular traits, for instance, exosomes
separated from gastric cancer cells with high CD97 (epidermal growth factor seven-
transmembrane subfamily) expression enhanced cancer cell proliferation and invasion via
exosome-mediated MAPK signaling pathway, and exosomal miRNAs may be contributing to
induction of the CD97-associated pathway (Li et al. 2015). A mutant epidermal growth
factor receptor (EGFRVIII), exists on the membrane of these nanovesicles originated from
glioblastoma cells, can trigger cells loss of this mutant form. The integration of EGFRVIII
within these cells caused by promotion of anti-apoptotic procedures and an augment in
capacity for anchorage-independent growth (Al-Nedawi et al. 2008). On the other hand, the
exosomes originated from pancreatic cancer cells enhance Bax expression, however,
reduce Bcl-2 expression, cause the leading to cancer cells of the mitochondrial apoptotic
pathway (Ristorcelli et al. 2008). This process illustrated that TD exosomes might act
pivotal anti-cancer role through triggering apoptosis in several tumors. Accordingly, the
determined or beneficial TD exosomes in vivo to their own survival relies on the cellular
traits and kinds of the cells, which more research needs to be clarified. Moreover, the bone
marrow mesenchymal stromal cells (BM-MSCs)-derived exosomes can support the
multiple tumor cell expansion and development in various human cancer cells (Figure 2).

7.2. Role of Exosomes in Tumor Angiogenesis. The angiogenic procedures induced
cancer cell progression can be activated through nutrient reduction, hypoxic, and in
addition, inflammatory responses, generally detected in epithelial cell carcinomas. The
neovascularization process from preexisting blood vessels associated with promoted
endothelial cell proliferation, migration, and budding (Dvorak 1986; Nazarenko et al.
2010). Vascular endothelial growth factors (VEGF), IL-8, transforming growth factor B
(TGF-β), and fibroblast growth factor (FGF) are some of the angiogenic factors that function
as endothelial cell proliferation and migration, can be necessary for the induction of tumor
angiogenesis. Also, the exosomal miR-92a derived from leukemic cells can regulate integrin
α5 to promote migration regulations and proliferation of endothelial cells and tube
formation (Umezu et al. 2013). By other research, exosomes originated from melanoma
cells including miR-9 were internalized through endothelial cells enhancing angiogenesis
and metastasis via activation of the JAK-STAT pathway (Gajos-Michniewicz et al. 2014).
Other report illustrated that CD-105-positive exosomes act an important role in
establishing a niche in the lung microenvironment of SCID mice through the elevate expression of MMP2, MMP9, and VEGFR1 (Grange et al. 2011). In addition, the exosomes originated from hypoxic brain tumor glioblastoma multiform cells were increased with IL-8 and PDGF as angiogenic stimulatory molecules (Kucharzewska et al. 2013).

7.3. Role of Exosomes in Tumor Metastasis. A major pathway in the metastatic cascade is tumor cell invasion and migration, missing the epithelial traits towards a more mesenchymal phenotype and the ability of the cell to attain a motile phenotype via changes in the cell to matrix interaction, disseminating tumor cells extravasate into remote sites and finally colonize secondary tissues and organs. There is an emerging report that shows tumor-derived exosomes are accomplished by tumor invasion and metastasis through regulating stromal cells, creating a pre-metastatic niche (Figure 3), remodeling the extracellular matrix (ECM) and inducing angiogenesis (Alderton 2012; Jung et al. 2009). Metastatic tumor cells dissemination enhanced level of miRNA by tumor-suppressor mechanism, that can indicate another procedure for the function of these nanovesicles in metastasis (Ostenfeld et al. 2014). The recent study illustrated that the exosomal proteins originated from tumor hypoxia of prostate cancer cells are associated with the process of adherens junctions in epithelial cells and cytoskeleton remodeling, including the enhanced metastasis and invasiveness in prostate cancer cells, is modulated through exosomes (Ramteke et al. 2015). Also, by recent investigate gastrointestinal stromal tumor cells (GISTs) secrete exosomes including protein tyrosine kinase to transform progenitor cell-derived smooth muscle cells to a premetastatic phenotype (Atay et al. 2014). Another report indicated that the Colorectal cancer cells with high invasive potential were detected to be significantly dependent on the concentration of exosomes including the signaling competent epidermal growth factor receptor (EGFR) ligand, inferring that exosome-mediated ligand shuttle causes cancer invasiveness and metastasis (Higginbotham et al. 2011). Exosome-modulated transferring of microRNA-221/222 from mesenchymal stem cells (MSCs) to gastric tumor cells significantly promotes migration and metastasis of these tumoral cells (Wang et al. 2014b).

7.4. Role of Exosomes in Tumor Immune Escape. The current researches represented that tumor-derived microvesicles may function as immunosuppressive effects. Exosome-
mediated communication among cancer cells and the immune system is triggered recruiting pro-cancerogenic immune cells (Figure 4). Also, tumor-derived exosomes are being utilized as an effective source of tumor antigen to induce dendritic cells (DCs), causing a shuttle of tumor antigens to DCs and including CD8+ T cell-related anti-tumor outcomes. The exosomal tumor-carried TGF-β1 deviated IL-2 modulates in favor of regulatory T cells and away from cytotoxic cells (Bu et al. 2011; Clayton et al. 2007). Also, tumor-derived exosomes can activate myeloid-derived suppressor cells (MDSC). The MDSCs by inhibiting the T cell reaction can apply immunosuppressive functions in cancer. Tumor-derived microvesicles from several tumor cell lines modulate synthesis of interleukin-6 (IL-6) in MDSCs via the activation of Toll-like receptor 2 through the membrane-associated heat shock protein 72 (HSP 72). Making of IL-6 outcomes in an autocrine phosphorylation of stat3 in MDSCs can enhance their immunosuppressive function (Chalmin et al. 2010; Nagaraj & Gabrilovich 2012). The miRNA shuttled via cancer cell-derived exosomes may function as ligands through attaching to the Toll-like receptors and activate the inflammation. Indeed, it was cleared that oncogenic miR-21 and miR-29a released from the exosomes derived from highly metastatic lung carcinoma cells can bind to the human and murine TLRs (Fabbri et al. 2012).

7.5. Role of Exosomes in Mediating Insensitivity to a Drug in Cancer. Exosomes via several mechanisms may play pivotal role in the progression of therapy resistance in cancer cells. Tumor-derived microvesicles can shuttle multi-drug resistance (MDR)-associated miRNAs and proteins to target cells. These illustrated that several major classes of anticancer drugs and their metabolites can be encapsulated and exported through exosomes outside of the cells, and shedding of these extracellular vesicles (EVs) is intimately associated with insensitivity to a drug (Figure 5) in various human cancer cells (Corcoran et al. 2012; Safaei et al. 2005; Shedden et al. 2003; Wei et al. 2014). Recently, emerging evidence illustrated that miR-21 was shuttled from cancer-associated adipocytes of fibroblasts to the various tumor cells, where it can inhibit ovarian cancer apoptosis and induce the Paclitaxel resistance through binding to its new direct target, apoptotic protease activating factor-1 (APAF1) (Yeung et al. 2016). Besides, there is plenty of interest in insensitivity to a drug through exosome-mediated shuttle of miRNAs. Several studies
suggested that breast cancer cells resistant to various drugs (Docetaxel-Adriamycin-Tamoxifen) may shuttle the resistance to sensitive cells in part via exosomal miRNA exchange (Chen et al. 2014). Moreover, PTEN is reduced in exosomes therefore applying biological acts in target cells. The loss of function of PTEN enhances resistance to sensitivity and chemotherapeutic of mTOR, which inhibits in breast cancer cells and, afterwards, PTEN exosomal shuttle, could be drew out as a shuttle mechanism or drug resistance changes (Steelman et al. 2008). Besides exosomes through regulating their binding to tumor cells may counteract the efficacy of antibody drugs. The exosomes-originated lymphoma carry CD20 can bind to the anti CD-20 antibody therapeutics and induce the preserving of target cells from antibody attack (Aung et al. 2011). Thus, the exosomes-derived cancer cells can be utilized as a procedure of cancer chemotherapy resistance of special cancer cells to special drugs.

7.6. Use of Exosomes as a Tumor Diagnostics and Biomarkers. The indicating of significant functional roles of exosomes in approximately all aspects of tumor cells was preparing the opportunities for enhancement of these nanovesicles as a considerable diagnostic biomarkers and remedial targets. The exosomes derived-human tumor cells are enriched with mRNA, proteins, and miRNA which are more plentiful in tumor than in healthy noncancerous cells (Roma-Rodrigues et al. 2014). One of the principal beneficiaries of the utilize of these nanovesicles as a valuable biomarkers is the feasibility of a fast pathology detection by minimally invasive procedures (Li & Bahassi 2013). The existence of exosomes in the blood circulatory system and shedding these nanovesicles into biological fluids such as urine, saliva, and ascites of exosomes containing biomarkers in several subtypes of human tumor cells can be obtained the minimally invasive “liquid biopsies” (for example blood collection) for clinical use (Zhang & Grizzle 2014). Also these microvesicles are really resistant under variant storage situations containing short-term storage at 4°C for 96 hours or long-term storage at -70°C (Taylor & Gercel-Taylor 2008). These quality attributes of the circulating serum exosomes can be used as a considerable biomarkers for early diagnostics of cancer cells and personalized cancer therapies. Several in vitro studies suggested that exosomes derived human tumor cells can be utilized as a remarkable biomarker to diagnose cancer cells through applying the methods of
proteomics and transcriptomics (Aushev et al. 2013; Dijkstra et al. 2014). Also, the enhanced levels of exosomes in blood plasma specimens of colon carcinoma patients was considerably linked to the weakly differentiated tumor cells and the declined entirely survival (Silva et al. 2012). Another study illustrated that exosomal EDIL3 and fibronectin in circulating EVs can utilize as pivotal biomarkers of early stage breast cancer through applying ELISA methods (Moon et al. 2016). Recent report showed that PCA3 and TMPRSS2:ERG, two established proteins exist in urinary exosomes from prostate cancer proteins which are detected as a potential biomarkers through label-free liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Nilsson et al. 2009). These finding show that the bodily fluids originated exosomes may be an important noninvasive marker for the early tumor detection.

7.7. Use of Exosomes as a Cancer Therapeutic. Emerging reports indicates that various clinical researches illustrated the role of exosomes as cancer remedies, and a few main adverse effects were identified for applying of these nanovesicles in cancer therapy. Figure 6 indicates the remedies that were proposed for therapy of cancers based on exosomes characteristics.

1) Trough secretion of exosomes, tumoral cells trigger the alteration of the local and systemic tumor environment to induce tumor growth promotion, metastasis and insensitivity to drugs. Thus, either the destruction of exosome-dissemination pathway through tumor cells or the removal of these nanovesicles from the blood circulatory system may create an effective method for cancer therapy. The Tinzaparin (a low-molecular-weight heparin) can trigger tissue factor pathway inhibitor (TFPI) secretion from cancer cells, and also the recombinant TFPI induce suppression of tumor-derived exosomes causing migration of tumor cells (Gamperl et al. 2016). Lately for the elimination of extracellular vesicles (EVs) from the blood circulatory system, a therapeutic hemofiltration process which is called ADAPT™ (adaptive dialysis-like affinity platform technology) is applied. Whenever the patient’s blood plasma samples transfer via ADAPT™ system, plasma specimens factors by the porous fibers are interacted with the immobilized affinity agents to that target molecules are particularly absorbed while unbound serum factors and blood cells can pass through this system (Logozzi et al. 2009).
2) As a delivery system, exosomes are considerably utilized as vehicles loaded with multiple anticancer drugs, siRNAs, and miRNAs for several cancer therapeutic cargos. The lipid bilayer membrane of these nanovesicles forms a natural protective shelter, thus enhances the cellular internalization of the encapsulated anti-cancer drugs. Regarding to the exosomes originated from autologous cancer cells, these nanovesicles can cause minimal toxicity when being shuttled into the target cells and can be less immunogenic than artificial delivery vehicles. Also their naturally small size permit them to elude phagocytosis through the mononuclear phagocyte system (MPS) and simplify their extravasation via tumor blood vessels and their subsequent release in target cancer tissues. The various researches illustrated that prosperous delivery and tumor inhibition utilizing this procedure. The enhancement of colorectal and breast xenograft cancers in vivo by applying the doxorubicin loaded into exosomes or using exosome-mimetic nanovesicles can be suppressed. Thus, the efficacy of doxorubicin was widely promoted through targeting the immature dendritic cell exosomes into cancer tissues (Jang et al. 2013; Tian et al. 2014). Also the another greatly utilized antimitotic chemotherapeutic drug is Paclitaxel that can be loaded into microvesicles through sonication, and these loaded microvesicles have 50 times more cytotoxicity than free Paclitaxel for drug resistance tumor cells in vitro. Besides the exosome-encapsulated paclitaxel can considerably block murine Lewis lung cancer pulmonary metastases and decline size of tumor in the mouse model (Kim et al. 2016).

3) Exosomes can be utilized to target special tissues or organs because of the having special cell tropism according to their traits. Applying the well-characterized exosomal membrane protein (Lamp2b) for expressing the targeting peptide instantly below the signal peptide sequence including the targeting peptides RVG and iRGD were prosperously inserted within these nanovesicles from immature dendritic cells to target either brain or cancer tissues. This method considerably promoted the cellular uptake of the nanovesicles in the tissue of interest, and enhanced the specificity of the remedy, and also reduced the toxicity of drugs delivered through exosomes (Jang et al. 2013; Tian et al. 2014).

4) In addition to anticancer therapeutic drugs, exosomes can likewise deliver several tumor antigens, nanobodies, apoptotic-containing proteins, proteasomes, deficient or mutant anti-
apoptosis proteins, tumor and tissue-specific peptides, transferrins, and lactoferrins within tumor cells for targeting remedy (Aspe et al. 2014; Cho et al. 2005; Hall et al. 2016; Hung & Leonard 2015; Kooijmans et al. 2016; Lai et al. 2012; Malhotra et al. 2016).

5) Also, exosomes based cell-free vaccines could indicate an alternating to dendritic cells (DCs) treatment for inhibiting tumor development through the function of exosomes in the immune system. So researchers detected that the nanovesicles originated from peptide-pulsed DCs, can present antigens to T cells to affect their immune response. These DC-originated exosomes include MHC-peptide complexes and co-stimulatory molecules on their membrane, that permit them to continue antigen presentation and increase immunization in mice comparing with antigen-presenting DCs (Luketic et al. 2007).

6) Moreover, miRNAs are widely detected in exosomes derived cancer cells or isolated from bodily fluids that contribute to exosome-mediated cell-cell communication, and induce anti-cancer traits. For instance EGFR-specific binding peptide GE11 can lead let-7a-containing exosomes to EGFR-positive cancer cells, that considerably suppressed EGFR-positive human breast tumor cell development in a heterograft mouse model (Ohno et al. 2013; Yu et al. 2015).

