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Review

Role of tumor-derived exosomes in cancer metastasis

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ABSTRACT

The highlights of cancer research include the discovery of exosomes, which are small (30-100 nm) sized vesicular nanoparticles released virtually by all cells. Tumor-derived exosomes (TDEs) are notoriously known for orchestrating the invasion-metastasis cascade via systemic pathways that we have previously proposed (1), resulting in a paradigm shift of our understanding about the pathobiology of metastases. In principle, exosomes serve as transport medium for proteins, mRNAs and miRNAs to transmit targeted cues from the primary cell to distant sites via horizontal transfer or cell-receptor interaction. In this chapter, we seek to explore in-depth the mechanisms engendering TDE in the metastatic cascade, along with experimental models to augment our understanding. The aforementioned has also paved way for parallel advancements in the therapeutic armamentarium, as evident from pronounced efforts to exploit the metastatic process for therapeutic targeting. In this light, we aim to examine potential anti-metastatic therapeutic opportunities derived from exosomal research. Lastly, exosomes may play a crucial role in the contemporary era of "liquid biopsies", given the array of molecular information with diagnostic and predictive indications. We thus intend to end this chapter off by exploring future applications of exosomes that could illuminate shortcomings and propel advancements in biomarker research.

1. Introduction

Our understanding of cancerous metastasis has traditionally hinged upon tumor-host interaction. This field of research has garnered renewed interest ever since the discovery of vesicle-based cell-cell communication mediated by exosomes. This concept was first propounded by Trams and colleagues in 1981 [1] as endocytic microvesicles exhibiting 5′-nucleotidase activity, which are released by virtually all cell lines including neoplastic variants. These nanoparticles are distinct in size between 30 and 100 nm, morphologically round or cup-shaped, with a density of between 1.13 and 1.19 g/ml [2]. The inceptive event to its secretion into the extracellular environs is the fusion of mutlti-vesicular endosomes with the cell surface or by reverse budding from the plasma membrane [2,3], giving rise to its distinct protein-lipid composition. Secretion of exosomes is pronounced in cancer cells, best attributed to a constellation of Rab3D overexpression [4]; other signaling cascades such as the Wnt pathway [5]; and a nurturing acidic microenvironment that further promotes cell fusion events [6].

Whilst exosomes have traditionally been understood to be cellular "trash bags", their enigmatic nature has warranted persistent research into their structure and functions as molecular Palkis. The highlights of recent research revealed the crucial role that tumor-derived exosomes (TDEs) may have in cancer inception, propagation, and metastasis [7,8]. TDEs are regarded as functional molecular cargos, carrying a myriad of more than 40,000 proteins reported to date, such as tubulin, actin, actin-binding molecules, cytosolic proteins, and those derived from plasma or endosomal membranes [9]. MHC class I [10] and heat shock protein (HSP 70 and 90) [3,9] are also key features present that are involved in antigen presentation. Tetraspanins are characteristically carried at high concentrations in exosomes; CD9 and CD82 in particular inhibit invasion and migration of cancer cells as well as interaction with integrins as reported in both in vitro and in vivo studies respectively [11]. Besides proteins, exosomes carry lipids, mRNA and DNA, all of which are adroit to ignite malignant changes and field cancerization.

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MiRNA constitutes the major component of RNA in exosomes [12,13]; it exhibits the dysregulated profile of cancer cells and serve as genetic messengers allowing for ubiquitous influence on gene expression of recipient target cells [14]. Lastly, long non-coding RNAs (LnRNAs) form an integral part of the molecular cargo, disrupting gene expression via histone complex modification or annealing with genomic DNA [15]. The actions of these oncogenic molecules potentially reflect the ever-changing pathognomonic pathways that cancer cells have adopted to ensure their own continuance.

It is of belief that TDEs are well involved in the invasion-metastasis cascade [8]. First, TDEs are capable of initiating the preliminary events of metastatic dissemination in the epithelial-mesenchymal transition (EMT) program by secreting autocrine and paracrine signals within the neoplastic environment. Penultimately once disseminated into the systemic circulation, TDEs may reside in distal organ tissues to attain a pre-metastatic niche where they either remain dormant (dormant tumor cells) or colonize to form micro-metastases and eventually macro-metastases [8]. Lastly, TDEs exhibit immune-modulating effects to escape immune surveillance and allow for unquenchable metastatic progression. A summary of the invasion-metastasis cascade is summarized in Fig. 1. In the following sections, we elucidate these mechanisms with contemporary evidence, which could form the basis for novel therapeutic tactics in the bid to outwit cancer.

