REVIEW   **Open Access**

Emerging roles and therapeutic value of exosomes in cancer metastasis

Miaowei Wu[1†], Guosheng Wang[2†], Weilei Hu[2], Yihan Yao[3] and Xiao-Fang Yu[1*]

Abstract

Exosomes are cell-derived vesicles of 30 to 150 nm that contain diverse proteins, nucleic acids, and lipids. These vesicles facilitate effective intercellular communication and trigger profound environmental changes. In recent years, many studies have identified diverse roles for exosomes in tumor metastasis, a major cause of cancer-related deaths; furthermore, circulating tumor-derived exosomes can drive the initiation and progression of metastasis and determine the specific target organs affected. Fortunately, our growing understanding of exosomes and relevant modification technology have provided new ideas for potential treatment of tumor metastases. Here we review recent advances concerning the role of exosomes in metastasis, focusing on their regulatory mechanisms and therapeutic targeting in advanced cancer.

Keywords: Cancer, Metastasis, Organ-specific metastasis, Exosome, Tumor microenvironment, Therapy

Background

In most cases, primary cancers can be cured by surgical resection and adjuvant treatment. However, metastatic cancers are difficult to completely alleviate and are the cause of about 90% of cancer-related deaths [1]. In 1889, Stephen Paget proposed the "seed and soil" hypothesis of metastasis, which claims that the distribution of cancers is not random [2]. Current evidence shows that several processes occur during tumor metastasis, including angiogenesis and an epithelial-to-mesenchymal transition (EMT) [3]. Although the mechanisms of organ-specific metastasis are not fully understood, the roles of intercellular communication and the molecular characteristics of tumor cells are important considerations [4]. Before metastasis, the target organs are specially modified to establish a microenvironment suitable for tumor cell growth, known as a pre-metastatic niche (PMN). The establishment of a pre-metastatic niche involves vascular leakage, modification of the stroma and extracellular matrix, and immune system changes [5]. Also, paracrine interactions with stromal cells facilitate

the formation of pre-metastatic niches and promote the growth of distant tumor cells [6, 7].

Growing evidence suggests that exosomes and their contents contribute to the formation of the pre-metastatic microenvironment and to non-random metastasis patterns [8–10]. Exosomes are constitutively secreted by almost all cell types and can transport proteins, lipids, DNA, and RNA to recipient cells [11]. They contain many of the important raft lipids found in cellular membranes, such as ceramides, sphingolipids, cholesterol, and glycerophospholipids [12]. The principal RNA of exosomes is miRNA [13, 14], but they also contain long non-coding RNAs (LncRNAs), which regulate gene expression in many ways [15]. The biological characteristics of exosomes are summarized in Fig. 1.
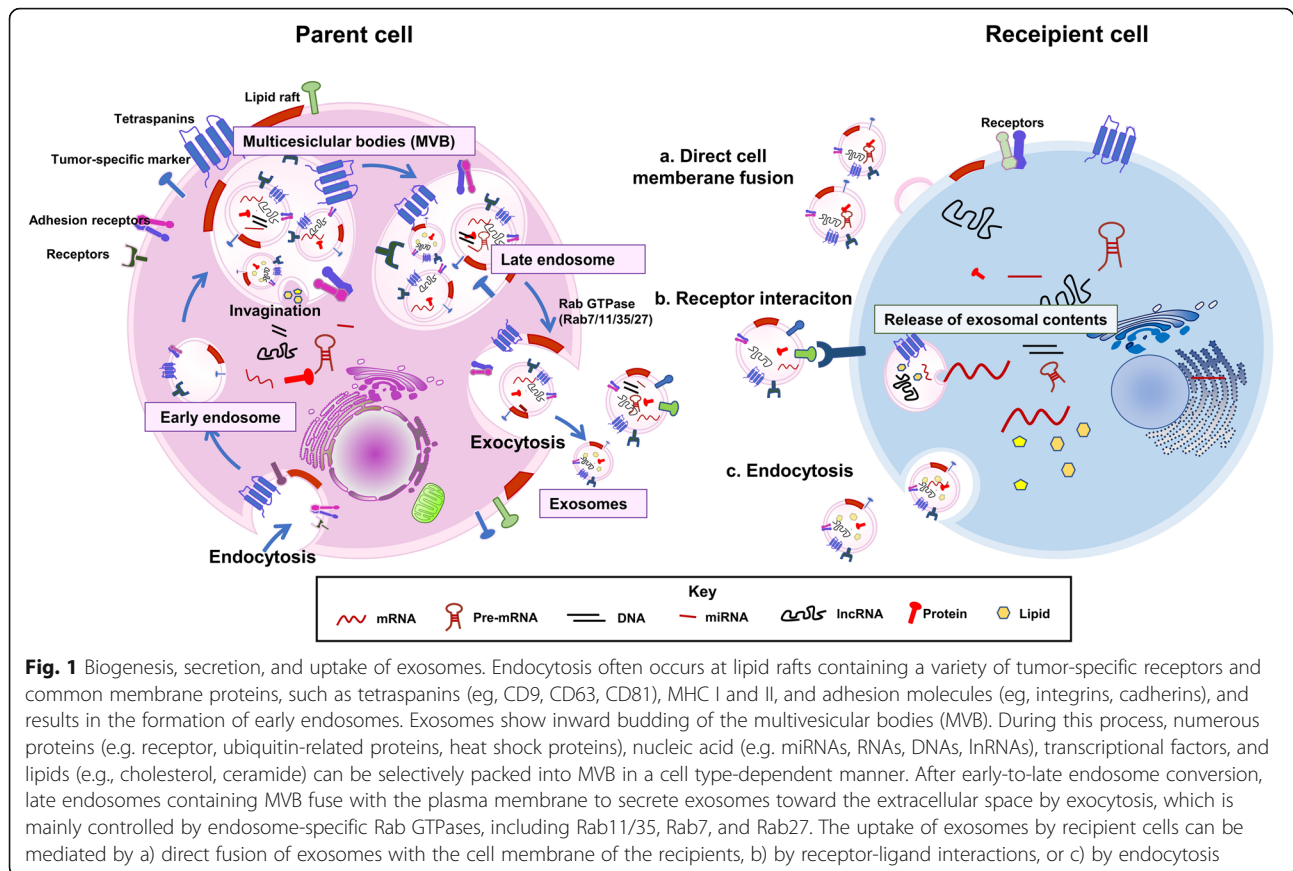
Tumor exosomal miRNAs have been shown to interfere with the miRNA profile of target cells at a distance, thereby contributing to the formation of pre-metastatic niches [16, 17]. In addition, exosomes secreted from cells in secondary microenvironments may enhance brain metastases by producing CCL2 and recruiting IBA1-expressing myeloid cells [18]. Of note, metastasis-associated exosomes include not only tumor-derived exosomes but also exosomes released from other cells, such as T cells and fibroblasts [19]. In contrast to these metastasis-promoting effects, CD9 and CD82 proteins in exosomes have been

* Correspondence: xfyu1@zju.edu.cn
†Miaowei Wu and Guosheng Wang contributed equally to this work.
¹Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, Ministry of Education), Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, People's Republic of China
Full list of author information is available at the end of the article

