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Review Articles

Revisiting the advances and challenges in the clinical applications of extracellular vesicles in cancer

Guangpeng He [a,b,1], Jiaxing Liu [a,b,1], Yifan Yu [a,b], Shibo Wei [a,b], Xueqiang Peng [a,b,**], Liang Yang [a,b,***], Hangyu Li [a,b,*]

[a] Department of General Surgery, The Fourth Affiliated Hospital, China Medical University, Shenyang, 110032, China
[b] Shenyang Clinical Medical Research Center for Diagnosis, Treatment and Health Management of Early Digestive Cancer, Shenyang, 110032, China

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ABSTRACT

Extracellular vesicles (EVs) have been the subject of an exponentially growing number of studies covering their biogenesis mechanisms, isolation and analysis techniques, physiological and pathological roles, and clinical applications, such as biomarker and therapeutic uses. Nevertheless, the heterogeneity of EVs both challenges our understanding of them and presents new opportunities for their potential application. Recently, the EV field experienced a wide range of advances. However, the challenges also remain huge. This review focuses on the recent progress and difficulties encountered in the practical use of EVs in clinical settings. In addition, we also explored the concept of EV heterogeneity to acquire a more thorough understanding of EVs and their involvement in cancer, specifically focusing on the fundamental nature of EVs.

1. Introduction

Extracellular vesicles (EVs) arise from all cells and are similar in topology to the cell, having a lipid bilayer membrane but no functional nucleus [1,2]. EVs naturally carry various bioactive materials (such as proteins, nucleic acids, glycoconjugates, lipids, etc.) and function as an important transport and signal transduction system in the body in pathophysiological processes (e.g. human reproduction, pregnancy, embryonic development, tumors [3]. The functions of EVs in the proliferation, metastasis, immune response, treatment resistance, and other cancer hallmarks are deciphered gradually and the reports related to the diverse roles of EVs in cancer are increasing explosively [4–6]. Among these EV-related researches, the therapeutic usage and functioning as biomarkers of EVs are gaining more and more attraction due to the huge value in potential clinical translation, which could transform present clinical practice [7–9]. EVs with natural or artificial advantages showed excellent potential as drug delivery tools [7–9]. The resources of EVs in therapeutic applications and the combination of EV modifications are various [7–9]. Besides, it's demonstrated effective in directly targeting EVs in tumor-bearing mice via several novel elimination methods [10,11]. In terms of functioning as biomarkers, analysis strategies are evolving from taking advantage of a single property or molecule, comprehensively analyzing multiple properties or molecules [12,13], and combining EV analysis with other liquid biopsies [14] to the application of machine learning [15,16] in EVs. However, despite the advances in the clinical application of EVs, the challenges remain outstanding. For instance, the heterogeneity of EVs not only benefits the applications of EVs but also blocks our understanding of EVs [6].

This study primarily focused on the crucial role of EV heterogeneity in our current understanding and recent advancements in the field of EV heterogeneity. Subsequently, we reassessed the diverse functions of EVs in cancer, focusing on their substantial role, which distinguishes this study from previous studies. Finally, we examined current progress in the utilization of EVs, particularly in the context of cancer. Nevertheless, we focused on common scenarios rather than including all associated research to establish a broad structure for the application of EVs in clinical settings.

* Corresponding author. Department of General Surgery, The Fourth Affiliated Hospital, China Medical University, Shenyang, 110032, China.
** Corresponding author. Department of General Surgery, The Fourth Affiliated Hospital, China Medical University, Shenyang, 110032, China.
*** Corresponding author. Department of General Surgery, The Fourth Affiliated Hospital, China Medical University, Shenyang, 110032, China
E-mail addresses: xqpeng@cmu.edu.cn (X. Peng), 20191264@cmu.edu.cn (L. Yang), sj_li_hangyu@sina.com (H. Li).
[1] These authors contributed equally: Guangpeng He, Jiaxing Liu.

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2. The EV heterogeneity

The most dominant feature of EVs is heterogeneities which not only represent the promising aspects of EVs but are also among the biggest obstacles to a better understanding of these organelles [6]. EVs is a general and heterogenous term referring to different kinds of vesicles from cells. At present, EVs can be divided into some main subtypes, including exosomes, ectosomes, and apoptotic bodies. The biogenesis of exosomes involves the formation of intraluminal vesicles (ILVs, pre-exosomes) in multivesicular bodies (MVBs) via ESCRT-dependent or ESCRT-independent mechanisms and the fusion of fusion of MVBs with the plasma membrane [3]. The ectosomes (also known as microvesicles) are directly shed from the plasma membrane [3] while apoptotic bodies are the products of cell apoptosis [7]. However, some new subtypes of EVs have been identified, including secretory autophagosomes/amphisomes which were generated by the fusion of vesicles originating from macroautophagy or MVB-autophagosome fusion, and the plasma membrane, migrasomes which were generated from retraction fibers when cell migrated [17], retractosomes resulting from broken-off retraction fibers [18], and so on. Besides, the biogenesis of these EVs is context-specific [19]and may vary in different cell types [9]. Due to the heterogeneity in EV biogenesis, these EVs also differ in structure. The most different aspect of the structure is EV size. Exosomes range from 30 to 150 nm, ectosomes range from 50 to 10, 000 nm, apoptotic bodies range from 50 to 5, 000 nm, migrasomes range from 500 to 3, 000 nm, and retractosomes range from 50 nm to 250 nm [9,18]. The cargoes (including lipids, proteins, nucleic acids, and other materials) of EVs also are both multifarious and heterogeneous. Taking exosomal proteins as an example, they include tetraspanins, heat shock proteins, lipid raft proteins, cytoskeletal proteins, as well as proteins integral to multivesicular body formation, membrane transport and fusion, antigen presentation, and adhesion [20]. These proteins actively engage in processes like cell membrane fusion and the release of exosomes [20].

Heterogeneities in the EV subtypes, biogenesis, origin, size, contents, and biofunctions of EVs also have been identified by most researchers [3,6]. Nevertheless, it is necessary to thoroughly examine additional physicochemical and biological characteristics, such as affinity/immunoaffinity, density, stiffness, bending modulus, osmotic pressure, electric charge, pH, and gravity. Consequently, we have incorporated these qualities into the heterogeneities of EVs. Interestingly, most available isolation methods for EVs are based on these basic properties such as ultracentrifugation or density gradient centrifugation based on size, mass, and density, filtration (ultrafiltration and sequential filtration), and size-exclusion chromatography based on size [21,22]. Recently, some new technologies have been developed that take advantage of different properties to isolate EVs. For example, cyclical electrical field flow fractionation (Cy-El-FFF) was developed to isolate exosomes based on both size and charge [23]. Understanding these characteristics can spur significant advancements in the EV space and help us identify the diverse heterogeneities connected to EVs. To understand the intricate communication mechanisms between cells in the context of various diseases, particularly cancer, we can use these fundamental characteristics to identify some significant subpopulations of EVs. In addition, these crucial features have a strong correlation with the phenotypes of the disease or tumor. Several previous studies (such as size and contents) [6] and recent advances have proven this aspect [24–27]. The stiffness and osmotic pressure are positively associated with the malignancy of the tumor-derived small extracellular vesicles (sEVs) and negative-related with the size of the tumor-derived sEVs but the bending modulus can significantly decrease with increasing malignancy of the sEVs and was found to be lower in smaller sEVs [24]. The corona of EVs, which is partially developed through electrostatic interactions, is another major advancement relative to these fundamental features. In essence, EV corona is cargo, but it also occurs outside and around EVs, supporting our theory that EV cargoes were more significant than their contents [6,25]. Besides this nonspecific electrostatic absorbance, ligand-receptor interactions and protein-protein interactions are also involved in the formation of corona [25]. The biological significance of the corona is enormous. The corona (EVs-LDL interaction) from the brain metastases cells can access the monocytes more easily and plays an important role in the formation of brain metastases niches [26], whereas the corona (WNT11 on exosome surface) derived from fibroblast can stimulate the activity, motility as well as invasion of the breast cancer cells [28]. In addition, a recent article demonstrated that the corona can significantly enhance angiogenesis, skin regeneration, and immunomodulation [27]. A recent study showed there were great differences in the electroactive cargo of EVs from different cell lines [29], which also indicated the great clinical translation potential of some EV properties that were not well-studied previously, although the differences between healthy people and cancer patients remained unknown. Hence, it is important to consider multiple aspects when studying the heterogeneities of EVs to fully comprehend their diverse and complex roles. Therefore, we should expand our awareness of the various dimensions of heterogeneities in EVs. We anticipate further research that examines additional characteristics of EVs, not just in mammalian cells but also in microorganisms.