2. Key players in EMT

The hallmark of the invasion-metastasis cascade is formation of macro-metastases in distant organs, and epithelial to mesenchymal transition (EMT) is a paramount process in the initiation phase. EMT is characterized by trans-differentiation from epithelial neoplastic cells into motile mesenchymal cells under the influence of cancer-associated fibroblasts (CAFs) [16] to create dramatic cell transpositions. The pronounced invasion and migratory properties of epithelial neoplastic cells occurs as a loss of polarized organization and cell-cell junctions [17]. Under EMT, there is diminished expression of E-cadherin as epithelial marker, heightened expression of vimentin as mesenchymal marker [18,19], and proteases such as matrix metalloproteinases (MMP) [20–26]. These enable epithelial cells to emanate cytoplasmic extensions and intrude the basement membrane. These protrusions then form new connections with the interstitial matrix and thereafter cytoskeleton contraction allows for cells to translocate into the stroma. The locomotion of invading cells depends heavily on repeated bouts of well-coordinated contractile and protrusive events.

A plethora of signaling pathways have been reported as crucial during the EMT program. The process depends on various putative signal transduction cascades that are mediated by SNAIL, zinc-finger *E*-box binding (ZEB), and TWIST factors [16,20,21,22,26]. Other novel development drivers include GATA family [27,28], SRY, and forkhead box (FOX), which modulate epithelial genes or polarity complexes, and to harmonize with SNAIL/SNAIL 2 to instigate EMT [29,30] . Given the hypoxic state that TDEs tend to shed in, there is increased propensity for TDEs to instigate metastasis due to enriched potency of EMT-transducing signaling molecules such as MMPs, tumor necrosis factor alpha, transforming growth factor beta (TGFβ), protein kinase B (AKT), interleukin-6 (IL-6), integrin-linked kinase, caveolin-1, platelet-derived growth factors (PDGFs), and β-catenin [31,32].

Recipient cells that have internalized TDEs undergo proteomic alterations consistent with EMT-like features [8]. Urothelial cells treated with exosomes isolated from muscle-invasive bladder urothelial cells displayed EMT-typical features with conspicuous migratory and invasive characteristics. Chief of these was an increased expression of vimentin, SNAIL and TWIST coupled with downregulation of E-cadherin and β-catenin expression via the TGF-β1 pathway [33]. These findings corroborated with another in-vivo study, where a nasopharyngeal carcinoma (NPC) cell line was co-cultured with exosomes isolated from a similar cell line, resulting in heightened expression of mesenchymal markers (vimentin and N-cadherin) and downregulation of E-cadherin [34]. Furthermore, functional assays of EBV-negative cells treated with LMP1 exosomes demonstrated EMT phenotypical features including tendency to invade and migrate [35]. These findings were further extrapolated in other neoplastic cell lines including lung, gastric and glioblastoma [36–38]. Pro-oncogenic mi-RNAs transported in exosomes appears to be key players orchestrating these processes. Inhibition of E-cadherin synthesis in lung cancer and melanoma cells is modulated by exosomal miR-23a, which perpetuates the EMT process via TGF-β pathway [39,40]. Other notable modulators of EMT include MiR-191 and let7a, which are found in melanoma, gastric and colorectal cancer [41–43].

Aside from EMT associated pathways, tight and adherens junctions serve as potential targets for TDEs [8]. They may exert direct influence on the invasive process into blood vessels by secreting miR-105. As manifested in breast cancer models, miR-105 has been shown to

