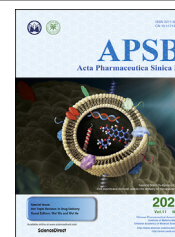




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## REVIEW

# Tumor-derived exosomes: Nanovesicles made by cancer cells to promote cancer metastasis



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Pre-metastatic niche;  
Therapeutic implications;  
Exosome targeting;  
Nanocarrier

**Abstract** Nanomedicine usually refers to nanoparticles that deliver the functional drugs and siRNAs to treat cancer. Recent research has suggested that cancer cells can also make nanoparticles that also deliver functional molecules in promoting cancer metastasis, which is the leading cause of various cancer mortalities. This nanoparticle is called tumor-derived vesicles, or better-known as tumor-derived exosomes (TEXs). TEXs are nanoscale membrane vesicles (30–140 nm) that are released continuously by various types of cancer cells and contain tumor-derived functional biomolecules, including lipids, proteins, and genetic molecules. These endogenous TEXs can interact with host immune cells and epithelial cells locally and systemically. More importantly, they can reprogram the recipient cells in favor of promoting metastasis through facilitating tumor cell local invasion, intravasation, immune evasion, extravasation, and survival and growth in distant organs. Growing evidence suggests that TEXs play a key role in cancer metastasis. Here, we will review the most recent findings of how cancer cells harness TEXs to promote cancer metastasis through modulating vascular permeability, suppressing systemic immune surveillance, and creating metastatic niches. We will also summarize recent research in targeting TEXs to treat cancer metastasis.

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## 1. Introduction

When cancer patients developed metastatic lesions in remote organs, the survival rate would be dramatically decreased compared to patients with local disease. Although it is known that cancer cells need to go through a series of steps to develop metastases, also known as the metastatic cascade<sup>1</sup>, the tumor-derived factors that modulate cancer cells to disseminate, survive in the circulation, and grow in the metastatic sites remain mostly unknown. Although several hypotheses about cancer metastasis have been proposed<sup>2–5</sup>, no theory can fully explain the whole metastatic process; more importantly, there is currently no effective way to cure metastatic disease, which causes more than 90% of all cancer-related deaths.

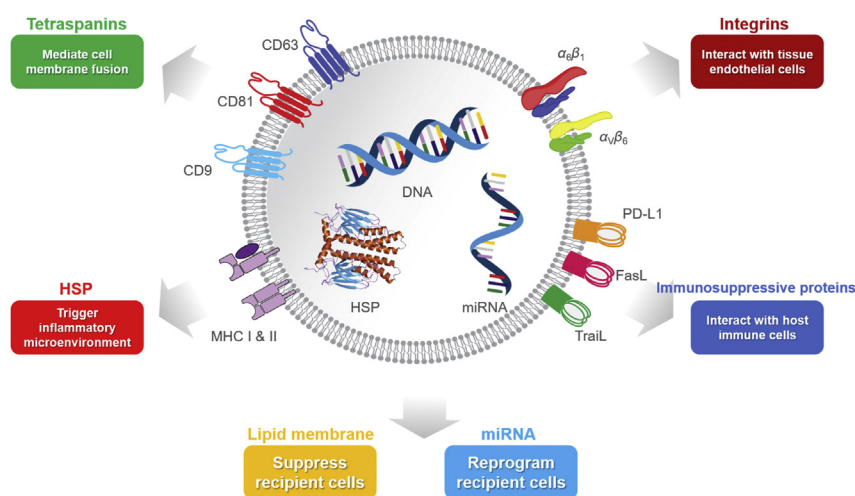
Recently, tumor-derived exosomes (TEXs) have surfaced as critical tumor-derived factors that play a critical role in the metastatic process. A growing body of evidence has suggested that TEXs can interact with host immune cells<sup>6,7</sup>, epithelial cells<sup>8,9</sup>, and tumor cells<sup>10,11</sup>, to alter and reprogram the recipient cells to facilitate tumor progression and cancer metastasis. For example, it was found that TEXs manipulate the leakage of vascular barriers to not only facilitate tumor cell escape from primary tumor tissues but also promote tumor cells to live and grow in metastatic organs<sup>8</sup>. Recent studies also found that TEXs carry immunosuppressive proteins such as program death ligand 1 (PD-L1) on their surfaces that can suppress cytotoxic T-cells locally and systemically<sup>12,13</sup>. Studies also found that TEXs activate epithelial cells through payload RNAs to recruit myeloid cells and develop permissive lung pre-metastatic niches<sup>9</sup>. Although previously viewed as “trash bags,” TEXs are now known to be important mediators in cancer metastasis. Here, we will review the recent progress in current understanding of how TEXs promote cancer metastasis through delivering exosomal genetic molecules, proteins, and lipids with a focus on the role of tumor-derived exosomes in each step of the metastatic cascade. Understanding how TEXs modulate immune suppression and facilitate tumor dissemination and outgrowth in distant organs through their

unique component will provide us novel insight to better design therapeutic strategies to fight against metastatic cancer.

## 2. TEXs and their interaction with host cells

TEXs are generally believed to be secreted from cancer cells by fusion of multivesicular bodies with the plasma membrane; a similar endocytic pathway is used for secreting exosomes by healthy cells<sup>14</sup>. However, due to TEXs' unique surface proteins, lipid composition, and contained genetic molecules, *e.g.*, tumor-derived microRNAs (miRNAs), messenger RNAs, and DNAs (Fig. 1), TEXs show entirely different characteristics from normal cell-derived exosomes toward their biological functions<sup>15</sup>. Therefore, due to their nano-ranged size (30–140 nm) and their active biological functions, TEXs are much like nanomedicine made by cancer cells, except that they are pro-metastatic. TEXs enter host cells *via* various mechanisms (*e.g.*, non-traditional endocytic pathways), depending on the target cells and the secreting cancer cells<sup>16</sup>. For example, glioblastoma cell-derived exosome uptake uses lipid Raft-mediated endocytosis and depends on undisturbed ERK1/2–HSP27 signaling<sup>17</sup>. Exosomes from brain-metastatic breast cancer cells use transcytosis to cross the brain endothelial cells<sup>18</sup> and utilize the “CDC42-dependent clathrin-independent carrier/GPI-AP-enriched compartment (CLIC/GEEC) endocytic pathway” to enter astrocytes<sup>19</sup>.

TEXs contain tumor-promoting content, both at the surface and in the lumen. For example, tetraspanins (which help mediate cell membrane fusion), MHC, integrins (which interact with tissue endothelial cells), and immunosuppressive proteins (*e.g.*, PD-L1, FasL, Trail) are in the phospholipid-bilayer membrane. HSPs (which trigger inflammatory microenvironments) and pro-tumor cytosolic proteins (*e.g.*, enzymes, cytokines, and oncoproteins) and receptors (*e.g.*, MET, gp130) are in the lumen<sup>20</sup>. TEXs also contain genetic materials (*e.g.*, DNAs, miRNAs, mRNAs,



**Figure 1** Schematic illustration of main characteristics of tumor-derived exosomes (TEXs). TEXs are membrane nanovesicles (30–140 nm) secreted by cancer cells. They carry specific tumor-derived integrins on their surfaces, which preferentially interact with certain endothelial cells. Once bound to the receptor cells, TEXs can deliver tumor-derived genetic molecules such as miRNAs and reprogram the recipient cells in the favor of cancer metastasis. TEXs also carry various immunosuppressive surface proteins such as PD-L1 that can directly interact with cytotoxic T-cells through PD-1/PD-L1 interaction locally and systemically, and inhibit T-cell anticancer function.

IncRNAs) that reprograms recipient cells. For example, exosomal miR-105, miR-25-3p, and miR-103 can compromise vascular integrity, and exosomal miR-1246 and miR-92 participate in immunosuppression (Table 1).

