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Review article

Tumor-derived extracellular vesicles: The metastatic organotropism drivers

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ABSTRACT

The continuous growing, spreading, and metastasis of tumor cells depend on intercellular communication within cells resident in a tissue environment. Such communication is mediated through the secretion of particles from tumor cells and resident cells known as extracellular vesicles (EVs) within a microenvironment. EVs are a heterogeneous population of membranous vesicles released from tumor cells that transfer many types of active biomolecules to recipient cells and induce physiologic and phenotypic alterations in the tissue environment. Spreading the 'seeds' of metastasis needs the EVs that qualify the 'soil' at distant sites to promote the progress of arriving tumor cells. Growing evidence indicates that EVs have vital roles in tumorigenesis, including pre-metastatic niche formation and organotropic metastasis. These EVs mediate organotropic metastasis by modifying the pre-metastatic microenvironment through different pathways including induction of phenotypic alternation and differentiation of cells, enrolment of distinct supportive stromal cells, up-regulation of the expression of pro-inflammatory genes, and induction of immunosuppressive status. However, instead of pre-metastatic niche formation, evidence suggests that EVs may mediate reawakening of dormant niches. Findings regarding EVs function in tumor metastasis have led to growing interests in the interdisciplinary significance of EVs, including targeted therapy, cell-free therapy, drug-delivery system, and diagnostic biomarker. In this review, we discuss EVs-mediated pre-metastatic niche formation and organotropic metastasis in visceral such as lung, liver, brain, lymph node, and bone with a focus on associated signaling, causing visceral environment hospitable for metastatic cells. Furthermore, we present an overview of the possible therapeutic application of EVs in cancer management.

1. Introduction

Extracellular vesicles (EVs) are heterogeneous lipid bilayer membranous vesicles released from most cells into the extracellular matrix (ECM) [1,2]. The main idea is that these vesicles are implicated in the intracellular communications as they contain different bioactive molecules and are present in most bodily fluids such as urine, blood, bile, breast milk, ascites, synovial, seminal, lacrimal, bronchoalveolar lavage fluids (BALF), and cerebrospinal fluid [1,2]. Through biofluids, EVs transfer their cargo and consequently modify the target cell's fate through paracrine, autocrine, and endocrine signaling. Cells produce EVs intrinsically and in response to different intrinsic and extrinsic stimuli including complement regulatory proteins, hypoxia, irradiation, cellular activation, environmental acidity, and injury [3–5]. Previous studies discussed that three classes of EVs are known as exosomes, microvesicles, and apoptotic bodies based on the mechanisms of biogenesis and their size [1,6] (Fig. 1). Exosomes, EVs with 30–150 nm size range, originate from the endosomal membrane system where invagination of multivesicular bodies (MVBs) membrane forms intraluminal vesicles (ILVs), subsequently, ILVs are called exosomes if MVBs fuse with the plasma membrane to release ILVs out of the cell. Alternatively, MVBs may fuse with lysosomes for the degradation of ILVs [1,6]. Different researchers have confirmed that various molecules and mechanisms contribute to the biogenesis, loading, and intracellular

