International Journal of Nanomedicine

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REVIEW

Cancer-Derived Exosomes: Their Role in Cancer Biology and Biomarker Development

This article was published in the following Dove Press journal:
International Journal of Nanomedicine

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Abstract: Exosomes are a subset of tiny extracellular vesicles manufactured by all cells and are present in all body fluids. They are produced actively in tumor cells, which are released and utilized to facilitate tumor growth. Their characteristics enable them to assist major cancer hallmarks, leveraged by cancer cells in fostering cancer growth and spread while implementing ways to escape elimination from the host environment. This review updates on the latest progress on the roles of cancer-derived exosomes, of 30–100 nm in size, in deregulating paracrine trafficking in the tumor microenvironment and circulation. Thus, exosomes are being exploited in diagnostic biomarker development, with its potential in clinical applications as therapeutic targets utilized in exosome-based nanoparticle drug delivery strategies for cancer therapy. Ongoing studies were retrieved from PubMed® and Scopus database and ClinicalTrials.gov registry for review, highlighting how cancer cells from entirely different cell lines rely on genetic information carried by their exosomes for homotypic and heterotypic intercellular communications in the microenvironment to favor proliferation and invasion, while establishing a pre-metastatic niche in welcoming cancer cells' arrival. We will elaborate on the trafficking of tumor-derived exosomes in fostering cancer proliferation, invasion, and metastasis in hematopoietic (leukemia and myeloma), epithelial (breast cancer), and mesenchymal (soft tissue sarcoma and osteosarcoma) cancers. Cancer-derived exosomal trafficking is observed in several types of liquid or solid tumors, confirming their role as cancer hallmark enabler. Their enriched genetic signals arising from their characteristic DNA, RNA, microRNA, and lncRNA, along with specific gene expression profiles, protein, or lipid composition carried by the exosomal cargo shed into blood, saliva, urine, ascites, and cervicovaginal lavage, are being studied as a diagnostic, prognostic, or predictive cancer biomarker. We reveal the latest research efforts in exploiting the use of nanoparticles to improve the overall cancer diagnostic capability in the clinic.

Keywords: tumor-released exosomes, carcinoma-associated fibroblasts, exosome cargo, exosome-induced chemoresistance, hallmarks of cancer, tumor-stromal communications

Introduction

There exist six well-researched cancer hallmarks, and they are as follows: (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) enabling replicative immortality, (4) activating invasion and metastasis, (5) inducing angiogenesis, and (6) resisting cell death. However, two emerging hallmarks were identified and added by Hanahan and Weinberg:[1,2] (7) deregulating cellular metabolism and (8) avoiding immune destruction. Cancer cells typically acquire these core capabilities through sustaining selective pressures and adopting alterations in specific and ubiquitous cellular function, defined as "enabling characteristics," to permit cancer hallmark

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DovePress
http://doi.org/10.2147/IJN.S272378

capabilities. Enabling characteristics, which foster cancer growth and spread, are typically adopted by cancer cells to survive, proliferate, and escape elimination by the host environment. In the next-generation review by Hanahan and Weinberg on cancer hallmarks' molecular mechanisms, they proposed two enabling characteristics that are present in cancer cells across the board, which are genome instability/mutation and tumor-promoting inflammation.[1]

In this review, we propose pathological trafficking of genetic materials carried by cancer-derived exosomes (CDE) cargo as a new enabling characteristic that fits the proposed criteria (Table 1). This CDE cargo phenomenon has been demonstrated to be present in virtually all kinds of cancer cell types, fostering the core hallmarks of cancer by effective homotypic or heterotypic intercellular (tumor-to-microenvironment) communications, which facilitate cancer invasion and metastasis as well as evasion from immune destruction. The second part of the review is dedicated to the updated status of CDE being evaluated as a valid diagnostic cancer biomarker in human studies.

This study aims to present a comprehensive overview of the roles of CDE in cancer progression and development, assimilated from the current literature and translational cancer research to stimulate cancer biologists, scientists, and oncologists who are interested in the involvement of CDE in cancer pathogenesis, cancer microenvironment, molecular mechanisms on cancer progression and who plan to apply the knowledge in developing more effective diagnostic strategies for various types of cancer. Ongoing research studies evaluating CDE as a cancer biomarker registered in the ClinicalTrials.gov were selected to enrich the discussion on the clinical application of CDE as a biological cancer marker and to indicate future opportunities for cross-disciplinary collaborations.

Table 1 Criteria for Classification as a Hallmark of Cancer or an Enabling Characteristic, According to Hanahan and Weinberg[1,2]

Hallmark of Cancer
• Essential alterations in cell physiology • An acquired functional capability that allows cancer cells to survive, proliferate, and spread • These alterations are acquired via distinct mechanisms and at various time points during multistep carcinogenesis • The characteristic should be present in most, if not all, tumor types
Enabling characteristic
• Fosters the acquisition of one or some hallmarks of cancer • Expedites the acquisition of the hallmarks of cancer • Can foster multiple hallmark functions • Should be present in most, if not all, tumor types

Literature Search

To ensure a comprehensive and unbiased literature review, we performed both electronic and manual literature search in the PubMed® and Scopus database to retrieve relevant original articles. We leveraged the use of PubMed Advanced Search Builder, Medical Subject Headings (MeSH), and Boolean logic to add terms or combine search terms using connector words, such as AND, OR, or NOT, as well as truncate terms. We used a controlled vocabulary to produce highly relevant search results. The search terms included exosome, exosomal cargo, cancer-derived or tumor-derived, cancer biology, proteasome, and cancer biology. Subsequently, we surveyed the ClinicalTrials.gov registry for clinical studies conducted in the United States and around the world.

Cancer-Derived Exosomes in Cancer Biology

The tumor microenvironment (TME) surrounding cancer cells is identified to be comprised of cancer-associated fibroblasts, blood vessels, nerve fibers, immune cells, other stromal cells, and extracellular vesicles containing various kinds of genetic signals. Considering all are functional in anticancerous immunosuppressive cells, the TME is known to create a milieu that prevents the free spread of the malignant cells.[3] The cancer cells, however, communicate with the neighboring stromal and immune cells, promoting immune evasion, and could also activate angiogenesis, tumor innervation, and epithelial-mesenchymal transition (EMT), in order to facilitate neoplastic growth. Recently, a phenomenon involving tumor-infiltrating innervation in the TME has also been proposed as a prerequisite for cancer cells of many types such as in prostate, gastric, pancreatic, and rectal cancers.[4–6] Tumors are capable of recruiting nerves via the release of neurotrophic factors and axonal guidance molecules, and, with the contribution of CDEs, induction of axonogenesis is initiated, whereby the communication between the tumor and potentially innervating nerves work in concert to promote tumor innervation.[4] Thus, it has been proposed that tumor innervation with neurite outgrowth (axonogenesis), just like angiogenesis, might be considered a new emerging hallmark of cancer.[7–13] Although research has demonstrated that angiogenesis is frequently associated with axonogenesis, more studies are eagerly required to elucidate the roles of

tumor-infiltrating innervation in tumor initiation, growth, and spread for this phenomenon to be widely accepted as a hallmark of cancer. Recent studies were able to confirm that the neurite recruitment/outgrowth and tumor innervation were promoted by the release of exosomes in the head and neck squamous cell carcinomas model[5] and human papillomavirus-positive uterine cervical cancer cell lines.[6]