Once TEXs are released out into the extracellular microenvironment, their biological function is determined upon how they can be recognized by the host cells. Previous research has suggested that due to their tumor-derived unique surface proteins and lipid composition as well as their virus-like hydrodynamic sizes, TEXs may be quickly captured by host immune cells and epithelial cells<sup>21–24</sup>. For example, using a luciferase-expressing mouse tumor cell line, Takahashi et al.<sup>21,22</sup> demonstrated that B16-BL6-derived exosomes administered to syngeneic mice could be quickly distributed from blood circulation to tissues like liver, spleen, and lung with a half-life of around 2 min. Consistently, fluorescence-labeled TEXs could be detected in the blood vessels of lungs 5 min after tail vein injection<sup>23</sup>. Rapid clearance of intravenously-injected exosomes, which are derived from human prostate adenocarcinoma and labeled with a radiotracer, was also observed in nude mice<sup>24</sup>. Interestingly, by comparing exosome clearance rate in 4T1 tumor-bearing mice with different stains, namely BALB/c, nude, and NOD/SCID mice, Smyth et al.<sup>24</sup> found that 4T1 exosomes were captured in liver and spleen as fast as 20 min in BALB/c mice after systemic administration and that the clearance rate is similar in both BALB/c mice and nude mice. However, the exosomes' clearance in immunocompromised mice was delayed, indicating that the innate immune system, not the adaptive immune system, plays a role in recognizing tumor exosomes in tumor-bearing hosts.

Given the similarity between TEXs and viruses, the human immune system might treat the constantly released TEXs as

viruses and develop B-cell-mediated humoral immune responses against them<sup>25</sup>, which may trigger inflammatory cytokine release. The induced cytokines, which are not able to eliminate TEXs, may have pro-metastatic effects. By using similar luciferase-expressing B16F10 tumor models and monitoring the interaction between endogenously produced tumor vesicles and host cells, Pucci et al.<sup>25</sup> found that tumor vesicles may be preferentially recognized by subcapsular sinus (SCS) CD169<sup>+</sup> macrophages in tumor-draining lymph nodes. As these vesicles disrupt the SCS macrophage layer, they can access the B-cell follicles and trigger pro-tumor humoral immune responses against the TEXs<sup>25</sup>. This study suggests that host body may initiate humoral immune responses and treat TEXs as invaded viruses. This study also suggests that the recognition of TEXs by host cells may occur through a receptor-mediated capture of TEXs rather than through non-specific interactions<sup>26</sup>. More importantly, how TEXs interact with the recipient cells may also explain why the contained genetic molecules, especially miRNAs, can enter the cytosol and remain functional instead of being digested in endosomes inside cells.

### 3. Biofunctional role of tumor-derived exosomes in metastatic cascade

#### 3.1. TEXs cause tumor vasculature leaky

One of the key questions in cancer biology is that why tumor cells can often leave primary tumor tissues regardless of their cellular origins, which results in the subsequent development of fatal metastasis. Recently, a pioneer study led by Dr. Wang has revealed that TEXs play a critical role in promoting tumor cell

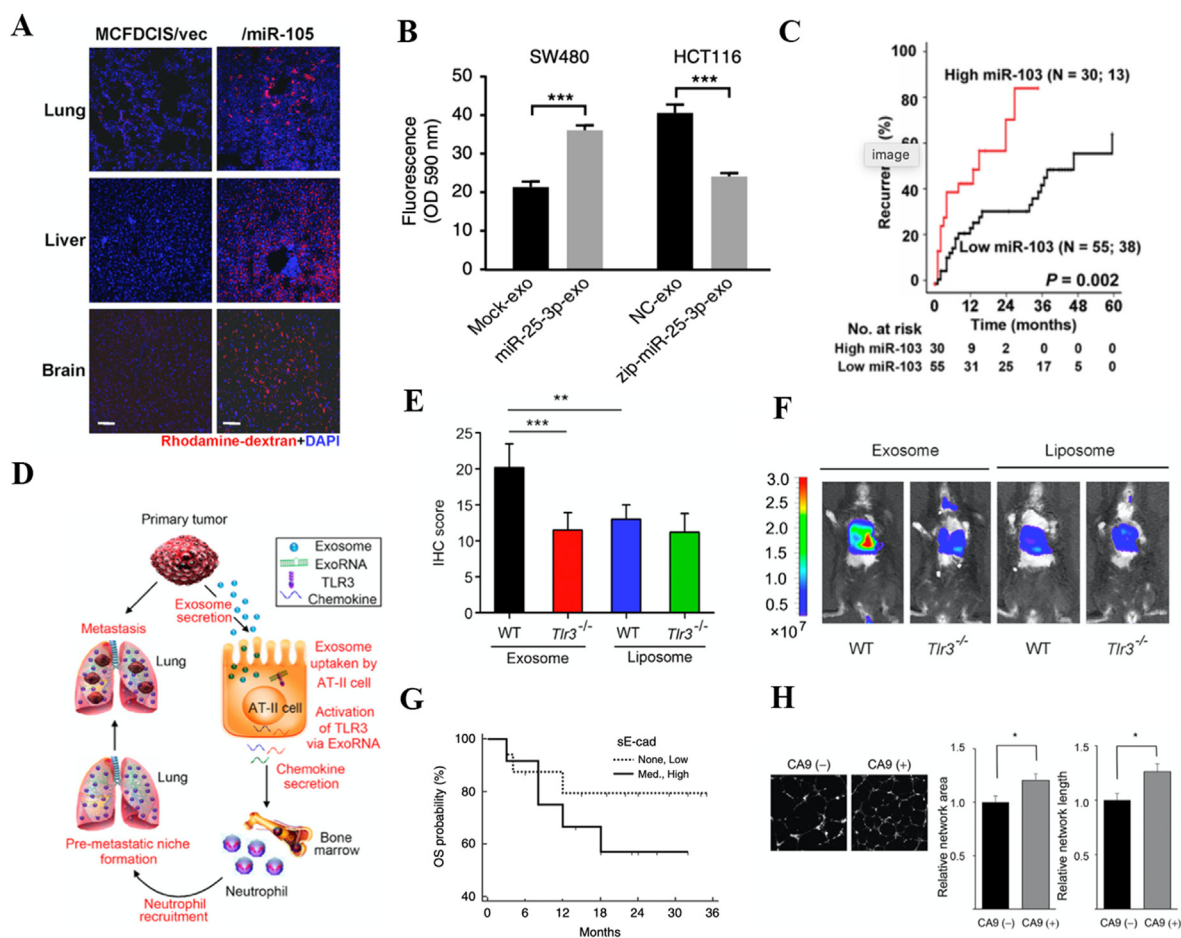
**Table 1** Summary of functional molecules in TEXs.