It is well-established that various cells, or even the same cell under different circumstances, can release diverse EVs into bodily fluids. This phenomenon is crucial for liquid biopsy using EVs since the EVs can transport a wide range of biomaterials. This characteristic of EVs can significantly improve the effectiveness of drug delivery [6]. This claim does not, however, imply that all EVs from the same cell are identical in every condition. Since the processes of cargo loading and biogenesis are dynamic, two EVs produced by a single cell under the same circumstances may differ from one another. Moreover, this argument does not preclude the existence of similarities between EVs derived from distinct cell types or the same cells under disparate environmental circumstances. Conversely, the fact that EVs are non-identifiable in several ways ensures that none of the platforms for separation in use today can collect entirely pure EV populations. One of the most exquisite studies by Smith et al. provides the best example for the following two points [30]. Raman spectroscopy was employed to detect exosomes at the individual vesicle level. The researchers used hierarchical clustering analysis to separate exosomes from seven different cell lines into four distinct subpopulations. This separation was based on the unique vibrational signatures of certain proteins, phospholipids, and cholesterol [30]. All exosomes from 3T3 cell lines fall into one cluster whereas six other cell lines (including A549, Huh-7, SKOV3, Kasumi-1, Jurkat, and IMR90) had exosomes in more than one cluster [30], which demonstrated the heterogeneities in one cell line. However, all four acquired exosome clusters were composed of exosomes from more than one cell line and A549, Huh-7, and 3T3 cells displayed similar exosome populations [30], which indicates that EVs from different cells or the same cells living under different conditions can share some similarities. Our primary objective is to investigate the biological similarities underlying these variations and subsequently implement them in clinical settings. Nevertheless, no two EVs are indeed completely identical. Due to the significant variations observed at the level of individual vesicles and the common practice of analyzing vesicles in large quantities or by averaged measurements [31], the development of a single-vesicle atlas can be considered a major advancement in the research of EVs. Traditional single EV analysis includes nanoparticle tracking analysis (NTA), atomic force microscopy (AFM), and microscopic imaging, which are lacking because of the low throughput and limited detectable information [31]. Other popular individual EV investigations include (nano-)flow cytometry and advanced microfluidic systems, which provide additional information and allow for the relatively high throughput isolation and identification of single EVs based on fluorescence staining. In addition, other available reviews provide relevant benefits and recommendations [32,33]. Fortunately, several novel single-vesicle technologies (including above Raman spectroscopy, PBA, and NP-SIMS) have significantly expanded our horizons regarding heterogeneities at the

single-vesicle level [31,34–36]. Nanoprojectile (NP) secondary ion mass spectrometry (NP-SIMS) could detect four markers at individual EVs, which enables to distinction of EVs from hepatocytes and liver cancer cells [36]. About 200 surface proteins on a single exosome can be profiled using the proximity barcoding assay (PBA), and single-exosome high-throughput analysis [34]. For example, there were 12 different exosome subpopulations identified in CRC patients and healthy people and two special subtypes were found. ITGAM$^+$ exosomes were enriched in healthy individuals and suppressed CRC development whereas ITGB3$^+$ exosomes were enriched in CRC patients with hepatic metastasis and promoted CRC progression [37]. The more practical single EV approaches, which allow for the detection of multiparametric even large numbers of components, are still required. Moreover, there is still a great deal of uncertainty and disagreement surrounding the spatial and temporal heterogeneity of EVs. Some research focused on the biodistribution of EVs *in vivo*, which impacted both the therapeutic usage and the biomarker function of EVs. On the one hand, the surface proteins on EVs and the administration method could impact the effect of the therapeutic EVs by affecting target organs or cell kinds *in vivo* (more details available in Part 4.1). One the other hand, the biodistribution of EVs *in vivo* determined which body liquid should be collected when using EVs as biomarkers (more details available in Part 4.2).

3. The role of EVs in tumors

All the cells release EVs into the tumor microenvironment (TME), forming an EV communication network (EVnet) in which both the malignant cells and non-malignant cells could experience autocrine self-communication or interact with each other [9,38]. A comprehensive elaboration about the roles of EVs in tumor biology appears to be very complicated [6] and hence we just provide a conceptualization of the role of EVs in tumor biology. When examining the potential involvement of EVs in any disease, including cancer, it is essential to adhere to the following fundamental principles: EVs function as vehicles or conveyors of biological information. Consequently, the inherent cargo of EVs derived from parental cells and the response of recipient cells determine whether EVs are beneficial or detrimental. The types of cells that EVs release and the types of cells they absorb in a specific condition can be compared to two speakers engaged in a situational discourse over the phone. The process of encoding and decoding the contents of EVs by these "speakers" is what eventually facilitates their biological tasks. By adhering to this framework, the diverse and disruptive impacts of EVs on carcinogenesis have become simpler and clearer to understand. Harm-avoiding and benefit-following tumor cells usually can absorb tumor-promoting EVs from the tumor cells themselves [39] and the various nonmalignant (including endothelial, stromal, and immune) cells, such as cancer-associated fibroblasts (CAFs) [40], TAMs [41,42], mesenchymal stem cells (MSCs) [43] as well as astrocytes [44]), and can then exclude the different unnecessary or tumor-inhibitory materials via EVs (such as tumor-suppressive circRHOBTB3 [45]) [46]. These crafty malign cells also share “candies” with their friends and administer “poisons” to their “enemies” via EVs. In this review, we used the TGF-β pathway, which is an anti-growth pathway, as an example for demonstration purposes. The presence of *c*-Myc in EVs formed from lung cancer can enhance the growth of lung bronchial cells. Similarly, circPACRGL in EVs from colorectal cancer cells can induce the transformation of pro-tumorigenic N2 neutrophils, which in turn suppresses the TGF-β pathway [39,47]. TGF-β1 in EVs from breast cancer cells can significantly impair T cell proliferation [48]. Since EVs are neither always beneficial nor harmful, they can also mediate a range of tumor-inhibitory effects. Better clinical outcomes have been associated with the stimulation of a tumor immune microenvironment by EVs carrying CSF-1 from triple-negative breast cancer [49]. EVs can also be used by tumor cells to handle unfavorable environments. When tumor cells are exposed to unfavorable anti-growth factors (such as TGF-β and liver kinase B1) they secrete more EVs to stimulate their compatriots to proliferate or migrate away from this terrible environment [50,51]. Therefore, taking into account the characteristics of tumors as well as other aspects, EVs can directly affect the majority of them. These aspects include regulating cell death and proliferation/anti-growth signal balance, mediating immune evasion or response, inducing angiogenesis, activating invasion or metastasis, modulating metabolism reprogramming and tumor-promoting inflammation, and affecting treatment resistance or response [6]. However, TME is always in a state of dynamic changes where various cells can experience concerted evolution, leading to a more pernicious outcome [52] and consequential changes in EVnet. The most intuitive evidence is the differences in EVs derived from different cancer stages [53]. While some tumor-inhibitory cells in TME, including M1 macrophages, can prevent tumor advancement through EVs, other cells in TME can facilitate tumor progression through EVs. A major unanswered topic is how tumor cells eventually disrupt the tumor-promoting/inhibiting balance by eluding tumor-inhibiting EVs, transforming the unfavorable TME into a tumor-promoting niche through EVs, and advancing toward malignancy. Therefore, more research is needed to gain a more thorough understanding of EVs’ function in tumor biology.

