Cancer Letters 460 (2019) 54–64

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Tumor-derived extracellular vesicles in breast cancer: From bench to bedside

Hong-Xia Wang[a,*], Olivier Gires[b,c]

[a] Department of Oncology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200080, PR China
[b] Department of Otorhinolaryngology, Grosshadern Medical Center, Ludwig-Maximilians-University of Munich, Marchioninistr. 15, 81377, Munich, Germany
[c] Clinical Cooperation Group Personalized Radiotherapy of Head and Neck Tumors, Helmholtz Zentrum München, Neuherberg, Germany

ARTICLE INFO

Keywords:
Extracellular vesicles
Exosomes
Microvesicles
Metastatic breast cancer
Liquid biopsy
Tumor microenvironment

ABSTRACT

Tumor-derived extracellular vesicles (TEVs) released from various tumor cell types comprise endosome-derived exosomes and microvesicles (MVs), which originate from plasma membrane budding. TEVs incorporate a myriad of biomolecules such as proteins, DNAs, metabolites and microRNAs, which can be transferred from cell-to-cell. Besides their role in the disposal of biomolecules, TEVs serve to orchestrate fundamental processes of normal and malignant development, including breast cancer (BC). As such, TEVs are important constituents of the tumor microenvironment (TME) that act as communication shuttles through transduction of encapsulated molecular cargos from a parent to a recipient cell and through direct interaction with target cells. Emerging evidence suggests that TEVs support BC development and disease progression by fostering invasion, angiogenesis, pre-metastatic niche preparation, escape from immune surveillance, and induction of resistance to treatment. Although there is a long way to go in order to translate the current knowledge into actual clinical applications, TEVs represent promising candidates for diagnostic biomarkers, therapeutic carriers and targets. In the present review, we will summarize the current knowledge on TEVs in BC.

1. Introduction

Breast cancer (BC) is the second leading cause of cancer-related death globally and is the most common invasive cancer in women [1]. In the past two decades, the mortality of BC has been declining, which is mainly a benefit from early detection and more effective treatment [2,3]. However, the 5-year survival rates are only 22% for those patients suffering from progressed metastatic breast cancer (MBC) [4]. The progression of BC is a dynamic process of clonal evolution and tumor cell selection in response to changing cues from the tumor microenvironment (TME). From the single cell of origin until the clinical detection of lumps, there is a constant acquisition and regulation of signal transduction abilities, intercellular communication, and genetic and epigenetic alterations. All these events eventually contribute to the generation of substantial inter- and intra-tumoral heterogeneity at the cellular level. To infer BC progression and explore potential therapeutic strategies to cope with the particularly poor prognosis of MBC, it is essential to understand the origins of inter- and intra-tumor heterogeneity and the drivers that boost cancer evolution.

In multicellular organisms, extracellular molecules, such as proteins, lipids, metabolites, short peptides, and nucleic acids including DNA, messenger RNAs (mRNAs) and microRNAs (miRs), are released by adjacent or remote cells into the intercellular space. These transmitters can act as ligands that bind to receptors on the recipient cells, modulating intracellular signaling, homeostasis, and adaptation to environmental cues. One way cells release the abovementioned biomolecules is through membrane-encapsulated vesicles termed extracellular vesicles (EVs). Although EVs were originally perceived as a “dustbin” for the removal of cellular waste, extensive research has demonstrated that EVs additionally are a means of specific cell-to-cell communication. This function of EVs was most intensively studied in cancers, where these vesicles are termed tumor-derived EVs (TEVs) [5–8]. As such, TEVs represent an informative readout for many types of cancers, including BC [9,10].

TEVs comprise particles of differing size, ranging from 30 to 2000 nm in diameter. TEVs furthermore differ in composition, incorporating molecular components of their cells of origin, and are actively secreted by different cell types into and by the TME [11]. Based on cell biological mechanisms of production and differing sizes, three main classes of EVs have been defined: exosomes (30–150 nm), shed micro-vesicles (MVs, 200–2000 nm; also termed ectosomes, microvesicles, microparticles, or oncosomes), and apoptotic bodies (ApoBs,

* Corresponding author.
E-mail addresses: whx365@126.com (H.-X. Wang), olivier.gires@med.uni-muenchen.de (O. Gires).

https://doi.org/10.1016/j.canlet.2019.06.012
Received 3 March 2019; Received in revised form 12 June 2019; Accepted 18 June 2019

400–2500 nm). Exosomes as such were first reported in 1987 by Johnstone and colleagues during their work on the maturation of sheep reticulocytes [12]. Exosomes are generated through consecutive steps of inward membrane budding, first during endocytosis and the formation of intracellular endosomes, and, second, through invagination of endosomal membranes. The resulting multivesicular bodies (MVBs), which contain intraluminal vesicles, fuse with the plasma membrane and thereby release exosomes in the extracellular space.

MVs were first described in 1967 as subcellular products of platelets that are present in serum and plasma [13]. In 1991, MVs were reported by Stein and Luzio as vesicles that are released following sublytic treatment of neutrophils with components of the complement system, and which serve as a means of cell protection [14]. Unlike exosomes, MVs are formed through ectocytosis, *i.e.* direct outwards budding of the plasma membrane, hence shedding a membrane-restricted cytoplasmic content [8,15]. Owing to the differences in origin and biogenesis between exosomes and MVs, and because enrichment methods primarily in use do not distinguish the various types of EVs, it is very difficult to pinpoint functional differences across EVs. Hence, although exosomes and MVs might have differing properties and functions, this question can currently not be finally answered [8].

Nonetheless, because of their facilitated retrieval from bodily fluids, and based on features of intercellular communication *via* autocrine, paracrine, and circulatory systems, TEVs are gaining increasing attention with respect to their quantification, expression pattern, and their function, as promising targets in the diagnosis and therapy of various diseases. Considering the relatively ubiquitous presence and abundance in bodily fluids (*e.g.* blood, urine, ascites, cerebrospinal fluid), TEVs, including MVs and exosomes, can offer unique superiority for longitudinal monitoring of disease development and as novel diagnostic biomarkers [16]. Furthermore, membrane-encapsulation can maintain the structural integrity and protect active cargos located within TEVs against degradation by external proteases and other enzymes in biofluid. These features qualify TEVs as candidates for highly stable and reconstructive “drug-loader” for long distance transportation or cancer gene therapies [6,17]. It must however be noted that the difficulty in selectively enriching the different subtypes of EVs, as well as open questions concerning the underlying mechanisms of specific sorting of cargos into EVs complicate a therapeutic application of EVs.

The present review summarizes the general characteristics of TEVs for BC patients, particularly exosomes and MVs, focusing on their biogenesis and secretion, the separation and purification method, their physiological roles and contribution to the pathogenesis and progression of BC, along with the challenges in future clinical application.

2. Biological characteristics, biogenesis, and secretion of TEVs

In spite of progress that has been made in understanding how extracellular vesicles are assembled and function, still, biogenesis, including the incorporation of specific cargos, and secretion mechanisms of TEVs are not completely deciphered. Available evidence supports that both, exosomes and MVs, are assembled by complex mechanisms, whereby their components are sorted into membranous sub-domains that eventually undergo budding. Although these mechanisms are not fully understood, however, it is well accepted that exosomes and MVs follow different processes of cargo sorting, under the influence of specified molecular machineries.

Exosomes are membrane vesicles that originate from inward budding within the endosomal/lysosomal pathway, and are released from the parental cells into the microenvironment upon fusion of multi-vesicular bodies (MVBs) with the plasma membrane [8,15]. A series of sequential molecular machineries involved in the complicated orchestration of these processes has been identified (Table 1, Fig. 1). These molecules are comprised of the endosomal sorting complex required for transport (ESCRT) [18], small Rab GTPase family members (especially Rab11, Rab27, and Rab35) [19–22], the vacuolar protein sorting 4 homolog A (VPS4A), itself an AAA-type ATPase [23,24], ceramide-generating sphingomyelinase [25,26], and the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) system [27]. These fine-tuned sorting molecules contribute to the various processes of exosome biogenesis, such as MVBs/intraluminal vesicle (ILV) biogenesis, cargo sorting, membrane invagination, and the release of exosomes, respectively. The process of biogenesis of exosomes and the actual nature of encapsulated cargos appear context-dependent and arise in response to bioactive triggers and/or tumor progression. Beyond that, multiple molecular switches are involved in the regulation of exosome release, including the degree of cholesterol or lysobisphosphatidic acid enrichment, and intracellular calcium changes [28] (see Table 2).

Furthermore, a link between exosome biogenesis and autophagy has been established, and shared molecular machineries have been described [29]. Activation of autophagy-promoting factors such as BECN1/Beclin 1, ULK1 (unc-51 like kinase 1) and ULK2 by a complex phosphorylation cascade downstream of AMP-activated protein kinase (AMPK) and MTOR (mechanistic target of rapamycin) results in distinct fates of MVBs through lysosomal degradation [30–34]. Hence, exosome formation and autophagy can be perceived as complementary routes to dispose of defective and unwanted cellular components. However, inhibition of autophagy under normal and pathological circumstances redirects MVBs from lysosomal degradation to exosome release [35].

In contrast to exosomes, it is accepted that MVs are ubiquitously generated by direct outward budding/blebbing from the plasma membrane followed by a fission event [36]. This biogenesis of MVs involves a series of tightly regulated processes, including asymmetric phosphatidylserine movement by aminophospholipid translocase [37,38], energy-dependent membrane curvature [39], cytoskeleton involvement, redistribution of molecular cargos [40] and ARF-6-mediated microvesicle shedding [41].

After release and transportation, TEVs interact with recipient cells through several different modes, including interaction of TEVs with a membrane receptor on target cells, direct membrane fusion, phagocytosis, or endocytosis.

Importantly, exosomes do not contain a random array of cargo proteins, but rather a specific array of proteins, which are frequently identified in exosomes and are compiled in exosome databases including *Exocarta* and *Vesiclepedia*, where a top 100 list of the most frequent proteins can be retrieved (http://exocarta.org/exosome_markers_new; http://microvesicles.org/extracellular_vesicle_markers). Currently, 9769 proteins, 3408 mRNAs, 2838 miRs, and 1116 lipids have been reported on *Exocarta*. These proteins originate from the plasma membrane (*e.g.* tetraspanins such as CD9, CD63, tetraspanin 6 and 8, amongst others, and cell adhesion molecules such as EpCAM, integrins, and ICAM), endocytic compartments (*e.g.* RNA-binding proteins), cytoskeleton-related (*e.g.* cofilin, profilin, actin, tubulin), and intracellular membrane transport (*e.g.* Rab GTPases), and signaling factors (*e.g.* ARF6, ROCK, K-Ras, EGFR, Src family kinases, amongst others), which are different from the content of ApoBs in biochemical composition [42].

These observations indicated that exosomes are actively secreted by live cells, supporting their proposed intra-endosomal origin, which may determine their target cell specificity. Furthermore, most exosomes contain ample bioactive cargos, such as diverse proteins (including oncoproteins, cytoskeletal proteins, transcriptional regulatory factors, heat-shock proteins, tumor suppressor proteins, numerous enzymes, splicing factors, etc.) [43–46], lipid raft-associated molecules (including cholesterol, sphingolipids, glycerophospholipids, prostaglandins, and ceramides) [46], messenger RNA (mRNA), microRNA (miRs), other non-coding RNAs, and mitochondrial DNA [47–49].