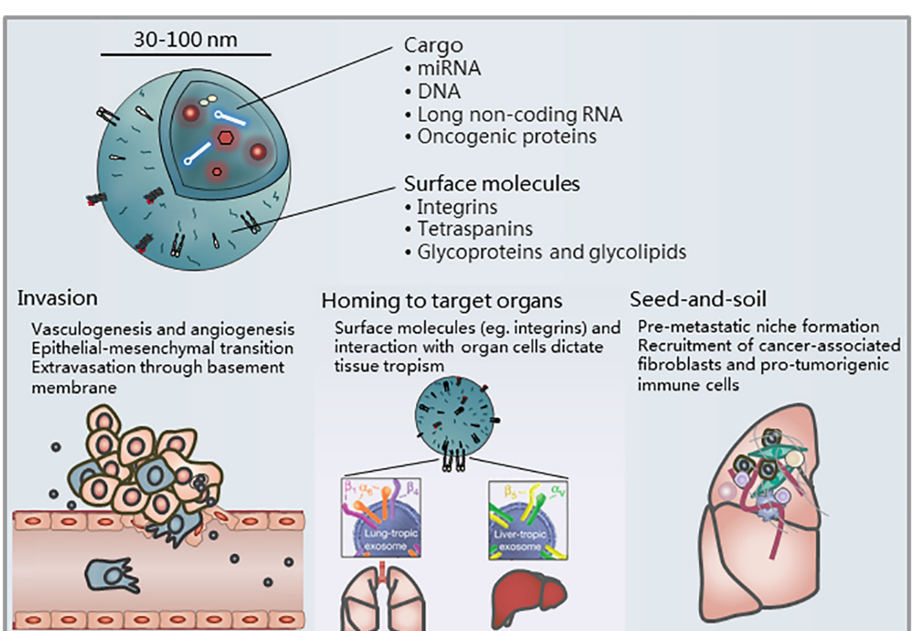

Fig. 1. – Contents of tumor derived exosomes (TDE) and invasion-metastasis cascade. TDEs are molecular cargos containing miRNA, DNA, long non-coding RNA, and oncogenic proteins. It is also bounded by surface molecules including integrins, tetraspanins, glyoproeins and glycolipids. TDEs are primarily responsible for initiating the epithelial-mesenchymal transition. Invasion first occurs through vasculogenesis and angiogenesis, and once disseminated into the systemic circulation, TDEs may reside in distal organ tissues to attain a pre-metastatic niche where they colonize to form micro-metastases and eventually macro-metastases. Immune-modulating effects occur due to recruitment of cancer-associated fibroblasts and pro-tumorigenic immune cells, allowing TDEs to escape immune surveillance.

interact with and downregulate the tight junction protein ZO-1 in endothelial monolayer cells, magnifying vascular permeability and accelerating formation of lung and brain metastases [14]. Implicitly, exosomal miR-105 serves as a prognostic marker for patients with breast carcinoma and serum levels reflect the level in the primary tumor itself [14]. However, it appears that exosomes secreted by non-neoplastic cells may have a role in driving cancer progression. In a salient study by Wrana and colleagues, fibroblast-secreted exosomes were reported to enhance the invasive activity and motility of breast cancer cell (BCC) via Wnt-planar cell polarity (PCP) signaling. Upon internalization of fibroblast-derived exosomes, BCCs repackaged the exosome cargo with Wnt11 and initiated activation of Wnt-PCP signaling in an autocrine fashion. Furthermore, co-culture of both BCC and fibroblasts promoted metastasis that was driven by both fibroblastic exosome tetraspanins CD81, and PCP signaling in BCC [44]. These fascinating findings provide mechanistic insights into the crosstalk between stromal cells and cancer cells to instigate metastasis.

Vice versa, TDEs have been shown to modulate stromal fibroblasts. In a comprehensive study by Webber and colleagues [45], they showed that TGFβ positive TDE were capable of elevating α-smooth muscle actin expression, converting mesenchymal stem cells into cancer-associated fibroblasts (CAFs). Just recently, the same group demonstrated that TDEs secreted by prostate cancer cells activated TGFβ1 dependent fibroblast differentiation to a unique myofibroblast phenotype, propagating tumor growth in vivo and reinforcing angiogenesis [46]. The aforementioned processes are specific to TDEs, as myofibroblasts induced using soluble TGFβ1 were not oncogenic. This exemplifies the indispensable role of cancer exosomes as key players in tumor-associated stromal differentiation.

3. Angiogenesis

Angiogenesis is a requisite process for tumor growth and metastasis [47]. Particularly in hypoxic conditions, exosomes function as key messengers between neoplastic and vascular cells during hypoxia-driven pro-angiogenic tumor responses [32]. Furthermore, TDEs secreted from melanoma cells can incite a proangiogenic signaling cascade characterized by endothelial spheroid formation in a dose-dependent fashion [48]. Elevated expression of Wnt5a in melanoma has also been correlated with the angiogenic marker ESAM during gene analysis, affecting extent of endothelial cell branching [5]. Just recently, a novel study by Asada and colleagues [49] reported that Delta-like 4 (DII4) containing exosomes resulted in loss of filopodia in capillary endothelial tip cells and blunted sprout formation. These findings suggest that DII4 containing exosomes elevate endothelial cell motility whilst suppressing their growth. Evidence is accumulating supporting the role of exosomal miRNA in regulating angiogenesis and its consequent impacts on tumor progression [50]. Grange and colleagues, for instance, reported that proangiogenic mRNAs and miRNAs released by CD105-positive renal cancer stem cells potentiated lung metastases [51]. In another instance, miR-214 – which controls endothelial cell function and angiogenesis – is responsible for exosome-mediated signaling between endothelial cells, resulting in ataxia telangiectasia mutated silencing in recipient cells [52].