Functional molecule	Summary of mechanism
Makes vasculature leaky	
miR-105	Lowers zonula occludens-1 (ZO-1) levels in endothelial cells
miR-25-3p	Silence Krüppel-like factor 2 (KLF2) and Krüppel-like factor 4 (KLF 4) in colorectal cancer
miR-103	In liver cancer, inhibits the expression of the VE-cadherin, P120, and ZO-1
Immunosuppression	
PD-L1	Binds to PD-1 on cytotoxic T-cells, blocks signaling downstream of the T-cell receptor (TCR), and hence inactivates them.
Noncoding Y RNA hY4	Helps reprogram monocytes in an immunosuppressive manner by activating endosomal TLR7/TLR8 signaling; increases TNF-alpha and PD-L1 expression
gp130	An IL-6 receptor; in breast cancer, stimulates STAT3 signaling in bone-marrow derived macrophages and triggers IL-6 secretion
MET	A hepatocyte growth factor receptor; educates bone-marrow derived cells to make them pro-vasculogenic and pro-migratory
miR-1246	Increases macrophages' TGF- $\beta$ activity and makes them more motile and invasive, undergo epithelial-to-mesenchymal transition, degrade the extracellular matrix, and recruit immunosuppressive Tregs
miR-92a	By suppressing SMAD7 protein, increases TGF- $\beta$ activity in hepatic stellate cells and deposition of extracellular matrix proteins in the pre-metastatic niche, promoting the recruitment of immunosuppressive BMDCs
Promotes metastatic niches	
Binding of disseminated tumor cells to metastatic niches	
ITG $\beta$ 4	On exosome's surface; binds to laminin in the laminin-rich lung microenvironment
ITG $\alpha$ 6 $\beta$ 4	On exosome's surface; binds to epithelial cells and fibroblasts that belong to the lung
ITG $\alpha$ v	May selectively adhere to fibronectin in the fibronectin-rich microenvironment in the liver
Angiogenesis in metastatic niche	
Soluble E-cadherin	Binds to VE-cadherin on endothelial cells and hence activates NF- $\kappa$ B and $\beta$ -catenin signaling
Carbonic anhydrase 9 (CA9)	Induces tube formation and migration and increases MMP2 production

dissemination<sup>8</sup>. This study found that exosomes derived from metastatic MDA-MB-231 breast cancer cells cause adjacent endothelial cells with a down-regulation of the expression of zonula occludens-1 (ZO-1) protein<sup>8</sup>, which has a tight junction function. The TEX-induced ZO-1 inhibition makes the vasculature in primary tumor tissue leaky and therefore facilitates breast cancer cells intravasation. They further revealed that the expression of a miRNA called miR-105 is significantly higher in exosomes derived from MDA-MB-231 breast cancer cells compared to exosomes derived from normal breast cells (*i.e.*, MCF-10A). Upon incubation with human microvascular endothelial cells (HMVECs), their data shows that exosomes with

higher expressions of miR-105 cause the recipient endothelial cells to lose the function for ZO-1 protein secretion, but exosomes containing low miR-105 do not have that effect (Fig. 2a). More importantly, these miR-105-containing exosomes can travel to distant organs and increase vascular permeability at future metastatic sites, thereby facilitating metastasis by promoting extravasation<sup>8</sup>. This work highlighted the importance of TEXs in vascular remodeling locally and remotely; they help tumor cells to not only escape from the primary site but also grow in future metastatic sites.

Similar effects were also reported in both colorectal cancer (CRC) and hepatocellular carcinoma (HCC). In CRC, exosomal



**Figure 2** TEXs support pre-metastatic niches by compromising vascular integrity, suppressing the immune system, and promoting tumor angiogenesis. (A) Exosomal miR-105 promotes vascular permeability, as indicated by the increased “appearance of intravenously injected rhodamine-dextran (red) in various organs.” Reprinted from Ref. 8. Copyright © 2014 Elsevier Inc. (B) In colorectal cancer, exosomal miR-25-3p increases vascular permeability, as indicated by the increased permeability of the human umbilical vein endothelial cell (HUVEC) monolayers to rhodamine-dextran after exposure to exosomal miR-25-3p. Reprinted from Ref 27. Copyright © 2018, The Author(s). (C) In hepatocellular carcinoma, exosomal miR-103 is linked to higher recurrence rates. Reprinted from Ref 29. Copyright © 2018 by the American Association for the Study of Liver Diseases. (D) In breast cancer, exosomal RNAs enters alveolar type II cells (AT-II cells) and causes them to secrete chemokines, hence causing neutrophil recruitment and pre-metastatic niche formation in the lungs. Reprinted from Ref. 9. Copyright © 2016 Elsevier Inc. (E) In breast cancer, TEXs increase fibronectin expression in the lungs in wild-type littermates but not in *Tlr3*<sup>-/-</sup> mice. Liposomes do not seem to significantly increase fibronectin expression. Reprinted from Ref. 9. Copyright © 2016 Elsevier Inc. (F) Quantification of lung metastasis of *Tlr3*<sup>-/-</sup> mice or WT littermates after exosome or liposome administration. Reprinted from Ref. 9. Copyright © 2016 Elsevier Inc. (G) In patients with ovarian cancer, high levels of exosomal soluble E-cadherin (sE-cad) are linked to worse prognoses. Reprinted from Ref. 84. Copyright © 2018, The Author(s). (H) Tube formation and angiogenesis increase when human umbilical vein endothelial cells (HUVECs) are treated with exosomes containing carbonic anhydrase-9. Reprinted from Ref. 85. Copyright © 2017 Elsevier Inc.

miR-25-3p can be transferred to vascular endothelial cells and silence Krüppel-like factor 2 (KLF2) and Krüppel-like factor 4 (KLF 4) in the receptor cells<sup>27</sup> (Fig. 2b). Decreased KLF2 cannot inhibit the promoter activity of vascular endothelial growth factor receptor 2 (VEGFR2) and induce angiogenesis; meanwhile, decreased KLF4 inhibits the expression of tight-junction related proteins like ZO-1, occludin, and Claudin5, which leads to vascular leakiness and metastasis<sup>27,28</sup>. In HCC, exosomal miR-103 promotes vascular permeability and metastasis<sup>29</sup> (Fig. 2c). Exosomal miR-103 is transferred to endothelial cells and inhibits the expression of VE-Cadherin, P120, and ZO-1, which are important in cell–cell adhesion and help maintain endothelial cell–cell contacts. When p120 expression is reduced, p120 cannot fulfill its normal function of stabilizing the E-cadherin (E-Cad)/ $\beta$ -catenin complex at the cell membrane to maintain intercellular adhesion<sup>29</sup>. Thus, vascular permeability, HCC cell migration, and the formation of hepatic and pulmonary metastases are promoted<sup>29</sup>.

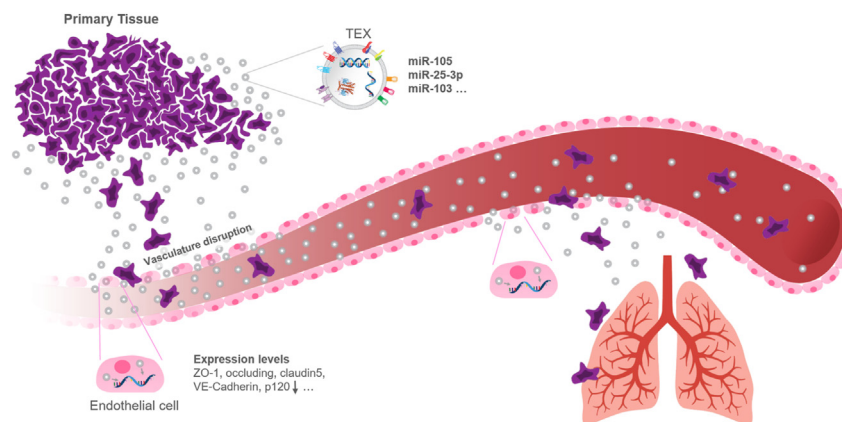
Together, these studies have demonstrated that miRNA encapsulated in the TEXs plays a critical role in vascular permeability and contributes to tumor cell invasion at the primary site and extravasation at secondary sites (Fig. 3). In addition to the tumor vasculature disruption, studies also found that exosomal miRNAs play various roles in promoting cancer metastasis, including suppressing glucose uptake by niche cells, causing PTEN loss, and activating Toll-like receptor (TLR) (Table 2). Although various specific miRNA molecules in different cancer types have been reported, it remains unclear whether there is a more general molecular mechanism that helps modulate recipient cell function and tumor vasculature, such as TEX-mediated RNA sensing regardless of specific miRNA molecules. In addition, leaky tumor vasculature has long been confirmed and has been widely harnessed for drug delivery using human-made nanomedicine *via* taking advantage of the well-known enhanced permeability and retention (EPR) effect<sup>37</sup>. It was generally believed that the leaky tumor vasculature is due to poorly aligned defective endothelial cells during fast cancer angiogenesis. Recent findings from TEXs may provide a completely novel molecular mechanism for the EPR effect.