Wu et al. Molecular Cancer   (2019) 18:53

Page 2 of 11

Fig. 1 Biogenesis, secretion, and uptake of exosomes. Endocytosis often occurs at lipid rafts containing a variety of tumor-specific receptors and common membrane proteins, such as tetraspanins (eg, CD9, CD63, CD81), MHC I and II, and adhesion molecules (eg, integrins, cadherins), and results in the formation of early endosomes. Exosomes show inward budding of the multivesicular bodies (MVB). During this process, numerous proteins (e.g. receptor, ubiquitin-related proteins, heat shock proteins), nucleic acid (e.g. miRNAs, RNAs, DNAs, lnRNAs), transcriptional factors, and lipids (e.g., cholesterol, ceramide) can be selectively packed into MVB in a cell type-dependent manner. After early-to-late endosome conversion, late endosomes containing MVB fuse with the plasma membrane to secrete exosomes toward the extracellular space by exocytosis, which is mainly controlled by endosome-specific Rab GTPases, including Rab11/35, Rab7, and Rab27. The uptake of exosomes by recipient cells can be mediated by a) direct fusion of exosomes with the cell membrane of the recipients, b) by receptor-ligand interactions, or c) by endocytosis

shown to restrain tumor cell metastasis via communication with integrins [20]. In the following sections, we will highlight the latest findings on metastatic mechanisms mediated by exosomes, and discuss their implications for metastasis management.
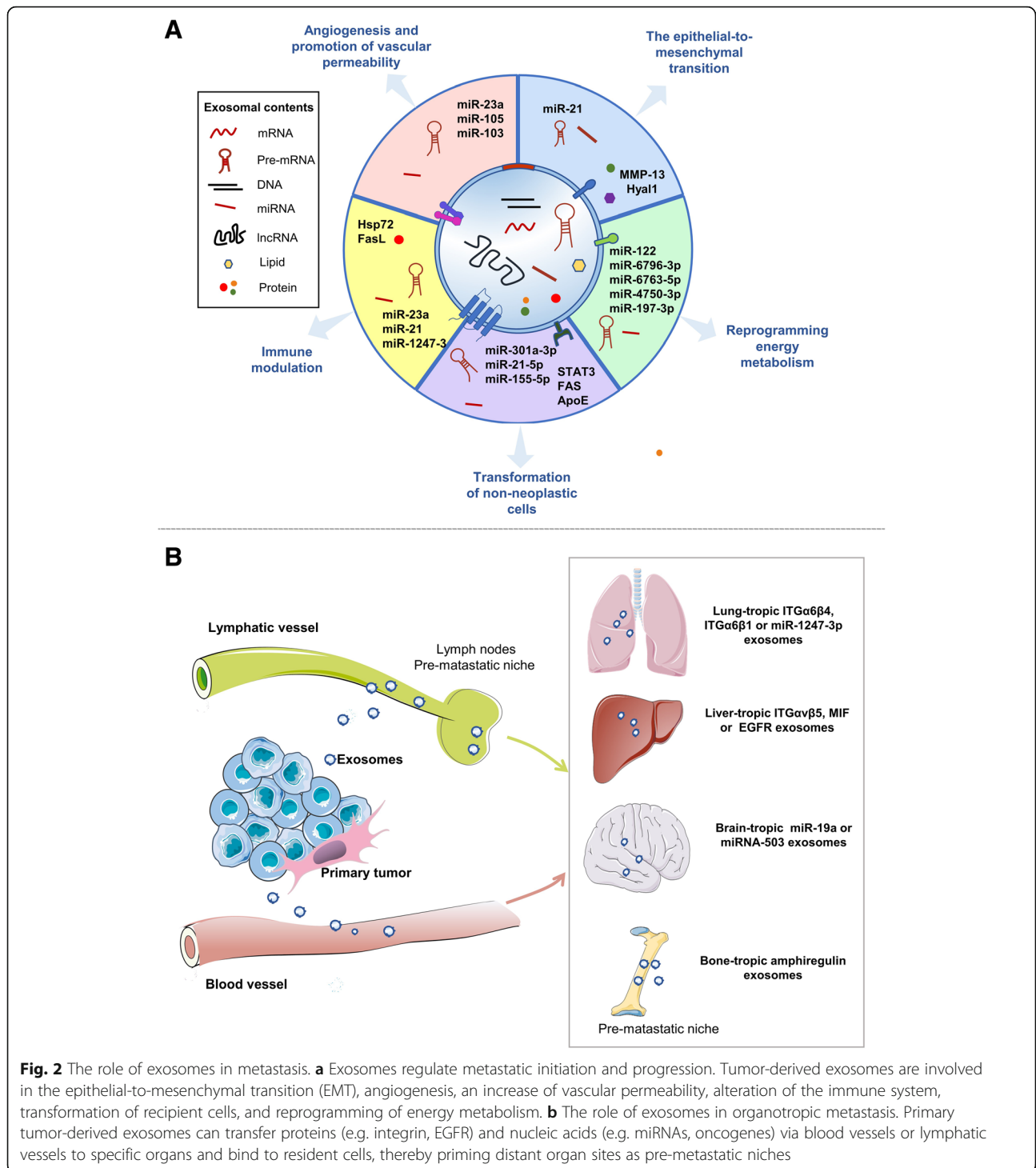
Exosomes regulate metastasis initiation and progression

Although only 0.01% of tumor cells in the blood stream can spread to distant sites, metastasis occurs frequently, and exosomes have been proposed to increase its success rate [21, 22]. Zomer et al. have reported that aggressive breast cancer cells promote the metastatic capacity of less-aggressive tumor cells, largely by transferring exosomes containing functional RNAs [23]. In the early stages of metastasis, exosomes participate in the sequential steps involved in both modulating tumor cells and establishing a distant pre-metastatic niche [24–26] (Fig. 2a).

The epithelial-to-mesenchymal transition (EMT)

EMT is a critical process in the initiation phase of metastasis. It is characterized by a loss of cell polarity and cell-cell adhesive ability in epithelial cells and an increase in the migratory and invasive ability that produces mesenchymal stem cells [27, 28]. Tumor-derived

exosomes can promote the initiation and progression of metastasis by targeting EMT-related factors, such as transforming growth factor beta (TGFβ), caveolin-1, hypoxia-inducible factor 1 alpha (HIF-1α), and β-catenin [10]. McAtee et al. have shown that prostate tumor cells can increase the mobility of stromal cells by secreting exosomes rich in hyaluronidase Hyal1, a lysosomal hyaluronidase involved in prostate cancer metastasis [29], and Chen et al. have demonstrated that exosomes from highly metastatic cells can promote the migratory ability of low-metastatic cells. Mechanistically, the exosomes can trigger an EMT process via MAPK/ERK signaling [30]. Of note, the authors of two recent studies have reported that under hypoxic conditions, tumor cells can release exosomes enriched in miR-21 and matrix metalloproteinase-13 (MMP-13), which lead to an increase in vimentin and decrease in E-cadherin in normoxic cells, thus enhancing metastases occurring via EMT [31, 32]. With regard to the effect of a hypoxic microenvironment, the authors further discovered that exosomal MMP-13, operating via HIF-1α and HIF-1α, can directly regulate MMP-13. Nevertheless, it would be interesting to explore how hypoxic conditions affect exosome-mediated EMT.

Wu *et al. Molecular Cancer*   (2019) 18:53

Page 3 of 11

Fig. 2 The role of exosomes in metastasis. **a** Exosomes regulate metastatic initiation and progression. Tumor-derived exosomes are involved in the epithelial-to-mesenchymal transition (EMT), angiogenesis, an increase of vascular permeability, alteration of the immune system, transformation of recipient cells, and reprogramming of energy metabolism. **b** The role of exosomes in organotropic metastasis. Primary tumor-derived exosomes can transfer proteins (e.g. integrin, EGFR) and nucleic acids (e.g. miRNAs, oncogenes) via blood vessels or lymphatic vessels to specific organs and bind to resident cells, thereby priming distant organ sites as pre-metastatic niches

Angiogenesis and promotion of vascular permeability

Compelling evidence shows that exosomes can deliver functional molecules to acceptor cells, thereby promoting angiogenesis and increasing vascular leakage [33, 34]. For example, one study has shown that exosomal miR-23a can induce angiogenesis in nasopharyngeal cancer [35]. In addition, Fang et al. have found that hepatoma cells generate exosomes rich in miR-103 that can promote tumor cell motility by increasing vessel permeability and targeting various endothelial junction proteins [36]. In a mouse model of melanoma, interactions between exosomes and the capillary wall have also been observed to increase vascular permeability, thereby leading to tumor cell leakage from blood vessels [37]. Similarly,

Wu *et al. Molecular Cancer*   (2019) 18:53

Page 4 of 11

tumor-derived exosomes carrying miR-105 can induce vascular permeability by specifically breaking tight junctions and natural barriers against metastasis, and tight junction protein ZO-1 has been shown to be the key target of exosomal miR-105 [17].