The name "tumor-derived exosomes" was coined in 1981, and this phenomenon has received extensive research attention over the past decade.[14] Exosomes are extracellular, membranous, cup-shaped microvesicles 30 to 100 nm in size, which are produced by most types of cells.[15–17] They originate from intracellular multivesicular bodies and are released by exocytosis into the extracellular microenvironment.[18] Exosome typically consists of a variety of genetic messengers such as DNA, mRNA, microRNA, cytosolic proteins, and lipids.[19–23] Exosomal markers such as tetraspanin proteins CD63, CD9, and CD81 allow sorting, selective recruitment, capturing, or profiling of CDEs.[24,25] Once the recipient cells internalize tumor-derived exosomes, the ensuing biological response is determined explicitly by the dedicated trafficking routes, the exosomal internalization pathway, and the complex surface molecules on the membrane of both the extracellular vesicle and the recipient cell. With the advent of theranostic nanotechnologies such as differential ultracentrifugation, nanofluidic technology, and the exosome total isolation chip (ExoTIC), a size-based extracellular vesicle isolation apparatus, researchers nowadays are now able to capture nano-sized CDE for further analyses.[26–28] The latest biosensing technologies, such as afterglow sensors with aptamer-based signal amplification, improve the limit of detection (LOD) that is nearly two orders of magnitude lower than that of fluorescence methods.[29] With the advent of these sensitive biosensors, the LOD can practically be improved to 10^2 exosomes per milliliter.

These exosomes, particularly those that are tumor-derived, act as signal transducers or messengers in the cell-cell communication.[5,30–33] The recipient cells respond to the exosomal contents (such as microRNA) by changing their phenotypes. microRNAs are considered an evolutionarily conserved family of molecules that bind to complementary sequences in the 3′-untranslated region (3′UTR) of their target mRNAs, post-transcriptionally repressing gene expression.[34] It has been demonstrated that in high-grade bladder cancer cell line, TCC-SUP, for example, exosomes promoted angiogenesis and migration of both cancer and endothelial cells.[35] In another study in prostate cancer, the malicious CDEs induced differentiation of the stromal mesenchymal stem cells toward alpha-smooth muscle actin-positive myofibroblasts, which secreted high levels of proangiogenic VEGF-A, pro-invasive HGF, MMP-1, MMP−3, and MMP−13.[36]

The role of CDEs as characteristic enablers of cancer hallmarks to facilitate organ-specific metastasis has been demonstrated by the proof-of-principle study conducted by Hoshino et al,[37] In their peripheral blood study of mouse and human cell lines, they claimed that during the metastatic cascade, organ-specific metastasis took place not by a random process but by somewhat predictable and trackable events. This happened through distinct integrin expression patterns contained in the CDEs, a phenomenon that now elucidates the mechanism of specific cancer organotropism adequately. The exosomal integrin αvβ5 was associated with hepatic metastasis, while exosomal integrins α6β4 and α6β1 were linked to lung metastasis.[37]

In the following sections, we will use three different cancer types: hematopoietic, epithelial, and sarcomatous malignancies (leukemia/myeloma, breast cancer, soft tissue sarcoma, and osteosarcoma) to prove, using compelling evidence, that cancer cells across the board leverage the pathological trafficking of exosomes to promote neoplastic growth, facilitate cancer spread through tumor-stromal interaction, and evade destruction by the host (Figure 1).

Exosomal Trafficking in Leukemia Pathogenesis

Although leukemia can reach every part of the host body through the ever-reaching blood vessels, recent research has reported that leukemic cells also employ paracrine exosome trafficking to achieve leukemogenesis, maintain leukemic persistence by shaping the leukemic niche and its progression, suppress hematopoiesis, modify anti-leukemic immunity, and evade destruction by chemotherapy. Table 2 illustrates these aspects taking acute myeloid leukemia (AML) as an example.

Patients with chronic lymphocytic leukemia (CLL) have been identified to have decreased T-cell immunity. A recent study showed that CLL induced myeloid-derived suppressor cells (MDSCs), which, in turn, suppressed T-cell activation and induced suppressive regulatory T cells (Treg) through exosomal miR-155 transfer.[38,39] This exosome-mediated transfer of microRNAs to monocytes could significantly contribute to CLL-related

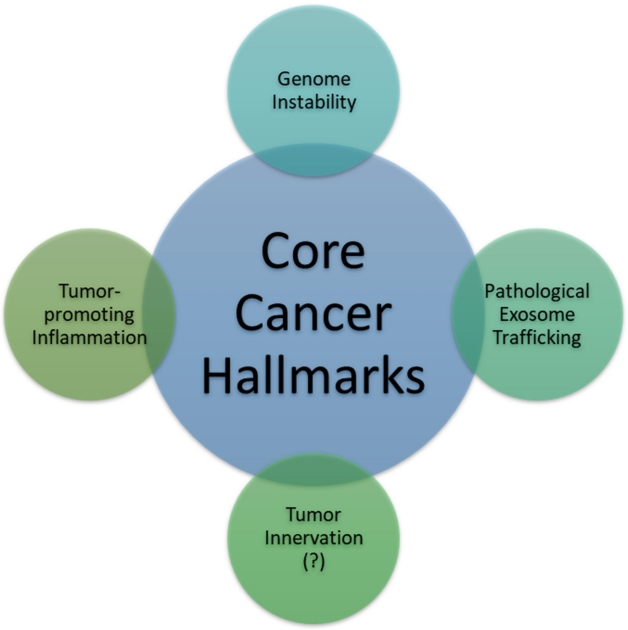

Figure 1 Two established enabling characteristics (genome instability or mutation and tumor-promoting inflammation): one investigating feature (tumor innervation) and one hitherto proposed enabling characteristic, that is, pathological exosome trafficking. Enabling characteristics are defined as the capabilities possessed by most cancer types to foster and/or expedite the acquisition of one or some core hallmarks of cancer.

