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Review

Exosomes in cancer development, metastasis, and immunity

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ABSTRACT

Exosomes play essential roles in intercellular communications. The exosome was discovered in 1983, when it was found that reticulocytes release 50-nm small vesicles carrying transferrin receptors into the extracellular space. Since then, our understanding of the mechanism and function of the exosome has expanded exponentially that has transformed our perspective of inter-cellular exchanges and the molecular mechanisms that underlie disease progression. Cancer cells generally produce more exosomes than normal cells, and exosomes derived from cancer cells have a strong capacity to modify both local and distant microenvironments. In this review, we summarize the functions of exosomes in cancer development, metastasis, and anti-tumor or pro-tumor immunity, plus their application in cancer treatment and diagnosis/prognosis. Although the exosome field has rapidly advanced, we still do not fully understand the regulation and function of exosomes in detail and still face many challenges in their clinical application. Continued discoveries in this field will bring novel insights on inter-cellular communications involved in various biological functions and disease progression, thus empowering us to effectively tackle accompanying clinical challenges.

1. Exosomes and other major types of extracellular vesicles

Exosomes are the most broadly investigated group among the three main subgroups (exosomes, microvesicles, and apoptotic vesicles, *i.e.,* ApoEVs) of extracellular vesicles (EVs) released from mammalian cells. Exosomes arise from the membranes of multivesicular bodies (MVB) [1,2] and are cup-shaped in morphology under electron microscopy, with diameters ranging from 50 nm to 150 nm [3]. After ultra-centrifugation (100,000*g*), relatively pure exosomes can be isolated by an additional sucrose gradient step, with exosomes at a sucrose density from 1.13 to 1.19 g/mL. Membrane protein CD63, ALG2-interacting protein X (ALIX), tumor susceptibility gene 101 protein (TSG101), and proteasome component HSC10 are highly enriched on exosomes [4]. Recently, Lyden and colleagues identified two exosome subpopulations: large exosome vesicles (Exo-L), sized 90-120 nm, and small exosome vesicles (Exo-S), sized 60-80 nm [5].

Microvesicles, sometimes called microparticles, are generated from the plasma membrane and have diameters ranging from 100 nm to 1000 nm [6]. Membrane proteins such as the integrin GPIB (CD42) and P-selectin are enriched on microvesicles [7]. While both exosomes and microvesicles are released from healthy cells, the ApoEVs are released from apoptotic cells or dying cells. The ApoEVs range in diameter from 1000 nm to 5000 nm, and they contain the nuclear protein histone and DNAs [8]. Besides the three main subtypes, other EVs include membrane particles, exosome-like vesicles, neutrophil-originating EVs (ectosomes) [9], prostate-originating EVs (prostasomes) [10,11], migrasomes [12], oncosomes [13], large oncosomes [14] and others [15].

While exosomes and other EV subtypes have different origins and take different cargos (Fig. 1), they all have the capacity to communicate with other cells and modulate local or distant microenvironments. Distinguishing the three main subtypes of EVs unambiguously on the basis of size, density, or morphology is difficult, because these properties overlap somewhat between the subtypes. In many published papers, therefore, all membrane-released EVs are referred to simply as EVs without attempts to distinguish them as exosomes, microvesicles, or other subtypes. However, strict criteria for distinguishing exosomes and other EV subtypes can help us understand their functions more thoroughly and are beneficial for reliably comparing and better

Abbreviations: EV, Extracellular vesicle; MVB, Multivesicular body; Exo-L, Large exosome; Exo-S, Small exosome; ApoEV, Apoptotic extracellular vesicle; ESCRT, Endosomal sorting complex required for transport; SNARE, Soluble NSF attachment protein receptor complex; miRNA, MicroRna; snRNA, Small nuclear RNA; CAF-DE, Cancer-associated fibroblast-derived exosome; lncRNA, Long non-coding RNA; ECM, Extracellular matrix; EMT, Epithelial-mesenchymal transition; BBB, Blood-brain barrier; NK, Natural killer; IL, Interleukin; MHC, Major histocompatibility complex; DC, Dendritic cell; MDSC, Myeloid-derived suppressor cell; siRNA, Small interfering RNA; shRNA, Short hairpin RNA; GM-CSF, Granulocyte-macrophage-colony-stimulating factor

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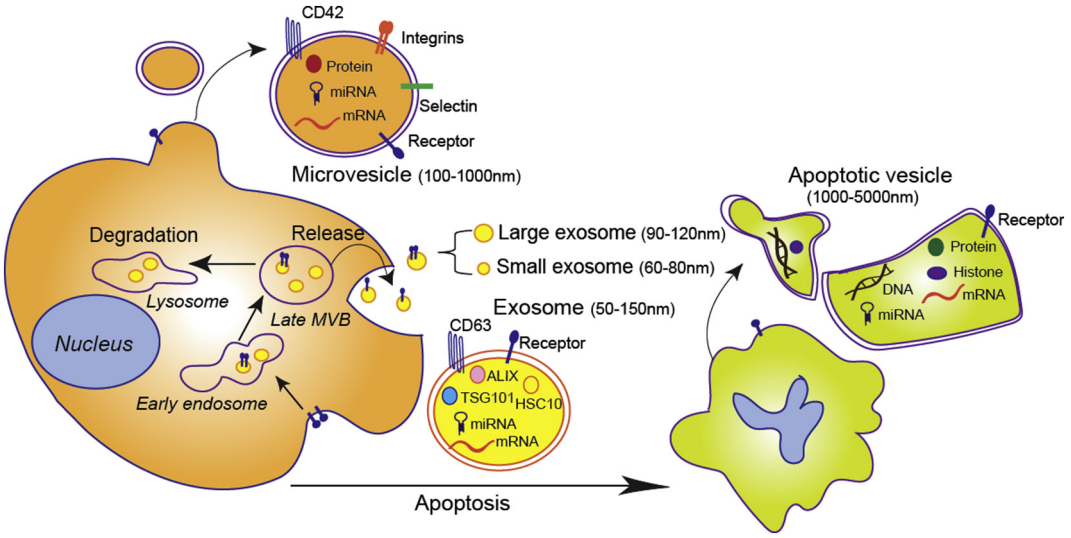

Fig. 1. Exosomes and other extracellular vesicles: biogenesis and secretion in eukaryotic cells. First, exosomes fuse into early endosomes and multivesicular bodies (MVBs). Late MVBs fuse with the plasma membrane to release exosomes or with lysosomes for degradation. Exosomes can be further categorized as large exosomes and small exosomes. CD63, ALIX, TSG101, and HSC10 are enriched in exosomes; many mRNAs, microRNAs (miRNAs), proteins, and receptors are also carried by exosomes. Microvesicles bud directly from the plasma membrane, not from MVBs. CD42, integrins, and selectin are enriched in microvesicles; microvesicles also carry multiple receptors, proteins, miRNAs, and mRNAs. Apoptotic vesicles are derived from apoptotic cells. They contain DNAs and histone besides proteins, receptors, mRNAs, and miRNAs.

understanding the findings from different study groups. In this review, we focus on exosomes' function in tumor development, metastasis, and immunity. Although other types of EVs, such as migrasomes, oncosomes, and large oncosomes have also been associated with cancer development [12,13,16], their contributions will not be extensively discussed in this review, in the interest of focus and space.

2. Exosome biogenesis, composition, and regulation

The process of exosome biogenesis involves several steps: 1) the inward budding of the plasma membrane forms early endosomes; 2) the early endosomes generate late endosomes and MVBs containing intraluminal vesicles, which are also called exosomes; and 3) upon fusion of late MVBs with the plasma membrane, exosomes are released; if these MVBs fuse with lysosomes, the MVBs are degraded [17] (Fig. 1). Generally, the endosomal sorting complex required for transport (ESCRT) machinery, such as ESCRT-0, -I, -II, and -III and accessory proteins, control exosome biogenesis and formation and vesicle scission [18–20]. Sphingolipid ceramide [21], the small GTPase ADP ribosylation factor 6 (ARF6), and its effector phospholipase D2 (PLD2) [22] also control exosome biogenesis, independent of the ESCRT machinery. In melanocytes, melanosomal protein PMEL [23] and tetraspanin CD63 [24] directly participate in ESCRT-independent endosomal sorting.

The RAB family, especially RAB27A, RAB27B, RAB11, RAB35, and RAB7, regulate exosome secretion [25–27]. The controlling of exosome secretion by various RAB proteins is dependent on the cellular context. For example, RAB27A and RAB27B control exosome release in HeLa cells [25], while RAB7 controls exosome release in MCF-7 breast cancer cells [27]. Vacuolar protein sorting protein 33b controls the maturation and secretion of exosomes in hematopoietic stem cells and leukemia-initiating cells [28]. Soluble N-ethyl- maleimide-sensitive factor (NSF)-attachment protein receptor complex (SNARE) [29,30] and the microenvironment pH [31] are also involved in the fusion between MVBs with the plasma membrane and thereby the regulation of exosome release. Unlike exosomes, microvesicles and ApoEVs bud directly from the plasma membrane, not from MVBs. The mechanism of microvesicle and ApoEV release are not well known, but ARF6 [32] and the small GTPase RHOA [33] were reported to control microvesicle shedding; caspase-3 was required for detachment of ApoEVs from the membrane [34].

Nearly all mammalian cells secrete and take up exosomes [35,36]. Besides cancer cells, cells such as adipocytes [37], immune cells [38,39], and brain resident cells [40,41] have been reported to release or take up exosomes in physiologic and pathologic conditions. Exosomes carry many cargos: proteins, lipids, nucleic acids (mRNA, microRNAs [miRNAs], and DNA), and metabolites [42–45]. The ExoCarta database (http://exocarta.org/) lists thousands of proteins, mRNAs, and miRNAs that are candidate exosomes cargos [46], but the cargos vary in different cell lines. The mechanism by which exosomes selectively package their cargos remains unclear. It has been reported that it required special sequences to sort mRNA or miRNAs in exosomes [47,48], but reliable criteria for the selection of proteins, lipids, and even RNAs into exosomes are unknown. Patton and colleagues found that colon cancer cells with mutant KRAS have different miRNA content in their exosomes than cells with wild-type KRAS: miR-10b was enriched in the exosomes from wild-type KRAS cancer cells, whereas miR-100 was enriched in the exosomes from mutant KRAS cancer cells [48]. This finding indicates that KRAS might control exosomal miRNA packaging. Lyden and colleagues showed that Exo-S specifically carries the proteins associated with endosomes and MVBs, while Exo-L carries proteins associated with the plasma membrane, cell-cell contact and junctions, late endosomes, and Golgi network proteins. Their findings further demonstrate that cargos are selectively packaged in exosomes [5].

Exosome uptake by recipient cells is processed by endocytosis, receptor-ligand interaction, or fusion with the cell membrane. The uptake of exosomes is not random, but depends on interactions between proteins on the surface of the exosomes and recipient cells. Several reports have suggested that adhesion-associated molecules on the surface of exosomes, such as tetraspanins, glycoproteins, and integrins, determine which cells accept exosomes [49,50]. For example, exosomes containing tetraspanin 8 (TSPAN8) and integrin α4 were easily taken up by $CD54^+$ pancreas cells [49]. TSPAN8-α4 integrin (CD49d) in exosomes contributed to exosome binding to and uptake by endothelial cells, thereby promoting angiogenesis [50]. Integrin CD47 expression in engineered exosomes facilitated uptake by tumor cells through micropinocytosis [51]. To demonstrate that recipient cells can take up mRNAs loaded in EVs *in vivo*, Rheenen and colleagues transfected Cre recombinase into malignant tumor cells such that the EVs released from

these cells contained Cre mRNA. After the reporter-expressing recipient benign tumor cells took up these EVs, their color switched from pre-labeled red to green due to Cre-LoxP recombination [52]. These data showed that mRNA in exosomes can be transferred to and function in recipient cells. Although the authors did not distinguish EVs from exosomes in this paper, it is very likely that exosomes also can transfer mRNAs between cells.

3. Exosome function in cancer development

Exosomes have been widely studied for their roles in intracellular communication, especially during tumor development. Exosome-associated RNAs, miRNAs, proteins, DNAs, and even metabolites can change the fate of recipient cells by autocrine and paracrine signaling. First, exosomal proteins can change the fate of exosome-releasing cells themselves via an autocrine pathway. For example, the exosomes derived from chronic myeloid leukemia cells contained a cytokine, TGFβ1, which binds to the TGFβ1 receptor on the leukemia cells, thereby promoting tumor growth through activation of ERK, AKT, and anti-apoptotic pathways in the producer cells [53]. Second, exosomal DNA changes cell survival itself. It has been debated whether exosomes carry DNA, because exosomes come from MVB in the cytoplasm but do not connect to the nucleus. However, several groups have detected double-stranded DNA fragments and DNA mutation in exosomes [54]. Immuno-gold labeling of double-stranded DNA detected by transmission electron microscopy revealed the presence of DNA in some exosomes that were still in cell plasma before their secretion [55]. If exosome secretion was inhibited, the accumulation of nuclear DNA in the cytoplasm resulted in cell cycle arrest or apoptosis through activation of reactive oxygen species-dependent DNA damage response. Thus, exosome secretion of harmful cytoplasmic DNA from cells supported cell survival and maintained cellular homeostasis [55].

Compared to these autocrine effects, paracrine mechanisms through which exosomes mediate intercellular interactions and modulate the microenvironment have been well studied. Cargos in exosomes or EVs serve as external stimuli for recipient cells, thereby modifying the signaling pathways in recipient cells. Because of the heterogeneity of cancer cells, exosomes or EVs from host cancer cells can activate the receptors or change miRNA or RNA expression in the neighboring cancer cells to alter their biological phenotypes. For example, glioma cells transferred EVs with the oncogenic receptor EGFRvIII to neighboring glioma cells lacking this receptor, thereby activating the AKT pathway in neighboring glioma cells and conferring on these cells the capacity for anchorage-independent growth [13]. Similarly, mutant KRAS, along with other oncogenes such as EGFR and SRC, can be transferred by exosomes to recipient colon cancer cells of wild-type KRAS, promoting tumor invasion [56]. Breast cancer cells released exosomes harboring PD-L1, allowing its transfer to other cancer cells expressing low- or no- PD-L1, promoting tumor evasion of immune surveillance [57]. Apoptotic vesicles also contribute to the survival of tumor cells. Apoptotic glioblastoma cells released ApoEVs to transfer spliceosomal proteins such as RBM11 and small nuclear RNAs (snRNAs) to change the mRNA splicing (MDM4, CCND1) in recipient cells, resulting in tumor aggressiveness and drug resistance [58].

Exosome not only transfer between cancer cells, but also transfer between cancer cells and stromal cells: stromal cells accept exosomes derived from cancer cells to generate a pro-tumor microenvironment; reciprocally, cancer cells take the exosomes released from stromal cells to facilitate cancer cell proliferation or invasion. For example, tumor-derived exosomes promoted endothelial cell proliferation and angiogenesis [50,59]. The exosomes from MDA-MB-231 breast cancer cells and U87 glioma cells endowed normal fibroblasts and epithelial cells with transformed characteristics of cancer cells, such as enhanced anchorage-independent growth and survival through the enzyme transglutaminase and its substrate fibronectin [60]. Breast cancer cell-derived exosomes triggered adipose-derived mesenchymal stem cells to transform to tumor-associated myofibroblasts via the TGFβ-SMAD-mediated signaling pathway [61]. Some cancer cells released $TGF\beta^{high}$ cancer exosomes, which trigged the transition of fibroblasts into α-smooth muscle actin positive myofibroblasts [62].