Immune modulation

In a variety of ways, exosomes can help metastatic cells to escape immune surveillance and induce a pre-metastatic microenvironment by transporting inflammatory factors [38]. In particular, tumor-derived exosomes expressing Hsp72 can restrain tumor immune surveillance by enhancing the activity of myeloid-derived suppressor cells [39]. In addition, exosomes released by breast cancer cells can decrease T-cell proliferation by targeting TGF-β [40]. It has been reported that tumor-derived exosomes expressing tumor antigens can inhibit T-cell activation and induce apoptosis of T-cells [41, 42]. Also, exosomes released from T cells have been shown to block the antitumor immune response by decreasing pMHC I expression in dendritic cells [43]. Furthermore, activated T-cell exosomes carrying bioactive FasL, a member of the tumor necrosis factor (TNF) family, may enhance the metastasis of melanoma and lung cancer cells by increasing the expression of MMP9 [44]. Andreola et al. have observed that tumor-derived exosomes expressing FasL can promote the apoptosis of lymphocytes [45]. Also, two studies have indicated that tumor-derived exosomes can block IL-2-mediated activation of NK cells and their cytotoxic activity [46, 47]. Furthermore, tumor-derived exosomes containing miR-23a may function as immunosuppressive factors by directly downregulating the expression of CD107a in NK cells [48].

More importantly, tumor-derived exosomes can precondition the tumor microenvironment for future metastasis by transporting inflammatory factors. For example, exosomes released from colorectal cancer cells are involved in formation of inflammatory pre-metastatic niches via the miR-21-TLR7-IL6 axis [49]. Furthermore, primary tumors can release exosomes carrying small nuclear RNAs to promote chemokine accumulation and neutrophil recruitment, thus aiding the formation of the pre-metastatic niche [50]. Interestingly, it has been found that TLR3 activation is crucial for metastasis but not essential for primary tumor growth; furthermore, the authors of this study confirmed that it is tumor-derived exosomal RNAs that activate TLR3, rather than tumor RNAs, indicating that the exosomal RNAs are selectively packed. These mechanisms need to be further explored [51]. Moreover, there may be other events that stimulate the formation of pre-metastatic niche, apart from TLR3 activation.

Exosomes also regulate the interaction between tumor cells and fibroblasts. Fibroblasts, in turn, produce exosomes containing pro-inflammatory cytokines that enhance tumor metastasis. For example, high-metastatic hepatocellular carcinoma cells release exosomes containing miR-1247-3p that target B4GALT3 and activate beta1-integrin-NF-kappaB signaling in fibroblasts, further boosting cancer progression by releasing pro-inflammatory cytokines, including IL-6 and IL-8 [52]. Exosomal IL-6 and IL-8 promote the local environmental changes that favor tumor metastasis.

Transformation of non-neoplastic cells

Tumor cells can affect recipient cells by releasing exosomes, thus promoting cancer metastasis, since exosomes can transfer molecules required for metastasis [53]. In particular, ovarian cancer cells can produce exosomes containing oncogenic proteins such as STAT3 and FAS, which increase the migratory ability of tumor cells [54]. Wang et al. have observed that pancreatic cancer cells can secrete exosomes containing miR-301a-3p to promote metastasis by inducing the M2 polarization of macrophages [55]. In addition, exosomes secreted from macrophages contribute to metastasis. For example, Lan et al. have discovered that M2 macrophages can release exosomes carrying miR-21-5p and miR-155-5p to promote colorectal cancer metastasis through the downregulation of BRG1 expression [56]. Similarly, M2 macrophages-secreted exosomes can promote the dissemination of gastric tumor cells by selectively transferring apolipoprotein E (ApoE), and ApoE can reshape cytoskeleton-supporting transportation by activating the PI3K-Akt signaling pathway [57].

Reprogramming energy metabolism

Metabolic adaptation allows cancer cells to adapt to an environment that lacks adequate nutrients [58, 59]. Recent studies have shown that tumor cells can alter their metabolic pattern to meet their energy needs under conditions of nutrient deprivation, giving them the ability to invade a hostile environment [60, 61]. In particular, exosomes function as metabolite carriers to promote tumor proliferation in nutrient-stressed microenvironments [62].

Extensive studies have shown that exosomal miRNAs can regulate the metabolic pathways associated with metastasis [63, 64]. For example, exosomes from CD105-positive renal cancer stem cells play a role in the formation of a premetastatic niche by transporting miRNAs. According to the enrichment analysis, miRNA takes the biggest proportion in the molecules affecting metabolic processes [65]. Furthermore, exosomal miR-122 s derived from breast cancer can modify the glucose metabolism of non-tumor cells in the

Wu *et al. Molecular Cancer*   (2019) 18:53

Page 5 of 11

premetastatic niche to promote metastasis. Mechanistically, tumor-derived exosomes carrying miR-122 can reduce glucose uptake via the downregulation of the glycolytic enzyme pyruvate kinase, thus increasing nutrient supplies [66]. More recently, Zhang et al. have found that exosomes released from pancreatic cancer cells can block the synthesis of GIP and GLP-1 in STC-1 cells in vitro by targeting PCSK1/3. These authors proposed that the miRNAs, including miR-6796-3p, miR-6763-5p, miR-4750-3p, and miR-197-3p, are critical for the process, although there may also be other undiscovered mechanisms involved [67]. In addition, exosomal miRNA-regulated metabolic reprogramming is a key mechanism that allows herpesviruses to form a tumor microenvironment. Also, exosomes from Kaposi's sarcoma-associated herpesvirus (KSHV)-infected cells can specifically transport viral miRNAs to nearby cells, ultimately causing a metabolic switch toward aerobic glycolysis in the recipient cells [68].

Organotropic metastasis

Metastasis is an organ-specific process in animal models that is not only dependent on the pattern of the vasculature or lymphatic vessels, but also on the tumor cell characteristics and host factors involved [69]. Many studies investigating the mechanisms of organ-specific metastasis have highlighted the roles of tumor cell traits, including the genes and pathways involved in the regulation of organotropism [17, 70–74]. Moreover, Fais and his peers have reported that microenvironmental pH is one of the factors influencing metastatic sites, since exosome fusion and interactions with the acceptor cells are affected by the microenvironmental pH [75, 76]. In the following sections, we will focus on the role of exosomes in organ-specific metastasis (Fig. 2b).

Lung and liver metastasis

It has been recognized that tumor-derived exosomes are involved in lung and liver metastasis. Initially, Hoshino et al. profiled a series of integrins expressed on tumor-derived exosomes because these integrins regulate the adhesion of exosomes to special tumor cell types and extracellular matrix (ECM) molecules in specific organs. Notably, they found that exosomes containing ITGαvβ5 specifically bind to Kupffer cells, thereby facilitating liver tropism, whereas exosomes expressing ITGα6β4 and ITGα6β1 favorably bind to lung-resident fibroblasts and epithelial cells, mediating lung tropism [8]. Also, Liu et al. have discovered that lung epithelial cells can sense tumor exosomal RNAs and be activated to recruit neutrophils by upregulating Toll-like receptor 3 (TLR3), thus facilitating the formation of a pre-metastatic niche [50]. However, neutrophils can also block metastasis [77], so it is necessary to identify the behavior of neutrophils at different stages and under different conditions. In addition, a recent study has revealed the mechanisms responsible for the lung metastasis of liver cancer: High-metastatic hepatocellular carcinoma cells can release exosomes containing miR-1247-3p to activate fibroblasts by targeting B4GALT3, and these activated fibroblasts can then release pro-inflammatory factors to boost lung metastasis [52]; this study has revealed a new molecular mechanism underlying the interaction between exosomal miRNAs and fibroblasts to promote lung metastasis.

Moreover, Costa-Silva et al. have reported that exosomes from pancreatic cancer can prime a liver pre-metastatic niche in the liver, with the exosomes enabling the Kupffer cells to alter growth factor β production and increase fibronectin secretion of hepatic stellate cells, thus shaping a fibrotic microenvironment with bone marrow-derived macrophages that facilitates metastasis. These authors further identified an exosomal protein, macrophage migration inhibitory factor (MIF), as an essential trigger of the pre-metastatic niche in the liver [78]. Although the study showed an important pattern of exosome-mediated metastasis, it remains unclear whether other components contained within exosomes of pancreatic cancer are needed to shape the liver's pre-metastatic niche. Furthermore, other authors have reported that exosomes from highly malignant pancreatic tumor cells can promote the establishment of a liver pre-metastatic niche [79]; they found 79 exosomal proteins that are differentially expressed between highly metastatic and less metastatic cells, but they did not extensively research the mechanisms involved. It has also been proposed that exosomes derived from gastric tumor cells promote the formation of a liver-like microenvironment and enhance liver-specific metastasis. Exosomes are known to deliver EGFR to the liver to upregulate hepatocyte growth factor [80]. Most recently, Shao Y et al. have found that exosomes released from colorectal cancer cells can be specifically delivered to liver tissue and increase liver metastasis. In addition, these researchers reported that exosomal miR-21 is a key factor in establishing an inflammatory premetastatic niche [49], a finding that provides a rationale for targeting certain components of exosomes.