8. Functions and Remedial Roles of Exosomes in different kinds of Human Cancers

The tumor exosomes originated from the ascites of a very aggressive murine T-cell lymphoma (EVs A) can effect on dendritic cells activity, thus disturbing the immune system to distinguish and destroy cancer cells. Also, the expression of marker-proteins including ALIX, TSG-101, CD63, CD81, and CD9 has detected in EVs A. This research illustrated that EVs A triggered both humoral and cellular immune reactions. Altogether, the outcomes indicated that the endosome-originated EVs secreted via an advanced-stage T-cell lymphoma, stimulated a special immune reaction (Menay et al. 2017). Besides, exosomes released through chronic myelogenous leukemia (CML) cells remedied with Curcumin, which originated from the plant Curcuma longa, has the anticancer effects, include a wide quantity of miR-21 that is transported into the endothelial cells in a biologically active
The treatment of HUVECs with CML Curcu-exosomes diminished RhoB expression and conversely modified endothelial cells motility. The research illustrated that the addition of CML control exosomes to HUVECs induced promotion of IL8 and VCAM1 levels, but Curcu-exosomes returned this efficacy, therefore, diminished their angiogenic properties. Overall, this research showed that besides Curcumin reduces the exosome's capability to enhance the angiogenic phenotype and to modify the endothelial barrier organization (Taverna et al. 2016). As exosomes emerge as a novel manner of intercellular communication the cargo includes through exosome is formed via somatic evolution. Regarding evaluating the effect of exosomes originated from several melanoma-related cell lines on primary CD8+ T cells act, exosomes from each cell line were different. The B16F0 exosomes dose-associated inhibited T-cell proliferation. Notwithstanding, Cloudman S91 exosomes enhanced T-cell proliferation and Melan-A exosomes load an insufficient impact on primary CD8+ T cells. Importantly, B16F0 exosomes suppressed T-cell proliferation through high-expressed of PTPN11 to tumor permeating lymphocytes would escape the extracellular control of the immune checkpoints (Wu et al. 2015). Regarding increasing evidence, extracellular vesicles (EVs) are inherently trigger intercellular relation through shuttling molecular information between cells. Therefore, the autologous cancer-cell originated EVs can be utilized as helpful carries of Paclitaxel to the prostate cancer cells, bringing the drug into the cells via an endocytic process and released into the cell cytosol leading to cell death. Most considerably, the EV-mediated delivery promoted the cytotoxic efficacy of the drug. This research suggested that the autologous EVs may be helpful for impressive transporting of chemotherapeutic agents to prostate cancer cells (Saari et al. 2015). It is noteworthy that the effective role of exosomes in relevance among cancer cells and surrounding stroma indicated that the TrkB expression in exosomes is necessitated including aggressiveness phenotype. In this report, the YKL-40 silencing contributes for reducing of TrkB, sortilin and P75NTR expression, related to a low aggressive phenotype. The release of TrkB in exosomes from normal glioma cells was able to relieve both migration and activation of YKL-40-inactivated cells. Furthermore, TrkB-containing exosomes may be remarked as a considerable biomarker for glioblastoma diagnosis (Pinet et al. 2016). Also, MVs originated from HLSC (MV-HLSC) can suppress the growth of hepatoma tumors through shuttling genetic information that mediates with deregulated
survival and proliferation of these cells. The antitumor effect of MV-HLSC was relevant to the decreased internalization, due to the lack of CD29 on MV-fibroblast or a decreased expression of antitumor miRNAs including miR-24, miR-31, miR-122, miR-125b, miR-223, and miR-451. Consequently, the promoted internalization was not relevant to an increased biological activity when MV-fibroblast expressing CD-29 were utilized. Hence, the various composition of miRNA content between MV-HLSC and MV-fibroblast was the remarkable reason for the various biological actions. Therefore, the transferring of these miRNAs through MVs originated from stem cells may suppress tumor growth development and stimulate apoptosis (Fonsato et al. 2012). Another research illustrated that exosomes originated from curcumin-pretreated H1299 cells were utilized to remedy BEAS-2B cells, which triggered proliferation, colony organization, and migration of BEAS-2B cells. Curcumin is a new drug lung cancer remedy. Although, the procedure associated with the antitumor effect of curcumin is related to the promoted expression of TCF-21, triggered through a low expression of DNMT1. Therefore, mechanism of curcumin is remarkable in cancer remedy, and create the pivotal biomarkers for developing cancer diagnostic and remedial procedures (Wu et al. 2016). The new research suggested that PSC (Pancreatic Stellate Cells)-originated exosomes can trigger and elevate the proliferation and migration of PANC-1 and SUIT-2. The exosomes differentially varied expression of a plethora of genes controlling multiple cellular procedures containing cell cycle, cellular assembly and organization, DNA replication, recombinant and repair, cell death and survival, cellular development, and growth in the recipient cancer cells. Also, three chemokines, CCL20, CXCL1, and CXCL2 were detected high expressed in exosome-treated cancer cells. Besides, glypican-1, a glycoprotein discovered in PSC exosomes, as a pivotal biomarker to distinguish PDAC (Pancreatic Ductal Adenocarcinoma), and as a tumor promoter shuttled among cells through exosomes (Ali et al. 2015; Charrier et al. 2014; Farrow et al. 2003). The exosome/staphylococcal enterotoxin B is a considerable sample for apopto-immunotherapy. The contribution of Exo and its lipid rafts in this structure assigns the feasibility of binding to pancreatic cancer cells. SEB and the characterized lipid rafts trigger the apoptotic signal both through extrinsic and mitochondria-dependent pathways. Also, the presence of tumor antigens associated with superantigen causing promotion of specific antitumor immune response (Mahmoodzadeh et al. 2014). Even more recent research
detected that gastric cancer cells may release exosomes for transferring apoptotic signals without direct cell-cell contact to anti-cancer T cells. The Cbl-b and Cbl-c of ubiquitin ligases might have a considerable role in exosome-induced apoptosis of Jurkat T cells through enhancing PI3K proteasome degradation, that causes inactivation of PI3K/Akt signaling, therefore led to activation of caspase 3, 8, and 9. Thus, relation among exosomes and immune response is presumably to assign considerable point of view through the process of tumor immune inhibition (Qu et al. 2009). Recently study illustrated that miR-375 promoted the growth inhibitory effect, Cell progression and dissemination of colon cancer through the Bcl-2 pathway. Therefore the miR-375 down-regulated in metastatic CRC, and it has important role for Bcl-2 blocking, with the significant minimally invasive prognostic biomarker for CRC through suppression of malignant proliferation and dissemination (Zaharie et al. 2015). Further, research reported that a HIV-Nef SMR-originated peptide suppressed the progress of human breast tumor cells through arresting cancer cell cycle and including blockade of exosome secretion. The SMR peptide inhibited the cancer cell cycle through G2/M phase boundary. While the SMR peptide and chemotherapeutic drugs were compound to remedy cancer cells, PEG-SMRwt-Clu synergically enhanced the anti-proliferative efficacies of drugs, considerably promoted the tumor cell growth suppression efficacy of drugs and inhibited exosomes secretion in breast cancer cell lines MCF-7 and MDA-MB-231 cells. Therefore the considerable usage of PEG-SMRwt-Clu peptide is pivotal process for the prevention and therapy of human breast tumor cells (Huang et al. 2017).

9. CONCLUSIONS

Prosperity in remedy against intricate cancers relies on our full comprehension of the complications among various components within tumors. The above studies supported the viewpoint that exosomes can play a pivotal role in the growth, and promotion of cancer cells via regulation of intercellular communication into the tumor microenvironment through the release of several biological molecules ranging from virions of mRNA, miRNA, protein, lipid, and DNA cargos. An overview of the roles of exosomes in different types of
cancer and the molecular mechanisms that have been used to evaluate the effect of EV in cancer progression and metastasis were presented in Table 1. The exploration of exosomes derived cancer cells contents may permit the progression of new diagnostic and remedial procedures, with minimally invasive approaches. Exosomes derived cancer cells also can cause cancer cell development and metastasis through suppressing the immune response and via enhancing chemoresistance by elimination of chemotherapeutic anti-cancer drugs. So they might be significant targets for remedial interventions through their alternation or elimination. The field of nanotechnology has widely benefitted exosome research to load nanovesicles with tiny molecules or drugs for cancer treatment because of their small size, lack of toxicity and target specificity toward prosperous immunotherapy in clinical trials. Besides exosomes can be considerable biomarkers for diagnosis of cancer and targeted remedy due to their nearly display the situation of their parental cells that are relatively constant in the blood circulation and could be feasibility obtained from body fluids. Most significantly the role of miRNA in the context of exosomes for targeting inactivation of cancer-causing miRNAs would probably provide a novel strategy for most cancers. It is expected that further researches on these microvesicles will not only determine great potential and hopeful effect on their functions in the pathogenesis of cancer but also will open the new strategies for cancer diagnosis and remedies.

ACKNOWLEDGMENTS

We thank Masoud Abak for the exact designing of figures that considerably improved the quality of this manuscript.
REFERENCES

Adams A. 1973. Concentration of Epstein-Barr virus from cell culture fluids with polyethylene glycol. *Journal of General Virology* 20:391-394.

Al-Nedawi K, Meehan B, Micaleff J, Lhotak V, May L, Guha A, and Rak J. 2008. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nature cell biology* 10:619.

Alderton GK. 2012. Metastasis: Exosomes drive premetastatic niche formation. *Nature Reviews Cancer* 12:447.

Ali S, Suresh R, Banerjee S, Bao B, Xu Z, Wilson J, Philip PA, Apte M, and Sarkar FH. 2015. Contribution of microRNAs in understanding the pancreatic tumor microenvironment involving cancer associated stellate and fibroblast cells. *American journal of cancer research* 5:1251.

Allenson K, Castillo J, San Lucas F, Scelo G, Kim D, Bernard V, Davis G, Kumar T, Katz M, and Overman M. 2017. High prevalence of mutant KRAS in circulating exosome-derived RNA from early-stage pancreatic cancer patients. *Annals of Oncology* 28:741-747.

Andaloussi SE, Lakhal S, Mäger I, and Wood MJ. 2013. Exosomes for targeted siRNA delivery across biological barriers. *Advanced drug delivery reviews* 65:391-397.

Arita T, Ichikawa D, Konishi H, Komatsu S, Shiozaki A, Ogino S, Fujita Y, Hiramoto H, Hamada J, and Shoda K. 2016. Tumor exosome-mediated promotion of adhesion to mesothelial cells in gastric cancer cells. *Oncotarget* 7:56855.

Arscott WT, Tandle AT, Zhao S, Shabason JE, Gordon IK, Schlaff CD, Zhang G, Tofilon PJ, and Camphausen KA. 2013. Ionizing radiation and glioblastoma exosomes: implications in tumor biology and cell migration. *Translational Oncology* 6:638IN635-648IN636.

Aspe JR, Diaz Osterman CJ, Jutzy JM, Deshields S, Whang S, and Wall NR. 2014. Enhancement of Gemcitabine sensitivity in pancreatic adenocarcinoma by novel exosome-mediated delivery of the Survivin-T34A mutant. *Journal of extracellular vesicles* 3:23244.

Atay S, Banskota S, Crow J, Sethi G, Rink L, and Godwin AK. 2014. Oncogenic KIT-containing exosomes increase gastrointestinal stromal tumor cell invasion. *Proceedings of the National Academy of Sciences* 111:711-716.

Aung T, Chapuy B, Vogel D, Wenzel D, Oppermann M, Lahmann M, Weinhage T, Menck K, Hupfeld T, and Koch R. 2011. Exosomal evasion of humoral immunotherapy in aggressive B-cell lymphoma modulated by ATP-binding cassette transporter A3. *Proceedings of the National Academy of Sciences* 108:15336-15341.

Aushev VN, Zborovskaya IB, Laktionov KK, Girard N, Cros M-P, Herceg Z, and Krutovskikh V. 2013. Comparisons of microRNA patterns in plasma before and after tumor removal reveal new biomarkers of lung squamous cell carcinoma. *PLoS One* 8:e78649.

Azmi AS, Bao B, and Sarkar FH. 2013. Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. *Cancer and Metastasis Reviews* 32:623-642.

Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, Ivarsson Y, Depoortere F, Coomans C, and Vermeiren E. 2012. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nature cell biology* 14:677.

Balaj L, Lessard R, Dai L, Cho Y-J, Pomeroy SL, Breakefield XO, and Skog J. 2011. Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nature communications* 2:180.

Bang C, and Thum T. 2012. Exosomes: new players in cell–cell communication. *The international journal of biochemistry & cell biology* 44:2060-2064.

Beach A, Zhang H-G, Ratajczak MZ, and Kakar SS. 2014. Exosomes: an overview of biogenesis, composition and role in ovarian cancer. *Journal of ovarian research* 7:14.
Bellavia D, Raimondo S, Calabrese G, Forte S, Cristaldi M, Patinella A, Memeo L, Manno M, Raccosta S, and Diana P. 2017. Interleukin 3-receptor targeted exosomes inhibit in vitro and in vivo Chronic Myelogenous Leukemia cell growth. *Theranostics* 7:1333.

Bhattacharya S, Pal K, Sharma AK, Dutta SK, Lau JS, Yan IK, Wang E, Elkanany A, Alkharfy KM, and Sanyal A. 2014. GAIP interacting protein C-terminus regulates autophagy and exosome biogenesis of pancreatic cancer through metabolic pathways. *PLoS One* 9:e114409.

Boelens MC, Wu TJ, Nabet BY, Xu B, Qiu Y, Yoon T, Azzam DJ, Twyman-Saint Victor C, Wiemann BZ, and Ishwaran H. 2014. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. *Cell* 159:499-513.

Bu N, Wu H, Sun B, Zhang G, Zhan S, Zhang R, and Zhou L. 2011. Exosome-loaded dendritic cells elicit tumor-specific CD8+ cytotoxic T cells in patients with glioma. *Journal of Neuro-Oncology* 104:659-667.

Cazzoli R, Butitta F, Di Nicola M, Malatesta S, Marchetti A, Rom WN, and Pass HL. 2013. microRNAs derived from circulating exosomes as noninvasive biomarkers for screening and diagnosing lung cancer. *Journal of Thoracic Oncology* 8:1156-1162.

Challagundla KB, Wise PM, Neviani P, Chava H, Murtadha M, Xu T, Kennedy R, Ivan C, Zhang X, and Vannini I. 2015. Exosome-mediated transfer of microRNAs within the tumor microenvironment and neuroblastoma resistance to chemotherapy. *JNCI: Journal of the National Cancer Institute* 107.

Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin J-P, Boireau W, Rouleau A, Simon B, and Lanneau D. 2010. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *The Journal of Clinical Investigation* 120:457.

Charrier A, Chen R, Chen L, Kemper S, Hattori T, Takigawa M, and Brigstock DR. 2014. Connective tissue growth factor (CCN2) and microRNA-21 are components of a positive feedback loop in pancreatic stellate cells (PSC) during chronic pancreatitis and are exported in PSC-derived exosomes. *Journal of Cell Communication and Signaling* 8:147-156.

Chen C, Skog J, Hsu C-H, Lessard RT, Balaj L, Wurdinger T, Carter BS, Breakefield XO, Tonner M, and Irimia D. 2010. Microfluidic isolation and transcriptome analysis of serum microvesicles. *Lab on a Chip* 10:505-511.

Chen W-x, Cai Y-q, Lv M-m, Chen L, Zhong S-l, Ma T-f, Zhao J-h, and Tang J-h. 2014. Exosomes from docetaxel-resistant breast cancer cells alter chemosensitivity by delivering microRNAs. *Tumor Biology* 35:9649-9659.

Chen W, Wang J, Shao C, Liu S, Yu Y, Wang Q, and Cao X. 2006. Efficient induction of antitumor T cell immunity by exosomes derived from heat-shocked lymphoma cells. *European Journal of Immunology* 36:1598-1607.

Chen Y, Zeng C, Zhan Y, Wang H, Jiang X, and Li W. 2017. Aberrant low expression of p85α in stromal fibroblasts promotes breast cancer cell metastasis through exosome-mediated paracrine Wnt10b. *Oncogene* 36:4692.

Cheruvank A, Zhou H, Pisitkun T, Kopp JB, Knepper MA, Yuen PS, and Star RA. 2007. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. *American Journal of Physiology-Renal Physiology* 292:F1657-F1661.

Cho JA, Yeo D, Son HY, Kim HW, Jung DS, Ko JK, Koh JS, Kim YN, and Kim CW. 2005. Exosomes: a new delivery system for tumor antigens in cancer immunotherapy. *International Journal of Cancer* 114:613-622.

Chun S, Ahn S, Yeom C-H, and Park S. 2016. Exosome Proteome of U-87MG Glioblastoma Cells. *Biology* 5:50.
Clayton A, Mitchell JP, Mason MD, and Tabi Z. 2007. Human tumor-derived exosomes selectively impair lymphocyte responses to interleukin-2. *Cancer research* 67:7458-7466.

Conde-Vancells J, Rodriguez-Suarez E, Gonzalez E, Berisa A, Gil D, Embade N, Valle M, Luka Z, Elortza F, and Wagner C. 2010. Candidate biomarkers in exosome-like vesicles purified from rat and mouse urine samples. *PROTEOMICS-Clincial Applications* 4:416-425.

Corcoran C, Rani S, O’Brien K, O’Neill A, Prencipe M, Sheikh R, Webb G, McDermott R, Watson W, and Crown J. 2012. Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer via exosomes. *Plos One* 7:e50999.

Corrado C, Raimondo S, Saieva L, Flugy AM, De Leo G, and Alessandro R. 2014. Exosome-mediated crosstalk between chronic myelogenous leukemia cells and human bone marrow stromal cells triggers an interleukin 8-dependent survival of leukemia cells. *Cancer letters* 348:71-76.

Damo M, Wilson DS, Simeoni E, and Hubbell JA. 2015. TLR-3 stimulation improves anti-tumor immunity elicited by dendritic cell exosome-based vaccines in a murine model of melanoma. *Scientific reports* 5:17622.

De Veirman K, Wang J, Xu S, Leleu X, Himpe E, Maes K, De Bruyne E, Van Valckenborgh E, Vanderkerken K, and Menu E. 2016. Induction of miR-146a by multiple myeloma cells in mesenchymal stromal cells stimulates their pro-tumoral activity. *Cancer letters* 377:17-24.

Desrochers LM, Antonyak MA, and Cerione RA. 2016. Extracellular vesicles: satellites of information transfer in cancer and stem cell biology. *Developmental Cell* 37:301-309.

Dijkstra S, Birker IL, Smit FP, Leyten GH, de Reijke TM, van Oort IM, Mulders PF, Jannink SA, and Schalken JA. 2014. Prostate cancer biomarker profiles in urinary sediments and exosomes. *The Journal of urology* 191:1132-1138.

Ding X, Ma M, Teng J, Teng RK, Zhou S, Yin J, Fonkem E, Huang JH, Wu E, and Wang X. 2015. Exposure to ALS-FTD-CSF generates TDP-43 aggregates in glioblastoma cells through exosomes and TNTs-like structure. *Oncotarget* 6:24178.

Dvorak HF. 1986. Tumors: wounds that do not heal. *New England Journal of Medicine* 315:1650-1659.

Dvorak HF, Weaver VM, Tlsty TD, and Bergers G. 2011. Tumor microenvironment and progression. *Journal of Surgical Oncology* 103:468-474.

Ekström EJ, Bergenfelz C, von Bülow V, Serifer F, Carlemalm E, Jönsson G, Andersson T, and Leandersson K. 2014. WNT5A induces release of exosomes containing pro-angiogenic and immunosuppressive factors from malignant melanoma cells. *Molecular cancer* 13:88.

El-Saghir J, Nassar F, Tawil N, and El-Sabban M. 2016. ATL-derived exosomes modulate mesenchymal stem cells: potential role in leukemia progression. *Retrovirology* 13:73.

Eldh M, Bagge RO, Lässer C, Svanvik J, Sjöstrand M, Mattssson J, Lindnér P, Choi D-S, Gho YS, and Lötvall J. 2014. MicroRNA in exosomes isolated directly from the liver circulation in patients with metastatic uveal melanoma. *BMC cancer* 14:962.

Escudier B, Dorval T, Chaput N, André F, Caby M-P, Novault S, Flament C, Leboulaire C, Borg C, and Amigorena S. 2005. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial. *Journal of Translational Medicine* 3:10.

Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, and Nuovo GJ. 2012. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. *Proceedings of the National Academy of Sciences* 109:E2110-E2116.

Farrow B, Rychahou P, Murillo C, O’connor KL, Iwamura T, and Evers BM. 2003. Inhibition of pancreatic cancer cell growth and induction of apoptosis with novel therapies directed against protein kinase A. *Surgery* 134:197-205.
Federici C, Petrucci F, Caimi S, Cesolini A, Logozzi M, Borghi M, D'Ilio S, Lugini L, Violante N, and Azzarito T. 2014. Exosome release and low pH belong to a framework of resistance of human melanoma cells to cisplatin. *PLoS One* 9:e88193.

Feng D-Q, Huang B, Li J, Liu J, Chen X-M, Xu Y-M, Chen X, Zhang H-B, Hu L-H, and Wang X-Z. 2013. Selective miRNA expression profile in chronic myeloid leukemia K562 cell-derived exosomes. *Asian Pacific Journal of Cancer Prevention* 14:7501-7508.

Février B, and Raposo G. 2004. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Current Opinion in Cell Biology* 16:415-421.

Fleming A, Sampey G, Chung MC, Bailey C, van Hoek ML, Kashanchi F, and Hakami RM. 2014. The carrying pigeons of the cell: exosomes and their role in infectious diseases caused by human pathogens. *Pathogens and disease* 71:109-120.

Fonsato V, Collino F, Herrera MB, Cavallari C, Deregibus MC, Cisterna B, Bruno S, Romagnoli R, Salizzoni M, and Tetta C. 2012. Human Liver Stem Cell-Derived Microvesicles Inhibit Hepatoma Growth in SCID Mice by Delivering Antitumor MicroRNAs. *Stem Cells* 30:1985-1998.

