Trends in Pharmacological Sciences

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Review

Exosome-Mediated Metastasis: From Epithelial–Mesenchymal Transition to Escape from Immunosurveillance

Nicholas Syn,[1,2] Lingzhi Wang,[1,3,*] Gautam Sethi,[3] Jean-Paul Thiery,[1,4,5,6] and Boon-Cher Goh[1,2,3]

Exosomes are extracellular signalosomes that facilitate eukaryotic intercellular communication under a wide range of normal physiological contexts. In malignancies, this regulatory circuit is co-opted to promote cancer cell survival and outgrowth. Tumour-derived exosomes (TDEs) carry a pro-EMT (epithelial–mesenchymal transition) programme including transforming growth factor beta (TGFβ), caveolin-1, hypoxia-inducible factor 1 alpha (HIF1α), and β-catenin that enhances the invasive and migratory capabilities of recipient cells, and contributes to stromal remodelling and premetastatic niche formation. The integrin expression patterns on TDEs appear to dictate their preferential uptake by organ-specific cells, implying a crucial role of this pathway in organotropic metastasis. Through the expression of immunomodulatory molecules such as CD39 and CD73, TDEs modify the immune contexture of the tumour microenvironment, which could have implications for immunotherapy. Hence, targeting TDE dysregulation pathways, such as the heparanase/syndecan-1 axis, could represent novel therapeutic strategies in the quest to conquer cancer.

Trends

Tumour-derived exosomes (TDEs) contain prodigious amounts of epithelial–mesenchymal transition (EMT) inducers, and transduce EMT characteristics in recipient epithelial cells.

Exosomes are being implicated in the aetiology of organotropic metastasis owing to their target-homing ability and capacity to form a premetastatic niche at specific organ sites.

Exosomes may be hijacked by tumour viruses and may confer oncogenic potential or induce malignant transformation in recipient cells.

TDEs have potent immunomodulatory effects that likely foster tumour escape from immunosurveillance.

Pharmacological agents that directly or indirectly modulate tumour exosome biogenesis, secretion, and function have also shown promising antimetastatic activity.

A Framework for Exosome-Mediated Metastasis

Metastatic outgrowths are the predominant cause of death from cancer. Since the late nineteenth century, when Paget formulated his enduring 'seed-and-soil' hypothesis [1], comparing disseminated tumour cells and the organ microenvironment with the 'seed' and 'soil', respectively, research on the mechanisms of cancerous metastasis has focused on the interaction between tumour and host. In recent years, this field has been enlivened with the exciting possibility that a newly described mode of intercellular crosstalk mediated by exosomes could have important and multifarious roles in local and distant failures, hence opening new possibilities for diagnostic, predictive, and therapeutic approaches.

Exosomes are small (30–100 nm) vesicular structures arising from the luminal membranes of multivesicular bodies (MVBs) and secreted into the extracellular milieu by most, if not all, cell types through fusion with the cell membrane. They may then diffuse to neighbouring cells or be carried via systemic transport to distant anatomic locations where they may induce signal transduction or mediate the horizontal transfer of information in specific recipient cells. Tumour-derived exosomes

[1]Cancer Science Institute of Singapore, Centre for Translational Medicine, National University of Singapore, 14 Medical Drive, #12-01, Singapore 117599, Singapore
[2]Department of Haematology–Oncology, National University Cancer Institute, 1E Kent Ridge Road, NUHS Tower Block, Level 7, Singapore 119228, Singapore

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(TDEs) carry a functional molecular cargo that can consist of oncogenic virus-derived molecules, various pathogenic miRNA, mRNA, DNA fragments, and proteins such as Dicer [2–8], which are capable of inducing malignant transformation and field cancerization [2,9,10]; potentially reflecting an evolutionary mechanism in which cancer cells repurpose the pathways that guard exosome homeostasis for their own survival and propagation.

In particular, contemporary evidence indicates that TDEs perform crucial roles in virtually all steps of the invasion–metastasis cascade (Figure 1, representative schematic). We propose the following framework. Firstly, TDEs provide autocrine and paracrine signals within the tumour ecosystem to activate an epithelial–mesenchymal transition (EMT) programme in neoplastic epithelial cells [6,11–14], which endows them with the ability to invade the tissue surrounding the primary tumour, intravasate, and enter the circulation. Secondly, TDEs are taken up in (distal) organ tissues and foster a premetastatic niche where metastatic cells may arrest, extravasate, and eventually colonise [15–17]. Thirdly, TDEs modulate the host immunity to allow unbridled disease progression, and even outwit immune players into fostering a prometastatic microenvironment by activating inflammation response pathways [4,18,19]. In this review, we illustrate these mechanistic insights with recent data, which is hoped may form the base of future pharmacological strategies against cancer.

Initiation of Metastasis: Epithelial–Mesenchymal Transition

The formation of life-threatening metastases at distant organs requires the invasion of primary tumours through the basement membrane and dissemination via the circulation. Epithelial cells at the invasive front of carcinoma surmount this physical barrier by acquiring migratory and invasive properties through EMT [20].