### 3.2. TEXs suppress anti-tumor immunity

#### 3.2.1. TEXs carry immunosuppressive PD-L1 on their surfaces and suppress activated T-cells

PD-L1 is a critical immunosuppressive protein that can bind to its receptor PD-1 on CD8<sup>+</sup> effector T-cells to inhibit antitumor immune responses<sup>38</sup>. When PD-L1 binds to PD-1, signaling downstream of the heterodimeric T-cell receptor (TCR) is inhibited<sup>39</sup>.

It was initially thought that only tumor cells or tumor-associated lymphocytes such as antigen-presenting cells and bone marrow-derived myeloid cells are the major immunosuppressive factors that can present PD-L1 to inhibit cytotoxic CD8<sup>+</sup> T-cell anticancer function<sup>38–40</sup>. Recent studies further found that TEXs in patient's blood also carry PD-L1 on their surfaces, revealing an entirely new understanding of TEX-mediated immune suppression<sup>12,13</sup>. For example, one group reported that exosomal PD-L1 has a significant immunosuppressive effect in melanoma patients<sup>12</sup>. By comparing PD-L1 expression on exosomes derived from a melanoma cell line, Chen et al.<sup>12</sup> discovered that exosomes from metastatic melanoma cells express much higher levels of PD-L1 than their primary tumor cells. Their study further showed that the level of PD-L1 in peripheral circulating exosomes from metastatic melanoma patients is significantly higher than those from healthy donors, and those PD-L1 highly expressed exosomes can effectively deactivate cytotoxic T-cells and hence prevent the immune system from fighting the cancer cells. This result is consistent with previous findings, indicating that TEXs inhibited the proliferation of activated CD8<sup>+</sup> effector T-cells<sup>41–46</sup>. More importantly, they discovered that higher levels of exosomal PD-L1 before a pembrolizumab therapy (an anti-PD-1 therapy) is predictive of greater tumor burden and higher levels of IFN- $\gamma$ , which are predictive of poor therapy outcomes<sup>12</sup>. Their results from clinical data further showed that high pretreatment levels of exosomal PD-L1 are indicative of immune dysfunction and severe exhaustion of T-cells, which makes them unable to be reinvigorated by the treatment<sup>12</sup>. This study further found that, in patients who responded to the therapy, the exosomal PD-L1 levels significantly increased (at least 2.43-fold) six weeks after the treatment started; this is likely because the treatment successfully



**Figure 3** Tumor-derived exosomes (TEXs) promote metastasis by promoting vasculature disruption. Exosomes from metastatic cancer cells contain specific miRNAs, which cause endothelial cells to reduce their expression of important tight-junction proteins like ZO-1, cadherin, and P120. TEXs from the primary tumors leak into the bloodstream and travel to the metastatic sites. Upon arrival to the metastatic sites, they get taken in by endothelial cells there, down-regulate the expression of tight-junction proteins, and hence destroy vascular integrity. The destruction of vascular integrity facilitates metastasis.

**Table 2** Various exosomal miRNAs in cancer metastasis.

Exosomal miRNA	Cancer type	Role in cancer metastasis	Ref.
miR-105	Breast cancer	Down-regulate tight junction protein ZO-1	8
miR-122	Breast cancer	Down-regulate glycolytic enzyme pyruvate kinase	30
miR-19	Breast cancer	PTEN loss	31
miR-939	Breast cancer	Inhibit VE-cadherin	32
miR-21	Lung cancer	Up-regulate STAT3 and VEGF	33
miR-23a	Lung cancer	Down-regulate ZO-1	34
miR-21/29a	Lung cancer	Activating TLR7 and TLR8	35
miR-25-3p	Color rector cancer	Down-regulate ZO-1, occludin, and claudin5	27
miR-103	Hepatocellular carcinoma	Down-regulate VE-Cadherin, p120, and ZO-1	29
miR-222-3p	Ovarian cancer	Down-regulate SOCS3	36

invigorated the cytotoxic T-cells, and the tumor cells released more PD-L1 in a futile attempt to inactivate cytotoxic T-cells adaptively<sup>12</sup>.

Almost at the same time, another group also reported the suppressive function of exosomal PD-L1 on antitumor immunity in prostate cancer<sup>13</sup>. Their study demonstrated that if the syngeneic TRAMP-C2 prostate cancer cells lose the capability of secreting exosomal PD-L1 *via* CRISPR/Cas9-mediated depletion of Rab27a and PD-L1, the tumor growth will be significantly inhibited compared to its wild-type cell line in immunocompetent B6 mice. The molecular and cellular mechanism studies further reveal that exosomal PD-L1 can suppress antitumor immunity in the draining lymph nodes. Their study also found that administering exosomes derived from the wild-type cell line can rescue tumor growth of Rab27 and PD-L1 deficient cancer cells, confirming the exosomal PD-L1 immune suppression toward anti-tumor immunity<sup>13</sup>.

### 3.2.2. *TEXs reprogram tumor-infiltrated immune cells into immunosuppressive phenotypes*

Tumor-associated immune cells play an important role in promoting cancer metastasis due to their immunosuppressive characteristics, which are strongly associated with the tumor microenvironment<sup>38–40</sup>. Recent studies have found that TEXs contribute to reprogramming the tumor-associated immune cells into pro-tumorigenic phenotypes<sup>6,7,36,47–52</sup>. For example, it was reported that when monocytes were treated with chronic lymphocytic leukemia (CLL)-derived exosomes, the uptake of the exosomes trigger the release of inflammatory cytokines and PD-L1 expression, and skew monocytes into pro-tumorigenic phenotypes<sup>6</sup>. It was further revealed that the exosomal noncoding Y RNA hY4 result in the monocyte reprogramming by binding to endosomal TLR7/TLR8<sup>6</sup>. This binding activates NF- $\kappa$ B expression, which promotes pro-tumor cytokine release (*e.g.*, TNF- $\alpha$  secretion) and increases PD-L1 expression in monocytes<sup>6</sup>. Exosomal hY4 also stimulates TNF- $\alpha$  secretion<sup>6</sup>, an important cytokine that promotes cancer metastasis<sup>53</sup>. Another recent study also found that the IL-6-receptor-beta gp130 in breast cancer TEXs stimulates STAT3 signaling in bone-marrow derived macrophages (BMDMs) and triggers IL-6 secretion<sup>52</sup>, which promotes cancer cell proliferation, cancer cell invasion, and metastasis<sup>54</sup>. It was concluded that TEXs dominate tumor-associated macrophage polarization toward immunosuppressive M2-type macrophages in breast cancer<sup>52,55</sup>.

Given the fact that neutrophils are the most abundant innate immune cells in our body and the high immunogenicity of TEXs, the interaction between neutrophils and TEXs actively take place,

and the exosome-mediated neutrophil activation may play a driving force for cancer metastasis progression<sup>56–59</sup>. This idea was further confirmed by two recent studies revealing that TEXs can polarize myeloid-derived neutrophils into pro-tumor phenotypes<sup>7,60</sup>. For instance, Zhang et al.<sup>7</sup> found that exosomes derived from gastric cancer can transport high mobility group box-1 (HMGB1) to neutrophils, which interacts with TLR4 and triggers neutrophil activation through NF- $\kappa$ B pathway. These findings demonstrated that TEXs play a role in polarizing tumor-associated neutrophils, which have been linked to the progression of cancer metastasis<sup>56,57</sup>.