Gajos-Michniewicz A, Duechler M, and Czyz M. 2014. MiRNA in melanoma-derived exosomes. *Cancer letters* 347:29-37.

Gamperl H, Plattfaut C, Freund A, Quecke T, Theophil F, and Gieseler F. 2016. Extracellular vesicles from malignant effusions induce tumor cell migration: inhibitory effect of LMWH tinzaparin. *Cell Biology International* 40:1050-1061.

Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B, and Camussi G. 2011. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. *Cancer research* 71:5346-5356.

Hall J, Prabhakar S, Balaj L, Lai CP, Cerione RA, and Breakefield XO. 2016. Delivery of therapeutic proteins via extracellular vesicles: review and potential treatments for Parkinson’s disease, Glioma, and Schwannoma. *Cellular and Molecular Neurobiology* 36:417-427.

Hannafon BN, and Ding W-Q. 2013. Intercellular communication by exosome-derived microRNAs in cancer. *International Journal of Molecular Sciences* 14:14240-14269.

Harding CV, Heuser JE, and Stahl PD. 2013. Exosomes: looking back three decades and into the future. *Journal of Cell Biology* 200:367-371.

Harshman SW, Canella A, Ciarlariello PD, Rocci A, Agarwal K, Smith EM, Talabere T, Efebera YA, Hofmeister CC, and Benson DM. 2013. Characterization of multiple myeloma vesicles by label-free relative quantitation. *Proteomics* 13:3013-3029.

Harshyne LA, Hooper KM, Andrews EG, Nasca BJ, Kenyon LC, Andrews DW, and Hooper DC. 2015a. Glioblastoma exosomes and IGF-1R/AS-ODN are immunogenic stimuli in a translational research immunotherapy paradigm. *Cancer Immunology, Immunotherapy* 64:299-309.

Harshyne LA, Nasca BJ, Kenyon LC, Andrews DW, and Hooper DC. 2015b. Serum exosomes and cytokines promote a T-helper cell type 2 environment in the peripheral blood of glioblastoma patients. *Neuro-Oncology* 18:206-215.

Hazan-Halevy I, Rosenblum D, Weinstein S, Bairey O, Raanani P, and Peer D. 2015. Cell-specific uptake of mantle cell lymphoma-derived exosomes by malignant and non-malignant B-lymphocytes. *Cancer letters* 364:59-69.

He M, Qin H, Poon TC, Sze S-C, Ding X, Co NN, Ngai S-M, Chan T-F, and Wong N. 2015. Hepatocellular carcinoma-derived exosomes promote motility of immortalized hepatocyte through transfer of oncogenic proteins and RNAs. *Carcinogenesis* 36:1008-1018.

Hedlund M, Nagaeva O, Kargl D, Baranov V, and Mincheva-Nilsson L. 2011. Thermal-and oxidative stress causes enhanced release of NKG2D ligand-bearing immunosuppressive exosomes in leukemia/lymphoma T and B cells. *PLoS One* 6:e16899.
Herrera M, Fonsato V, Gatti S, Deregibus M, Sordi A, Cantarella D, Calogero R, Bussolati B, Tetta C, and Camussi G. 2010. Human liver stem cell-derived microvesicles accelerate hepatic regeneration in hepatectomy-treated rats. *Journal of Cellular and Molecular Medicine* 14:1605-1618.

Higginbotham JN, Beckler MD, Gephart JD, Franklin JL, Bogatcheva G, Kremers G-J, Piston DW, Ayers GD, McConnell RE, and Tyska MJ. 2011. Amphiregulin exosomes increase cancer cell invasion. *Current Biology* 21:779-786.

Hong C-S, Muller L, Whiteside TL, and Boyiadzis M. 2014a. Plasma exosomes as markers of therapeutic response in patients with acute myeloid leukemia. *Frontiers in Immunology* 5.

Hong CS, Muller L, Boyiadzis M, and Whiteside TL. 2014b. Isolation and characterization of CD34+ blast-derived exosomes in acute myeloid leukemia. *PLoS One* 9:e103310.

Hood JL, San RS, and Wickline SA. 2011. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. *Cancer research* 71:3792-3801.

Hosseini HM, Fooladi AAI, Soleimanirad J, Nourani MR, Davaran S, and Mahdavi M. 2014. Staphylococcal enterotoxin B anchored exosome induces apoptosis in negative estrogen receptor breast cancer cells. *Tumor Biology* 35:3699-3707.

Huan J, Hornick NI, Shurtleff MJ, Skinner AM, Goloviznina NA, Roberts CT, and Kurre P. 2013. RNA trafficking by acute myelogenous leukemia exosomes. *Cancer research* 73:918-929.

Huang M-B, Gonzalez RR, Lillard J, and Bond VC. 2017. Secretion modification region-derived peptide blocks exosome release and mediates cell cycle arrest in breast cancer cells. *Oncotarget* 8:11302.

Hung ME, and Leonard JN. 2015. Stabilization of exosome-targeting peptides via engineered glycosylation. *Journal of Biological Chemistry* 290:8166-8172.

Hwang I. 2013. Cell-cell communication via extracellular membrane vesicles and its role in the immune response. *Molecules and Cells* 36:105-111.

Iessi E, Logozzi M, Lugini L, Azzarito T, Federici C, Spugnini EP, Mizzoni D, Di Raimo R, Angelini DF, and Battistini L. 2017. Acridine Orange/exosomes increase the delivery and the effectiveness of Acridine Orange in human melanoma cells: A new prototype for theranostics of tumors. *Journal of Enzyme Inhibition and Medicinal Chemistry* 32:648-657.

Jang SC, Kim OY, Yoon CM, Choi D-S, Roh T-Y, Park J, Nilsson J, Lötvall J, Kim Y-K, and Gho YS. 2013. Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. *ACS nano* 7:6988-7710.

Jeppesen DK, Nawrocki A, Jensen SG, Thorsen K, Whitehead B, Howard KA, Dyrskjaøt L, Ørntoft TF, Larsen MR, and Ostenfeld MS. 2014. Quantitative proteomics of fractionated membrane and lumen exosome proteins from isogenic metastatic and nonmetastatic bladder cancer cells reveal differential expression of EMT factors. *Proteomics* 14:699-712.

Joanne LY, May L, Lhotak V, Shahrzad S, Shirasawa S, Weitz Ji, Coomber BL, Mackman N, and Rak JW. 2005. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. *Blood* 105:1734-1741.

Jung T, Castellana D, Klingbeil P, Hernández IC, Vitacolonna M, Orlicky DJ, Roffler SR, Brodt P, and Zöller M. 2009. CD44v6 dependence of premetastatic niche preparation by exosomes. *NeoPlasia* 11:IN13-IN17.

Kalluri R, and Zeisberg M. 2006. Fibroblasts in cancer. *Nature reviews Cancer* 6:392.

Kim MS, Haney MJ, Zhao Y, Mahajan V, Deygen I, Klyachko NL, Inskeo E, Piroyan A, Sokolsky M, and Okolie O. 2016. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. *Nanomedicine: Nanotechnology, Biology and Medicine* 12:655-664.

Ko S-F, Yip H-K, Zhen Y-Y, Lee C-C, Lee C-C, Huang C-C, Ng S-H, and Lin J-W. 2015. Adipose-derived mesenchymal stem cell exosomes suppress hepatocellular carcinoma growth in a rat model:
apparent diffusion coefficient, natural killer T-cell responses, and histopathological features. Stem cells international 2015.

Koch R, Demant M, Aung T, Diering N, Cicholas A, Chapuy B, Wenzel D, Lahmann M, Güntsch A, and Kiecke C. 2014. Populational equilibrium through exosome-mediated Wnt signaling in tumor progression of diffuse large B-cell lymphoma. Blood 123:2189-2198.

Kogure T, Lin WL, Yan IK, Braconi C, and Patel T. 2011. Intercellular nanovesicle-mediated microRNA transfer: A mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology 54:1237-1248.

Kong JN, He Q, Wang G, Dasgupta S, Dinkins MB, Zhu G, Kim A, Spassieva S, and Bieberich E. 2015. Guggulsterone and bexarotene induce secretion of exosome-associated breast cancer resistance protein and reduce doxorubicin resistance in MDA-MB-231 cells. International journal of cancer 137:1610-1620.

Kooijmans SA, Aleza CG, Roffler SR, van Solinge WW, Vader P, and Schiffelers RM. 2016. Display of GPI-anchored anti-EGFR nanobodies on extracellular vesicles promotes tumour cell targeting. Journal of extracellular vesicles 5:31053.

Kore RA, and Abraham EC. 2014. Inflammatory cytokines, interleukin-1 beta and tumor necrosis factor-alpha, upregulated in glioblastoma multiforme, raise the levels of CRYAB in exosomes secreted by U373 glioma cells. Biochemical and Biophysical Research Communications 453:326-331.

Kowal J, Tkach M, and Théry C. 2014. Biogenesis and secretion of exosomes. Current Opinion in Cell Biology 29:116-125.

Krarup S, Elmageed ZYA, Hawke DH, Wörner PM, Jansen DA, Abdel-Mageed AB, Alt EU, and Izadpanah R. 2014. Molecular characterization of exosome-like vesicles from breast cancer cells. BMC cancer 14:44.

Kucharszewska P, and Belting M. 2013. Emerging roles of extracellular vesicles in the adaptive response of tumour cells to microenvironmental stress. Journal of extracellular vesicles 2:20304.

Kucharszewska P, Christianson HC, Welch JE, Svensson KJ, Fredlund E, Ringnér M, Mörgelin M, Bourseau-Guilmain E, Bengzon J, and Belting M. 2013. Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. Proceedings of the National Academy of Sciences 110:7312-7317.

Lai RC, Arslan F, Lee MM, Sze NSK, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, and El Oakley RM. 2010. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem cell research 4:214-222.

Lai RC, Tan SS, Teh BJ, Sze SK, Arslan F, De Kleijn DP, Choo A, and Lim SK. 2012. Proteolytic potential of the MSC exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome. International journal of proteomics 2012.

Lau CS, and Wong DT. 2012. Breast cancer exosome-like microvesicles and salivary gland cells interplay alters salivary gland cell-derived exosome-like microvesicles in vitro. PloS One 7:e33037.

Lazar I, Clement E, Dauvillier S, Milhas D, Ducoux-Petit M, LeGonidec S, Moro C, Soldan V, Dalle S, and Balor S. 2016. Adipocyte exosomes promote melanoma aggressiveness through fatty acid oxidation: A novel mechanism linking obesity and cancer. Cancer research 76:4051-4057.

Lehmann BD, Paine MS, Brooks AM, McCubrey JA, Renegar RH, Wang R, and Terrian DM. 2008. Senescence-associated exosome release from human prostate cancer cells. Cancer research 68:7864-7871.

Lespagnol A, Duflaut D, Beekman C, Blanc L, Fiucci G, Marine J, Vidal M, Amson R, and Telerman A. 2008. Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice. Cell Death and Differentiation 15:1723.
Lewis GD, and Metcalf TG. 1988. Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. Applied and Environmental Microbiology 54:1983-1988.

Li C, Liu D-R, Li G-G, Wang H-H, Li X-W, Zhang W, Wu Y-L, and Chen L. 2015. CD97 promotes gastric cancer cell proliferation and invasion through exosome-mediated MAPK signaling pathway. World Journal of Gastroenterology: WJG 21:6215.

Li J, Chen Y, Guo X, Zhou L, Jia Z, Peng Z, Tang Y, Liu W, Zhu B, and Wang L. 2017. GPC1 exosome and its regulatory miRNAs are specific markers for the detection and target therapy of colorectal cancer. Journal of Cellular and Molecular Medicine 21:838-847.

Li J, Liu A, and Wang Y. 2014. β-Elemene against human lung cancer via up-regulation of P53 protein expression to promote the release of exosome. Lung Cancer 86:144-150.

Li Y, and Bahassi EM. 2013. Biofluid-based circulating tumor molecules as diagnostic tools for use in personalized medicine. Journal of Molecular Biomarkers & Diagnosis 5:157-163.

Liao J, Liu R, Shi Y-J, Yin L-H, and Pu Y-P. 2016. Exosome-shuttling microRNA-21 promotes cell migration and invasion-targeting PDCD4 in esophageal cancer. International journal of oncology 48:2567-2579.

Lim JW, Mathias RA, Kapp EA, Layton MJ, Faux MC, Burgess AW, Ji H, and Simpson RJ. 2012. Restoration of full-length APC protein in SW480 colon cancer cells induces exosome-mediated secretion of DKK-4. Electrophoresis 33:1873-1880.

Liu J, Zhang Y, Liu A, Wang J, Li L, Chen X, Gao X, Xue Y, Zhang X, and Liu Y. 2016. Distinct dasatinib-induced mechanisms of apoptotic response and exosome release in imatinib-resistant human chronic myeloid leukemia cells. International Journal of Molecular Sciences 17:531.

Liu W-h, Ren L-n, Wang X, Wang T, Zhang N, Gao Y, Luo H, Navarro-Alvarez N, and Tang L-j. 2015. Combination of exosomes and circulating microRNAs may serve as a promising tumor marker complementary to alpha-fetoprotein for early-stage hepatocellular carcinoma diagnosis in rats. Journal of Cancer Research and Clinical Oncology 141:1767-1778.

Liu Z-M, Wang Y-B, and Yuan X-H. 2013. Exosomes from murine-derived GL26 cells promote glioblastoma tumor growth by reducing number and function of CD8+ T cells. Asian Pacific Journal of Cancer Prevention 14:309-314.

Logozzi M, De Milito A, Lugini L, Borghi M, Calabro L, Spada M, Perdicchio M, Marino ML, Federici C, and Lessi E. 2009. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. PLoS One 4:e5219.

Lötvall J, Hill AF, Hochberg F, Buzás EI, Di Vizio D, Gardiner C, Gho YS, Kurochkin IV, Mathivanan S, and Quesenberry P. 2014. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. Taylor & Francis.

Luketic L, Delanghe J, Sobol PT, Yang P, Frottet E, Mossman KL, Gauldie J, Bramson J, and Wan Y. 2007. Antigen presentation by exosomes released from peptide-pulsed dendritic cells is not suppressed by the presence of active CTL. The Journal of Immunology 179:5024-5032.

Lv L-H, Wan Y-L, Lin Y, Zhang W, Yang M, Li G-L, Lin H-M, Shang C-Z, Chen Y-J, and Min J. 2012. Anticancer drugs cause release of exosomes with heat shock proteins from human hepatocellular carcinoma cells that elicit effective natural killer cell antitumor responses in vitro. Journal of Biological Chemistry 287:15874-15885.

Madhavan B, Yue S, Galli U, Rana S, Gross W, Müller M, Giese NA, Kalthoff H, Becker T, and Büchler MW. 2015. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. International journal of cancer 136:2616-2627.
Mahmoodzadeh HH, Ali IFA, Soleimanirad J, Reza NM, and Mahdavi M. 2014. Exosome/staphylococcal enterotoxin B, an anti tumor compound against pancreatic cancer. *Journal of BU ON: official journal of the Balkan Union of Oncology* 19:440-448.

Malhotra H, Sheokand N, Kumar S, Chauhan AS, Kumar M, Jakhar P, Boradia VM, Raje CI, and Raje M. 2016. Exosomes: tunable nano vehicles for macromolecular delivery of transferrin and lactoferrin to specific intracellular compartment. *Journal of biomedical nanotechnology* 12:1101-1114.

Mathivanan S, Ji H, and Simpson RJ. 2010. Exosomes: extracellular organelles important in intercellular communication. *Journal of Proteomics* 73:1907-1920.

McKiernan J, Donovan MJ, O’Neill V, Bentink S, Noerholm M, Belzer S, Skog J, Kattan MW, Partin A, and Andrei G. 2016. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. *JAMA oncology* 2:882-889.

Menay F, Herschlik L, De Toro J, Coccoza F, Tsacalian R, Gravisaco MJ, Di Sciullo MP, Vendrell A, Waldner CI, and Mongini C. 2017. Exosomes isolated from ascites of T-cell lymphoma-Bearing Mice expressing surface CD24 and hsP-90 induce a Tumor-specific immune response. *Frontiers in Immunology* 8.

Mineo M, Garfield SH, Taverna S, Flugy A, De Leo G, Alessandro R, and Kohn EC. 2012. Exosomes released by K562 chronic myeloid leukemia cells promote angiogenesis in a Src-dependent fashion. *Angiogenesis* 15:33-45.

Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, and Nagata S. 2007. Identification of Tim4 as a phosphatidylserine receptor. *Nature* 450:435.

Moon P-G, Lee J-E, Cho Y-E, Lee SJ, Jung JH, Chae YS, Bae H-I, Kim Y-B, Kim I-S, and Park HY. 2016. Identification of developmental endothelial locus-1 on circulating extracellular vesicles as a novel biomarker for early breast cancer detection. *Clinical Cancer Research* 22:1757-1766.

Muhsin-Sharafaldine M-R, Saunderson SC, Dunn AC, Faed JM, Kleffmann T, and McLellan AD. 2016. Procoagulant and immunogenic properties of melanoma exosomes, microvesicles and apoptotic vesicles. *Oncotarget* 7:56279.

Munoz JL, Bliss SA, Greco SJ, Ramkisson SH, Ligon KL, and Rameshwar P. 2013. Delivery of functional anti-miR-9 by mesenchymal stem cell–derived exosomes to glioblastoma multiforme cells conferred chemosensitivity. *Molecular Therapy—Nucleic Acids* 2:e126.

Nagaraj S, and Gabrilovich DI. 2012. Regulation of suppressive function of myeloid-derived suppressor cells by CD4+ T cells. Seminars in Cancer Biology: Elsevier. p 282-288.

Nazarenko I, Rana S, Baumann A, McAlear J, Hellwig A, Trendelenburg M, Lochnit G, Preissner KT, and Zöller M. 2010. Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation. *Cancer research* 70:1668-1678.

Nilsson J, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield X, and Widmark A. 2009. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. *British journal of cancer* 100:1603.

Ohno S-i, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyma N, Fujita K, Mizutani T, Ohgi T, and Ochiya T. 2013. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Molecular Therapy* 21:185-191.

Ohshima K, Kanto K, Hatakeyama K, Ide T, Wakabayashi-Nakao K, Watanabe Y, Sakura N, Terashima M, Yamaguchi K, and Mochizuki T. 2014. Exosome-mediated extracellular release of polyadenylate-binding protein 1 in human metastatic duodenal cancer cells. *Proteomics* 14:2297-2306.

