ORIGINAL PAPER

Exosomes in Cancer Microenvironment and Beyond: have we Overlooked these Extracellular Messengers?

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Abstract Cancer is a complex organ whose behavior is not only influenced by genetic and epigenetic changes in cancer cells but also by stromal cells, local extracellular matrix and specific tissue architecture. Intercellular communications within the cancer microenvironment are critical to coordinate the assembly of multiple cell types for an amalgamated form and function of a cancer. Exosomes are small membrane vesicles with an endosome origin that are released by cells into the extracellular environment. They carry a cargo of proteins, lipids, and nucleic acids and transfer their cargo to recipient cells and altering the recipient cells' biochemical composition, signaling pathways, and gene regulation. Exosomes can thus serve as extracellular messengers mediating cell-cell communication. Both cancer cells and stromal cells release exosomes not only into the cancer microenvironment but also into the circulation. In this review, we summarize the research done so far on cancer-derived exosomes and assess their roles as extracellular messengers facilitating cancer progression and metastasis.

Keywords Exosome · Cancer microenvironment · Extracellular messenger · Stroma

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Introduction

Exosomes

Exosomes are small extracellular membrane vesicles with a mean size of 40–100 nm that are released by cells either spontaneously or by induction. They were first described as exfoliated membrane vesicles that contain 5′-nucleotidase activity and released from neoplastic cell lines [1]. Soon after, other groups reported the secretion of small vesicles that were originated from endosomes in cultured reticulocytes and were present inside multivesicular bodies (MVBs). These small vesicles were shown to have a function in clearing the transferrin receptor and other obsolete proteins during the maturation of reticulocytes to erythrocytes [2–6]. Exosomes represent a subset of secreted membrane vesicles and are generally identified by having a buoyant density of 1.10 to 1.21 g/ml in sucrose, a size range from 30 nm to 200 nm, a cup shaped appearance by electron microscopy and sedimentation by ultracentrifugation at the speed of at least $100,000 \times g$ with prior centrifugation at a lower speed to remove larger membrane vesicles [7]. Other types of secreted membrane vesicles include microvesicles (MVs) also called microparticles (MPs) or ectosomes which are generated from most cell types by plasma membrane shedding/budding/apoptotic blebbing with a wide size range from 50 nm to 1 μm. MVs are sometimes also used as a general term to describe all secreted membrane vesicles including exosomes. As there is yet any definite nomenclature for the different types of vesicles, in this review we will use MVs to indicate membrane vesicles released by membrane shedding/budding/apoptotic blebbing. The larger MVs are generally isolated by centrifugation at a speed of 10,000 - $20,000 \times g$. MVs with sizes in the range of exosomes cannot be easily distinguished from exosomes through the

differential centrifugation method. Further analysis of biochemical markers such as endosomal markers Alix, CD9 and CD63 are thus required to confirm exosome identity. Thus, the membrane vesicle populations in vitro and in vivo are often mixtures of exosomes plus MVs. Exosome preparations prepared through ultracentrifugation at $100{,}000 \times g$ commonly used in various published studies most likely also contain other small MVs. Hence, stringent purification methods and consensus characteristics for identifying exosomes need to be established [8, 9].

Exosomes come from the intraluminal vesicles (ILVs) of MVBs that are formed through the inward budding of late endosome membrane (Fig. 1). When MVBs fuse with plasma membrane, exosomes are released as part of their cargo. During the formation of exosomes, there are two membrane inversion events. The first occurs at the plasma membrane during endocytic internalization and the second occurs when the endosome membrane bud inwardly, encapsulating some of cell's cytoplasm including RNA, protein, and even DNA. As a result, molecules are retained in the same inside-outside configuration (positive orientation) in ILVs, allowing exosomes to have the same orientation as the cell membrane upon their release to the extracellular environment (summarized in [10, 11]). (Note: There are many excellent reviews on exosomes in the past few years. We apologize that we could not cite all of them.)

Many cell types have been described to release exosomes into the extracellular medium in vitro such as hematopoietic cells (B cells, T cells, dendritic cells, mast cells, platelets), intestinal epithelial cells, Schwann cells, neuronal cells,

adipocytes, fibroblasts, tumour cells, etc. They are also found in vivo in several biological fluids such as urine, plasma, epididymal fluid, amniotic liquid, malignant and pleural effusions of ascites, bronchoalveolar lavage fluid, synovial fluid and breast milk.