4. Clinical applications of EVs in tumor

EVs are associated with great expectations in clinical oncology due to their beneficial properties although numerous efforts are urgently required before implementation of successful translational in medical practices. We will discuss this from the following two aspects.

4.1. Therapeutic applications

In the TME, the pro- or anti-tumor effects of EVs are contingent on intracellular genetic and metabolic properties and environmental conditions at the time of their biogenesis. Consequently, the primary and most immediate approach is to decrease the pro-tumor EVs (Table 1). For example, reducing the release of melanoma exosomes, which can significantly enhance the pro-metastatic phenotype of bone marrow progenitor cells (BMDC), by targeting Rab27a, which is one of the key proteins involved in exosome production, can effectively prevent both tumor invasion and metastasis in mice which were injected with these highly metastatic melanoma cells [53]. GW4869, a compound inhibiting EV release, was also found to display substantial anti-tumoral effects in different cell experiments [54,55]. Antiviral α-helical peptide (AH-D)

Table 1
Examples of targeting EVs in cancers discussed in this review.

Cancer	In vivo/ vitro	Treatments	Effects
ovarian cancer [55]	In *vivo* and in *vitro*	GW4869 or *RAB27A* knockdown	Reducing the release of EVs in *vitro*; Decreasing the peritoneal dissemination and promoting macrophage infiltration in *vivo*
prostate cancer [54]	in *vitro*	GW4869	Reducing the release of EVs and reducing tumor growth in *vitro*
Melanoma [53]	In *vivo* and in *vitro*	*RAB27A* knockdown	Reducing the release of EVs in *vitro*; Inhabiting tumor growth and metastasis in *vivo*
Melanoma and mammary carcinoma [11]	In *vivo* and in *vitro*	a 27-mer amphipathic, α-helical (AH-D) peptide	Disrupt tumor-derived exosomes in *vitro*; Preventing tumor-derived exosome-mediated T cell exhaustion and enhancing antitumor efficacy of PD-1 blockade in *vivo*

can disrupt tumor-derived exosomes and acidic properties of TME can enhance this disruption, thus rendering it suitable for targeting tumors *in vivo*. Notably, AH-D peptide can improve PD-1 blockade therapy, which contributes to developing an environment that fights against tumors and prevents the formation of premetastatic niches mediated by tumor exosomes [11]. Another similar research developed a blood purification equipment (pro-metastatic derivatives eliminator, PMDE) to remove circulating tumor cells and tumor-derived exosomes via EpCAM and EGFR on the surface of CTCs and exosomes [10]. Remodeling the TME or reprogramming the intracellular genetic properties from a state that induces pro-tumor EVs to a state that favors the production of anti-tumor EVs appears to be another good strategy (Fig. 1). Exosomes derived from the cancer cells in acidic [56], hypoxic [6], or stiff [57] environments can promote tumor progression, whereby decreased number of released EVs have been shown to regulate these chemical or physical properties of TME to inhibit tumors in different cell lines. However, concerning Rab27a alone, it can control the exocytosis of various cells (such as neutrophils [58,59] and cytotoxic T lymphocytes [60]). Therefore, directly targeting the release of EVs in the human body remains a major challenge, as non-specific targeting of key genes may also impair the antitumor effect of EVs and lead to side or off-effects. Furthermore, most studies, if not all, simply examined them through cellular experiments. Because of the difficulty in directly modulating the acidic, hypoxic, or rigid TMEs *in vivo*, most studies did not reduce EV release via these ways to suppress tumors. This reflects the challenges in targeting EV release *in vivo* and the broad implications of the chemical or physical properties of TMEs. Therefore, it is crucial to preserve physiologically healthy EVs when especially focusing on combating harmful EVs or enhancing the generation of advantageous EVs. Thus, to transform EVs into direct therapeutic targets in living organisms, advancements in various biological fields are necessary. These include achieving a comprehensive understanding of the diverse characteristics of tumors and EVs, developing a more accurate and specialized gene editing technology, and effectively manipulating the chemical or physical properties of the TME.

In contrast to directly targeting EVs, a wide range of studies focusing on using EVs as therapeutic tools are growing almost exponentially because targeting tumors via EVs offers many unique advantages partly based on EV heterogeneity (Table 2). First, EV heterogeneity meant that some EVs could be more specifically delivered to the target cells. For example, it has been reported that EVs possess the same tropisms as the cells from which their membranes originate [61], and thus the tumor cells tend to adhere to each other [62], which reflects EV heterogeneity in origin and uptake. Besides, exosomes reprogrammed with low-pH/hypoxia had a better affinity towards low-pH treated and hypoxic tumor cells respectively [63], which reflected EV heterogeneity in context-specificity. Interestingly, taking advantage of this affinity phenomenon, researchers have developed a smart drug delivery system LP-exos$^{Alp+Dox}$ through loading low-pH reprogrammed exosomes (LP-exos) with a hydrophilic photosensitizing compound Al (III) phthalocyanine chloride tetrasulfonic acid (Alp) and the hydrophobic chemo drug doxorubicin (Dox). LP-exos$^{Alp+Dox}$ can effectively kill the tumor cells under both *in vitro* and *in vivo* settings [63]. Moreover, compared to cell therapy, the application of EVs can significantly reduce carcinogenesis or toxic risks [7]. Second, EVs can reach where conventional drugs cannot, such as crossing the blood-brain barrier [64] or the blood-testis barrier [65]. Third, the membrane of EVs can also deceive immune cells and prevent immune clearance due to their cell-mimicking properties, which results in the prolongation of the half-life of EVs. The three virtues mentioned above can significantly improve the bioavailability of EVs. Last but not least, the structure of EVs is extremely suitable for drug delivery because they can naturally carry lipids, proteins, and nucleic acids, which reflect EV heterogeneity in cargo. Therefore, EVs can load both lipophilic and hydrophilic drugs. More importantly, EVs can enable the delivery of multiple drugs in an appropriate proportion, such as LP-exos$^{Alp+Dox}$ [63]. Hence, due to these important merits, EVs serve as ideal "transport tools".