Oksvold MP, Kullmann A, Forfang L, Kierulf B, Li M, Brech A, Vlassov AV, Smeland EB, Neurauter A, and Pedersen KW. 2014. Expression of B-cell surface antigens in subpopulations of exosomes released from B-cell lymphoma cells. *Clinical Therapeutics* 36:847-862. e841.
Ostenfeld MS, Jeppesen DK, Laurberg JR, Boysen AT, Bramsen JB, Primdal-Bengtson B, Hendrix A, Lamy P, Dagnaes-Hansen F, and Rasmussen MH. 2014. Cellular disposal of miR23b by RAB27-dependent exosome release is linked to acquisition of metastatic properties. *Cancer research* 74:5758-5771.

Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G, Savina A, Moita CF, Schauer K, Hume AN, and Freitas RP. 2010. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nature cell biology* 12:19.

Paggetti J, Haderk F, Seiffert M, Janji B, Distler U, Ammerlaan W, Kim YJ, Adam J, Lichter P, and Solary E. 2015. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. *Blood* 126:1106-1117.

Pant S, Hilton H, and Burczynski ME. 2012. The multifaceted exosome: biogenesis, role in normal and aberrant cellular function, and frontiers for pharmacological and biomarker opportunities. *Biochemical Pharmacology* 83:1484-1494.

Park J, Hwang M, Choi B, Jeong H, Jung J-h, Kim HK, Hong S, Park JH, and Choi Y. 2017. Exosome Classification by Pattern Analysis of Surface-enhanced Raman Spectroscopy Data for Lung Cancer Diagnosis. *Analytical Chemistry* 921.

Park S, Ahn ES, and Kim Y. 2015. Neuroblastoma SH-SYSY cell-derived exosomes stimulate dendrite-like outgrowths and modify the differentiation of A375 melanoma cells. *Cell Biology International* 39:379-387.

Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, Coscia C, Iessi E, Logozzi M, and Molinari A. 2009. Microenvironmental pH is a key factor for exosome traffic in tumor cells. *Journal of Biological Chemistry* 284:34211-34222.

Patel SJ, Darie CC, and Clarkson BD. 2016. Exosome mediated growth effect on the non-growing pre-B acute lymphoblastic leukemia cells at low starting cell density. *American Journal of Translational Research* 8:3614.

Peinado H, Alečković M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, García-Santos G, and Ghajar CM. 2016. Corrigendum: Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nature Medicine* 22:1502.

Peinado H, Alečković M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, García-Santos G, and Ghajar CM. 2012. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nature Medicine* 18:883-891.

Philley JV, Kannan A, Griffith DE, Devine MS, Benwill JL, Wallace JRJ, Brown-Elliott BA, Thakkar F, Taskar V, and Fox JG. 2017. Exosome secretome and mediated signaling in breast cancer patients with nontuberculous mycobacterial disease. *Oncotarget* 8:18070.

Pinet S, Bessette B, Vedrenne N, Lacroix A, Richard L, Jauberteaux M-O, Battu S, and Laloué F. 2016. TrkB-containing exosomes promote the transfer of glioblastoma aggressiveness to YKL-40-inactivated glioblastoma cells. *Oncotarget* 7:50349.

Qu J-L, Qu X-J, Qu J-L, Qu X-J, Zhao M-F, Teng Y-E, Zhang Y, Hou K-Z, Jiang Y-H, and Yang X-H. 2009. The role of cbl family of ubiquitin ligases in gastric cancer exosome-induced apoptosis of Jurkat T cells. *Acta Oncologica* 48:1173-1180.

Qu L, Ding J, Chen C, Wu Z-J, Liu B, Gao Y, Chen W, Liu F, Sun W, and Li X-F. 2016a. Exosome-transmitted IncARS promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. *Cancer cell* 29:653-668.

Qu Z, Wu J, Wu J, Luo D, Jiang C, and Ding Y. 2016b. Exosomes derived from HCC cells induce sorafenib resistance in hepatocellular carcinoma both in vivo and in vitro. *Journal of Experimental and Clinical Cancer Research* 35:159.
Que R-s, Lin C, Ding G-p, Wu Z-r, and Cao L-p. 2016. Increasing the immune activity of exosomes: the effect of miRNA-depleted exosome proteins on activating dendritic cell/cytokine-induced killer cells against pancreatic cancer. Journal of Zhejiang University SCIENCE B 17:352-360.

Rabinowits G, Gerçel-Taylor C, Day JM, Taylor DD, and Kloeker GH. 2009. Exosomal microRNA: a diagnostic marker for lung cancer. Clinical Lung Cancer 10:42-46.

Ragusa M, Barbagallo C, Statello L, Caltabiano R, Russo A, Puzzo L, Avitabile T, Longo A, Toro MD, and Barbagallo D. 2015. miRNA profiling in vitreous humor, vitreal exosomes and serum from uveal melanoma patients: Pathological and diagnostic implications. Cancer Biology & Therapy 16:1387-1396.

Raimondo F, Morosi L, Chinello C, Magni F, and Pitto M. 2011. Advances in membranous vesicle and exosome proteomics improving biological understanding and biomarker discovery. Proteomics 11:709-720.

Raimondo S, Saieva L, Corrado C, Fontana S, Fluty A, Rizzo A, De Leo G, and Alessandro R. 2015. Chronic myeloid leukemia-derived exosomes promote tumor growth through an autocrine mechanism. Cell Communication and Signaling 13:8.

Ramteke A, Ting H, Agarwal C, Mateen S, Somasagara R, Hussain A, Graner M, Frederick B, Agarwal R, and Deep G. 2015. Exosomes secreted under hypoxia enhance invasiveness and stemness of prostate cancer cells by targeting adherens junction molecules. Molecular carcinogenesis 54:554-565.

Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, and Geuze HJ. 1996. B lymphocytes secrete antigen-presenting vesicles. Journal of Experimental Medicine 183:1161-1172.

Record M, Carayon K, Poirot M, and Silvente-Poirot S. 2014. Exosomes as new vesicular lipid transporters involved in cell–cell communication and various pathophysiologies. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1841:108-120.

Ristorcelli E, Beraud E, Verrando P, Villard C, Lafitte D, Sbarra V, Lombardo D, and Verine A. 2008. Human tumor nanoparticles induce apoptosis of pancreatic cancer cells. The FASEB Journal 22:3358-3369.

Rodriguez M, Silva J, López-Alfonso A, López-Muñiz MB, Peña C, Domínguez G, García JM, López-González A, Méndez M, and Provencio M. 2014. Different exosome cargo from plasma/bronchoalveolar lavage in non-small-cell lung cancer. Genes, Chromosomes and Cancer 53:713-724.

Roma-Rodrigues C, Fernandes AR, and Baptista PV. 2014. Exosome in tumour microenvironment: overview of the crosstalk between normal and cancer cells. Biomed research international 2014.

Ruiz-Martinez M, Navarro A, Marrades RM, Viñolas N, Santasusagna S, Muñoz C, Ramirez J, Molins L, and Monzo M. 2016. YKT6 expression, exosome release, and survival in non-small cell lung cancer. Oncotarget 7:51515.

Saari H, Lazaro-Ibanez E, Viitala T, Vuorimaa-Laukkanen E, Siljander P, and Yliperttula M. 2015. Microvesicle-and exosome-mediated drug delivery enhances the cytotoxicity of Paclitaxel in autologous prostate cancer cells. Journal of Controlled Release 220:727-737.

Saeki M, Egusa H, Kamo Y, Kakihara Y, Houry WA, Yatani H, Moguchi S, and Kamisaki Y. 2013. Exosome-bound WD repeat protein Monad inhibits breast cancer cell invasion by degrading amphiregulin mRNA. PLoS One 8:e67326.

Safaei R, Larson BJ, Cheng TC, Gibson MA, Otani S, Naerdemann W, and Howell SB. 2005. Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. Molecular cancer therapeutics 4:1595-1604.
Sagar G, Sah RP, Javeed N, Dutta SK, Smyrk TC, Lau JS, Giorgadze N, Tchkonia T, Kirkland JL, and Chari ST. 2015. Pathogenesis of pancreatic cancer exosome-induced lipolysis in adipose tissue. *Gut*:gutjnl-2014-308350.

Segura E, Nicco C, Lombard B, Véron P, Raposo G, Batteux F, Amigorena S, and Théry C. 2005. ICAM-1 on exosomes from mature dendritic cells is critical for efficient naive T-cell priming. *Blood* 106:216-223.

Sharma S, Das K, Woo J, and Gimzewski JK. 2014. Nanofilaments on glioblastoma exosomes revealed by peak force microscopy. *Journal of The Royal Society Interface* 11:20131150.

Shedden K, Xie XT, Chandaroy P, Chang YT, and Rosania GR. 2003. Expulsion of Small Molecules in Vesicles Shed by Cancer Cells. *Cancer research* 63:4331-4337.

Shigoka M, Tsuchida A, Matsudo T, Nagakawa Y, Saito H, Suzuki Y, Aoki T, Murakami Y, Toyoda H, and Kumada T. 2010. Deregulation of miR-92a expression is implicated in hepatocellular carcinoma development. *Pathology International* 60:351-357.

Silva J, Garcia V, Rodriguez M, Compte M, Cisneros E, Veguillas P, Garcia J, Dominguez G, Campos-Martin Y, and Cuevas J. 2012. Analysis of exosome release and its prognostic value in human colorectal cancer. *Genes, Chromosomes and Cancer* 51:409-418.

Singh R, Pochampally R, Watabe K, Lu Z, and Mo Y-Y. 2014. Exosome-mediated transfer of miR-10b promotes cell invasion in breast cancer. *Molecular cancer* 13:256.

Steelman LS, Navolanic PM, Sokolosky ML, Taylor JR, Lehmann BD, Chappell WH, Abrams SL, Wong EW, Stadelman KM, and Terrian DM. 2008. Suppression of PTEN function increases breast cancer chemotherapeutic drug resistance while conferring sensitivity to mTOR inhibitors. *Oncogene* 27.

Stope MB, Klinkmann G, Diesing K, Koensgen D, Burchardt M, and Mustea A. 2017. Heat Shock Protein HSP27 Secretion by Ovarian Cancer Cells Is Linked to Intracellular Expression Levels, Occurs Independently of the Endoplasmic Reticulum Pathway and HSP27's Phosphorylation Status, and Is Mediated by Exosome Liberation. *Disease markers* 2017.

Sugimachi K, Matsumura T, Hirata H, Uchi R, Ueda M, Ueo H, Shinden Y, Iguchi T, Eguchi H, and Shirabe K. 2015. Identification of a bona fide microRNA biomarker in serum exosomes that predicts hepatocellular carcinoma recurrence after liver transplantation. *British journal of cancer* 112:532-538.

Sund M, and Kalluri R. 2009. Tumor stroma derived biomarkers in cancer. *Cancer and Metastasis Reviews* 28:177-183.

Swartz MA, Iida N, Roberts EW, Sangaletti S, Wong MH, Yull FE, Coussens LM, and DeClerck YA. 2012. Tumor microenvironment complexity: emerging roles in cancer therapy. AACR.

Takahashi Y, Nishikawa M, Shinotsuka H, Matsui Y, Ohara S, Imai T, and Takakura Y. 2013. Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. *Journal of Biotechnology* 165:77-84.

Taverna S, Flaig Y, Saieva L, Kohn EC, Santoro A, Meraviglia S, De Leo G, and Alessandro R. 2015. Identification of a bona fide microRNA biomarker in serum exosomes that predicts hepatocellular carcinoma recurrence after liver transplantation. *British journal of cancer* 112:532-538.

Taverna S, Monteleone F, Pucci M, Saieva L, De Caro V, Cardinale VG, Gialloblomo M, Vicario E, and Rolfo C. 2016. Curcumin modulates chronic myelogenous leukemia exosomes composition and affects angiogenic phenotype via exosomal miR-21. *Oncotarget* 7:30420.

Taylor DD, and Gercel-Taylor C. 2008. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecologic Oncology* 110:13-21.

Taylor DD, and Gercel-Taylor C. 2011. Exosomes/microvesicles: mediators of cancer-associated immunosuppressive microenvironments. *Seminars in Immunopathology*: Springer. p 441-454.
Théry C, Amigorena S, Raposo G, and Clayton A. 2006. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Current Protocols in Cell Biology*: 3.22. 21-23.22. 29.

Théry C, Zitvogel L, and Amigorena S. 2002. Exosomes: composition, biogenesis and function. *Nature reviews Immunology* 2:569.

Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, Wei J, and Nie G. 2014. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. *Biomaterials* 35:2383-2390.

Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brügger B, and Simons M. 2008. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 319:1244-1247.

Trams EG, Lauter CJ, Salem JN, and Heine U. 1981. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 645:63-70.

Umezu T, Ohyashiki K, Kuroda M, and Ohyashiki J. 2013. Leukemia cell to endothelial cell communication via exosomal miRNAs. *Oncogene* 32:2747.

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, and Lötvall JO. 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature cell biology* 9:654.

Vlassov AV, Magdaleno S, Setterququist R, and Conrad R. 2012. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1820:940-948.

Wang H, Hou L, Li A, Duan Y, Gao H, and Song X. 2014a. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. *BioMed research international* 2014.

Wang J, De Veirman K, Faict S, Frassanito MA, Ribatti D, Vacc a A, and Menu E. 2016. Multiple myeloma exosomes establish a favourable bone marrow microenvironment with enhanced angiogenesis and immunosuppression. *The Journal of pathology* 239:162-173.

Wang K, Zhang S, Weber J, Baxter D, and Galas DJ. 2010. Export of microRNAs and microRNA-protective protein by mammalian cells. *Nucleic acids research* 38:7248-7259.

Wang M, Zhao C, Shi H, Zhang B, Zhang L, Zhang X, Wang S, Wu X, Yang T, and Huang F. 2014b. Deregulated microRNAs in gastric cancer tissue-derived mesenchymal stem cells: novel biomarkers and a mechanism for gastric cancer. *British journal of cancer* 110:1199.

Wei Y, Lai X, Yu S, Chen S, Ma Y, Zhang Y, Li H, Zhu X, Yao L, and Zhang J. 2014. Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. *Breast cancer research and treatment* 147:423-431.

Wojtuszkiewicz A, Schuurhuis GJ, Kessler FL, Piersma SR, Knol JC, Pham TV, Jansen G, Musters RJ, van Meerloo J, and Assaraf YG. 2016. Exosomes secreted by apoptosis-resistant acute myeloid leukemia (AML) blasts harbor regulatory network proteins potentially involved in antagonism of apoptosis. *Molecular & Cellular Proteomics* 15:1281-1298.

Wollert T, and Hurley JH. 2010. Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. *Nature* 464:864.

Worst TS, von Hardenberg J, Gross JC, Erben P, Schnöller M, Hauesser I, Bugert P, Michel MS, and Boutros M. 2017. Database-augmented Mass Spectrometry Analysis of Exosomes Identifies Claudin 3 as a Putative Prostate Cancer Biomarker. *Molecular & Cellular Proteomics* 16:998-1008.

Wu H, Zhou J, Zeng C, Wu D, Mu Z, Chen B, Xie Y, Ye Y, and Liu J. 2016. Curcumin increases exosomal TCF21 thus suppressing exosome-induced lung cancer. *Oncotarget* 7:87081.
Wu Y, Deng W, McGinley E, and Klinke DJ. 2015. B16F0 melanoma exosomes deliver a unique and complex biological payload that includes Ptpn11 to suppress T lymphocyte function. Journal for immunotherapy of cancer 3:P270.

Wu Y, Deng W, McGinley EC, and Klinke DJ. 2017. Melanoma exosomes deliver a complex biological payload that upregulates PTPN11 to suppress T lymphocyte function. Pigment cell & melanoma research 30:203-218.

Xiang X, Poliakov A, Liu C, Liu Y, Deng Zb, Wang J, Cheng Z, Shah SV, Wang GJ, and Zhang L. 2009. Induction of myeloid-derived suppressor cells by tumor exosomes. International journal of cancer 124:2621-2633.

Xiao W, Dong W, Zhang C, Saren G, Geng P, Zhao H, Li Q, Zhu J, Li G, and Zhang S. 2013. Effects of the epigenetic drug MS-275 on the release and function of exosome-related immune molecules in hepatocellular carcinoma cells. European Journal of Medical Research 18:61.

Xie Y, Zhang X, Zhao T, Li W, and Xiang J. 2013. Natural CD8+ 25+ regulatory T cell-secreted exosomes capable of suppressing cytotoxic T lymphocyte-mediated immunity against B16 melanoma. Biochemical and Biophysical Research Communications 438:152-155.

Yamamoto KR, Alberts BM, Benzinger R, Lawhorne L, and Treiber G. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology 40:734-744.

Yamashita T, Kamada H, Kanasaki S, Maeda Y, Nagano K, Abe Y, Inoue M, Yoshioka Y, Tsutsumi Y, and Katayama S. 2013. Epidermal growth factor receptor localized to exosome membranes as a possible biomarker for lung cancer diagnosis. Die Pharmazie-An International Journal of Pharmaceutical Sciences 68:969-973.

Yang T, Martin P, Fogarty B, Brown A, Schurman K, Phipps R, Yin VP, Lockman P, and Bai S. 2015. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. Pharmaceutical Research 32:2003-2014.

Yao Y, Wang C, Wei W, Shen C, Deng X, Chen L, Ma L, and Hao S. 2014. Dendritic cells pulsed with leukemia cell-derived exosomes more efficiently induce antileukemic immunities. PLoS One 9:e91463.

Yao Y, Wei W, Sun J, Chen L, Deng X, Ma L, and Hao S. 2015. Proteomic analysis of exosomes derived from human lymphoma cells. European Journal of Medical Research 20:8.

Yeung CLA, Tsuruga T, Yeung T-L, Kwan S-Y, Leung CS, Li Y, Lu ES, Kwan K, Wong K-K, and Schmandt R. 2016. Exosomal transfer of stroma-derived miR21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. Nature communications 7:11150.

Yoon C, Kim J, Park G, Kim S, Kim D, Hur DY, Kim B, and Kim YS. 2016. Delivery of miR-155 to retinal pigment epithelial cells mediated by Burkitt’s lymphoma exosomes. Tumor Biology 37:313-321.

Yu S, Cao H, Shen B, and Feng J. 2015. Tumor-derived exosomes in cancer progression and treatment failure. Oncotarget 6:37151.

Yu X, Harris SL, and Levine AJ. 2006. The regulation of exosome secretion: a novel function of the p53 protein. Cancer research 66:4795-4801.