Recently there have been compelling suggestions that TDEs may serve as conduit for EMT-initiating signals, owing to the observations that they (i) appear to deliver prodigious amounts of known and putative EMT inducers, and (ii) epithelial cells within the tumour stroma that have taken up TDEs manifest distinct biochemical and morphological changes that are consistent with EMT. Molecular characterisation studies of the TDE cargo have revealed appreciable levels of transcriptional regulators, which may influence diverse signalling pathways (Figure 2A), and EMT drivers such as Notch-1, matrix metalloproteinases (MMPs), miR-100, LMP1 [from Epstein–Barr virus (EBV)-infected nasopharyngeal cancer (NPC) exosomes], hypoxia-inducible factor alpha (HIFα), casein kinase IIα, and Annexin A2 [6,11,13,21–24]. Provocatively, tumour exosomes shed under hypoxia, a state associated with EMT and elevated risk of metastasis, further exhibit enrichment of potent EMT-transducing signalling molecules such as transforming growth factor beta (TGFβ), MMPs, tumour necrosis factor alpha (TNFα), interleukin-6 (IL-6), protein kinase B (AKT), integrin-linked kinase 1 (ILK1), caveolin-1, platelet-derived growth factors (PDGFs), and β-catenin compared with exosomes secreted under a normoxic state [25,26].

Subsequently, having internalised TDEs, recipient cells demonstrate physiological changes associated with alterations of their cell transcriptome and proteome that are symptomatic of EMT [6,7,11,12,23,27–32]. For instance, the co-culture of a NPC cell line (CNE-2) with exosomes from the same cell line acted in an autocrine manner to induce EMT, as evidenced by increased expression of N-cadherin and vimentin, and reduced expression of E-cadherin (Figure 2B) [11]. Changes in EMT markers were also seen when LMP1-negative NPC cells were treated with exosomes from LMP1-expressing cells [6]. Ovarian cancer exosomes have also been shown to induce EMT and spindle-like morphology in mesothelial cells (reflecting a loss of cell polarity), which resulted in clearance of the mesothelial barrier [32]. Finally, urothelial cells exposed to exosomes isolated from the urine or bladder barbotage of patients with muscle invasive bladder cancer exhibited increased expression of mesenchymal markers (α-smooth muscle actin, S100A4, and Snail), contractility, and amoeboid-like migration [12].

[3]Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117600, Singapore
[4]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117596, Singapore
[5]UMR 7057 Matter and Complex Systems University Paris Denis Diderot, Paris, France
[6]Comprehensive Cancer Center Institut Gustave Roussy, Villejuif, France

*Correspondence: csiwl@nus.edu.sg (L. Wang).