In the same way, the choice of proteins that will be incorporated into MVs is a highly selective process. A variety of proteins without signal peptides, nucleic acids, and lipids are selectively transported *via* MVs and transferred to recipient cells through different mechanisms.

Table 1
Significance of molecular machineries involved in biogenesis and secretion of TEVs.

Exosomes	Function	Ref.
ESCRT	Control MVBs formation and sorting of ubiquitinated membrane proteins to endosome	[18]
Small Rab GTPases	Rab11, Rab27, and Rab35 are involved in exosome release by contributing to MVB trafficking and docking with the plasma membrane	[19,21,82]
	Rab11 is involved in the fusion of MVBs with plasma membrane	[19]
VPS4A	Central regulator of early endosome trafficking	[23]
Sphingomyelinase	Regulates cargo packaging into exosomes	[25]
SNARE	Membrane fusion of MVBs with the plasma membrane	[27]
MVs	**Function**	**Ref.**
APLTs	Membrane curvature	[39]
	Asymmetric phosphatidylserine movement	[37]
	Cytoskeleton involvement	[40]
ARF-6	Redistribution of molecular cargos	[41]
	ARF6-GTP-dependent activation of phospholipase D promotes the recruitment of ERK to the plasma membrane	[39]
ESCRT	Catalyzes membrane budding and fission	[18]

ESCRT, endosomal sorting complex required for transport complex; VPS4A, vacuolar protein sorting 4 homolog A; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor system; APLTs, amino-phospholipid translocases.

Although the mode of incorporation of specific molecules into TEVs remains only partly understood, systems biology and metabolomic studies of exosomes or MVs are underway and will eventually broaden our understanding of the subject matter.

3. Isolation and purification of different types of TEVs

The isolation of TEVs from cell suspensions or body fluids was inevitably interfused with undesirable contaminants, such as protein aggregates and oligomers, and fragments of apoptotic/dead cells. Thus, no reference method has been established, despite various attempts from different research groups to standardize the procedure [50,51]. These efforts have included differential centrifugation, density-gradient ultracentrifugation, size-exclusion chromatography, gel filtration, flow-field fractionation, commercial kits using polymer-based precipitation, and immunoaffinity-based capture to achieve the purification of TEVs [52]. Up to date, the most commonly used approach is differential ultracentrifugation (up to 2×10^5 g, > 10 h) [53–55]. Another option relies on affinity purification that is based on the binding of specific antibodies to target proteins that are enriched on the surface of TEVs. These targets, also termed exosome-associated antigens, include cluster of differentiation (CD) molecules CD63, CD81, CD82, CD9, epithelial cell adhesion molecule (EpCAM), and Ras-related protein 5 (Rab5) [56]. Taking advantages of the enriched expression of such exosome-associated antigens, next generation techniques capable of isolating and characterizing TEVs have been developed. These techniques additionally implemented affinity-binding beads [57], microfluidic immune-capture [58], and alternating current electrohydrodynamic-induced nanoshearing for more efficient capture of TEVs [59]. Several other techniques such as nanoparticle tracking analysis, resistive pulse sensing, and small-angle X-ray scattering are currently under further development [60]. Although approaches using optical and non-optical methods such as electron microscopy can provide information on size, concentration, and morphology of TEVs, these approaches cannot quantify levels of TEVs markers. Size and density features are overlapping between exosomes and MVs, highlighting the difficulties in

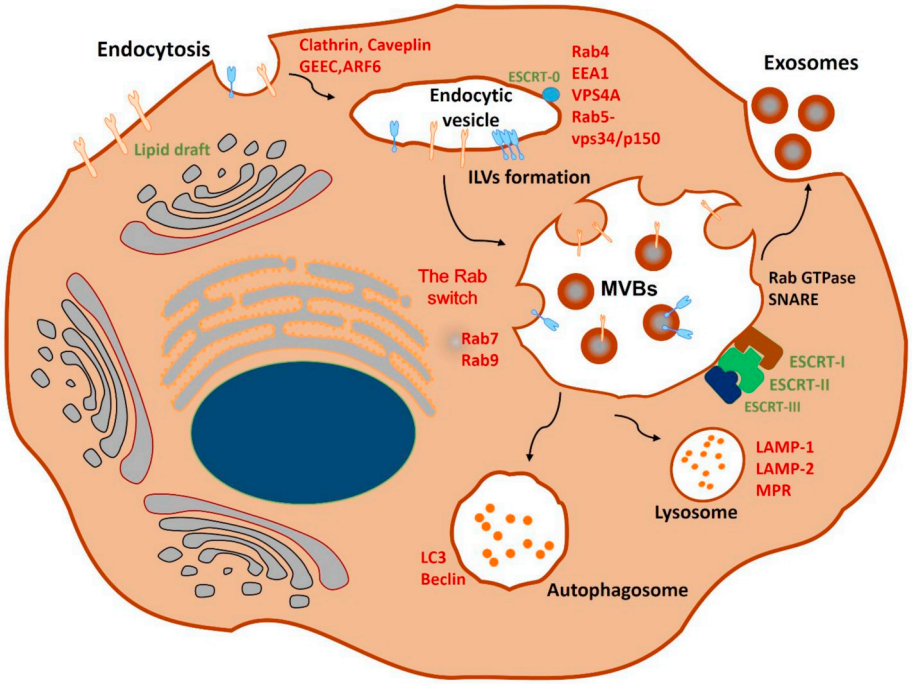

Fig. 1. Diagram of biogenesis and secretion of TEVs.

Table 2
Biological characteristics, isolation, and identification of different types of TEVs.

Characteristics	Exosomes	MVs	Apo Bodies
Parent cells	Live/stimulated cells	Live/stimulated cells	Dying cells
Biogenic origins	MVBs	Plasma membrane	Plasma membrane
Molecular machineries for biogenesis/release	Inward budding within endosomal pathway, release upon fusion of MVBs with the plasma membrane	Outward budding from the plasma membrane followed by a fission	Outward blebbing of cell membrane
Release	Delayed	Seconds	/
Density (g/ml)	1.13–1.19	1.032–1.068	1.16–1.28
Size (nm)	30–150	200-2000	400-2500
Morphology	Cup-shaped	Cup-shaped	Heterogeneous
Molecular cargos	Proteins, lipid raft-associated molecules, mRNA, miRs, mtDNAs, non-coding RNAs, metabolites	Nucleic acids, lipids, variety of proteins without signal peptides	Nuclear fractions, cellular organelles
Isolation	• Ultracentrifugation (100,000 × *g*) • Commercial kit precipitation • Polymer precipitation • Immunomagnetic beads • Microfluidic Chip	Ultracentrifugation (10,000 × *g*)	/
Associated markers	CD63, CD81, CD82, CD9, Alix, annexin	TyA, C1q	/
Interaction with target cells	Direct membrane fusion	Direct membrane fusion	/

MVs, shed micro-vesicles; MVBs, multivesicular bodies.

attempting to clearly distinguish the two classes of TEVs (Table 2). Considering that exosomes and MVs display more specified surface markers, the development of technologies to discriminate both types of TEVs should however become feasible. For example, it was reported that exosomes contained proteins associated with the extracellular matrix, cell surface receptors, heparin-binding receptors, and cell adhesion functions, whereas MVs were characterized by the presence of proteins associated with the endoplasmatic *reticulum*, mitochondria, and the proteasome [61].

4. Functional roles of TEVs in breast cancer

Although long regarded as cellular debris and a means of disposal of cellular components, recent studies demonstrated that TEVs play critical roles in tumor initiation, progression, and metastasis formation [62,63]. Significant differences have been observed between TEVs derived from cancer and normal cells, where TEVs from cancer cells possess the ability to induce proliferation of neighboring normal cells [64]. TEVs released by BC and from other cancer cells were reported to induce malignant transformation, rapid cell proliferation, or oncogene amplification in target cells [65,66]. In addition to cancer initiation, TEVs promote processes favorable to cancer progression, such as cancer cell migration and invasion, remodeling of the extracellular matrix, induction of angiogenesis, and priming of the pre-metastatic niche [67–69]. TEVs furthermore help tumor cells to evade clearance through the immune system and foster therapy resistance through the regulation of drug sensitivity [70]. Most intensely investigated are the roles of TEVs in tumor immunity, specifically in the modulation of immune cell differentiation, expansion of myeloid-derived suppressor cells (MDSCs), and tumor-induced immune suppression [71–73]. Initial studies have indicated that exosomes secreted by BC cells could block the differentiation of myeloid precursor cells into dendritic cells [74]. These findings imply that BC-derived TEVs could open an opportunity in cancer immunotherapy.

Exosomes and TEVs in general are additionally instrumental in regulating metastases formation in BC. For instance, exosomes derived from aggressive subclones of the triple-negative BC (TNBC) cell line Hs578T (*i.e.* $Hs578Ts(i)_8$) readily transferred the more aggressive phenotype to a panel of BC cell lines, as well as pro-angiogenic signals to human endothelial cells [75]. These results were further corroborated by the fact that exosomes enriched from the serum of TNBC patients were capable to transfer an invasive phenotype to target cells. When silencing the expression of the central regulator of TEVs secretion in BC, *i.e.* the small GTPase Rab27a, reduced tumor growth and metastases formation were observed [76]. Furthermore, TEVs derived from BC cells educate bone marrow-derived metastatic progenitor cells through the regulation of mesenchymal-to-epithelial transition (MET), which is believed to be required for the outgrowth of disseminated tumor cells at distant sites [77].

Besides their potential to directly trigger target cells through interactions with membrane-tethered receptor/ligand pairs, TEVs transfer mRNAs and miRs to target cells, and thereby regulate gene expression. TEV-encapsulated miRs can be internalized by target cells in the primary TME and in pre-/metastatic niches. TEVs carrying multiple angiogenesis-related proteins enhance vessel formation and directly promote endothelial cell growth. Secreted miRs are selectively packaged into TEVs and actively delivered into recipient cells to modulate downstream target genes expression and enhance targeted endothelial cell migration [78]. Lin et al. [79] reported that astrocyte-derived exosomes mediate an intercellular transfer of miR-19a to modulate PTEN down-regulation in favor of brain metastasis of BC cells. TEV-mediated transfer of oncogenic EGFR [80] or cancer-secreted miR-105 [81] efficiently destroys tight junctions and the integrity of endothelial cells barriers against metastasis, enhancing the expression of VEGF, and promoting tumor angiogenesis. Such transferred miRs can be functional both in *vitro* [82] and, importantly, in *vivo* [83].