Zaharie F, Muresan M-S, Petrushev B, Berce C, Gafencu G-A, Seliancuc S, Jurj A, Cojocneanu-Petric R, Lisencu C-I, and Pop L-A. 2015. Exosome-carried microRNA-375 inhibits cell progression and dissemination via Bcl-2 blocking in colon cancer. Journal of Gastrointestinal and Liver Diseases 24:435-443.

Zeringer E, Barta T, Li M, and Vlassov AV. 2015. Strategies for isolation of exosomes. Cold Spring Harbor Protocols 2015:pdb. top074476.

Zhang H-G, and Grizzle WE. 2014. Exosomes: a novel pathway of local and distant intercellular communication that facilitates the growth and metastasis of neoplastic lesions. The American journal of pathology 184:28-41.
Zhang H, Deng T, Liu R, Bai M, Zhou L, Wang X, Li S, Wang X, Yang H, and Li J. 2017. Exosome-delivered EGFR regulates liver microenvironment to promote gastric cancer liver metastasis. *Nature communications* 8.

Zhao Z, Yang Y, Zeng Y, and He M. 2016. A microfluidic ExoSearch chip for multiplexed exosome detection towards blood-based ovarian cancer diagnosis. *Lab on a Chip* 16:489-496.
Figure 1

Schematic of exosomes derived cancer cell biogenesis and secretion

Exosomes can secrete through cells while intracellular organs called multivesicular bodies (MVBs) fuse with the plasma membrane. The MVBs formation occurs through invaginations of late endosomes, which increased molecules from the Golgi apparatus (e.g., MHC class II molecules) or the cell surface (e.g., growth factor receptors). Subsequently, exosomes could be enriched in several materials including sphingomyelin, intracellular protein, ceramide, cholesterol, transmembrane receptors, mRNA, and miRNA. The exosomes secreted from human tumor cells can affect the local tumor microenvironment, alter the extracellular matrix, and enhance the angiogenesis, thrombosis and cancer cell proliferation.
Figure 2

Exosome recruitment of bone marrow-derived cells

Exosomes transform the tumor microenvironment (TME) and dispose of distant tissue sites for metastasis. The efficacies of exosomes at distant tumor sites necessitate that exosomes migrate through the blood or lymph. They dispose tissue sites for metastasis or transform the bone marrow (BM) environment, and making a pre-metastatic niche to enhance tumor invasion and development. Thus the tumor-derived exosomes can cause recruiting bone marrow-derived cells to the tumor and pre-tumor tissue where they function as cancer development and support the multiple tumor cell expansion and development in various human cancer cells.
Mobilisation of BMDC (Bone marrow driven cells)

Bone Marrow
Figure 3

Exosomes drive pre-metastatic niche formation

The formation of the pre-metastatic niche is required for organ-specific metastatic tropism. The exosomes can move to the distant location for increasing the formation of pre-metastatic niche. The complementation of angiogenesis and induction of stromal and epithelial cell differentiation can be associated with a pro-tumor environment. Tumor-derived exosomes provide a pre-metastatic niche, through the polarization of tissue macrophage, Suppression of dendritic cell maturation, Induction of CAF (cancer-associated fibroblasts) via differentiation of fibroblasts to myofibroblasts. This effect can be performed via the mediation of intercellular cross-talk and subsequent adjustment of both local and distant microenvironments in an autocrine and paracrine fashion.
Manuscript to be reviewed

Induces M2 macrophage polarization

Suppress DC (dendritic cell) differentiation

Enhances of angiogenesis and thrombosis

Induces CAF (cancer associated fibroblasts) through differentiation of fibroblasts to myofibroblasts

pre-metastatic niche formation
Figure 4

Regulation of immune responses by extracellular vesicles

The tumor-derived microvesicles may function as immunosuppressive effects. Exosome-mediated communication among cancer cells and the immune system is triggered recruiting pro-cancerogenic immune cells. The regulation of immune response in a procedure of prevention tumor diagnosis and anti-tumoral immune functions through impairing the function of effector T cells and natural killer cells (NK cells), can induce mobilization of neutrophils, and differentiate T-helper cells toward a T-regulatory cell phenotype.
Manuscript to be reviewed

**Immune System**

**Neutrophils**
- Induces mobilization

**Exosome**
- Induces apoptosis of cytotoxic T cells
- Decrease proliferation of NK cells and suppress cytotoxicity
- Differentiation of T-helper to T-reg and enhances expression and function
Figure 5

Exosomes as mediators of drug resistance

Drug resistance applies for a critical role in various cancer treatments. There are various mechanisms of drug resistance even multi-drug resistance (MDR) such as drug efflux, triggered by extracellular vesicles, which can make the defeat of the whole remedy. The tumor-derived exosomes can induce tumor cells to promote drug resistance through sending out the tumor drugs or inhibiting antibody-based drugs.
Drug resistance

Exosome
Antibody based drug target
Antibody based drug
Tumoural drug
miRNAs

Drug exportation

Target cell

Antibody inhibition

Tumoural drug
Figure 6

The main groups of exosomes based therapies

This overview includes impairing the secretion of exosomes via cancer cells and removing cancer derived exosomes, including bioactive molecules, from the blood (or other body fluids) of cancer patients; Using exosomes, naturally-equipped nanocarriers, including microRNA (miRNA), small interference RNA (siRNA), and/or anticancer drugs for targeting delivery to tumor cells; The exosomes molecular composition indicates their cells of origin, may confer special cell or tissue tropism; applying exosomes as potent cell-free peptide-based vaccine demonstrate an remarkable strategy to inhibit tumor development; exosomal miRNAs can contribute to exosome-mediated cell-cell communication and induce anticancer features.
Exosomes and Cancer Therapy

- Applying the exosomes as vehicles loaded with various anti-cancer drugs, siRNA, and miRNA for several cancer therapeutic cargos
- Applying the exosomes as target special tissues or organs because of the having special cell tropism according to their traits
- Using the exosomes based cell free vaccines can represent cell development for inhibiting tumor and development in the immune system
- Impairing the secretion of exosomes through cancer cells or isolating from bodily fluids can contribute to exosome mediated cell-cell communication, and induce anti-cancer traits
- Manuscript to be reviewed
Table 1 (on next page)

Overview of the role of exosomes in cancer
| Exosomal Cargos          | Cancer Cell Types                                                                 | Methods                                                                 | Clinical Values                                                                                                                                                                                                 | References       |
|-------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| ECM1, APN, APOC4, and AZGP1 | Serum samples were collected from normal, and healthy women, women with NTMnb, and women with BrCa-NTMnb. The HMLE and SUM149 cell lines. | FACS analysis, Western blotting and, Immunofluorescence analyses.       | APOC4, APN, and AZGP1 as additive factors might possibly increase NTM (Bronchiectasis and nontuberculous mycobacterial disease) susceptibility via the modulation of immune function and triggering lipolysis.            | (Philley et al. 2017) |
| PEG-SMRwt-CLU peptide    | The human breast cancer cell lines MCF-7 cell line, a noninvasive estrogen receptor positive (ER+), and MDA-MB-231 cell line ER negative. The MCF-10A cell line, a non-tumorigenic epithelial cell line. | Exosomes characterization by acetylcholinesterase (AchE) assay, Exosome nanoparticles tracking analysis (NTA), and Western blotting. | The SMR peptide inhibited breast cancer cell growth, reduced exosome secretion without increasing the cytotoxic effects of chemotherapy or promoting apoptosis. | (Huang et al. 2017) |
| MiR-10b                 | The human breast cancer cell lines MCF-7 and MDA-MB-231 cells.                   | qRT-PCR analysis, and Western blotting                                  | MiR-10b as an exosomal miRNA that elevated cell invasion in HMLE cells through targeting HOXD10 and KLF4, indicating the invasive tumor cells may utilize exosomal miRNAs as a means for their advance. | (Singh et al. 2014) |
| MiR-198, MiR-26a, MiR-34a, MiR-49a, let-7a, MiR-328, MiR130a, MiR-149, MiR-602, MiR-92b | The human breast cancer cell lines, culture supernatants from MCF7, and MDA-MB231 cells. The human mammary epithelial cell lines MCF-10A, and HMLE cells. The human embryonic kidney cell line HEK-293T cells. | qRT-PCR analysis, and Western blotting. | The extracellular vesicles carry oncogenic proteins and miRNAs, which may further be applicable for early detection of breast malignancy as well as delineating the possible role of extracellular vesicles in tumorigenesis and metastasis. | (Kruger et al. 2014) |
| C6 Ceramide             | The human breast cancer cell line MDA-MB-231 cells.                               | qRT-PCR analysis, and Immunocytochemistry assay.                        | Exogenous C6 ceramide, a sphingolipid known to induce exosome secretion, also induced secretion of BCRP-associated exosomes, while siRNA-mediated knockdown or GW4869-mediated inhibition of neutral sphingomyelinase 2 (nSMase2), an enzyme generating ceramide, restored cellular BCRP. | (Kong et al. 2015) |
| HCV RNA (exoRNA)        | The human breast cancer cell lines (IRDs Responders) 1833, MDA-MB-231, Hs578T, MDA-MB-436, MDA-MB-157, and HCC1937. The human breast cancer cell lines (IRDs non-Responders) SKBr3, T47D, MCF-7, HCC70, and MDA-MB-468. | Chromatin immunoprecipitation and primary transcript analysis, and Mammosphere analysis. | Stromal cells orchestrate an intricate cross-talk with BrCa cells by utilizing exosomes to instigate anti-viral signaling. This expands BrCa subpopulations adept at resisting therapy and re-initiating tumor growth. | (Boelens et al. 2014) |
| **Hsp70 (an exosomal protein marker)** | **The epithelial like breast cancer cell line MDA MB-231 cells** | **qRT-PCR analysis, and Western blotting** | **The EXO/SEB, two immune inducer substances, was able to induce cytostatic events through apoptosis in insensitive human ER− breast cell line. The EXO/SEB considerably decreased the cell proliferation and stimulated apoptosis via increasing the expression level of bak, and bax, and raised the activity of caspase-3 and caspase-9. (Hosseini et al. 2014)** |
|---|---|---|---|
| **RPL27A, GDF11, EPS15L1, NUDT16, TRAK2, CCDC11, BEND6, ZNF114, IFNAR1, PITPNM3, ENSA, ALKBH7, APLP2, VAPA, SNRPN, SAR1B, DCAF16, FAM134B, GJC1, and MSLN** | **The human metastatic mammary gland epithelial adenocarcinoma cell line MDA-MB-231, and human submandibular gland (HS) cells Western blot analysis** | **The breast cancer-derived exosome-like microvesicles are capable of interacting with salivary gland cells, altering the composition of their secreted exosome-like microvesicles. (Lau & Wong 2012)** |
| **OIP2** | **The human breast cancer cell lines MDA-MB-231 cells, and MCF-7 cells** | **qRT-PCR analysis, and Enzyme-linked Immunosorbent Assay (ELISA)** | **Monad-mediated degradation is one of the mechanisms that determine the stability of amphiregulin mRNA and that Monad-amphiregulin axis plays an essential role in the invasion of breast cancer cells. (Saeki et al. 2013)** |
| **Wnt10b** | **The immortalized WT mouse embryonic fibroblasts (MEFs) and the p85α− /− MEFs** | **qRT-PCR analysis, and Western blotting** | **Paracrine Wnt10b from p85α-deficient fibroblasts can promote cancer progression via EMT induced by the canonical Wnt pathway. Moreover, exosomes have a key role in paracrine Wnt10b transport from fibroblasts to breast cancer epithelial cells. Thus p85α expression in stromal fibroblasts has a pivotal role in regulating breast cancer tumorigenesis and progression. (Chen et al. 2017)** |
| **ERG, PCA3, and SPDEF** | **The urine samples of prostate cancer (PCA)-free men 50 years or older** | **qRT-PCR analysis** | **The ExoDx Prostate IntelliScore is a validated, easy to administer, noninvasive urine exosome gene expression assay gene signature is derived from genes known to play a pivotal role in prostate cancer initiation and development including ERG, PCA3, and SPDEF with the potential to decrease the total number of biopsies performed in men with a suspicion of prostate cancer. (McKiernan et al. 2016)** |
| **Paclitaxel (PtX), a widely used antimitotic cancer therapeutic** | **The human prostate cancer cell lines LNCaP and PC-3 Pca cells** | **Nanoparticle tracking analysis (NTA), and Western blotting** | **Cancer cell-derived EVs can be utilized as beneficial carriers of Paclitaxel to their parental cells, bringing the drug into the cells via an endocytic pathway and promoting its cytotoxicity. Thus, autologous EVs may have potential for effective delivery of chemotherapeutics to cancer cells. (Saari et al. 2015)** |
| **Claudin 3 (CLDN3)** | **The human metastatic PC3 and benign PNT1A prostate cell lines** | **Immunoblotting, Enzyme-linked immunosorbent assay (ELISA), and Western blotting** | **CLDN3 is an exemplary exosome-based circulating biomarker which candidate for prostate cancer from in vitro profiling of cancer exosomes over in silico identification and in vitro retesting to clinical validation. Besides, CLDN3 plasma levels were considerably increased in patients with high Gleason score, pointing to a potential predictive value of this marker. (Worst et al. 2017)** |
**B7-H3 (CD276)**

- The human prostate cancer cell lines (androgen-responsive: LNCaP, 22RV1 and -irrespective: DU145)
- Normal human dermal fibroblasts (NHDF)

**Western Blot Analysis**

The release of exosome-like microvesicles can promote during proliferative senescence in normal human diploid fibroblasts. Moreover, these exosomes were enriched in B7-H3 protein, a recently identified diagnostic marker for prostate cancer and an abundance of exosomal shuttle RNA. (Lehmann et al. 2008)

**The immunomodulatory cytokine IL-6, and the pro-angiogenic factors IL-8, VEGF, and MMP2**

- The malignant melanoma cell lines, Mewo, SKmel28, A2058, A375, and HTB63 (HT-144)
- MS1 murine endothelial cells

**qRT-PCR analysis, Enzyme-linked immunosorbent assay (ELISA), and Western blotting**

The non-canonical Wnt protein WNT5A signaling, induces a Ca2+-dependent release of exosomes containing the immunomodulatory and pro angiogenic proteins IL-6, IL-8, VEGF, and MMP2 in melanoma cells. (Ekström et al. 2014)

**Histones (H2A, H2B, H3.1 and H4), heat shock proteins (GRP78 and HSC71), and the tetraspanin CD81**

- The C57BL/6 derived melanoma cell lines B16-F1, and B16-OVA (B16-F0 cell line)
- The C57BL/6 derived thymoma derived EL4 cell line

**Flow cytometric analyses, and Western blotting**

Extracellular vesicles (EV) have been implicated in thrombotic events (the second highest cause of death in cancer patients) and tumor vesicles contribute to the anti-cancer immune response. (Muhsin-Sharafaldine et al. 2016)

**CD9, CD63, CD81, Cluster 1 (MiR-216a, MiR-217, MiR-129-5p, and MiR-203), Cluster 2 (MiR-9, MiR-125a-5p, MiR-25, MiR-125b, MiR-335, and MiR-19a), Cluster 3 (MiR-370, MiR-210, MiR-320a, MiR-124, MiR-107, and MiR-486-5p)**

- Metastatic melanoma cell lines Me 30966

**Flow cytometric analyses, and Western blotting**

The enhanced drug delivery time of Exo-AO to melanoma cells as compared to the free AO, improving the cytotoxicity of AO. Thus, Exo-AO have a great potential for a real exploitation as a novel theranostic approach against tumors based on AO delivered through the exosomes. (Iessi et al. 2017)

**CD9, CD63, CD81, Cluster 1**

- The blood plasma samples of patients with isolated liver metastases from uveal melanoma
- The human malignant melanoma cell lines A375, and MML-1
- The human breast cancer cell line, HTB-133
- The human lung carcinoma cell line, HTB-177
- The human mast cell line, HMC-1.2

**Flow cytometry assay, qRT-PCR analysis, and Western blotting**

Melanoma exosomes are released into the liver circulation in metastatic uveal melanoma, and is associated with higher concentrations of exosomes in the systemic circulation. The exosomes isolated directly from liver circulation contain miRNA clusters that are different from exosomes from other cellular sources. (Eldh et al. 2014)

**MAGE A3 (168–176) / class I, MAGE A3 (247–258) / class II, tetanus toxoid / class III, MAGE A3 (168–176) / class I, MAGE A3 (247–258) / class II, MAGE A3 (204–218) / class I, MAGE A3 (204–218) / class II, MAGE A3 (204–218) / class I, MAGE A3 (204–218) / class II**

- Fifteen patients bearing melanoma (stage IIIB and IV, HLA-A1+, or -B35+ and HLA-DP4+/class II, tetanus toxoid / class II, MAGE A3 (168–176) / class I, MAGE A3 (247–258) / class II, MAGE A3 (204–218) / class I, MAGE A3 (204–218) / class II, MAGE A3 (204–218) / class I, MAGE A3 (204–218) / class II)