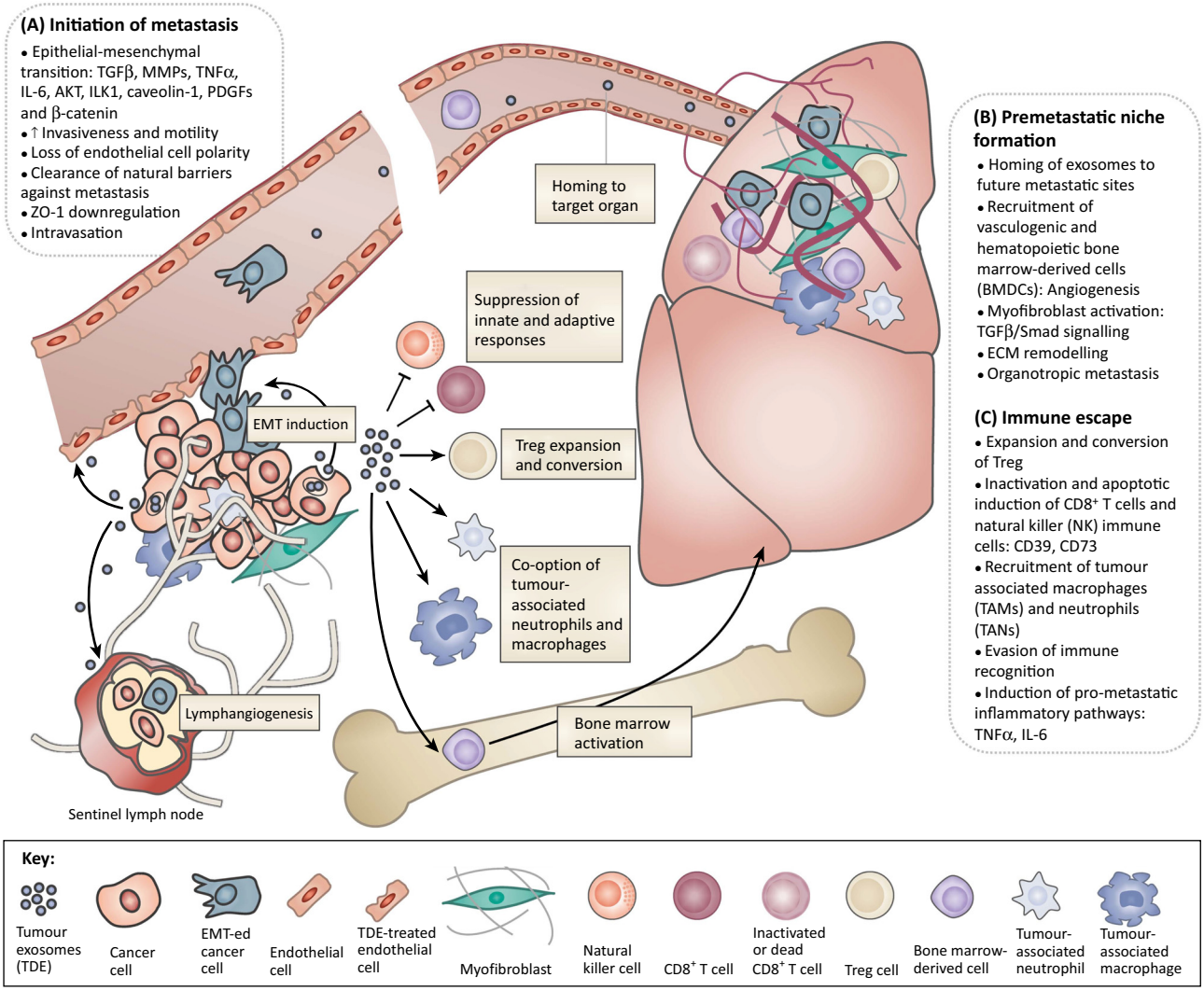

Figure 1. Exosome-Mediated Metastasis. Exosomes are small vesicular structures that are shed by tumour cells and provide various autocrine and paracrine signalling cues that culminate in the formation of metastases at secondary sites. TDEs are involved in (A) the initiation of metastasis, which may co-opt EMT pathways to enhance the invasiveness and motility of neoplastic cells and clearance of natural barriers against metastases; (B) the preparation of a premetastatic niche, via the recruitment of BMDCs, myofibroblast activation, and induction of ECM remodelling and angiogenic processes; and (C) the escape of tumour cells from immunosurveillance, which may occur via the suppression of the innate and adaptive arms of the host immunity, and conversion of reactive tumour infiltrates into accomplices in malignancy. Abbreviations: Treg, regulatory T cell; TGFβ, transforming growth factor beta; MMPs, matrix metalloproteinases; TNFα, tumour necrosis factor alpha; IL-6, interleukin-6; AKT, proto-oncogene Akt; ILK1, integrin-linked kinase 1; PDGF, platelet-derived growth factor; ZO-1, tight junction protein 1; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; BMDCs, bone marrow-derived cells; TDEs, tumour-derived exosomes.

Alternatively, exosomes could also promote the initiation of the invasion–metastasis cascade by directly targeting the tight and adherens junctions. Breast cancer exosomes, for instance, were demonstrated by Zhou and colleagues to downregulate the expression of the tight junction protein ZO-1 in endothelial monolayer cells via exosomal miR-105, which led to increased vascular permeability and lung and brain metastases [5]. Another exosome-mediated mechanism implicates exosomes derived from non-neoplastic tumour-associated cells. For instance, fibroblast-secreted exosomes were shown by Luga and colleagues to drive invasive behaviour in breast cancer cells the via Wnt–planar cell polarity (PCP) signalling pathway [33]. For further study, it would be instructive to better characterise the unique exosomal molecular cargo of