immune escape via PD-L1 expression. Colleagues from the University of Liverpool verified that CLL-derived exosomes encapsulate small RNAs, and the encapsulated microRNA miR-202-3p enhanced the expression of a Hedgehog signaling intermediate.[40] An enriching body of evidence shows that the TME created by the bone marrow significantly favors the survival, growth, and proliferation of leukemic cells. For example, CLL leukemic cells can establish their favorable leukemic niche in the TME. Paggetti et al have discovered that CLL-derived exosomes could affect bone marrow stromal cells in adopting a cancer-associated fibroblast phenotype, which would contribute to a tumor-supportive microenvironment.[41] In the chronic myeloid leukemia (CML) model, CML-derived exosomal paracrine trafficking stimulated bone marrow stromal cells to produce interleukin (IL)-8.[42,43] Further, another study demonstrated that exosomes released from CML cells affect the endothelium directly to modify the neovascularization process.[44]

Exosomal Trafficking in Multiple Myeloma (MM) Pathogenesis

In a murine MM model, the myeloma exosomes were identified to have a proangiogenic function to enhance the viability of bone marrow endothelial cells; besides, an in vivo experiment demonstrated that these exosomes increased the presence of bone marrow MDSCs and changed their subsets to a more tumorigenic profile.[55] MM-derived exosomes could modify the bone marrow microenvironment to facilitate myeloma progression. Conversely, the bone marrow stromal cells could, reciprocally, also release certain exosomes to be taken up by MDSCs through the STAT3 and STAT1 pathways, which leads to increased immunosuppression, thereby inducing MM expansion.[56] Initially, investigators from the Dana-Farber Cancer Institute demonstrated that there were significant differences in microRNA profiling between normal and bone marrow mesenchymal stromal cell-derived exosomes in MM.[57] A recent study in patients with MM using small RNA sequencing of circulating exosomes from ten patient samples confirms that microRNAs are the most predominant small RNAs present in MM exosomes.[58] Meanwhile, investigators from the Karolinska Institute have examined the human bone marrow stromal cell

Table 2 Paracrine Exosome Trafficking Employed by Acute Myeloid Leukemia (AML) as an Example and Its Specific Functional Outcomes

Leukemogenesis	Immunosuppression	Suppression of Hematopoiesis	Chemotherapy Resistance	Leukemic
Persistence/Progression AML-derived bone marrow mesenchymal stromal cells release exosomes that can affect gene regulatory networks.[45]	AML-derived exosomes carry immunosuppressive molecules responsible for immune cell deregulation.[46,47]	Blast-derived exosomes remodel the bone marrow niche into a leukemia growth-permissive microenvironment.[48,49]	Blast-derived exosomes propel bone marrow stromal cells to generate IL-8, which regulates chemo- cytotoxicity.[50] AML cells secrete VEGF/VEGFR-containing exosomes that induce glycolysis in endothelial cells, leading to vascular remodeling and chemoresistance.[51]	May have a broader role in shaping the leukemic niche.[52–54]

line L88 and verified that caspase-3 is activated by the stroma cell–released exosomes, which can cleave the anti-apoptotic protein Bcl-xL, localized on the outer exosomal membrane. Through the cleavage of Bcl-xL, these exosomes could then be internalized using plasma cell myeloma, which led to their increased proliferation.[59]

Finally, in another study performed at Tokyo Medical University, researchers established a hypoxia-resistant MM cell model to mimic the in vivo hypoxic microenvironment induced by the rapid proliferation of MM in the bone marrow. Their experiment showed that under normoxic or acute hypoxic settings, the hypoxia-resistant MM cells produced more exosomes than the parental cells, and the major functional protein in the exosomal cargo was identified to be miR-135b. This protein directly suppressed factor-inhibiting hypoxia-inducible factor 1 (FIH-1) in bone marrow endothelial cells.[60] Hence, further studies are needed to test if miR-135b could be used as a target for therapeutically avert angiogenesis in MM.

Exosomal Trafficking in Breast Cancer Pathogenesis

A study using plasma exosomal microRNAs as a diagnostic biomarker in breast cancer patients demonstrated that these molecules have outstanding power to distinguish breast cancer patients from normal counterparts. Zhai et al used a nucleic acid-functionalized Au nanoflare probe, which are known to have the ability to directly enter plasma exosomes and generate quantitative fluorescent signals for successful in situ detection of exosome-located microRNA-1246. At its best cutoff point, the in situ detection of the exosomal miRNA-1246 in the peripheral blood was able to distinguish 46 breast cancer patients from 28 healthy controls with 100% sensitivity and 93% specificity.[61] Another clinical study on the exosomal microRNA signatures of 20 healthy women and 435 breast cancer patients discovered that 10 miRNAs in the entire breast cancer patient cohort, 13 in the HER2-positive subgroup (211 patients), and 17 in the triple-negative subgroup (224 patients) were significantly deregulated in comparison to those in healthy women, indicating different underlying aspects of cancer biology in different breast cancer types.[62] These different exosomal microRNA signatures are associated with the clinicopathological features of each subgroup. In addition, exosomes that are released by breast cancer cells could modify TME through direct suppression of T-cell proliferation and inhibition of NK cell cytotoxicity, thus dampening the anticancer immune response in pre-metastatic organs.[63]

Another hallmark of cancer is the transfer of chemoresistant or hormone-resistant propensity from breast cancer stem cells to the daughter cells, explored in the study of Santos et al, who demonstrated that miR-155 was upregulated in breast cancer stem cells and chemoresistant cells and was involved in the EMT. An enrichment in miR-155 was noted in exosomes isolated from stem-like breast cancer stem and chemoresistant cells. Moreover, the experiments demonstrated the capability of the horizontal transfer of miR-155 from the chemoresistant cells' exosomal cargo to the recipient sensitive cells.[64] This study supports the presence of exosome-mediated chemoresistance and EMT in refractory cancer. Estrogen receptor (ER)-positive cancers are found to transition from an endocrine sensitive/dormant state to a resistant one, acquiring host mitochondrial DNA, which promoted oxidative phosphorylation (OXPHOS) and signaled the transition from metabolic quiescence toward hormonal therapy resistance.[65] Further, functional studies have identified cancer-associated fibroblast-derived extracellular vesicles containing whole genomic mitochondrial DNA in patients and xenograft models.