**Flow cytometry assay, qRT-PCR analysis, and Enzyme-linked immunosorbent assay (ELISA)**

The case report of MART1 antigen spreading and MHC class I loss variant suggested that exosomes mediated bioactivity in vivo, supporting to conduct Phase II clinical trials. Thus, the first exosome Phase I trial highlighted the possibility of large scale exosome production and the safety of exosome administration. (Escudier et al. 2005)
| Housekeeping proteins (CD63 and Rab-5b) and a tumor-associated marker (caveolin-1) | The human metastatic melanoma cell lines Me501, and MeBS cells | Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | Plasma exosomes expressing CD63 or caveolin-1 were significantly promoted in melanoma patients as compared to healthy donors. Moreover, caveolin-1+ plasma exosomes were remarkably increased with respect to CD63+ exosomes in the patients group. (Logozzi et al. 2009) |
| The osteosarcoma (SaOS-2) and colon carcinoma cell lines | The blood plasma samples of melanoma patients | |
| The blood plasma samples of melanoma patients | Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | |
| MiR-21, MiR-34 a, and MiR-146a | The blood serum sampling of Uveal melanoma (UM) patients and healthy donors | Flow cytometry assay, and qRT-PCR analysis | MiRNAs differentially expressed in UM patients comparing with healthy donors. Most alterations were common to vitreous humor (VH), and vitreal exosomes (upregulation of miR-21, -34 a, -146a). Interestingly, miR-146a, miR-34a, and miR-146a were upregulated in the serum of UM patients, as well as in serum exosomes. (Ragusa et al. 2015) |
| Tyrosinase related protein-2 (TYRP2), very late antigen 4 (VLA-4), heat shock protein 70 (HSP70), an HSP90 isoform, and MET oncoprotein | The human peripheral blood samples of melanoma patients | Flow cytometry assay, qRT-PCR analysis, and Western blotting | Decreasing Met expression in exosomes reduced the pro-metastatic behavior of BM cells. Interestingly, MET expression was increased in circulating CD45$^-$ C-KIT$^/$ TIE2$^+$ BM progenitors from metastatic melanoma subjects. RAB1a, RAB5b, RAB7, and RAB27a were highly expressed in melanoma cells and Rab27a RNA interference diminished exosome production, preventing BM education, tumor growth and metastasis. (Peinado et al. 2012) |
| 8–10 week-old C57Bl/6 female mice | The human breast cancer cell lines MCF-7, SkBr3, and MDA-MB-231 | The cellosaurus cell line AsPC-1 | The Lewis Lung carcinoma cell line LLC |
| The colon carcinoma cell lines SW480, and SW620 | The human melanoma cell lines B16-F10, and B16-F1 |
| Superparamagnetic iron oxide nanoparticles 5 (SPION5) | The C57BL/6 mouse model | MRI analysis | The melanoma exosomes appear to be trafficking to a particular microanatomical destination in lymph nodes known as the subcapsular sinus. Thus, SPION5 loaded exosomes might be particularly tailored through endogenous molecular cell based nanofactories and/or exogenous synthetic exosome modification to simultaneously detect and treat pathogenic microenvironments. (Peinado et al. 2016) |
| The mouse B16-F10 (CRL 6475) melanoma cells | | |
| The mouse B16-F10 (CRL 6475) melanoma cells | qRT-PCR analysis | |
| Male 6- to 8-week old albino C57/BL6 mice | Melanoma exosomes are capable of directly tuning a remote lymph node toward a microenvironment that facilitates melanoma growth and metastasis in lymph nodes even in the local absence of tumor cells. (Hood et al. 2011) | |
| **PTPN11** | Eight- to 12-week-old transgenic B6.Cg-Thylac/Tg (TcrαTcrβ)Rest/J female mice | Flow cytometry assay, qRT-PCR analysis, and Western blotting | The tumor-derived exosomes can upregulate PTPN11, which is a phosphatase involved in immune checkpoint pathways, to suppress T cell proliferation and are sized to accumulate within the tumor microenvironment. | (Wu et al. 2017) |
|-----------|-----------------------------------------------------------------|-------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| **Fatty acid oxidation (FAO)** | The murine 3T3-F442A preadipocyte line | The murine 3T3-F442A preadipocyte line | Fatty acid oxidation (FAO) | (Lazar et al. 2016) |
| | Eight week old C57BL/6J male mice | Nano-LC MS/MS analysis, and Western blotting | The adipocyte exosomes stimulate melanoma cell migration and invasion. These exosomes, particularly enriched in proteins implicated in fatty acid oxidation (FAO), induce metabolic reprogramming in tumor cells in favor of FAO, enhancing aggressiveness. | |
| | The human adipose tissue samples | | | |
| | The human melanoma cell line SK-MEL-28 | | | |
| | The human metastatic melanoma cell line 1205Lu | | | |
| | | The murine B16-BL6 melanoma cell line | qRT-PCR analysis, and Dynamic light scattering, zeta potential assay | Through designing a fusion protein consisting of Gaussia luciferase and a truncated lactadherin, gLuc-lactadherin, and constructing a plasmid expressing the fusion protein, sequential in vivo imaging indicated that the B16-BL6-exosome-derived signals distributed first to the liver and then to the lungs which is helpful for tracing exosomes in vivo and that B16-BL6 exosomes. | (Takahashi et al. 2013) |
| | Five-week-old male C57BL/6 and BALB/c mice | | | |
| **LAMP-1, and CD9** | The female C57BL/6 mice | Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The natural CD8+25+ Tr cell-secreted EXOs are capable of suppressing in vivo DC-induced CTL responses and antitumor immunity, indicating that CD8+25+ Tr-released exosomal molecules may play a pivotal role in Tr cell-mediated immune suppression. CD4+25+ Tr cell suppression has been found to be related with cell-surface inhibitory LAG-3, Gal-1, Nrp-1 and TIGI molecules. | (Xie et al. 2013) |
| | The highly lung metastatic OVA expressing B16 melanoma cell line B16-L6_OVA | | | |
| | The naïve CD8+ T cells and ovalbumin (OVA)-pulsed splenic dendritic cells (DC_OVA) | | | |
| | The C57BL/6 female mice and CD45.2+ OT-1 transgenic female mice (8 to 12 weeks of age) | Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The therapeutic vaccination targeted to the tumor-draining lymph nodes (tDLNs) of B16F10 melanoma-bearing mice with Dexo released by DGs co-cultured with oxidized necrotic B16F10 cells as source of melanoma antigens and matured with poly (I:C) (Dexo (B16 + pIC)) raised both melanoma-specific effector CD8+ T cells in the tDLNs, spleen and tumor mass and tumor-infiltrating NK and NKT cells, significantly reducing tumor growth and increasing the survival rate of diseased mice. | (Damo et al. 2015) |
| Exosome Marker | Origin | Methods | Findings |
|---------------|--------|---------|----------|
| cisplatin (CisPt) | The human breast cancer cell line MCF7, The human metastatic melanoma cell lines Me30966 and Me501, The human colon carcinoma cell line SW480, The Human PBMC (Peripheral Blood Mononuclear Cells), Female CB.17 SCID/SCID mice aged 4–5 weeks | Enzyme-linked immunosorbent assay (ELISA) | CisPt uptake by human tumor cells was markedly impaired by low pH conditions. Moreover, exosomes purified from supernatants of these cell cultures contained various amounts of CisPt, which correlated to the pH conditions of the culture medium. (Federici et al. 2014) |
| MiR-21 | The imatinib-sensitive CML cell lines K562-s and LAMA84-s, The human Umbilical Vein Endothelial Cells (HUVEC) | qRT-PCR analysis, Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The exosomes released by chronic myelogenous leukemia (CML) cells after Curcumin remedy deeply changed their molecular composition, acquiring antiangiogenic properties. Curcu-exosomes were enriched in miR-21 which was then shuttled in endothelial cells as a biologically active form. (Taverna et al. 2016) |
| ALIX/PDCD6IP, TSG101, HLA-DR, RAB5A, CD63, CD81, MiR-21, MiR-155, MiR-146a, MiR-148a, and let-7g, human leukocyte antigen (HLA)-DR molecules, B cell-specific markers (CD19 and CD20) and tetraspanins (CD37, CD53, and CD82) | The blood plasma of patients with chronic lymphocytic leukemia (CLL), The eight-week-old NSG mice, The Bone Marrow-Derived Mesenchymal Stem Cells (BM-MSCs), The BM-derived stromal cell line H5-S5, and the endothelial cell line HMEC-1, The primary PKH67-labeled CLL cells | Flow cytometry assay, qRT-PCR analysis, and Western blotting | Exosome uptake by endothelial cells promoted angiogenesis ex vivo and in vivo, and coinjection of CLL-derived exosomes and CLL cells enhanced tumor growth in immunodeficient mice. Also, the results showed a-smooth actin-positive stromal cells in lymph nodes of CLL patients. (Paggetti et al. 2015) |
| MiR-1908, and MiR-298 | The human myeloid leukemia (CML) cell line K562 | qRT-PCR analysis, and Western blotting | The expression level of miRNAs were different among K562 cells and K562 cell-derived exosomes. Thus, Selectively expressed miRNAs in exosomes may promote the development of CML via effects on interactions (e.g. adhesion) of CML cells with their microenvironment. (Feng et al. 2013) |
| CD81, Alix, Tsg101, and TGF-β1 | The human chronic myeloid leukemia cell line LAMA84 cells, The four-to-five weeks old NOD/SCID mice | qRT-PCR analysis, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The exosome-treated LAMA84 cells there is the reduction of BAD and BAX proteins, as well as an increase in the protein levels of BCL-xL, BCL-w. Moreover, CML exosomes stimulate the proliferation and survival of the producer cells via the activation of ERK, Akt and NF-kB. (Raimondo et al. 2015) |
| Cargo of Interest | Source | Methods | Findings |
|------------------|--------|---------|----------|
| VEGF, Tax, CXCR4, Nanog, MMP-9, N-Cadherin, α-SMA, MiR-21, and MiR-155 | The leukemic cell lines HTLV-I negative (Molt-4) or positive (C81 and HuT-102), The peripheral blood plasma of acute ATL patients, The human mesenchymal stem cell line MSCs | qRT-PCR analysis, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The cargo of HuT-102-derived exosomes included of miR-21, miR-155 and vascular endothelial growth factor. Also, HuT-102-derived exosomes not only deliver Tax to recipient MSCs, but also induce NF-κB activation leading to an alteration in cellular morphology, promote in proliferation and the induction of gene expression of migration and angiogenic markers. |
| TGFβ1, latency-associated protein (LAP), CD9, CD34, and CD117 | The blood plasma of acute myeloid leukemia (AML) patients at diagnosis, post-induction CT, during consolidation CT, in long-term remission, and from healthy volunteers | Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The changes in total exosomal protein levels and the presence of various forms of transforming growth factor-beta1 (TGF-b1) carried by AML exosomes reflect effects of remedy and might serve as indicators of leukemic relapse in AML patients. Besides, AML exosomes carrying an active form of TGF-b1 induced down-regulation of NKG2D expression in normal natural killer (NK) cells. |
| HSP70, and ABL | The DBA/2 mice (Dilute Brown Non-Agouti), The chronic myeloid leukemia (CML) cell line K562, The mouse lymphocytic leukemia cells L1210, The DBA/2 mouse leukemia cell line The Menogaril-resistant mouse leukemia P388 cells | Flow cytometry assay, and Western blotting | The EXOK562-pulsed DCs activate CTLs in vitro, which kill target cells more powerfully than CTLs induced by EXOK562 alone or by DCs pulsed with cell lysates. Moreover, LEXs induce antileukemic immunity and that LEX-pulsed DCs have the more potent antigen-specific antileukemic effects, because all mice injected with non-pulsed DCs developed tumors. |
| CD63, CD81, CD34, CD200, CD44, and CD105 | The human CD34+ leukemic cell line, The blood plasma samples of newly-diagnosed AML patients and from healthy volunteers | Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The blast-derived exosomes can be quantitatively ameliorated from AML patients’ plasma and that their molecular profile recapitulates that of the blasts. These isolated exosomes are biologically-active, trigger immune inhibition and might be helpful for AML diagnosis and prognosis. |
| CD63, CD81, and Tsg101 | The chronic myeloid leukemia (CML) cell line K562, The human umbilical vein endothelial cells (HUVEC), Four-week-old BALB/c nude mice | Immunoblot analysis, Endothelial tube formation assay, XTT cell viability assay, and Matrigel plug assay | Exosomes released by K562 CML cells are internalized via endothelial cells during tubular differentiation on Matrigel and are shuttled to neighboring cells via the formation of nanotubular structures connecting the cells. Also, these exosomes stimulate tube formation in endothelial cells via Src activation. While both imatinib and dasatinib reduced exosome release from K562 cells, only dasatinib blocked exosome effect on endothelial cells. |
| Exosomal Protein(s) | Cell Type(s) | Assays Used | Results |
|---------------------|--------------|-------------|---------|
| Interleukin-8 (IL-8) | The human vascular endothelial cells (HUVECs) | qRT-PCR analysis, Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), Immunoprecipitation assay and Western blotting | LAMA84 CML cells and illustrated that addition of exosomes to human vascular endothelial cells (HUVEC) induces an increase of both ICAM-1 and VCAM-1 cell adhesion molecules and interleukin-8 expression. Also, the treatment with exosomes from CML cells caused an increase in endothelial cell motility accompanied by a loss of VE-cadherin and β-catenin from the endothelial cell surface. (Taverna et al. 2012) |
| Alix, CD81, Tsg101, Interleukin 3 (IL3), and Lamp2b | The human embryonic kidney cell line HEK293T cells | qRT-PCR analysis, Atomic Force Microscopy (AFM) assay, and Western blotting | The HEK293T cells was engineered to express the exosomal protein Lamp2b, fused to a fragment of Interleukin 3 (IL3). The modified exosomes, including IL3-Lamp2B, which loaded with Imatinib, are able to particularly target tumor cells in vivo, causing the decrease in tumor size. Thus, the modified exosomes are able to deliver functional BCR-ABL siRNA towards Imatinib-resistant CML cells. (Bellavia et al. 2017) |
| GATA1, FOXP3, SHIP1, ID1, E2F1, CEBP-a and -b, Myc, and MEF2C, specifically, nucleophosmin 1 (NPM1), FLT3, CXCR4, MMP9, IGF-IR, Let-7a, MIR-9, MIR-99h, MIR-150, MIR-155, MIR-191, MIR-223, MIR-146a, and MIR-150 | The acute myelogenous leukemia (AML) cell line HEL, HL-60, Molm-14, and U937 | qRT-PCR analysis, Flow cytometry assay, and Western blotting | Profiling the mRNA content of these microvesicles indicated the presence of transcripts relevant to AML prognosis (FLT3-ITD, NPM1), treatment (FLT3-ITD, IGF-IR, CXCR4), and niche function (IGF-IR, CXCR4, MMP9). Also, both miR-150 and CXCR4 mRNA are present in AML exosomes, miR-150 is highly enriched therein, and exosome transfer to Ba/F3 progenitor cells was associated with a loss of CXCR4 surface expression and consequent reduce in cell migration toward SDF-1a. (Huan et al. 2013) |
| Statistical analysis indicated that out of a total of 4,232 proteins 729 were considerably up-regulated in high AAI exosomes and 498 were up-regulated in low AAI exosomes (Wojtuszkiewicz et al. 2016) | The blood plasma of AML patient | Immunocytochemistry assay, Flow cytometry analysis | The expression of apoptosis-regulating proteins (B-cell CLL/Lymphoma 2 - BCL-2, Myeloid Cell Leukemia 1 -MCL-1, BCL-2 like 1 - BCL-X and BCL-2-associated X protein - BAX) in AML blasts at diagnosis is associated with disease-free survival. The intraindividual ex vivo apoptosis-related profiles of normal lymphocytes and AML blasts within the bone marrow of AML patients were increasingly correlated. Also, apoptosis-resistant primary AML blasts, as opposed to apoptosis-sensitive cells, were able to up-regulate BCL-2 expression in sensitive AML blasts in contact cultures. (Wojtuszkiewicz et al. 2016) |
| - | The human p190BCR-ABL driven ALL cells line (ALL3) | [H]Thymidine incorporation assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The HD ALL3 cells are able to secret exosomes in large-quantities and that they are capable of trigger the growth of the LD ALL3 cells without which they will not survive. Direct stimulation of non-growing LD ALL3 cells using purified exosomes shows that the ALL3 cells can also communicate with each other by means of exchange of exosomes independently of direct cell-cell contacts or diffusible soluble stimulatory factors secreted by HD ALL3 cells. (Patel et al. 2016) |
| - | The chronic myeloid leukemia (CML) cell line K562, R10(-), Mo7, and CML CD34+ cells | qRT-PCR analysis, Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The HD ALL3 cells are able to secret exosomes in large-quantities and that they are capable of trigger the growth of the LD ALL3 cells without which they will not survive. Direct stimulation of non-growing LD ALL3 cells using purified exosomes shows that the ALL3 cells can also communicate with each other by means of exchange of exosomes independently of direct cell-cell contacts or diffusible soluble stimulatory factors secreted by HD ALL3 cells. (Patel et al. 2016) |
| **TGF-β1, Hsc70, and NKG2D** | The chronic myeloid leukemia (CML) cell line K562 | Flow cytometry analysis, and Western blotting | The Dasatinib promotes cellular apoptosis via suppression of Akt/mTOR activities, and prevents exosomal release via downregulation of beclin-1 and Vps34-dependent autophagic activity, containing distinct dasatinib-induced mechanisms of apoptotic response and exosomes release in imatinib-resistant CML cells. | (Liu et al. 2016) |
|---|---|---|---|---|
| **Interleukin-8 (IL 8)** | The chronic myelogenous leukemia cell line LAMA84 cells | qRT-PCR analysis, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | Serum IL 8 levels enhanced in hematologic malignancies compared to healthy controls and promoted expression of IL 8 and its receptors has been indicated in cancer cells and stromal cells illustrating that IL 8 may modulate tumors microenvironment. Thus, LAMA84-derived exosomes are able to activate bone marrow stromal cells which in turn release IL 8 acting as an in vitro and in vivo pro survival factor for chronic myelogenous leukemia cells. | (Corrado et al. 2014) |
| **The NKG2D ligands (MICA/B, ULBP1, ULBP2), and HSP70** | The human T cell leukemia Jurkat- and B cell leukemia/lymphoma Raji cell lines | qRT-PCR analysis, Flow cytometry assay, and Western blotting | The NKG2DL-carrying exosomes abrogate NKG2D-mediated NK-cell cytotoxicity and, thus, might contribute to the immune evasion of leukemia/lymphoma cells T- and B-cell lines Jurkat and Raji as hematopoietic malignancy models. | (Hedlund et al. 2011) |
| **CD40, CD86, HSP60, HSP70, HSP90, RANTES, and IL-1b** | The six-to-eight week old female BALB/c (H-2d) and C57BL/6j (H-2b) mice | Antigen presentation assay, Flow cytometry analysis, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The exosomes derived from heat-shocked lymphoma cells contain more HSP60 and HSP90 and increased amounts of molecules involved in immunogenicity including MHC class I, MHC class II, CD40, CD86, RANTES and IL-1b Consistent with the in vitro results the H5-Exo exhibit a more potent antitumor effect than control exosomes in prophylaxis and therapeutic in vivo lymphoma models. | (Chen et al. 2006) |
| **HLA class I and II molecules such as HLA-B, HLA-C histocompatibility antigen, B-15 alpha chain, B-39 alpha chain, A-26 alpha Chain, HLA-DQAI MHC class II antigen, HLA class II histocompatibility antigen, DQ(1) beta Chain, HLA-C antigen, Cw-4 alpha and Cw-3 alpha chain, HLA-DRB1 major histocompatibility complex, class II, DP beta1, CD19, CD20, CD22, CD81, CD82 antigen and intercellular adhesion molecule 1, etc.** | The human B cell leukemia/lymphoma Raji cell lines | Mass spectrometry assay, and Western blotting | The lymphoma cell-derived exosomes (LCEXs) expressed a discrete set of proteins involved in antigen presentation and cell migration and adhesion, indicating that LCEXs play a significant role in the regulation of immunity and interaction between lymphoma cells and their microenvironment. | (Yao et al. 2015) |
| ALIX, TSG-101, CD63, CD9, CD81, CD24, HSP70, and HSP90 |
| The syngeneic BALB/c T-cell lymphoma cell line LBC (H-2d) cells |
| Six- to ten-week-old female immunocompetent BALB/c mice |
| Flow cytometry analysis, Enzyme-linked immunosorbent assay (ELISA), Dot blot and Western blotting |
| T-cells from EVs A-immunized mice secreted IFN-γ in response to tumor stimulation. Thus, tumor-specific CD4+ and CD8+ IFN-γ secreting cells could be effectively expanded from mice immunized with EVs A, indicating that a T helper 1 response is associated with tumor rejection. |
| (Menay et al. 2017) |
| CD63, CD81, CD19, CD20, CD22, CD23, CD24, CD37, CD40, and CD45 |
| The B-cell lymphoma cell lines Ramos, SUDHL-4, SUDHL-6, and Ros-50 cells |
| The colon adenocarcinoma SW480 cell line |
| qRT-PCR analysis, Flow cytometry assay, Electron microscopy assay, and Western blotting |
| The several B-cell surface antigens including CD19, CD20, CD24, and HLA-DR, but not CD22, CD23, CD40, and CD45 are expressed on exosomes from B-cell lymphoma cell lines with large heterogeneity among the different B-cell lymphoma cell lines. Interestingly, these B-cell lymphoma–derived EVs are able to rescue lymphoma cells from rituximab-induced complement-dependent cytotoxicity. |
| (Oksvold et al. 2014) |
| CD63, CD81, CD19, MCL4, MCL8, and MCL7 |
| The Mantle cell lymphoma cell lines Jeko-1, and Mino cells |
| The J Urgut human acute T cell leukemia cell line and HS-5 human bone marrow derived stroma cell line |
| The blood plasma of MCL patients and healthy donors |
| qRT-PCR analysis, Flow cytometry assay, Electron microscopy assay, Nanoparticle tracking analysis (NTA), and Western blotting |
| The MCL exosomes are quickly and preferentially internalized via B-lymphocytes. Only minor fraction of exosomes was internalized into T-cell leukemia and bone marrow stroma cell lines, when these cells were co-cultured with MCL cells. Thus, exosome internalization was not suppressed by specific siRNA against caveolin1 and clathrin but was found to be mediated by cholesterol-dependent pathway. |
| (Hazan-Halevy et al. 2015) |
| MiR-9, MiR-146a, and MiR-155 |
| The human Burkitt’s lymphoma cell lines Raji, and Ramos cells |
| The retinal pigment epithelial cell line ARPE-19 cells |
| The human umbilical vein endothelial cells (HUVECs) |
| qRT-PCR analysis, Enzyme-linked immunosorbent assay (ELISA), and Western blotting |
| Raji-exosome mediated delivery of miR-155 inhibitor diminished endogenous and secreted levels of VEGFA in ARPE-19 cells. Also, a significant increase in cellular levels of miR-9, miR-146a, and miR-155 in co-cultures of Raji cell compared with EBV-negative B cells was detected. |
| (Yoon et al. 2016) |
| Wnt3a, and SFRP4 |
| The diffuse large B-cell lymphoma (DLBCL) cell lines SIDDHL4, U2932, OCI Iy1, OCI Iy3, and Karpas 422 cells |
| The human B-lymphocytic lymphoma cell lines BALM-3 cells |
| The spontaneously immortalized cell line L-Wnt3a cells |
| qRT-PCR analysis, Flow cytometry assay, and Western blotting |
| The diffuse large B-cell lymphomas possessed a self-organized infrastructure comprising side population (SP) and non-SP cells, where transitions between clonogenic states are modulated by exosome mediated Wnt signaling. Lymphoma SP cells displayed autonomous clonogenicity and exported Wnt3a via exosomes to neighboring cells, thus modulating population equilibrium in the tumor. |
| (Koch et al. 2014) |
| MiR-96-5p, MiR-182-5p, and MiR-149 |
| The human colon carcinoma cell lines HT-29 and HCT-116 cells |
| The peripheral fasting blood specimens of colon carcinoma patients |
| The human colon carcinoma tissues and normal tissue samples |
| qRT-PCR analysis, Flow cytometry assay, and Western blotting |
| The considerably promoted GPC1+ exosomes are present in the plasma of CRC patients and can be released from CRC tumor cells. The high expression of miR-96-5p and miR-149 significantly decreased cell viability and enhanced cell apoptosis in HT-29 and HCT-116 cells, and suppressed the growth of xenograft HT-29 and HCT-116 tumors. |
| (Li et al. 2017) |
| Dataset | Sample Details | Analysis Methods | Summary |
|---------|----------------|------------------|---------|
| DKK4    | The human colon carcinoma cell lines SW480 and SW480APC | qRT-PCR analysis, Electron microscopy assay, and Western blotting | The secretion of Wnt antagonist, dickkopf-related protein 4 (DKK4) enhanced in SW480APC colon carcinoma cells derived exosomes. In addition, the promoter region of the DKK4 gene appears to have decreased methylation in SW480APC cells, comparing with the paternal SW480 cells, as well as reduced expression of DNA methyltransferase 3a (DNMT-3a). (Lim et al. 2012) |
| TSAP6   | The human colon carcinoma cell line HCT-116 TP53-wt, and HCT-116 TP53-null cells | qRT-PCR analysis, Flow cytometry assay, and Western blotting | The expression of TSAP6 is not related with release of exosomes; and regulation of TSAP6 through P53 was not detected either in tumor samples or in HCT-116 cell lines. Besides, it was not shown that the P53/TSAP6 pathway regulates the release of exosomes into the plasma of colorectal cancer patients. (Silva et al. 2012) |
| CD9, CD97, ERK, JNK, p38, HSP70, MiR-2861, MiR-4734, MiR-4728-5p, MiR-6165 | The stomach adenocarcinoma cell line SGC-7901 cells | qRT-PCR analysis, Electron microscopy assay, and Western blotting | CD97 elevates gastric cancer cell proliferation and invasion in vitro via exosome-mediated MAPK signaling pathway, and also exosomal miRNAs including miR-2861 and miR-4734 are probably involved in activation of the CD97-associated pathway. (Li et al. 2015) |
| FN1, and LAMC1 | The human gastric cancer cell lines KatoIII, MKW45, and MDK74 cells | qRT-PCR analysis, and Western blotting | The expression of adhesion-related molecules, including fibronectin 1 (FN1) and laminin gamma 1 (LAMC1), were promoted in mesothelial cells after internalization of tumor-derived exosomes (TEX) from gastric cancer cell line and malignant pleural effusion. (Arita et al. 2016) |
| EGFR    | The human GC liver metastatic and paired adjacent non-cancerous tissues Male nude mice (BALB/c-nu, 6 to 8 weeks) The human gastric adenocarcinoma cell line SGC7901 cells The primary mouse liver cells were obtained from the livers of C57BL/6J mice (6–8 weeks of age) | qRT-PCR analysis, Enzyme-linked immunosorbent assay (ELISA), Electron microscopy assay, Nanoparticle tracking analysis (NTA), and Western blotting | The EGFR-containing exosomes derived from cancer cells is demonstrated to impressively activate hepatocyte growth factor (HGF) by inhibiting miR-26a/b expression. In addition, the high expressed of paracrine HGF, which binds the c-MET receptor on the migrated cancer cells, provides fertile 'soil' for the 'seed', simplifying the landing and proliferation of metastatic cancer cells. (Zhang et al. 2017) |
| HLA-A, and CD9 | The human gastric adenocarcinoma cell line SGC7901 and Jurkat T cells | qRT-PCR analysis, Electron microscopy assay, Western blotting and Immunoprecipitation | The Cbl family of ubiquitin ligases might be involved in regulation of exosome-induced apoptosis of Jurkat T cells by promoting PI3K proteasome degradation, inactivation of PI3K/Akt signaling, and mediating some effects of caspase activation. (Qu et al. 2009) |
| **MiR-203, MiR-212-3p** | The human pancreatic carcinoma epithelial like cell line PANC-1 cells | Liquid chromatography-electro spray ionization mass spectrometry/mass spectrometry (LC-ESIMS/MS) analysis, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The pancreatic cancer (PC)-derived exosomes downregulated the expression of TRL4 in dendritic cells (DCs) through miR-203, including immune tolerance. Therefore, the PC-derived exosomal miRNAs can down regulate the anti-tumor activity of DC/cytokine-induced killer cells (CIKs) and that depletion of exosomal miRNAs can promote the anti-tumor activity of DC/CIKs. (Que et al. 2016) |
| **CD63, TSG101, and Alix** | The pancreatic cancer patient-derived cell lines 6741-1 (MCPAN014), 6413-1 (MCPAN013), 5822-1 (MCPAN008), 7135-1, 7426-1, and 7291-1 cells, Murine 3T3-L1 preadipocytes | Enzyme-linked immunosorbent assay (ELISA), Immunoprecipitation, and Western blotting | Lipolysis in 3T3-L1 cells and in human adipocytes enhanced upon exposure to PC-exosomes. Increase in lipolysis is attributed to adrenomedullin (AM) contained within PC-exosomes, as AM receptor blockade led to abrogation of the effect of exosomes and activation of ERK1/2 and p38 MAPKs in both murine and human adipocytes. (Sagar et al. 2015) |
| **HSP70** | The MIA PaCa-2, an epithelial-like pancreatic cancer cell line | Electron microscopy assay, Western blotting | The EXO/SEB is a novel model or apoto-immunotherapy, being able to induce apoptosis in addition to specific immune responses. The enhanced expression of antiapoptotic genes including bax, bak and fas in cells treated with the EXO/SEB causes promotion of apoptosis. In addition, EXOs released from pancreatic cancer cells can trigger the mitochondrial-dependent apoptosis and increase the caspase-3 and caspase-9 activities. (Mahmoodzadeh et al. 2014) |
| **Alix, TSG101, CHMP4B, and ATP-binding cassette sub-family G member 2 (ABCG2)** | The human pancreatic cancer cell lines AsPC-1 and PANC-1 | qRT-PCR analysis, Electron microscopy assay, Western blotting and Proteomics analysis | The involvement of GIPC on metabolic stress pathways regulating autophagy and microvesicular shedding, and observed that GIPC status determines the loading of cellular cargo in the exosome. Thus, the detection showed the overexpression of the drug resistance gene ABCG2 in exosomes from GIPC-depleted pancreatic cancer cells. (Bhattacharya et al. 2014) |
| **KRAS** | Blood plasma samples of 39 early-stage pancreatic ductal adenocarcinoma (PDAC) patients and 82 age matched healthy controls | qRT-PCR analysis, Flow cytometry assay, Electron microscopy assay, and Western blotting | By comparing exoDNA to cfDNA in liquid biopsies of patients with pancreatic ductal adenocarcinoma, the higher exoKRAS mutant allele frequency, but not CA19-9, was associated with disease free survival in patients with localized disease. (Allenson et al. 2017) |
| **CD44v6, Tspan8, EpCAM, MET, CD104, CD184, Tspan8, CD24, CD133, CD9, CD63, CD151, MIR-1246, MIR-4644, MIR-3976, and MIR-4306** | Blood collection samples from 131 PaCa, 25 chronic pancreatitis (CP), 22 benign pancreatic tumor and 12 patients with non-PaCa, and 30 volunteers | qRT-PCR analysis, Flow cytometry assay, and Microarray miRNA analysis | MiR-1246, miR-4644, miR-3976, and miR-4306 were significantly upregulated in 83% of PaCa serum-exosomes, but rarely in control groups. These miRNA were also elevated in exosome-depleted serum of patients with PaCa, but at a low level. Also, the expression of the PaCEx markers CD24, CD44v6, CD104, Tspan8, EpCAM, MET, and CD151 and the common exosome markers CD9 and CD63 was based on high expression in tumor tissue. (Madhavan et al. 2015) |
| IncARSR (lncRNA Activated in RCC with Sunitinib Resistance), HSC70, ALIX, CD43, heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1), TSPAN8, VPS36, and CD63 |
|---|
| The nude mice grafted with 786-O and ACHN cells |
| The renal cell carcinoma (RCC) cell lines 786-O and ACHN |
| The sunitinib-resistant RCC cells 7Su3rd, 771S, 771R, ACSu3rd |
| qRT-PCR analysis, Electron microscopy assay, and Western blotting |
| In sunitinib-resistant renal cell carcinoma (RCC) cells, IncRNA activated in RCC with sunitinib resistance (IncARSR) elevates sunitinib resistance by competitively binding miR-34 and miR-449, leading to the increased expression of AXL/c-MET and reactivation of STAT3, AKT, and ERK signaling. Moreover, IncARSR can be packaged into exosomes and secreted from sunitinib-resistant RCC cells, transferring resistance to recipient-sensitive cells. |
| (Qu et al. 2016a) |