Brain metastasis

In the case of organ-tropic metastasis to the brain, several studies have reported a few brain-derived factors, such as secretory proteins and exosomal miRNAs, that alter the brain microenvironment to promote the colonization of brain metastasis [66, 81]. Camacho et al. have shown that exosomes derived from brain metastatic cells can transport metastasis-related proteins and miRNAs to non-brain metastatic cells, leading to increased

cell-adhesive and invasive capability [82]. Zhang and colleagues have found that both human and mouse tumor cells with a normal expression of PTEN, an important tumor suppressor, have a decreased PTEN expression level after moving into the brain. Furthermore, they observed that the expression of PTEN in these brain metastatic tumor cells was restored after they were removed from the brain microenvironment. Finally, they showed that astrocytes can produce exosomes containing miR-19a, which decreases the expression of PTEN in circulating tumor cells in the brain microenvironment, thus leading to metastasis [18]. Astrocytes are a main stromal cell type in the brain, and their abnormal increase is associated with brain metastases in animal models and human patients [83]. These findings reveal that tumor cells can be remolded by their specific metastatic microenvironment, which to a large extent depends on exosomes. Similarly, a recent study on breast cancer has revealed that exosomal miRNA-503 can block T-cell production by increasing immune-suppressive cytokines, and this inhibition was only observed in brain metastasis [84].

Bone metastasis
Tumor-derived exosomes participate in bone metastasis by affecting bone metabolism, since tumor-derived exosomes can destroy the cycle of bone remodeling that occurs during the onset of a bone lesion [85], and exosomal miRNAs may be important regulators of bone metastasis [86]. Interestingly, Valencia et al. have found that exosomes carrying miR-192 can lessen bone metastasis [87], but they did not uncover the underlying mechanisms involved. In addition, Hashimoto et al. have identified eight miRNAs that are highly expressed in exosomes from prostate cancer cells with metastatic potential to bone, further demonstrating that exosomal hsa-miR-940 can enhance the osteoblastic phenotype of a bone metastatic microenvironment by targeting ARHGAP1 and FAM134A [88]. The functions of the remaining seven exosomal miRNAs are still unclear. Karlsson et al. have compared the exosomes from prostate tumor cells and fibroblastic cells and found that the exosomes from cancer cells can remarkably affect osteoclast formation by inhibiting the maturation of monocytic osteoclast precursors, which may represent a exosome-regulated abnormal formation of bone cells at the metastatic site [89]. Despite these revealing studies, the process of bone metastasis still needs to be studied in greater detail.

Implications of exosomes in metastatic cancer therapy
There are biological vulnerabilities for tumor cells at various stages of metastasis, and these vulnerabilities provide opportunities for blocking the metastatic process. Because exosomes are involved in metastatic cascades and possess appealing properties for therapeutic delivery [90], we will comprehensively review below the current exosome-based therapeutic approaches with promising clinical applications.

Decreasing production and uptake of exosomes
Blocking secretion and uptake of exosomes is a potential approach for inhibiting metastasis. Preclinical and clinical studies have suggested that metastatic cancers can be at least partly alleviated by targeting the heparanase/syndecan-1 axis [91] or syndecan heparan sulfate proteoglycans, which are involved in the formation of exosomes and tumor cell dissemination [92, 93]. For instance, Sento et al. have found that heparin can remarkably block metastasis by decreasing uptake of tumor-derived exosomes in oral squamous carcinoma [94]. Furthermore, Nishida-Aoki and colleagues have designed a therapeutic antibody targeted at reducing the production of tumor-derived exosomes, leading to a decrease in distant metastasis of breast cancer in a mouse model [95]. These findings indicate that therapeutic antibodies targeting metastasis-related exosomes constitute a potential treatment approach. Also, targeted antibodies have been reported to significantly reduce cancer metastasis by promoting elimination of tumor-derived exosomes by macrophages [95]. In addition, a novel device has been designed to block or divert the spread of tumor cells: Creating an artificial pre-metastatic niche by implanting tumor exosomes in a 3D scaffold and then transplanting the scaffold into the peritoneal cavity of a mouse has led to the capture of ovarian tumor cells within the peritoneum and a redirecting of the tumor cells from their original target sites. This approach remarkably suppressed tumor metastasis at a distance [96]. However, this therapeutic strategy still needs to be tested in further in vivo experiments [22].

Increasing evidence has shown that extracellular acidity may influence the generation of exosomes by cancer cells. Initially, melanoma cells cultured under acidic conditions (pH 6.7) were found to secrete a larger number of exosomes than did the same cells cultured under physiological conditions (pH 7.4) [75, 97]. This phenomenon was later confirmed in other human tumor types, such as prostate cancer, melanoma, osteosarcoma, colon cancer, and breast cancer [76]. The mechanisms responsible for the increased secretion of exosomes in acidic environments are incompletely characterized, although some researchers have pointed out that producing more exosomes under low-pH conditions may be a way of relieving the intracellular accumulation of toxics material [76]. Based on this theory, comparable proton-pump inhibitors have been developed to decrease plasmatic exosome levels in xenograft models [98]. Thus,

Wu *et al. Molecular Cancer*   (2019) 18:53

Page 7 of 11

the alkalinizing approach may be a potential anti-tumor strategy for patients with tumor metastasis [85].

Exosomal miRNAs

Exosomes contain a variety of miRNA that can be targeted to restrain exosome-mediated metastasis [99]. In colorectal cancer (CRC), CRC-derived exosomal miRNAs have been shown to promote tumor cell proliferation [90, 100], making it possible to use gene therapy to suppress tumor metastasis by engineering exosomal miRNA. Furthermore, miR-379 in CRC-derived exosomes has been found to down-regulate the migration of CRC cells, and transfer of these engineered miR-379-overexpressing exosomes to recipient cells reduced their migration [101]. Similarly, Zaharie et al. have demonstrated that exosomal microRNA-375 inhibits tumor cell dissemination by blocking Bcl-2 in colon cancer, suggesting that exosomal microRNA-375 can be considered a potential therapeutic target [102]. Furthermore, it has been suggested since there is a positive correlation between exosomal miR-193a expression and liver metastasis of colon cancer, the major vault protein (MVP) can possibly decrease the level of circulating exosomal miR-193a, providing a new therapeutic approach for metastatic colon cancer [103].

Vaccination

Overwhelming evidence demonstrates the immune-suppressive function of exosomes. For instance, they can inhibit the cytotoxicity of NK cells and differentiation of dendritic cells (DC), as well as induce apoptosis of cytotoxic T-cells and M2 polarization of macrophages, all of which promote tumor metastasis [9, 24]. In the first phase I trial of exosome therapy, among 15 metastatic melanoma patients receiving exosome vaccinations, only WHO grade II toxicity was present and one patient exhibited a partial response, suggesting that it is feasible to produce large-scale exosomes for therapeutic purpose because of their good safety profiles [104]. Notably, in the second Phase I trial performed at Duke University in advanced non-small cell lung cancer, a high efficacy of autologous dendritic cell (DC)-derived exosomes and MHC class I peptides led to long-term survival [105].

Exosomes as promising delivery systems

Given their high physicochemical stability and biocompatibility, exosomes can be chemically or biologically modified to yield delivery systems that can enhance the treatment outcomes of chemotherapeutic agents, as well as reduce drug toxicity [106–110]. One study has found that exosomes can be used to deliver doxorubicin specifically to tumor tissues through intravenous injection, decreasing tumor growth without overt toxicity [111]. In addition, Ohno et al. have shown that exosomes can efficiently carry let-7a miRNA to EGFR-expressing breast cancer cells in animal models [112]. Genetically engineered exosomes have also been shown to inhibit schwannoma tumor progression by delivering suicide mRNAs and proteins [113]. Finally, research in zebrafish has demonstrated that exosomes can increase the uptake of drugs in the brain, demonstrating their capacity to function as delivery systems for anticancer drugs targeting brain metastasis [114].

Conclusions

In conclusion, exosomes act as an important regulator in metastatic cascades, including the initiation, progression, and colonization of metastasis in distant organs, by delivering functional molecules and directly affecting target cells. Undoubtedly, targeting exosomes associated with metastasis represents a new approach to the development of novel, effective anti-tumor therapeutic agents.