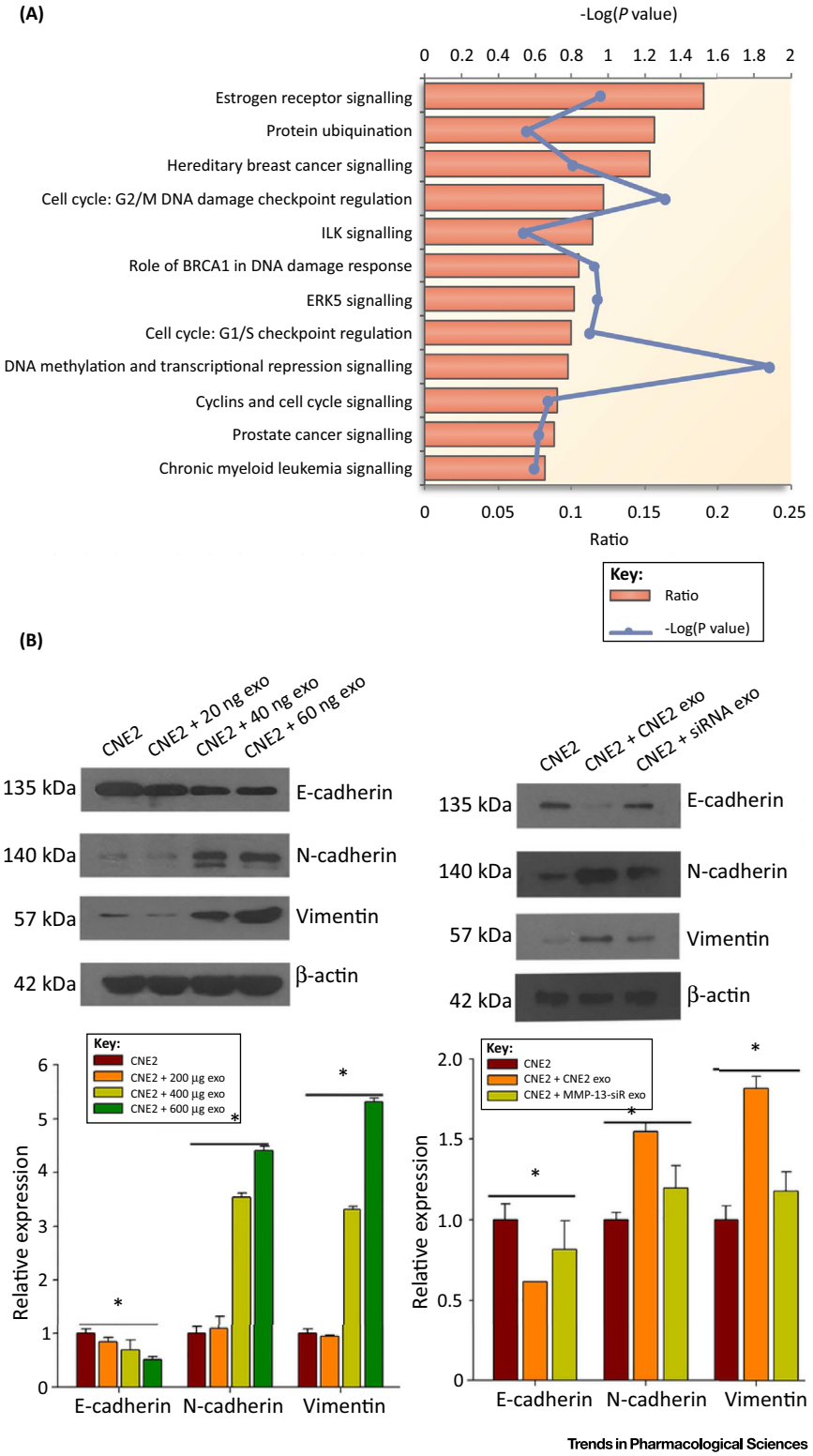

Figure 2. Biochemical Changes and Epithelial–Mesenchymal Transition in Recipient Cells. (A) Comprehensive proteomic analyses of tumour-derived exosomes reveal multiple canonical pathways that may be potentially transduced by tumour-derived exosomes. The ratio indicates the fraction of molecules that map to the respective canonical pathway in the

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different cell types in the tumour stroma, their mechanism of action, and relative contribution to the initiation of metastasis.

Organotropic Metastasis: New Leads to an Old Mystery

Organotropic metastasis–the proclivity of certain primary tumours to spawn secondary neoplasia in specific organs–has been an age-old enigma in cancer biology [1]. Whereas EMT may support the dissemination of metastatic cells, incoming tumour stem cells would then need to engraft in a permissive foreign tissue microenvironment to proliferate and establish successful secondary outgrowths. Several recent studies suggest that cancers engender this congenial turf through exosomes, which in turn display differential affinity for different target organs, thus mediating nonrandom patterns of dissemination (Figure 3).

Target Cell Specificity

Evidence that exosomes may bias the metastatic efficiency to different target organs derives from their own avidity for specific recipient cells. Specifically, TDEs have been reported to home to future colonisation sites and other tumour-associated cells with characteristic specificity [15–17,34–38]. For example, in an early study on this trafficking behaviour, Hood *et al.* injected fluorescently labelled B16-F10 melanoma exosomes into the footpads of mice, and demonstrated that the exosomes preferentially localised to regional lymph nodes closest to the injection site, whereas similarly sized liposomes were evenly distributed in regional and distant lymph nodes [16]. Furthermore, melanoma exosomes subsequently initiated a premetastatic niche in regional lymph nodes [16], reminiscent of how sentinel lymph nodes downstream of melanomas undergo reactive lymphangiogenesis prior to metastasis [39]. Apart from trafficking to premetastatic organs, exosomes from melanoma cells home to the bone marrow to 'educate' and mobilise vasculogenic and hematopoietic bone marrow progenitor cells [15], a step that may be important for vascular proliferation and immunosuppression within the premetastatic niche [40,41].

The precise targeting of exosomes to their specific recipient cells and their subsequent internalisation is probably dependent on the exosomal repertoire of membrane proteins and lipids, especially those related to extracellular matrix (ECM) and adhesion [28,33,35,37,42]. For example, primary tumours destined to home to lung tissue secrete exosomes expressing the integrins $\alpha 6\beta 4$ and $\alpha 6\beta 1$, whereas integrin $\alpha v\beta 5$ directs metastasis to the liver [35]. Nonetheless, the integrin repertoire is probably not large enough to promote organ-specific metastasis, hence there are likely to be other exosomal determinants waiting to be discovered. The subsequent entry of TDEs into recipient cells could engage heterogeneous endocytic pathways such as clathrin, lipid raft, and caveolin-mediated uptake [14,43–45].