| MiR-34a, MiR-134, MiR-135a, MiR-135b, and MiR-370 |
|---|
| The human lung adenocarcinoma cell lines A549 cells |
| The collection of non-small cell lung cancer (NSCLC) patient tissue samples |
| qRT-PCR analysis, Electron microscopy assay, and Western blotting |
| YKT6 downregulation is associated with a remarkable reduction in exosome release in an NSCLC cell line and that low YKT6 expression is associated with better clinical outcome in NSCLC patients. Thus, YKT6 is a SNARE protein in the regulation of exosome release in lung cancer cells and is in turn accurately regulated by miR-134 and miR-135b. |
| (Ruiz-Martinez et al. 2016) |

| MiR-378a, MiR-379, MiR-139-5p, MiR-200b-5p, MiR-200b-3p, MiR-629, MiR-100, and MiR-154-3p |
|---|
| Plasma samples from patients with lung adenocarcinoma and a control group without known lung cancer or other active cancer |
| Microarray analysis |
| The considerable difference in total exosome and miRNA levels between lung cancer patients and controls, and the similarity between the circulating exosomal miRNA and the tumor-derived miRNA patterns, suggest that circulating exosomal miRNA might be useful as a screening test for lung adenocarcinoma. |
| (Rabinowits et al. 2009) |

| Curcumin (anti-cancer drug for lung cancer remedy) |
|---|
| The human lung cancer cell lines BEAS-2B, A549, PC9, and H1299 cells |
| qRT-PCR analysis, and Western blotting |
| The anti-cancer effects of Curcumin are associated with upregulation of transcription factor 21 (TCF21), mediated by downregulation of DNMT1. Also, TCF21 overexpression and knockdown was introduced to H1299 cells through lentiviral system, which led to suppression and promotion of lung tumor growth, respectively. |
| (Wu et al. 2016) |

| CD63, flotillin-1, and HSP70 |
|---|
| The human lung adenocarcinoma cell lines A549, and H460 cells |
| BALB/c nude male mice |
| qRT-PCR analysis, Flow cytometry assay, Electron microscopy assay, and Western blotting |
| The β-elemene significantly suppressed growth and induced apoptosis in lung cancer cells. The levels of the anti-apoptotic genes Bcl-2 and Bcl-xl in A549 cells decreased, while expression of P53 and production of exosomes, and the exosome markers CD63, flotillin-1, and HSP70 increased after β-elemene remedy. |
| (Li et al. 2014) |

| Alix, CD9, CD63, CD81, syntenin, calreticulin, calpain 1, VDAC1, vimentin, hepatoma-derived growth factor, casein kinase IIα, and annexin A2 |
|---|
| The human urinary bladder transitional cell carcinoma cell lines T24, FL3, and SLT4 cells |
| LC-MS/MS analysis, Electron microscopy assay, and Western blotting |
| The several proteins lead to EMT was detected in bladder carcinoma cells, including enhanced abundance of vimentin and hepatoma-derived growth factor in the membrane, and casein kinase IIα and annexin A2 in the lumen of exosomes, respectively, from metastatic cells. The change in exosome protein abundance correlated little, although significant for FL3 versus T24, with alters in cellular mRNA expression. |
| (Jeppesen et al. 2014) |