TEXs also reprogram other types of tumor-infiltrated immune cells like tumor-associated macrophages. For example, colon cancer cells with specific mutp53 proteins secrete exosomes containing miR-1246 that reprogram neighboring macrophages, increasing their TGF-beta activity and hence their anti-inflammatory immunosuppressive activities. These macrophages become more motile and invasive, undergo epithelial–mesenchymal transition (EMT), degrade the extracellular matrix, have diminished phagocytotic abilities, and recruit immunosuppressive regulatory T-cells (Tregs). Their matrix metalloproteinase 9 (MMP-9) and vascular non-inflammatory molecule 1 (VNN-1) secretion also increases<sup>61</sup>. Mechanistically, exosomal miR-1246 originates from RNU2-1 degradation and its generation is dependent on neither *Drosha* nor *Dicer*<sup>62</sup>.

TEXs also suppress activity of tumor-infiltrated cytotoxic T lymphocytes. For example, the HSP70 on exosomes from renal cancer cells interact with TLR2 on the membrane of myeloid-derived suppressor cells (MDSCs). This interaction causes activation of downstream factors including MyD88, TRAF6, P38, and AP1; hence, factors like arginase, reactive oxygen species (ROS), and nitric oxide (NO) that suppress T-cell activity and promote antigen-specific tumor immune escape are upregulated. This interaction also allows the exosomes to be uptaken by these MDSCs<sup>63</sup>.

### 3.2.3. *TEXs suppress NK cell function*

It was recently found that TEXs play a role in suppressing NK cells in the tumor microenvironment<sup>64</sup>. NK cells are believed to be important innate immune cells in controlling cancer metastasis<sup>65,66</sup>. NK cells can exert robust anti-metastatic functions *via* different pathways including the secretion of IFN- $\gamma$ , granules, and the exposure of death inducing ligands such as Fas ligand (FasL) and TNF-related apoptosis inducing ligand (TRAIL)<sup>65</sup>. Upon coculturing, NK cells have been shown to be able to kill cancer cell lines of different histological origins<sup>67</sup>. Improved NK cell cytotoxicity has been linked with good prognosis in patients

bearing primary prostate carcinomas<sup>68</sup>. However, research found that NK cells have only a minimal inhibitory effect on established micro metastasis and these NK cells in the tumor microenvironment have been functionally suppressed<sup>64,66,69</sup>. Study further showed that TEXs derived from a highly-metastatic pancreatic cancer cell line inhibited the anti-tumor cytotoxicity of NK cells by downregulating their expression of NK group 2D (NKG2D) (an NK-cell activating receptor) and secretion of cytokines including TNF- $\alpha$  and IFN- $\gamma$ <sup>64</sup>. Studies from other groups further confirmed that TEXs can deliver TGF- $\beta$  to the surface of NK cells, and the engagement between TGF- $\beta$  and its receptor on NK cells decreases NKG2D expression and suppresses NK cell cytotoxicity<sup>70,71</sup>, which has been found to contribute to the progression of cancer metastasis in various types of tumors<sup>65,69,72</sup>.

Taken together, these studies found that TEXs play a significant role in contributing to immunosuppression including enhancing exosomal immunosuppressive protein expression, suppressing antitumor immunity, polarizing tumor-associated immune cells into pro-tumor phenotype, and activating immunosuppressive population at primary tumor sites and remote metastatic organs (Fig. 4). So, disseminated tumor cells will be less likely to be caught by the immune system and hence will be able to settle, thrive, and proliferate in the metastatic sites. Recent studies even found that TEXs released from dying tumor cells following chemotherapy could promote Ly6C<sup>+</sup>CCR2<sup>+</sup> monocyte expansion in pre-metastatic niche, thereby to facilitate cancer metastasis<sup>73</sup>.

### 3.3. TEXs promote pre-metastatic niche formation

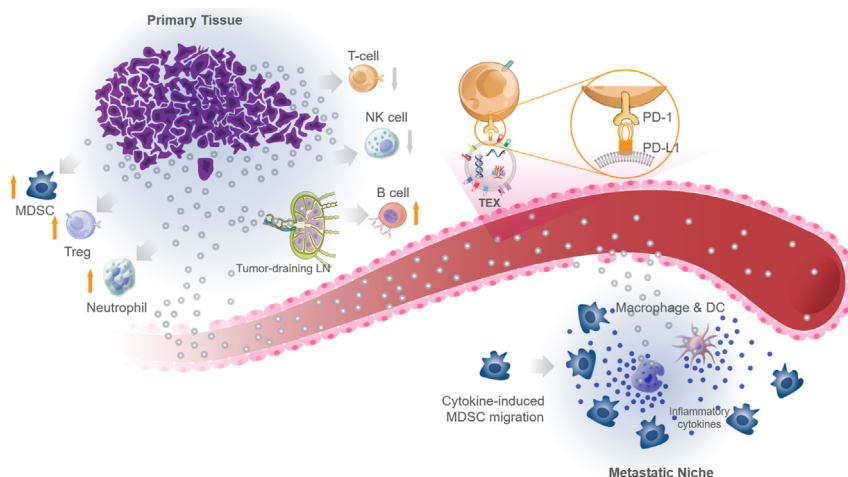
#### 3.3.1. Exosomes promote metastatic niches by recruiting and educating immune cells

Pre-metastatic niche formation was first proposed and investigated by a study led by Dr. Kaplan<sup>74</sup>. In their pioneer study, they found that bone marrow-derived dendritic cells (BMDCs) that express vascular endothelial growth factor receptor 1 (VEGFR1) home to pre-metastatic sites before the arrival of tumor cells. These

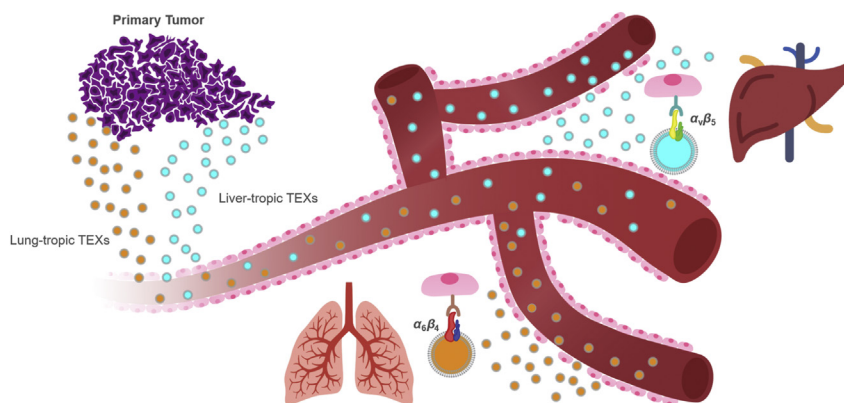
VEGFR1<sup>+</sup> cells express VLA-4 (also known as integrin  $\alpha 4\beta 1$ ); meanwhile, tumor-specific growth factors increase the expression of fibronectin (a VLA-4 ligand) in resident fibroblasts<sup>74</sup>. The interaction between fibronectin and VLA-4 enables BMDCs to home toward the pre-metastatic sites where these BMDCs produce proteinases like matrix metalloproteinase 9 (MMP9) that release Kit-ligand and VEGF-A<sup>74</sup>. Thereby, a permissive environment (pre-metastatic niche) for disseminated cancer cells to live and grow is prepared.

Later, another study further found that metastatic carcinomas like Lewis lung carcinoma (LLC) produce factors that induce bone marrow-derived dendritic cell (BMDC) production of pro-metastasis TNF- $\alpha$  through the activation of TLR2:TLR6 complexes<sup>53</sup>. TNF- $\alpha$  increases recruitment of leukocytes (in part because TNF- $\alpha$  increases vascular permeability); these leukocytes are later educated by the cancer cells and become pro-tumor. Hence, pre-metastatic niches become more tumor friendly<sup>39</sup>. But, the tumor-derived factors that caused the BMDCs to become activated and home to pre-metastatic niches remained unidentified in those studies<sup>53,74</sup>.