Premetastatic Niche Formation

After homing to their target tissue, exosomes may play a role in the activation of a reactive, myofibroblast-rich stroma and thus promote a host of tumour-supportive processes such as ECM remodelling, proliferation, and angiogenesis [8,15,28,32,41,46–50]. For example, TDEs internalised by myofibroblast progenitors (mesenchymal stem cells and normal stromal fibroblasts) have been shown to enhance their recruitment [15,49] and trigger their differentiation into myofibroblast-like cells [41,46–48]. Exosomal-transduced TGFβ/Smad signalling has been shown to underlie the differentiation process [41,46–48]. Interestingly, a number of experiments have showed that exosomal TGFβ appears to consistently generate myofibroblasts that are

Ingenuity Pathway Analysis database. Adapted from [21]. (B) In a nasopharyngeal cancer, cells co-cultured with exosomes from the same cell line (CNE-2) dose-dependently induced downregulation of E-cadherin and increased expression of N-cadherin and vimentin, which are indicative of EMT. Exosomes from CNE-2 cells transfected with MMP13 siRNA secreted exosomes with lower levels of MMP13 and had reduced tendency to induce EMT in recipient cells. Reproduced from [11]. Abbreviations: MMP, matrix metalloproteinase; EMT, epithelial–mesenchymal transition.

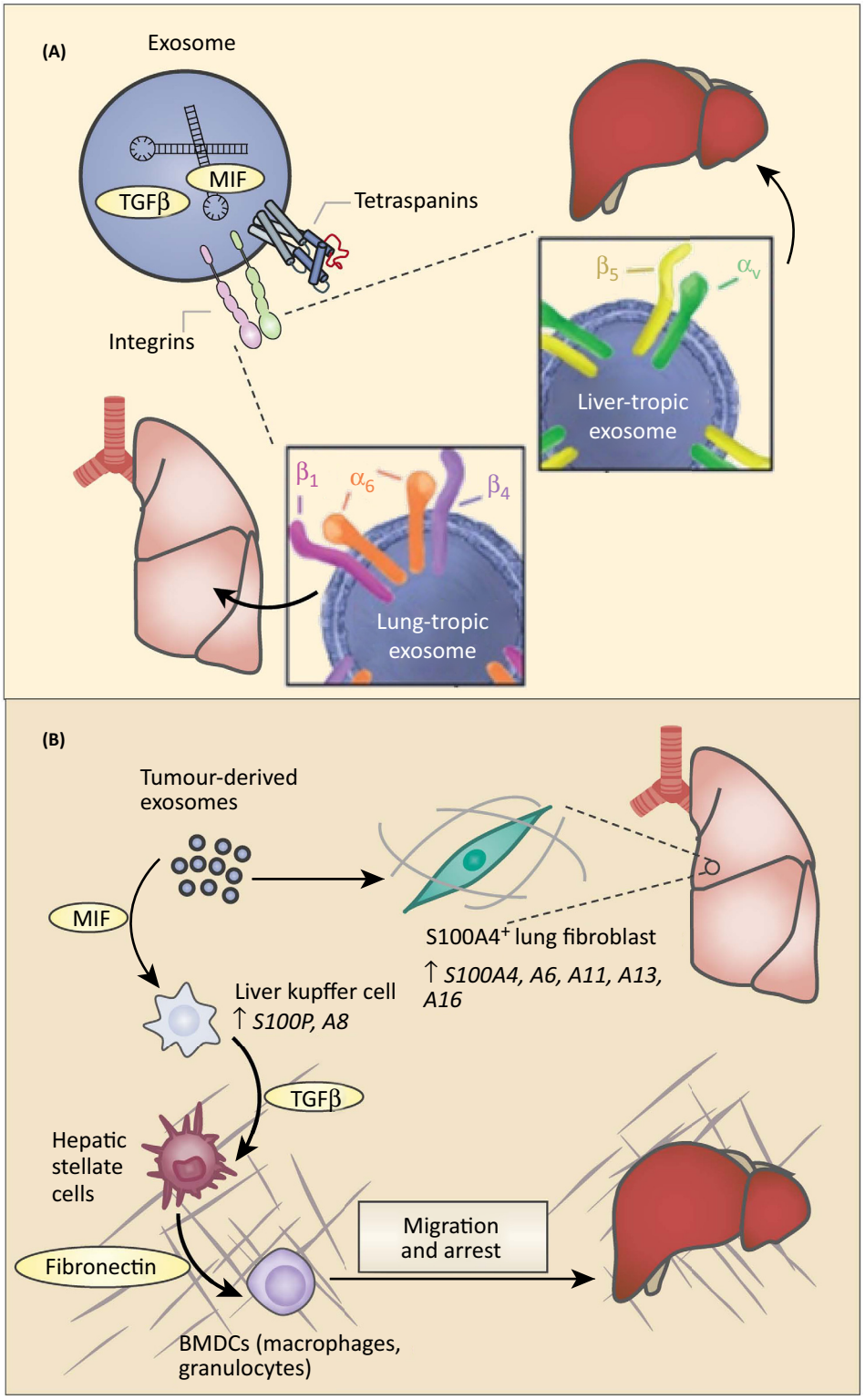

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Figure 3. Mechanisms by which Tumour-Derived Exosomes Direct Organotropism. (A) The specific repertoire of exosomal surface molecules dictates their homing to their target cell types. The inner panels that depict the liver- and

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phenotypically and biochemically distinct [e.g., more proangiogenic and heightened basic fibroblast growth factor (FGF2) responsiveness] to those induced by soluble TGFβ [41,46,47], although the reason for this disparity is not presently clear.