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trafficking of exosomes. Molecules lonely or in form of different complexes named ESCRT machinery mediate exosomes biogenesis [6,7]. The miscellaneous and many cargo molecules including RNAs, proteins, and lipids associated with exosomes originate from endomembrane compartments, cytoplasm, and Golgi apparatus. Exosomes are associated with typical protein markers like CD81, CD9, CD63, and TSG101 for assistance in the isolation, identification, and characterization methods [6,7]. Recent developments in the field of exosomes have confirmed the heterogeneity of the exosomes population, with distinct subtypes as small exosomes (60–80 nm) and large exosomes (90–120 nm) [8]. Researches on the heterogeneity of exosomes are presently an ongoing area with a substantial interest in exosomes isolation, purification, and characterization methods. Microvesicles or ectosomes are occasionally so-called shedding vesicles that originate from most cells through outward budding of the plasma membrane with a size between 0.1 and 2 μm [9,10]. Apoptotic bodies, the biggest EVs, are produced by apoptotic cells through apoptotic cells fractionalization [11]. EVs can target cells through different mechanisms such as docking on the surface receptors of recipient cells or directly fusing to the plasma membrane or entering cells through endocytosis [12] (Fig. 1). Through these routes, EVs possibly will induce signal transduction or facilitate the horizontal transfer of signals/cargo in distinct target cells [12]. Recent trends in EVs biology have led to a proliferation of studies that suggest EVs can be classified based on their size into large (lEVs) and small EVs (sEVs) [13–15], where microvesicles (200 nm–1 μm), apoptotic bodies (1–5 μm) and oncosomes (1–10 μm) can be named lEVs, however smaller vesicles like exosomes and exomeres are named as sEVs [13–15]. Recent studies in EVs have heightened their crucial roles in cancer progressions such as pre-metastatic niche development and metastasis [16,17]. Tumor cells release more EVs with distinct biological cargo when compared to their non-cancerous equivalent. EVs as functional mechanisms of the tumor cell secretome can induce tumor formation at local and secondary metastasis locations [18,19]. EVs from tumor cells content various signaling molecules that orchestrate tumorigenesis in secondary site by pathways, including i: inducing epithelial-mesenchymal transition (EMT) to cells [20]; ii: synchronization of pre-metastatic niche through the enrolment and edification of stem cells from bone marrow, transdifferentiation of fibroblasts into myofibroblasts, angiogenesis and vascular remodeling, and rearrangement of matrix structure; iii: modulation of cancer immunogenicity and immunosurveillance [21,22]. In this review, to avoid misunderstanding, we cautiously describe the class of EVs as consisting of all populations of exosomes and microvesicles, furthermore, EVs discussed throughout the text refer to EVs derived from tumor cells. Throughout this review, we aim to present a comprehensive and updated review with a detailed focus on the role of EVs in tumor metastasis toward different organs.