Some studies even proposed that TEVs regulate the selective tropism of cancer cells for specific target organs during the formation of metastases. It was demonstrated that TEVs fuse preferentially with resident cells at their predicted destination to prepare the pre-metastatic niche. In fact, TEVs from tumor cells with a tropism for the lung were capable to re-direct the specific tropism of tumor cells with a bone tropism. This specificity is achieved through differing integrin expression profiles, where the exosomal integrins α6β4 and α6β1 were linked with pulmonary metastases and integrin αvβ5 with liver metastases [84]. Furthermore, the dynamic intercellular crosstalk that is mediated by TEVs mobilizes oncogenic factors, relocalizes cancer-associated fibroblasts (CAFs) to tumor sites, and *vice versa* shapes the TEM and sustains metastasis [85]. For example, Yan et al. reported that BC cells-secreted, TEVs-encapsulated miR-105 is activated through MYC-dependent signaling in cancer cells, and, in turns, activates the MYC signaling pathway in CAFs to induce a metabolic program [81]. Conversely, it was demonstrated that EVs secreted from CAFs alter the protrusive and invasive behavior, enhance glucose and glutamine metabolism, and increase glycolysis and glutamine-dependent reductive carboxylation when nutrients are sufficient, to fuel neighboring BC cells [86]. Oppositely, under low-nutrient conditions CAFs primed by BC-derived TEVs are able to detoxify the accumulating metabolic by-products, thereby promoting tumor growth. In addition, hypoxic conditions within the TME can induce tumor cells to secrete enhanced amounts of

TEVs [87]. Presumably, the release of TEVs by BC is established as an early event during tumorigenesis, and the resulting crosstalk of tumor cells and the TME potentially supports and sustains tumor progression.

In this respect, it is worth mentioning that current studies revealed that EVs not only act as vectors of molecular information, but also possess intrinsic biological activity. Vardaki et al., for example, demonstrated that caspase-3 can be activated within bone marrow stroma cell–derived exosomes, thereby inducing the cleavage of exosomal Bcl-xL and leading to increased cancer cell proliferation [88]. Melo et al. reported that precursor miRs present in BC-derived TEVs are processed to fully mature miRs *via* the presence of Dicer, AGO2, and TRBP proteins within TEVs, thus in a cell-independent manner [89]. A major advance in our understanding of exosomal miRs biology was the finding that heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) directs the loading of specific miRs into exosomes, through recognition of specific short GGAG motifs present in exosomal miRs [90]. Moreover, the authors could demonstrate that SUMOylating of hnRNPA2B1 in exosomes represents a second layer of regulation that controls the binding of hnRNPA2B1 to miRs. Hence, the biological activity of exosomes is the result of a regulated and specific incorporation of effector molecules, including proteins, mRNAs, and miRs.

5. Role of TEVs in maintaining stemness

Normal stem cells (SCs), tumor cells and, more particularly, cancer stem cells (CSCs) are characterized by a high degree of plasticity that is in great parts determined by micro-environmental stimuli and extracellular cues. Based on their biological properties, it has been speculated that EVs might act as paracrine factors that modulate phenotypic changes of recipient cells and generate a functional link between normal SCs and the resident niche under non-pathological conditions [91]. Ratajczak et al. reported that EVs derived from normal embryonic SCs (ESCs) contain Wnt-3 protein and are selectively enriched in mRNA for several pluripotency transcription factors, supporting self-renewal and expansion of hematopoietic progenitor cells [92]. The expression of a key subunit of the exosome complex, EXOSC9, was found to promote self-renewal and to prevent premature differentiation of epidermal progenitor cells [93]. Soon afterwards similar mechanisms were unraveled for CSCs and their microenvironment, when several reports described exosomes as CSC-specific molecule carriers, including in BC [94,95]. In this regard, Sansone et al. described the transfer of exosomal miR-221 by CAFs to luminal BC cells, which, in combination with hormone therapy, induced the formation of therapy resistant tumor cells characterized by high expression of the CSC marker CD133 [95].

Mesenchymal stromal cells (MSCs) are another group of SCs that have multi-lineage differentiation potential and are ubiquitously present in various tissues. MSCs have the ability of differentiating into osteoblasts, chondrocytes, myocytes, adipocytes, and tumor stromal cells, the latter cells being commonly considered as pro-tumorigenic. Several research groups investigated the mutual influences of MSCs, tumor cells, and their respectively secreted TEVs, in initiating tumor phenotypes and a wide range of biological processes [96]. Wang et al. demonstrated that exosomes from BC cells can convert adipose tissue-derived MSCs into myofibroblast-like cells [97]. Grange et al. found that TEVs released from renal cancer-derived MSC induced angiogenesis, matrix remodeling, and lung pre-metastatic niche preparation [98]. Eirin et al. characterized the RNA cargo of TEVs from adipose tissue-derived MSCs by high-throughput RNA sequencing (RNA-seq.) [99], demonstrating that TEVs are selectively enriched for distinct classes of mRNAs for transcription factors and genes involved in angiogenesis (*e.g.* HGF, HES1, TCF4) and adipogenesis (*e.g.* CEBPA, KLF7). Furthermore, TEVs from bone marrow-derived MSCs transport tumor regulatory miRs and metabolites, and activate signaling pathways by transmitting TEV-encapsulated growth factors such as VEGF and c-KIT, to enhance BC growth [100,101]. However, current studies also revealed that MSC-derived TEVs can exert dual regulatory functions on tumor cells, demonstrating that TEVs can have tumor-promoting and tumor-controlling effects. For example, TEVs derived from human bone marrow MSCs act as negative regulators of the cell cycle and inhibit tumor cell growth [102]. Exosomes from bone marrow-derived MSCs contain miRs such as miR-23b and promote BC cell dormancy in the metastatic niche [103]. Furthermore, BC angiogenesis can be suppressed by exosomes derived from MSC through miR-mediated down-regulation of VEGF expression [104]. However, the exact mechanisms behind these contradicting effects remain unclear. Eventually, the net effects of TEVs to either favor tumor development, or to inhibit progression of established tumors, will depend on the tumor entity and primordially on the state of the TME [105].

6. Significance of predicting and reversing drug resistance for breast cancer therapy

Cytotoxic chemotherapy is a standard therapy for invasive BC. However, a substantial proportion of BC patients is characterized by treatment failure due to drug resistance. High-throughput proteomics and transcriptomics analyses have revealed that TEVs do not contain a random set of intracellular molecules, but rather a specific array of very highly selective cargos from intracellular compartments [106]. Keklikoglou et al. demonstrated that chemotherapeutic agents elicit the secretion of TEVs with enhanced pro-metastatic capacity to facilitate the establishment of lung metastasis [107]. Wang et al. reported that transient receptor potential channel 5 (TRPC5) could be transferred to chemosensitive BC cells through the release of TEVs from chemoresistant cells, resulting in acquired chemoresistance in the recipient cell [108]. The study further showed that TEV-encapsulated TRPC5 ensured intercellular transfer of TRPC5 to recipient cells, and consequently stimulated multidrug efflux transporter P-glycoprotein expression, conferring chemoresistance to non-resistant cells [109]. Sun et al. reported that genotoxic stress could induce WNT16B expression, which is regulated by nuclear factor of κ light polypeptide gene enhancer in B cells 1 (NF-κB) after DNA damage, and subsequent paracrine signals to activate the canonical Wnt program in tumor cells [110]. Moreover, some studies have shown that TEVs contain miRs that could alter chemo-susceptibility, which is partly attributed to the successful intercellular transfer of multidrug resistance (MDR)-specific miRs such as β-elemene [111]. In this respect, Kong et al. reported that ceramide induces BC resistance protein (BCRP/ABCG2) secretion and reduces MDR, which may be useful for sensitizing BC cells to drug treatment [112].

7. TEVs as a source of breast cancer diagnostic and predictive biomarkers

Nearly 50% of BC patients develop distant metastasis even after receiving systemic therapy, with a 5-year survival rate of only ~20% [4]. Current findings concerning TEVs and their central regulatory mechanism in tumor development and metastasis formation has disclosed them as promising sources for the discovery of highly specific diagnostic biomarkers [113–116], even before physiological detection of a tumor or the onset of symptoms [117]. Utilization of TEVs as a diagnostic, prognostic, or predictive tool has additional advantages, such as a minimally invasive assessment and a reduced influence by intratumoral heterogeneity [118]. Recent attempts have been made to determine the potential of a series of exosomal proteins or miRs for the non-invasive detection of biomarkers for BC patients in different settings, ranging from early detection, recurrence prediction, metastasis hazard, and therapeutic outcomes [119]. Hildonen et al. developed an LC-MS/MS-based proteomic analysis method of urinary exosomal proteins and identified 49 proteins that were considered significantly enriched, 30 of which had been associated to various diseases, including pancreatic cancer [120]. Hence, this method might allow to further determine potential candidates for diagnostic, prognostic, or predictive

Table 3
Predictive value of TEVs in breast cancer patients.

Study	Primary Samples	Function	Biomarker	Study outcome	Ref.
Ciravolo et al., 2012	Sera of $HER2^+$ BC patients (n = 22)	Resistance to therapy	HER2	$HER2^+$ exosomes restrain the therapeutic efficacy of Trastuzumab *in vivo*	[150]
Ma et al., 2014	BC tissues (n = 26) and peripheral blood specimens (n = 33)		TrpC5	TrpC5-containing TEVs transfer chemo-resistance	[151]
Martinez et al., 2017	Serum of patients in trial NCT01485926		TGFß1	TEVs-associated TGFß1 as a predictive biomarker of treatment response	[152]
Wang et al., 2017	Plasma from n = 131 BC patients, tumor tissues from n = 54 patients		TRPC5	CcirExo-TRPC5 as a non-invasive chemo-resistance marker	[153]
O'Brien et al., 2015	GSE26659: n = 77 BC specimens and n = 17 NBTs		miR-134	Biomarker and potential therapeutic target	[154]
	GSE40525: n = 61 BC specimens and n = 56 NBTs				
Yang et al., 2017	Peripheral blood from n = 30BC patients, n = 42 BC tissues		GSTP1	Transfers drug resistance and predicts chemo-resistance	[155]
Miyoshi et al., 2010	n = 100 BC tissues and n = 24NBTs	Diagnosis and prognosis	UCH-L1 and–L3 mRNA	Exosomal UCH-L1/UCH-L3 mRNA are associated with pathogenesis and progression of BC	[156]
Wang et al., 2019	Tissues from n = 80 BC patients and n = 80 patients with benign diseases		CD82	Exosomal CD82 levels to evaluate metastatic potential and predict prognosis	[157]
Yoshikawa et al., 2018	Plasma from n = 185 BC patients		miR-223–3p	Exosomal miR-223–3p level is associated with BC malignancy	[158]
Ding et al., 2018	BC patients (n = 19 cases with lymphatic metastasis and n = 19 cases without)		miR-222	Exosomal miR-222 levels are correlated with BC metastasis	[159]
Li et al., 2018	Plasma samples from n = 200 BC patients and n = 200 HCs		miR-106a-363	MiR-106a-363 as biomarker for the diagnosis of BC	[160]
Gao et al., 2017	n = 259 patients, including BC patients and others		miR-155	miR-155 as a biomarker for detection of early stage BC	[161]
Ning et al., 2017	Blood samples from n = 93 BC patients		UCH-L1	$UCH\text{-}L1^+$ exosomes level was significantly negatively correlated with prognosis	[162]
Eichelser et al., 2014	Serum of n = 168 BC patients		miR-373	Exosomal miR-373 levels are associated with TNBC	[163]

HCs, healthy controls; BC, breast cancer; NBTs, normal breast tissues; TNBC, triple-negative breast cancer.

biomarkers in urine sample of cancer patients.

Deregulated miRs such as miR-105 [81] were revealed to be associated with progression and distant metastasis. In particular, several studies revealed that exosomal miRs are associated with a specific molecular subtype, for instance, miR-373 [121] and miR-939 [122] are linked to triple-negative and more aggressive BC.