There are many challenges in the field of exosomes, and many worthwhile directions for future studies to pursue. First, it is important to develop insights that will allow for a standardization of the classification of exosomes. Among the challenges to be met: 1) More efficient methods and reproducible criteria are needed for isolating and characterizing pure groups of specific subtypes of exosomes according to their biophysical and biological features. 2) For different subtypes of exosomes, their specific target cells and the consequences that ensue after they reach acceptor cells need to be further explored. 3) the process of exosome uptake and cargo delivery into the cytosol of receptor cells need to be more fully understood [115].

Second, the tumor microenvironment is linked to the progression of various stages of tumors [116]. It will be important to explore how components of the tumor microenvironment affect exosome behavior [117, 118]. It is also pivotal to learn more about the roles of exosomes in metabolic changes related to metastasis [58]. For example, Abhinav and colleagues have devised an experimental and computational platform to analyze exosomes with different metabolite components in various environments, which is helpful for understanding the metabolic alterations in tumor microenvironment components; however, the platform still needs to be optimized [119]. Consequently, it will be interesting to determine whether exosomes can be used as markers for disease progression and treatment [120–122]. Third, the precise roles of exosomes in organ metastasis need to be further defined. The knowledge of which component of exosomes is responsible for tissue specific targeting, local environment modification, and possibly immune alteration should be investigated. In particular, the mechanism involved in brain metastasis should be a research focus because of the unique repercussions of the

blood-brain barrier for cancer and the potential for future therapeutic development. Recently, He C et al. have proposed that RNAs are responsible for the major functions of exosomes, a conclusion that suggests new directions for research into the functional contents of exosomes [123].

Although therapeutics based on exosomes are promising, there are several significant issues that must be addressed before exosomes can be tested clinically: 1) Standard guidelines for the manufacture, purification, storage, usage, duration, and dosage of exosome-based drugs remain to be established, since it is reported that surgical intervention may trigger local hypoxia and inflammatory response, both of which are relevant to metastasis [124–127]. 2) It must be verified that the sources of the exosomes are safe for clinical use [75]. 3) How the acceptor cells can be protected by removing any carcinogenic components of the exosomes must be investigated [128]. 4) How interactions between therapeutic exosomes and unexpected cells can be avoided must also be examined [129]. 5) Is it feasible that only autologous exosomes be used? Under what circumstances can heterologous be explored? 6) Is it promising to design exosome-based drugs targeting the hallmarks of cancer metabolism [130]? 7) Since many studies have focused on the application of exosome-based vaccines as alternative approaches to suppress tumor growth [131], is it meaningful to develop exosome-based vaccines to prevent metastasis? Significant progress has been made in characterizing the role of exosomes in tumor metastasis, and the therapeutic potential of using exosomes or their derived vesicles is entering an uncertain but exciting stage.

Abbreviations
B4GALT3: Beta-1,4-galactosyltransferase 3; BRG1: ATP-dependent chromatin remodeler SMARCA4; CCL2: Chemokine (C-C motif) ligand 2; c-MET: Tyrosine-protein kinase Met; CRC: Colorectal cancer; DC: Dendritic cells; ECM: Extracellular matrix; EGFR: Epidermal growth factor receptor; EMT: Epithelial to mesenchymal transition; ERK: Extracellular signal-regulated kinases; FAS: Fatty acid synthase; FasL: Fas ligand; GIP: Glucose-dependent insulinotropic peptide; GLP-1: Glucagon-like peptide-1; HIF-1α: Hypoxia-inducible factor 1 alpha; IBA1: Ionized calcium-binding adapter molecule 1; IL-2: Interleukin-2; KSHV: Kaposi's sarcoma-associated herpesvirus; LncRNAs: Long non-coding RNAs; M2: macrophages Alternatively activated macrophages; MAPK: Mitogen-activated protein kinases; MIF: Migration inhibitory factor; MMP-13: Matrix metalloproteinases-13; MMP9: Matrix metallopeptidase 9; MVP: Major vault protein; NK: Natural killer; NSCLC: Non-small cell lung cancer; PCSK1/3: Proprotein convertase subtilisin/kexin type 1/3; pMHC I: Peptide-MHC-I complexes; PMNs: Pre-metastatic niches; PTEN: Phosphatase and tensin homolog; STAT3: Signal transducer and activator of transcription 3; TGFβ: Transforming growth factor beta; TLR3: Toll-like receptor 3; TNF: Tumor necrosis factor

Availability of data and materials
Not applicable.

Disclosure statement
The authors have nothing to disclosure.

Authors' contributions
MW, YY and XFY drafted the manuscript and carried out the final editing. Guosheng Wang participated in the design of the review, and WH drafted the figures. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
[1]Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, Ministry of Education), Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, People's Republic of China. [2]Inst Translat Med, School of Medicine, Zhejiang University, Hangzhou 310029 Zhejiang, People's Republic of China. [3]Department Surg Oncol, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, People's Republic of China.

References
1. Obenauf AC, Massague J. Surviving at a distance: organ specific metastasis. Trends Cancer. 2015;1:76–91.
2. Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 1989;8:98–101.
3. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
4. Chen W, Hoffmann AD, Liu H, Liu X. Organotropism: new insights into molecular mechanisms of breast cancer metastasis. NPJ Precis Oncol. 2018;2:4.
5. Peinado H, Zhang H, Matei IR, Costa-Silva B, Hoshino A, Rodrigues G, Psaila B, Kaplan RN, Bromberg JF, Kang Y, et al. Pre-metastatic niches: organ-specific homes for metastases. Nat Rev Cancer. 2017;17:302–17.
6. McAllister SS, Weinberg RA. The tumour-induced systemic environment as a critical regulator of cancer progression and metastasis. Nat Cell Biol. 2014;16:717–27.
7. Cammarota F, Laukkanen MO. Mesenchymal Stem/Stromal Cells in Stromal Evolution and Cancer Progression. Stem Cells Int. 2016;2016:4824573.
8. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527:329–35.
9. Lobb RJ, Lima LG, Moller A. Exosomes: Key mediators of metastasis and pre-metastatic niche formation. Semin Cell Dev Biol. 2017;67:3–10.
10. Syn N, Wang L, Sethi G, Thiery JP, Goh BC. Exosome-Mediated Metastasis: From Epithelial-Mesenchymal Transition to Escape from Immunosurveillance. Trends Pharmacol Sci. 2016;37:606–17.
11. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002;2:569–79.
12. Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Mobius W, Hoernschemeyer J, Slot JW, Geuze HJ, Stoorvogel W: Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem 2003, 278:10963–10972.

Wu *et al. Molecular Cancer*   (2019) 18:53

Page 9 of 11

13. Redis RS, Calin S, Yang Y, You MJ, Calin GA. Cell-to-cell miRNA transfer: from body homeostasis to therapy. Pharmacol Ther. 2012;136:169–74.

14. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9:654–9.

15. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev. 2009;23:1494–504.

16. Shurtleff MJ, Temoche-Diaz MM, Karfilis KV, Ri S, Schekman R. Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. Elife. 2016;5.

17. Zhou W, Fong MY, Min Y, Somlo G, Liu L, Palomares MR, Yu Y, Chow A, O'Connor ST, Chin AR, et al. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. Cancer Cell. 2014;25:501–15.

18. Zhang L, Zhang S, Yao J, Lowery FJ, Zhang Q, Huang WC, Li P, Li M, Wang X, Zhang C, et al. Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth. Nature. 2015;527:100–4.

19. Luga V, Zhang L, Viloria-Petit AM, Ogunjimi AA, Inanlou MR, Chiu E, Buchanan M, Hosein AN, Basik M, Wrana JL. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell. 2012;151:1542–56.

20. Zoller M. Tetraspanins: push and pull in suppressing and promoting metastasis. Nat Rev Cancer. 2009;9:40–55.

21. Shah R, Patel T, Freedman JE. Circulating Extracellular Vesicles in Human Disease. N Engl J Med. 2018;379:2180–1.

22. Tkach M, Thery C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. Cell. 2016;164:1226–32.

23. Zomer A, Maynard C, Verweij FJ, Kamermans A, Schafer R, Beerling E, Schiffelers RM, de Wit E, Berenguer J, Ellenbroek SIJ, et al. In Vivo imaging reveals extracellular vesicle-mediated phenocopying of metastatic behavior. Cell. 2015;161:1046–57.