The composition of exosomes varies depending on the cell type of origin. Over the years, various techniques such as Western blotting, mass spectrometry, fluorescence-activated cell sorting, and immunoelectron microscopy have been used to analyze the content of exosomes. They contain RNAs, lipids and 350–400 proteins [12]. Common exosome proteins include annexins that regulate membrane cytoskeleton dynamics and membrane fusion events; Rab family of GTPases which promote docking and membrane fusion events; and ESCRT (endosomal sorting complex required for transport) proteins [13–15]. Other proteins include tetraspanins (CD81, CD63, CD9) and heat shock proteins (HSP60, HSP70, HSP90) which can facilitate peptide loading onto major histocompatibility complex (MHC) I and II [16, 17]. Exosomes also express cell-specific markers such as MHC class I and II, co-stimulatory proteins (CD80 and CD86) on antigen presenting cell-derived exosomes, integrin CD41a and Von Willebrand factor on platelet-derived exosomes, and perforin and granzyme on cytotoxic T-lymphocyte-derived exosomes [18–20]. A number of adhesion molecules have also been reported in exosomes including CD146, CD9, EGFRvIII, CD18, CD11a, CD11b, CD11c, CD166 and LFA-3/CD58 [21, 22]. In addition, metabolic enzymes such as peroxidases, pyruvate kinase, lipid kinases and enolase-1 are also reported to be present in exosomes [12].
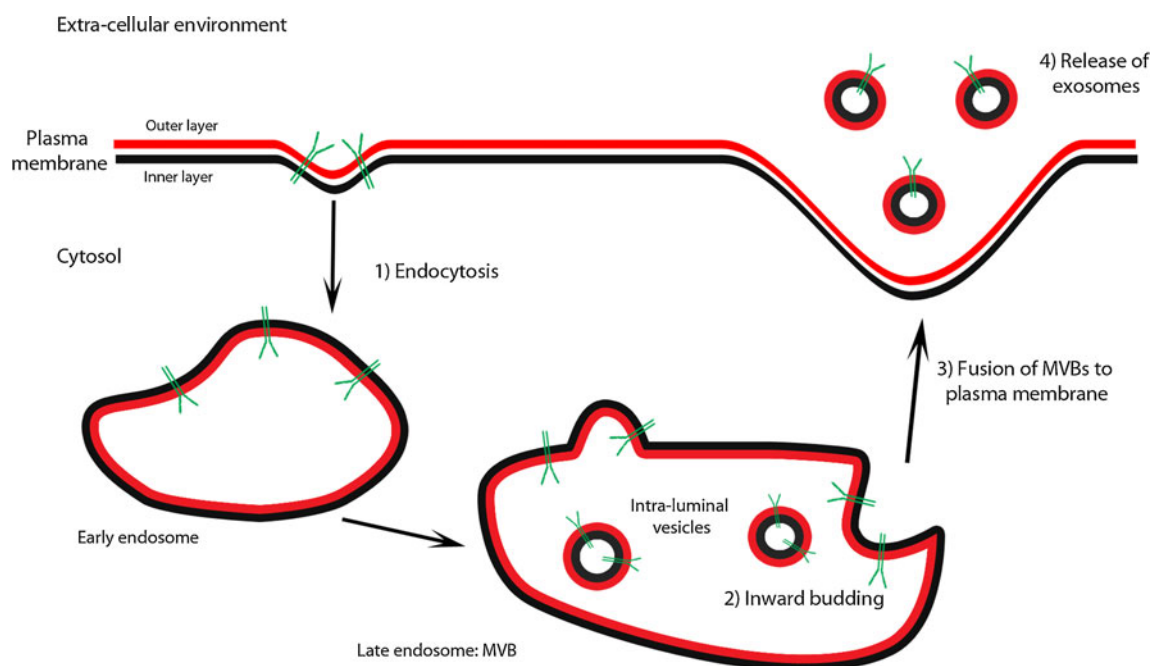

Fig. 1 A schematic diagram depicting the formation of exosome through ILV of MVB

Similarly, the composition of MVs also varies depending on the cell type of origin [23]. The membrane composition of MVs is distinct from its parental cell plasma membrane with significant structural remodelling. For example, in MVs, phosphatidylserine is relocated to the outer membrane leaflet while the topology of membrane proteins remains intact [24–26]. This is a distinct feature of MVs that is different from exosomes. In addition, MVs also in general do not contain endosomal proteins.