TEVs cargos (proteins and miRs) have been highlighted as novel diagnostic and prognostic biomarkers of BC, although more convincing evidence in large-scale cohort designs is still required (Table 3). Despite promising results, identification of adequately sensitive platforms and biomarkers with high-specificity is still an urgent need. Analyzing candidate exosomal biomarkers in routine clinical settings is challenging given the unresolved technical difficulties, mainly due to the lacking standardization of fast assay, sources of variability, and methodological issues [123]. According to the unique characteristics of different types of body fluids (*e.g.* viscosity and composition), standardized sample preparation protocols (*e.g.* centrifugation or use of anticoagulant, sample collection and storage, freeze/thaw cycle) should be governed by strict standard operating protocols to guarantee yield and/or size distribution of TEVs [124]. Flow cytometry provides an option of high-throughput detection, yet tends to miss a portion of small vesicles (< 200 nm) because of weak light scattering [125]. Though particle tracking or dynamic light scattering-based approaches showed more accurate particle counts ($> 10^3$ - fold higher as compared with flow cytometry), these methods yield limited molecular information of the vesicles analyzed [125]. Exosomal genomic information such as miRs, mRNAs, and DNAs incorporated in TEVs can be measured by PCR or next generation sequencing, exosomal proteins can be detected by conventional molecular assays (*e.g.* enzyme-linked immunosorbent assay [126] and Western blot). However, TEVs have to be lysed before the detection of genetic and protein material, and have shortcomings, including poor sensitivity and requirement of large amounts of clinical samples.

8. Bioinspired exos-mimetic nanovesicles as a drug carrier for BC therapy

In order to decrease side effects and achieve more specific targeted transfer of therapeutics, nanocarriers have been designed for drug wrapping and were fabricated from various materials such as liposomes, nanoparticles, and polymeric particles [127]. The features of TEVs delivering ligands, receptors, and miRs in the local TME have significantly fueled the assumption that TEVs could be exploited for the delivery of proteins, miRs, or genetic material to target cells for treatment purposes [128]. In this respect, neurodegenerative and cardiovascular diseases have provided a proof-of-concept, where selective protein delivery or gene therapy approaches have already yielded encouraging preclinical results [129,130]. Exosomes were recently introduced as a novel bio-inspired system for drug delivery with many of the desirable advantages: multiple drug loading, passive targeting, enhanced permeability and retention effect, biocompatibility, low harmful immunogenicity, and enhancement of endocytosis [131].

Exploiting TEVs as an efficient source of carriers of therapeutics to address several potential issues increases the feasibility to introduce targeting moieties to specific tissues and the optimal choice of donor cell type. Donor cells should produce TEVs with low or no immunogenicity and prevent immune responses after administration, while also being stable to be protected from degradation during their therapeutic delivery. Numerous cell types have been chosen as exosome factories, such as HeLa and HEK-293 [132,133], immature dendritic cells [134], MSCs [135,136], and others. However, some researchers advocated for a cautious note on MSCs as donor cells, on account of the reported cancer-stimulating properties of MSC-derived TEVs [137]. Immature dendritic cells have favorable properties concerning immunogenicity. However, upscaling of the production of TEVs from these cells and enhancing yields of exosomes are major issues related to

Table 4
TEVs as drug delivery vehicles in breast cancer.

Exosome source	Setting	Therapeutic agent	Study outcome	Ref.
MSCs	*In vivo, in vitro*	LNA-modified anti-miR-142–3p oligonucleotides	Reduce miR-142–3p and miR-150 levels, increase transcription of APC and P2X7R target genes	[164]
MSCs	*In vitro*	Doxorubicin (DOX)	Preferentially uptaken by $HER2^+$ cells as an efficient drug delivery system	[165]
Kidney cells (HEK293)	*In vivo*	GE11 peptide + Let-7a	Suppressed tumor growth	[166]
Dendritic cells	*In vivo*	Lamp2b fused to αγ iRGD peptide + DOX	Significantly inhibited tumor growth with no overt toxicity	[167]
Dendritic cells	*In vivo, in vitro*	AS1411-modified EVs loaded with let-7 miRs	Inhibited tumor growth with no evident side effects	[168]
Red blood cells (RBC)	*In vivo*	Antisense oligonucleotides, Cas9 mRNA, and guide RNAs	Successfully used to target a specific onco-miR gene, with no observable cytotoxicity	[169]
Chinese hamster ovary (CHO) cells (EGF^+)	*In vitro*	Anti-eGFP	Represents the first example of EVs targeting using multiple types of protein ligands	[170]
Breast cancer (SKBR-3 and EFM-192A)	*In vivo*	Trastuzumab emtansine (T-DM1)	Tumor growth inhibition and activation of caspases 3 and/or 7	[171]
Breast Cancer (MCF10A)	*In vivo, in vitro*	CDK4-siRNA	Downregulation of CDK4 mRNA and protein expression	[172]
Dendritic cells	*In vivo*	HChrR6 mRNA	Targeting and arresting tumor growth	[173]
Mouse B cell myeloma cells (J558L)	*In vivo, in vitro*	miR-335	Downregulation of SOX4 mRNA	[174]
Mouse peripheral blood	*In vivo*	DOX or paclitaxel (PTX)	Enhanced therapeutic efficiency for lung metastases	[175]

TEVs, tumor-derived extracellular vesicles; MSCs, mesenchymal stem cells.

their clinical use [134].

Extensive research has been done using TEVs as delivery vehicles for small molecules, proteins, nucleic acids, small interference RNA (siRNA), or miRs (Table 4). Phase I studies of dendritic cell-derived TEVs as immunotherapies in patients suffering from solid cancers were performed more than ten years ago [138,139]. Another phase I clinical trial of autologous ascites-derived exosomes (Aex) combined with GM-CSF was launched for the treatment of colorectal cancer, showing a favorable cytotoxic T cell response directed against the tumor [140]. Rivoltini et al. reported that TRAIL-armed exosomes can induce apoptosis in cancer cells and control tumor progression in *vivo* [141]. Ohno et al. showed that BC-derived exosomes can efficiently deliver let-7a miR to $EGFR^-$positive cancer cells [132]. The blood-brain barrier (BBB) is a fortress blocking therapeutic agents to pass through and to be transported to the brain [142]. Several research projects try to solve these problems by encapsulating anticancer drugs such as paclitaxel and doxorubicin into exosomes, showing the potential of exosomes for brain delivery across the BBB [143]. Alvarez-Erviti et al. showed that rabies viral glycoprotein-targeted exosomes can deliver siRNA to the murine brain, resulting in a gene-specific knockdown [144]. All of these exploratory studies (on-going or completed) exhibit proper safety profiles of TEV-based targeted therapies in a clinical setting, supporting further development of TEV-based drug delivery systems.

TEVs-mediated delivery of tumor suppressor cargos has a broad scope in future clinical application. This treatment strategy offers a new remedial approach targeting multiple tumor-regulatory pathways, such as HER2 signaling pathways, the Wnt and Hedgehog pathways, PI3K/AKT signaling pathways, angiogenic pathways (the VEGF and kinase pathways). Furthermore, quantification and qualitative analyses of TEVs, as well, could represent novel strategies to monitor tumor growth. For example, Gernapudi et al. reported that targeting exosomes from pre-adipocytes inhibits the potent signaling axis miR-140/SOX2/SOX9, which regulates CSCs signaling and epithelial-to-mesenchymal transition, could be targeted to obstruct tumor progression in early stage BC [145].

Successful delivery of substantial amounts of therapeutic cargos by TEVs highly relies on an efficient loading methods [146,147]. Usually, the adopted approaches included electroporation, chemical-based transfection, transfection of exosome-producing cells, or incubation of exosomes with cargo.

9. Conclusion

The past few decades represented the beginning of a new chapter in intracellular signal transmission amongst cancer cells, which now include membrane dynamics and the release of specific TEVs as means of communication. More and more evidences showed that TEVs are closely related to the metastatic cascade processes of BC including proliferation, angiogenesis, invasion, and pro-metastatic niche preparation. Unlike soluble factors, the encapsulating membrane of TEVs protects the concentrated bioactive molecules from degradation not only locally, but also during long-distance transportation. The protein composition of TEVs has been analyzed extensively, predominantly by mass spectrometry to reveal a defined subset of cellular proteins common to TEVs originating from a variety sources [148,149].

The emerging evidence that TEVs possess special characteristics qualified them as biomarkers for BC diagnosis, with a specificity and sensitivity mostly superior to traditional serum markers. Meanwhile, establishment of TEV-based drug delivery system superior to synthetic drug carriers is part of ongoing research work. However, there is a long way to go from the theory to practical applications, with some existing obstacles that need to be overcome to reach maximum potential in the clinic. This restriction and open questions include the definition of optimal donor cells, bioactive cargo loading type, administration routes, the quantity and quality control of loading, and surface modifications of targeting peptides of TEVs. In addition, the use of TEVs as

cancer specific biomarkers is technically limited by their heterogeneity and the need for extensive purification and labelling.

Although compelling studies have identified that TEVs contribute to the plasticity of cancer cells and multiple stages of cancer progression, experimental evidence and mechanical elaboration to define TEVs as an important communicator is only just coming to light.

Conflicts of interest

The authors report no conflicts of interest.

Funding

This work was supported by the National Natural Science Foundation of China (Project number: 81772802), Shanghai Science and Technology Innovation Action Plan (Project number: 17JC1404400), Shanghai Science and Technology Commission (Project number: 16ZR1427400,16DZ1910200), the Special Funds for Technological Innovation of Shanghai Jiao Tong University (Project number: YG2016MS22), all to HXW. The work was further supported by funds from the Deutsche Forschungsgemeinschaft to OG (Project number: Gi 540/3-2).