In a breast cancer cell line, recipient cells treated with exosomes from stemness-related breast cancer CXCR4-positive cells showed an increase in the same oncogenic abilities.[66] This experiment has also demonstrated that inoculating exosomes derived from CXCR4-positive cells into immunocompromised mice can stimulate primary tumor proliferation and metastatic potential. The same investigators also discovered a "stemness and metastatic" signature in the exosomes of patients with worse prognoses after comparing exosomal nucleic acid contents.[66]

Exosomal Trafficking in the Pathogenesis of Soft Tissue Sarcoma

In 2013, for the first time, a study has showed that exosome-mediated pathogenesis, similar to epithelial carcinoma and hematopoietic malignancy, was also present in Ewing sarcoma.[67] Microarray analysis of exosomes shed by the Ewing sarcoma cell line revealed that their exosomal content shared a transcriptional signature potentially involved in the modification of the surrounding microenvironment via G-protein-coupled signaling, neurotransmitter signaling, and stemness.[67]

A recently published study used both patient plasma samples and cell lines to demonstrate that liposarcoma cells secreted miR-25-3p and miR-92a-3p in exosomes. Subsequently, it stimulated the secretion of the proinflammatory cytokine, interleukin (IL)-6, in tumor-associated macrophages through a TLR7/8-dependent mechanism, which can ultimately cause liposarcoma progression.[68] In another study using patient-derived Ewing sarcoma cells, miR-34a, an inhibitor of Notch-NFκB signaling, was enriched and secreted through exosomes shed by CD99-silenced (by small interfering RNA) cells.[69] CD99 has been identified as a cell surface molecule involved in cell differentiation, migration, and death. In Ewing sarcoma cells, it is pro-oncogenic due to its effect on the prevention of NFκB-mediated neural differentiation and is continuously present at high levels. The horizontal transfer of miR-34a through exosomes to recipient cells enhanced neural differentiation in Ewing sarcoma.[69]

Moreover, another study demonstrated that the membrane-type 1 matrix metalloproteinase (MT1-MMP, MMP14) was released by exosomes of cultured human fibrosarcoma (HT-1080) cells.[70] MT1-MMP is a crucial metalloproteinase that facilitates tumor invasion by remodeling the extracellular matrix. Pathological sarcomatous exosomal trafficking carrying MT1-MMP could be detrimental to the host by providing a favorable microenvironment for sarcoma.

Exosomal Trafficking in Osteosarcoma Pathogenesis

Osteosarcomas are known as malignant mesenchymal-derived bone tumors and the most common bone cancers in children and adolescents. Emerging evidence has also shed light on the exosomal trafficking employed by osteosarcoma cells to shape its supporting TME and facilitate growth, as well as hematogenous spread. Among the specific exosomal contents, miR-148a and miR-21-5p are known to help shape the TME.[71] The microRNA, miR-21, is a common oncological molecule taking part in the pathogenesis of various types of malignancies.[71–80] Take esophageal cancer as an example; it has been demonstrated in a human esophageal carcinomas cell line co-cultivation experiment that miR-21 in the CDE shuttled from donor cells significantly promoted the migration and invasion capability of recipient cells by activating c-Jun N-terminal kinase signaling pathway.[74] A recent multi-omics study observed that the progression from localized to metastatic osteosarcoma was accompanied by an elevation of the levels of urokinase plasminogen activator (uPA) and uPA receptor in the metastatic cells' exosomal cargo.[81] The impact of abundant miR-25-3p in the liposarcoma-derived exosomes on the surrounding microenvironment was similar to what was observed in osteosarcoma cases.[82] Jerez et al conducted a gene ontology analysis of predicted targets for the miRNAs present in osteosarcoma-derived extracellular vesicles. Their bioinformatics analysis indicated that miRNAs derived from osteosarcoma cell lines might regulate metastatic potential by inhibiting a network of genes involved in apoptosis and cell adhesion.[83] Further research is needed to provide more evidence on the details and importance of exosomal trafficking in osteosarcoma pathogenesis and to determine it as a core hallmark of cancer.

Other Molecules (Proteins, Enzymes, Receptors, Ligands, or Signaling Molecules) That Can Exert Neoplastic Functional Activities Carried by Exosomes

Certainly, exosomal cargoes are not limited by miRNAs only but also by a lot of other candidate molecules such as proteins, lipids, enzymes, signaling molecules, which can exert their functional activities far from the exosome-producing cells.[84] Table 3 demonstrates that CDE cargoes could contain basically any purpose-built loaded nano-molecules for its ultimately release from the parental cells. It is evident that horizontal or paracrine transfer of these molecules, when received by the specific recipient cells in the TME or any distant metastatic niches, could facilitate the progression, invasion, and metastatic spread of cancer cells. Some of these exosomal molecules have the potential to serve as valid biomarkers, and, thus, there should be worthwhile testing for cancer detection and/or diagnosis.

In summary, it is evident that cancer cells from entirely different lineages, such as those from leukemia to osteosarcoma, rely on their exosomes to carry the genetic information for homotypic and heterotypic intercellular communications in the TME (Figure 2). This communication creates a favorable environment for cell proliferation and invasion and further establishes a pre-metastatic niche that is readily welcoming for the arrival of cancer cells when they carry the correct form of exosomal integrins. Therefore, these CDEs and their pathological trafficking

Table 3 Representative Exosomal Cargoes Other Than miRNAs (Such as Proteins, Lipids, Signaling Molecules, DNA, Mitochondrial DNAs, circRNAs, lncRNAs, Integrin, and Enzyme), Which are Involved in Cancer Progression or in the Interplay in Anticancer Immunity or Served as a Biomarker in Various Cancer Types

Exosomal Cargo	Cancer Type	Functions (Mechanism)/ Usage	Measurement	Reference (First Author/ Year Published)
Proteins associated with cell adhesion, extracellular matrix, and some signaling molecules (EGFR, GRB2, and SRC)	KRAS-activated or EGFR-activated NSCLC/two cell lines	Abundance differences in exosomal protein cargo detected between two NSCLC cell lines and non-cancer cell lines	Triple SILAC quantitative proteomic strategy	Clark, D.J./ 2016[85]
Exosome proteome	Four epithelial ovarian cancer cell lines	Signaling biology and biomarker discovery	Mass spectrometry-based proteomics	Sinha, A./ 2014[86]
Proteins	Several mouse breast tumor lines with a different metastatic propensity	Protein cargo varies significantly between nonmetastatic and metastatic cell-derived exosomes	Comparative proteomic analysis	Gangoda, L./2017[87]
EGF-like repeats and discoidin I-like domain-3 (EDIL-3) protein	Human bladder cancer cell lines and urine of patients with high-grade bladder cancer	Facilitates bladder cancer progression; potential for therapeutic target	Mass spectrometry analysis	Beckham, C. J./2014[35]
Epithelial cell adhesion molecule (EpCAM) glycoprotein	Pancreatic ductal carcinoma (patients)	Liquid biopsy for EpCAM quantification as prognostication	High sensitivity enzyme linked immunoassay (ELISA)	Giampieri, R./2019[88]
Myoferlin	Breast and pancreatic cancer cell lines	Promotes cancer cell migration and invasion	Proteomic analysis	Blomme, A./ 2016[89]
Exosomal lipid profiles	Lung cancer/patients' plasma	Lipid profiles successfully distinguish early-stage lung cancer from healthy subjects	Ultrahigh-resolution Fourier transform mass spectrometry (UHR-FTMS)	Fan, T.W.M./ 2018[90]
A total of 162 lipids such as diacylglycerol, triacylglycerol, and phosphatidylglycerol	Urinary exosomes from prostate cancer patients	Potentially be used as a prostate cancer biomarker	Flow field-flow fractionation and nanoflow liquid chromatography-tandem mass spectrometry	Yang, J.S./ 2017[91]
27-Hydroxycholesterol	ER+ breast cancer cell line (MCF-7)	Possibility of diagnostic value	Capillary liquid chromatography-mass spectrometry	Roberg-Larsen, H./ 2017[92]
Lipid composition of urinary exosomes (phosphatidylserine 18:1/18:1, phosphatidylserine 18:0–18:2, and lactosylceramide d18:1/16:0)	Prostate cancer patients	Prostate cancer urinary biomarkers	High-throughput mass spectrometry quantitative lipidomic analysis	Skotland, T./ 2017[93]
Esophageal cancer related gene-4 (ECRG4) mRNA (tumor suppressor)	Oral squamous cell carcinoma patients	Suppresses cell proliferation and inhibits cancerous growth	Ultracentrifugation method	Mao, L./ 2018[94]

(*Continued*)

Table 3 (Continued).