24. Weidle UH, Birzele F, Kollmorgen G, Ruger R. The Multiple Roles of Exosomes in Metastasis. Cancer Genomics Proteomics. 2017;14:1–15.

25. Steinbichler TB, Dudas J, Riechelmann H. Skvortsova, II: The role of exosomes in cancer metastasis. Semin Cancer Biol. 2017;44:170–81.

26. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 2005;438:820–7.

27. Ye X, Brabletz T, Kang Y, Longmore GD, Nieto MA, Stanger BZ, Yang J, Weinberg RA. Upholding a role for EMT in breast cancer metastasis. Nature. 2017;547:E1–3.

28. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011;147:275–92.

29. McAtee CO, Booth C, Elowsky C, Zhao L, Payne J, Fangman T, Caplan S, Henry MD, Simpson MA. Prostate tumor cell exosomes containing hyaluronidase Hyal1 stimulate prostate stromal cell motility by engagement of FAK-mediated integrin signaling. Matrix Biol. 2018.

30. Chen L, Guo P, He Y, Chen Z, Chen L, Luo Y, Qi L, Liu Y, Wu Q, Cui Y, et al. HCC-derived exosomes elicit HCC progression and recurrence by epithelial-mesenchymal transition through MAPK/ERK signalling pathway. Cell Death Dis. 2018;9:513.

31. Li L, Li C, Wang S, Wang Z, Jiang J, Wang W, Li X, Chen J, Liu K, Li C, Zhu G. Exosomes Derived from Hypoxic Oral Squamous Cell Carcinoma Cells Deliver miR-21 to Normoxic Cells to Elicit a Prometastatic Phenotype. Cancer Res. 2016;76:1770–80.

32. Shan Y, You B, Shi S, Shi W, Zhang Z, Zhang Q, Gu M, Chen J, Bao L, Liu D, You Y. Hypoxia-Induced Matrix Metalloproteinase-13 Expression in Exosomes from Nasopharyngeal Carcinoma Enhances Metastases. Cell Death Dis. 2018;9:382.

33. Steenbeek SC, Pham TV, de Ligt J, Zomer A, Knol JC, Piersma SR, Schelfhorst T, Huisjes R, Schiffelers RM, Cuppen E, et al. Cancer cells copy migratory behavior and exchange signaling networks via extracellular vesicles. Embo j. 2018;37.

34. Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis. J Biol Chem. 2013;288:10849–59.

35. Bao L, You B, Shi S, Shan Y, Zhang Q, Yue H, Zhang J, Zhang W, Shi Y, Liu Y, et al. Metastasis-associated miR-23a from nasopharyngeal carcinoma-derived exosomes mediates angiogenesis by repressing a novel target gene TSGA10. Oncogene. 2018;37:2873–89.

36. Fang JH, Zhang ZJ, Shang LR, Luo YW, Lin YF, Yuan Y, Zhuang SM. Hepatoma cell-secreted exosomal microRNA-103 increases vascular permeability and promotes metastasis by targeting junction proteins. Hepatology. 2018;68:1459–75.

37. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, Garcia-Santos G, Ghajar C, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med. 2012;18:883–91.

38. Robado de Lope L, Alcibar OL, Amor Lopez A, Hergueta-Redondo M, Peinado H: Tumour-adipose tissue crosstalk: fuelling tumour metastasis by extracellular vesicles. Philos Trans R Soc Lond B Biol Sci 2018, 373.

39. Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, Boireau W, Rouleau A, Simon B, Lanneau D, et al. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. J Clin Invest. 2010;120:457–71.

40. Rong L, Li R, Li S, Luo R. Immunosuppression of breast cancer cells mediated by transforming growth factor-beta in exosomes from cancer cells. Oncol Lett. 2016;11:500–4.

41. Abusamra AJ, Zhong Z, Zheng X, Li M, Ichim TE, Chin JL, Min WP. Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis. Blood Cells Mol Dis. 2005;35:169–73.

42. Taylor DD, Gercel-Taylor C. Tumour-derived exosomes and their role in cancer-associated T-cell signalling defects. Br J Cancer. 2005;92:305–11.

43. Xie Y, Zhang H, Li W, Deng Y, Munegowda MA, Chibbar R, Qureshi M, Xiang J. Dendritic cells recruit T cell exosomes via exosomal LFA-1 leading to inhibition of CD8+ CTL responses through downregulation of peptide/MHC class I and Fas ligand-mediated cytotoxicity. J Immunol. 2010;185:5268–78.

44. Cai Z, Yang F, Yu L, Yu Z, Jiang L, Wang Q, Yang Y, Wang L, Cao X, Wang J. Activated T cell exosomes promote tumor invasion via Fas signaling pathway. J Immunol. 2012;188:5954–61.

45. Andreola G, Rivoltini L, Castelli C, Huber V, Perego P, Deho P, Squarcina P, Accornero P, Lozupone F, Lugini L, et al. Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. J Exp Med. 2002;195:1303–16.

46. Liu C, Yu S, Zinn K, Wang J, Zhang L, Jia Y, Kappes JC, Barnes S, Kimberly RP, Grizzle WE, Zhang HG. Murine mammary carcinoma exosomes promote tumor growth by suppression of NK cell function. J Immunol. 2006;176:1375–85.

47. Zhang HG, Kim H, Liu C, Yu S, Wang J, Grizzle WE, Kimberly RP, Barnes S. Curcumin reverses breast tumor exosomes mediated immune suppression of NK cell tumor cytotoxicity. Biochim Biophys Acta. 1773;2007:1116–23.

48. Berchem G, Noman MZ, Bosseler M, Paggetti J, Baconnais S, Le Cam E, Nanbakhsh A, Moussay E, Mami-Chouaib F, Janji B, Chouaib S. Hypoxic tumor-derived microvesicles negatively regulate NK cell function by a mechanism involving TGF-beta and miR23a transfer. Oncoimmunology. 2016;5:e1062968.

49. Shao Y, Chen T, Zheng X, Yang S, Xu K, Chen X, Xu F, Wang L, Shen Y, Wang T, et al. Colorectal cancer-derived small extracellular vesicles establish an inflammatory premetastatic niche in liver metastasis. Carcinogenesis. 2018;39:1368–79.

50. Liu Y, Gu Y, Han Y, Zhang Q, Jiang Z, Zhang X, Huang B, Xu X, Zheng J, Cao X. Tumor Exosomal RNAs Promote Lung Pre-metastatic Niche Formation by Activating Alveolar Epithelial TLR3 to Recruit Neutrophils. Cancer Cell. 2016;30:243–56.

51. Antonopoulos D, Balatsos NAA, Gourgoulianis KI. Cancer's smart bombs: tumor-derived exosomes target lung epithelial cells triggering pre-metastatic niche formation. J Thorac Dis. 2017;9:969–72.

52. Fang T, Lv H, Lv G, Li T, Wang C, Han Q, Yu L, Su B, Guo L, Huang S, et al. Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. Nat Commun. 2018;9:191.

53. Naito Y, Yoshioka Y, Yamamoto Y, Ochiya T. How cancer cells dictate their microenvironment: present roles of extracellular vesicles. Cell Mol Life Sci. 2017;74:697–713.

54. Dorayappan KDP, Wanner R, Wallbillich JJ, Saini U, Zingarelli R, Suarez AA, Cohn DE, Selvendiran K. Hypoxia-induced exosomes contribute to a more aggressive and chemoresistant ovarian cancer phenotype: a novel mechanism linking STAT3/Rab proteins. Oncogene. 2018;37:3806–21.

55. Wang X, Luo G, Zhang K, Cao J, Huang C, Jiang T, Liu B, Su L, Qiu Z. Hypoxic Tumor-Derived Exosomal miR-301a Mediates M2 Macrophage

Polarization via PTEN/PI3Kgamma to Promote Pancreatic Cancer Metastasis. Cancer Res. 2018;78:4586–98.

56. Lan J, Sun L, Xu F, Liu L, Hu F, Song D, Hou Z, Wu W, Luo X, Wang J, et al. M2 macrophage-derived exosomes promote cell migration and invasion in colon cancer. Cancer Res. 2018;79(1):146–58.

57. Zheng P, Luo Q, Wang W, Li J, Wang T, Wang P, Chen L, Zhang P, Chen H, Liu Y, et al. Tumor-associated macrophages-derived exosomes promote the migration of gastric cancer cells by transfer of functional Apolipoprotein E. Cell Death Dis. 2018;9:434.