In addition, exosomes derived from non-neoplastic cell types may facilitate the adaptation of disseminated tumour cells to the foreign soil, thus reflecting a dynamic and bidirectional crosstalk within the metastatic microenvironment. A recent study demonstrated that miRNAs in brain astrocyte-derived exosomes epigenetically deplete PTEN expression in brain-tropic metastatic cells [51]. This induced the secretion of the chemokine CCL2, which subsequently recruited tumour-promoting AIF1-expressing myeloid cells [51].

Finally, TDEs may also prime the metastatic niche by modulating tumour-infiltrating lymphocytes to foster immune evasion and suppression within the metastatic microenvironment, the mechanisms of which are discussed later. These findings provide examples of how exosomes abet a milieu of local cell types to enhance the acclimatisation of engrafted tumour cells to the drastically different metastatic microenvironments.

Direct Malignant Transformation and Oncogenic Viruses

In some instances, TDEs have been shown to confer oncogenic potential to untransformed cells [2,9,10]. For example, tumour-tropic patient-derived adipose stem cells (pASCs) can be induced to acquire cytogenetic aberrations, undergo mesenchymal-to-epithelial transition (MET) and develop aggressive prostate-like secondary tumours upon conditioning with prostate cancer-derived exosomes [9], which may explain the known link between adiposity and prostate cancer progression. In addition, human mammary epithelial MCF10A cells implanted into the mammary fat pads of mice formed tumours when co-injected with breast cancer MDA-MB-231-derived exosomes, accompanied by distinct changes in their miRNA and expression profile, such as miR-21 and miR-10b upregulation and the corresponding downregulation of their target transcripts *PTEN* (*phosphatase and tensin homolog*) and *HOXD10* (*homeobox protein HoxD10*) [2].

Human tumour viruses such as EBV and Kaposi's sarcoma herpesvirus (KSHV) have also been demonstrated to utilise the host exosomal apparatus for intercellular communication and exert protumourigenic signalling in recipient cells [3,23,44,52]. For instance, NPC-secreted exosomes contain the Epstein–Barr viral oncoprotein LMP1 and viral miRNAs (several of which are enriched compared with intracellular levels), which induces epidermal growth factor receptor (EGFR) expression, extracellular signal-regulated kinases (ERK), and AKT signalling in EBV-uninfected epithelial cells [6,23,52]. Hence, these points draw attention to additional hitherto unappreciated mechanisms exploited by cancers to promote their outgrowth.

Immune-Modulating Effects

The notion that the successful proliferation of disseminated clones to clinically manifest outgrowths hinges on the ability of tumour cells to escape natural or therapy-induced immunosurveillance has found widespread acceptance, and in this section it is our goal to assemble some of the emerging insights that implicate tumour exosomes in cancer immunoediting and subversion.

Firstly, TDEs arbitrate the generation of an immunosuppressive environment by blunting the response of immune effector cells and triggering the expansion of immune suppressor cells

lung-tropic exosomes are adapted, with permission, from Macmillan Publishers Ltd: Nature [35], © 2015. (B) Liver- and lung-tropic exosomes induce the upregulation of certain S100 family proteins, which are known to promote metastasis, in their target cells. Liver-tropic exosomes express MIF, which induces liver Kupffer cells to release TGFβ and in turn activate fibronectin production by hepatic stellate cells. The fibrotic environment induces the migration and arrest of various tumour-supporting BMDCs in the liver, thus initiating the premetastatic niche. Abbreviations: TGFβ, transforming growth factor beta; MIF, macrophage inhibitory factor; S100, S100 calcium binding protein family; BMDCs, bone marrow-derived cells.

[18,19,53–56]. For example, TDEs from patients with solid tumours or acute myelogenous leukaemia (AML) were shown to drive the apoptosis of $CD8^+$ T cells and expansion of regulatory T cells (Tregs), and decrease the cytotoxic activity of natural killer (NK) cells [54]. NPC exosomes were also shown to mediate Treg recruitment and expansion [18,56] and inhibit T cell proliferation and T helper 1 (Th1) and Th17 differentiation [18], but further recruited $CD4^+CD25^-$T cells and facilitated their conversion into inhibitory $CD4^+CD25^{high}$ T cells [56]. Several mechanisms of T cell suppression have been proposed, including enzymatic production of adenosine by functional CD39 and CD73 present on TDEs [19], as well as the transcriptional regulation of immune-related genes in recipient cells [55].

Secondly, TDEs help malignant cells evade immune recognition by employing decoy mechanisms. Their ability to efficiently bind and sequester opsonising antibodies may attenuate NK cell-mediated antibody-dependent cytotoxicity (ADCC) [57]. Furthermore, exosomal proteins may also bind therapeutic monoclonal antibodies (mABs) and hence contribute to the initial 'sink' effect whereby high doses of mABs are sometimes required to achieve optimal plasma levels. For instance, in B cell lymphomas [58], such as chronic lymphocytic leukaemia [50], exosomal CD20 has been shown to effectively intercept the anti-CD20 antibody rituximab and reduce its deposition on target cells. Hence, the potential implications of TDEs on the efficacy of immunotherapy is an important area for further research.