Exosomal Cargo	Cancer Type	Functions (Mechanism)/ Usage	Measurement	Reference (First Author/ Year Published)
The signaling molecule, Wnt5b	Lung adenocarcinoma cells (A549)	Promotes cancer cell migration and proliferation	MALDI mass spectrometry and electrospray ionization mass spectrometry	Harada, T./ 2017[95]
Double-stranded DNA	Chronic myeloid leukemia (K-562), colorectal carcinoma (HCT116), and murine melanoma (B16-F10) cell lines	Novel potential biomarker for cancer detection as a surrogate for tumor tissues	dsDNA-specific shrimp DNase and atomic force microscopy (AFM)	Thakur, B. K./2014[21]
Tumor cell-derived DNA	Murine breast cancer cell line E0771 post topotecan treated	Activate dendritic cells via STING signaling	Purified DNA was stained with SYBR Gold following agarose gel electrophoresis and visualized with a UV transilluminator	Kitai, Y./ 2017[96]
Mitochondrial DNA	ER+ breast cancer xenograft from metastatic hormonal therapy-resistant patient	Promotes exit from dormancy of therapy-induced cancer stem-like cells	Whole-mtDNA amplification and sequencing assays	Sansone, P./ 2017[65]
Circular RNAs (circRNAs)	Liver cancer cells (MHCC-LM3)	circRNAs able to bind to miRNA; exosome-based cancer biomarkers	RNA-seq analyses	Li, Y./2015[22]
Long noncoding (lnc) RNA ZFAS1	Gastric cancer cell lines	Enhances gastric cancer cell proliferation and migration	Transmission electron microscopy, Nanoparticle Tracking Analysis (NTA), and Western blot.	Pan, L./ 2017[97]
Alphavbeta3 integrin	Prostate metastatic PC3 and CWR22Pc cancer cells	Promotes a migratory and metastatic phenotype	Nanoparticle Tracking Analysis; BCA followed by immunoblotting	Singh, A./ 2016[98]
GSTP1 in exosomes from patient's serum	Anthracycline/taxane-based neoadjuvant chemotherapy-treated breast cancer	Confers drug resistance	Confocal microscopy images; Western blot analyses	Yang, S. J./ 2017[99]

capabilities should be considered as an emerging enabling characteristic for the well-established hallmarks of cancer.

Cancer-Derived Exosomes in Biomarker Development

The first half of the review has exemplified the role of exosome in specific cancer types. Shifting gear to discuss the potential clinical application, this review will examine and discuss how we can develop cancer biomarkers based on characteristics such as exosomal cargo contents, detection methods, and localization of these exosomes in peripheral blood, pleural effusions, ascites, or urine. Further clinical studies are eagerly awaited to establish and validate the usefulness of specific CDE biomarkers in the different clinical setting during the cancer management. For example, in breast cancer, we can leverage the characteristics of exosomal microRNA signatures and exosomal nucleic acid contents in assisting in breast cancer subtyping or discovering a stemness and metastatic signature, as mentioned previously. Similarly, in the case of soft