Tumor cell-derived exosomes can also regulate endothelial cell characteristics to promote angiogenesis, especially in hypoxic conditions. Exosomes presenting tetraspanins can promote tumor growth by increasing angiogenesis [63]. For example, cancer cell-derived exosomes enriched with TSPAN8 and integrin subunit α4 enhanced endothelial cell proliferation and angiogenesis through upregulation of angiogenesis-related genes [50]. NOTCH ligand Delta-like 4 (DLL4) presented by cancer cell-derived exosomes increased vessel density and branching *in vivo* [64]. Soluble E-cadherin, a potent inducer of angiogenesis, was expressed at greater levels in the exosomes of ovarian cancer cells. Soluble E-cadherin carried by exosome was heterodimerized with vascular-endothelial cadherin on endothelial cells to active β-catenin and NF-kB signaling for angiogenesis [65]. Hypoxic conditions stimulated tumor cells, such as glioblastoma, to release exosomes, which enhanced angiogenesis by upregulating protease-activated receptor 2 (PAR2) in epithelial cells [66]. Under hypoxic conditions, lung cancer cells produced more exosomes enriched with miR-23a, which suppressed its target prolyl hydroxylases 1 and 2 (PHD1 and PHD2), resulting in the accumulation of hypoxia-inducible factor-1-alpha (HIF1A) in endothelial cells. Exosomal miR-23a also targeted to the tight junction protein ZO1 to increase vascular permeability and cancer migration [67]. In the hypoxic bone marrow, multiple myeloma-derived exosomal miR-135b inhibited its target, factor-inhibiting hypoxia-inducible factor 1 (FIH1AN), in endothelial cells, thereby enhancing endothelial tube formation under hypoxic conditions [68].

Stromal cells also change the fate of tumor cells via exosomes. Activated stromal cells around breast cancer cells were found to release exosomes containing cytoplasmic unshielded RNA RN7SL1, which activated the viral RNA pattern recognition receptor RIG-1 signaling, resulting in an inflammatory response and tumor progression [69]. Cancer-associated fibroblast-derived exosomes (CAF-DEs) containing abundant ADAM10 enhanced cancer cell motility through the GTPase RHOA and maintained stem cell status through Notch signaling in cancer cells [70]. In addition, CAF-DEs carried metabolic cargos, including amino acids, lipids, and TCA-cycle intermediates. After prostate and pancreatic cancers took in CAF-DEs, glycolysis and glutamine-dependent reductive carboxylation were increased in cancer cells, thereby promoting tumor growth under nutrient deprivation or nutrient-stressed conditions [45,71].

4. Exosomes induce drug resistance in cancers

Exosomes and EVs have robust impacts on drug resistance and induce drug resistance through multiple mechanisms. First, exosomes released from tumor cells can help the cells expel cytotoxic drugs, as has been observed in melanoma and ovarian cancer [72–75]. Second, drug-sensitive cells become drug resistant by taking up exosomes derived from drug-resistant cells. For example, a multidrug-resistant leukemia subline transferred exosomes containing P-glycoprotein to drug-sensitive cells [76]. MiRNAs such as miR-30a, miR-222, or miR-100-5p carried by exosomes induced drug-sensitive cells to become resistant possibly through regulating MAPK or mTOR pathway [77,78]. Expression of glutathione S-transferase P1 (GSTP1), an enzyme that has been reported to detoxify several anticancer drugs by conjugating them with glutathione [79], was much higher in exosomes derived from doxorubicin-resistant cells. When exosomal GSTP1 was transferred to sensitive cells, it conferred drug resistance to sensitive cells, and numbers of circulating GSTP1-containing exosomes were negatively correlated with the clinical outcome of chemotherapy in breast cancer patients [79]. Exosomal long-non-coding RNA (lncRNA) mediated sunitinib drug resistance in renal cell carcinoma, since lncRNA competed for binding of miR-34 and miR-449 to their target RNAs, thereby

increasing the expression of AXL and MET in sensitive cells to spread sunitinib resistance [80]. EVs released by HER2$^+$ cells that are resistant to HER2-targeted drugs contained immune-regulated proteins TGFβ1 and PD-L1, which made cells that had been sensitive to HER2-targeted drugs resistant. In fact, TGFβ1 expression was higher in EVs isolated from the serum of patients with HER2$^+$ breast cancer that did not respond to HER2-targeted drugs trastuzumab or lapatinib [81]. Third, stromal exosomes can also induce drug resistance in cancer cells. For example, exosomes were transferred from the TME stroma to breast cancer cells to expand therapy-resistant tumor-initiating cells by exosome-RNA mediated activation of the STAT1-NOTCH3 pathway in the cancer cells [82]. Macrophage-derived exosomes decreased the sensitivity of pancreatic cancer cells to gemcitabine, an effect mediated by transfer of miR-365, which activated the enzyme cytidine deaminase to make pancreatic cancer cells resistant to this chemotherapy agent [83]. The other mechanisms of EV-based drug resistance have been comprehensively reviewed by McNamee and O'Driscoll [84].

5. Exosome function in cancer metastasis

The cancer metastatic process comprises several steps. It begins with local invasion by cancer cells, then cancer cells enter the circulation (intravasation) via the lymphatic system or the blood vessel. In the circulation, the cancer cells need to survive and disseminate to different organs, exiting the circulation to enter the parenchyma of distant organs (extravasation). Finally, extravasated cancer cells can die, remain dormant or outgrown to succeed in colonization in distant organs. Exosomes have an extensive impact on each step of metastasis.

5.1. Exosomes in cancer cell migration and invasion

First, exosomes control cell polarity and directional cell movement. Fibrosarcoma cells secreted exosomes contain fibronectin that bound with cellular integrin receptors to facilitate integrin clustering and strong adhesion formation at the leading edge to promote cell migration [85]. Cancer-associated fibroblast–secreted CD81-positive exosomes were loaded with WNT ligand WNT11 and were taken up by breast cancer cells. The CD81/WNT11-positive exosomes promoted breast cancer cell protrusion, invasion, and metastasis via activating autocrine WNT-planar cell polarity signaling [86]. Second, exosomes derived from cancer cells could modulate the extracellular matrix (ECM) to promote cell invasion and metastasis. For example, CD151/TSPAN8-positive exosomes derived from the rat pancreatic adenocarcinoma cell line (ASML) recruited integrins and proteases, which degraded collagen and fibronectin to modify ECM [87]. Exosomes from RAB27B high-expressing metastatic breast cancer cells contained activated matrix metallopeptidase 2 (MMP2), a protease that degrades the ECM [88]. Third, exosomes could unlock the tight junctions to enhance tumor cells intravasation. Exosomes containing miR-105 from breast cancer reduced the expression of ZO1 in endothelial cells, thus destroyed the tight junctions of endothelial cells [89]. Fourth, exosomes derived from cancer cells could promote recipient cells' epithelial-mesenchymal transition (EMT), which is an essential process for cancer invasion and metastasis. Many EMT factors delivered by exosomes, such as HIF1α, matrix metalloproteinase 13 (MMP13), casein kinase II α (CKIIA), annexin A2, and latent membrane protein 1 (LMP1), contributed to the metastatic features of recipient cells [90–93]. Exosomes also may contain anti-metastasis cargos. For example, bladder carcinoma cell exosomes contained miR-23b, which deterred cell invasion and metastasis. However, the release of these miR-23b–containing exosomes reduced miR-23b level inside the parental cells, promoting their metastasis [94].

Other EVs have similar capacities to promote cancer cell invasion. For example, less malignant tumor cells that took up EVs from highly malignant tumor cells showed enhanced migratory behavior and metastatic capacity by *in vivo* imaging [52]. The secretion of EVs by breast cancer cells can help metastatic cancer cell extravasation across the blood-brain- barrier (BBB). These EVs from brain metastatic cancer cells transferred miR-181c into endothelial cells of the BBB, resulting in the destruction of cell-cell contacts and therefore the breakdown of the BBB to allow brain metastasis [95]. Large oncosomes, which are a subgroup of gigantic EV (size > 1,000 nm to > 10,000 nm), were reported to promote amoeboid migration of metastatic prostate cancer cells [16]. Migrasomes are migration-dependent EVs that are assembled on retraction fibers, present at the trailing edge of migrating cells. As cells migrate, the migrasomes are released into the extracellular environment and are taken up by surrounding cells, but the exact function of the migrasomes in the recipient cells is unclear [12].

5.2. Exosomes in pre-metastatic niches

Primary tumors release systemic signals, such as cytokines or exosomes, to prepare metastatic sites. The importance of exosomes in building these pre-metastatic niches for disseminated cancer cells was revealed by two reports that tumor cell-derived exosomes conditioned lymph nodes or lung tissue to become favorable niches for the metastatic colonization and outgrowth of melanoma cells [96,97]. Hood et al. reported that melanoma-derived exosomes can travel to sentinel lymph nodes through lymphatic trafficking. These exosomes prepared lymph nodes for melanoma metastasis via the activation of molecular signals that modulated ECM deposition and vascular proliferation [96]. The exosomes from pancreatic cancer possessed increased levels of miRNAs, such as miR-494 and miR-542-3p, which downregulate cadherin-17, thereby increasing proteases, adhesion molecules, and other proteins to prepare pre-metastatic niches in lungs or lymph nodes for tumor cell hosting [97]. In prostate cancer, exosomes released by the cancer cells impaired osteoclast differentiation but promoted osteoblast activity to regulate the microenvironment of bone metastases [98,99]. However, some exosomes cannot reach the pre-metastatic organ without the help of the soluble fraction or the adhesive matrix. Specifically, exosomes from rat pancreatic cancer had to combine with the cell-released soluble fraction, such as CD44v, c-Met, UPAR, and C3, to establish pre-metastatic niches within the lung or lymph node [100]. In addition, microvesicles released by CD105$^+$ renal cell carcinoma stem cells triggered angiogenesis and promoted the formation of a pre-metastatic niche in the lung [101]. The CD105$^+$ microvesicles contained a selected pattern of miRNAs that induce endothelial cell growth, invasion of the matrix, and resistance to apoptosis [101].

Lyden and colleagues have advanced our understanding of the novel function of exosomes in pre-metastatic niches. They found that exosomes from melanoma cells delivered MET, a receptor tyrosine kinase, to bone marrow progenitor cells. After taking up the MET+ exosomes, bone marrow progenitor cells activated hepatocyte growth factor (HGF)-MET signaling to support tumor metastasis to bones [102]. The exosomes from pancreatic cancer cells were taken up by Kupffer cells, contributing to the establishment of pre-metastatic niches in the liver. Pancreatic cancer cell-derived exosomes deliver macrophage-inhibitory factor (MIF) that made Kupffer cells release more TGFβ, which in turn increased fibronectin production and recruited bone marrow-derived macrophages for liver pre-metastatic niche formation [103]. Strikingly, exosomes derived from breast cancer contained different integrin patterns, which predetermined the future metastatic organ. Specifically, high expression of integrins $\alpha_6\beta_4$ and $\alpha_6\beta_1$ in breast cancer-derived exosomes primed for lung metastasis, while high expression of integrin $\alpha_v\beta_5$ in exosomes primed for liver metastasis [104].

Additionally, it was reported that snRNAs in exosomes derived from lung cancer or melanoma promoted lung pre-metastatic niches by activating TLR3 and releasing cytokines which recruited neutrophils into the lung [105]. Another striking new mechanism for facilitation of metastasis is an increase of nutrient availability to cancer cells in the pre-metastatic niche by exosomal miR-122. Glucose uptake by niche cells such as lung fibroblasts and brain astrocytes were suppressed by

miR-122 through the downregulation of the glycolytic enzyme pyruvate kinase, thereby increasing nutrient availability for metastatic cancer cell growth [106].

In contrast, exosomes from poorly metastatic melanoma cells lack the capacity to generate pre-metastatic niches in the bone but inhibit cancer cell in the secondary organ sites. These "non-metastatic" exosomes stimulate an innate immune response in the bone marrow through pigment epithelium-derived factor (PEDF) on the outer surface of the exosome. This exosome-induced immune response caused cancer cell clearance at the pre-metastatic niche via recruitment of monocytes, natural killer (NK) cells, and macrophages [107]. In another study, exosomes derived from parental lung cancer cells contained miR-192, which inhibited interleukin 8 (IL8), intercellular adhesion molecules, and CXCL1 in the endothelial precursor cells of the bone microenvironment, thus preventing effective metastatic angiogenesis and impairing colonization. However, the exosomes from highly metastatic subpopulations harbored less miR-192, promoting bone-metastasis niches [108].

5.3. Stromal exosomes in metastatic dormancy and outgrowth

In metastatic organ sites, stroma-derived exosomes can regulate survival, dormancy or outgrowth of disseminated cancer cells. Bone marrow mesenchymal stem cell-released exosomes transferred miR-23b to metastatic cancer cells, resulting in cancer cell dormancy through the suppression of MARCKS, which promotes cell cycling and motility [109]. In contrast, astrocytes in the brain microenvironment can release exosomes containing miR-19, which downregulated PTEN expression in metastatic tumor cells, thereby promoting the outgrowth of brain metastasis [41].

The non-random pattern of metastasis can be explained by the “seed and soil” hypothesis, which indicates successful metastasis depends not only on the intrinsic factors of cancer cells (the “seed”) but also on the preferential microenvironment of select organs (the “soil”) that allow cancer cells to survive and metastases can only prosper when the appropriate seed was implanted in its suitable soil [110]. The effect of exosomes on educating the stromal cells in the distant organs for building pre-metastatic niches complements the “seed and soil” hypothesis, revealing that the cancer cells release exosomes to modify the selected “soils” before they arrive. Meanwhile, the exosome interactions between cancer cells and stromal cells in the distant organs also highlight the bi-directional (seed-soil) co-evolution during the metastasis process. Fig. 2 and 3 briefly summarizes various functions of exosomes in cancer development and metastasis.

6. Exosome function in cancer immunity

Besides the strong capacity to regulate tumor metastasis, another important function of exosomes in cancer is modulating tumor immune response. The evidence for exosome-mediated intercellular antigen transfer was first reported by Thery and colleagues 20 years ago [38,39]. They showed that Epstein-Barr virus-transformed B cells released exosomes containing major histocompatibility complex class (MHC)-II, which activated $CD4^+$ T cells, whereas dendritic cell (DC)-secreted exosomes expressed MHC-I, which activated $CD8^+$ T cells *in vitro*. Recently, the success of immune checkpoint therapy in several cancer types has boosted the interest in further exploration of immune dysregulation in tumors, including modification of tumor immunity by exosomes.

6.1. Anti-tumor immune response

Tumor-derived exosomes or EVs have been reported to activate immune responses. Exosomes derived from tumor cells present neoantigens and/or MHC-peptide complexes to prime and activate T cells by the direct presentation and cross-presentation through DCs, or directly activate NK cells or macrophages [111–115]. For example, tumor-derived exosomes transferred tumor antigens heat-shock proteins (HSP70-80) and MHC-I molecules to DCs, which induced potent $CD8^+$ T cell-dependent antitumor effects on mouse tumors [111,116]. HSP70 on the exosome surface also stimulated NK cell migration and cytolytic activity [117], macrophage activity [118] and induced stronger anticancer immune responses of helper T cells [119]. Exosomes derived from RAB27A-overexpressing tumor cells were capable of upregulating MHC-II, CD80, and CD86 on DCs, in turn promoting $CD4^+$ T cell proliferation [120]. Exosomes from patients’ melanoma delivered MART1 tumor antigens to DCs for cross-presentation to cytotoxic T lymphocytes specific to MART1 [121].

Exosomal DNA regulates tumor-associated inflammation and immunity. Guan and colleagues reported nucleic acid-rich EVs released from Hippo pathway kinase LATS1/2-depleted tumor cells induced antitumor immunity by stimulating the TLR-IFN pathway, whereas cancer cells with high LATS1/2 expression suppressed cancer immunity [122]. Treatment of breast cancer cells with the antitumor agent topotecan that triggered DNA double-strand breaks can induce exosome release. Consequently, exosomal DNA derived from topotecan-treated cancer cells triggered DC activation and subsequent $CD8^+$ T cell activation via CGAS-STING signaling [123].

The capacity of exosomes to express antigens and MHC complexes and induce helper T cell immune responses raises the possibility that exosomes could be used as anticancer vaccines. Remarkably, ApoEVs from melanoma B16-ovalbumin provided the highest antitumor protection compared to microvesicles and exosomes. Mice immunized with ovalbumin-pulsed ApoEVs vaccine had significantly longer tumor-free survival than mice immunized with ovalbumin-pulsed exosome vaccine or ovalbumin-pulsed microvesicle vaccine. Protection by ApoEVs against melanoma challenge might be related to their expression of proteins associated with “immunogenic cell death,” such as high-mobility group box protein B1 (HMGB1) and calreticulin, on their surface [124].