2. EVs cargoes

Different EVs from different sources harbor diverse biological cargo,

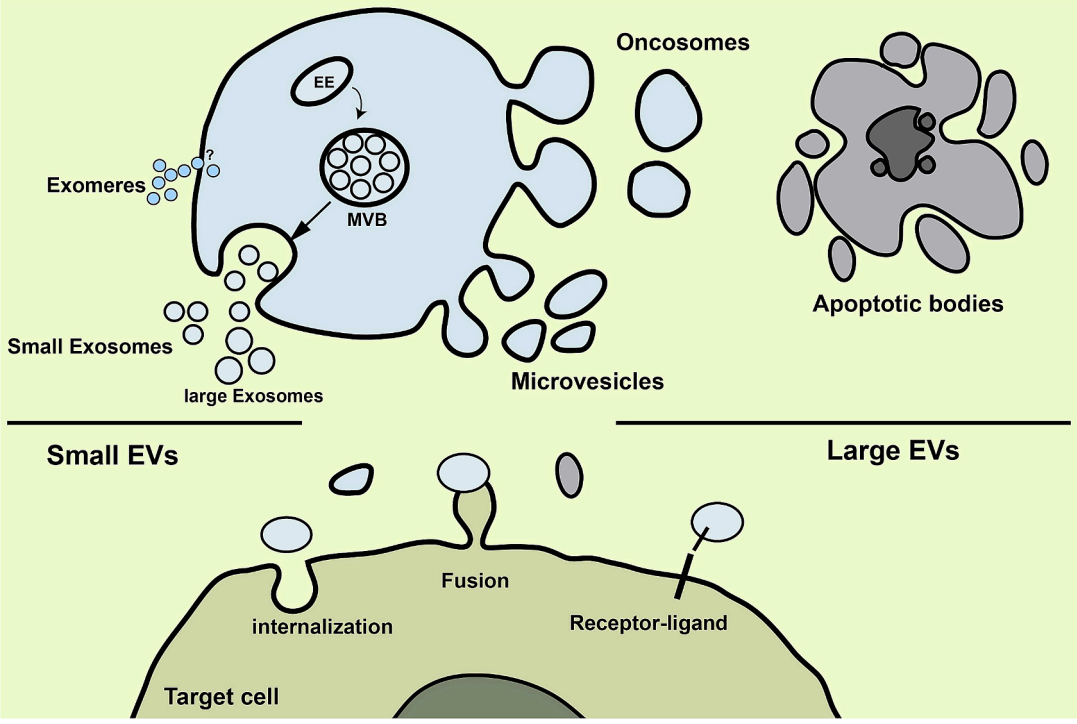

Fig. 1. Types of extracellular vesicles (EVs). According to literatures, EVs can be classified into two classes as Small EVs (sEVs) and Large EVs (lEVs) based on their origin and size. sEVs comprises exosomes and exomeres, while lEVs are microvesicles (MVs), oncosomes, and apoptotic bodies (ABs). (A) Exosomes biogenesis and release are synchronized through distinct intracellular compartments and pathways. The endocytosis pathway and endosomal compartment along with different complexes and molecules contribute to biogenesis and release exosomes. Exosomes initially are formed in multivesicular bodies (MVBs) as intraluminal vesicles (ILVs) through the invagination of MVBs. Scientists believe that these compartments are temporary vesicles that may fuse with the plasma membrane to release ILVs out of cells, subsequently named exosomes or fuse with the lysosome, leading to the degradation of their cargo. The pathways involved in exomeres biogenesis and secretion are mostly unknown. (B) MVs, oncosomes, are formed through a distinct pathway. Generation of MVs may involve reorganization of the cytoskeleton of the plasma membrane components, the contraction and disassembly of the cytoskeleton, finally the plasma membrane shedding. (C) ABs are formed from cells undergoing apoptosis. Formation of ABs is a several-staged process, comprising condensation of chromatin, splitting of nuclear and generation of micronuclei, then blebbing membrane, and finally segmentation of the cell into various membranous vesicles containing biomolecules. EVs can transport various nucleic acids, proteins, and lipids to target cells that may mediate signaling pathways. EVs can deliver their cargo to cells *via* different types of internalization (phagocytosis, macropinocytosis, and endocytosis) or directly fusion of the EVs membrane with the plasma membrane of target cells (Fusion). In parallel, communication of EVs and target cells occurs by interactions between different ligands and receptors located on EVs (receptor-ligand). It seems the common or/and specific molecules facilitate this interaction in various EVs and cells. EE: early endosome; MVB: multivesicular body.

however, EVs express common cargo that sometimes is considered as distinct EVs markers [23]. Recent advancements in the field of EVs isolation, identification, and characterization attracted substantial interest in the discovery of EVs cargo since their cargo increases our information on detailed mechanisms deal with biogenesis and function of EVs; especially EVs molecules may function as biomarkers for diagnosis, even prognosis of diseases and use as therapeutic tools for targeted treatment [24,25]. To support, modernize, standardize, and simplify EVs-related studies, the International Society for Extracellular Vesicles (ISEV) prepares and releases minimum guidelines [26]. Fortunately, different databases were established in recent years to promote and support further research, including ExoCarta, Vesiclepedia, EVmiRNA, and EVpedia [27–30]. EXOcarta (http://www.exocarta.org/credits) is a web-based collection that simply gathered the exosomal genetic components. In this database, data of exosomal cargo from 286 exosome-based studies presented including 41,860 proteins, around 7540 RNAs, and 116 lipids. Another web is Vesiclepedia (http://www.microvesicles.org/) containing data on proteins, RNAs, lipids, and metabolites of EVs. This database is voluntarily accessible for download and motivates scientists to contribute their discoveries, confirming observations are up-to-date. These data were categorized from 1254 studies, which studied 349,988 proteins, 10,520 miRNAs, 27,646 mRNA, and 639 lipids. Vesiclepedia is more comprehensive, however, it includes all EVs-based studies and does not effort on EVs distinct subclasses. EVmiRNA database (http://bioinfo.life.hust.edu.cn/EVmiRNA) repositories specific exosomal cargo originates in EVs. This database focuses on reporting miRNA in EVs and entirely affords complete miRNA expression outlines in EVs. In this regard, 462 small RNA examples of EVs were prepared from 17 studies and diseases, which were manually collected in this database. Another available database is EVpedia (https://omictools.com/evpedia-tool) participating vesicular module for EVs investigation. Data are composed of 2879 studies integrating 172,080 vesicular molecules from 163 comprehensive data collection. This data is most viewed since 2015, demonstrating its high potential as a source for EVs-based studies. Certainly, these databases provide data for simply and quickly download to assist EVs-related enquiry and applications, comprising biomarker documentation for various diseases and function analysis of genetic loads. Many of these databases have been collected to enable EVs-based researchers, however, no consensus has been obtained for the isolation, purification, and characterization of specific EVs subpopulations, and therefore researchers must cautiously use these databases. The difficulty in isolation, purification, and characterization of a pure EVs population led to slow down extra advances in the EVs field [31,32]. A survey of the literature shows that different methods are used to isolate and characterize EVs by different laboratories especially studies done before 2014 and 2018 ISEV's guidelines, which causes concern about the validity and reliability of the data [26,33]. Efficient isolation methods and precise characterization need to state that finding resulted uniquely from one class of EVs. Thus, exact characterization and outlining of proteomic, transcriptomic, genomic, and metabolomic cargo in EVs would increase understanding of their biomedical application and also intercellular communications in various cancers.