4. Organotropic metastasis

The penchant of primary tumors to disseminate to specific secondary sites is understood to be a result of intricate, well-coordinate tumor-stroma interaction in the host organ. This formed the crux of the "soil and seed" hypothesis that was first theorized by Paget in 1889, where primary tumor cells tended to form metastasis only at compatible secondary organ sites [53]. Fidler and colleagues later bolstered these findings by showing that metastasis was site-specific regardless of the vasculature or lymphatic vessels that drain the primary tumor site [54]. This set forth the concept that a conducive microenvironment, or niche, is paramount for circulating tumor cells to engraft at distant organ sites. Bone marrow derived cells, endothelial progenitor cells and mesenchymal cells play key roles in establishing the pre-metastatic niche [55]. Robust evidence to date comes from melanoma-derived exosomes, which are capable of "educating" bone marrow progenitors – through the receptor tyrosine kinase MET – to become pro-vasculogenic [56]. This is perpetrated by enhancement of vascular leakiness and upregulation of pro-inflammatory molecules at metastatic sites [56].

As astutely described by Rak as "organ-seeking vesicles" [57], there is mounting evidence accumulated across cancer types suggesting that the biodistribution of exosomes commensurate with pattern of organotropic metastatic spread, including breast, melanomas, and pancreatic carcinomas [8,48,58,59]. Mouse model studies for pancreatic ductal adenocarcinoma (PDAC) demonstrated that TDEs are important in promoting engraftment of metastatic pancreatic cells in the liver by interaction with Kupffer-cells [60]. Through migration inhibitory factor (MIF) signaling, hepatic stellate cells were found to upregulate fibronectin production and TGFβ secretion upon internalization of PDAC-derived exosomes [60]. Similar observations were made in malignant melanomas, where exosomes had greater propensity to harbor in the interstitial space of the kidney, liver, bone marrows and lungs, all of which are organotropic sites for melanoma metastasis. This consequently leads to an upregulation of genes like S100A8 and S100A9 involved in inflammation and extracellular matrix remodeling (ECM); annexins A6 and CD44v6 potentiating metastasis; and HSPs (HSP 90 and HSP 70) [61–63].

The integrin repertoire is thought to be – at least in part – responsible for the biased distribution of exosomes. For instance, Integrin beta 5 enhances exosome adhesion in the liver whilst integrin beta 4 directs metastasis to the lungs [59]. However, it is probably not the only means to the end – the uptake of TDEs into recipient cells could trigger a myriad of other endocytic processes including lipid raft, clathrin, and caveolin-mediated internalization [64–67].

5. Homing of neoplastic cells to their target tissue

After "seeding" has taken place in the new foreign soil, exosomes are able to foment tumor-supportive processes including proliferation, angiogenesis, and ECM remodeling. Uptake of TDEs by myofibroblast progenitors can enhance recruitment [64,68] and augment differentiation into myofibroblast-like cells via TGFβ/Smad signaling [45,46,69]. Intriguingly, it appears that the homing process occurs in a bidirectional fashion, where exosomes secreted by non-neoplastic cells may also promote adaption of circulating tumor cells. The remarkable plasticity of the tumor microenvironment was further demonstrated by Zhang and colleagues, who reported a reduction of PTEN expression after metastasis to the brain [70]. This was epigenetically mediated by microRNAs from brain astrocytes, which release exosomes to orchestrate an intercellular transfer of PTEN-specific microRNAs to metastatic tumor cells. The consequent loss of PTEN expression resulted in increased secretion of chemokine CCL2, consequently recruiting IBA1-expressing myeloid cells that promoted the development of brain metastatic tumor cells [70].