The current general consensus seems to be that the major differences between exosomes and other secreted membrane vesicles are the mechanism of secretion and size. However, due to the overlap in sizes of exosomes and small MVs generated through membrane shedding/budding, some previously reported findings regarding exosomes or microvesicles may actually involve a mixture of exosomes and MVs. In this review, we will use the term "exosomes" for studies involving extracellular membrane vesicles isolated through ultracentrifugation at 100,000 x g even though in some cases, these preparations may contain heterogeneous types of vesicles.

Exosomes can be internalized by recipient cells through mechanisms such as endocytosis, phagocytosis, micropinocytosis or even direct membrane lipid fusion [27–30]. Uptake of exosomes by recipient cells rely on a variety of recipient cell surface receptors as well as exosome surface proteins such as integrins, annexins, galectin, and ICAM1 [27, 31]. Not only proteins (both cytoplasmic and cell surface transmembrane proteins) but also mRNA and miRNA are transferred to the recipient cells by exosomes [32–34]. Exosomes can thus alter the recipient cell's molecular profile, signalling pathways and gene regulation and serve as extracellular messengers that can transfer information from the donor cell to the recipient cell.

Cancer Microenvironment

It is now well accepted that a cancer is a complex organ whose behavior is not only influenced by genetic and epigenetic changes in the cancer cells but also by stromal cells, local extracellular matrix (ECM) and specific tissue architecture [35]. Conceivably, the cancer microenvironment is influenced by both the cancer cells as well as stromal cells. Cancer cells maneuver the cancer microenvironment to one that favors cancer progression and metastasis by influencing stromal cells and the ECM. On the other hand, the stromal cells carry the local tissue influences and architectural cues which also influence cancer behavior. Hence, intercellular communication is critical in the cancer microenvironment to coordinate the assembly of multiple cell types for an amalgamated form and function of a cancer [36].

The massive cancer cell proliferation in cancer quickly lead to a cancer microenvironment that is hypoxic, acidic, and deprived of nutrients. Such hostile microenvironment put on a powerful selection pressure which select cells that are hypoxia-resistant, highly invasive, genetically unstable, and with an altered metabolism re-programming to metabolize glucose by aerobic glycolysis rather than by oxidative phosphorylation (reviewed in [36–38]). Hypoxia activates the hypoxia-inducible factor 1 (HIF1) which stimulate the expression of vascular endothelial growth factor (VEGF) and many other angiogenesis growth factors and chemokines. Altered glycolytic metabolism lead to extracellular acidity with a selection of tumor cells armed to avoid intracellular accumulation of acids. Maintaining the intracellular pH is crucial to normal cell function because many cellular biochemical processes have a narrow pH optimum. To counteract cytosolic acidification, tumor cells mobilize proton pumps to pump H^+ into the extracellular environment, thus generating an acidic microenvironment.

Cancer microenvironment is also rich in cytokines and chemokines which attract large number of immune cells into the cancer microenvironment. The cancer-localized immune cells in turn release more cytokines and chemokines which influence cancer cell survival and migration. In addition, cancer extracellular environment is also rich in matrix metalloproteinases which help to promote angiogenesis, cancer cell invasion and metastasis. High level of extracellular adenosine level in cancer due to metabolic changes also suppress immune function and help cancer to evade immune surveillance (reviewed in [37].

Thus, cancer microenvironment is unique and it changes according to the oncogene/tumor suppressor gene mutations and host factors. Cells in cancer (parenchymal cancer cells and stromal cells) communicate with and influence each other by releasing soluble factors and ECM proteins. In addition, cells also release lipid membrane enclosed vesicles such as exosomes and other secreted membrane vesicles, which play important roles as extracellular messengers not only within the cancer and but also between the cancer and the host environment.