Thirdly, TDEs may engage prometastatic inflammatory processes to convert reactive stromal infiltrates into accomplices in malignancy [4,15,17,59,60]. For example, the uptake of pancreatic cancer exosomes (which highly express macrophage inhibitory factor) by hepatic Kupffer cells activated fibronectin production, which promoted the arrest of bone marrow-derived macrophages and neutrophils in the liver, thus establishing the premetastatic niche [17]. In another example, exosomal miRNAs (miR-21 and miR-29a) may bind to Toll-like receptors (TLRs; murine TLR7 and human TLR8), leading to TLR-mediated NF-κB activation and secretion of prometastatic inflammatory cytokines TNFα and IL-6, which manifested as greater lung metastatic burden in a murine model [4].

In essence, it now appears clear that tumour exosomes have many pathogenic properties that likely promote distant progression and treatment failure. The pertinent question then, is whether insights gleaned from a better understanding of exosomal dysregulation in cancer will prove useful to the development of cancer treatments.

Pharmacological Strategies against Exosomal Dysregulation

As might be inferred from the preceding discussions, exosomal-mediated metastasis encompasses an intricate sequence of coordinated events, each of which may be amenable to therapeutic targeting (Figure 4), and collectively potentially represent a new paradigm to guide future development of antimetastatic therapeutic strategies.

Pharmacological Agents that Affect Exosome Biogenesis and Secretion

Several components of the machinery that produce and secrete exosomes have been identified as important regulators of metastasis. These include the heparanase/syndecan axis, vacuolar ATPases, and several members of the *Rab* family. The heparanase enzyme, for example, is upregulated in aggressive tumours and drives robust exosome secretion [61,62]. The exact role of heparanase in exosome biogenesis has not been established, but it is conceivable that it remodels the heparin sulfate chains on syndecan-1 to enhance the formation of syndecan–syntenin–ALIX and hence the intraluminal budding of endosomal membranes [63]. Heparanase inhibitors, modified heparins, and heparin mimetics such as PG545 and M402 are being investigated as experimental anticancer agents and have demonstrated antimetastatic activity in animal models [63].

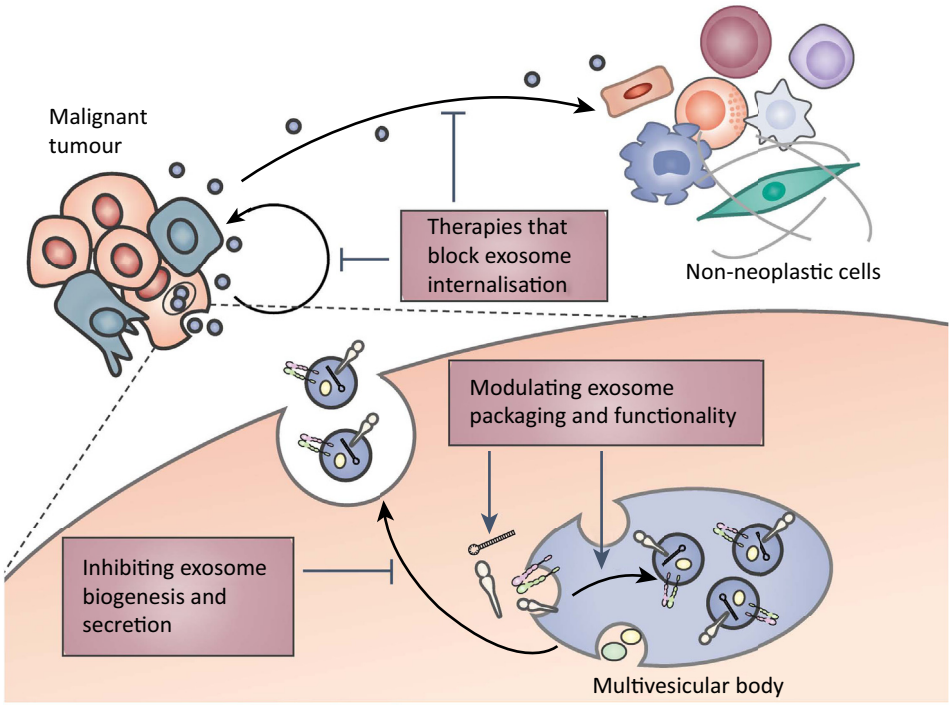

Figure 4. Targeting Exosomal Dysregulation for Therapeutic Modulation of Metastasis. Owing to the contributions of dysregulated exosomal pathways to experimental metastasis, therapies that affect exosome biogenesis, packaging, trafficking, and internalisation by recipient cells may prove to be clinically effective for the prevention and treatment of advanced cancers.

In addition, vacuolar H^+-ATPases (V-ATPases) are overexpressed in cancer cells with metastatic potential [64] and may enhance the secretion of exosomes by fusion of MVBs with the plasma membrane [65]. V-ATPases may be implicated in aberrant vesicular trafficking, and have been shown to mediate the sequestration of cytotoxic drugs such as cisplatin into exosomes [66]. Treatment with proton pump inhibitors (PPIs) has been shown to interfere with exosome release and could have the dual effect of ameliorating drug resistance [66,67]. This is compatible with *in vivo* findings that demonstrate reversal of chemoresistance, enhanced sensitivity of drug-sensitive cells to anticancer agents, as well as single agent antitumour activity of PPIs [64].