References

[1] C. Desantis, J. Ma, L. Bryan, A. Jemal, Breast cancer statistics, 2013, CA: Canc. J. Clin. 64 (2014) 52–62.
[2] P. Autier, M. Boniol, C. Lavecchia, L. Vatten, A. Gavin, C. Héry, M. Heanue, Disparities in breast cancer mortality trends between 30 European countries: retrospective trend analysis of WHO mortality database, BMJ 341 (2010) 1201-1201.
[3] C.E. Desantis, F. Bray, J. Ferlay, J. Lortettieulent, B.O. Anderson, A. Jemal, International variation in female breast cancer incidence and mortality rates, Canc. Epidemiol., Biomark. Prevent.: Publ. Am. Assoc. Canc. Res., Cospons. Am. Soc. Prevent. Oncol. 24 (2015) 1495–1506.
[4] A. Terjung, S. Kummer, M. Friedrich, Simultaneous 24 h-infusion of high-dose 5-fluorouracil and sodium-folinate as alternative to capecitabine in advanced breast cancer, Anticancer Res. 34 (2014) 7233.
[5] R. Kalluri, The biology and function of exosomes in cancer, J. Clin. Investig. 126 (2016) 1208.
[6] C. Théry, L. Zitvogel, S. Amigorena, Exosomes: composition, biogenesis and function, Nat. Rev. Immunol. 2 (2002) 569–579.
[7] J. De Toro, L. Herschlik, C. Waldner, C. Mongini, Emerging roles of exosomes in normal and pathological conditions: new insights for diagnosis and therapeutic applications, Front. Immunol. 6 (2015) 203.
[8] G. Van Niel, G. D'Angelo, G. Raposo, Shedding light on the cell biology of extracellular vesicles, Nat. Rev. Mol. Cell Biol. 19 (2018).
[9] A. Hesari, M. Sag, A. Siasi, M. Rahmani, N. Behboodi, A. Rastgar-Moghadam, G.A. Ferns, F. Ghasemi, A. Avan, Tumor-derived exosomes: potential biomarker or therapeutic target in breast cancer? J. Cell. Biochem. 119 (6) (2018 Jun) 4236–4240.
[10] K. Nobuyoshi, Y. Yusuke, T. Naoomi, H. Keitaro, K. Takeshi, O. Takahiro, Dark side of the exosome: the role of the exosome in cancer metastasis and targeting the exosome as a strategy for cancer therapy, Future Oncol. 10 (2014) 671–681.
[11] A. O'Loghlen, Role for extracellular vesicles in the tumour microenvironment, Phil. Trans. Biol. Sci. 373 (2018) 20160488.
[12] R.M. Johnstone, M. Adam, J.R. Hammond, L. Orr, C. Turbide, Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes), J. Biol. Chem. 262 (1987) 9412–9420.
[13] P. Wolf, The nature and significance of platelet products in human plasma, Br. J. Haematol. 13 (1967) 269–288.
[14] J.M. Stein, J.P. Luzio, Ectocytosis caused by sublytic autologous complement attack on human neutrophils. The sorting of endogenous plasma-membrane proteins and lipids into shed vesicles, Biochem. J. 274 (Pt 2) (1991) 381.
[15] C. Tricarico, J. Clancy, C. D'Souzaschorey, Biology and biogenesis of shed microvesicles, Small GTPases 8 (2017) 220–232.
[16] R. Francesca, M. Lavinia, C. Clizia, M. Fulvio, P. Marina, Advances in membranous vesicle and exosome proteomics improving biological understanding and biomarker discovery, Proteomics 11 (2011) 709–720.
[17] T. Clotilde, O. Matias, S. Elodie, Membrane vesicles as conveyors of immune responses, Nat. Rev. Immunol. 9 (2009) 581.
[18] W. Thomas, J.H. Hurley, Molecular mechanism of multivesicular body biogenesis by ESCRT complexes, Nature 464 (2010) 864–869.
[19] A. Savina, C.M. Fader, M.T. Damiani, M.I. Colombo, Rab11 promotes docking and fusion of multivesicular bodies in a calcium-dependent manner, Traffic 6 (2010) 131–143.
[20] O. Matias, N.B. Carmo, K. Sophie, F. Isabelle, R. GraA, S. Ariel, C.F. Moita, S. Kristine, A.N. Hume, P. Rui, Freitas, Rab27a and Rab27b control different steps of the exosome secretion pathway, Nat. Cell Biol. 12 (2010) 11–13 19-30;sup.
[21] C. Hsu, Y.S. Morohashi, H.N. Manrique, S. Jung, M.A. Lauterbach, M. Bakhti, M. Gronborg, W. Mobius, J. Rhee, F.A. Barr, Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C, JCB (J. Cell Biol.) 189 (2010) 223–232.
[22] B. Short, Rab35 drives exosome secretion, JCB (J. Cell Biol.) 189 (2010) 190-190.
[23] I. Akrap, A. Thavamani, A. Nordheim, Vps4A-mediated Tumor Suppression upon Exosome Modulation? (2016).
[24] M. Babst, T.K. Sato, L.M. Banta, S.D. Emr, Endosomal transport function in yeast requires a novel AAA-type ATPase, Vps4p, EMBO J. 16 (2014) 1820–1831.
[25] B.B. Guo, S.A. Bellingham, A.F. Hill, The neutral sphingomyelinase pathway regulates packaging of the prion protein into exosomes, J. Biol. Chem. 290 (2015) 3455–3467.
[26] M.B. Dinkins, J. Enasko, C. Hernandez, G. Wang, J. Kong, I. Helwa, Y. Liu, T.A. Jr, E. Bieberich, Neutral sphingomyelinase-2 deficiency ameliorates alzheimer's disease pathology and improves cognition in the 5XFAD mouse, J. Neurosci. Off. J. Soc. Neurosci. 36 (2016) 8653.
[27] R.W. Baker, F.M. Hughson, Chaperoning SNARE assembly and disassembly, Nat. Rev. Mol. Cell Biol. 17 (2016) 465.
[28] K. Denzer, M.J. Kleijmeer, H.F. Heijnen, W. Stoorvogel, H.J. Geuze, Exosome: from internal vesicle of the multivesicular body to intercellular signaling device, J. Cell Sci. 113 Pt 19 (2000) 3365–3374.
[29] F. Baixauli, C. López-Otín, M. Mittelbrunn, Exosomes and Autophagy: Coordinated Mechanisms for the Maintenance of Cellular Fitness vol. 5, (2014).
[30] M. Hamasaki, S.T. Shibutani, T. Yoshimori, Up-to-date membrane biogenesis in the autophagosome formation, Curr. Opin. Cell Biol. 25 (2013) 455–460.
[31] R.C. Russell, T. Ye, Y. Haixin, P. Hyun Woo, C. Yu-Yun, K. Joungmok, K. Haerin, T.P. Neufeld, D. Andrew, G. Kun-Liang, ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase, Nat. Cell Biol. 15 (2013) 741–750.
[32] C. Fader, M. Colombo, Autophagy and multivesicular bodies: two closely related partners, Cell Death Differ. 16 (2009) 70–78.
[33] E.J. Lee, C. Tournier, The requirement of uncoordinated 51-like kinase 1 (ULK1) and ULK2 in the regulation of autophagy, Autophagy 7 (2011) 689–695.
[34] T. Xu, D. Singh, J. Liu, H. Li, S. Peng, L.J. Rizzolo, S.B. Wang, Neferine, is not inducer but blocker for macroautophagic flux targeting on lysosome malfunction, Biochem. Biophys. Res. Commun. 495 (2017) S0006291X17323471.
[35] J. Xu, R. Camfield, S.M. Gorski, The interplay between exosomes and autophagy - partners in crime, J. Cell Sci. 131 (2018).
[36] B.T. Kreger, A.L. Dougherty, S.G. Kai, R.A. Cerione, M.A. Antonyak, Microvesicle cargo and function changes upon induction of cellular transformation, J. Biol. Chem. 291 (2016) jbc.M116.725705.
[37] D.L. Daleke, Regulation of transbilayer plasma membrane phospholipid asymmetry, JLR (J. Lipid Res.) 44 (2003) 233.
[38] W.B. Huttner, J. Zimmerberg, Implications of lipid microdomains for membrane curvature, budding and fission: Commentary, Curr. Opin. Cell Biol. 13 (2001) 478–484.
[39] R. Beck, Z. Sun, F. Adolf, C. Rutz, J. Bassler, K. Wild, I. Sinning, E. Hurt, B. Brügger, J. Béthune, Membrane curvature induced by Arf1-GTP is essential for vesicle formation, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 11731–11736.
[40] A. Holzinger, S. Monajembashi, K.O. Greulich, U. Lã¼Tz-Meindl, Impairment of cytoskeleton-dependent vesicle and organelle translocation in green algae: combined use of a microfocused infrared laser as microbeam and optical tweezers, J. Microsc. 208 (2010) 77–83.
[41] V. Muralidharan-Chari, J. Clancy, C. Plou, M. Romao, P. Chavrier, G. Raposo, C. D'Souza-Schorey, ARF6-Regulated shedding of tumor cell-derived plasma membrane microvesicles, Curr. Biol. Cb 19 (2009) 1875–1885.
[42] G. van Niel, G. D'Angelo, G. Raposo, Shedding light on the cell biology of extracellular vesicles, Nat. Rev. Mol. Cell Biol. 19 (2018) 213–228.
[43] S. Keerthikumar, L. Gangoda, M. Liem, P. Fonseka, I. Atukorala, C. Ozcitti, A. Mechler, C.G. Adda, C.S. Ang, S. Mathivanan, Proteogenomic analysis reveals exosomes are more oncogenic than ectosomes, Oncotarget 6 (2015) 15375–15396.
[44] Z. Chen, L. Yang, Y. Cui, Y. Zhou, X. Yin, J. Guo, G. Zhang, T. Wang, Q.Y. He, Cytoskeleton-centric protein transportation by exosomes transforms tumor-favorable macrophages, Oncotarget 7 (2016) 67387–67402.
[45] G. Nina, H. Urban, M. Linus, L. Göran, R. Gunnar, W. Anders, M. Stellan, Growth factor stimulation of cardiomyocytes induces changes in the transcriptional contents of secreted exosomes, J. Extracell. Vesicles 2 (2013) 1–8.
[46] N.A. Garcia, J. Moncayoarlandi, P. Sepulveda, A. Diezjuan, Cardiomyocyte exosomes regulate glycolytic flux in endothelium by direct transfer of GLUT transporters and glycolytic enzymes, Cardiovasc. Res. 109 (2015) 397.
[47] S. Johan, W. Tom, V.R. Sjoerd, D.H. Meijer, G. Laura, S.E. Miguel, W.T. Curry, B.S. Carter, A.M. Krichevsky, X.O. Breakefield, Glioblastoma Microvesicles Transport RNA and Proteins that Promote Tumour Growth and Provide Diagnostic Biomarkers, (2008).
[48] H. Valadi, K. Ekstrom, A. Bossios, M. Sjostrand, J.J. Lee, J.O. Lotvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (6) (2007 Jun) 654–659.
[49] Z. Wang, J.Q. Chen, J.L. Liu, L. Tian, Exosomes in tumor microenvironment: novel transporters and biomarkers, J. Transl. Med. 14 (2016) 297.
[50] D.S. Choi, D.K. Kim, Y.K. Kim, Y.S. Gho, Proteomics, transcriptomics and lipidomics of exosomes and ectosomes, Proteomics 13 (2013) 1554–1571.
[51] D.S. Choi, D.K. Kim, Y.K. Kim, Y.S. Gho, Proteomics of extracellular vesicles: exosomes and ectosomes, Mass Spectrom. Rev. 34 (2015) 474–490.
[52] A.S. Haqqani, Method for isolation and molecular characterization of extracellular microvesicles released from brain endothelial cells, Fluids Barriers CNS 10 (2013)