The tumor-derived factors were later identified as TEXs by different groups<sup>9,75</sup>. For instance, Liu et al.<sup>9</sup> found that TEXs activate alveolar epithelial cells in a TLR3-dependent pathway and trigger myeloid cell attractive chemokine secretion, which leads to myeloid cell recruitment and pre-metastatic niche formation (Fig. 2d-f). In addition to epithelial cell activation, researchers also found that TEXs can trigger NF- $\kappa$ B pathway activation in a TLR2-or MyD88-dependent pathway in tissue-resident macrophages, which results in the secretion of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, G-CSF, and CCL2<sup>76</sup>. Also, pancreatic cancer-derived exosomes, which have migration inhibitory factor (MIF), are phagocytosed by Kupffer cells in the liver and release MIF into these cells<sup>76</sup>. So, these cells secrete more transforming growth factor- $\beta$  (TGF- $\beta$ ) and produce more fibronectin, a contributor to the recruitment of bone marrow-derived macrophages, which contribute to the metastasis-supportive nature of hepatic pre-metastatic niches<sup>76</sup>.



**Figure 4** TEXs have various immunosuppressive functions. Cancer cells release tumor-derived exosomes (TEXs). TEXs suppress the activities of T-cells and natural killer (NK) cells. Exosomal PD-L1 (programmed death-ligand 1) suppresses T-cell activity by binding to PD-1 (programmed cell death protein 1) on T-cells. TEXs activate myeloid-derived suppressor cells (MDSCs), regulatory T-cells, and neutrophils in a pro-tumor manner. TEXs invade tumor-draining lymph nodes and disrupt SCS cells, which form the protective outer layer of the lymph nodes; then access B-cell zone to activate B-cells in a tumor-supportive manner. Next, TEXs leak into the blood stream and eventually develop the metastatic niche. In the metastatic sites, TEXs are uptaken by tissue resident epithelial cells and macrophages, which in turn release inflammatory cytokines. These cytokines cause MDSCs to migrate to the metastatic niche and hence make it more tumor-friendly.



**Figure 5** TEX surface integrins determine organotropic metastasis. Exosomes derived from different type of cancer cells can display different integrin proteins on their surfaces and those exosomal integrins determine which tissue endothelial cells can preferentially recognize them and develop pre-metastatic niche in that specific organ. For instance, ITG $\alpha$ 6 $\beta$ 4-expressing TEXs preferentially interact with epithelial cells in the lungs, and ITG $\alpha$ v $\beta$ 5-expressing TEXs preferentially being recognized by Kupffer cells in the liver. This finding revealed how certain cancer tends to develop organ-specific metastasis.

Furthermore, in melanoma, TEXs have MET (a hepatocyte growth factor receptor), which makes bone-marrow derived cells (BMDCs) pro-vasculogenic and pro-migratory. Then, these BMDCs exit the bone marrow and migrate to the pre-metastatic niches, where they promote vascular leakiness and secrete pro-inflammatory cytokines and chemokines<sup>23</sup>.

### 3.3.2. Exosomes determine organotropism (i.e., organ-specific metastasis)

It is a consistent clinical observation that patients with certain cancers tend to develop organ-specific metastasis. But what factors determine the metastasis organotropism has been a scientific mystery for a long time. Back in 1889, an English surgeon named Stephen Paget was asking this question<sup>77</sup>, “What is it that decides what organs shall suffer in a case of disseminated cancer?” Recently, Dr. Lyden’s group found that TEXs prepare metastatic niches in specific organs and that exosomal surface integrins determine the organ-specific metastasis<sup>78</sup>. Remarkably, they found that exosomes derived from different cancer models interact preferentially with resident cells at the same future metastatic organs as their cell of origin, thus prepare the pre-metastatic niche<sup>78</sup>. Also, before being uptaken by the target cells, these exosomes first increase vascular leakiness at the pre-metastatic niche. For instance, in mice treated with 4175-LuT-derived exosomes (LuT, Lung-tropic), these exosomes increased the capability of 4175-LuT tumors to metastasize to the lungs and redirected the bone-tropic tumor cells to develop metastasis in the lung<sup>78</sup>. Treatment with liver-tropic exosomes derived from the pancreatic cancer cell line BxPC-3 increased their uptake in the liver and hence promoted liver metastases<sup>78</sup>.

There is more evidence to support that exosomal surface integrins (ITGs) dictate metastatic organotropism by binding to specific ECM-rich areas in the target regions. For instance, ITG $\beta$ 4 is expressed in especially high amounts in pre-metastatic breast cancer patients who later developed lung metastases. ITG $\beta$ 4 (on the exosome’s surface) binds to laminin in the laminin-rich lung microenvironment. Hence, the exosomes are preferentially uptaken by the lung, and lung metastases are promoted. Exosomal ITG $\alpha$ v (at time of diagnosis) is expressed in especially high amounts in pre-metastatic pancreatic cancer patients who later developed liver metastasis within 3 years of diagnosis. These

exosomes may selectively adhere to fibronectin in the fibronectin-rich microenvironment in the liver. Exosomal ITG $\alpha$ 6 $\beta$ 4 and ITG $\alpha$ 6 $\beta$ 1 bind to epithelial cells and fibroblasts that belong to the lung<sup>78</sup>. Hence, they direct or redirect metastases to the lung. S100A4 helps cancer spread to the lungs<sup>79</sup> and is regulated by ITG $\alpha$ 6 $\beta$ 4<sup>80</sup>; so, in lung fibroblasts and when pre-metastatic niches are made, ITG $\alpha$ 6 $\beta$ 4 likely activates the Src-S100A4 axis<sup>78</sup>. These data suggest that TEXs express unique surface integrins that determine which organ recognizes the tumor-exosomes; therefore, pre-metastatic niches are preferentially prepared in that organ (Fig. 5).

### 3.3.3. Exosomes promote metastatic niches by increasing vascular permeability

For metastasis to occur, one of the key steps is to cross the endothelial barrier in the potential metastatic organs. TEXs help cancer cells cross the barrier by increasing vascular permeability in the metastatic organs. For example, metastatic breast cancer cells secrete miR-105-containing TEXs to pre-metastatic niche cells and hence increase vascular permeability there. Due to the TEXs, miR-105 levels in the pre-metastatic niche cells (which are endothelial cells) increased dramatically, and the expression of TJP1 (tight junction protein 1; also known as ZO-1) was down-regulated<sup>8</sup>. Consequently, these endothelial cells became better at migrating; the vascular integrity of endothelial layers became severely compromised<sup>8</sup>. Destruction of vascular integrity (mediated by miRNAs like miR-105) increases the penetration of pro-tumor exosome cargos (the particles that the exosomes contain), which leak into the pre-metastatic niches; hence, the cellular physiology of these organs becomes more metastasis-promoting and tumor-supportive<sup>8</sup>.