6.2. Pro-tumor immune reaction

Tumor exosomes also have exhibited strong pro-tumor immune reactions. Tumor cell-derived exosomes could inhibit T cell and NK cell activation and promote regulatory T cell function [115,125]. For example, cancer cell-derived exosomes expressed TGFβ and ligands for NKG2D to downregulate the surface NKG2D expression on NK cells and $CD8^+$ T cells, thus blocked their activity [126,127]. Exosomes that present FAS ligand, TNF-related apoptosis-inducing ligand, or galectin-9 induced the apoptosis of activated T cells [128–130]. Exosomes released by melanoma cells stimulated high levels of reactive oxygen species (ROS) in neighboring T cells to inhibit their function [131]. TGFβ in cancer exosomes upregulated the generation of $Foxp3^+$ T regulatory cells [132].

Three recent studies simultaneously showed cancer cell-derived exosomes or EVs harboring PD-L1 which inhibited T cell functions, thereby promoting tumor growth. One reported that breast cancer cell-derived exosomes carried PD-L1, which was transferred to cancer cells that lacked or expressed only low levels of PD-L1 and also blocked T cell activity through interaction with programmed death protein, PD1 [57]. Another showed that PD-L1 was expressed on the surface of some glioblastoma-derived EVs. PD-L1-containing EVs in the serum or plasma of glioblastoma patients were positively correlated with tumor burden [133]. The third reported that exosomes from human melanoma, breast cancer, or lung cancer carried PD-L1 on their surface, and that increased circulating exosomal PD-L1 can be used to predict patient response to anti-PD1 therapy [134].

Besides regulating T cells, cancer-derived exosomes inhibit the activity of DCs and increase the expansion of myeloid-derived suppressor cells (MDSCs). Pancreatic cancer-derived exosomes were found to inhibit DC cytokines via downregulation of TLR4 expression by miR-203

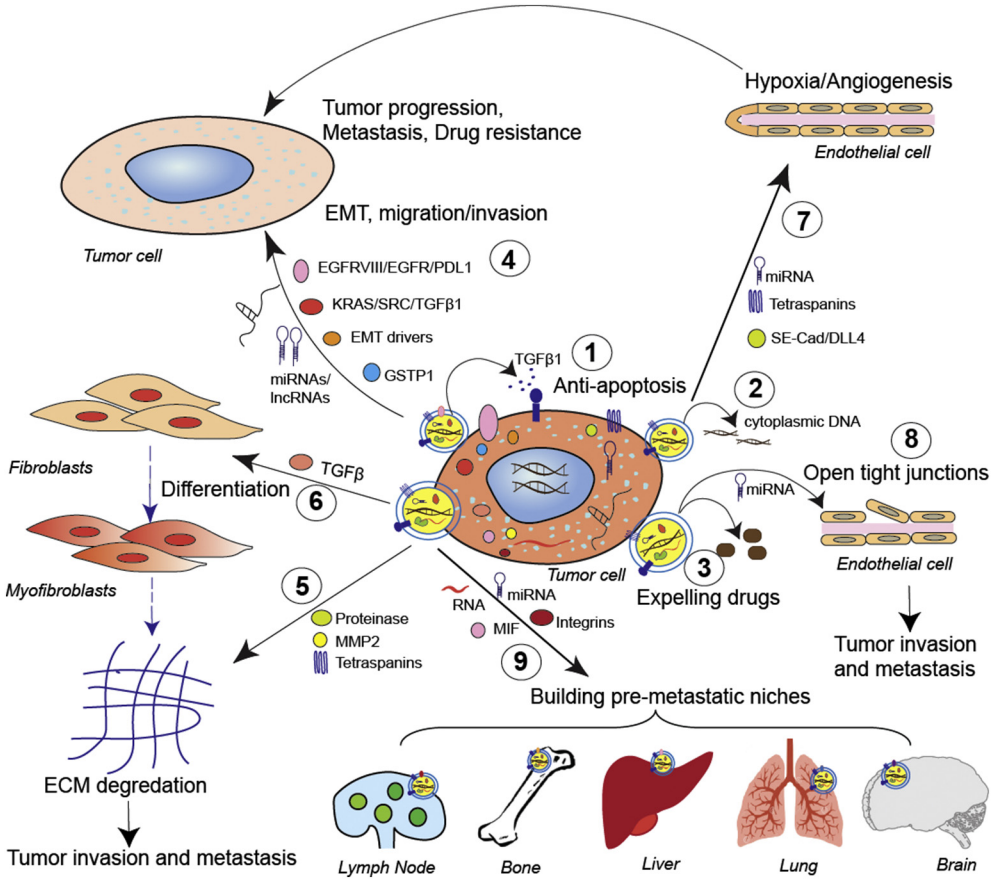

Fig. 2. Functions of cancer cell-derived exosome in tumor progression and metastasis. Tumor-derived exosomes (1) inhibit apoptosis of tumor cells through secretion of TGFβ1 or other ligands and (2) pump cytoplasmic DNA out for cellular homeostasis. (3) Exosomes expel cytotoxic drugs, resulting in tumor cell drug resistance. (4) Tumor-derived exosomes transfer their cargos (such as EGFRVIII, KRAS, SRC, TGFβ1, EMT drivers, PDL1, GSTP1, lncRNAs, or miRNAs) to other tumor cells to induce EMT, migration, and invasion, or drug resistance in recipient cells, thereby promoting tumor progression and metastasis. Tumor exosomes can (5) reprogram the ECM through proteinase, MMP2, or tetraspanins, or (6) induce fibroblast differentiation to myofibroblasts through TGFβ1, which further induces ECM degradation; they also can (7) enhance endothelial cell proliferation and angiogenesis by transferring soluble E-cadherin (SE-Cad), DLL4, tetraspanins, or miRNAs, and (8) open tight junctions in endothelial cells by miRNAs, resulting in tumor progression and metastasis. (9) Tumor exosomes carry specific integrins, macrophage-inhibitory factor, mRNAs, or miRNAs, which allow them to establish pre-metastatic niches in lymph nodes, bone, liver, lung, and brain.

[135]. Lung cancer-derived exosomes blocked DC differentiation via downregulation of surface markers, including CD80, MHC-II, and CD86, but upregulated CD11B and PD-L1 expression [136]. Tumor exosomes converted myeloid cells to MDSCs by secreting PGE2 and TGFβ [137], or IL6, TNFα, and CCL2 [138]. They also activated MDSCs and triggered their suppressive function in an HSP72/TLR2-STAT3–dependent manner [139,140]. In glioma and lung carcinoma mouse models, exosome-recipient cells were mainly $CD45^+$ leukocytes; among them, $CD11b^+Gr1^+$ MDSCs is the major subpopulation. After taking up cancer-derived exosomes, the immunosuppressive function of the MDSCs was enhanced [141]. The hypoxia-inducible expression of miR-10a and miR-21 in glioblastoma exosomes mediated MDSC expansion and activation through downregulation of RAR-related orphan receptor alpha (RORA) and PTEN in MDSCs [142].

Tumor cell-derived exosomes also stimulate the polarization of macrophages towards the cancer-promoting M2 phase. For example, breast cancer-derived exosomes altered macrophage polarization to M2 phase via glycoprotein 130/STAT3 signaling. Specifically, glycoprotein 130 was enriched in the cancer-derived exosomes, which activated the STAT3 pathway in bone marrow-derived macrophages, resulting in IL6 secretion and polarization to M2 phase [143]. SNAIL-expressing human head and neck cancer cells produced miR-21-enriched exosomes, which were engulfed by $CD14^+$ human monocytes, in turn, suppressed the expression of M1 markers and increased that of M2 markers [144]. In hypoxic conditions, pancreatic cancer cell-derived exosomes induced macrophages to the M2 phenotype in a HIF1α or HIF2α-dependent manner, thereby facilitating the migration, invasion, and EMT of cancer cells [145]. Colon cancer cells harboring mutant p53 selectively shed miR-1246-enriched exosomes, which triggered neighboring macrophages into the cancer-promoting M2 phase with increased activity of TGFβ [146]. Breast cancer cell-derived exosomes transferred activated EGFR to host macrophages, which inhibited their production of type I interferons and antiviral immunity, resulting in compromise of innate immunity [147]. Additionally, tumor cell-derived exosomes also induced PD-L1 expression in macrophages. Gastric cancer-derived exosomes effectively induced generation of $PD\text{-}L1^+$ tumor-associated macrophages and impaired $CD8^+$ T cell function via IL10 [148], while glioblastoma-derived exosomes traversed to the monocytes, the precursor to macrophages, and skewed them toward the immune-suppressive M2 phenotype, inducing PD-L1 expression via activation of STAT3 or phosphorylated p70S6 kinase and ERK1/2 [149]. A complex report indicated that oral squamous cell carcinomas-derived exosomes transferred TSP1 to polarize macrophages to the M1-like phenotype, which were activated through P38 MAPK, AKT, and SAPK/JNK signaling at the early phase. However, it was found that exosome-activated M1 macrophages still had a pro-tumor effect because they significantly facilitated the migration of the cancer cells [150]. Similarly, melanoma- or breast cancer-derived exosomes activated M1 macrophages to secrete pro-inflammatory cytokines through stimulation of the NF-B pathway, but these macrophages still facilitated cancer metastasis and immune escape [151,152].

Therefore, exosomes exert both immune-activating and immune-suppressive functions in cancer. The effect of activating immunity mainly depends on antigen presentation by exosomes, while the effect of exosomes' immune inhibition mainly depends on their carried ligands, proteins, and miRNAs, which inhibit the activity of cytotoxic T cells or increase immune-suppressive cells (Fig. 4). Understanding the underlying mechanisms of both functions should benefit efforts to target or utilize exosomes in cancer treatment.

7. Exosomes as therapeutic tools

Exosomes are a type of natural nanoparticle bio-vehicle, which are stable, membrane-permeable, and can even pass through the BBB.

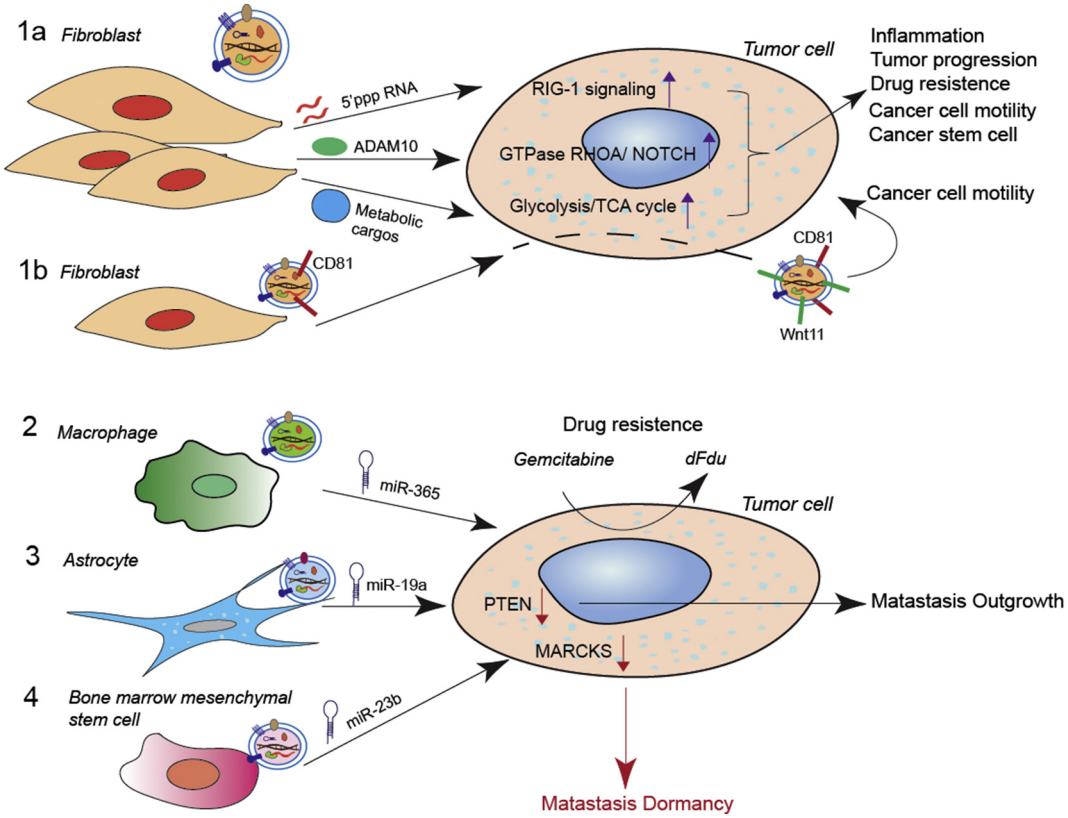

Fig. 3. Examples of stromal cell-derived exosomes in tumor progression and metastasis. 1a) Activated fibroblasts secrete exosomes that are taken up by cancer cells and that transfer unshielded RNAs, protein ADAM10, metabolic cargos, and other molecules, to induce inflammation, tumor growth, drug resistance, cancer cell motility and/or cancer stem cell phenotypic traits. 1b) Fibroblast-secreted CD81-positive exosomes are loaded with WNT11 by cancer cells, and these exosomes promote cancer cell motility and metastasis through an autocrine mechanism. 2) Macrophage-derived exosomes promote pancreatic cancer cell resistance to gemcitabine through miR-365. 3) Astrocytes promote the outgrowth of brain metastasis through exosomal miR-19a. 4) Bone marrow mesenchymal stem cells induce bone metastasis dormancy through exosomal miR-23b.

Because exosomes "recognize" specific cells, delivery of therapeutic cargos by exosomes can have better efficacy and fewer off-target effects than other bio-vehicles, such as liposomes. Therefore, exosomes have become a promising tool for delivery and transfer of drugs, miRNAs, small-interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), and other compounds that remain stable in exosomes for the treatment of cancer and other diseases [153]. Several strategies for improving exosomes' tumor cell-targeting specificity and uptake by tumors have been reported; for example, exosomes were engineered to express target ligands such as lysosome-associated membrane protein 2b (LAMP2B) and a tumor-targeting integrin [154]. An impressive example of exosomes delivering siRNAs or shRNAs that depleted oncogenes to inhibit tumor growth was reported by Kalluri and colleagues. They purified $CD47^{+}$ exosomes from the supernatants of human fibroblast cultures and then introduced siRNA or short hairpin RNA (shRNA) that targeted oncogene $KRAS^{G12D}$ [51]. This kind of engineered exosome (iExosomes) have a reduced clearance from monocytes and macrophages while increasing the uptake by the tumor cells. In a study, treatment of pancreatic tumor-bearing mice with iExosomes that specifically target KRAS-mutant tumor cells suppressed metastasis and dramatically prolonged survival [51], demonstrating the potential of exosomal miRNA-based approaches for effective targeting of KRAS or other mutations in cancer patients.

Strikingly, exosome-mediated delivery of drugs or siRNAs can pass the BBB. DC-derived exosomes delivered siRNAs to the brain in mice. Alvarez-Erviti et al. transduced the DCs to express exosomal membrane protein LAMP2B, which was fused to the neuron-specific RVG peptide. Purified DC exosomes were loaded with exogenous siRNA targeting BACE1, which is important in the pathogenesis of Alzheimer disease; these iExosomes were then intravenously injected into mice. The exosomes specifically entered neurons, microglia, and oligodendrocytes in the brain, resulting in knockdown of the BACE1 gene in the mouse model. This study demonstrated the feasibility of exosome-specific systemic delivery and, more importantly, shows that exosome can pass biological barriers, shedding light on the possibility of new RNAi-based therapy for brain tumors, brain metastases, and neurodegenerative diseases [155].