6. Oncogenic transformation of non-neoplastic cells

Non-neoplastic cells are at risk of undergoing oncogenic transformation in the presence of TDEs. For instance, an in vivo study involving patient adipose-derived stem cells (pASCs) acquired cytogenetic aberrations and mesenchymal-to-epithelial (MET) transition when primed with prostate cancer cells. Aside from the downregulation of large tumor suppressor homolog2 and the programmed cell death protein 4, the oncogenic transformation potential was attributed to a host of exosomes carrying oncogenic factors, such as miR-125B, miR-130b, miR-155, H-ras, K-ras, Rab1a, Rab1b and Rab11a [71]. This phenomenon is also evident in breast cancer cells, where human mammary epithelial MCF10A cells underwent neoplastic changes when co-cultured with breast cancer MDA-MB-231 derived exosomes. This was further characterized by downregulation of target transcripts PTEN and homeobox protein (HOXD1) and reciprocal upregulation of miR-21 and miR-10b [72].

Oncogenic viruses are also capable of conferring pro-oncogenic signaling in recipient cells by exploiting the host exosomal apparatus [66,70,73,74]. Epstein-Barr virus positive nasopharyngeal cancer cells (EBV positive NPC) secrete exosomes containing EBV oncoproteins LMP1 and viral miRNAs, which activates AKT, extracellular signal-regulated kinases (ERK), and epidermal growth factor receptor (EGFR) expression in normal epithelial cells [35,73,74]. However, this field is still in its relative infancy, and much remains to be explored regarding mechanisms exploited by viral cancers to amplify their progression.

7. Immune modulation

Immunosurveillance is perhaps the host's main defense mechanism against proliferation of circulating clones; unfortunately, metastasis remains a common phenomenon reflecting the success of neoplastic cells in evading the immune system. We seek to uncover the various mechanisms in which cancer cells have employed to ensure their longevity.

Since some cancers spread via hematogenous routes, it is crucial for these cells to be able to survive in the blood circulation and not succumb to immune surveillance. In this regard, platelets appear to be a key player ever since the first link between platelets and cancer was inaugurated by Trousseau in 1865 [75]. Exosomes secreted by platelets consist of P-selectin and GP IIb-IIIa, which interact with cancer cells, endothelial cells and leukocytes [76,77]. The relationship between platelets and cancer is akin to deadly allies; platelets appear to be chaperons to cancer cells, accompanying them in the blood stream and facilitating adhesion at the vessel endothelium via P-selectin, resulting in extravasation of neoplastic cells into the premetastatic niche [78–80]. Mice model studies reinstated the crucial role of platelets, where platelet depletion inhibited metastasis whilst recovery of these platelets was associated with metastatic activity [81]. Besides vessel adhesion, platelets function as "guardians" of these neoplastic cells against immune cell activity, such as natural killer (NK) cell mediated antibody-dependent cytotoxicity [82,83]. Coating cancer cells with fibrin appears to be the key mechanism responsible for repudiating NK cell activity due to impaired recognition of coated cells [79]. A second plausible mechanism hinges on the secretion of TGFβ1 by hypoxic tumor cells, blunting expression of NK cell activating receptor (NKG2D) [84,85]. This further activates the EMT-associated TGFβ1 pathway, which is a key component of the metastatic cascade [86].

Upon arrival the secondary organ site, TDEs activate immunosuppressive pathways to combat the foreign microenvironment in order to ensure tumor cell proliferation. This is achieved by inhibiting effector cells and stimulation of regulatory T cells [87]. An in vitro culture model study of acute myeloid leukemia showcased the reduction of CD8+ T cells by TDEs via activation of Fas/Fas ligand mediated apoptosis [88,89]. This was coupled with proliferation and conversion of CD4 + T cells into regulatory T cells (CD4+, CD25+, FOXP3+) characterized by expression of interleukin-10, TGFβ1, cytotoxic lymphocyte antigen −4 (CTLA-4) and granzyme, all of which curtail the cytotoxic effect of NK cells [90,91]. Furthermore, TDEs were shown to activate human myeloid derived suppressor cells – immature myeloid cells that suppress T cell activation – through HSP72/TLR-2 via IL-6 expression and STAT3 signaling pathway [92,93]. The immunosuppressive effects of TDEs could possibly stem from miRNAs as evident from an in vitro model study of NPC, where immunosuppressive miRNAs (hsa-miR-24-3p, hsa-miR-891a, hsa-miR-106a-5p, hsa-miR-20a-5p, and hsa-miR-1908) were present in TDEs [94,95]. TDEs were also capable of converting CD4 + CD25- T cells into inhibitory CD4 + CD25^{high} T cells [94]. One of the many proposed mechanisms of T cell suppression include extracellular adenosine production by TDEs positive for CD39 and CD73 [96], which may occur due to epigenetic changes conferred by TDEs to T cells. For instance, TDEs can downregulate regulatory T cell genes modulating the adenosine pathway leading to pronounced expression of CD39 and adenosine production [97]. This portends the possibility that TDEs exuberate a myriad of immunosuppressive mechanisms to ensure neoplastic survival.