Exosomes as Extracellular Messengers in the Cancer Microenvironment

It is now believed that many types of cells can release exosomes including cells in cancer, although the amount released in vivo is not clear. Here we examine the possible roles exosomes play in cancer microenvironment.

Exosomes Released by Cancer Cells

The intercellular exchange of proteins and nucleic acids within the cancer microenvironment could be an effective local communication route that plays important roles in

promoting cancer progression and metastasis. Exosomes released by cancer cells can not only transmit signals to other cancer cells, but also to stromal cells within the cancer microenvironment, thus impacting the cancer ECM architecture and generating cancer microenvironment.

Cancer-derived Exosomes Promote Cancer Growth

Glioblastoma-derived exosomes were reported to stimulate glioma cell proliferation and promote cancer growth in a self-promoting manner [34] (Although this paper described the membrane vesicles (MVs) used as microvesicles, these MVs are most likely exosomes judging from the isolation method used). In another study, the anti-apoptotic protein survivin was shown to be released into exosomes by cervical carcinoma cells. In particular, this survivin exosomal release is significantly upregulated after proton irradiation-induced stress, suggesting that exosomes may be involved in cancer cell self protection under stress. Furthermore, extracellular survivin was able to enhance proliferation, survival and cancer cell invasion, indicating the significance of exosome-mediated survivin release in cancer progression [39]. Significantly, ovarian carcinoma patients were found to release exosomes into the ascites and blood circulation and become systemic. Application of malignant ascites-derived exosomes to cancer bearing mice resulted in augmented cancer growth, indicating that cancer-derived exosomes promote cancer progression [40]. In yet another study, human breast and colorectal cancer cells were shown to release exosomes containing signaling-competent EGFR ligands [41]. By analyzing exosomes released from cultured MDCK cells expressing individual EGFR ligand, exosomes carrying amphiregulin (AREG) were found to most potently enhance the invasiveness of breast cancer cells. Significantly, AREG exosomes displayed 5-times more invasive potential over equivalent amounts of recombinant AREG. AREG exosomes are shown to be rapidly internalized by recipient cells [41]. Furthermore, the authors revealed that exosomes from colon cancer cells with a mutant KRAS allele exhibited both higher AREG levels and greater invasive potential than exosomes from isogenically matched, nontransformed cells in which mutant KRAS was eliminated by homologous recombination. Hence, EGFR ligand can signal via exosomes and this may contribute to cancer progression and metastasis.

The cancer microenvironment is long believed to be unique as a result of mutual influences between cancer cells and stromal cells. A recent report indicated that the cancer microenvironment also influences the components and functions of cancer-derived exosomes [42]. In that report, it was demonstrated that co-culture of leukocytes isolated from breast cancer tissue led to uptake of fibronectin (FN) into the cancer exosomes. The uptake of FN into exosomes is cancer tissue derived and leukocyte specific, as leukocytes isolated from the peripheral blood of naive mice failed to induce FN uptake by cancer exosomes. They further identified the CD25(+)and Gr-1(+) subset of cancer-associated leucocytes which are mainly responsible for promoting FN uptake by cancer exosomes. In response to absorbing of FN (+) exosomes, focal adhesion kinase/Src-dependent signaling pathways are activated, and the production of proinflammatory cytokines and metalloproteinase 9 is enhanced, causing enhanced cancer cell invasion in vitro and in vivo. This work also highlighted the fact that exosomes released from in vitro cultured cells may be functionally different from exosomes in the in vivo tissue environment.