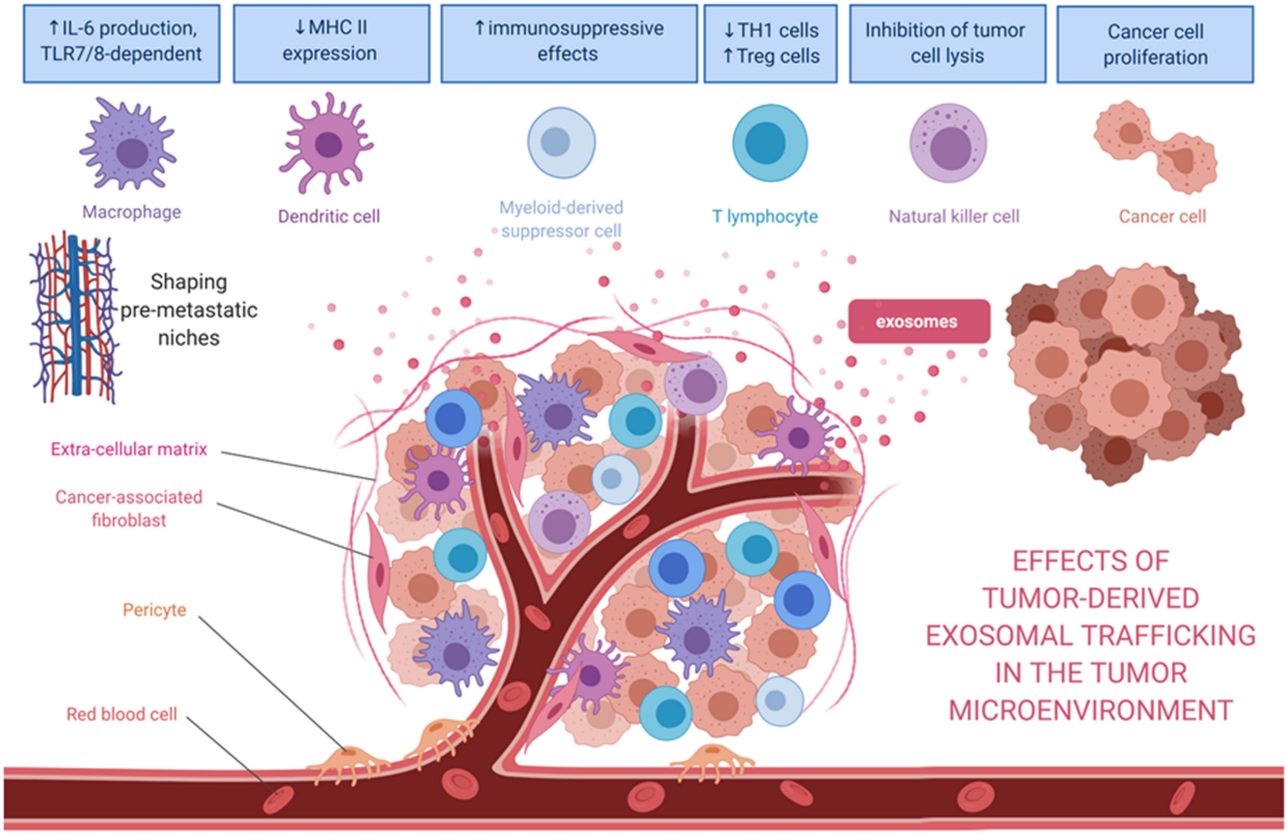

Figure 2 Effects of tumor-derived exosomes and their horizontal paracrine trafficking to impact on the tumor microenvironment. For example, breast cancer-derived exosomes modify the TME through the suppression of T-cell proliferation and NK cell cytotoxicity. Also, the exosomal content (eg, miR-1246 or miR-155) might contribute to the chemoresistance or hormone-resistance in tumor cells. Exosomes secreted by liposarcoma cells containing miR-25-3p and miR-92a-3p have been found to stimulate IL-6 secretion in tumor-associated macrophages, leading to liposarcoma progression. miR-34a in the released exosomes enhances the neural differentiation of Ewing sarcoma. Myeloma-derived exosomes could modify the microenvironment, affecting various recipient cells such as bone marrow endothelial cells or myeloid-derived suppressor cells. In the case of myeloma, these exosomal cargoes include miR-135b, miR18a, and let7b. After being internalized by recipient cells, miRs could bind to their target genes and trigger numerous pathways to facilitate tumor progression.

tissue sarcoma, in recognition of the role of exosomal cargo transcriptional signatures or microRNA profiling including miR-25-3p, miR-92a-3p, miR-34a, MT1-MMP, and MMP-14 playing in the promotion of sarcoma progression, remodeling extra-sarcomatous matrix to facilitate tumor invasion, and establishing a favorable TME for sarcoma growth, we can develop a practical analysis to investigate these soft tissue sarcoma-derived exosomes in assisting diagnosis or monitoring of disease along with the treatment milestones for a patient.

Although the technology for developing exosome-encapsulated therapeutics as targeted drug delivery is still in infancy, with the help of improving detection methods, rapid application of the analytic tests for specific exosomal cargoes for diagnostic purposes has become feasible, facilitating exosomal biomarker development. Several characteristics of CDEs, previously discussed in the cancer biology section, such as analyzable cancer-specific and stage-specific genetic contents in the cargo of CDEs, allow us to capture, profile and quantify using the current nanoanalytical technology. The phenomenon of pathological exosomal trafficking during cancer development and progression can be utilized in cancer diagnosis, prognostication, and treatment strategies. Studies have demonstrated that CDEs containing enriched genetic signals involved in cancer initiation and progression are shed by cancer cells into the blood, saliva, urine, ascites, and even cervicovaginal lavage. In clinical oncology, a cancer biomarker can be used for a diagnostic purpose, for example, in differentiating cancer from the non-cancer conditions. It can also be used for disease monitoring during antineoplastic therapy or follow-ups, for prognosticating a patient's survival, and for predicting a tumor response after anticancer treatment.

Current sophisticated purification techniques offer an opportunity to utilize isolated exosomal cargoes to assist in

Table 4 Published Clinical Studies on Exploiting Exosomes as Diagnostic, Prognostic, or Predictive Biomarkers in Various Types of Cancer

Biomarker Function	Exosomes/ Exosomal Cargo Content	Specimen Origin	Cancer Type	Utilization	References (First Author, Year)
DIAGNOSTIC					
	Exosomal lncRNAs	Cervicovaginal lavage	Uterine cervix	Differentiate cervical cancer from normal controls	Zhang J, 2016[103]
	ExomiR-1246	Serum	Breast	100% sensitivity and 92.9% specificity	Zhai LY, 2018[61]
	miRNAs from the miR-106a-363 cluster	Plasma and serum	Breast	Serve as potential diagnostic biomarkers	Li M, 2018[102]
	Exosomal phosphatidylserine	Serum	Ovary	AUC of 1.0 for predicting malignant against normal	Lea J, 2017[104]
	Urinary 3-gene expression assay	Urine	Men with elevated PSA levels	Discriminate between Gleason score (GS)7 and GS6 prostate cancer and benign disease on initial biopsy	McKiernan J, 2016;[100] McKiernan J, 2018[105]
	Exosomal shuttle RNA pattern	Urine	Clear cell renal cell carcinoma	Provide a noninvasive test to diagnose clear cell RCC	De Palma G, 2016[106]
	Exosomal miR-25-3p	Serum	Osteosarcoma	Reflect tumor burden	Fujiwara T, 2017;[82] Yoshida A, 2018[107]
	Exosomal lncRNA PRINS	Serum	Multiple myeloma or monoclonal gammopathies	Differentiate from healthy donors: sensitivity 84.9% and specificity 83.3%	Sedlarikova L, 2018[108]
	Tumor-derived exosomal miRNAs	Plasma	Early-stage NSCLC	Differentiate adenocarcinoma from squamous cell carcinoma	Jin X, 2017[109]
	Exosomal microRNA-191, - 21, −451a	Serum	Pancreas	Differentiate cancer and IPMN from normal	Goto T, 2018[72]
	Exosomal RNA cargo	Serum	Pancreas	Differentiate cancer from healthy controls in blinded studies	Ko J, 2017[27]
	Exosomal miRNA-21 and miRNA-181a-5p	Serum	Thyroid	Differentiate follicular from papillary thyroid cancer	Samsonov R, 2016[101]
	Phosphatidylserine-expressing CDEs	Blood of tumor-bearing mice	Early-stage malignancies	Detect very early-stage cancers before clinical evidence of disease in four mouse models.	Sharma R, 2017[110]
PROGNOSTIC					
	Exosomal miR-21	Serum	Pediatric hepatoblastoma	Predict event-free survival	Liu W, 2016[75]
	Exosomal cancer stem cell-like marker CD133	Ascites	Pancreas	Western blot revealed enhanced expression of CD133 in exosomes from pancreatic cancer patients	Sakaue, T. 2019[111]
	Exosomal miR-638	Serum	Hepatocellular carcinoma	Lower levels of serum exosomal miR-638 associates with poor overall survival	Shi M, 2018[112]
PREDICTIVE					
	Chimeric GOLM1-NAA35 RNA	Saliva	Esophageal squamous cell carcinoma	Changes in chimeric RNA levels predict PFS after chemoradiation.	Lin Y 2019[113]

(*Continued*)

Table 4 (Continued).