Besides modulating cellular immune responses, the humoral response – specifically antibody-dependent cytotoxicity (ADCC) [98] – falls victim to TDEs as well, which in fact is the major of the three response mechanisms including complement-mediated cytotoxicity. For instance, in breast cancer cells, TDEs can express human epidermal growth factor receptor 2 (Her2) and epithelial cell adhesion molecule (EpCAM), therefore binding and sequestering antibodies exerting ADCC on neoplastic cells positive for these antigens. Hence, therapeutic response against Trastuzumab (mammalian Her2-antibody) is diminished [99]. A similar observation was made in B cell lymphomas, where exosomes carrying CD20 intercepted the anti-CD20 antibody (Rituximab), diminishing the membrane attack complex mediated by binding of Rituximab at the B cell surface. Paradoxically, administration of Rituximab increased secretion of CD20 positive exosomes whereas depletion of these exosomes improved therapeutic responses of Rituximab [100]. These findings hint at the impending need for future research to investigate potential immunotherapy pitfalls associated with TDEs [87].

Inflammation is a paramount process necessary for tumor growth and adaption to the "foreign soil" [55]. Tumor-associated macrophages (TAM) promote invasion, ECM remodeling and angiogenesis [87,101]. Histological evidence of macrophages in high density has been correlated with poorer prognosis in breast cancer specimens [102]. It has been established now that TDEs activate TAMs via nuclear factor-κB and interaction with toll-like receptors (TLRs) found on the macrophage cell surface [103]. Alternatively, this could be achieved by binding of exosomal miRNAs (mi-R21, mi-R29a) as ligands to receptors of TLRs (TLR-7, TLR-8) in a paracrine fashion [104], resulting in a pro-metastatic inflammatory response involving cytokines TNFα and IL-6. The relationship between TDEs and inflammation is also evident in pancreatic ductal adenocarcinoma, where the uptake of TDEs by liver Kupffer cells lead to heightened fibronectin production by hepatic stellate cells. This in part is mediated by macrophage migratory inhibition factor (MIF) potentiating the release of TGFβ1 by Kupffer cells [60]. Deposition of fibronectin in the liver subsequently fosters arrest of bone marrow-derived macrophages hence creating a pre-metastatic niche.

8. Pharmacological developments

Alluding to the aforementioned discussions, it is now discernible that tumor derived exosomes exert a spectrum of oncogenic mechanisms that instigate tumor growth, progression and metastasis. Every stage along this multi-level process are potential therapeutic targets to be exploited. It is the aim of the next few paragraphs to comprehensively review the development of the therapeutic armamentarium that could target these pathways.

The biogenesis of exosomes constitutes several components, some of which are regarded as fundamental regulators of metastasis. Heparanase, for instance, has been identified as one potential therapeutic target. The role of heparanase in exosome biogenesis remains to be elucidated, but it functions primarily as an endoglucuronidase that cleaves heparan sulfate chains on syndecan-1 propagating formation of syndecan-syntenin-ALIX complex [105]. Syndecan-syntenin-ALIX plays a key role in membrane transport and signaling processes, hence altering exosome composition; promoting exosome production; and driving exosome secretion [106,107]. In this light, investigations in animal models are now underway to explore the potential of heparanase inhibitors, heparin mimetics and modified heparins as anticancer therapeutics [105].

Other potential targets include RAB genes, which are highly expressed in metastatic tumors and are responsible for secreting innumerable amounts of exosomes [56]. Blockade of GTPase RAB27a, for example, has been demonstrated in mouse models to downregulate exosomal secretion and metastatic dissemination, hence suggesting a role for RAB27a in exosome-dependent mechanisms that promote oncogenic progression and modify the tumor microenvironment [56,108]. In the same vein, a synergistic interaction has been discovered between invadopodia biogenesis and exosome secretion. The invasive actin structural nature of invadopodia allows for docking and secretion of CD63- and RAB27a positive exosomes [109]. Hence targeting of key proteins in these structures (N-WASP, TKS4, TKS5, MT1MMP) could spur therapeutic strategies aimed at bridling exosome biogenesis and secretion [110]. One other key target that could be exploited to inhibit TDE release is neutral sphingomyelinase 2 (nSMase2), a key regulator of ceramide biosynthesis. In vitro inhibition of nSMase2 with GW4869 has been shown to inhibit TDE release, hence suggesting that ceramide plays a weighty role in the dissemination of exosomes [111].