Post-generation or naturally modified EVs derived from engineered or natural cells can be successfully applied to treatments [66] (Fig. 1). Currently, the cells that are being produced for therapies include red

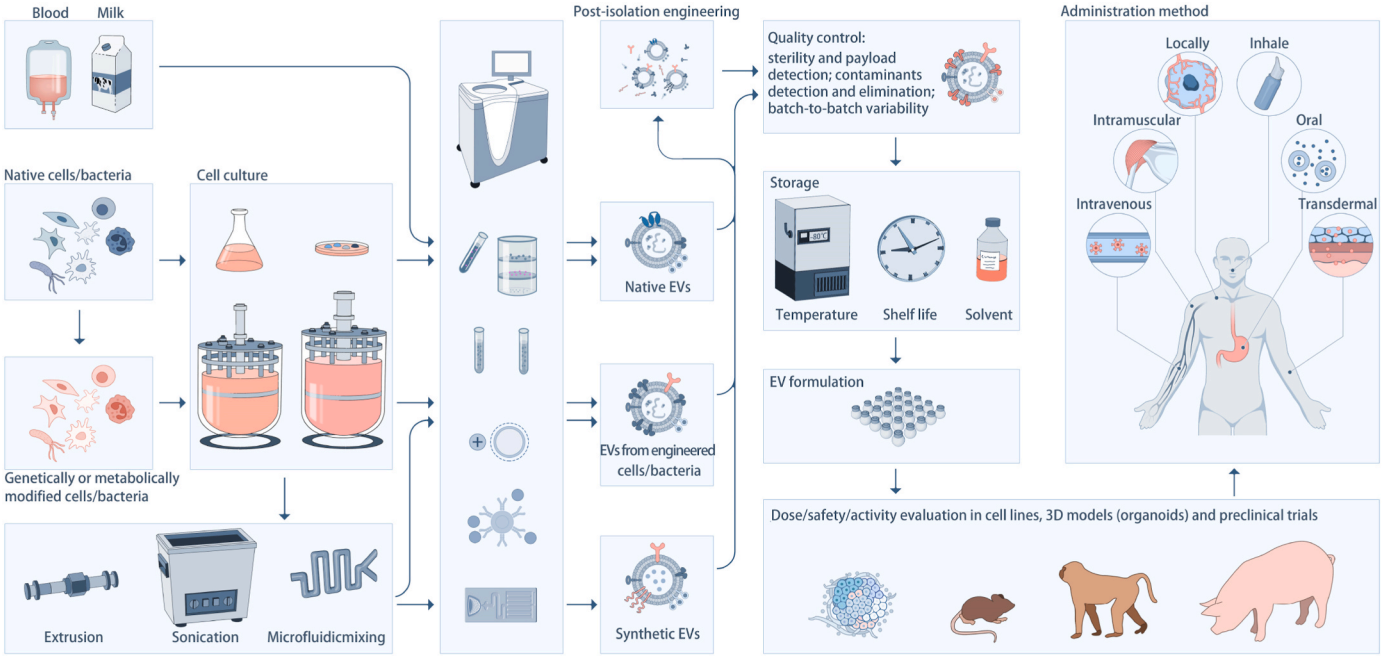

Fig. 1. Production process of therapeutic EVs. Native EVs are collected from milk, blood, and native cells/bacteria cultures. Engineered cells/bacteria can also be used as EV sources. In this condition, EVs are directly collected from cultures or manually generated as synthetic EVs. All kinds of EVs are modified after isolation or directly enter the quality control stage. EV products are stored under the most suitable conditions so that stable EV formulations can be developed. Evaluating the safety, activity and suitable dose of the EV formulation in various models, including cell lines, 3D models (organoids), animals and other preclinical trials identifies the best EV formulation for the clinical application. Finally, the most suitable administration method is selected according to patients' diseases.

Table 2
Examples of therapeutic EVs in cancers discussed in this review.

Therapeutic EVs	Parental cells	Function materials	Targeting cells/tissues	Effects	Function model
LPexos$^{Alp+Dox}$ [63]	Tumor cells in low pH (LP)	Alp, Dox	Tumor cells	Killing tumor cells in *vitro* and in *vivo*	1): exosomes from tumor cells living in low pH targeted tumor cells; 2): Alp enhanced the irradiation effects and deconstructed the exosome for the release of Dox; 3): Dox-induced chemotherapy
Responsive exosome nano-bioconjugates [69]	M1 macrophages	The immune-regulating cargoes in exosomes from M1 macrophages; aCD47 and aSIRPα; pH-sensitive benzoic-imine bonds.	macrophages	Reprogramming M2 towards M1 phenotype in *vitro* and in *vivo*; inhabiting tumor in *vivo*	1): M1 exosomes reprogramed M2 macrophages; 2): A pH-sensitive benzoic-imine bond promoted the release of aCD47 and aSIRPα in acidic TME; 3): aCD47 and aSIRPα improved the phagocytosis of macrophages.
miR-146b-bearing exosomes [71]	marrow stromal cell	miR-146b	Tumor cells	Reducing glioma growth in *vitro* and in *vivo*	miR-146b inhibited EGFR expression
aMT-exos: chimeric exosomes [72]	macrophage-tumor hybrid cells	Various immunostimulatory factors (details unknown)	lymph nodes and tumor tissue	Preventing immunosuppressive TME in *vivo*; inhabiting tumor growth, metastasis, and postsurgical recurrence in *vivo*	aMT-expos formed an anti-tumor immune microenvironment but details were unknown.
iExosomes [75]	fibroblast-like mesenchymal cells	siRNA or shRNA targeting *Kras*^{G12D} (and CD47)	Tumor cells	Inhibiting tumor growth and metastasis in *vivo*	1): CD47 presence on exosomes facilitated iExosome efficacy; 2): iExosomes reduced *Kras*^{G12D} mRNA and phosphorylated-ERK protein levels
Exosomes hybrid with liposomes [77]	Tumor cells	Triptolide (TP), miR497, cRGD	Tumor cells and macrophages	Overcoming the cisplatin-resistance of tumor cells and inducing M2 to M1 polarization of macrophages both in *vitro* and in *vivo*	1): Tumor-derived exosome and cRGD targeted tumor sites; 2): miR497 and TP inhibited the PI3K/AKT/mTOR signaling pathway; 3): TP depleted GSH and elevated intracellular ROS; 4): TP regulated macrophages (but details were unknown)
PASEVs [78]	M1 macrophages	siRNA targeting PAK4, ROS, EVs from M1 macrophages	Tumor cells	Inhabiting tumors and regulating immune responses in *vitro* and in *vivo*; cooperating with immunotherapy to prevent tumors.	1): Laser irradiation generated ROS and induced the release of siPAK4; 2); siPAK4 silenced PAK4 and blocked Wnt/β-catenin signaling; 3): EVs from M1 macrophages targeted tumor; 4): ROS triggered a robust ICD cascade accompanied by the release of DAMPs; 5): EVs from M1 macrophages could regulate immune cells.
PARP-1 sgR/Cas9-loaded exosomes [82]	Tumor cells	PARP-1 sgR/Cas9 which targets and inhabits PARP-1	Tumor cells	Cooperating with cisplatin to induce tumor cell death in *vitro* and in *vivo*	1): exosomes from tumor cells accumulated in tumor cells. 2): PARP-1 sgR/Cas9 inhabited PARP1
γδ-T-Exos [79]	γδ-T cells	details unknown	Tumor cells	Cooperating with radiotherapy to inhabit tumor in *vivo*	1): γδ-T-Exos induced tumor cell apoptosis via death receptor ligation and inhibited tumor progression 2): γδ-T-Exos promoted T-cell migration; 3): Radiotherapy increased the accumulation of γδ-T-Exos in tumors
IL12 mRNA load EVs [92]	HEK293T cells	IL12 mRNA	Cancer cells and immune cells	Activating tumor immune microenvironment *in vivo*	1): the expansion of tumor cytotoxic immune effector cells, including $CD8^+$ T cells, $CD4^+$ T cells, NK cells, and NKT cells; 2): the decrease of immune-suppressive Treg cells and MDSCs; 3): the formation of immune memory 4): improved antigen presentation and tumor-specific T-cell priming