Cancer-derived Exosomes Stimulate Angiogenesis

Exosomes released by cancer cells have been shown to promote angiogenesis. Exosomes produced by mouse B16 melanoma cells were shown to promote endothelial tubule formation and stimulate production of endothelial spheroids. In addition, B16 exosomes stimulated pro-angiogenic cytokine production in endothelial cells (ECs) such as IL-1α, FGF, GCS-F, TNFα, Leptin, TGFα, and VEGF while high doses of exosomes suppressed pro-inflammatory cytokines including TREM-1, I-TAC, IL-3, and IL-16 [43]. Exosomes derived from glioblastoma also promoted angiogenesis and mRNAs carried by glioblastoma exosomes can be translated in the recipient ECs [34]. Colorectal cancer cell-derived exosomes are shown to be enriched in cell cycle-related mRNAs and promoted the proliferation of ECs [44]. In another study, MVs produced by human cancer cells harboring activated EGFR (A431, A549, DLD-1) were shown to be taken up by cultured ECs and elicit EGFR-dependent responses in the recipient ECs including activation of MAPK and Akt pathways, initiation of VEGF expression and activation of key VEGF signalling receptor VEGF receptor-2 [45]. The MVs used in this study were obtained from the normal method of exosome isolation (ultracentrifugation at 100,000 × g with prior removal of large microvesicles by low speed centrifugation) and it is conceivable that these MVs contain exosomes [45]. In another study, cancer-derived exosomes containing the tetraspanin Tspan8 (also called D6.1A) were shown to efficiently induce angiogenesis in cancers by stimulating angiogenic factor transcription such as VEGF and VEGFR [46]. Tspan8 exosomes bind to ECs through Tspan8-CD49d complex and become internalized, leading to enhanced EC proliferation, migration, sprouting, and maturation of EC progenitors [47]. Under hypoxia, cancer cells were found to secrete many cytoplasmic and membrane proteins involved in promoting angiogenesis and metastasis through the release of exosomes that enhance the angiogenic and metastatic potential and modulate its cancer microenvironment [48]. The

transmembrane notch ligand delta like 4 (Dll4) has been demonstrated to be incorporated into cancer cell exosomes [49]. These exosomes can transfer the Dll4 protein to ECs and incorporate it into their cell membrane. This results in inhibition of Notch signaling and confers a tip cell phenotype to the recipient ECs and an increase in angiogenesis. Transfer of Dll4 was also shown in vivo from cancer cells to host endothelium through exosomes. All together, these results indicate that cancer cell derived exosomes play an important role in cancer growth and metastasis by facilitating angiogenesis.

Cancer-derived Exosomes Modulate Stromal Cell Function

It has been shown that some cancer-derived exosomes could trigger fibroblast differentiation into myofibroblasts and elicit a significant upregulation of FGF2 expression in the recipient cells [50]. These exosomes express TGF-β at their surface in association with the transmembrane proteoglycan betaglycan and can elicit SMAD-dependent signalling in the recipient fibroblasts. Enrichment of myofibroblasts in solid cancers represents an alteration of the stroma into one that typically supports cancer growth, vascularization and metastasis.

Cancer-derived Exosomes Modify the ECM

The exosomes isolated from malignant ascitic fluid of ovarian carcinoma patients were shown to contain gelatinolytic enzymes and the presence of these exosome-associated enzymes in the cancer vicinity might augment cancer invasion [51]. Cancer-derived exosomes also carry extracellular matrix remodelling enzymes such as MMP2, MMP9 and urokinase plasminogen activator (uPA), leading to increased cancer ECM degradation and cancer invasiveness [52–54].

The L1 adhesion molecule (CD171) is overexpressed in human ovarian and endometrial carcinomas and is associated with poor prognosis. Although expressed as a transmembrane molecule, L1 is released from carcinoma cells in soluble form. Soluble L1 is present in serum and ascites of ovarian carcinoma patients and is a potent inducer of cell migration. It may also stimulate cell proliferation by activating extracellular signal-regulated kinase (ERK). Cancer-derived exosomes are shown to be an important source of soluble L1 [55]. Exosomes derived from ovarian carcinoma cells contain transmembrane metalloprotease such as ADAM10 that cleaves the transmembrane L1 and facilitates the release of the cleaved cytoplasmic L1 fragments into the extracellular space. The cleavage occurs in both the endosomal compartment and released exosomes [56].

Cancer-derived Exosomes Promote Metastasis

Cancer-derived exosomes have been shown to facilitate the formation of premetastatic niche, the early changes in the local microenvironment of a distant organ in preparation for metastatic cancer cell embedding and growth. In a rat pancreatic adenocarcinoma model, cancer-derived exosomes were shown to function together with a soluble matrix to generate the premetastatic niche for pancreatic cancer metastasis [57]. Neither exosome nor the soluble matrix alone is sufficient for this premetastatic niche formation.