Plant-derived EVs are also used to efficiently deliver drugs, siRNAs, or proteins to specific cells or tissue such as intestine, colon or liver [156]. Plant-derived EVs have several benefits: they are edible, low toxicity and easily scaled up for mass production. It was reported that grapefruit-derived EVs delivered STAT3 inhibitor JSI-124 to prevent mouse glioblastoma tumor growth, when grapefruit-derived EVs were intranasally administrated. Grapefruit-derived EVs also can co-deliver paclitaxel with folic acid to increase the targeting efficiency to CT26 or SW620 colon cancer cells which express folate receptors. These EVs inhibited colon cancer growth in the mouse model [157].

Another promising area for exosomes is in anticancer vaccination, because exosomes can deliver or present tumor-derived antigens that activate cytotoxic T cells. One good example is the DC exosome vaccine. DC-derived exosomes express MHC-I and MHC-II molecules and induced antitumor immunity. Compared to DC vaccine, which is rapidly eliminated by antigen-specific cytotoxic T lymphocytes, the DC-derived exosome vaccine is relatively long-lived, and thus is considered an alternative to and replacement for DC vaccine. DC-derived exosome vaccines have been tested in several phase I clinical trials [158–161]. In these clinical trials, no grade 2 or greater toxicity was observed, indicating the safety of exosome administration. In a follow-up phase II

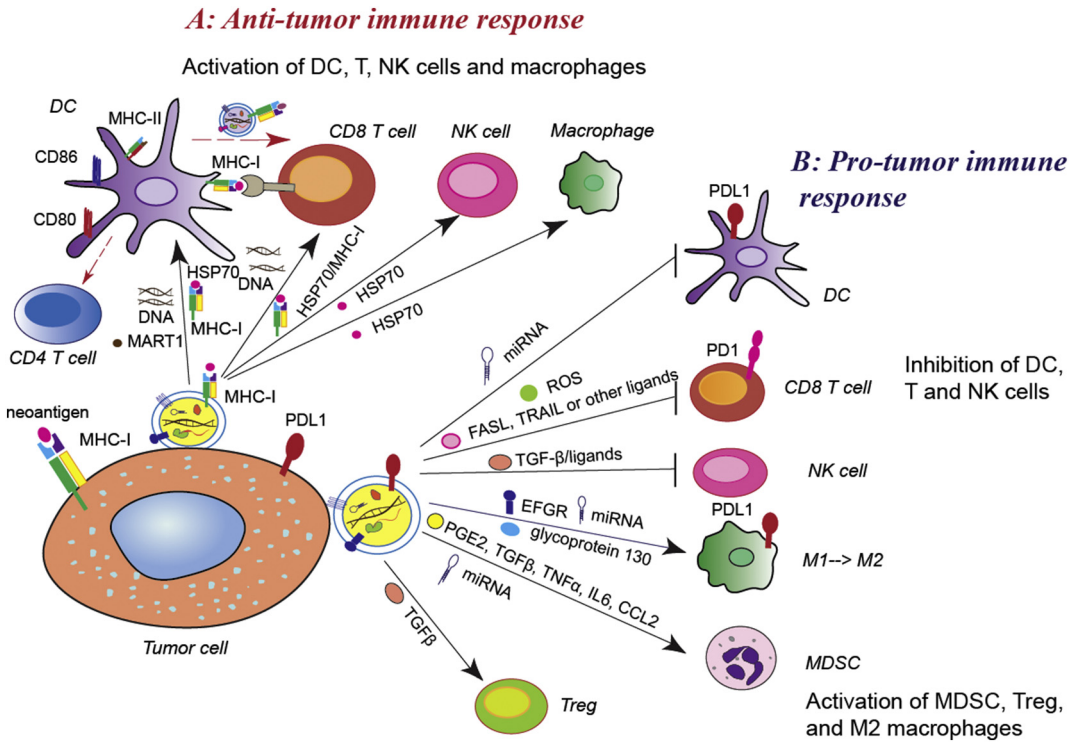

Fig. 4. Exosomes' functions in tumor immunity. A. Anti-tumor immune response: Tumor exosomes present neo-antigens (such as HSP70, MART1) with MHC-I complex to dendritic cells (DCs) or directly to activate T cells. Tumor exosomes increase CD80, CD86, and MHC-II expression in DCs, which further activates $CD4^+$ T cells. Exosomal DNAs trigger activation of DCs and $CD8^+$ T cells. Tumor exosomes induce the activation of natural killer (NK) cells and macrophages by transferring HSP70. DCs release exosomes containing the antigens and MHC-I complex to activate cytotoxic T cells to inhibit tumor growth. B. Pro-tumor immune response: tumor exosomes also repress the function of DCs, T cells, and NK cells, enhancing the populations of myeloid-derived suppressive cells (MDSCs) and regulatory T cells (Treg) and skewing macrophage function toward the M2 phenotype through various signaling pathways. Tumor exosomes carry PD-L1 from tumor cells and transfer it to DCs or macrophages, and then block T cell function.

clinical trial testing the clinical benefit of DC exosomes for patients with non-small cell lung cancer after chemotherapy cessation, patients were administered granulocyte macrophage–colony-stimulating factor (GM-CSF) and IL4 to stimulate production of DCs from monocytes; the DCs were then induced to mature with IFNγ and loaded with MHC-I and MHC-II restricted cancer antigens. Exosomes were isolated from these modified DCs and injected into the patients. No objective tumor response nor detectable T cell response was identified in this clinical trial, but NK cells were activated [162]. Although the phase II clinical result is not as favorable as expected, hope remains that DC-derived exosomes, after further optimization, represent a viable new vaccine strategy for cancer immunotherapy. In one of the phase I clinical trials, exosomes isolated from ascites of patients with colon cancer were injected into the patients as a vaccine. Administered with GM-CSF, the ascites-derived exosomes were safe and well tolerated and yielded a tumor-specific antitumor cytotoxic T cell response [160]. However, as already described, tumor-derived exosomes carry numerous oncogenes, mRNAs, and miRNAs, which induce tumor progression and metastasis, besides tumor antigens, the safety of tumor-derived exosome vaccine is still uncertain.

Given that cancer cell-derived exosomes can induce angiogenesis, promote metastasis, and modify pre-metastatic niches [104], a third potential clinical application of exosomes in cancer is depleting tumor exosomes from the circulatory system in order to block cancer metastasis. Inhibiting exosome assembly and release from tumor cells via inhibitors [139] or shRNAs [25], or eliminating exosomes from cancer patients' circulation by extracorporeal purification, reduces numbers of exosomes in the circulation. For example, the ADAPT system is designed to selectively capture and remove circulating $HER2^+$ exosomes [163]. Recently Datta et al. screened 4580 compounds to identify those that modulate exosome biogenesis and/or release by aggressive prostate cancer cells. Twenty-two of these compounds were found to be either potent activators or inhibitors of exosomes. The potent inhibitors, including tipifarnib, neticonazole, climbazole, ketoconazole, and triadimenol, might be utilized to deplete exosomes in cancer patients [164].

In summary, substantial evidence supports the application of exosomes for cancer treatment based on experiments in cell cultures or experimental models. Currently, however, only a few clinical trials of exosome for cancer therapy are underway. The main challenges of exosome application in the treatment of cancer or other diseases include, but are not limited to, 1) how to efficiently load exogenous miRNAs, siRNAs, shRNAs, or drugs into exosomes and further increase cell-specific delivery; 2) how to prevent immune reactions when utilizing non-autologous exosomes, which carry MHC-I or II; 3) how to increase cytotoxic T cell activation when using DC exosomes as a vaccine; 4) how to prolong the half-life of exosome vaccines or bioengineered exosomes in the body and avoid rapid clearance by immune cells, liver, or kidney; 5) how to prevent, when depleting tumor-derived exosomes from the blood of patients, the loss of non-tumor-promoting exosomes and their physiological function in the whole body; and 6) how to control the quality of exosomes to be administered to patients and the technological challenges related to clinical grade production [165]. Because of the complexity of exosome biology and these clinical challenges, carefully developing standard criteria for exosome quality and improving their efficacy in vivo are crucial before widely employing exosomes in clinical trials.

8. Exosomes as biomarkers

Besides the treatment of disease, other significant applications of exosomes include their use as biomarkers for disease diagnosis and

prognosis. Because exosomes can be detected in bodily fluids such as blood, urine, saliva, and cerebrospinal fluid, they represent ideal non-invasive or less-invasive biomarkers for cancer diagnosis. For example, double-stranded DNAs in exosomes can be used as clinical biomarkers for cancer diagnosis because they reflect mutations in primary and/or metastatic cancer cells [44]. Circulating exosomal DNA enabled the detection of $KRAS^{G12D}$ and $TP53^{R273H}$ mutations, which are potential biomarkers of pancreatic cancer [166]. Additionally, PD-L1 level on exosomes from plasma was associated with disease progression in head and neck cancers [167,168] and predicted melanoma patient response to treatment with PD1 antibody [134]. Furthermore, certain proteins or miRNAs in exosomes isolated from cancer patient plasma are associated with tumor metastasis or relapse [169,170]. Elevated expression of miRNA-191, -21, and -451a in serum exosomes seems to be a biomarker of pancreatic cancer [171]. miRNA analysis of EVs or exosomes from cerebrospinal fluid showed that miR-21 can serve as a biomarker for glioblastoma development and prediction of tumor recurrence or metastasis [172,173]. Circulating exosomal miR-17-5p and miR-92a-3p were associated with pathologic stage and grade of colon cancers [174].

Although numerous publications present various exosomal miRNAs as potential biomarkers of breast cancer, prostate cancer, pancreatic cancer, melanoma, and other cancers, their utilization as clinical biomarkers still faces challenges. Most of these exosomal miRNA studies were conducted in a small patient cohort or only in a mouse model. In these studies, miRNA levels in plasma exosomes varied widely in the single cohort, and results from different groups were heterogeneous even when studying the same cancer type. The methods of exosome isolation from plasma and miRNA extraction from exosomes were not identical in the various study groups, and the studies lacked a common endogenous miRNA control for quantification of exosomal miRNAs. These problems affect the reliability of circulating exosomal miRNAs as cancer biomarkers in clinical diagnosis or prognosis.

Kalluri and colleagues discovered a serum exosomal biomarker for pancreatic cancer [175]. The exosomes from pancreatic cancers expressed at high levels a cell surface proteoglycan, glypican-1 (GPC1), which can be detected in serum as a highly sensitive and specific biomarker of pancreatic cancer, better than classic biomarker CA19-9 [175]. $GPC1^+$ exosomes in serum can distinguish patients with pancreatic cancer from normal individuals and from patients with benign pancreatic disease. $GPC1^+$ exosome level was found to be associated with tumor burden and patient survival [175]. Following this finding, however, Lai et al. compared circulating exosomal GPC1 and miRNA levels in healthy subjects and in patients with pancreatic carcinoma or chronic pancreatitis and found that circulating exosomal GPC1 was not a good diagnostic marker for carcinoma. They found, in contrast, that high levels of miR-10b, miR-21, miR-30c, and miR-181a and a low level of miR-let7a in exosomes more reliably and promptly differentiated pancreatic carcinoma from normal controls and patients with benign pancreatic disease in their cohort relative small cohort [176]. The discrepancies between these two reports might be due to different methods used to detect circulating exosomal GPC1. The first group quantified exosomal GPC1 by an antibody-based assay, whereas the second group used liquid chromatography-tandem mass spectrometry. While these findings exemplify the power of utilizing exosomes for cancer diagnosis, the inconsistency indicates that GPC1 as a biomarker of pancreatic cancer requires further validation in larger patient cohorts and also highlights the importance of standardized methodologies for identifying exosomal biomarkers.

Another good example of exosomal protein as a biomarker is the urine exosome gene expression assay for prostate cancer. This assay detects RNA expression of *ERG*, *PCA3*, and *SPDEF*, which have known functions in prostate cancer initiation and progression. As a screening test, the urine exosome gene expression assay result combined with standard clinical data, comprising of prostate-specific antigen (PSA) level, age, race, and family history, can improve identification of patients with higher-grade prostate cancer over elevated prostate-specific antigen level alone, thereby reducing the number of unnecessary biopsies [177]. A urine-based liquid biopsy platform for the exosome gene expression assay, ExoDx Prostate (IntelliScore), has been developed for non-invasive detection of prostate cancer and there is an is ongoing clinical trial investigating the reliability of this urine test in predicting high-grade prostate cancer at the time of initial prostate biopsy.

In summary, applying exosome biomarkers in cancer diagnosis requires high sensitivity and specificity. Many miRNAs or proteins carried by exosomes have been considered as potential biomarkers, but the sensitivity and specificity of these candidates were not as good as the classic serum biomarkers of cancer, and most have not shown a prognostic or diagnostic significance in large patient cohorts. The technology of isolating exosomes from the serum, urine, or other bodily fluids and the methods of quantifying miRNAs or proteins also need to be further standardized.

Since research on these applications of exosomes is on the fast track, diagnostic applications of exosomes in cancer has an optimistic future.

9. Conclusion

Looking back over the more than 35-year history of exosome investigations, it is remarkable how rapidly our knowledge of exosomes has expanded. A multitude of studies have explored the functions of exosomes under different physiologic and pathologic conditions. Several hallmarks of exosomes have emerged, especially in cancer biology. First, extensive evidence shows that exosomes are not merely waste particles but rather critical mediators of intercellular communications. The cells control the cargos inside exosomes, with the effect of changing their own fate or that of other cells. As satellites of host cells, exosomes, contain substantial bio-information and function beyond initial expectations. Second, exosomes have a robust impact on tumor progression and metastasis. Strikingly, exosomes can predict sites of metastasis and build pre-metastatic niches, depending on their interactions with stromal cells. Third, exosomes can induce both effective pro-tumor and antitumor immune responses, but cancer cell-derived exosomes show much stronger immune suppression than immune activation in the advanced stage. Exosome vaccines, based on exosomes carrying antigens, are being tested in clinical trials. Given the specific characters of exosomes, their use as a new platform for cancer therapy and as cancer biomarkers is promising despite the technical challenges. Deep understanding of the mechanisms underlying exosome function should advance their application in the clinic. It should be noted that in many preclinical studies, the function of exosomes on tumor progression or immunity was mainly determined by “gain-of-function” experiments, such as the adoptive transfer of exosomes, and this may not accurately represent the actual physiological function of exosomes. Although blocking exosome secretion by knocking down RAB27A or RAB27B was used for “loss-of-function” exosome phenotypes in some studies, more rigorous "loss-of-function" techniques may be required to thoroughly uncover the essential function of exosomes and move the field further ahead. We anticipate that advances made in the exosome field will lead to breakthroughs in clinical applications to benefit patients.

Note

We apologize for not being able to cite all the relevant original research and review articles due to space limitation.