LP-exos$^{Alp+Dox}$: loading Alp and Dox into exosomes from tumor cells living in low pH; LP: low pH; Alp: hydrophilic photosensitizer compound Al(III) phthalocyanine chloride tetra sulfonic acid; Dox: doxorubicin; Responsive exosome nano-bioconjugates: Azide-modified exosomes derived from M1 macrophages are conjugated with dibenzocyclooctyne-modified antibodies of CD47 and SIRPα (aCD47 and aSIRPα) through pH-sensitive linkers; iExosomes: Exosomes with siRNA or shRNA targeting

Kras^{G12D}; PASEVs: EVs fusing with cores (PAS) which were engineered by complexing siPAK4 with a photo-activated reactive-oxygen-species (ROS)-sensitive polymer TKPEI–Ce6 (PA); γδ-T-Exos: exosomes fromγδ-T cells.

blood cells (RBCs), platelets, MSCs, fibroblasts, dendritic cells (DCs), macrophages, neutrophils, natural killer cells (NK cells), T cells, epithelial cells, cancer cells, bacteria and plant cells [7,61,67]. In addition, there is also an increased focus on various EVs obtained from the bioliquid [68] as well as milk and factitious biomimetic EVs produced by physical extrusion or sonication. The different intrinsic properties inherited from their parental cells can cultivate EVs with some beneficial characteristics, such as non-immunogenic EVs from RBCs, immunogenic EVs from the platelets, MSCs, and other immune cells, tumor-homing EVs from the cancer cells, etc. [7,61,63]. Despite the advantages of these innate characteristics, natural EVs frequently fall short of treatment requirements because of factors including low yield, inadequate payload, possible pathogenicity, etc. Therefore, to address these limitations, either separately or in combination, engineering or external stimuli in the cells and post-generation alterations in EVs are being applied. A typical example that utilizes all of these strategies is pH-responsive exosome nano-bioconjugates [69]. It was found that the external stimulus that Mn^{2+} was used to treat RAW264.7 cells can induce the tumor-inhabiting M1 phenotype to produce the various immunomodulatory and anti-tumoral exosomes. Additionally, the membrane of RAW264.7 cells was modified using azide-choline incubation to indirectly enhance the exosome membrane. This modification allows the exosome membrane to have azide groups, which serve as a connecting bridge. The pH-sensitive benzoic-imine bond was also incorporated between anti-CD47/anti-SIRPα and DBCO to synthesize the amplifier effect. It has been documented that free anti-CD47 and anti-SIRPα can work in concert to strengthen macrophages' immune response against tumor cells, while the pH-sensitive benzoic-imine bond may substantially boost the release of these bioconjugates' free anti-CD47 and anti-SIRPα in acidic TME. Moreover, DBCO was mixed with azide groups on the surface of exosomes to produce pH-responsive exosome nano-bioconjugates that can effectively inhibit tumor growth in both tumor-bearing mice and *in vitro* settings [69]. In this case, characteristics of EVs, including the natural macrophage-reprogramming properties inherited from the parental cells, the ability acquired from the manipulation to regulate the phagocytosis of macrophages against the tumor cells, and TME-targeting through the acidic tropism, function in a comprehensive manner. Long noncoding RNAs and miRNAs are relatively stable because they are predominantly secreted in EVs, or in complex with other proteins. Furthermore, it has been designed to genetically modify different cells in order to include protein- and RNA-based therapeutic drugs and/or targeting ligands into EVs. This process mainly relies on the cellular machinery responsible for producing EVs [8,70]. For example, miR-146b-containing exosomes from the marrow stromal cells transfected with miR-146b expression plasmid can significantly decrease the glioma xenograft growth in a rat model of the primary brain tumor [71]. EVs derived from hybrid cells, which result from the integration of two distinct cell types, can possess additional advantages. Hybrid cells, known as activated macrophage-tumor (aMT) cells, were produced through the process of macrophages engulfing the nuclei of tumor cells and being stimulated by lipopolysaccharide (LPS) [72]. Exosomes from aMT cells (aMT-exo) were demonstrated to inherit the tumor tropism from the tumor cells and pronounced immunostimulatory effects from LPS-induced M1-like macrophages [72]. Macrophage-tumor chimeric exosomes can accumulate in tumors as well as lymph nodes. They then trigger immune responses and help reverse the immunosuppressive tumor microenvironment (TME), which prevents the progression of the primary tumor, distant tumor metastases, and postoperative tumor recurrence [72]. Adoptive T-cell therapy, such as chimeric antigen receptor T-cell (CAR-T) and engineered T cell receptor T cells (TCR-T) immunotherapy, was at the forefront of cancer therapy [73]. EVs from these T cells also showed great potential in cancer treatment. And CAR-T cell-derived exosomes inhabited tumor growth via the chimeric antigen receptor (CAR) on their surface and didn't express immunosuppressive PD1 [74]. Furthermore, electroporation was widely used. For example, in an elegant study, Kamerkar et al. loaded *KRAS*^{G12D} siRNA into exosomes from the normal fibroblast-like mesenchymal cells via electroporation. These exosomes containing *KRAS*^{G12D} siRNA were able to successfully suppress tumor growth in multiple mouse models of pancreatic cancer and significantly increased overall survival [75]. EVs can also fuse with other nanoparticles. EVs fused with the liposomes can facilitate the generation of EV-inspired liposomes that gain the versatile properties of the liposomes (tunable lipid and protein composition, surface functionalization, lumen loading, etc.) and the functionality of EVs (natural targeting properties, low immunogenicity, anti-inflammatory properties, etc.) [76]. For example, Li et al. fused CD47-expressing tumor exosomes and cRGD-modified liposomes to deliver miR497 and triptolide [77]. These exosome-liposome hybrid nanoparticles could overcome cisplatin-resistant ovarian cancer and induce M2 macrophage reprogramming toward M1 macrophages [77]. When discussing the functioning of EVs, it is important to note that they can also serve as adjuvant therapy to improve the effectiveness of other treatment methods. Additionally, they have the potential to be used as drugs in cancer treatment. For example, photoactivatable silencing extracellular vesicles can effectively sensitize cancer immunotherapy [78]; a combination of exosomes derived from γδ-T cells with radiotherapy can augment the immunosuppressive nasopharyngeal carcinoma [79]. Delivering gene-editing tools (such as CRISPR/Cas9) through EVs constitutes another new therapeutic for cancer. The PARP protein functions in various biological processes, such as DNA damage response, chromatin remodeling, cell cycle regulation, and cell death [80,81]. Targeting PARP in cancer treatments to overcome drug resistance has been developed [80,81]. A recent study showed that targeted inhibition of PARP-1 by CRISPR/Cas9-loaded exosomes can substantially enhance the chemosensitivity of SKOV3 cells (an ovarian cancer cell line) to cisplatin and exhibit marked antitumor activity in SKOV3 xenograft mice [82]. EVs encapsulated into an extended-release hydrogel can maintain a sustained treatment [83,84].