In breast cancer, TEXs can breach the blood–brain barrier (BBB) *via* transcytosis in endothelial cells. Although transcytosis is normally uncommon, they decrease Rab7 expression, hence facilitating their transport. The clathrin-dependent pathway and micropinocytosis, but not the caveolin-dependent pathway, were involved in the transcytosis<sup>18</sup>. Later on, the same group found out that, in breast cancer, after undergoing transcytosis in endothelial cells, these exosomes can enter astrocytes *via* the “Cdc42-dependent clathrin-independent carrier/GPI-AP-enriched compartment (CLIC/GEEC) endocytic pathway”<sup>19</sup>. The miR-301a



**Table 3** Therapeutic implications *via* targeting TEXs.

Mechanism	Case	Ref.
Inhibition of TEXs release	Targeting Rab27a	23,87,88
	Targeting Munc13-4	89
	Targeting nSMase2	90,91
Removal of circulating TEXs	Aethlon Hemopurifier®	92
	Anti-CD9/anti-CD63 antibodies	77,93
Blocking interaction of TEXs with recipient cells	Targeting fibronectin/heparan interaction	11,94
	Targeting HSP70/TLR2 interaction	95
	Targeting exosome endocytosis	17,96
Harnessing TEXs as therapeutic carriers to treat cancer and its metastasis	TEXs delivery with nucleic acids ( <i>e.g.</i> , siRNA against RAD51 and RAD52)	97
	TEXs delivery with chemotherapeutics ( <i>e.g.</i> , doxorubicin, MTX, cisplatin and paclitaxel)	98–101
	TEXs delivery with nanoparticles ( <i>e.g.</i> , iron oxide nanoparticles)	102
	TEXs to boost immune response	103–105

in these exosomes downregulates tissue inhibitor of metalloproteinase-2 (TIMP-2) and upregulates ECM-remodelling proteins in astrocytes, contributing to astrocyte migration and the metastatic niche in the BBB<sup>19</sup>. Note that TEX uptake mechanisms are different in endothelial cells *versus* in astrocytes, suggesting that different cells have different exosome uptake mechanisms<sup>19</sup>.

Other studies support the role of TEXs in brain metastases. For example, Tominaga et al.<sup>81</sup> discovered that, in breast cancer, TEXs trigger BBB destruction. The exosomal miR-181c downregulates PDPK1 and hence decreases phosphorylated cofilin, causing increased actin filament disassembly and disorganization and leading to BBB destruction. Exosomes derived from brain-metastatic breast cancer cells are taken up by brain microvascular endothelial cells (BMECs) and have lncRNA GS1-600G8.5, which downregulates tight-junction proteins like ZO-1, N-cadherin, and claudin-5, and hence increases BBB permeability<sup>82</sup>. In breast and lung cancer, exosomal CEMIP (after uptake by microglia and brain endothelial cells) increases expression of the PTGS2, TNF, and CCL/CXCL cytokines, leading to increased branching of endothelial cells and inflammation in the brain's vascular niche<sup>83</sup>.

### 3.3.4. Exosomes promote metastatic niches by increasing angiogenesis

In ovarian cancer patients, exosomal soluble E-cadherin promotes tumor angiogenesis by binding to VE-cadherin on endothelial cells (Fig. 2g–h); this event activates NF- $\kappa$ B and  $\beta$ -catenin signaling, which contributes to angiogenesis<sup>84</sup>.

In cancers such as renal cell carcinoma, TEXs overexpress carbonic anhydrase 9 (CA9)<sup>85</sup>. Its production is triggered by hypoxia-inducible factor 1 (HIF1), which is produced in response to hypoxic conditions<sup>85</sup>. Due to interacting with bicarbonate receptors in lamellipodia of tumor cells<sup>86</sup>, CA9 induces tube formation and migration and increases MMP2 production; hence, angiogenesis is promoted<sup>85</sup>. The formation of new blood vessels in the pre-metastatic niche helps tumor-derived secreted factors and pro-tumor extracellular vesicles to reach it. Hence, the microenvironment there becomes more tumor-friendly<sup>78</sup>.

Together, these studies have demonstrated that cancer cells do not randomly form metastases. They are more likely to make metastases when pre-metastatic niches are formed, where the microenvironments are tumor-friendly. TEXs promote pre-metastasis niche formation by increasing vascular permeability

(in the pre-metastatic niche) and recruiting immune cells (*e.g.*, bone marrow-derived cells, neutrophils).

## 4. Therapeutic implications *via* targeting TEXs

Based on these uncovered functional roles of TEXs in cancer metastasis, various strategies have been developed to target TEXs and to block exosome-mediated metastasis. In this section, we will mainly review the pilot studies in targeting TEXs to improve therapeutic efficacy (as summarized in Table 3). Previous reviews have summarized how to harness TEXs as therapeutic delivery cargos or as a liquid biopsy<sup>106–108</sup>.

### 4.1. Inhibition of TEXs release

Given the fact that tumor cells send out exosomes to help them survive and spread, the first idea is to inhibit the release of TEXs. Since Rab family proteins are well-described modulators of exosomes biogenesis<sup>109</sup>, Roma-Rodrigues and colleagues developed gold nanoparticles functionalized with thiolated oligonucleotides anti-Rab27a for selective silencing of Rab27a to decrease exosomes release in two breast cancer cell lines MCF-7 and MDA-MD-453, respectively<sup>87</sup>. Their results from *in vitro* studies showed an 80% decrease in exosome release after successful Rab27a gene silencing. However, this study did not provide the *in vivo* antitumor efficacy assessment. Using mouse models, Bobrie et al.<sup>88</sup> reported that the Rab27a blockade in mammary carcinoma cells resulted in a decreased secretion of TEXs. Their results further indicate that the blockade of Rab27a in metastatic 4T1 cells significantly delayed tumor growth and lung metastases. In another study, Rab27a knockdown significantly decreased exosome secretion in melanoma cells and led to a decrease in tumor growth and lung metastasis<sup>23</sup>. In addition to increasing Rab protein-mediated exosome release, the protein Munc13-4 plays an essential role in promoting Ca<sup>2+</sup>-stimulated exosome release in highly aggressive breast carcinoma MDA-MB-231 cells, suggesting that Munc13-4 could be another molecular target for intervention in exosome-mediated metastasis<sup>89</sup>. It has also been reported that targeting neutral sphingomyelinase 2 (nSMase2) can inhibit cancer cell exosome secretion effectively<sup>90,91</sup>. Using xenograft breast cancer models, Kosaka et al.<sup>90</sup> demonstrated that nSMase2 knockdown in cancer cells resulted in a significant decrease in lung metastasis, though not primary tumor growth, when compared with the parental cell line. nSMase2 knockdown

inhibits exosomal miR-210 transfer to epithelial cells and diminishes the exosome-mediated angiogenic activity of endothelial cells in inoculated tumors.

#### 4.2. Removal of circulating TEXs

Given the challenge of inhibiting TEX biogenesis, removal or disruption of circulating exosomes may serve as an alternative therapeutic strategy to inhibit cancer metastasis. It has been proposed that a device called Aethlon Hemopurifier® can capture and remove TEXs from the plasma of breast cancer patients<sup>92</sup>. The device comprises a hollow-fiber plasma separator cartridge containing a lectin affinity matrix or other affinity reagents, such as aptamers and proteins ligands, and can be integrated into standard dialysis machines. The idea is when the patient's blood goes through the device, the immobilized affinity reagents capture the targets such as TEXs. One of the biggest challenges associated with this technology lies in how to capture only TEXs while leaving normal cell-derived exosomes intact as there is currently no specific way to differentiate them. In addition, the entire blood dialysis strategy is complicated and leaves many uncertainties.

Alternatively, Nishida-Aoki et al.<sup>93</sup> showed that the circulating exosomes in human breast cancer xenograft models could be depleted through administering human-specific anti-CD9 or anti-CD63 antibodies. They found this antibody treatment led to a significant decrease in lung and lymph node metastases though it did not affect inoculated tumor growth. Their study further showed that the antibody-tagged exosomes could be preferentially recognized and cleared by macrophages. Despite the promising effect, some questions remained. For instance, would antibody binding change the interaction of exosomes with host immune cells and epithelial cells? Why does antibody binding cause recognition of macrophages but not of other myeloid suppressive cells? The detailed molecular and cellular mechanisms that are responsible for the observed metastasis inhibition may be worthy of further investigation.