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References

[1] B.T. Pan, K. Teng, C. Wu, M. Adam, R.M. Johnstone, Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes, J. Cell Biol. 101 (1985) 942–948.
[2] C. Harding, J. Heuser, P. Stahl, Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes, J. Cell Biol. 97 (1983) 329–339.
[3] C. Thery, M. Ostrowski, E. Segura, Membrane vesicles as conveyors of immune responses, Nat. Rev. Immunol. 9 (2009) 581–593, https://doi.org/10.1038/nri2567.
[4] A. Bobrie, M. Colombo, S. Krumeich, G. Raposo, C. Thery, Diverse subpopulations of vesicles secreted by different intracellular mechanisms are present in exosome preparations obtained by differential ultracentrifugation, J. Extracell. Vesicles 1 (2012), https://doi.org/10.3402/jev.v1i0.18397.
[5] H. Zhang, D. Freitas, H.S. Kim, K. Fabijanic, Z. Li, H. Chen, M.T. Mark, H. Molina, A.B. Martin, L. Bojmar, J. Fang, S. Rampersaud, A. Hoshino, I. Matei, C.M. Kenific, M. Nakajima, A.P. Mutvei, P. Sansone, W. Buehring, H. Wang, J.P. Jimenez, L. Cohen-Gould, N. Paknejad, M. Brendel, K. Manova-Todorova, A. Magalhaes, J.A. Ferreira, H. Osorio, A.M. Silva, A. Massey, J.R. Cubillos-Ruiz, G. Galletti, P. Giannakakou, A.M. Cuervo, J. Blenis, R. Schwartz, M.S. Brady, H. Peinado, J. Bromberg, H. Matsui, C.A. Reis, D. Lyden, Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation, Nat. Cell Biol. 20 (2018) 332–343, https://doi.org/10.1038/s41556-018-0040-4.
[6] M. Colombo, G. Raposo, C. Thery, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (2014) 255–289, https://doi.org/10.1146/annurev-cellbio-101512-122326.
[7] H.F. Heijnen, A.E. Schiel, R. Fijnheer, H.J. Geuze, J.J. Sixma, Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules, Blood 94 (1999) 3791–3799.
[8] E. van der Pol, A.N. Boing, P. Harrison, A. Sturk, R. Nieuwland, Classification, functions, and clinical relevance of extracellular vesicles, Pharmacol. Rev. 64 (2012) 676–705, https://doi.org/10.1124/pr.112.005983.
[9] O. Gasser, C. Hess, S. Miot, C. Deon, J.C. Sanchez, J.A. Schifferli, Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils, Exp. Cell Res. 285 (2003) 243–257.
[10] G. Ronquist, M. Hedstrom, Restoration of detergent-inactivated adenosine triphosphatase activity of human prostatic fluid with concanavalin A, Biochim. Biophys. Acta 483 (1977) 483–486.
[11] G. Ronquist, I. Brody, A. Gottfries, B. Stegmayr, An Mg2+ and Ca2+-stimulated adenosine triphosphatase in human prostatic fluid–part II, Andrologia 10 (1978) 427–433.
[12] L. Ma, Y. Li, J. Peng, D. Wu, X. Zhao, Y. Cui, L. Chen, X. Yan, Y. Du, L. Yu, Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration, Cell Res. 25 (2015) 24–38, https://doi.org/10.1038/cr.2014.135.
[13] K. Al-Nedawi, B. Meehan, J. Micallef, V. Lhotak, L. May, A. Guha, J. Rak, Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells, Nat. Cell Biol. 10 (2008) 619–624, https://doi.org/10.1038/ncb1725.
[14] B. Meehan, J. Rak, D. Di Vizio, Oncosomes - large and small: what are they, where they came from? J. Extracell. Vesicles 5 (2016) 33109, , https://doi.org/10.3402/jev.v5.33109.
[15] M.Y. Konoshenko, E.A. Lekchnov, A.V. Vlassov, P.P. Laktionov, Isolation of extracellular vesicles: general methodologies and latest trends, Biomed. Res. Int. 2018 (2018) 8545347, , https://doi.org/10.1155/2018/8545347.
[16] D. Di Vizio, J. Kim, M.H. Hager, M. Morello, W. Yang, C.J. Lafargue, L.D. True, M.A. Rubin, R.M. Adam, R. Beroukhim, F. Demichelis, M.R. Freeman, Oncosome formation in prostate cancer: association with a region of frequent chromosomal deletion in metastatic disease, Cancer Res. 69 (2009) 5601–5609, https://doi.org/10.1158/0008-5472.CAN-08-3860.
[17] R. Xu, A. Rai, M. Chen, W. Suwakulsiri, D.W. Greening, R.J. Simpson, Extracellular vesicles in cancer - implications for future improvements in cancer care, Nat. Rev. Clin. Oncol. 15 (2018) 617–638, https://doi.org/10.1038/s41571-018-0036-9.
[18] W.M. Henne, H. Stenmark, S.D. Emr, Molecular mechanisms of the membrane sculpting ESCRT pathway, Cold Spring Harb. Perspect. Biol. 5 (2013), https://doi.org/10.1101/cshperspect.a016766.
[19] D.J. Katzmann, M. Babst, S.D. Emr, Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I, Cell 106 (2001) 145–155.
[20] P.I. Hanson, A. Cashikar, Multivesicular body morphogenesis, Annu. Rev. Cell Dev. Biol. 28 (2012) 337–362, https://doi.org/10.1146/annurev-cellbio-092910-154152.
[21] K. Trajkovic, C. Hsu, S. Chiantia, L. Rajendran, D. Wenzel, F. Wieland, P. Schwille, B. Brugger, M. Simons, Ceramide triggers budding of exosome vesicles into multivesicular endosomes, Science 319 (2008) 1244–1247, https://doi.org/10.1126/science.1153124.
[22] R. Ghossoub, F. Lembo, A. Rubio, C.B. Gaillard, J. Bouchet, N. Vitale, J. Slavik, M. Machala, P. Zimmermann, Syntenin-ALIX exosome biogenesis and budding into multivesicular bodies are controlled by ARF6 and PLD2, Nat. Commun. 5 (2014) 3477, https://doi.org/10.1038/ncomms4477.
[23] M. Babst, MVB vesicle formation: ESCRT-dependent, ESCRT-independent and everything in between, Curr. Opin. Cell Biol. 23 (2011) 452–457, https://doi.org/10.1016/j.ceb.2011.04.008.
[24] G. van Niel, S. Charrin, S. Simoes, M. Romao, L. Rochin, P. Saftig, M.S. Marks, E. Rubinstein, G. Raposo, The tetraspanin CD63 regulates ESCRT-independent and -dependent endosomal sorting during melanogenesis, Dev. Cell 21 (2011) 708–721, https://doi.org/10.1016/j.devcel.2011.08.019.
[25] M. Ostrowski, N.B. Carmo, S. Krumeich, I. Fanget, G. Raposo, A. Savina, C.F. Moita, K. Schauer, A.N. Hume, R.P. Freitas, B. Goud, P. Benaroch, N. Hacohen, M. Fukuda, C. Desnos, M.C. Seabra, F. Darchen, S. Amigorena, L.F. Moita, C. Thery, Rab27a and Rab27b control different steps of the exosome secretion pathway, Nat. Cell Biol. 12 (2010) 19–30 sup pp 11-13 https://doi.org/10.1038/ncb2000.
[26] C. Hsu, Y. Morohashi, S. Yoshimura, N. Manrique-Hoyos, S. Jung, M.A. Lauterbach, M. Bakhti, M. Gronborg, W. Mobius, J. Rhee, F.A. Barr, M. Simons, Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C, J. Cell Biol. 189 (2010) 223–232, https://doi.org/10.1083/jcb.200911018.
[27] M.F. Baietti, Z. Zhang, E. Mortier, A. Melchior, G. Degeest, A. Geeraerts, Y. Ivarsson, F. Depoortere, C. Coomans, E. Vermeiren, P. Zimmermann, G. David, Syndecan-syntenin-ALIX regulates the biogenesis of exosomes, Nat. Cell Biol. 14 (2012) 677–685, https://doi.org/10.1038/ncb2502.
[28] H. Gu, C. Chen, X. Hao, C. Wang, X. Zhang, Z. Li, H. Shao, H. Zeng, Z. Yu, L. Xie, F. Xia, F. Zhang, X. Liu, Y. Zhang, H. Jiang, J. Zhu, J. Wan, C. Wang, W. Weng, J. Xie, M. Tao, C.C. Zhang, J. Liu, G.Q. Chen, J. Zheng, Sorting protein VPS33B regulates exosomal autocrine signaling to mediate hematopoiesis and leukemogenesis, J. Clin. Invest. 126 (2016) 4537–4553, https://doi.org/10.1172/JCI87105.
[29] K. Zylbersztejn, T. Galli, Vesicular traffic in cell navigation, FEBS J. 278 (2011) 4497–4505, https://doi.org/10.1111/j.1742-4658.2011.08168.x.
[30] C.M. Fader, D.G. Sanchez, M.B. Mestre, M.I. Colombo, TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways, Biochim. Biophys. Acta 1793 (2009) 1901–1916, https://doi.org/10.1016/j.bbamcr.2009.09.011.
[31] I. Parolini, C. Federici, C. Raggi, L. Lugini, S. Palleschi, A. De Milito, C. Coscia, E. Iessi, M. Logozzi, A. Molinari, M. Colone, M. Tatti, M. Sargiacomo, S. Fais, Microenvironmental pH is a key factor for exosome traffic in tumor cells, J. Biol. Chem. 284 (2009) 34211–34222, https://doi.org/10.1074/jbc.M109.041152.
[32] 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. 19 (2009) 1875–1885, https://doi.org/10.1016/j.cub.2009.09.059.
[33] B. Li, M.A. Antonyak, J. Zhang, R.A. Cerione, RhoA triggers a specific signaling pathway that generates transforming microvesicles in cancer cells, Oncogene 31 (2012) 4740–4749, https://doi.org/10.1038/onc.2011.636.
[34] M.R. Muhsin-Sharafaldine, A.D. McLellan, Tumor-derived apoptotic vesicles: with death they do part, Front. Immunol. 9 (2018) 957, https://doi.org/10.3389/fimmu.2018.00957.
[35] J. Kowal, M. Tkach, C. Thery, Biogenesis and secretion of exosomes, Curr. Opin. Cell Biol. 29 (2014) 116–125, https://doi.org/10.1016/j.ceb.2014.05.004.
[36] P. Munson, A. Shukla, Exosomes: Potential in Cancer Diagnosis and Therapy, Medicines (Basel) 2 (2015) 310–327, https://doi.org/10.3390/medicines2040310.
[37] T. Thomou, M.A. Mori, J.M. Dreyfuss, M. Konishi, M. Sakaguchi, C. Wolfrum, T.N. Rao, J.N. Winnay, R. Garcia-Martin, S.K. Grinspoon, P. Gorden, C.R. Kahn, Adipose-derived circulating miRNAs regulate gene expression in other tissues, Nature 542 (2017) 450–455, https://doi.org/10.1038/nature21365.
[38] G. Raposo, H.W. Nijman, W. Stoorvogel, R. Liejendekker, C.V. Harding, C.J. Melief, H.J. Geuze, B lymphocytes secrete antigen-presenting vesicles, J. Exp. Med. 183 (1996) 1161–1172.
[39] L. Zitvogel, A. Regnault, A. Lozier, J. Wolfers, C. Flament, D. Tenza, P. Ricciardi-Castagnoli, G. Raposo, S. Amigorena, Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes, Nat. Med. 4 (1998) 594–600.
[40] Y. Zhang, M.S. Kim, B. Jia, J. Yan, J.P. Zuniga-Hertz, C. Han, D. Cai, Hypothalamic stem cells control ageing speed partly through exosomal miRNAs, Nature 548 (2017) 52–57, https://doi.org/10.1038/nature23282.
[41] L. Zhang, S. Zhang, J. Yao, F.J. Lowery, Q. Zhang, W.C. Huang, P. Li, M. Li, X. Wang, C. Zhang, H. Wang, K. Ellis, M. Cheerathodi, J.H. McCarty, D. Palmieri, J. Saunus, S. Lakhani, S. Huang, A.A. Sahin, K.D. Aldape, P.S. Steeg, D. Yu, Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth, Nature 527 (2015) 100–104, https://doi.org/10.1038/nature15376.
[42] 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 (2007) 654–659, https://doi.org/10.1038/ncb1596.
[43] G. Raposo, W. Stoorvogel, Extracellular vesicles: exosomes, microvesicles, and friends, J. Cell Biol. 200 (2013) 373–383, https://doi.org/10.1083/jcb.201211138.
[44] B.K. Thakur, H. Zhang, A. Becker, I. Matei, Y. Huang, B. Costa-Silva, Y. Zheng, A. Hoshino, H. Brazier, J. Xiang, C. Williams, R. Rodriguez-Barrueco, J.M. Silva,

W. Zhang, S. Hearn, O. Elemento, N. Paknejad, K. Manova-Todorova, K. Welte, J. Bromberg, H. Peinado, D. Lyden, Double-stranded DNA in exosomes: a novel biomarker in cancer detection, Cell Res. 24 (2014) 766–769, https://doi.org/10.1038/cr.2014.44.

[45] H. Zhao, L. Yang, J. Baddour, A. Achreja, V. Bernard, T. Moss, J.C. Marini, T. Tudawe, E.G. Seviour, F.A. San Lucas, H. Alvarez, S. Gupta, S.N. Maiti, L. Cooper, D. Peehl, P.T. Ram, A. Maitra, D. Nagrath, Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism, Elife 5 (2016) e10250, , https://doi.org/10.7554/eLife.10250.

[46] S. Mathivanan, C.J. Fahner, G.E. Reid, R.J. Simpson, ExoCarta 2012: database of exosomal proteins, RNA and lipids, Nucleic Acids Res. 40 (2012) D1241–D1244, https://doi.org/10.1093/nar/gkr828.

[47] A.O. Batagov, V.A. Kuznetsov, I.V. Kurochkin, Identification of nucleotide patterns enriched in secreted RNAs as putative cis-acting elements targeting them to exosome nano-vesicles, BMC Genomics 12 (Suppl. 3) (2011) S18, https://doi.org/10.1186/1471-2164-12-S3-S18.

[48] D.J. Cha, J.L. Franklin, Y. Dou, Q. Liu, J.N. Higginbotham, M. Demory Beckler, A.M. Weaver, K. Vickers, N. Prasad, S. Levy, B. Zhang, R.J. Coffey, J.G. Patton, KRAS-dependent sorting of miRNA to exosomes, Elife 4 (2015) e07197, , https://doi.org/10.7554/eLife.07197.

[49] S. Rana, S. Yue, D. Stadel, M. Zoller, Toward tailored exosomes: the exosomal tetraspanin web contributes to target cell selection, Int. J. Biochem. Cell Biol. 44 (2012) 1574–1584, https://doi.org/10.1016/j.biocel.2012.06.018.

[50] I. Nazarenko, S. Rana, A. Baumann, J. McAlear, A. Hellwig, M. Trendelenburg, G. Lochnit, K.T. Preissner, M. Zoller, Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation, Cancer Res. 70 (2010) 1668–1678, https://doi.org/10.1158/0008-5472.CAN-09-2470.

[51] S. Kamerkar, V.S. LeBleu, H. Sugimoto, S. Yang, C.F. Ruivo, S.A. Melo, J.J. Lee, R. Kalluri, Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer, Nature 546 (2017) 498–503, https://doi.org/10.1038/nature22341.

[52] A. Zomer, C. Maynard, F.J. Verweij, A. Kamermans, R. Schafer, E. Beerling, R.M. Schiffelers, E. de Wit, J. Berenguer, S.I. Ellenbroek, T. Wurdinger, D.M. Pegtel, J. van Rheenen, In Vivo imaging reveals extracellular vesicle-mediated phenocopying of metastatic behavior, Cell 161 (2015) 1046–1057, https://doi.org/10.1016/j.cell.2015.04.042.

[53] S. Raimondo, L. Saieva, C. Corrado, S. Fontana, A. Flugy, A. Rizzo, G. De Leo, R. Alessandro, Chronic myeloid leukemia-derived exosomes promote tumor growth through an autocrine mechanism, Cell Commun. Sign. CCS 13 (2015) 8, https://doi.org/10.1186/s12964-015-0086-x.

[54] R. Kalluri, V.S. LeBleu, Discovery of double-stranded genomic DNA in circulating exosomes, Cold Spring Harb. Symp. Quant. Biol. 81 (2016) 275–280, https://doi.org/10.1101/sqb.2016.81.030932.

[55] A. Takahashi, R. Okada, K. Nagao, Y. Kawamata, A. Hanyu, S. Yoshimoto, M. Takasugi, S. Watanabe, M.T. Kanemaki, C. Obuse, E. Hara, Exosomes maintain cellular homeostasis by excreting harmful DNA from cells, Nat. Commun. 8 (2017) 15287, , https://doi.org/10.1038/ncomms15287.

[56] M. Demory Beckler, J.N. Higginbotham, J.L. Franklin, A.J. Ham, P.J. Halvey, I.E. Imasuen, C. Whitwell, M. Li, D.C. Liebler, R.J. Coffey, Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS, Mol. Cell. Proteomics 12 (2013) 343–355, https://doi.org/10.1074/mcp.M112.022806.

[57] Y. Yang, C.W. Li, L.C. Chan, Y. Wei, J.M. Hsu, W. Xia, J.H. Cha, J. Hou, J.L. Hsu, L. Sun, M.C. Hung, Exosomal PD-L1 harbors active defense function to suppress T cell killing of breast cancer cells and promote tumor growth, Cell Res. 28 (2018) 862–864, https://doi.org/10.1038/s41422-018-0060-4.