The potential of EV-based treatments is immense, as are the accompanying obstacles. The diverse nature of EVs poses a complex obstacle in the implementation of EV treatments, however, this heterogeneity additionally provides them certain advantages in specific areas [6]. The heterogeneity in the size and content of EVs makes their purification and identification difficult. The different EV subtypes sharing similar characteristics (such as size and density) could be co-isolated [85]. The absence of a comprehensive term "extracellular vesicle" is due to the inability to ensure the exclusion of all potential impurities from a certain subtype of EV, as EVs are just a diverse and varied population [2]. These problems require much better isolation and analysis methods to solve. In addition, more studies related to the capture [86] and analysis [37] of EVs at the single-vesicle level are urgent and could be very helpful [6,31]. The content heterogeneity adds another layer of challenge in EV therapeutics since both the active substances and non-active components should be declared from a pharmaceutical perspective and it is impossible to describe all the materials in EVs [87]. However, clear content is a prerequisite for the safety of EV therapeutics. For example, although stem cell-derived EVs can minimize the inherent disadvantages (e.g. oncological complications, fusion toxicity, etc.) inherent to their parental stem cells, the potential tumor-promoting activity (e.g. proangiogenesis) of these EVs still needs more attention [66,88]. Moreover, the exclusion of oncogenic effects of tumor-derived EVs (or the pathogenic functions of EVs from bacteria) is also the predominant requirement when exploiting their affinity for tumors (or yield advantages). The primary objective is to detect and subsequently eliminate compounds that have the potential to cause

cancer, have negative effects, are disease-causing, or are unwanted. In order to accomplish this, the parental cells must undergo EV editing or gene editing after a thorough characterization of EV payloads. However, due to the context-dependent heterogeneity in origin [6,19], fine-tuning changes can have a large impact on the production of EVs so the "batch-to-batch" variations in EV production compared to the other synthetic formulations will be a larger conundrum. Therefore, the production standard of EVs for therapeutic purposes must be stricter and more complex in comparison to other synthetic formulations. The autologous use of EVs, which refers to collecting EVs from patients for re-administration, could have advantages related to compatibility and other aforementioned aspects. However, this autologous manner lacks sufficient clinic safety data and is difficult for a streamlined qualified product and acute diseases [66] (Table 3). Although EVs derived from blood- or plasma-derived are readily available [66], the long-term application of autologous EVs for therapy may require a large volume of blood, which can cause harm to the patient's body. As mentioned above, the biodistribution of EVs *in vivo* also impacted the therapeutic usage of EVs. When injected intravenously, EVs tend to accumulate in the liver and cancer *in vivo*, meaning that many EVs cannot reach the targeted tissues, and there is no evidence as to whether EVs act primarily systemically rather than locally [89]. Whereas subcutaneously injected into the tail base and the tumor tissue, EVs accumulated in the lymph nodes (LNs), rather than in the liver and the spleen [90]. A recent study showed that both the EV source and the dose had determined impacts on the cellular response to EVs [91]. Another recent study developed inhalable EVs loading IL-12 mRNA via electroporation targeting lung cancer [92]. These inhalable EVs showed an excellent superior lung distribution and better tumor specificity in C57BL/6 mice bearing LL/2 lung carcinoma cells. Treating tumor-bearing mice with inhalable EVs three times resulted in an increase in some immune-active cells and a decrease in some immune-suppressive cells [92]. Compared with previous intravenous injections of lipid nanoparticles carrying IL-12 RNA, which resulted in hepatic toxicity and weight loss [93], drug administration of IL12 mRNA-containing EVs and IL mRNA-containing liposomes via inhalation didn't lead to weight loss and hepatotoxicity. Therefore, what are the suitable administration methods [66,94] and the amount [89] of therapeutical EVs for different diseases? Besides, the storage condition, such as temperature and solvent, also impacts the stability of EV products. For example, the EV structure would be damaged and EV aggregated if EVs were stored at 4 °C [95]. At present, more researches are needed although −80 °C is the recommended storage temperature [66]. The experiments revealed significant disparities in the quantity of released EVs, proteins, and glycans between EVs derived from gastric cancer cells cultured in 2D and 3D environments [96]. These findings also suggested the potential variations in cellular response to therapeutic EVs between 2D and 3D settings, as well as between *in vivo* and *in vitro* experiments, and among different animal models. Current research often uses cell lines, but exploring 3D microenvironments (such as organoids) and other complex animal models (not limited to mice or small animals) to evaluate the effects of EVs could be more convincing [9,89].

4.2. Functioning as biomarkers

Another important clinical application of EVs is to use them as potential biomarkers for cancer diagnosis (Table 4), patient stratification, monitoring progress, and guiding treatment through inducing changes in EV cargo (including proteins [13,97], RNA [98,99], DNA [12], lipid, glycan, etc. [100]) or physicochemical properties (such as size [12], concentration [12]). EVs acting as biomarkers can be collected from various body liquids, such as blood [12,13,97,98], urine [99,101], saliva [102], cerebrospinal fluid, and ascites [100] because EVs from different cancer may enrich in different body liquids, which reflected that the heterogeneity in EV biodistribution impacted the biomarker function of EVs (Fig. 2). Therefore, EVs together with the cell-free DNA (cfDNA)/-circulating tumor DNA (ctDNA) and CTCs in the body fluids are the three main research subjects in liquid biopsy. cfDNA has a shorter half-life of approximately an hour or less [103], which is due to the unstable nature of ctDNA and is easily degraded in the body fluids [104,105], whereas CTC is very rarely detectable. Therefore, EVs have unique advantages, as they are relatively abundant and have a longer half-life for detection.

Finding a single critical ingredient or a significant physicochemical characteristic that strongly corresponds with the advancement of the tumor and the response to therapy is the main strategy for employing EVs from bodily fluids as biomarkers. For example, an individual with positive *KRAS* mutations in exosomal DNA is 8.17 times more likely to develop early-stage pancreatic cancer than a cancer-free person and the mean *KRAS* mutations found in exosomal DNA were higher in the metastatic compared with localized samples [12]; Circulating exosomes carrying glypican-1 were enriched in pancreatic ductal adenocarcinoma (PDAC) patients in comparison to the healthy donors [13]. Nevertheless, the identification of a single key component is considerably challenging, and its diagnostic accuracy or specificity may not meet the required standards. As a result, (multi)omics or combinations of marker molecules have been commonly employed [97,98]. Numerous diagnostic,

Table 3
Clinical trials investigating the use of EVs in the treatment of various cancers.