#### 4.3. Blocking TEXs interaction with recipient cells

Studies revealed that TEXs may interact with recipient cells through specific binding followed by receptor-mediated endocytosis<sup>11,110</sup>, which provides a rationale to block exosome-mediated communication. It has been found that fibronectin on the surface of myeloma cell-derived exosomes acts as a ligand that can effectively bind to its receptor heparan sulfates on recipient cells<sup>11,94</sup>. Indeed, exosome uptake was specifically inhibited in the presence of free heparin sulfate, and exosome-mediated stimulation of glioblastoma cell migration was significantly reduced when the cells were treated with heparin<sup>94</sup>. TEXs can bind to Toll-like receptors on myeloid cells to induce myeloid cell activation and polarization. Using A8 peptide aptamer that can bind to heat shock protein 70 (HSP70), Gobbo et al.<sup>95</sup> demonstrated that targeting TEXs can result in a decreased number of MDSCs in the spleen in B16F10 tumor-bearing C57BL/6 mice and a suppressed tumor progression. Other molecules, such as methyl- $\beta$ -cyclodextrin (M $\beta$ CD)<sup>17</sup> and dynasore<sup>96</sup>, have also been reported to inhibit TEXs uptake by receptor cells.

#### 4.4. Harnessing TEXs as therapeutic carriers to treat cancer and its metastasis

TEXs, as the nature reservoir of proteins or RNAs (siRNA, miRNA), are excellent carriers to solve suboptimal pharmaceutical properties of anti-cancer biomolecules such as instability, off-target toxicity, inefficient cell-uptake and so on<sup>111–113</sup>. In order to protect the degradation of siRNAs against RAD51 and RAD52, exosomes from HeLa and ascites have been used as the carriers and cause better post-transcriptional gene silencing in recipient cells<sup>97</sup>. siRNA/shRNA which are specifically targeted to oncogenic Kras<sup>G12D</sup> have also demonstrated to have a longer circulation time and stronger inhibition against pancreatic cancer when delivered in exosomes<sup>114</sup>.

Another advantage to utilize TEXs as therapeutic carriers is their tumor targeting efficiency either by its intrinsic cell/organ tropism or extrinsic surface modification<sup>115</sup>. TEXs as summarized in this review have the ability to promote metastatic niches formation. Therapeutic cargos can be delivered easily to those niches by TEXs. Tang et al.<sup>98</sup> isolated TEXs from HT1080 or HeLa and found these exosomes not only fuse preferential with parent cancer cells *in vitro* but also show increased anti-cancer drugs (doxorubicin) in tumor lesion<sup>101</sup>. Another study also finds that exosomes from H22 cells can efficiently deliver chemotherapeutic drug molecules into tumor cells. It is known that pre-metastatic niche (PMN) in secondary organs create conducive environment for tumor metastasis. Zhao et al.<sup>116</sup> developed biomimetic nanoparticles with exosome membranes and found higher lung PMN delivery. By incorporating siS100A4, these nanoparticles exhibited outstanding gene-silencing effects and suppressed post-operative breast cancer metastasis. Other strategies like active targeting of TEXs are also feasible due to easy protein/peptide expression, chemical conjugation or hydrophobic insertion on the surface. Researchers obtained large amounts of exosomes carrying iRGD by overexpressing iRGD-Lamp2b fused protein in cells. These exosomes are shown to specifically deliver doxorubicin to breast cancer cells through recognizing the surface  $\alpha$ V integrin<sup>117</sup>. The ligand-based delivery system is capable of specific carry of siRNA to cells and block tumor growth in prostate, breast and colorectal cancers<sup>118</sup>.

It is also appealing to use TEXs as vaccines for immunotherapy, since large amount of tumor exosomes can be found in malignant effusions. These TEXs are able to carry and transfer tumor specific antigens to DCs, which is shown to induce potent cytotoxic antitumor effects. TS/A, MC38 and P815 cancer derived exosomes were studied to be loaded with BMDCs and injected back to established tumor models, promoting significantly tumor growth delay and up to 40% cure rate<sup>104</sup>. Morishita et al.<sup>103</sup> modified the surface of TEXs with streptavidin and mixed with biotinylated CpG DNA to form antigens-adjuvant codelivery. They find stronger antitumor effects in B16BL6 mice after immunization with combined TEXs vaccines. Another study demonstrates that exosomes derived from HepG2 cells which are treated with resistant anticancer drug can induce superior HSP-specific NK cell responses<sup>105</sup>. A completed phase I clinical trial for recurrent malignant gliomas took patients' own tumor cells and treated with antisense drug to induce exosomes generation when re-implanted into patients. These exosomes are supposed to activate the immune system against tumor as they slowly diffused

out from small chambers. However, optimism of applying TEXs in immunotherapy should be conservative or at least the positive results should be treated case by case. There are other studies discover the immunosuppressive function of TEXs including impairment of monocyte differentiation and induction of a pro-inflammatory microenvironment. Under which condition that TEXs can be used to amplify the immune response should be treated cautiously.

Artificial nanoparticles used in medicine are largely known for its ability to deliver small molecule anti-cancer drugs, like doxorubicin (Doxil®) and paclitaxel (Abraxane®). Similarly, as a natural nanoparticle, TEXs have also been used as carriers for these small molecule drugs. Tang et al.<sup>98</sup> incubate tumor cells (H22 or A2780 cells) with chemotherapeutics like doxorubicin, MTX, cisplatin and then irradiated with UVB to release TEXs. These exosomes are found to be more effective through efficiently delivering to tumors, inducing the formation of additional drug-packaging TEXs and enhancing tumor cell susceptibility to chemotherapy. Another study by the same group demonstrates that TEXs containing anti-tumor drugs are able to reverse drug resistance of tumor-repopulating cells through interfering with drug efflux and increasing nuclear uptake<sup>99</sup>. In a study to compare the difference of microvesicle- and exosome-mediated paclitaxel delivery, researchers found both TEXs with chemo-drugs are also uptook by the recipient cells through endocytosis<sup>100</sup>. Several clinical trials are ongoing to test the safety and effectiveness of TEXs loaded with chemotherapeutic drugs against malignant pleural effusion [NCT01854866 and NCT02657460].

## 5. Conclusions

Growing evidence has revealed that tumor-derived exosomes (TEXs), as a powerful nanoparticle secreted by cancer cells, play various roles in promoting cancer metastasis. First, TEXs can induce endothelial cells to down-regulate tight junction protein expression and hence cause leaky tumor vasculature, which contributes to cancer cells' local invasion and intravasation into the lymphatic system and the blood circulation. Various exosomal miRNAs contribute to cancer cell invasion and metastasis. Second, TEXs can suppress antitumor immunity to help tumor cells survive in circulation and metastatic sites. Third, TEXs can prepare tumor-friendly environments in distant organs to facilitate tumor cell extravasation into the parenchymal tissues (metastatic niche). Knowing these pro-metastatic roles of TEXs, scientists developed various strategies to target them, which include inhibiting the secretion of TEXs, removing circulating TEXs, and blocking TEXs' interactions with recipient cells. It is worth noting that we are still at the infant stages of targeting TEXs to interfere with their pro-tumor purposes. However, we believe that this is the right direction to pursue in order to find effective ways to cure cancer metastasis.

## Future perspectives

A variety of molecular components of TEXs have been reported to be involved in cancer metastasis. However, many issues regarding the role of TEXs in cancer metastasis, immune regulation, and treatment resistance remain unanswered. Some important questions that need to be addressed include: (1) What is the key factor that regulates the interaction between TEXs and host immune cells and epithelial cells? (2) How exosomal miRNAs modulate

the recipient cells? (3) How does the host immune system differentiate TEXs from normal cell-derived exosomes? (4) How to block TEXs communications with host immune cells to restore patient antitumor immunity?

## Author contributions

Hongwei Chen conceived the idea and wrote the manuscript. Venkata Chengalvala wrote the manuscript. Hongxiang Hu re-graphed the Figures, wrote part of the manuscript and partially revised the manuscript. Duxin Sun reviewed the manuscript.

## Conflicts of interest

The authors have no conflicts of interest to declare.

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