[58] M.S. Pavlyukov, H. Yu, S. Bastola, M. Minata, V.O. Shender, Y. Lee, S. Zhang, J. Wang, S. Komarova, J. Wang, S. Yamaguchi, H.A. Alsheikh, J. Shi, D. Chen, A. Mohyeldin, S.H. Kim, Y.J. Shin, K. Anufrieva, E.G. Evtushenko, N.V. Antipova, G.P. Arapidi, V. Govorun, N.B. Pestov, M.I. Shakhparonov, L.J. Lee, D.H. Nam, I. Nakano, Apoptotic cell-derived extracellular vesicles promote malignancy of glioblastoma via intercellular transfer of splicing factors, Cancer Cell 34 (2018) 119–135 e110, https://doi.org/10.1016/j.ccell.2018.05.012.

[59] J. Skog, T. Wurdinger, S. van Rijn, D.H. Meijer, L. Gainche, M. Sena-Esteves, W.T. Curry Jr., B.S. Carter, A.M. Krichevsky, X.O. Breakefield, Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers, Nat. Cell Biol. 10 (2008) 1470–1476, https://doi.org/10.1038/ncb1800.

[60] M.A. Antonyak, B. Li, L.K. Boroughs, J.L. Johnson, J.E. Druso, K.L. Bryant, D.A. Holowka, R.A. Cerione, Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 4852–4857, https://doi.org/10.1073/pnas.1017667108.

[61] 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) 130–138, https://doi.org/10.3892/ijo.2011.1193.

[62] J. Webber, R. Steadman, M.D. Mason, Z. Tabi, A. Clayton, Cancer exosomes trigger fibroblast to myofibroblast differentiation, Cancer Res. 70 (2010) 9621–9630, https://doi.org/10.1158/0008-5472.CAN-10-1722.

[63] S. Gesierich, I. Berezovskiy, E. Ryschich, M. Zoller, Systemic induction of the angiogenesis switch by the tetraspanin D6.1A/CO-029, Cancer Res. 66 (2006) 7083–7094, https://doi.org/10.1158/0008-5472.CAN-06-0391.

[64] H. Sheldon, E. Heikamp, H. Turley, R. Dragovic, P. Thomas, C.E. Oon, R. Leek, M. Edelmann, B. Kessler, R.C. Sainson, I. Sargent, J.L. Li, A.L. Harris, New mechanism for Notch signaling to endothelium at a distance by Delta-like 4 incorporation into exosomes, Blood 116 (2010) 2385–2394, https://doi.org/10.1182/blood-2009-08-239228.

[65] M.K.S. Tang, P.Y.K. Yue, P.P. Ip, R.L. Huang, H.C. Lai, A.N.Y. Cheung, K.Y. Tse, H.Y.S. Ngan, A.S.T. Wong, Soluble E-cadherin promotes tumor angiogenesis and localizes to exosome surface, Nat. Commun. 9 (2018) 2270, https://doi.org/10.1038/s41467-018-04695-7.

[66] K.J. Svensson, P. Kucharzewska, H.C. Christianson, S. Skold, T. Lofstedt, M.C. Johansson, M. Morgelin, J. Bengzon, W. Ruf, M. Belting, Hypoxia triggers a proangiogenic pathway involving cancer cell microvesicles and PAR-2-mediated heparin-binding EGF signaling in endothelial cells, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13147–13152, https://doi.org/10.1073/pnas.1104261108.

[67] Y.L. Hsu, J.Y. Hung, W.A. Chang, Y.S. Lin, Y.C. Pan, P.H. Tsai, C.Y. Wu, P.L. Kuo, Hypoxic lung cancer-secreted exosomal miR-23a increased angiogenesis and vascular permeability by targeting prolyl hydroxylase and tight junction protein ZO-1, Oncogene 36 (2017) 4929–4942, https://doi.org/10.1038/onc.2017.105.

[68] T. Umezu, H. Tadokoro, K. Azuma, S. Yoshizawa, K. Ohyashiki, J.H. Ohyashiki, Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1, Blood 124 (2014) 3748–3757, https://doi.org/10.1182/blood-2014-05-576116.

[69] B.Y. Nabet, Y. Qiu, J.E. Shabason, T.J. Wu, T. Yoon, B.C. Kim, J.L. Benci, A.M. DeMichele, J. Tchou, J. Marcotrigiano, A.J. Minn, Exosome RNA unshielding couples stromal activation to pattern recognition receptor signaling in cancer, Cell 170 (2017) 352–366 e313, https://doi.org/10.1016/j.cell.2017.06.031.

[70] M. Shimoda, S. Principe, H.W. Jackson, V. Luga, H. Fang, S.D. Molyneux, Y.W. Shao, A. Aiken, P.D. Waterhouse, C. Karamboulas, F.M. Hess, T. Ohtsuka, Y. Okada, L. Ailles, A. Ludwig, J.L. Wrana, T. Kislinger, R. Khokha, Loss of the Timp gene family is sufficient for the acquisition of the CAF-like cell state, Nat. Cell Biol. 16 (2014) 889–901, https://doi.org/10.1038/ncb3021.

[71] A. Achreja, H. Zhao, L. Yang, T.H. Yun, J. Marini, D. Nagrath, Exo-MFA - A 13C metabolic flux analysis framework to dissect tumor microenvironment-secreted exosome contributions towards cancer cell metabolism, Metab. Eng. 43 ((2017) 156–172, https://doi.org/10.1016/j.ymben.2017.01.001.

[72] F. Luciani, M. Spada, A. De Milito, A. Molinari, L. Rivoltini, A. Montinaro, M. Marra, L. Lugini, M. Logozzi, F. Lozupone, C. Federici, E. Iessi, G. Parmiani, G. Arancia, F. Belardelli, S. Fais, Effect of proton pump inhibitor pretreatment on resistance of solid tumors to cytotoxic drugs, J. Natl. Cancer Inst. 96 (2004) 1702–1713, https://doi.org/10.1093/jnci/djh305.

[73] K.G. Chen, J.C. Valencia, B. Lai, G. Zhang, J.K. Paterson, F. Rouzaud, W. Berens, S.M. Wincovitch, S.H. Garfield, R.D. Leapman, V.J. Hearing, M.M. Gottesman, Melanosomal sequestration of cytotoxic drugs contributes to the intractability of malignant melanomas, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 9903–9907, https://doi.org/10.1073/pnas.0600213103.

[74] R. Safaei, B.J. Larson, T.C. Cheng, M.A. Gibson, S. Otani, W. Naerdemann, S.B. Howell, Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells, Mol. Cancer Ther. 4 (2005) 1595–1604, https://doi.org/10.1158/1535-7163.MCT-05-0102.

[75] J. Yin, X. Yan, X. Yao, Y. Zhang, Y. Shan, N. Mao, Y. Yang, L. Pan, Secretion of annexin A3 from ovarian cancer cells and its association with platinum resistance in ovarian cancer patients, J. Cell. Mol. Med. 16 (2012) 337–348, https://doi.org/10.1111/j.1582-4934.2011.01316.x.

[76] M. Bebawy, V. Combes, E. Lee, R. Jaiswal, J. Gong, A. Bonhoure, G.E. Grau, Membrane microparticles mediate transfer of P-glycoprotein to drug sensitive cancer cells, Leukemia 23 (2009) 1643–1649, https://doi.org/10.1038/leu.2009.76.

[77] W.X. Chen, X.M. Liu, M.M. Lv, L. Chen, J.H. Zhao, S.L. Zhong, M.H. Ji, Q. Hu, Z. Luo, J.Z. Wu, J.H. Tang, Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of microRNAs, PLoS ONE 9 (2014) e95240, , https://doi.org/10.1371/journal.pone.0095240.

[78] X. Qin, S. Yu, L. Zhou, M. Shi, Y. Hu, X. Xu, B. Shen, S. Liu, D. Yan, J. Feng, Cisplatin-resistant lung cancer cell-derived exosomes increase cisplatin resistance of recipient cells in exosomal miR-100-5p-dependent manner, Int. J. Nanomedicine 12 (2017) 3721–3733, https://doi.org/10.2147/IJN.S131516.

[79] 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, https://doi.org/10.1016/j.gene.2017.04.031.

[80] L. Qu, J. Ding, C. Chen, Z.J. Wu, B. Liu, Y. Gao, W. Chen, F. Liu, W. Sun, X.F. Li, X. Wang, Y. Wang, Z.Y. Xu, L. Gao, Q. Yang, B. Xu, Y.M. Li, Z.Y. Fang, Z.P. Xu, Y. Bao, D.S. Wu, X. Miao, H.Y. Sun, Y.H. Sun, H.Y. Wang, L.H. Wang, Exosome-Transmitted lncARSR Promotes Sunitinib Resistance in Renal Cancer by Acting as a Competing Endogenous RNA, Cancer Cell 29 (2016) 653–668, https://doi.org/10.1016/j.ccell.2016.03.004.

[81] 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, , https://doi.org/10.1080/2162402X.2017.1362530.

[82] M.C. Boelens, T.J. Wu, B.Y. Nabet, B. Xu, Y. Qiu, T. Yoon, D.J. Azzam, C. Twyman-Saint Victor, B.Z. Wiemann, H. Ishwaran, P.J. Ter Brugge, J. Jonkers, J. Slingerland, A.J. Minn, Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways, Cell 159 (2014) 499–513, https://doi.org/10.1016/j.cell.2014.09.051.

[83] Y. Binenbaum, E. Fridman, Z. Yaari, N. Milman, A. Schroeder, G. Ben David, T. Shlomi, Z. Gil, Transfer of miRNA in macrophage-derived exosomes induces drug resistance in pancreatic adenocarcinoma, Cancer Res. 78 (2018) 5287–5299, https://doi.org/10.1158/0008-5472.CAN-18-0124.

[84] N.M. Namee, L. O'Driscoll, Extracellular vesicles and anti-cancer drug resistance, Biochim. Biophys. Acta Rev. Cancer 1870 (2018) 123–136, https://doi.org/10.