A complementary strategy to inhibiting exosome release is blockage of exosome internalization pathways [8]. Through competitive inhibition, persistent treatment with heparin and heparan sulfate chains likewise are capable of inhibiting TDE uptake and consequent oncogenic progression in oral squamous cell carcinoma and glioblastoma [33,37,112], hence lending further support to the pharmacological development of heparanase-targeting agents.

In addition, the functionality of TDEs are amenable to alterations, as evident from dietary studies of polyphenol curcumin. Curcumin was shown to reverse the TDE-mediated suppression of NK cell lysis in breast cancer models [113]. In chronic myelogenous leukemia (CML), the addition of curcumin as an adjunct resulted in a dose-dependent increase of PTEN, a target of miR-21. A consequent reduction of miR-21 in CML cells and exosomal packaging was observed. This was accompanied by downregulation of VEGF expression and release, as well as AKT phosphorylation [114].

A novel therapeutic paradigm involving the use of exosomes as immunologic stimulants has also been propounded [115]. Exosomes derived from antigen-presenting cells (APCs) are functional cargos of MHC/tumor antigens complexes, which can subsequently be presented to dendritic cells in vivo to prime cytotoxic T cells for T cell-dependent response against tumor cells. Hence, exosome-based cell-free vaccines may be an appealing anti-cancer therapeutic alternative [115,116]. However, there are shortcomings that might impede therapeutic advancement in this field. First and foremost, APCs are technically difficult to culture along with the need for specific tumor antigens to be transferred to APCs. Secondly, such an approach cannot be generalized to all patients as MHC-1 haplotype matching is warranted given the MHC-1 dependent antitumor response [117]. In addition, APC-exosomes pale in comparison to TDEs as the latter is able to carry shared tumor antigens hence conferring protection against different cancer types. TDEs also abolish the need for MHC-1 haplotype matching since they exert antitumor immunogenicity without expressing MHC-1 molecules [118]. Human Mucin 1 (hMUC1), for example, is a shared tumor antigen which can be incorporated into exosomes to be employed as anti-cancer vaccination against MUC1-expressing cancers [119]. In another instance, in vitro and in vivo murine studies reported that HSP70-enriched TDEs released by heat-treated tumors potentiated stronger Th1 immune response in comparison to normal TDEs [117]. This was accompanied by direct activation of NK cell mediated apoptosis of tumor cells [120]. Dendritic cells too, have shown to be primed by exosomes isolated from ascites in cancer patients, resulting in T cell mediated antitumor response [121]. Considerable progress has been made, as success from these experimental models have now translated into phase I clinical trials, two of which have demonstrated encouraging results in patients with non-small cell lung cancer [122] and late-stage malignant melanoma [123].

The lipid bilayer membrane-encapsulated structure of exosomes prohibits degradation and RNase damage, potentially serving as delivery systems for traditional chemotherapeutic agents, which reduces chemotherapy associated toxicity [115,124,125]. Intriguingly, cytotoxicity increased more than 50-fold in multi-drug resistant cancer upon amalgamation of paclitaxel with exosomes [126]. In another instance, the exosome-based delivery of certain miRNAs into lung cancer cells can increase the sensitivity to taxanes. This has been shown in the delivery of miR-181a into lung adenocarcinoma cells to increase its sensitivity to paclitaxel and platinum-based chemotherapy [127]. Aside from its unique structure, the small size of exosomes allows for easy passage through different biological barriers, such as the blood-brain barrier [128]. Therefore, exosomes may have the potential to bolster classical chemotherapeutic regimens, by improving its efficacy and safety profile [115].

The use of exosomes in vaccine development has heightened interest as well. Together with dendritic cell-derived exosomes (Dex), TDEs can migrate to lymph nodes to activate $CD4^+$ and $CD8^+$ T cells to initiate immunological reactions [129]. In a Phase IIB trial, the administration of TG4010 vaccine enhanced the effect of chemotherapy in patients with advanced non-small cell lung cancer (NSCLC), with a superior 6-month progression free survival (PFS) of 43.2% compared to just 35.1% in the chemotherapy only group [130]. In another Phase II trial, the generation of Dex increased the PFS of NSCLC patients with defective expression of NKp30 [129].