Biomarker Function	Exosomes/ Exosomal Cargo Content	Specimen Origin	Cancer Type	Utilization	References (First Author, Year)
	Exosomal EpCAM protein	Plasma	Pancreas (on palliative chemotherapy)	Exosomal EpCAM increase during treatment was associated with better PFS	Giampieri, R. 2019[88]
	Exosomal glutathione S-transferase P1	Serum	Breast (on neoadjuvant anthracycline/taxane)	GSTP1 from the PD/SD group was significantly higher than those in the PR/CR group	Yang SJ 2017[99]

Abbreviations: AUC, area under the curve; ExomiR, exosomal microRNA; lncRNAs, long noncoding RNAs; IPMN, intraductal papillary mucinous neoplasm; NSCLC, non-small cell lung cancer; PSA, prostate-specific antigen; PFS, progression-free survival.

differentiating the type of cancer and high tumor grade from low-grade cancer.[61,75,100–102] In a recent study, using a urinary exosome 3-gene signature obtained from the ExoDx Prostate IntelliScore urine exosome assay, the investigators can differentiate high-grade (Gleason's score > 7) vs low-grade prostate cancer and benign prostatic hyperplasia.[100] This noninvasive urine testing implies that many unnecessary invasive transrectal biopsies could be avoided. As was aforementioned in the review, in patients with multiple myeloma, serum exosomal miRNAs could add to the risk stratification in identifying newly diagnosed multiple myeloma with particularly poor outcomes.[58] Table 3 presents the select representations of exosomal cargo other than miRNAs, namely, proteins, lipids, signaling molecules, DNA, mitochondrial DNAs, circRNAs, lncRNAs, integrin, and enzyme, which potentially serve as a biomarker in various cancer types. Laboratory analytic methods to measure these contents in the research included triple SILAC quantitative proteomic analysis, mass spectrometry (MS)-based proteomic assays, lectin blotting, NP-HPLC analysis, ultrahigh-resolution Fourier transform MS, shotgun and targeted molecular quantitative lipidomic assays, capillary liquid chromatography-MS, MALDI MS, electrospray ionization MS, dsDNA-specific shrimp DNase and atomic force microscopy, RNA-seq analysis, transmission electron microscopy, nanoparticle tracking analysis, etc., depending on the study design (Table 3).

One of the advantages of investigating the CDEs as either a diagnostic, prognostic, or predictive biomarker is that physicians can obtain a specimen for CDE testing from a patient via relatively noninvasive methods. The shed CDEs into body secretion or discharges such as saliva, ascites, and cervicovaginal lavage can now be noninvasively or microinvasively assessed. In the past 5 years, clinical studies on exploiting CDEs as a clinical biomarker reported some promising results in various types of cancer (Table 4). The CDE cargo tested include lncRNAs, microRNAs, exosomal phosphatidylserine, urinary 3-gene expression profile, shuttle RNA pattern, RNA cargo, exosomal cancer stem cell-like marker CD133, exosomal EpCAM protein, and exosomal glutathione S-transferase P1 (Table 4).

As of this writing, there have been several dozens of prospective observational studies being carried out to investigate the role of specific exosomal cargo as a cancer biomarker in various cancers and their diagnostic performance in a particular clinical setting (Table 5).

Rapidly evolving nanotechnologies provide an opportunity to exploit and engineer exosomes for therapeutic purposes, which is gradually becoming a new class of cell-free nanomedicine. Therapeutic blockade of the exosome biogenesis to halt cancer progression at specific stages of the disease could be enticing in the development of cancer therapeutics.[80,114,115] The potential application of responsive exosome nano-bioconjugates for cancer therapy has also been confirmed in a recent study; the nano-bioconjugates can actively target tumors through the specific recognition on the surface of tumor cell and abolished signaling and improved phagocytosis of macrophages.[116] There are growing interests in investigating engineered exosomes as potential therapeutic vehicles or an active drug delivery system.[117–123] Making use of the exosomal organotropic characteristics, exosomes loaded with therapeutic compounds could be employed to target a recipient cell to carry out gene therapy selectively.

The following examples shall illustrate how the application of exosomal engineering technology may enhance cancer therapeutics. Targeting the immune cells in the TME as an adjunct of anticancer treatment has been becoming a hot research area. In the application of nanomedicine, various forms of nanoparticles-bioconjugate exosomes have been synthesized and tested

Table 5 Ongoing Human Studies Investigating Exosomes as a Biomarker in Various Types of Cancer, as Registered in ClinicalTrials.gov

ClinicalTrials.gov ID	Phase of Study	Cancer Type and Setting	Objectives	Outcome Measures
Lung Cancer				
NCT03542253	Observational (Observ.)	Early lung cancer	Combined diagnosis of computerized tomography and exosome	Exosomal micro-A was highly expressed in early-stage lung cancer tissues
NCT02890849	Prospect. cohort observ.	Non-small cell lung cancer	Consistency analysis of PD-L1 in cancer tissue and plasma exosome	Match rate of PD-L1 protein expression in cancer tissue and PD-L1 mRNA expression in exosome
NCT02921854	Prospect. cohort observ.	Non-small cell lung cancer after radiotherapy and chemotherapy	Detection of circulating biomarkers of immunogenic cell death (ICD)	Research to see if exosomal markers of anti-tumor immunity can be detected in the serum
Breast Cancer				
NCT04288141	Prospect. cohort observ.	HER2+ breast cancer on HER2 targeted therapies	Measure the expression of the HER2-HER3 dimer in the blood (exosomes)	Compare HER2 expression in blood exosomes by protein detection assays; correlate with change in HER2-HER3 dimer expression after HER2-directed therapy
NCT03974204	Multicenter prospective single-arm observ.	Breast cancer patients suspected of leptomeningeal metastasis	Analyses of exosomes in the cerebrospinal fluid	Evaluate the use of proteomic profiles issued from cerebrospinal fluid exosomes
Gastrointestinal Cancer				
NCT01779583	Prospect. case-control	Advanced gastric cancer on first-line chemotherapy	Circulating exosomes as potential prognostic and predictive biomarkers	Characterization of the molecular profile in cancer-derived exosomes
NCT03581435	Prospect. case-control	Gallbladder carcinoma	Study of circulating exosome proteomics	Proteomics studies will be done in both tumor tissue and the circulating exosome
NCT03102268	Prospect. cohort observ.	Cholangiocarcinoma patients without any anticancer therapy	Characterization of the noncoding RNAs in cancer-derived exosomes	Correlation of exosomes-derived ncRNAs and time-to-event end-points
NCT02393703	Prospect. cohort observ.	Pancreatic cancer	Exosome-mediated intercellular signaling	Prospective cohort; exosomes purification for downstream proteomic and RNA sequencing
NCT03821909	Prospect. cohort observ.	Patients suspected to have pancreatic masses undergoing diagnostic workup	Endoscopic ultrasound-guided portal venous blood sampling	Compare the expression of specific exosomal mRNA markers between portal venous and peripheral blood
NCT03874559	Prospect. cohort observ.	Locally advanced rectal cancer on neoadjuvant chemoradiation	Characterize exosomal biomarker levels	Compare rates of exosomal expression before during and after chemoradiation therapy with pathological response rates

(*Continued*)

Table 5 (Continued).