Exosomes Released by Cancer Stroma Cells

It is known that the cargo carried by exosomes is determined by the donor cells. Conceivably, stroma cells also release exosomes either constitutively or under induction by environmental stimulation that can impact cancer cells and/or other stromal cells [58]. The stromal cells in cancer include ECs, fibroblasts, leukocytes (macrophages, dentritic cells, T- and B-cells etc.), pericytes and smooth muscle cells, as well as mesenchymal stem cells (MSCs) and other blood precursor cells from the blood circulation.

ECs are known to release exosomes as well. The transmembrane notch ligand Dll4 has been demonstrated to be incorporated not only into cancer cell exosomes but also into endothelial exosomes [49]. These exosomes can transfer Dll4 protein to other endothelial cells and cancer cells, incorporate it into the recipient cell membrane, which results in inhibition of Notch signaling. This observation suggests that endothelial exosomes can transfer signal molecules to other ECs as well as cancer cells in the cancer microenvironment to influence cancer behavior.

It is well known that solid cancers are infiltrated by immune cells of both innate immunity and adaptive immunity [59–61]. Exosome production by various types of immune cells has been well established [11, 62]. It is conceivable that exosomes produced by cancer infiltrated immune cells could mediate cell-cell communication between immune cells and cancer cells as well as other stromal cells to modulate cancer form and function.

Up to now, there has been no report of exosome release by pericytes and smooth muscle cells. These are cells associated with blood vessels in cancer. Therefore, the contribution of these two cell types to cancer microenvironment through exosomes is unknown at the moment.

Exosomes from the Circulation and their Influence on Cancer Microenvironment

Exosomes have been found in various biological fluids such as blood, urine, sperm, milk and others [11]. Exosomes from platelets have been shown to induce lung cancer metastasis

and angiogenesis as well as promote breast cancer metastasis [63, 64]. Exosome release by bone marrow derived mesenchymal stem cells (MSCs) and other precursor cells of the blood lineages, e.g. endothelial precursor cells (EPCs) have been reported. MVs derived from EPCs are able to promote EC survival, proliferation and capillary morphogenesis in vitro and stimulate angiogenesis in vivo. These proangiogenic effects were shown to be mediated by mRNA transfer from EPCs to ECs involving the PI3K/AKT signaling pathway [65]. Judging from the isolation method used, the MVs in this study are most likely exosomes. In addition, cancer is known to be in a prothrombotic state, with an increased prevalence of venous thromboembolism. Plasma tissue factor positive microparticles (MPs) have been postulated to contribute to venous thromboembolism since its level is significantly higher in cancer patients with thromboembolism [66]. Whether circulating exosomes also contribute to the prothrombotic state of cancer is unclear at the moment. Furthermore, as all the data on exosomes obtained so far are based on exosomes purified and concentrated in vitro, it is therefore unclear whether physiological levels of circulating exosomes perform similar functions.

Exosomes as a Cancer Defense Mechanism

Exosomes are able to influence both the innate immune response and the adaptive immune system. Although exosomes have been shown to suppress inflammation [67, 68], most work on cancer exosomes have focused on their ability to manipulate lymphocytes. Cancer exosomes are able to influence the proliferation of cytotoxic effector cells and regulatory T (Treg) cells. In addition, they are also able to influence the activation of naïve T-cells. We will present a brief summary here. For more elaborate coverage in this area, refer to other recent reviews in this area [11, 69–71].