However, as discussed in Section 7, exosome-mediated immunosuppression may be the Achilles' heel of pharmaceutical developments. In this regard, the use of TDEs as a therapeutic tool may serve as a double-edged sword. Strategies are still needed to overcome TDE-mediated apoptosis of $CD8^+$ T cells, as well as inhibition of effector T cell proliferation. Aside, TDEs may alter the immunologic receptors and ligands of receptor cells similar to that of oncocytes, disrupt the immune function of these cells, or even sequester therapeutic antibodies. This has been show in a TDE-mediated transfer of epidermal growth factor receptor to macrophages, resulting in compromised innate immunity [131,132].

9. Biomarkers

It is understood that the blood concentration of exosomes in cancer patients is twice that of a normal health adult, as cancer cells have a higher propensity to generate more exosomes [13,133,134]. Unfortunately, technical limitations related to isolation cost and technique have impeded adoption of these biomarkers in clinical practice. This warrants the need to develop a robust method capable of minimize co-isolating protein aggregates and membranous particles.

Within the cargo that exosomes carry, miRNA serves as a useful source for predictive biomarkers, given that tumors tend to alter their miRNA expression profiles making it distinctive from normal cells [13,135]. Biomarkers have been studied heavily in prostatic carcinoma patients, where exosomal miR-141 and miR-375 were present in superior concentrations after radical prostatectomy [136–138]. Furthermore, varying levels of miRNAs may also help to distinguish high grade from low grade tumors, allowing for early risk identification amongst patients [138].

Similar observations were made with exosomal protein markers [13]. The most robust evidence to date comes from pancreatic patients, where Glypican-1 carrying exosomes were detected with 100% sensitivity and 100% specificity in comparison to health donors or patients with benign pancreatic lesions. Furthermore, the concentration of Glypican-1 correlated with tumor load hence facilitating prompt diagnosis of relapse post-surgery or metastatic spread. Whilst impressive, the study's sample size (n = 31 in total) warrants the need for larger studies to establish these conclusions, particularly for early detection of pancreatic cancer (pancreatic cancer precursor lesions, PCPL) in which only 5 cases were included in the discovery cohort but none in the validation cohort. Aside from pancreatic cancer, the same study also showed elevated Glypican-1 carrying exosomes in patients with breast cancer (24/32, 75%) compared to healthy donors. Hence, the 100% specificity of Glypican-1 as a pancreatic diagnostic biomarker could be greatly compromised [134]. Exosomal transmembrane256 has also been implicated in patients with prostate cancer, with relative success in terms of sensitivity and specificity [139]. Evidence for other cancer types including gastric and colorectal cancer [140] are also emerging in support of exosomes functioning as novel diagnostic biomarkers [141]. In the surge of the "liquid biopsy" era, exosomes may prove to be promising biomarkers for diagnostic, prognostic and predictive purposes in the near future [142].

10. Conclusion and future directions

Whilst TDEs hold significant potential in offering advanced therapeutic options, it may serve as a double-edged sword. On the one hand, TDEs hold strong potential for pharmaceutical development, be it as a vector or biological target. On the other hand, they are key players in the oncogenic-metastatic cascade, further impeding pharmaceutical developments through immune-modulation rendering these therapies ineffective. Nevertheless, several questions remain unanswered. The integration of exosomes and CRISPR-Cas genome-editing systems, for instance, is an unexplored area for future research and advancements in precision oncology. Similarly, can exosomes be integrated and loaded with multiple tumoricidal compounds? Next, it is unclear whether the route of administration (subcutaneous, intravenous etc), affects the therapeutic efficacy of exosome-based treatments.

From a legislative standpoint, there is yet to be an established TDE-specific guideline for therapeutic development. Nonetheless, it is expected that aspects of regulatory and safety requirements for pharmaceutical development and clinical application will be derived from current legislature on cell-based guidelines [129,143]. In the same vein, logistical issues regarding processing and manufacturing of exosomes may pose a challenge given the large amount exosomes needed to overcome the poor therapeutic efficacy of nanoparticle administration [144]. Ultimately, in the bid to offer patients with newer, advanced, and more effective treatment modalities, a multidisciplinary team effort consisting of scientists, clinicians, and engineers, is first needed to collectively address these challenges.

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Conflicts of interest

The authors declare no conflict of interest.

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