Several *RAB* genes (e.g., *RAB1A*, *RAB5B*, *RAB7*, and *RAB27A*) are also overexpressed in highly metastatic cell lines that also shed copious quantities of exosomes [15]. Silencing the expression of the small GTPase Rab27a reduces exosomal secretion and the metastatic burden in mice [15,68], implying that Rab27a may play an important role in the invasive and metastatic characteristics of cancer cells. In triple negative breast cancer, siRNA knockdown of ESCRT-1 (endosomal sorting complexes required for transport) complex subunit TSG101 has also been shown to impede release of TDEs [69]. Exosome secretion is also enhanced by actin-rich dynamic protrusions known as invadopodia, which are formed by invasive cancer cells, and in turn induce or stabilise invadopodia to establish a positive feedback loop [70]. Hence, targeting the canonical regulators of invadopodia formation such as N-WASp and Tks5 [71] could further represent valuable strategies of suppressing tumour exosome biogenesis and release.

Inhibition and Modulation of Exosome Function

Recent studies suggest that the dietary polyphenol curcumin may be able to modulate the cargo and functionality of cancer exosomes to repress their diverse pathogenic roles [72–74]. In

chronic myelogenous leukaemia cells, curcumin treatment induced selective packaging of the PTEN-targeting miRNA, miR-21, into exosomes and decreased Akt phosphorylation and vascular endothelial growth factor (VEGF) expression [74]. In breast cancer, curcumin reverses the TDE-mediated suppression of NK cell cytotoxicity in a dose-dependent manner [73]. However, the *in vivo* capacity of curcumin to regulate TDE function is less clear. The histone deacetylase (HDAC) inhibitor vorinostat has also been shown to induce HSP60 nitration in lung cancer cells and the packaging of nitrated HSP60 into exosomes [75], which may in turn stimulate potent NK cell-mediated antitumour immunogenicity [76].

Targeting Exosome Internalisation

Blockade of exosome uptake pathways is another potential strategy against exosomal dysregulation. Heparin application has been shown to effectively inhibit TDE internalisation and TDE-mediated cancer progression in glioblastoma and oral squamous cell carcinoma models, possibly through competitive inhibition with cell surface heparan sulfate proteoglycan (HSPG) receptors for TDE binding and internalisation [12,77,78]. In fact, consistent with this model of competitive inhibition, heparan sulfate chains have also been shown to impede TDE internalisation in a dose-, size-, and charge density-dependent manner [77], thus reinforcing the potential pharmacological relevance of heparanase-targeting agents [63].

Collectively, these studies suggest that exosomal dysregulation is an eminently exploitable facet of tumour biology, which can be targeted at several levels to curb distant cancer progression.

Concluding Remarks

Recent developments in cancer biology require us to reconsider long-held assumptions about the pathobiology of metastases. Exosomes are evidently versatile and critical intercellular messengers employed by tumours to architect the local and distant microenvironment. These extrinsic signalling cues orchestrate the initiation of metastasis, which may occur through EMT, the synchronised preparation of a premetastatic niche, as well as escape from immunosurveillance to allow tumours to propagate and flourish. In hindsight, it is indeed remarkable how much progress has been made only in the past 5 years. Nevertheless, a number of gaps in our knowledge exist and will be especially illuminating to address (see Outstanding Questions).

In this review, we elaborated a conceptual framework for exosome-mediated metastasis and potential pharmacological strategies against it. Strategies targeting TDEs will be critically important for improving outcomes of cancer patients, because as mentioned at the outset, overt metastases are responsible for the majority of cancer mortality. The examples highlighted earlier provide proof-of-concept of the antimetastatic effects of TDE inhibition and modulation, although that some were achieved using RNA-based knockdown strategies as opposed to conventional pharmacological inhibition. Yet, ironically enough, it is possible to envision applications in which exosomes are harnessed for their tissue specificity to deliver such therapeutic nucleic acid drugs to tumour cells [79]. Other possibilities for future applications also spring to mind, including 'liquid biopsies', which capture and profile the exosomal cargo for diagnostic, prognostic, and predictive biomarkers [80]. Ultimately, such a varied array of research directions will propel our understanding of exosome-mediated crosstalk in malignancies and the translation of these discoveries and insights to the oncology clinic to yield benefits for patients with advanced diseases.

Outstanding Questions

The differentiation and proliferation of engrafted cancer cells at secondary sites is thought to involve MET, the reverse of EMT. Does the premetastatic niche, in turn, impose pro-MET signals on disseminated tumour cells via exosomes?

What are the relative contributions of exosomes secreted by different cell types to the formation of metastases? What are their unique exosomal cargo and mechanisms of action?

What are the somatic molecular and genetic determinants of exosomal dysregulation in cancers?

Might the host immunity play a role in the immunoselection of neoplastic cells with aberrant exosomal homeostasis?

Pharmacological modulation of exosome biogenesis, secretion, and function by tumour cells could alter exosome homeostasis in immune cells, which highly rely on exosomes for intercellular communication. What will be the implications of these, if any, on antitumour immunity?

What other therapeutic windows (arising from the mechanisms of exosome biogenesis, uptake, and target cell modulation) between normal and cancer cells can be exploited to reduce the off-target effects of pharmacological modulation?

Acknowledgments

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