4-4.
[53] K. Agarwal, M. Saji, S.M. Lazaroff, A.F. Palmer, M.D. Ringel, M.E. Paulaitis, Analysis of exosome release as a cellular response to MAPK pathway inhibition, Langmuir Acs J. Surf. Colloids 31 (2015) 5440–5448.
[54] L. Grasso, R. Wyss, L. Weidenauer, A. Thampi, D. Demurtas, M. Prudent, N. Lion, H. Vogel, Molecular screening of cancer-derived exosomes by surface plasmon resonance spectroscopy, Anal. Bioanal. Chem. 407 (2015) 5425–5432.
[55] L. Tiancheng, D.E. Mendes, C.E. Berkman, Functional prostate-specific membrane antigen is enriched in exosomes from prostate cancer cells, Int. J. Oncol. 44 (2014) 918–922.
[56] Y.T. Kang, Y.J. Kim, J. Bu, Y.H. Cho, S.W. Han, B.I. Moon, High-purity capture and release of circulating exosomes using an exosome-specific dual-patterned immunofiltration (ExoDIF) device, Nanoscale 9 (2017) 13495.
[57] M. Kosuke, T. Riyako, K. Koji, K. Taku, H. Kengo, T. Tomohiro, S. Kensaku, E. Hidetoshi, F. Yasunori, K. Kyojiro, Isolation of prostate cancer-related exosomes, Anticancer Res. 34 (2014) 3419.
[58] C. Chen, J. Skog, C.H. Hsu, R.T. Lessard, L. Balaj, T. Wurdinger, B.S. Carter, X.O. Breakefield, M. Toner, D. Irimia, Microfluidic isolation and transcriptome analysis of serum microvesicles, Lab Chip 10 (2010) 505–511.
[59] R. Vaidyanathan, L.M.V. Leeuwen, S. Rauf, M.J.A. Shiddiky, M. Trau, A multiplexed device based on tunable nanoshearing for specific detection of multiple protein biomarkers in serum, Sci. Rep. 5 (2015) 9756.
[60] E. Pol, Van Der, F. Coumans, Z. Varga, M. Krumrey, R. Nieuwland, Innovation in detection of microparticles and exosomes, J. Thromb. Haemost. Jth 11 (2013) 36–45.
[61] R.A. Haraszti, M.C. Didiot, E. Sapp, J. Leszyk, S.A. Shaffer, H.E. Rockwell, F. Gao, N.R. Narain, M. Difiglia, M.A. Kiebish, High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources, J. Extracell. Vesicles 5 (2016) 32570.
[62] A. Abak, A. Abhari, S. Rahimzadeh, Exosomes in cancer: small vesicular transporters for cancer progression and metastasis, biomarkers in cancer therapeutics, Peerj 6 (2018) e4763.
[63] X. Zhang, X. Yuan, H. Shi, L. Wu, H. Qian, W. Xu, Exosomes in cancer: small particle, big player, J. Hematol. Oncol. 8 (2015) 83.
[64] A.N. Khalid, M. Brian, M. Johann, L. Vladimir, M. Linda, G. Abhijit, R. Janusz, Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells, Nat. Cell Biol. 10 (2008) 619–624.
[65] M. Angela, A.T. Larregina, W.J. Shufesky, S. Donna Beer, M.L.G. Sullivan, J.M. Karlsson, C.J. Baty, G.A. Gibson, E. Geza, W. Zhiliang, Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes, Blood 119 (2012) 756–766.
[66] L. Jing, Z. Yujing, L. Yuchen, D. Xin, L. Wenyang, C. Xing, Y. Yuan, W. Qiang, X. Yunxing, W. Cheng, Microvesicle-mediated transfer of microRNA-150 from monocytes to endothelial cells promotes angiogenesis, J. Biol. Chem. 288 (2013) 23586–23596.
[67] P. Héctor, A.K. Ma?A, L. Simon, M. Irina, C.S. Bruno, M.B. Gema, H.R. Marta, W. Caitlin, G.S. Guillermo, G. Cyrus, Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET, Nat. Med. 18 (2012) 883.
[68] J.L. Hood, S. Roman Susana, S.A. Wickline, Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis, Cancer Res. 71 (2011) 3792.
[69] X. Xiang, Y. Liu, X. Zhuang, S. Zhang, S. Michalek, D.D. Taylor, W. Grizzle, H.G. Zhang, TLR2-mediated expansion of MDSCs is dependent on the source of tumor exosomes, Am. J. Pathol. 177 (2010) 1606–1610.
[70] M.C. Lowry, W.M. Gallagher, O.D. Lorraine, The role of exosomes in breast cancer, Clin. Chem. 61 (2015) 1457–1465.
[71] H.G. Zhang, W.E. Grizzle, Exosomes and cancer: a newly described pathway of immune suppression, Clin. Cancer Res. 17 (2011) 959–964.
[72] J. Wang, K.D. Veirman, N.D. Beule, K. Maes, E.D. Bruyne, E.V. Valckenborgh, K. Vanderkerken, E. Menu, The bone marrow microenvironment enhances multiple myeloma progression by exosome-mediated activation of myeloid-derived suppressor cells, Oncotarget 6 (2015) 43992–44004.
[73] D.D. Taylor, C. Gercel-Taylor, Exosomes/microvesicles: mediators of cancer-associated immunosuppressive microenvironments, Semin. Immunopathol. 33 (2011) 441–454.
[74] S. Yu, C. Liu, K. Su, J. Wang, Y. Liu, L. Zhang, C. Li, Y. Cong, R. Kimberly, W.E. Grizzle, C. Falkson, H.G. Zhang, Tumor exosomes inhibit differentiation of bone marrow dendritic cells, J. Immunol. 178 (2007) 6867–6875.
[75] K. O'Brien, S. Rani, C. Corcoran, R. Wallace, L. Hughes, A.M. Friel, S. Mcdonnell, J. Crown, M.W. Radomski, L. O'Driscoll, Exosomes from triple-negative breast cancer cells can transfer phenotypic traits representing their cells of origin to secondary cells, Eur. J. Cancer 49 (2013) 1845–1859.
[76] A. Bobrie, S. Krumeich, F. Reyal, C. Recchi, L.F. Moita, M.C. Seabra, M. Ostrowski, C. Théry, Rab27a supports exosome-dependent and -independent mechanisms that modify the tumor microenvironment and can promote tumor progression, Cancer Res. 72 (2012) 4920.
[77] H. Peinado, M. Alečković, S. Lavotshkin, I. Matei, B. Costasilva, G. Morenobueno, M. Herguetaredondo, C. Williams, G. Garcíasantos, C.M. Ghajar, Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET, Nat. Med. 18 (2012) 883.
[78] G. Zhuang, X. Wu, Z. Jiang, l Kasman, J. Yao, Y. Guan, J. Oeh, Z. Modrusan, C. Bais, D. Sampath, N. Ferrara, Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway, EMBO J. 31 (2012) 3513–3523.
[79] Z. Lin, Z. Siyuan, Y. Jun, F.J. Lowery, Z. Qingling, H. Wen-Chien, L. Ping, L. Min, W. Xiao, Z. Chenyu, Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth, Nature 527 (2015) 100–104.
[80] A.N. Khalid, M. Brian, R.S. Kerbel, A.C. Allison, R. Janusz, Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 3794–3799.
[81] W. Yan, X. Wu, W. Zhou, M.Y. Fong, M. Cao, J. Liu, X. Liu, C.H. Chen, O. Fadare, D.P. Pizzo, Cancer-cell-secreted exosomal miR-105 promotes tumour growth through the MYC-dependent metabolic reprogramming of stromal cells, Nat. Cell Biol. 20 (2018).
[82] W. Ting, D.M. Gilkes, T. Naoharu, X. Lisha, L. Weibo, C.J. Bishop, C. Pallavi, J.J. Green, G.L. Semenza, Hypoxia-inducible factors and RAB22A mediate formation of microvesicles that stimulate breast cancer invasion and metastasis, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) E3234.
[83] Z. Naseri, R.K. Oskuee, M.R. Jaafari, M. Forouzandeh Moghadam, Exosome-mediated delivery of functionally active miRNA-142-3p inhibitor reduces tumorigenicity of breast cancer in vitro and in vivo, Int. J. Nanomed. 13 (2018) 7727–7747.
[84] H. Ayuko, C.S. Bruno, S. Tang-Long, R. Goncalo, H. Ayako, T.M. Milica, M. Henrik, K. Shinji, D.G. Angela, C. Sophia, Tumour exosome integrins determine organotropic metastasis, Nature 527 (2015) 329–335.
[85] Q. Michael, T.S. Ping, T. Hiroyuki, G. Tamas, S.S.W. Wang, T. Shigeo, B. Gwang Ho, S. Wataru, D. Bethany, K.S. Betz, Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth, Cancer Cell 19 (2011) 257–272.
[86] Y. Chen, C. Zeng, Y. Zhan, H. Wang, X. Jiang, W. Li, Aberrant low expression of p85α in stromal fibroblasts promotes breast cancer cell metastasis through exosome-mediated paracrine Wnt10b, Oncogene 36 (2017) 4692–4705.
[87] H.W. King, M.Z. Michael, J.M. Gleadle, Hypoxic enhancement of exosome release by breast cancer cells, BMC Canc. 12 (2012) 421.
[88] I. Vardaki, C. Sanchez, P. Fonseca, M. Olsson, D. Chioureas, G. Rassidakis, A. Ullén, B. Zhivotovsky, M. Björkholm, T. Panaretakis, Caspase-3-dependent cleavage of Bcl-xL in the stroma exosomes is required for their uptake by hematological malignant cells, Blood 128 (2016) 2655–2665.
[89] S. Melo, H. Sugimoto, J. O'Connell, N. Kato, A. Villanueva, A. Vidal, L. Qiu, E. Vitkin, L. Perelman, C. Melo, Cancer exosomes perform cell-independent MicroRNA biogenesis and promote tumorigenesis, Cancer Cell 26 (2014) 707–721.
[90] C. Villarroyabeltri, C. Gutiérrezvázquez, F. Sánchezcabo, D. Pérezhernández, J. Vázquez, N. Martincofreces, D.J. Martinezherrera, A. Pascualmontano, M. Mittelbrunn, F. Sánchezmadrid, Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs, Nat. Commun. 4 (2013) 2980.
[91] P.J. Quesenberry, M.S. Dooner, J.M. Aliotta, Stem cell plasticity revisited: the continuum marrow model and phenotypic changes mediated by microvesicles, Exp. Hematol. 38 (2010) 581–592.
[92] J. Ratajczak, K. Miekus, M. Kucia, J. Zhang, R. Reca, P. Dvorak, M.Z. Ratajczak, Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery, Leukemia 20 (2006) 847–856.
[93] D. Mistry, Y. Chen, G. Sen, Progenitor function in self-renewing human epidermis is maintained by the exosome, Cell Stem Cell 11 (2012) 127–135.
[94] C. Arthit, D.L. Smith, P. Pierre, H. Michael, M.J. Caplan, Exosome release of β-catenin: a novel mechanism that antagonizes Wnt signaling, JCB (J. Cell Biol.) 190 (2010) 1079–1091.
[95] P. Sansone, M. Berishaj, V.K. Rajasekhar, C. Ceccarelli, Q. Chang, A. Strillacci, C. Savini, L. Shapiro, R. Bowman, C. Mastroleo, Evolution of cancer stem-like cells in endocrine-resistant metastatic breast cancers is mediated by stromal microvesicles, Cancer Res. 77 (2017) 1927–1941.
[96] S. Bruno, F. Collino, A. Iavello, G. Camussi, Effects of mesenchymal stromal cell-derived extracellular vesicles on tumor growth, Front. Immunol. 5 (2014) 382.
[97] J.A. Cho, H. Park, E.H. Lim, K.W. Lee, Exosomes from breast cancer cells can convert adipose tissue-derived mesenchymal stem cells into myofibroblast-like cells, Int. J. Oncol. 40 (2012) 130.
[98] R.S. Lindoso, F. Collino, G. Camussi, Extracellular vesicles derived from renal cancer stem cells induce a pro-tumorigenic phenotype in mesenchymal stromal cells, Oncotarget 6 (2015) 7959–7969.
[99] A. Eirin, S.M. Riester, X.Y. Zhu, H. Tang, J.M. Evans, D. O'Brien, A.J.V. Wijnen, L.O. Lerman, MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells, Gene 551 (2014) 55–64.
[100] W. Zhu, L. Huang, Y. Li, X. Zhang, J. Gu, Y. Yan, X. Xu, M. Wang, H. Qian, W. Xu, Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth in vivo, Cancer Lett. 315 (2012) 28–37.
[101] K.C. Vallabhaneni, P. Patrice, D. Santosh, G. Francois, K.V. Adams, M.Y. Yuan, X. Rui, L. Yiming, W. Kounosuke, M.C. Vemuri, Extracellular vesicles from bone marrow mesenchymal stem/stromal cells transport tumor regulatory microRNA, proteins, and metabolites, Oncotarget 6 (2015) 4953–4967.
[102] S. Bruno, F. Collino, M.C. Deregibus, C. Grange, C. Tetta, G. Camussi, Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth, Stem Cell. Dev. 22 (2013) 758–771.
[103] M. Ono, N. Kosaka, N. Tominaga, Y. Yoshioka, F. Takeshita, R.U. Takahashi, M. Yoshida, H. Tsuda, K. Tamura, T. Ochiya, Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells, Sci. Signal. 7 (2014) ra63.
[104] J.K. Lee, S.R. Park, B.K. Jung, Y.K. Jeon, Y.S. Lee, M.K. Kim, Y.G. Kim, J.Y. Jang, C.W. Kim, Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells, PLoS One 8 (2013) e84256.
[105] A.N. Shirazi, S. Mozaffari, R.T. Sherpa, R. Tiwari, K. Parang, Efficient intracellular