EV Origin	Deliver Drug	Cancer Type	Study Phase	Study Title	NCT Number
DC	–	Lung cancer	Phase 2	Trial of a Vaccination With Tumor Antigen-loaded Dendritic Cell-derived Exosomes	NCT01159288
DC	–	Lung cancer	Phase 1	A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer [113]	–
Plant	Curcumin	Colon Cancer	Phase 1	Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	NCT01294072
Plant	–	Head and Neck Cancer	Phase 1	Edible Plant Exosome Ability to Prevent Oral Mucositis Associated With Chemoradiation Treatment of Head and Neck Cancer	NCT01668849
MSC	KrasG12D siRNA	Pancreas Cancer	Phase 1	iExosomes in Treating Participants With Metastatic Pancreas Cancer With KrasG12D Mutation	NCT03608631
APC-tumor chimeric cell	–	Bladder Cancer	Early Phase 1	An Open, Dose-escalation Clinical Study of Chimeric Exosomal Tumor Vaccines for Recurrent or Metastatic Bladder Cancer	NCT05559177
–	CDK-004 and STAT6 antisense oligonucleotide	Advanced (HCC); GC Metastatic to Liver; CRC Metastatic to Liver	Phase 1	A Study of exoASO-STAT6 (CDK-004) in Patients With Advanced Hepatocellular Carcinoma (HCC) and Patients With Liver Metastases From EIther Primary Gastric Cancer or Colorectal Cancer (CRC)	NCT05375604

DC: dendritic cell; APC: antigen-presenting cells, including dendritic cell or macrophage; MSC: mesenchymal stem cell; HCC: hepatocellular carcinoma; GC: gastric cancer; CRC: colorectal cancer.

Table 4
Examples of using EVs as biomarkers in cancers discussed in this review.

Methods	Judgment indicators	Cancers	Exosome sources
single marker	KRAS mutation	PDAC [12]	blood
single marker	Glypican-1	PDAC [13]	blood
(multi)omics or marker combination	Combination for poor prognosis: COMP, GNAI2, and CFAI; Combination for good prognosis: COMP, ACTN1, MYCT1, and PF4V.	Cholangiocarcinoma [97]	blood
(multi)omics or marker combination	ERG, PCA3, and SPDEF	Prostate cancer [99]	urine
(multi)omics or marker combination	miRNA-21, miRNA-16, miRNA-10b, miRNA-155, miRNA-1246, and miRNA-196a	Pancreatic cancer [98]	blood
(multi)omics or marker combination	tRNA-GlyGCC-5 and small RNA RESE	ESCC [102]	saliva
EVs combine with another liquid biopsy	Exosomal RNA/DNA combined with cfDNA	NSCLC [14]	blood
physicochemical properties	the number of EVs	PDAC [12]	blood
physicochemical properties	the size of EVs	PDAC [12]	blood
ML/AL	Using ML to analyze SERS signals	early-stage cancers* [15]	blood
ML/AL	Using ML to analyze the results from simultaneously detecting 4 mRNAs (PGR, ESR1, ERBB2 and GAPDH) via 4-plex droplet digital PCR	breast cancer [16]	blood

AI: artificial intelligence; ML: machine-learning; PDAC: pancreatic ductal adenocarcinoma; ESCC: esophageal squamous cell carcinoma; NSCLC: non-small cell lung cancer; SERS: surface-enhanced Raman spectroscopy. *: 6 types of early-stage cancers (lung, breast, colon, liver, pancreas, and stomach).

developmental, and prognostic indicators for cholangiocarcinoma were discovered by high-throughput proteomics of EVs. The best protein combinations for poor prognosis (COMP, GNAI2, and CFAI) and excellent prognosis (ACTN1, MYCT1, and PF4V) were identified among the prognostic biomarkers [97]. A combination of three RNA genes (*ERG*, *PCA3*, and *SPDEF*) from urine exosomes could effectively discriminate high-grade (Gleason 7 and higher) from low-grade (Gleason 6) prostate cancer and benign disease [99]. Interestingly, evidence about the EVs from the plasma samples obtained from the pancreatic cancer patients demonstrated that the diagnostic accuracy of a combination of six miRNAs (miRNA-21, miRNA-16, miRNA-10b, miRNA-155, miRNA-1246, and miRNA-196a) in EVs (98 %) was significantly better than a single miRNA in EVs (e.g., 73 % for miRNA-155) [98]. Furthermore, research on the physicochemical characteristics of EVs has demonstrated a correlation between the quantity and size of EVs and the advancement of cancer. In order to meet the requirements of cell-to-cell communication, tumor cells release more EVs than healthy cells [6]. For instance, more exosomes in the blood represented a worse median survival in PDAC patients [12].

Furthermore, average exosome size was greater among patients with PDAC in comparison with the healthy controls [12]. However, another study showed that the plasma exosomes from prostate cancer patients were substantially smaller than those from individuals with no signs of urological disease [106]. The conflicting impact of EV size in cancer patients may be attributable to changes in circumstances. Additional data is required about the physicochemical characteristics of EVs functioning as biomarkers. To improve detection, researchers have also examined the use of EVs in combination with another liquid biopsy. This is because the cargo carried by EVs may compensate for the lack of ctDNA or CTC. The combination of exosomal RNA/DNA and cfDNA for T790 M mutation detection was reported to have higher sensitivity and specificity compared with the historical cohorts using cfDNA alone [14]. However, it was difficult to select the best combination as a cancer-specific or therapy-specific signature. Computer technologies, such as artificial intelligence (AI) or machine learning (ML), can be beneficial to solve this problem. To concurrently diagnose six cancer types by label-free analysis of plasma exosomes, surface-enhanced Raman spectroscopy (SERS) and artificial intelligence (AI) were combined in a research study. With a sensitivity of 89.4 % and a specificity of 96.3 % at the ideal cutoff, this method demonstrated an accuracy of 97 % (AUC) for all cancer types. AUC values by cancer type were 0.936, 0.984, 0.972, 0.978, 0.992, and 0.999 for lung, breast, colorectal, liver, pancreatic, and stomach cancer respectively. When analyzed by cancer stage, the sensitivity for patients with advanced-stage cancer was 97.5 % but early-stage cancer patients were detectable at a sensitivity of 88.1 % [15].

Another study also revealed that ML can significantly improve the diagnostic performance of EV liquid biopsy. For example, when distinguishing the breast cancer patients from the healthy group, the AUC with ML (0.9167 for PGR mRNA alone, 0.8929 for ESR1 mRNA alone, and 0.8553 for ERBB2 mRNA alone) was remarkably better than the AUC without ML (0.8697 for PGR mRNA alone, 0.8270 for ESR1 mRNA alone and 0.7948 for ERBB2 mRNA alone). Additionally, as the number of biomarkers increased, the diagnostic accuracy also increased substantially. Among these biomarkers, the combination of the three mentioned markers resulted in an AUC value of 1.000 (95 % CI = 1.000–1.000), with a sensitivity of 94.74 % (95 % CI = 75.36–99.73) and a specificity of 100 % (95 % CI = 60.97–100.0) [16].