1016/j.bbcan.2018.07.003.
[85] B.H. Sung, T. Ketova, D. Hoshino, A. Zijlstra, A.M. Weaver, Directional cell movement through tissues is controlled by exosome secretion, Nat. Commun. 6 (2015) 7164, https://doi.org/10.1038/ncomms8164.
[86] V. Luga, L. Zhang, A.M. Viloria-Petit, A.A. Ogunjimi, M.R. Inanlou, E. Chiu, M. Buchanan, A.N. Hosein, M. Basik, J.L. Wrana, Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration, Cell 151 (2012) 1542–1556, https://doi.org/10.1016/j.cell.2012.11.024.
[87] S. Yue, W. Mu, U. Erb, M. Zoller, The tetraspanins CD151 and Tspan8 are essential exosome components for the crosstalk between cancer initiating cells and their surrounding, Oncotarget (6) (2015) 2366–2384, https://doi.org/10.18632/oncotarget.2958.
[88] A. Hendrix, D. Maynard, P. Pauwels, G. Braems, H. Denys, R. Van den Broecke, J. Lambert, S. Van Belle, V. Cocquyt, C. Gespach, M. Bracke, M.C. Seabra, W.A. Gahl, O. De Wever, W. Westbroek, Effect of the secretory small GTPase Rab27B on breast cancer growth, invasion, and metastasis, J. Natl. Cancer Inst. 102 (2010) 866–880, https://doi.org/10.1093/jnci/djq153.
[89] W. Zhou, M.Y. Fong, Y. Min, G. Somlo, L. Liu, M.R. Palomares, Y. Yu, A. Chow, S.T. O'Connor, A.R. Chin, Y. Yen, Y. Wang, E.G. Marcusson, P. Chu, J. Wu, X. Wu, A.X. Li, Z. Li, H. Gao, X. Ren, M.P. Boldin, P.C. Lin, S.E. Wang, Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis, Cancer Cell 25 (2014) 501–515, https://doi.org/10.1016/j.ccr.2014.03.007.
[90] M. Aga, G.L. Bentz, S. Raffa, M.R. Torrisi, S. Kondo, N. Wakisaka, T. Yoshizaki, J.S. Pagano, J. Shackelford, Exosomal HIF1alpha supports invasive potential of nasopharyngeal carcinoma-associated LMP1-positive exosomes, Oncogene 33 (2014) 4613–4622, https://doi.org/10.1038/onc.2014.66.
[91] Y. You, Y. Shan, J. Chen, H. Yue, B. You, S. Shi, X. Li, X. Cao, Matrix metalloproteinase 13-containing exosomes promote nasopharyngeal carcinoma metastasis, Cancer Sci. 106 (2015) 1669–1677, https://doi.org/10.1111/cas.12818.
[92] D.K. Jeppesen, A. Nawrocki, S.G. Jensen, K. Thorsen, B. Whitehead, K.A. Howard, L. Dyrskjot, T.F. Orntoft, M.R. Larsen, M.S. Ostenfeld, 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 (2014) 699–712, https://doi.org/10.1002/pmic.201300452.
[93] T. Yoshizaki, S. Kondo, N. Wakisaka, S. Murono, K. Endo, H. Sugimoto, S. Nakanishi, A. Tsuji, M. Ito, Pathogenic role of Epstein-Barr virus latent membrane protein-1 in the development of nasopharyngeal carcinoma, Cancer Lett. 337 (2013) 1–7, https://doi.org/10.1016/j.canlet.2013.05.018.
[94] M.S. Ostenfeld, D.K. Jeppesen, J.R. Laurberg, A.T. Boysen, J.B. Bramsen, B. Primdal-Bengtson, A. Hendrix, P. Lamy, F. Dagnaes-Hansen, M.H. Rasmussen, K.H. Bui, N. Fristrup, E.I. Christensen, I. Nordentoft, J.P. Morth, J.B. Jensen, J.S. Pedersen, M. Beck, D. Theodorescu, M. Borre, K.A. Howard, L. Dyrskjot, T.F. Orntoft, Cellular disposal of miR23b by RAB27-dependent exosome release is linked to acquisition of metastatic properties, Cancer Res. 74 (2014) 5758–5771, https://doi.org/10.1158/0008-5472.CAN-13-3512.
[95] N. Tominaga, N. Kosaka, M. Ono, T. Katsuda, Y. Yoshioka, K. Tamura, J. Lotvall, H. Nakagama, T. Ochiya, Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood-brain barrier, Nat. Commun. 6 (2015) 6716, https://doi.org/10.1038/ncomms7716.
[96] J.L. Hood, R.S. San, S.A. Wickline, Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis, Cancer Res. 71 (2011) 3792–3801, https://doi.org/10.1158/0008-5472.CAN-10-4455.
[97] S. Rana, K. Malinowska, M. Zoller, Exosomal tumor microRNA modulates premetastatic organ cells, Neoplasia 15 (2013) 281–295.
[98] T. Karlsson, M. Lundholm, A. Widmark, E. Persson, Tumor cell-derived exosomes from the prostate cancer cell line TRAMP-C1 impair osteoclast formation and differentiation, PLoS ONE 11 (2016) e0166284, , https://doi.org/10.1371/journal.pone.0166284.
[99] Y. Ye, S.L. Li, Y.Y. Ma, Y.J. Diao, L. Yang, M.Q. Su, Z. Li, Y. Ji, J. Wang, L. Lei, W.X. Fan, L.X. Li, Y. Xu, X.K. Hao, Exosomal miR-141-3p regulates osteoblast activity to promote the osteoblastic metastasis of prostate cancer, Oncotarget 8 (2017) 94834–94849, https://doi.org/10.18632/oncotarget.22014.
[100] T. Jung, D. Castellana, P. Klingbeil, I. Cuesta Hernandez, M. Vitacolonna, D.J. Orlicky, S.R. Roffler, P. Brodt, M. Zoller, CD44v6 dependence of premetastatic niche preparation by exosomes, Neoplasia 11 (2009) 1093–1105.
[101] C. Grange, M. Tapparo, F. Collino, L. Vitillo, C. Damasco, M.C. Deregibus, C. Tetta, B. Bussolati, G. Camussi, Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche, Cancer Res. 71 (2011) 5346–5356, https://doi.org/10.1158/0008-5472.CAN-11-0241.
[102] H. Peinado, M. Aleckovic, S. Lavotshkin, I. Matei, B. Costa-Silva, G. Moreno-Bueno, M. Hergueta-Redondo, C. Williams, G. Garcia-Santos, C. Ghajar, A. Nitadori-Hoshino, C. Hoffman, K. Badal, B.A. Garcia, M.K. Callahan, J. Yuan, V.R. Martins, J. Skog, R.N. Kaplan, M.S. Brady, J.D. Wolchok, P.B. Chapman, Y. Kang, J. Bromberg, D. Lyden, Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET, Nat. Med. 18 (2012) 883–891, https://doi.org/10.1038/nm.2753.
[103] B. Costa-Silva, N.M. Aiello, A.J. Ocean, S. Singh, H. Zhang, B.K. Thakur, A. Becker, A. Hoshino, M.T. Mark, H. Molina, J. Xiang, T. Zhang, T.M. Theilen, G. Garcia-Santos, C. Williams, Y. Ararso, Y. Huang, G. Rodrigues, T.L. Shen, K.J. Labori, I.M. Lothe, E.H. Kure, J. Hernandez, A. Doussot, S.H. Ebbesen, P.M. Grandgenett, M.A. Hollingsworth, M. Jain, K. Mallya, S.K. Batra, W.R. Jarnagin, R.E. Schwartz, I. Matei, H. Peinado, B.Z. Stanger, J. Bromberg, D. Lyden, Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver, Nat. Cell Biol. 17 (2015) 816–826, https://doi.org/10.1038/ncb3169.
[104] A. Hoshino, B. Costa-Silva, T.L. Shen, G. Rodrigues, A. Hashimoto, M. Tesic Mark, H. Molina, S. Kohsaka, A. Di Giannatale, S. Ceder, S. Singh, C. Williams, N. Soplop, K. Uryu, L. Pharmer, T. King, L. Bojmar, A.E. Davies, Y. Ararso, T. Zhang, H. Zhang, J. Hernandez, J.M. Weiss, V.D. Dumont-Cole, K. Kramer, L.H. Wexler, A. Narendran, G.K. Schwartz, J.H. Healey, P. Sandstrom, K.J. Labori, E.H. Kure, P.M. Grandgenett, M.A. Hollingsworth, M. de Sousa, S. Kaur, M. Jain, K. Mallya, S.K. Batra, W.R. Jarnagin, M.S. Brady, O. Fodstad, V. Muller, K. Pantel, A.J. Minn, M.J. Bissell, B.A. Garcia, Y. Kang, V.K. Rajasekhar, C.M. Ghajar, I. Matei, H. Peinado, J. Bromberg, D. Lyden, Tumour exosome integrins determine organotropic metastasis, Nature 527 (2015) 329–335, https://doi.org/10.1038/nature15756.
[105] Y. Liu, Y. Gu, Y. Han, Q. Zhang, Z. Jiang, X. Zhang, B. Huang, X. Xu, J. Zheng, X. Cao, Tumor exosomal RNAs promote lung pre-metastatic niche formation by activating alveolar epithelial TLR3 to recruit neutrophils, Cancer Cell 30 (2016) 243–256, https://doi.org/10.1016/j.ccell.2016.06.021.
[106] M.Y. Fong, W. Zhou, L. Liu, A.Y. Alontaga, M. Chandra, J. Ashby, A. Chow, S.T. O'Connor, S. Li, A.R. Chin, G. Somlo, M. Palomares, Z. Li, J.R. Tremblay, A. Tsuyada, G. Sun, M.A. Reid, X. Wu, P. Swiderski, X. Ren, Y. Shi, M. Kong, W. Zhong, Y. Chen, S.E. Wang, Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis, Nat. Cell Biol. 17 (2015) 183–194, https://doi.org/10.1038/ncb3094.
[107] M.P. Plebanek, N.L. Angeloni, E. Vinokour, J. Li, A. Henkin, D. Martinez-Marin, S. Filleur, R. Bhowmick, J. Henkin, S.D. Miller, I. Ifergan, Y. Lee, I. Osman, C.S. Thaxton, O.V. Volpert, Pre-metastatic cancer exosomes induce immune surveillance by patrolling monocytes at the metastatic niche, Nat. Commun. 8 (2017) 1319, https://doi.org/10.1038/s41467-017-01433-3.
[108] K. Valencia, D. Luis-Ravelo, N. Bovy, I. Anton, S. Martinez-Canarias, C. Zandueta, C. Ormazabal, I. Struman, S. Tabruyn, V. Rebmann, J. De Las Rivas, E. Guruceaga, E. Bandres, F. Lecanda, miRNA cargo within exosome-like vesicle transfer influences metastatic bone colonization, Mol. Oncol. 8 (2014) 689–703, https://doi.org/10.1016/j.molonc.2014.01.012.
[109] 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, https://doi.org/10.1126/scisignal.2005231.
[110] I.J. Fidler, The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited, Nat. Rev. Cancer 3 (2003) 453–458, https://doi.org/10.1038/nrc1098.
[111] J. Wolfers, A. Lozier, G. Raposo, A. Regnault, C. Thery, C. Masurier, C. Flament, S. Pouzieux, F. Faure, T. Tursz, E. Angevin, S. Amigorena, L. Zitvogel, Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming, Nat. Med. 7 (2001) 297–303, https://doi.org/10.1038/85438.
[112] S. Dai, T. Wan, B. Wang, X. Zhou, F. Xiu, T. Chen, Y. Wu, X. Cao, More efficient induction of HLA-A*0201-restricted and carcinoembryonic antigen (CEA)-specific CTL response by immunization with exosomes prepared from heat-stressed CEA-positive tumor cells, Clin. Cancer Res. Offi. J. Am. Asso. Cancer Res. 11 (2005) 7554–7563, https://doi.org/10.1158/1078-0432.CCR-05-0810.
[113] S. Dai, X. Zhou, B. Wang, Q. Wang, Y. Fu, T. Chen, T. Wan, Y. Yu, X. Cao, Enhanced induction of dendritic cell maturation and HLA-A*0201-restricted CEA-specific CD8(+) CTL response by exosomes derived from IL-18 gene-modified CEA-positive tumor cells, J. Mol. Med. 84 (2006) 1067–1076, https://doi.org/10.1007/s00109-006-0102-0.
[114] H.G. Zhang, X. Zhuang, D. Sun, Y. Liu, X. Xiang, W.E. Grizzle, Exosomes and immune surveillance of neoplastic lesions: a review, Biotech. Histochem. Offi. Publ. Biol. Stain Comm. 87 (2012) 161–168, https://doi.org/10.3109/10520291003659042.
[115] P. Filipazzi, M. Burdek, A. Villa, L. Rivoltini, V. Huber, Recent advances on the role of tumor exosomes in immunosuppression and disease progression, Semin. Cancer Biol. 22 (2012) 342–349, https://doi.org/10.1016/j.semcancer.2012.02.005.
[116] S.L. Altieri, A.N. Khan, T.B. Tomasi, Exosomes from plasmacytoma cells as a tumor vaccine, J. Immunother. 27 (2004) 282–288.
[117] R. Gastpar, M. Gehrmann, M.A. Bausero, A. Asea, C. Gross, J.A. Schroeder, G. Multhoff, Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells, Cancer Res. 65 (2005) 5238–5247, https://doi.org/10.1158/0008-5472.CAN-04-3804.
[118] V.L. Vega, M. Rodriguez-Silva, T. Frey, M. Gehrmann, J.C. Diaz, C. Steinem, G. Multhoff, N. Arispe, A. De Maio, Hsp70 translocates into the plasma membrane after stress and is released into the extracellular environment in a membrane-associated form that activates macrophages, J. Immunol. 180 (2008) 4299–4307.
[119] J.A. Cho, Y.S. Lee, S.H. Kim, J.K. Ko, C.W. Kim, MHC independent anti-tumor immune responses induced by Hsp70-enriched exosomes generate tumor regression in murine models, Cancer Lett. 275 (2009) 256–265, https://doi.org/10.1016/j.canlet.2008.10.021.
[120] W. Li, D. Mu, F. Tian, Y. Hu, T. Jiang, Y. Han, J. Chen, G. Han, X. Li, Exosomes derived from Rab27aoverexpressing tumor cells elicit efficient induction of anti-tumor immunity, Mol. Med. Rep. 8 (2013) 1876–1882, https://doi.org/10.3892/mmr.2013.1738.
[121] F. Andre, N.E. Schartz, M. Movassagh, C. Flament, P. Pautier, P. Morice, C. Pomel, C. Lhomme, B. Escudier, T. Le Chevalier, T. Tursz, S. Amigorena, G. Raposo, E. Angevin, L. Zitvogel, Malignant effusions and immunogenic tumour-derived exosomes, Lancet 360 (2002) 295–305, https://doi.org/10.1016/S0140-6736(02)09552-1.
[122] T. Moroishi, T. Hayashi, W.W. Pan, Y. Fujita, M.V. Holt, J. Qin, D.A. Carson, K.L. Guan, The hippo pathway kinases LATS1/2 suppress cancer immunity, Cell 167 (2016) 1525–1539 e1517, https://doi.org/10.1016/j.cell.2016.11.005.
[123] Y. Kitai, T. Kawasaki, T. Sueyoshi, K. Kobiyama, K.J. Ishii, J. Zou, S. Akira, T. Matsuda, T. Kawai, DNA-containing exosomes derived from cancer cells treated with topotecan activate a STING-dependent pathway and reinforce antitumor