| MiR-17-3p, MiR-21, MiR-106a, MiR-146, MiR-155, MiR-191, MiR-192, MiR-203, MiR-205, MiR-210, MiR-212, and MiR-214 |
|---|
| Plasma samples from patients with lung adenocarcinoma and a control group without known lung cancer or other active cancer |
| Microarray analysis |
| The considerable difference in total exosome and miRNA levels between lung cancer patients and controls, and the similarity between the circulating exosomal miRNA and the tumor-derived miRNA patterns, suggest that circulating exosomal miRNA might be useful as a screening test for lung adenocarcinoma. |
| (Rabinowits et al. 2009) |
| CD63, Calnexin, MiR-122, MiR-126, MiR-128, MiR-143, MiR-144, MiR-302a, MiR-302c | The consecutive series of blood and bronchoalveolar lavage (BAL) samples from 30 non-small cell lung cancer (NSCLC) patients and 75 patients with non-tumor pathology | qRT-PCR analysis, MicroRNA Quantitative PCR Array, and Western blotting | Exosome levels were considerably higher in plasma than in bronchoalveolar lavage (BAL) samples in both groups of patients. Also, in tumor patients the number of miRNAs with high expression was greater in the exosomes released to plasma than in those released to the airway. (Rodríguez et al. 2014) |
| --- | --- | --- | --- |
| Epidermal Growth Factor Receptor (EGFR) | The human lung carcinoma cell lines HARA, HARA-B, A549, RERF-LC-MS and LU165 cells Primary human pulmonary alveolar epithelial cell line HPAEpiC cells Blood plasma samples from tumor-bearing mice, and the human blood plasma specimens from both healthy individuals and cancer patients Six-week-old male BALB/c Sc-nu/nu mice | Enzyme-linked immunosorbent assay (ELISA), Electron microscopy assay, and Western blotting | The secretion of exosomes in plasma that express high levels of EGFR are clearly derived from tumor tissue samples. Also, the exosomal EGFR detection could potentially be applied in blood tests to diagnose lung cancer because the exosomal EGFR level was higher in lung cancer patients than in normal individuals. (Yamashita et al. 2013) |
| CD9, and CD63 | The human lung carcinoma cell lines H1299 and H522 cells The human pulmonary alveolar epithelial cells | Principal component analysis (PCA), Enzyme-linked immunosorbent assay (ELISA), Electron microscopy assay, and Western blotting | The experiment showed the successful segregation of NSCLC-derived exosomes from normal alveolar cell-derived exosomes using the noninvasive method of SERS accompanied with PCA. Therefore, the Raman signals of lung cancer cell derived exosomes and normal alveolar cell-derived exosomes are well distinguished through PCA. (Park et al. 2017) |
| Polyadenylate-binding protein 1 (PABP1) | The human duodenal cancer cell line HuTu 80 cells The human gastric cancer cell line AZ-521 cells, and the metastatic gastric cancer cell line AZ-P7a cells | qRT-PCR analysis, and Western blotting | The PABP1 is predominantly abundant in exosomes from a metastatic duodenal cancer cell line even though its intracellular expression levels do not vary among cell lines. Thus, AZ-P7a cells do not tolerate intracellular PABP1 accumulation and are thus supported into the extracellular milieu through the exosome-mediated pathway. (Ohshima et al. 2014) |
| MiR-21 | The blood plasma samples of esophageal squamous cell carcinoma (ESCC) and healthy volunteers The human esophageal cancer cell line EC9706 cells | qRT-PCR analysis, Flow cytometry assay, Microarray analysis, and Western blotting | The Cy3-labeled miR-21 mimics could be transferred between esophageal cancer cells by exosomes. Thus, the miR-21 mimics could affect migration and invasion of recipient cells partly via modulation of its target gene PDCD4 and its downstream-signaling molecules, MMP-2 and MMP-9 by using the cell co-culture system. Also, miR-21 was upregulated significantly in plasma from esophageal cancer patients and indicated a significant risk association for esophageal cancer. (Liao et al. 2016) |
| HSP27 | The human ovarian cancer (OC) cell lines OVCAR-3 and SK-OV-3 cells | Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The heat shock protein HSP27 has been correlated in OVCAR-3 and SK-OV-3 cells ovarian cancer cell lines by exosomes with aggressiveness and chemoresistance and, thus, represents a promising potential biomarker for OC diagnosis, prognosis, and treatment response. (Stope et al. 2017) |
| Markers | Cell Type | Methods | Description |
|--------|-----------|---------|-------------|
| CA-125, EpCAM, and CD24 | The blood plasma samples of ovarian cancer (OC) patients | Flow cytometry assay, Enzyme-linked immunosorbent assay (ELISA), Electron microscopy assay, and Western blotting | Through the exosome analysis enabled by the ExoSearch chip has been applied for ovarian cancer diagnosis via quantifying a panel of tumor markers from exosomes in a small-volume of blood plasma (20 μL), which indicated significant diagnostic accuracy and was comparable with standard Bradford assay. (Zhao et al. 2016) |
| MiR-584, MiR-517c, MiR-378, MiR-520f, MiR-518d, MiR-215, MiR-376a, MiR-133b, and MiR-367 | The human Hepatocellular carcinoma (HCC) cell lines Hep3B, HepG2, and PLC/PRF/5 cells | qRT-PCR analysis, Flow cytometry assay, and Electron microscopy assay | The HCC cell-derived exosomes can modulate β activated kinase-1 (TAK1) expression and associated signaling and promote transformed cell growth in recipient cells. Loss of TAK1 has been implicated in hepatocarcinogenesis and is a biologically plausible target for intercellular modulation. (Kogure et al. 2011) |
| HSP60, HSP70, and HSP90 | The human hepatocellular carcinoma cell line HepG2 and PLC/PRF/5 cells The erythromyeloblastoid leukemia cell line K562 cells The blood plasma samples of hepatocellular carcinoma cells (HCC) patients and healthy donors | Enzyme-linked immunosorbent assay (ELISA), Flow cytometry assay, Electron microscopy assay, and Western blotting | The anti-cancer drugs (Paclitaxel, Etoposide, Carboplatin, Irinotecan hydrochloride, Mitoxantrone hydrochloride, Epirubicin hydrochloride, Cisplatin, Mitomycin, Fluorouracil, Oxlipatin, and Gemcitabine hydrochloride) can efficiently up-regulate the expression of HSPs (HSP60, HSP70, and HSP90) on the human hepatocellular carcinoma cell-derived exosomes and the ability of exosomal HSPs as a tumor vaccine to significantly induce NK cells reacts that lead to eliciting an anti-tumor immune response in vivo. (Lv et al. 2012) |
| CD10, CD26, CD81, PrPc, and Slc3A1 | The urine samples obtained from experimental models of mouse, and male Wistar rats, 14 week of age | NanoLC-MS/MS analysis, Electron microscopy assay, and Western blotting | The enhancement in the level of CD10 protein was detected in urinary exosomes obtained from glycine N-methyltransferase knockout mice, an animal model of chronic liver injury associated with steatosis, fibrosis, and human Hepatocellular carcinoma (HCC). In addition, the proteome of different vesicle populations indicates several biomarkers including PrPc, CD26, Slc3a1, Ed81, and Ed10 that are detected in urinary vesicles and may be useful for diagnostic purposes. (Conde-Vancells et al. 2010) |
| MiR-16-1, MiR-21, MiR-24, MiR-31, MiR-122, MiR-125b, MiR-223, MiR-410, CD29, and CD44 | The human adult liver stem cells (HLSC) were isolated from human cryopreserved normal hepatocytes The normal human hepatocytes and human fibroblasts The hepatoma cell line HepG2 cells | qRT-PCR analysis, and Western blotting | The microvesicles (MVs) derived from HLSC suppressed the growth of hepatoma tumors and cell line HepG2 cells by transferring the genetic information and delivering anti-tumor miRNAs that interfered with the deregulated survival and proliferation of these cells. (Fonsato et al. 2012) |
| EIF2C2 (AGO2), CHEK2, CDK2, and MATK | The human adult liver stem cells (HLSC) were isolated from human cryopreserved normal hepatocytes Rat liver tissue samples | qRT-PCR analysis, Enzyme-linked immunosorbent assay (ELISA), and Western blotting | The microvesicles (MVs) derived from HLSC may activate a proliferative program in remnant hepatocytes after hepatectomy through a horizontal transfer of specific mRNA subsets. The MVs-mediated transfer of mRNA from HLSC to hepatocytes can display a procedure that contribute to liver regulation and that could be extracted in regenerative medicine. (Herrera et al. 2010) |
| **MIR-92a, and MIR-638** | The hepatocellular carcinoma (HCC) cell lines HepG2, OR6 and SN1a cells  

The blood plasma samples of hepatocellular carcinoma cells (HCC) patients and healthy donors | qRT-PCR analysis, and MTT assay | The miR-92a is highly expressed in hepatocellular carcinoma (HCC). Thus, the expression level of miR-92a affects the proliferation of hepatoma cell lines HepG2, OR6 and SN1a cells. Also, the ratio of miR-92a/miR-638 decreased in the plasma samples from the HCC patients compared with healthy donors. (Shigoka et al. 2010) |
|---|---|---|---|
| **CD9, and CD63** | The human HCC cell lines SMMC-7721, MHCC-97H, MHCC-97 L, and LO2 cells  

4 to 6 week old male BALB/c nu/nu mice | MTT assay, Enzyme-linked immunosorbent assay (ELISA), Electron microscopy assay, Fluorescence-activated cell sorting (FACS) analysis, and Western blotting | The HCC cell-derived exosomes mediate sorafenib resistance in HCC cells in vitro, and exosomes derived from highly invasive tumors have greater effects than those derived from less invasive tumors. Also, HCC cell-derived exosomes exerted their functions through enhancing the level of proteins associated with sorafenib resistance, protecting tumor cells from sorafenib-induced apoptosis and activating the HGF/c-Met/Akt signaling pathways in vitro. (Qu et al. 2016b) |
| **CD63, tumor susceptibility gene-101 (TSG-101)** | Male Fischer-344 (F344) rats  

N1S1 rat HCC cells (hepatocellular carcinoma cells)  

Blood plasma samples from the Lateral tail vein of the Rat | Electron microscopy assay, and Western blotting | The adipose-derived mesenchymal stem cells (ADMSCs) derived exosomes enhanced natural killer T-cell (NKT) cell anti-tumor response in rats, through facilitating HCC inhibition, early apparent diffusion coefficient (ADC) increase, and low-grade tumor differentiation. (Ko et al. 2015) |
| **ACTB, TUBA1A, FN1, FNLA, CD61, HLA-A, LGALS3BP, Alix, RAB5B, RAB5C, SDCBP, VPF37B, CLTC, ARF1, ANXA2, ANXA5, HSC70, HBA1, tumor susceptibility gene-101 (TSG-101), and Grp94** | The inhouse established human HCC cell lines HKCI-C3 and HKCI-8 cells  

The hepatocellular carcinoma (HCC) cell line MHCC97L cells  

The immortalized hepatocyte cell line MIHA cells | qRT-PCR analysis, Ion Torrent Next-Generation Sequencing, and Western blotting | The internalization of exosomes could activate PI3K/AKT and MAPK signaling pathway, and promoted secretion of MMP-2 and MMP-9 that favored cell invasion. Also, by proteome analysis syndecan–syntenin–ALIX is known to support biogenesis of exosomes and the segregation of signaling cargo to these vesicles. The research also detected the components of endosomal protein sorting complex, such as VPS28 and VPS37, whose functions are required for exosome cargo sorting Process. (He et al. 2015) |
| **MIR-718, and MIR-1246** | Six cases that underwent living donor liver transplantation (LDLT)  

Blood plasma samples from patients that underwent living donor liver transplantation (LDLT)  

The hepatocellular carcinoma (HCC) cell lines Huh-7, and PLC/PRF/5 cells | qRT-PCR analysis, Electron microscopy assay, MTT assay, MicroRNA microarray analysis, and Western blotting | The specific biomarker miR-718 showed significantly different expression in the serum exosomes of HCC cases with recurrence after LT compared with those without recurrence. Decreased expression of miR-718 was associated with HCC tumor aggressiveness in the validated cohort series. (Sugimachi et al. 2015) |
| **MIR-21, CD63, and tumor susceptibility gene-101 (TSG-101)** | Blood plasma samples from the hepatocellular carcinoma (HCC), and hepatitis B (CHB) patients | qRT-PCR analysis, Electron microscopy assay, and Western blotting | The expression of serum exosomal miR-21 was higher in patients with HCC than in patients with CHB and healthy volunteers, the sensitivity of detection is much lower than using exosomal miR-21. These findings indicate that miR-21 is enriched in serum exosomes which provides increased sensitivity of detection than whole serum. (Wang et al. 2014a) |
| HSP70, major histocompatibility complex (MHC) class I, polypeptide-related sequence A (MICA) and MICB | Peripheral blood samples of hepatocellular carcinoma (HCC) patients | qRT-PCR analysis, Electron microscopy assay, and Western blotting | MS-275 (one of the histone deacetylase inhibitor (HDACi) drugs) modified exosomes enhance the cytotoxic effect of NK cells significantly through upregulating the expression of MICA, MICB and HSP70. |
|---|---|---|---|
| MIR-10b, MIR-21, MIR-122, and MIR-200a | The hepatocellular carcinoma (HCC) tissues and blood plasma samples of 108 male fisher 344 rats | qRT-PCR analysis, Electron microscopy assay, Flow cytometry assay, and Western blotting | The changing in the expression of both exosomes and miRNAs (miR-10b, miR-21, miR-122, and miR-200a) was observed during cirrhosis, which in contrast with alpha-fetoprotein (AFP) starts showing up until the early HCC stage. Therefore, the combination of circulating miRNAs and exosomes might serve as promising biomarkers for non-virus infected HCC screening and cirrhosis discrimination. |
| Transactive response DNA-binding protein of 43 kDa (TDP-43) | The human glioma cell line U251 cells | Western blot analysis | The ALS-FTD-CSF incubation with U251 cells generate TDP-43 mislocalization, prion-like propagation of TDP-43 aggregates, and the cell-cell transmission of TDP-43 accumulates is mediated through exosome and TNTs-like structure. Thus, incubation of ALS-CSF and ALS-FTD-CSF with U51 causes toxic to the cells. |
| TrkB, P75NTR, sortilin, HSP90, CD63, and CD9 | The human GBM cell line U87-MG cells | qRT-PCR analysis, Electron microscopy assay, Flow cytometry assay, and Western blotting | The loss of aggressiveness in YKL-40-silenced cells significantly reduced TrkB, p75NTR and sortilin expression. Thus, the release of TrkB in exosomes from control glioma cells, was able to rescue both migration and activation of YKL-40-inactivated cells. |
| MIR-9, CD44, CD45, CD105 | The glioblastoma multiforme (GBM) tissue samples | qRT-PCR analysis, Flow cytometry assay, and Western blotting | The promotion of miR-9 promotes temozolomide (TMZ)-resistant GBM cells. To block miR-9, methods were developed with Cy5-tagged anti-miR-9. Dye-transfer studies indicated intracellular communication between GBM cells and MSCs. This occurred by gap junctional intercellular communication and the release of microvesicles. Thus, anti-miR-9 was transferred from MSCs to GBM cells. |

(Xiao et al. 2013)  
(Liu et al. 2015)  
(Ding et al. 2015)  
(Pinet et al. 2016)  
(Liu et al. 2013)  
(Munoz et al. 2013)
| Protein(s) | Cell Lines | Assays | Description | References |
|------------|------------|--------|-------------|------------|
| CRYAB (crystallin, alpha B), CD9, CD63 | The human glioma cell line U373 cells | Enzyme-linked immunosorbent assay (ELISA), Electron microscopy assay, and Western blotting | Increase in CRYAB levels in GBM coupled with its secretion via exosomes points to an important mode of intercellular communication which, in GBM, may confer resistance to apoptosis in surrounding cells following radiation and chemo-therapies. Proinflammatory cytokines also bring about profound changes in the proteome of the exosome. | (Kore & Abraham 2014) |
| CD11b, CD14, CD16, and CD163 | The glioblastoma multiforme (GBM) tissue samples | Enzyme-linked immunosorbent assay (ELISA), Flow cytometry assay, and Luminex Analysis | The M2-like monocytes expressing CD14+ and CD163+, another indicator of Th2 bias, are promoted in GBM patient blood and associated with high serum concentrations of colony 2 stimulating factor 2 and 3, as well as interleukin-2, -4, and -13, the latter 2 cytokines being hallmarks of Th2 immunity. Fractionation of GBM patient sera into samples enriched for exosomes or soluble factors proved that both fractions are capable of inducing CD163 expression in normal monocytes. | (Harshyne et al. 2015b) |
| Nanofilament | The human glioblastoma cell lines U87 and U251 cells | Piezoresponse force microscopy (PFM) | Compared with normal exosomes, glioblastoma exosomes displayed numerous nanofilaments, and the nanofilaments were trypsin- and RNase-resistant. Based on in vitro uptake assays, glioblastoma exosomes indicated a significantly higher uptake in cells compared with normal exosomes. | (Sharma et al. 2014) |
| Actin, CD9, CTGF, tumor susceptibility gene 101 (TSG-101), apoptosis-linked gene 2-interacting protein x (Alb), IGFBP2, phospho-/total TrkA, phospho-/total FAK, phospho-/total Paxillin | The glioblastoma multiforme (GBM) cell lines LN18, U87MG, and U251cells | qRT-PCR analysis, Flow cytometry assay, Electron microscopy assay, and Western blotting | CTGF mRNA and IGFBP2 protein levels were elevated, and coculture of nonirradiated cells with exosomes isolated from irradiated cells increased CTGF protein expression in the recipient cells. Besides, these exosomes promoted the activation of TrkA, FAK, Paxillin, and Src in recipient cells, molecules involved in cell migration. | (Arscott et al. 2013) |
| CD63, CD71, CD81, and AS-ODN (Antisense oligodeoxynucleotide) | The human glioblastoma cell line U118 cells | Flow cytometry assay, Electron microscopy assay, and Enzyme-linked immunosorbent assay (ELISA) | The exosomes released from brain endothelial cells delivered anticancer drug across the blood-brain barrier (BBB), which subsequently exerted cytotoxic efficacy against brain cancer. Also, the high presence of CD63 in beND.3 exosomes indicates that these exosome nanovesicles might be differently implicated in receptor-mediated transport across the BBB. | (Yang et al. 2015) |

Notes:
- U373, U87, U251: Human glioma cell lines
- U118: Human glioblastoma cell line
- U87MG, U251: Human glioblastoma cell lines
- LN18: Human glioblastoma cell line
- U87: Human glioblastoma cell line
- U251: Human glioblastoma cell line
- LN18: Human glioblastoma cell line
- U87MG, U251: Human glioblastoma cell lines
- U118: Human glioblastoma cell line
- beND.3: Mouse brain endothelial cell line
|    |    |    |    |
|----|----|----|----|
| collagen type VI alpha 1, putative RNA-binding protein 15B chain A, substrate induced remodeling of the active site regulates HTRA1, coatomer protein complex subunit beta 2, myosin-heavy chain 1, keratin-type I cytoskeletal 9, HSP90, and CD63 | The brain neuronal glioblastoma astrocytoma cell line U-87 MG cells | Electron microscopy assay, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis, and Western blotting | Through the proteome analysis of U-87MG exosome the Hsp90 was promoted in exosomes exposed to a low temperature compared with exosomes incubated under normal conditions. Also, there was detected an increase expression of calcium-dependent secretion activator 2 isoform b, hGG1B7425, armadillo repeat-containing protein 4, and immunoglobulin heavy variable 5-a in low temperature-exposed proteome. Besides, The proteins that were reduced on the L.T. gel were collagen alpha-1(VI), DNA topoisomerase I, titin, mitochondrial isoform 2, RNA-binding protein 15B, phosphoserine aminotransferase isoform 2, and Chain A, Substrate Induced Remodeling Of The Active Site Regulates HTRA1 Activity. (Chun et al. 2016) |
| MiR-21, MiR-155, and CD163 | The neuroblastoma primary tissue samples | qRT-PCR analysis, Flow cytometry assay, and Luciferase reporter assay | The result indicated a new exosomal miR-21/TLR8/NF-κB/exosomal miR-155/TERF1 axis triggered regardless of M1- or M2- polarization, but not in dendritic cells involved in resistance to chemotherapy in NBL, and identifies exosomes within the TME as important molecular targets to restore drug sensitivity. (Challagundla et al. 2015) |
| Major histocompatibility complex II (MHC II), Hsp90 and flotillin-1 | The human neuroblastoma cell line SH-SY5Y cells | Electron microscopy assay, and Western blotting | The SH-SY5Y neuroblastoma-derived exosomes comprised of MHC II, Hsp90 and flotillin-1, whereas other cargo proteins or neuron specific proteins, such as actin or tau, NeuN, Sv2, are not released. Moreover, the results showed that, when applied extracellularly, exosomes released from neuronal cells modulated differentiation of melanoma cells. (Park et al. 2015) |