Cancer exosomes are involved in cancer immune evasion by suppressing the host immune system. Immune suppression can be accomplished by several means; mechanisms involved in eliciting an immune response could be hindered; or those involved in immune suppression bolstered. Cancer exosomes have been shown to be able to impair the function of Natural Killer (NK) and Cytotoxic CD8+ T-cells by reducing the expression of the receptor NKG2D, which is required for their activation, via exosomal TGFβ1 [72]. In addition, cancer exosomes impaired CD4+ T cell mediated NK and CD8+ T cell proliferation in response to IL-2 [73]. Cancer exosomes also induce apoptosis in CD8+ T-cells whilst inducing the proliferation and generation of the immunosuppressive Treg cells [74]. Cancer exosomes have also been shown to mediate the conversion of CD4+ T cells into Treg cells, promote Treg suppressor function and increase their resistance to apoptosis [75]. Another function of cancer exosomes is to influence the functions of myeloid-derived suppressor cells (MDSCs) which are known to negatively regulate immune responses during cancer and other diseases [76]. Cancer exosomes induce the accumulation of these immune-suppressing MDSCs. Upon exosome uptake, MDSCs increase the production of inflammatory cytokines and promote tumor growth [77]. Hence cancer exosomes are able to interfere with the immune system and aid in cancer immune evasion.

On the other hand, cancer exosomes carry a subset of antigens from their host cells. Hence it is possible that antigen presenting cells (APCs) are able to process exosomes and present the tumor antigens to CD4+ T cells, eventually triggering an anti-tumor immune response. It has been demonstrated that dendritic cells (DCs), when pulsed with cancer exosomes, promote the activation of CD8+ T-cell-dependant anti-tumor response [20]. In contrast, cancer exosomes inhibited DC maturation and conferred immunosuppression specific to antigens that are present on the exosomes [78]. Furthermore, exosomes that bear tumor antigens are shown to sequester tumor reactive antibodies, thus reducing the susceptibility of tumor to antibody dependant cellular cytotoxicity [79].

It seems that cancer exosomes can work both for and against tumor survival by influencing different cells in the immune system. Further studies are needed to elucidate the physiological relevance and the extent that cancer exosomes contribute to the various immune responses to clarify the significance of exosomes in cancer immune evasion.

Exosomes as Diagnostic and Prognostic Markers for Cancer

The importance of finding diagnostic markers for cancer patients is not a new topic. Over the past few years, many approaches have been reported for replacing the invasive approach of diagnosing cancer by tissue biopsies with much simpler and non-invasive approaches. The existence of circulatory proteins and nucleic acids in bodily fluids of cancer patients in form of exosomes has presented an opportunity to develop novel diagnostic markers through non-invasive means. One advantage of using exosomes as biomarkers are the enrichment of markers within exosomes which would otherwise constitute only a small fraction of the total secreted proteins. The presence of highly enriched protein in body fluids normally diminish the minor presence of relevant biomarkers that could provide vital information into disease or pathology [80].

It has been shown that the number of exosomes in body fluids is elevated in cancer patients. The presence of tumor-derived exosomes was initially reported as small membrane fragments of endocytic origin in the peripheral circulation of

women with ovarian cancer [81]. Subsequent study by the same group using exosomes purified through immunoaffinity purification against epithelial cell adhesion molecule EpCAM showed that ovarian cancer derived exosomes increase with progressive stages of ovarian cancer development [82]. In this study, the identity of the purified exosomes was validated by electron microscopy and Western blot with exosome markers. In patients with lung adenocarcinoma, circulating exosomes were significantly higher than in control group [83]. Furthermore, in glioblastoma, the tumour-specific EGFRvIII splice variant was detected in serum exosomes from some glioblastoma patients by nested RT-PCR but not from exosomes derived from normal control serum, indicating the potential of exosomes as cancer diagnostic markers [34].

Proteomic studies on urinary exosomes have generated a long list of molecular markers that offers a potential for diagnostic and prognostic discovery. Exosomes derived from cultured bladder cancer cells contain more than 350 proteins, some of them were found in urinary exosomes of a bladder cancer patient [84]. Moreover, analysis of urinary exosomes from prostate cancer patients has revealed two known tumor biomarkers, PCA-3 and TMPRSS2:ERG, indicating the potential of using exosomes for prostate cancer diagnostics [85].

More extensive identifications of cancer biomarkers are required in order to offer more reliable diagnosis for cancer. However, despite all the work and effort that has been done so far, this area of research remains to be challenging. The small size of exosomes is still a barrier in identifying the mechanism of their interactions with other cells and delivery in a dynamic fashion. The low level of exosomes from biological fluid is also a major limitation for exosome proteins to be reliable cancer markers in all patients. The discovery of mRNA and miRNA in exosomes offer the opportunity to develop the sensitive PCR-based diagnostic and prognostic methods for cancer clinical applications.