58. Payen VL, Porporato PE, Baselet B, Sonveaux P. Metabolic changes associated with tumor metastasis, part 1: tumor pH, glycolysis and the pentose phosphate pathway. Cell Mol Life Sci. 2016;73:1333–48.

59. Caneba CA, Bellance N, Yang L, Pabst L, Nagrath D. Pyruvate uptake is increased in highly invasive ovarian cancer cells under anoikis conditions for anaplerosis, mitochondrial function, and migration. Am J Physiol Endocrinol Metab. 2012;303:E1036–52.

60. Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell. 2010;140:49–61.

61. Yang L, Moss T, Mangala LS, Marini J, Zhao H, Wahlig S, Armaiz-Pena G, Jiang D, Achreja A, Win J, et al. Metabolic shifts toward glutamine regulate tumor growth, invasion and bioenergetics in ovarian cancer. Mol Syst Biol. 2014;10:728.

62. Zhao H, Yang L, Baddour J, Achreja A, Bernard V, Moss T, Marini JC, Tudawe T, Seviour EG, San Lucas FA, et al. Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. Elife. 2016;5:e10250.

63. Chau BN, Xin C, Hartner J, Ren S, Castano AP, Linn G, Li J, Tran PT, Kaimal V, Huang X, et al. MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. Sci Transl Med. 2012;4:121ra118.

64. Zhu H, Shyh-Chang N, Segre AV, Shinoda G, Shah SP, Einhorn WS, Takeuchi A, Engreitz JM, Hagan JP, Kharas MG, et al. The Lin28/let-7 axis regulates glucose metabolism. Cell. 2011;147:81–94.

65. Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B, Camussi G. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res. 2011;71:5346–56.

66. Fong MY, Zhou W, Liu L, Alontaga AY, Chandra M, Ashby J, Chow A, O'Connor ST, Li S, Chin AR, et al. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. Nat Cell Biol. 2015;17:183–94.

67. Zhang Y, Huang S, Li P, Chen Q, Li Y, Zhou Y, Wang L, Kang M, Zhang B, Yang B, et al. Pancreatic cancer-derived exosomes suppress the production of GIP and GLP-1 from STC-1cells in vitro by down-regulating the PCSK1/3. Cancer Lett. 2018;431:190–200.

68. Yogev O, Henderson S, Hayes MJ, Marelli SS, Ofir-Birin Y, Regev-Rudzki N, Herrero J, Enver T. Herpesviruses shape tumour microenvironment through exosomal transfer of viral microRNAs. PLoS Pathog. 2017;13:e1006524.

69. Hart IR, Fidler IJ. Role of organ selectivity in the determination of metastatic patterns of B16 melanoma. Cancer Res. 1980;40:2281–7.

70. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001;410:50–6.

71. Lu X, Kang Y. Organotropism of breast cancer metastasis. J Mammary Gland Biol Neoplasia. 2007;12:153–62.

72. Weilbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. Nat Rev Cancer. 2011;11:411–25.

73. Chang Q, Bournazou E, Sansone P, Berishaj M, Gao SP, Daly L, Wels J, Theilen T, Granitto S, Zhang X, et al. The IL-6/JAK/Stat3 feed-forward loop drives tumorigenesis and metastasis. Neoplasia. 2013;15:848–62.

74. Cox TR, Rumney RMH, Schoof EM, Perryman L, Hoye AM, Agrawal A, Bird D, Latif NA, Forrest H, Evans HR, et al. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. Nature. 2015;522:106–10.

75. Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, Coscia C, Iessi E, Logozzi M, Molinari A, et al. Microenvironmental pH is a key factor for exosome traffic in tumor cells. J Biol Chem. 2009;284:34211–22.

76. Logozzi M, Mizzoni D, Angelini DF, Di Raimo R, Falchi M, Battistini L, Fais S. Microenvironmental pH and Exosome Levels Interplay in Human Cancer Cell Lines of Different Histotypes. Cancers (Basel). 2018;10.

77. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. Nat Rev Cancer. 2016;16:431–46.

78. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, Becker A, Hoshino A, Mark MT, Molina H, et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol. 2015;17:816–26.

79. Yu Z, Zhao S, Ren L, Wang L, Chen Z, Hoffman RM, Zhou J. Pancreatic cancer-derived exosomes promote tumor metastasis and liver pre-metastatic niche formation. Oncotarget. 2017;8:63461–83.

80. Zhang H, Deng T, Liu R, Bai M, Zhou L, Wang X, Li S, Wang X, Yang H, Li J, et al. Exosome-delivered EGFR regulates liver microenvironment to promote gastric cancer liver metastasis. Nat Commun. 2017;8:15016.

81. Valiente M, Obenauf AC, Jin X, Chen Q, Zhang XH, Lee DJ, Chaft JE, Kris MG, Huse JT, Brogi E, Massague J. Serpins promote cancer cell survival and vascular co-option in brain metastasis. Cell. 2014;156:1002–16.

82. Camacho L, Guerrero P, Marchetti D. MicroRNA and protein profiling of brain metastasis competent cell-derived exosomes. PLoS One. 2013;8:e73790.

83. Kodack DP, Askoxylakis V, Ferraro GB, Fukumura D, Jain RK. Emerging strategies for treating brain metastases from breast cancer. Cancer Cell. 2015;27:163–75.

84. Xing F, Liu Y, Wu SY, Wu K, Sharma S, Mo YY, Feng J, Sanders S, Jin G, Singh R, et al. Loss of XIST in Breast Cancer Activates MSN-c-Met and Reprograms Microglia via Exosomal miRNA to Promote Brain Metastasis. Cancer Res. 2018;78:4316–30.

85. Zhao H, Achreja A, Iessi E, Logozzi M, Mizzoni D, Di Raimo R, Nagrath D, Fais S. The key role of extracellular vesicles in the metastatic process. Biochim Biophys Acta Rev Cancer. 1869;2018:64–77.

86. Probert C, Dottorini T, Speakman A, Hunt S, Nafee T, Fazeli A, Wood S, Brown JE, James V. Communication of prostate cancer cells with bone cells via extracellular vesicle RNA; a potential mechanism of metastasis. Oncogene. 2018. Published online October 23, 2018.

87. Valencia K, Luis-Ravelo D, Bovy N, Anton I, Martinez-Canarias S, Zandueta C, Ormazabal C, Struman I, Tabruyn S. Rebmann V, et al: miRNA cargo within exosome-like vesicle transfer influences metastatic bone colonization. Mol Oncol. 2014;8:689–703.

88. Hashimoto K, Ochi H, Sunamura S, Kosaka N, Mabuchi Y, Fukuda T, Yao K, Kanda H, Ae K, Okawa A, et al. Cancer-secreted hsa-miR-940 induces an osteoblastic phenotype in the bone metastatic microenvironment via targeting ARHGAP1 and FAM134A. Proc Natl Acad Sci U S A. 2018;115:2204–9.

89. Karlsson T, Lundholm M, Widmark A, Persson E. Tumor Cell-Derived Exosomes from the Prostate Cancer Cell Line TRAMP-C1 Impair Osteoclast Formation and Differentiation. PLoS One. 2016;11:e0166284.

90. Cheshomi H, Matin MM. Exosomes and their importance in metastasis, diagnosis, and therapy of colorectal cancer. J Cell Biochem. 2018.

91. Ramani VC, Purushothaman A, Stewart MD, Thompson CA, Vlodavsky I, Au JL, Sanderson RD. The heparanase/syndecan-1 axis in cancer: mechanisms and therapies. Febs j. 2013;280:2294–306.

92. Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, Ivarsson Y, Depoortere F, Coomans C, Vermeiren E, et al. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. Nat Cell Biol. 2012;14:677–85.

93. Thompson CA, Purushothaman A, Ramani VC, Vlodavsky I, Sanderson RD. Heparanase regulates secretion, composition, and function of tumor cell-derived exosomes. J Biol Chem. 2013;288:10093–9.

94. Sento S, Sasabe E, Yamamoto T. Application of a Persistent Heparin Treatment Inhibits the Malignant Potential of Oral Squamous Carcinoma Cells Induced by Tumor Cell-Derived Exosomes. PLoS One. 2016;11:e0148454.

95. Nishida-Aoki N, Tominaga N, Takeshita F, Sonoda H, Yoshioka Y, Ochiya T. Disruption of Circulating Extracellular Vesicles as a Novel Therapeutic Strategy against Cancer Metastasis. Mol Ther. 2017;25:181–91.