delivery of cell-impermeable cargo molecules by peptides containing tryptophan and histidine, Molecules 23 (2018) 1536.
[106] M. Pietrowska, S. Funk, M. Gawin, Ł. Marczak, A. Abramowicz, P. Widłak, T. Whiteside, Isolation of exosomes for the purpose of protein cargo analysis with the use of mass spectrometry, Methods Mol. Biol. 1654 (2017) 291.
[107] I. Keklikoglou, C. Cianciaruso, E. Guc, M.L. Squadrito, L.M. Spring, S. Tazzyman, L. Lambein, A. Poissonnier, G.B. Ferraro, C. Baer, A. Cassara, A. Guichard, M.L. Iruela-Arispe, C.E. Lewis, L.M. Coussens, A. Bardia, R.K. Jain, J.W. Pollard, M. De Palma, Chemotherapy elicits pro-metastatic extracellular vesicles in breast cancer models, Nat. Cell Biol. 21 (2019) 190–202.
[108] T. Wang, K. Ning, T.X. Lu, X. Sun, L. Jin, X. Qi, J. Jin, D. Hua, Increasing circulating exosomes-carrying TRPC5 predicts chemoresistance in metastatic breast cancer patients, Cancer Sci. 108 (2017) 448.
[109] M. Xin, C. Zhen, H. Dong, H. Dongxu, W. Linjun, Z. Peng, W. Junqi, C. Yanfei, G. Caiji, Z. Xiaodong, Essential role for TrpC5-containing extracellular vesicles in breast cancer with chemotherapeutic resistance, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 6389–6394.
[110] S. Yu, C. Judith, H. Celestia, T.M. Beer, P. Peggy, C. Ilsa, T. Lawrence, P.S. Nelson, Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B, Nat. Med. 18 (2012) 1359–1368.
[111] J. Zhang, H.D. Zhang, Y.F. Yao, S.L. Zhong, J.H. Zhao, J.H. Tang, Beta-elemene reverses chemoresistance of breast cancer cells by reducing resistance transmission via exosomes, Cell. Physiol. Biochem.: Int. J. Exp. Cell. Phys., Biochem. Pharm. 36 (2015) 2274–2286.
[112] J.N. Kong, Q. He, G. Wang, S. Dasgupta, M.B. Dinkins, G. Zhu, A. Kim, S. Spassieva, E. Bieberich, Guggulsterone and bexarotene induce secretion of exosome associated breast cancer resistance protein and reduce doxorubicin resistance in MDA-MB-231 cells, Int. J. Cancer 137 (2015) 1610–1620.
[113] S.A. Melo, L.B. Luecke, K. Christoph, A.F. Fernandez, S.T. Gammon, K. Judith, V.S. Lebleu, E.A. Mittendorf, W. Juergen, R. Nuh, Glypican-1 identifies cancer exosomes and detects early pancreatic cancer, Nature 523 (2015) 177–182.
[114] S. Boukouris, S. Mathivanan, Exosomes in bodily fluids are a highly stable resource of disease biomarkers, Proteonomics Clin. Appl. 9 (2015) 358–367.
[115] J. Li, C.A. Sherman-Baust, M. Tsai-Turton, R.E. Bristow, R.B. Roden, P.J. Morin, Claudin-containing exosomes in the peripheral circulation of women with ovarian cancer, BMC Canc. 9 (2009) 244.
[116] P.A. Gonzales, T. Pisitkun, J.D. Hoffert, D. Tchapyjnikov, R.A. Star, R. Kleta, N.S. Wang, M.A. Knepper, Large-scale proteomics and phosphoproteomics of urinary exosomes, J. Am. Soc. Nephrol. 20 (2009) 363–379.
[117] M. Saraswat, S. Joenväära, L. Musante, H. Peltoniemi, H. Holthofer, R. Renkonen, N-linked (N-) glycoproteomics of urinary exosomes, Mol. Cell. Proteom (2015) 2298–2298.
[118] H. Shao, J. Chung, L. Balaj, A. Charest, D.D. Bigner, B.S. Carter, F.H. Hochberg, X.O. Breakefield, R. Weissleder, H. Lee, 443 protein typing of circulating microvesicles allows real-time monitoring of glioblastoma therapy, Nat. Med. 48 (2012) 137–138.
[119] Y. Dong, W.K.K. Wu, C.W. Wu, J.J.Y. Sung, J. Yu, S.S.M. Ng, MicroRNA dysregulation in colorectal cancer: a clinical perspective, Br. J. Canc. 104 (2011) 893–898.
[120] S. Hildonen, E. Skarpen, T.G. Halvorsen, L. Reubsaet, Isolation and mass spectrometry analysis of urinary extraexosomal proteins, Sci. Rep. 6 (2016) 36331.
[121] E. Corinna, S. Isabel, M. Volkmar, M.L. Karin, W. Harriet, P. Klaus, S. Heidi, Increased serum levels of circulating exosomal microRNA-373 in receptor-negative breast cancer patients, Oncotarget 5 (2014) 9650–9663.
[122] M.M. Di, V. Regondi, M. Sandri, M.V. Iorio, A. Zanetti, E. Tagliabue, P. Casalini, T. Triulzi, Breast cancer-secreted miR-939 downregulates VE-cadherin and destroys the barrier function of endothelial monolayers, Cancer Lett. 384 (2017) 94–100.
[123] A. Liga, A.D. Vliegenthart, W. Oosthuyzen, J.W. Dear, M. Kersaudy-Kerhoas, Exosome isolation: a microfluidic road-map, Lab Chip 15 (2015) 2388.
[124] K.W. Witwer, E.I. Buzás, L.T. Bemis, A. Bora, C. Lässer, J. Lötvall, N.T. Hoen, M.G. Piper, S. Sivaraman, J. Skog, Standardization of sample collection, isolation and analysis methods in extracellular vesicle research, J. Extracell. Vesicles 2 (2013) 1–25.
[125] d.P.E. Van, M.J. van Gemert, A. Sturk, R. Nieuwland, T.G. van Leeuwen, Single vs. swarm detection of microparticles and exosomes by flow cytometry, J. Thromb. Haemost. 10 (2012) 919–930.
[126] V. Ciravolo, V. Huber, G.C. Ghedini, E. Venturelli, F. Bianchi, M. Campiglio, D. Morelli, A. Villa, P.D. Mina, S. Menard, Potential role of HER2-overexpressing exosomes in countering trastuzumab-based therapy, J. Cell. Physiol. 227 (2011) 658–667.
[127] A.S. Lawrie, A. Albanyan, R.A. Cardigan, I.J. Mackie, P. Harrison, Microparticle sizing by dynamic light scattering in fresh-frozen plasma, Vox Sang. 96 (2010) 206–212.
[128] E.A. Murphy, B.K. Majeti, L.A. Barnes, M. Milan, S.M. Weis, L.F. Kimberly, W. Wolfgang, D.A. Cheresh, Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 9343–9348.
[129] M.J. Haney, N.L. Klyachko, Y. Zhao, R. Gupta, E.G. Plotnikova, Z. He, T. Patel, A. Piroyan, M. Sokolsky, A.V. Kabanov, Exosomes as drug delivery vehicles for Parkinson's disease therapy, J. Control. Release Off. J. Control. Release Soc. 207 (2015) 18–30.
[130] C. Emanueli, A.I.U. Shearn, G.D. Angelini, S. Sahoo, Exosomes and exosomal miRNAs in cardiovascular protection and repair, Vasc. Pharmacol. 71 (2015) 24–30.
[131] M.E. Davis, C. Zhuo, M.S. Dong, Nanoparticle therapeutics: an emerging treatment modality for cancer, Nat. Rev. Drug Discov. 7 (2008) 771–782.
[132] O. Shin-Ichiro, T. Masakatsu, S. Katsuko, U. Shinobu, I. Akio, M. Nagahisa, F. Koji, M. Takayuki, O. Tadaaki, O. Takahiro, Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells, Mol. Ther. 21 (2013) 185–191.
[133] A. Mizrak, M.F. Bolukbasi, G.B. Ozdener, G.J. Brenner, S. Madlener, E.P. Erkan, T. Ströbel, X.O. Breakefield, O. Saydam, Genetically engineered microvesicles carrying suicide mRNA/protein inhibit schwannoma tumor growth, Mol. Ther. 21 (2013) 101–108.
[134] J.M. Pitt, C. Mélinda, V. Sophie, A. Fabrice, B. Benjamin, C. Nathalie, Z. Laurence, Dendritic cell-derived exosomes as immunotherapies in the fight against cancer, J. Immunol. 193 (2014) 1006–1011.
[135] H. Xin, Y. Li, B. Buller, M. Katakowski, Y. Zhang, X. Wang, Exosome-mediated transfer of mir-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth, Stem Cell. 30 (2012) 1556–1564.
[136] J.L. Munoz, S.A. Bliss, S.J. Greco, S.H. Ramkissoon, K.L. Ligon, P. Rameshwar, Delivery of functional anti-mir-9 by mesenchymal stem cell–derived exosomes to glioblastoma multiforme cells conferred chemosensitivity, Mol. Ther. Nucleic Acids 2 (2013) e126.
[137] M. Katakowski, B. Buller, X. Zheng, Y. Lu, T. Rogers, O. Osobamiro, W. Shu, F. Jiang, M. Chopp, Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth, Cancer Lett. 335 (2013) 201–204.
[138] B. Escudier, T. Dorval, N. Chaput, F. André, M.P. Caby, S. Novault, C. Flament, C. Leboulaire, C. Borg, S. Amigorena, Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial, J. Transl. Med. 3 (1) (2005) 10 (2005-03-02), 3.
[139] M.A. Morse, J. Garst, T. Osada, S. Khan, A. Hobeika, T.M. Clay, N. Valente, R. Shreeniwas, M.A. Sutton, A. Delcayre, A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer, J. Transl. Med. 3 (2005) 9.
[140] D. Shengming, W. Dong, W. Zhen, Z. Xiangyang, W. Xiaomou, H. Haixin, L. Guisheng, Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer, Mol. Ther. 16 (2008) 782–790.
[141] L. Rivoltini, C. Chiodoni, P. Squarcina, M. Tortoreto, A. Villa, B. Vergani, M. Bürdek, L. Botti, I. Arioli, A. Cova, TNF-related apoptosis-inducing ligand (TRAIL)-armed exosomes deliver pro-apoptotic signals to tumor site, Clin. Canc. Res. Off. J. Am. Assoc. Canc. Res. 22 (2016) 3499–3512.
[142] W.M. Pardridge, Drug transport across the blood-brain barrier, J. Cereb. Blood Flow Metab. 32 (2012) 1959–1972.
[143] T. Yang, P. Martin, B. Fogarty, A. Brown, K. Schurman, R. Phipps, V.P. Yin, P. Lockman, S. Bai, Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio, Pharm. Res. (N. Y.) 32 (2015) 2003–2014.
[144] L. Alvarezerviti, Y. Seow, H.F. Yin, C. Betts, S. Lakhal, M.J.A. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes, Nat. Biotechnol. 29 (2011) 341.
[145] G. Ramkishore, Y. Yuan, Z. Yongshu, W. Benjamin, R. Sanchita, D. Nadire, E. Gabriel, Y. Peixin, Z. Qun, Targeting exosomes from preadipocytes inhibits preadipocyte to cancer stem cell signaling in early-stage breast cancer, Breast Canc. Res. Treat. 150 (2015) 685–695.
[146] J.L. Hood, M.J. Scott, S.A. Wickline, Maximizing exosome colloidal stability following electroporation, Anal. Biochem. 448 (2014) 41–49.
[147] S.A.A. Kooijmans, S. Stremersch, K. Braeckmans, S.C.D. Smedt, H. An, M.J.A. Wood, R.M. Schiffelers, K. Raemdonck, P. Vader, Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles, J. Control. Release 172 (2013) 229–238.
[148] C. Théry, A. Regnault, J. Garin, J. Wolfers, L. Zitvogel, P. Ricciardi-Castagnoli, G. Raposo, S. Amigorena, Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73, JCB (J. Cell Biol.) 147 (1999) 599–610.
[149] S. Fontana, F. Simona, L. Saieva, S. Laura, S. Taverna, T. Simona, R. Alessandro, A. Riccardo, Contribution of proteomics to understanding the role of tumor-derived exosomes in cancer progression: state of the art and new perspectives, Proteomics 14 (2013) 1581–1594.
[150] V. Ciravolo, V. Huber, G.C. Ghedini, E. Venturelli, F. Bianchi, M. Campiglio, D. Morelli, A. Villa, P. Della Mina, S. Menard, P. Filipazzi, L. Rivoltini, E. Tagliabue, S.M. Pupa, Potential role of HER2-overexpressing exosomes in countering trastuzumab-based therapy, J. Cell. Physiol. 227 (2012) 658–667.
[151] X. Ma, Z. Chen, D. Hua, D. He, L. Wang, P. Zhang, J. Wang, Y. Cai, C. Gao, X. Zhang, Essential role for TrpC5-containing extracellular vesicles in breast cancer with chemotherapeutic resistance, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 6389–6394.
[152] V.G. Martinez, S. O'Neill, J. Salimu, S. Breslin, A. Clayton, J. Crown, L. O'Driscoll, Resistance to HER2-targeted anti-cancer drugs is associated with immune evasion in cancer cells and their derived extracellular vesicles, OncoImmunology 6 (2017) e1362530.
[153] T. Wang, K. Ning, T.X. Lu, X. Sun, L. Jin, X. Qi, J. Jin, D. Hua, Increasing circulating exosomes-carrying TRPC5 predicts chemoresistance in metastatic breast cancer patients, Cancer Sci. 108 (2017) 448.
[154] K. O'Brien, M.C. Lowry, C. Corcoran, V.G. Martinez, M. Daly, S. Rani, W.M. Gallagher, M.W. Radomski, R.A. MacLeod, L. O'Driscoll, miR-134 in extracellular vesicles reduces triple-negative breast cancer aggression and increases drug sensitivity, Oncotarget 6 (2015) 32774–32789.
[155] S.J. Yang, D.D. Wang, J. Li, H.Z. Xu, H.Y. Shen, X. Chen, S.Y. Zhou, S.L. Zhong, J.H. Zhao, J.H. Tang, Predictive role of GSTP1-containing exosomes in chemotherapy-resistant breast cancer, Gene 623 (2017) 5–14.