Conclusion and Future Perspectives

Increasing number of studies has indicated that exosomes play important roles in shaping the form and function of a cancer. By serving as intercellular messengers, exosomes transmit signaling pathways and even genetic materials not only between cancer cells and stromal cells within the primary cancer microenvironment, but also between cancer and distant host organs. The main functions of exosomes in cancer microenvironment are: (1) promote primary cancer growth; (2) stimulate angiogenesis; (3) activate stromal fibroblasts; (4) sculpture cancer ECM; (5) generate premetastatic niche; (6) suppress host immune responses (Fig. 2). The linkage of cancer-derived exosomes in blood circulation specifically with the presence and extent of cancer development is very promising for developing exosomes into reliable clinical diagnostic and prognostic markers for cancers.
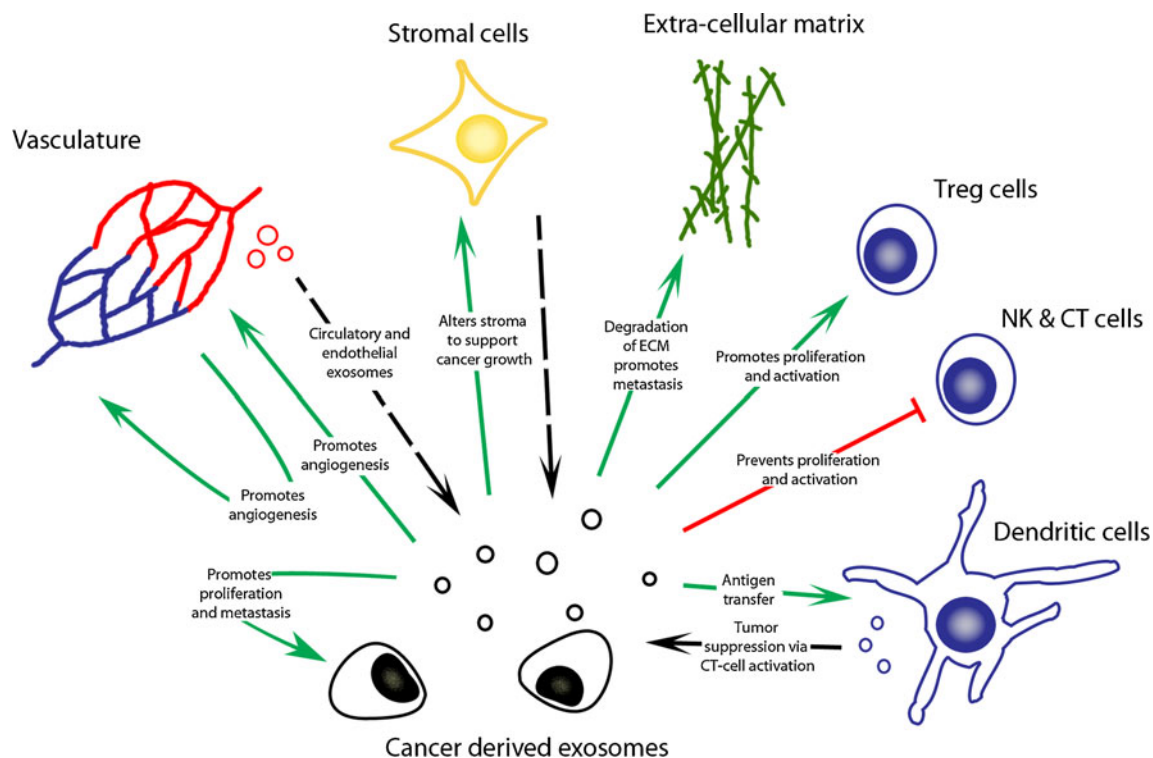

Fig. 2 A schematic diagram showing the various functions of exosomes in cancer microenvironment

Further development and standardization of the technologies and methods for exosomes isolation, analysis, specific inhibition, removal from blood circulation as well as in depth understanding of the molecular regulations of exosomes biogenesis and signal transduction are essential for exosomes to serve as important tools in advancing cancer biology and medicine.

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