immunity, J. Immunol. 198 (2017) 1649–1659, https://doi.org/10.4049/jimmunol.1601694.
[124] M.R. Muhsin-Sharafaldine, S.C. Saunderson, A.C. Dunn, J.M. Faed, T. Kleffmann, A.D. McLellan, Procoagulant and immunogenic properties of melanoma exosomes, microvesicles and apoptotic vesicles, Oncotarget 7 (2016) 56279–56294, https://doi.org/10.18632/oncotarget.10783.
[125] A. Bobrie, M. Colombo, G. Raposo, C. Thery, Exosome secretion: molecular mechanisms and roles in immune responses, Traffic 12 (2011) 1659–1668, https://doi.org/10.1111/j.1600-0854.2011.01225.x.
[126] M. Lundholm, M. Schroder, O. Nagaeva, V. Baranov, A. Widmark, L. Mincheva-Nilsson, P. Wikstrom, Prostate tumor-derived exosomes down-regulate NKG2D expression on natural killer cells and CD8+ T cells: mechanism of immune evasion, PLoS ONE 9 (2014) e108925, , https://doi.org/10.1371/journal.pone.0108925.
[127] A. Clayton, J.P. Mitchell, J. Court, S. Linnane, M.D. Mason, Z. Tabi, Human tumor-derived exosomes down-modulate NKG2D expression, J. Immunol. 180 (2008) 7249–7258.
[128] M. Iero, R. Valenti, V. Huber, P. Filipazzi, G. Parmiani, S. Fais, L. Rivoltini, Tumour-released exosomes and their implications in cancer immunity, Cell Death Differ. 15 (2008) 80–88, https://doi.org/10.1038/sj.cdd.4402237.
[129] A.J. Abusamra, Z. Zhong, X. Zheng, M. Li, T.E. Ichim, J.L. Chin, W.P. Min, Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis, Blood Cells Mol. Dis. 35 (2005) 169–173, https://doi.org/10.1016/j.bcmd.2005.07.001.
[130] J. Klibi, T. Niki, A. Riedel, C. Pioche-Durieu, S. Souquere, E. Rubinstein, S. Le Moulec, J. Guigay, M. Hirashima, F. Guemira, D. Adhikary, J. Mautner, P. Busson, Blood diffusion and Th1-suppressive effects of galectin-9-containing exosomes released by Epstein-Barr virus-infected nasopharyngeal carcinoma cells, Blood 113 (2009) 1957–1966, https://doi.org/10.1182/blood-2008-02-142596.
[131] A. Soderberg, A.M. Barral, M. Soderstrom, B. Sander, A. Rosen, Redox-signaling transmitted in trans to neighboring cells by melanoma-derived TNF-containing exosomes, Free Radic. Biol. Med. 43 (2007) 90–99, https://doi.org/10.1016/j.freeradbiomed.2007.03.026.
[132] A. Clayton, J.P. Mitchell, J. Court, M.D. Mason, Z. Tabi, Human tumor-derived exosomes selectively impair lymphocyte responses to interleukin-2, Cancer Res. 67 (2007) 7458–7466, https://doi.org/10.1158/0008-5472.CAN-06-3456.
[133] F.L. Ricklefs, Q. Alayo, H. Krenzlin, A.B. Mahmoud, M.C. Speranza, H. Nakashima, J.L. Hayes, K. Lee, L. Balaj, C. Passaro, A.K. Rooj, S. Krasemann, B.S. Carter, C.C. Chen, T. Steed, J. Treiber, S. Rodig, K. Yang, I. Nakano, H. Lee, R. Weissleder, X.O. Breakefield, J. Godlewski, M. Westphal, K. Lamszus, G.J. Freeman, A. Bronisz, S.E. Lawler, E.A. Chiocca, Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles, Sci. Adv. 4 (2018) eaar2766, , https://doi.org/10.1126/sciadv.aar2766.
[134] G. Chen, A.C. Huang, W. Zhang, G. Zhang, M. Wu, W. Xu, Z. Yu, J. Yang, B. Wang, H. Sun, H. Xia, Q. Man, W. Zhong, L.F. Antelo, B. Wu, X. Xiong, X. Liu, L. Guan, T. Li, S. Liu, R. Yang, Y. Lu, L. Dong, S. McGettigan, R. Somasundaram, R. Radhakrishnan, G. Mills, Y. Lu, J. Kim, Y.H. Chen, H. Dong, Y. Zhao, G.C. Karakousis, T.C. Mitchell, L.M. Schuchter, M. Herlyn, E.J. Wherry, X. Xu, W. Guo, Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response, Nature 560 (2018) 382–386, https://doi.org/10.1038/s41586-018-0392-8.
[135] M. Zhou, J. Chen, L. Zhou, W. Chen, G. Ding, L. Cao, Pancreatic cancer derived exosomes regulate the expression of TLR4 in dendritic cells via miR-203, Cell. Immunol. 292 (2014) 65–69, https://doi.org/10.1016/j.cellimm.2014.09.004.
[136] Y. Ning, K. Shen, Q. Wu, X. Sun, Y. Bai, Y. Xie, J. Pan, C. Qi, Tumor exosomes block dendritic cells maturation to decrease the T cell immune response, Immunol. Lett. 199 (2018) 36–43, https://doi.org/10.1016/j.imlet.2018.05.002.
[137] X. Xiang, A. Poliakov, C. Liu, Y. Liu, Z.B. Deng, J. Wang, Z. Cheng, S.V. Shah, G.J. Wang, L. Zhang, W.E. Grizzle, J. Mobley, H.G. Zhang, Induction of myeloid-derived suppressor cells by tumor exosomes, Int. J. Cancer 124 (2009) 2621–2633, https://doi.org/10.1002/ijc.24249.
[138] Y. Liu, X. Xiang, X. Zhuang, S. Zhang, C. Liu, Z. Cheng, S. Michalek, W. Grizzle, H.G. Zhang, Contribution of MyD88 to the tumor exosome-mediated induction of myeloid derived suppressor cells, Am. J. Pathol. 176 (2010) 2490–2499, https://doi.org/10.2353/ajpath.2010.090777.
[139] F. Chalmin, S. Ladoire, G. Mignot, J. Vincent, M. Bruchard, J.P. Remy-Martin, W. Boireau, A. Rouleau, B. Simon, D. Lanneau, A. De Thonel, G. Multhoff, A. Hamman, F. Martin, B. Chauffert, E. Solary, L. Zitvogel, C. Garrido, B. Ryffel, C. Borg, L. Apetoh, C. Rebe, F. Ghiringhelli, Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells, J. Clin. Invest. 120 (2010) 457–471, https://doi.org/10.1172/JCI40483.
[140] J. Diao, X. Yang, X. Song, S. Chen, Y. He, Q. Wang, G. Chen, C. Luo, X. Wu, Y. Zhang, Exosomal Hsp70 mediates immunosuppressive activity of the myeloid-derived suppressor cells via phosphorylation of Stat3, Med. Oncol. 32 (2015) 453, https://doi.org/10.1007/s12032-014-0453-2.
[141] K. Ridder, A. Sevko, J. Heide, M. Dams, A.K. Rupp, J. Macas, J. Starmann, M. Tjwa, K.H. Plate, H. Sultmann, P. Altevogt, V. Umansky, S. Momma, Extracellular vesicle-mediated transfer of functional RNA in the tumor microenvironment, Oncoimmunology 4 (2015) e1008371, , https://doi.org/10.1080/2162402X.2015.1008371.
[142] X. Guo, W. Qiu, Q. Liu, M. Qian, S. Wang, Z. Zhang, X. Gao, Z. Chen, H. Xue, G. Li, Immunosuppressive effects of hypoxia-induced glioma exosomes through myeloid-derived suppressor cells via the miR-10a/Rora and miR-21/Pten Pathways, Oncogene 37 (2018) 4239–4259, https://doi.org/10.1038/s41388-018-0261-9.
[143] S. Ham, L.G. Lima, E.P.Z. Chai, A. Muller, R.J. Lobb, S. Krumeich, S.W. Wen, A.P. Wiegmans, A. Moller, Breast Cancer-Derived Exosomes Alter Macrophage Polarization via gp130/STAT3 Signaling, Front. Immunol. 9 (2018) 871, https://doi.org/10.3389/fimmu.2018.00871.
[144] C.H. Hsieh, S.K. Tai, M.H. Yang, Snail-overexpressing cancer cells promote M2-like polarization of tumor-associated macrophages by delivering MiR-21-abundant exosomes, Neoplasia 20 (2018) 775–788, https://doi.org/10.1016/j.neo.2018.06.004.
[145] X. Wang, G. Luo, K. Zhang, J. Cao, C. Huang, T. Jiang, B. Liu, L. Su, Z. Qiu, Hypoxic tumor-derived exosomal miR-301a mediates M2 macrophage polarization via PTEN/PI3Kgamma to promote pancreatic cancer metastasis, Cancer Res. 78 (2018) 4586–4598, https://doi.org/10.1158/0008-5472.CAN-17-3841.
[146] T. Cooks, I.S. Pateras, L.M. Jenkins, K.M. Patel, A.I. Robles, J. Morris, T. Forshew, E. Appella, V.G. Gorgoulis, C.C. Harris, Mutant p53 cancers reprogram macrophages to tumor supporting macrophages via exosomal miR-1246, Nat. Commun. 9 (2018) 771, https://doi.org/10.1038/s41467-018-03224-w.
[147] L. Gao, L. Wang, T. Dai, K. Jin, Z. Zhang, S. Wang, F. Xie, P. Fang, B. Yang, H. Huang, H. van Dam, F. Zhou, L. Zhang, Tumor-derived exosomes antagonize innate antiviral immunity, Nat. Immunol. 19 (2018) 233–245, https://doi.org/10.1038/s41590-017-0043-5.
[148] F. Wang, B. Li, Y. Wei, Y. Zhao, L. Wang, P. Zhang, J. Yang, W. He, H. Chen, Z. Jiao, Y. Li, Tumor-derived exosomes induce PD1(+) macrophage population in human gastric cancer that promotes disease progression, Oncogenesis 7 (2018) 41, https://doi.org/10.1038/s41389-018-0049-3.
[149] K. Gabrusiewicz, X. Li, J. Wei, Y. Hashimoto, A.L. Marisetty, M. Ott, F. Wang, D. Hawke, J. Yu, L.M. Healy, A. Hossain, J.C. Akers, S.N. Maiti, S. Yamashita, Y. Shimizu, K. Dunner, M.A. Zal, J.K. Burks, J. Gumin, F. Nwajei, A. Rezavanian, S. Zhou, G. Rao, R. Sawaya, G.N. Fuller, J.T. Huse, J.P. Antel, S. Li, L. Cooper, E.P. Sulman, C. Chen, C. Geula, R. Kalluri, T. Zal, A.B. Heimberger, Glioblastoma stem cell-derived exosomes induce M2 macrophages and PD-L1 expression on human monocytes, Oncoimmunology 7 (2018) e1412909, , https://doi.org/10.1080/2162402X.2017.1412909.
[150] M. Xiao, J. Zhang, W. Chen, W. Chen, M1-like tumor-associated macrophages activated by exosome-transferred THBS1 promote malignant migration in oral squamous cell carcinoma, J. Exp. Clin. Cancer Res. 37 (2018) 143, https://doi.org/10.1186/s13046-018-0815-2.
[151] A. Marton, C. Vizler, E. Kusz, V. Temesfoi, Z. Szathmary, K. Nagy, Z. Szegletes, G. Varo, L. Siklos, R.L. Katona, V. Tubak, O.M. Howard, E. Duda, J. Minarovits, K. Nagy, K. Buzas, Melanoma cell-derived exosomes alter macrophage and dendritic cell functions in vitro, Immunol. Lett. 148 (2012) 34–38, https://doi.org/10.1016/j.imlet.2012.07.006.
[152] A. Chow, W. Zhou, L. Liu, M.Y. Fong, J. Champer, D. Van Haute, A.R. Chin, X. Ren, B.G. Gugiu, Z. Meng, W. Huang, V. Ngo, M. Kortylewski, S.E. Wang, Macrophage immunomodulation by breast cancer-derived exosomes requires Toll-like receptor 2-mediated activation of NF-kappaB, Sci. Rep. 4 (2014) 5750, https://doi.org/10.1038/srep05750.
[153] K.B. Johnsen, J.M. Gudbergsson, M.N. Skov, L. Pilgaard, T. Moos, M. Duroux, A comprehensive overview of exosomes as drug delivery vehicles - endogenous nanocarriers for targeted cancer therapy, Biochim. Biophys. Acta 1846 (2014) 75–87, https://doi.org/10.1016/j.bbcan.2014.04.005.
[154] 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, https://doi.org/10.1016/j.biomaterials.2013.11.083.
[155] L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhal, M.J. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes, Nat. Biotechnol. 29 (2011) 341–345, https://doi.org/10.1038/nbt.1807.
[156] M. Zhang, E. Viennois, C. Xu, D. Merlin, Plant derived edible nanoparticles as a new therapeutic approach against diseases, Tissue Barriers 4 (2016) e1134415, , https://doi.org/10.1080/21688370.2015.1134415.
[157] Q. Wang, X. Zhuang, J. Mu, Z.B. Deng, H. Jiang, L. Zhang, X. Xiang, B. Wang, J. Yan, D. Miller, H.G. Zhang, Delivery of therapeutic agents by nanoparticles made of grapefruit-derived lipids, Nat. Commun. 4 (2013) 1867, https://doi.org/10.1038/ncomms2886.
[158] M. Bourderioux, T. Nguyen-Khoa, C. Chhuon, L. Jeanson, D. Tondelier, M. Walczak, M. Ollero, S. Bekri, B. Knebelmann, E. Escudier, B. Escudier, A. Edelman, I.C. Guerrera, A new workflow for proteomic analysis of urinary exosomes and assessment in cystinuria patients, J. Proteome Res. 14 (2015) 567–577, https://doi.org/10.1021/pr501003q.
[159] M.A. Morse, J. Garst, T. Osada, S. Khan, A. Hobeika, T.M. Clay, N. Valente, R. Shreeniwas, M.A. Sutton, A. Delcayre, D.H. Hsu, J.B. Le Pecq, H.K. Lyerly, A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer, J. Transl. Med. 3 (2005) 9, https://doi.org/10.1186/1479-5876-3-9.
[160] S. Dai, D. Wei, Z. Wu, X. Zhou, X. Wei, H. Huang, G. Li, Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer, Mol. Ther. 16 (2008) 782–790, https://doi.org/10.1038/mt.2008.1.
[161] B. Escudier, T. Dorval, N. Chaput, F. Andre, M.P. Caby, S. Novault, C. Flament, C. Leboulaire, C. Borg, S. Amigorena, C. Boccaccio, C. Bonnerot, O. Dhellin, M. Movassagh, S. Piperno, C. Robert, V. Serra, N. Valente, J.B. Le Pecq, A. Spatz, O. Lantz, T. Tursz, E. Angevin, L. Zitvogel, Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial, J. Transl. Med. 3 (2005) 10, https://doi.org/10.1186/1479-5876-3-10.
[162] B. Besse, M. Charrier, V. Lapierre, E. Dansin, O. Lantz, D. Planchard, T. Le Chevalier, A. Livartoski, F. Barlesi, A. Laplanche, S. Ploix, N. Vimond, I. Peguillet, C. Thery, L. Lacroix, I. Zoernig, K. Dhodapkar, M. Dhodapkar, S. Viaud, J.C. Soria, K.S. Reiners, E. Pogge von Strandmann, F. Vely, S. Rusakiewicz, A. Eggermont,

J.M. Pitt, L. Zitvogel, N. Chaput, Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC, Oncoimmunology 5 (2016) e1071008, , https://doi.org/10.1080/2162402X.2015.1071008.

[163] A.M. Marleau, C.S. Chen, J.A. Joyce, R.H. Tullis, Exosome removal as a therapeutic adjuvant in cancer, J. Transl. Med. 10 (2012) 134, https://doi.org/10.1186/1479-5876-10-134.

[164] A. Datta, H. Kim, L. McGee, A.E. Johnson, S. Talwar, J. Marugan, N. Southall, X. Hu, M. Lal, D. Mondal, M. Ferrer, A.B. Abdel-Mageed, High-throughput screening identified selective inhibitors of exosome biogenesis and secretion: A drug repurposing strategy for advanced cancer, Sci. Rep. 8 (2018) 8161, https://doi.org/10.1038/s41598-018-26411-7.

[165] T. Lener, M. Gimona, L. Aigner, V. Borger, E. Buzas, G. Camussi, N. Chaput, D. Chatterjee, F.A. Court, H.A. Del Portillo, L. O'Driscoll, S. Fais, J.M. Falcon-Perez, U. Felderhoff-Mueser, L. Fraile, Y.S. Gho, A. Gorgens, R.C. Gupta, A. Hendrix, D.M. Hermann, A.F. Hill, F. Hochberg, P.A. Horn, D. de Kleijn, L. Kordelas, B.W. Kramer, E.M. Kramer-Albers, S. Laner-Plamberger, S. Laitinen, T. Leonardi, M.J. Lorenowicz, S.K. Lim, J. Lotvall, C.A. Maguire, A. Marcilla, I. Nazarenko, T. Ochiya, T. Patel, S. Pedersen, G. Pocsfalvi, S. Pluchino, P. Quesenberry, I.G. Reischl, F.J. Rivera, R. Sanzenbacher, K. Schallmoser, I. Slaper-Cortenbach, D. Strunk, T. Tonn, P. Vader, B.W. van Balkom, M. Wauben, S.E. Andaloussi, C. Thery, E. Rohde, B. Giebel, Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper, J. Extracell. Vesicles 4 (2015) 30087, , https://doi.org/10.3402/jev.v4.30087.

[166] S. Yang, S.P. Che, P. Kurywchak, J.L. Tavormina, L.B. Gansmo, P. Correa de Sampaio, M. Tachezy, M. Bockhorn, F. Gebauer, A.R. Haltom, S.A. Melo, V.S. LeBleu, R. Kalluri, Detection of mutant KRAS and TP53 DNA in circulating exosomes from healthy individuals and patients with pancreatic cancer, Cancer Biol. Ther. 18 (2017) 158–165, https://doi.org/10.1080/15384047.2017.1281499.

[167] M.N. Theodoraki, S.S. Yerneni, T.K. Hoffmann, W.E. Gooding, T.L. Whiteside, Clinical significance of PD-L1(+) exosomes in plasma of head and neck cancer patients, Clin. Cancer Res. 24 (2018) 896–905, https://doi.org/10.1158/1078-0432.CCR-17-2664.

[168] S. Ludwig, T. Floros, M.N. Theodoraki, C.S. Hong, E.K. Jackson, S. Lang, T.L. Whiteside, Suppression of lymphocyte functions by plasma exosomes correlates with disease activity in patients with head and neck cancer, Clin. Cancer Res. 23 (2017) 4843–4854, https://doi.org/10.1158/1078-0432.CCR-16-2819.

[169] M. An, I. Lohse, Z. Tan, J. Zhu, J. Wu, H. Kurapati, M.A. Morgan, T.S. Lawrence, K.C. Cuneo, D.M. Lubman, Quantitative proteomic analysis of serum exosomes from patients with locally advanced pancreatic cancer undergoing chemoradiotherapy, J. Proteome Res. 16 (2017) 1763–1772, https://doi.org/10.1021/acs.jproteome.7b00024.

[170] A. Becker, B.K. Thakur, J.M. Weiss, H.S. Kim, H. Peinado, D. Lyden, Extracellular vesicles in cancer: Cell-to-Cell mediators of metastasis, Cancer Cell 30 (2016) 836–848, https://doi.org/10.1016/j.ccell.2016.10.009.

[171] T. Goto, M. Fujiya, H. Konishi, J. Sasajima, S. Fujibayashi, A. Hayashi, T. Utsumi, H. Sato, T. Iwama, M. Ijiri, A. Sakatani, K. Tanaka, Y. Nomura, N. Ueno, S. Kashima, K. Moriichi, Y. Mizukami, Y. Kohgo, T. Okumura, An elevated expression of serum exosomal microRNA-191, - 21, -451a of pancreatic neoplasm is considered to be efficient diagnostic marker, BMC Cancer 18 (2018) 116, https://doi.org/10.1186/s12885-018-4006-5.

[172] J.C. Akers, V. Ramakrishnan, R. Kim, J. Skog, I. Nakano, S. Pingle, J. Kalinina, W. Hua, S. Kesari, Y. Mao, X.O. Breakefield, F.H. Hochberg, E.G. Van Meir, B.S. Carter, C.C. Chen, MiR-21 in the extracellular vesicles (EVs) of cerebrospinal fluid (CSF): a platform for glioblastoma biomarker development, PLoS ONE 8 (2013) e78115, , https://doi.org/10.1371/journal.pone.0078115.

[173] R. Shi, P.Y. Wang, X.Y. Li, J.X. Chen, Y. Li, X.Z. Zhang, C.G. Zhang, T. Jiang, W.B. Li, W. Ding, S.J. Cheng, Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients, Oncotarget 6 (2015) 26971–26981, https://doi.org/10.18632/oncotarget.4699.

[174] F. Fu, W. Jiang, L. Zhou, Z. Chen, Circulating exosomal miR-17-5p and miR-92a-3p predict pathologic stage and grade of colorectal cancer, Transl. Oncol. 11 (2018) 221–232, https://doi.org/10.1016/j.tranon.2017.12.012.

[175] S.A. Melo, L.B. Luecke, C. Kahlert, A.F. Fernandez, S.T. Gammon, J. Kaye, V.S. LeBleu, E.A. Mittendorf, J. Weitz, N. Rahbari, C. Reissfelder, C. Pilarsky, M.F. Fraga, D. Piwnica-Worms, R. Kalluri, Glypican-1 identifies cancer exosomes and detects early pancreatic cancer, Nature 523 (2015) 177–182, https://doi.org/10.1038/nature14581.

[176] X. Lai, M. Wang, S.D. McElyea, S. Sherman, M. House, M. Korc, A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer, Cancer Lett. 393 (2017) 86–93, https://doi.org/10.1016/j.canlet.2017.02.019.

[177] J. McKiernan, M.J. Donovan, V. O'Neill, S. Bentink, M. Noerholm, S. Belzer, J. Skog, M.W. Kattan, A. Partin, G. Andriole, G. Brown, J.T. Wei, I.M. Thompson Jr., P. Carroll, A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy, JAMA Oncol 2 (2016) 882–889, https://doi.org/10.1001/jamaoncol.2016.0097.