[156] Y. Miyoshi, S. Nakayama, Y. Torikoshi, S. Tanaka, H. Ishihara, T. Taguchi, Y. Tamaki, S. Noguchi, High expression of ubiquitin carboxy-terminal hydrolase-L1 and -L3 mRNA predicts early recurrence in patients with invasive breast cancer, Cancer Sci. 97 (2010) 523–529.
[157] X. Wang, W. Zhong, J. Bu, Y. Li, R. Li, R. Nie, C. Xiao, K. Ma, X. Huang, Y. Li, Exosomal protein CD82 as a diagnostic biomarker for precision medicine for breast cancer, Mol. Carcinog. 58 (5) (2019) 674–685.
[158] M. Yoshikawa, H. Iinuma, Y. Umemoto, T. Yanagisawa, A. Matsumoto, H. Jinno, Exosome-encapsulated microRNA-223-3p as a minimally invasive biomarker for the early detection of invasive breast cancer, Oncology Letters 15 (2018).
[159] J. Ding, Z. Xu, Y. Zhang, C. Tan, W. Hu, M. Wang, Y. Xu, J. Tang, Exosome-mediated miR-222 transferring: an insight into NF-κB-mediated breast cancer metastasis, Exp. Cell Res. 369 (2018) S0014482718302763.
[160] H.J. Ji, Y. Tada, D. Bornstaedt, C. Wahlquist, M. Mercola, Y. Woo, P. Yang, Exosomal miR-106a-363 cluster from the hypoxic human ipsc-derived cardiomyocytes restore the ischemic myocardium, J. Am. Coll. Cardiol. 71 (2018) A14.
[161] S. Gao, Y. Wang, M. Wang, Z. Li, Z. Zhao, R.X. Wang, R. Wu, Z. Yuan, R. Cui, K. Jiao, MicroRNA-155, induced by FOXP3 through transcriptional repression of BRCA1 , is associated with tumor initiation in human breast cancer, Oncotarget 8 (2017) 41451–41464.
[162] K. Ning, T. Wang, X. Sun, P. Zhang, Y. Chen, J. Jin, D. Hua, UCH-L1-containing exosomes mediate chemotherapeutic resistance transfer in breast cancer, J. Surg. Oncol. 115 (2017).
[163] C. Eichelser, I. Stückrath, V. Müller, K. Mildelangosch, H. Wikman, K. Pantel, H. Schwarzenbach, Increased serum levels of circulating exosomal microRNA-373 in receptor-negative breast cancer patients, Oncotarget 5 (2014) 9650–9663.
[164] Z. Naseri, R. Kazemi Oskuee, M.R. Jaafari, M. Forouzandeh, Exosome-mediated delivery of functionally active miRNA-142-3p inhibitor reduces tumorigenicity of breast cancer in vitro and in vivo, Int. J. Nanomed. 13 (2018) 7727–7747.
[165] H. Gomari, M. Forouzandeh Moghadam, M. Soleimani, Targeted cancer therapy using engineered exosome as a natural drug delivery vehicle, OncoTargets Ther. 11 (2018) 5753–5762.
[166] T.D. Clemons, R. Singh, A. Sorolla, N. Chaudhari, A. Hubbard, K.S. Iyer, Distinction between active and passive targeting of nanoparticles dictate their overall therapeutic efficacy, Langmuir :ACS J. Surf. Colloids 34 (2018) 15343–15349.
[167] Y. Tian, S. Li, J. Song, T. Ji, M. Zhu, G.J. Anderson, J. Wei, G. Nie, A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy, Biomaterials 35 (2014) 2383–2390.
[168] Y. Wang, X. Chen, B. Tian, J. Liu, L. Yang, L. Zeng, T. Chen, A. Hong, X. Wang, Nucleolin-targeted extracellular vesicles as a versatile platform for biologics delivery to breast cancer, Theranostics 7 (2017) 1360–1372.
[169] W.M. Usman, T.C. Pham, Y.Y. Kwok, L.T. Vu, V. Ma, B. Peng, Y.S. Chan, L. Wei, S.M. Chin, A. Azad, A.B. He, A.Y.H. Leung, M. Yang, N. Shyh-Chang, W.C. Cho, J. Shi, M.T.N. Le, Efficient RNA drug delivery using red blood cell extracellular vesicles, Nat. Commun. 9 (2018) 2359.
[170] C. Zhao, D.J. Busch, C.P. Vershel, J.C. Stachowiak, Multifunctional transmembrane protein ligands for cell-specific targeting of plasma membrane-derived vesicles, Small (Weinheim an der Bergstrasse, Germany) 12 (2016) 3837–3848.
[171] M. Barok, M. Puhka, G. Vereb, J. Szollosi, J. Isola, H. Joensuu, Cancer-derived exosomes from HER2-positive cancer cells carry trastuzumab-emtansine into cancer cells leading to growth inhibition and caspase activation, BMC Canc. 18 (2018) 504.
[172] Z. Yang, J. Xie, J. Zhu, C. Kang, C. Chiang, X. Wang, X. Wang, T. Kuang, F. Chen, Z. Chen, A. Zhang, B. Yu, R.J. Lee, L. Teng, L.J. Lee, Functional exosome-mimic for delivery of siRNA to cancer: in vitro and in vivo evaluation, J. Control. Release :Off. J. Control. Release Soc. 243 (2016) 160–171.
[173] J.H. Wang, A.V. Forterre, J. Zhao, D.O. Frimannsson, A. Delcayre, T.J. Antes, B. Efron, S.S. Jeffrey, M.D. Pegram, A.C. Matin, Anti-HER2 scFv-directed extracellular vesicle-mediated mRNA-based gene delivery inhibits growth of HER2-positive human breast tumor xenografts by prodrug activation, Mol. Cancer Ther. 17 (5) (2018) 1133–1142.
[174] S. Zhang, K.H. Kim, U.H. Jin, C. Pfent, H. Cao, B. Amendt, X. Liu, H. Wilsonrobles, S. Safe, Aryl hydrocarbon receptor agonists induce microrna-335 expression and inhibit lung metastasis of estrogen receptor negative breast cancer cells, Mol. Cancer Ther. 11 (2012) 108–118.
[175] Q.L. Wang, X. Zhuang, M.K. Sriwastva, J. Mu, Y. Teng, Z. Deng, L. Zhang, K. Sundaram, A. Kumar, D. Miller, J. Yan, H.G. Zhang, Blood exosomes regulate the tissue distribution of grapefruit-derived nanovector via CD36 and IGFR1 pathways, Theranostics 8 (2018) 4912–4924.