ClinicalTrials.gov ID	Phase of Study	Cancer Type and Setting	Objectives	Outcome Measures
Genitourinary Tract Cancer				
NCT04053855	Prospect. cohort observ.	Clear cell renal cell carcinoma post partial or total nephrectomy	Evaluation of urinary exosomes	Study CD9+ and CA9+ exosomes by electron microscopy technique
NCT04155359	Prospect. cohort observ.	Urinary bladder cancer (UBC) in participants presenting with hematuria and another cohort of UBC patients	To establish the performance characteristics of a urine exosome-based diagnostic test to identify bladder cancer	Results compared to that of cystoscopy
NCT04357717	Prospect. cohort observ.	Elevated PSA between 2 and 10 ng/mL and at least one prior negative prostate biopsy	Correlation of the ExoDx Prostate test results with the outcome of prostate biopsies in a prior negative repeat biopsy patient cohort	Clinical Evaluation of ExoDx™ Prostate (IntelliScore)
NCT03911999	Prospect. cohort observ.	Prostate cancer	Exosomal microRNA in predicting the aggressiveness of prostate cancer in Hong Kong Chinese patients	To compare the differences in miRNA expression
NCT03694483	Prospect. case-control	Prostate cancer	Genetic analysis for the detection of prostasomes	Determine the sensitivity and specificity of the prostasome purification methodology
NCT02702856	Prospective (Prospect.) cohort observ.	Prostate cancer (for first-time biopsy patients in the PSA Gray zone of 2.0–10 ng/mL)	Validation of a urinary exosome gene signature in men suspicious of prostate cancer	Correlate signature with the presence or absence of high-grade prostate cancer biopsy
NCT03236688	Prospect. cohort observ.	Advanced metastatic castrate-resistant prostate cancer	Detection of ARv7 splice variant transcripts from exosomes in circulation	Correlate ARv7 status with PSA response and correlate non-Arv7 with clinical outcomes
NCT04167722	Prospect. case-control	Prostate cancer status post robotic radical prostatectomy	Understanding the role of exosomal communication in lean vs obese patients	Collecting prostate and fat tissue from radical prostatectomy participants for culture
Sarcoma				
NCT03800121	Prospect. cohort observ.	Soft tissue sarcoma	Study of exosomes in monitoring patients with sarcoma	To quantify circulating exosomes and analyze their protein and RNA content
NCT03108677	Prospect. case-control	Primary High-Grade Osteosarcoma	Study if the profile of circulating exosomal RNA can be used as a biomarker for lung metastases	Differences in the levels and profiles of circulating exosome RNA from patients with or without lung metastasis
Hematological Cancer				
NCT03985696	Prospect. cohort observ.	Aggressive non-Hodgkin B-cell lymphoma (B-NHL)	Evaluation of peripheral exosomes can be used as novel biomarkers in B-NHL	Evaluate if a high expression of CD20 and PD-L1 on exosomes may allow tumor cells to evade immunotherapy

(*Continued*)

Table 5 (Continued).

ClinicalTrials.gov ID	Phase of Study	Cancer Type and Setting	Objectives	Outcome Measures
Other Types of Cancer				
NCT02862470	Prospect. cohort observ.	Newly diagnosed thyroid papillary, follicular and anaplastic thyroid cancer	Pilot prognostic study via urine exosomal biomarkers	Collect urine samples before an operation, immediately after surgery, postoperative 3, 6, and 12 months.
NCT02147418	Prospect. case-control	Human papillomavirus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC)	Exosome testing as a screening modality for HPV-positive OPSCC	To develop a new test that can detect specific HPV proteins in the blood or saliva to help improve detection of OPSCC
NCT03738319	Prospect. case-control	High-grade serous ovarian carcinoma (HGSOC)	Analyze the expression of miRNA and lncRNA by next-generation sequencing	Candidate miRNA/lncRNA will be validated as a biomarker for the detection and prognosis of HGSOC
NCT03895216	Prospect. cohort observ.	Cancer patients with bone metastases	To identify deregulated miRNAs within the circulating exosomes	Changes in miRNAs content of circulating tumor exosomes

to target specific immune cells in the acidic TME. Recent research has demonstrated that anti-tumoral M1 macrophages-derived exosomes conjugated with CD47 and SIRPα antibodies effectively reprogrammed the macrophages from M2 to M1 phenotype in the TME.[116] In another study in the living mice, cancer-associated fibroblasts in the TME can be specifically targeted by activated fibroblasts whose cell membrane was coated with semiconducting polymer nanoagents aiming to enhance multimodal cancer theranostics.[124]

MicroRNA-21 is a well-known microRNA that over-expresses in almost all cancer types, where its upregulation promotes cell proliferation, invasion, and metastasis.[69–78] MiR-21 derived from the exosomes of MSCs regulates the death and differentiation of neurons in patients with spinal cord injury. Recent efforts involve utilizing an exosomal transfer of miRNAs or anti-miRNAs to tumor cells as a new approach for the therapeutic application of miRNAs to combat the most aggressive form of glioma, glioblastoma multiforme. Monfared and coworkers recently attempted to down-regulate miR-21 expression in glioma cell lines, U87-MG and C6, and rat glioblastoma models treated with miR-21-sponge exosomes and demonstrated a decline in tumor cell proliferation, a dramatic enhancement of apoptotic rate, and a significant reduction in tumor volume.[125]

Conclusion

Cancer-derived exosomal trafficking is observed in almost all types of liquid or solid tumors, including leukemia, soft tissue sarcoma, and osteosarcoma, which supports its role as an enabling characteristic for cancer hallmarks. The cargoes carried by CDEs contain enriched genetic signals in the form of DNA, RNA, microRNA, lncRNA, protein, lipid composition, or specific gene expression profiles, which are shed into blood, saliva, urine, effusions, ascites, and cervicovaginal lavage. There are a growing number of studies that investigate CDE as either a diagnostic, prognostic, or predictive nano-biomarker in various kinds of cancer. Out of the published clinical studies on exploiting CDE as a cancer biomarker, 70% of them were looking at the CDE as a diagnostic biomarker. In contrast, the rest of the studies were testing the role of CDE as a prognostic or predictive biomarker. Not surprisingly, only a few of them have reached the state of validation trials. In the near future, we shall expect to see more prospective clinical trials to validate the performance of these nanoparticle biomarkers aiming to improve the overall cancer diagnostic capability in the clinic.

Acknowledgments

We are grateful that part of this review has been critically reviewed by Prof. Ed Harlow of Harvard Medical School, Boston, in the High-Impact Cancer Research: Cancer Biology and Therapeutics program, where Prof. Robert Weinberg was also a faculty member. A significant amount of time was dedicated to the hallmarks of cancer. The authors of this review took part in the program as students and this review is a result of these activities.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article and revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

This work received no external fundings.

Disclosure

The authors report no conflicts of interest.

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