However, there also are huge challenges with EV liquid biopsy. A major problem is how to extract EVs from the different biofluids and which one of the EV subtypes is most suitable for cancer detection. For example, EV-LPP (lipoproteins particle) complexes can form spontaneously in physiological solutions (such as blood plasma) and affect the identification and detection of CD9, one of the EV markers [107]. This study demonstrated that the natural contaminants in bioliquid could influence the accuracy of EV biopsy [107]. Certain vital information may be lost if some EVs are purposefully or inadvertently neglected. International collaboration and rigorous standardization are particularly vital in this expanding sector because variations among laboratories worldwide might also impede future clinical translation. Another issue is how to identify the few biomolecules in EVs because of the restricted space. For instance, the average amount of miRNA per EV was significantly lower than a given amount [108]. Therefore, improving the detection sensitivity in EV biopsy is urgent. Recently, some methods have occurred, such as EXTRA-CRISPR (Endonucleolytically Exponentiated Rolling Circle Amplification with CRISPR–Cas12a) which is a method based on Cas12 [109]. This EXTRA-CRISPR has a sensitivity comparable to RT-qPCR testing when detecting key markers in EVs and a more simplified and convenient detection procedure [109]. In addition, an ideal test method should possess high sensitivity and specificity, as even a minor proportion of inaccurate positive or negative test outcomes could lead to irreversible harm to the patients. False-negative test findings can lead to the advancement of cancer into an incurable stage, while false-positive test results can cause significant psychological distress and unnecessary treatments that may have adverse effects. Preclinical data and advancements in other areas are necessary before EV diagnostics may be employed in a clinical setting.

5. Conclusions

The heterogeneity of EVs hinders our understanding of EVs while providing them with promising applications. Thus, focusing on EV

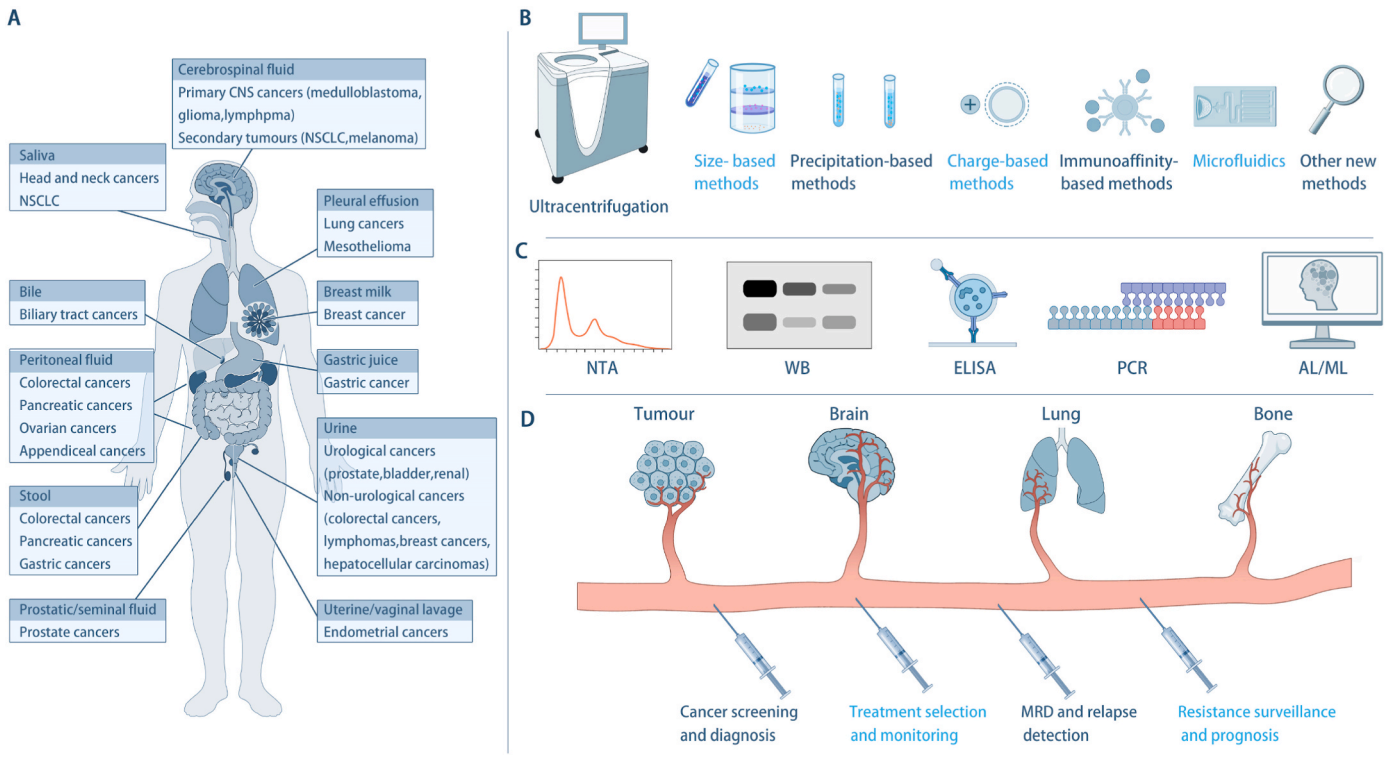

Fig. 2. Illustration of EV diagnosis process. A, Body liquids are enriched with EVs. B, Present EV isolation methods. C, Current EV detection methods. D, The clinical applications of EV diagnosis.

heterogeneity with a broader horizon can benefit the clinical application of EVs. A detailed understanding of EV heterogeneity broadly and at the single-vesicle level remains the foreland in EV research. Currently, the clinical application of EVs includes EV treatment and EV biopsy. Along with the development of precision medicine and personalized treatment, EV research my benefit cancer patients [110,111]. On the one hand, the therapeutic usage of EVs could provide the next-generation therapeutic platform and especially target organs or cells [7]. On the other hand, the biomarker function of EVs could guide cancer treatment, especially precision medicine and personalized treatment, which could handle the following important problems: the correct time to treat cancers, which target is the best, how to personally detect cancer progression, etc. [112]. While EV therapy and EV biopsy have distinct benefits, their thorough clinical translation is fraught with complications. Advanced technologies are needed for EV research to overcome these challenges. These days, multidisciplinary teamwork offers countless benefits and sources of inspiration. EV research has been greatly enhanced by experiences from other domains, such as gene editing technology, nanomedicine, biomaterials, pharmacology, precision or personality medicine, AI, etc. To overcome current obstacles, the therapeutic use of EVs in cancers will move further. We can eventually achieve the EV-everything world if we can successfully overcome these obstacles.

LIST OF ABBREVIATIONS

AI	Artificial intelligence
BMDC	Bone marrow progenitor cells
CAFs	Cancer-associated fibroblasts
cfDNA	Cell-free DNA
CRC	Colorectal carcinoma
CTCs	Circulating tumor cells
ctDNA	Circulating tumor DNA
DCs	Dendritic cells
EVnet	Extracellular vesicle communication network
EVs	Extracellular vesicles
LNs	Lymph nodes
ML	Machine learning
MSCs	Mesenchymal stem cells
NK	cells Natural killer cells
PDAC	Pancreatic ductal adenocarcinoma
RBCs	Red blood cells
SERS	Surface-enhanced Raman spectroscopy
sEVs	Small extracellular vesicles
TAMs	Tumor-associated macrophages
TME	Tumor microenvironment

(*continued on next column*)

(*continued*)

6. Ethics approval and consent to participate

Not applicable.

7. Consent for publication

Not applicable.

Availability of data and materials

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Guangpeng He: Writing – review & editing, Writing – original draft. **Jiaxing Liu:** Writing – review & editing, Writing – original draft. **Yifan Yu:** Supervision, Investigation. **Shibo Wei:** Supervision, Conceptualization. **Xueqiang Peng:** Supervision, Funding acquisition. **Liang Yang:** Validation, Supervision, Funding acquisition. **Hangyu Li:** Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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