96. de la Fuente A, Alonso-Alconada L, Costa C, Cueva J, Garcia-Caballero T, Lopez-Lopez R, Abal M. M-Trap: Exosome-Based Capture of Tumor Cells as a New Technology in Peritoneal Metastasis. J Natl Cancer Inst. 2015;107:djv184.

97. Logozzi M, Angelini DF, Iessi E, Mizzoni D, Di Raimo R, Federici C, Lugini L, Borsellino G, Gentilucci A, Pierella F, et al. Increased PSA expression on prostate cancer exosomes in in vitro condition and in cancer patients. Cancer Lett. 2017;403:318–29.

98. Federici C, Petrucci F, Caimi S, Cesolini A, Logozzi M, Borghi M, D'Ilio S, Lugini L, Violante N, Azzarito T, et al. Exosome release and low pH belong to a framework of resistance of human melanoma cells to cisplatin. PLoS One. 2014;9:e88193.

Wu *et al. Molecular Cancer*   (2019) 18:53

Page 11 of 11

99.   Zhang Y, Yang P, Wang XF. Microenvironmental regulation of cancer metastasis by miRNAs. Trends Cell Biol. 2014;24:153–60.

100. Hong BS, Cho JH, Kim H, Choi EJ, Rho S, Kim J, Kim JH, Choi DS, Kim YK, Hwang D, Gho YS. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. BMC Genomics. 2009;10:556.

101. Clancy C, Khan S, Glynn CL, Holian E, Dockery P, Lalor P, Brown JA, Joyce MR, Kerin MJ, Dwyer RM. Screening of exosomal microRNAs from colorectal cancer cells. Cancer Biomark. 2016;17:427–35.

102. Zaharie F, Muresan MS, Petrushev B, Berce C, Gafencu GA, Selicean S, Jurj A, Cojocneanu-Petric R, Lisencu CI, Pop LA, et al. Exosome-Carried microRNA-375 Inhibits Cell Progression and Dissemination via Bcl-2 Blocking in Colon Cancer. J Gastrointestin Liver Dis. 2015;24:435–43.

103. Teng Y, Ren Y, Hu X, Mu J, Samykutty A, Zhuang X, Deng Z, Kumar A, Zhang L, Merchant ML, et al. MVP-mediated exosomal sorting of miR-193a promotes colon cancer progression. Nat Commun. 2017;8:14448.

104. Escudier B, Dorval T, Chaput N, Andre F, Caby MP, Novault S, Flament C, Leboulaire C, Borg C, Amigorena S, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial. J Transl Med. 2005;3:10.

105. Morse MA, Garst J, Osada T, Khan S, Hobeika A, Clay TM, Valente N, Shreeniwas R, Sutton MA, Delcayre A, et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med. 2005;3:9.

106. Boulikas T. Clinical overview on Lipoplatin: a successful liposomal formulation of cisplatin. Expert Opin Investig Drugs. 2009;18:1197–218.

107. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharm Sin B. 2016;6:287–96.

108. Kim MS, Haney MJ, Zhao Y, Mahajan V, Deygen I, Klyachko NL, Inskoe E, Piroyan A, Sokolsky M, Okolie O, et al. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. Nanomedicine. 2016;12:655–64.

109. Armstrong JP, Holme MN, Stevens MM. Re-Engineering Extracellular Vesicles as Smart Nanoscale Therapeutics. ACS Nano. 2017;11:69–83.

110. Dos Anjos PB, da Luz Andres Cordero F, Socorro Faria S, Peixoto Ferreira de Souza L, Cristina Brigido Tavares P, Alonso Goulart V, Fontes W, Ricardo Goulart L, Jose Barbosa Silva M. The multifaceted role of extracellular vesicles in metastasis: Priming the soil for seeding. Int J Cancer. 2017;140:2397–407.

111. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, Wei J, Nie G. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials. 2014;35:2383–90.

112. Ohno S, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, Mizutani T, Ohgi T, Ochiya T, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. Mol Ther. 2013;21:185–91.

113. Mizrak A, Bolukbasi MF, Ozdener GB, Brenner GJ, Madlener S, Erkan EP, Strobel T, Breakefield XO, Saydam O. Genetically engineered microvesicles carrying suicide mRNA/protein inhibit schwannoma tumor growth. Mol Ther. 2013;21:101–8.

114. Yang T, Martin P, Fogarty B, Brown A, Schurman K, Phipps R, Yin VP, Lockman P, Bai S. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. Pharm Res. 2015;32:2003–14.

115. Mathieu M, Martin-Jaular L, Lavieu G, Thery C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol. 2019;21:9–17.

116. Belli C, Trapani D, Viale G, D'Amico P, Duso BA, Della Vigna P, Orsi F, Curigliano G. Targeting the microenvironment in solid tumors. Cancer Treat Rev. 2018;65:22–32.

117. Mazzocca A, Ferraro G, Misciagna G, Fais S. Moving the systemic evolutionary approach to cancer forward: Therapeutic implications. Med Hypotheses. 2018;121:80–7.

118. Aleckovic M, Kang Y. Welcoming Treat: Astrocyte-Derived Exosomes Induce PTEN Suppression to Foster Brain Metastasis. Cancer Cell. 2015; 28:554–6.

119. Achreja A, Zhao H, Yang L, Yun TH, Marini J, Nagrath D. Exo-MFA - A 13C metabolic flux analysis framework to dissect tumor microenvironment-secreted exosome contributions towards cancer cell metabolism. Metab Eng. 2017;43:156–72.

120. Olmeda D, Cerezo-Wallis D, Riveiro-Falkenbach E, Pennacchi PC, Contreras-Alcalde M, Ibarz N, Cifdaloz M, Catena X, Calvo TG, Canon E, et al. Whole-body imaging of lymphovascular niches identifies pre-metastatic roles of midkine. Nature. 2017;546:676–80.

121. Hyenne V, Lefebvre O, Goetz JG. Going live with tumor exosomes and microvesicles. Cell Adh Migr. 2017;11:173–86.

122. Chen C, Zong S, Wang Z, Lu J, Zhu D, Zhang Y, Cui Y. Imaging and Intracellular Tracking of Cancer-Derived Exosomes Using Single-Molecule Localization-Based Super-Resolution Microscope. ACS Appl Mater Interfaces. 2016;8:25825–33.

123. He C, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. Theranostics. 2018;8:237–55.

124. Lim C, Broqueres-You D, Brouland JP, Merkulova-Rainon T, Faussat AM, Hilal R, Rouquie D, Eveno C, Audollent R, Levy BI, Pocard M. Hepatic ischemia-reperfusion increases circulating bone marrow-derived progenitor cells and tumor growth in a mouse model of colorectal liver metastases. J Surg Res. 2013;184:888–97.

125. Govaert KM, Emmink BL, Nijkamp MW, Cheung ZJ, Steller EJ, Fatrai S, de Bruijn MT, Kranenburg O, Borel Rinkes IH. Hypoxia after liver surgery imposes an aggressive cancer stem cell phenotype on residual tumor cells. Ann Surg. 2014;259:750–9.

126. Jiao SF, Sun K, Chen XJ, Zhao X, Cai N, Liu YJ, Xu LM, Kong XM, Wei LX. Inhibition of tumor necrosis factor alpha reduces the outgrowth of hepatic micrometastasis of colorectal tumors in a mouse model of liver ischemia-reperfusion injury. J Biomed Sci. 2014;21:1.

127. van der Bilt JD, Kranenburg O, Nijkamp MW, Smakman N, Veenendaal LM, Te Velde EA, Voest EE, van Diest PJ, Borel Rinkes IH. Ischemia/reperfusion accelerates the outgrowth of hepatic micrometastases in a highly standardized murine model. Hepatology. 2005;42:165–75.

128. Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009;9:274–84.

129. Armstrong JPK, Stevens MM. Strategic design of extracellular vesicle drug delivery systems. Adv Drug Deliv Rev. 2018;130:12–6.

130. Yang L, Venneti S, Nagrath D. Glutaminolysis: A Hallmark of Cancer Metabolism. Annu Rev Biomed Eng. 2017;19:163–94.

131. Xu R, Rai A, Chen M, Suwakulsiri W, Greening DW, Simpson RJ. Extracellular vesicles in cancer - implications for future improvements in cancer care. Nat Rev Clin Oncol. 2018;15:617–38.