3. EVs in metastatic organotropism

Metastasis is the travel of cancer cells from the first site formed (primary cancer) through the blood or lymph system to the secondary site and form new tumors (metastatic tumors) [34]. The metastatic tumor has resembled the primary tumor. This process is a crucial characteristic of malignant tumors and is the deadliest feature that is responsible for more than 90% of cancer-related mortality [35]. Organotypic metastasis is a feature of primary tumors to dictate secondary tumor at the metastatic location in specific organs and consist of a series of cell-biological events, known collectively as the invasion-metastasis event [35,36]. It seems that each type of tumor shows remarkable discrepancies in kinetics (time to relapse) of metastatic development, the temporal level (relapse to different organs) of metastasis, and the rigorousness of metastasis in key organs [37]. The microenvironment at the secondary site is imperative for developing organ-specific metastatic cells with a complete ability for aggressive establishment. The brain, bone marrow, liver, and lung execute diverse selective pressures for the formation of metastatic residents [38]. These pressures come from the different physiological barriers, distinctive arrangement of the microenvironment of specific organs, and also haematogenous features like circulation arrangements and blood vessel's wall availability [39]. Following the specific pressure of the host microenvironment, tumor cells acquire vigorous metastatic competency features through epigenetic and genetic modifications [37]. Besides the genetic changes, the intrinsic capabilities of the metastatic tumor cells to interact with the new microenvironment offers a specific benefit to tumor cells for adaptation to the second place [40]. EVs-based metastasis has been reported to play an essential function for adoption mechanisms of tumor cells in the new microenvironment as well as allow the redistribution of metastasis [41,42] (Fig. 2). Studies show that EVs prefer metastatic efficacy to various target organs because of their affinity for individual target cells, consequently facilitating planned arrangements of spreading [43,44]. For instance, integrins of EVs dictate specific organ targeting [43], regulating organotropism to distinct organs, specifically the liver, brain, and lung. In detail, integrins α6β4 and integrins α6β1 postulate primary lung metastasis, whereas integrins αvβ5 targets metastasis to the liver and integrins Gβ3 to the brain. Integrins α6β1 and integrins α6β4 stimulate tumor-EVs adhesion to lung epithelial cells and fibroblasts, consequently leading to lung tropism, although integrins αvβ5 supports adhesion to Kupffer cells, thus inducing liver tropism. Integrins also network with cell-associated ECM components that may arbitrate EVs internalization in specific organs. Moreover, integrins can induce cellular signaling like the proto-oncogene tyrosine-protein kinase Src [43], which in turn can activate pro-inflammatory and pro-migratory signaling by increasing expression of the S100 gene in certain cells within distant target microenvironments [43]. In support, integrins participate in organotropism where integrins Gα2β from EVs promote brain metastasis, and that of integrins Gα4 help lymph node (LN) metastasis [45]. In general, the contribution of EVs in signaling/communication between tumor cells and the secondary microenvironment supports the formation of the pre-metastatic niche and increases tumor development (Fig. 2).

3.1. Seed and soil hypothesis

For the first time, Paget published an article suggesting the hypothesis "**Seed** and **Soil**" that describes why BC cells display a tendency for metastasis to specific organ sites [46]. This hypothesis is generally cited and established, and it offers weighty directions in cancer research until today. According to the seed and soil hypothesis, the primary tumor contributes to the preparation of secondary sites for the influx and development of tumor cells, well-known as the pre-metastatic niche (PMN) [22]. EVs released by tumor cells have been suggested as vital players in this process. Indeed, EVs prime the distant site "**Soil**" for metastatic tumor cell "**Seed**". In this context, EVs prime and rearrange the particular organ niches to expedite the metastasis process even for tumor cells with humble colonization ability for those microenvironments [22,42] (Fig. 2). EVs train the distant "soil" for a tumor-growing microenvironment and tumorigenesis. For example, BC cells such as MDA-MB-231 and MCF7 cells produce EVs that promote the differentiation of adipose-derived MSCs into myofibroblasts like cells and induce secretion of factors including VEGF, SDF-1, TGFβ, and CCL5 which are included in promoting tumor development and metastasis [47]. Similarly, Costa-Silva et al. showed that EVs from pancreatic ductal adenocarcinomas (PDACs) contain abundantly macrophage migration inhibitory factor (MIF) that induce liver pre-metastatic niche formation [48]. In the next sections, we have described the roles of EVs in tumor metastasis in different organs. However, in this section we should

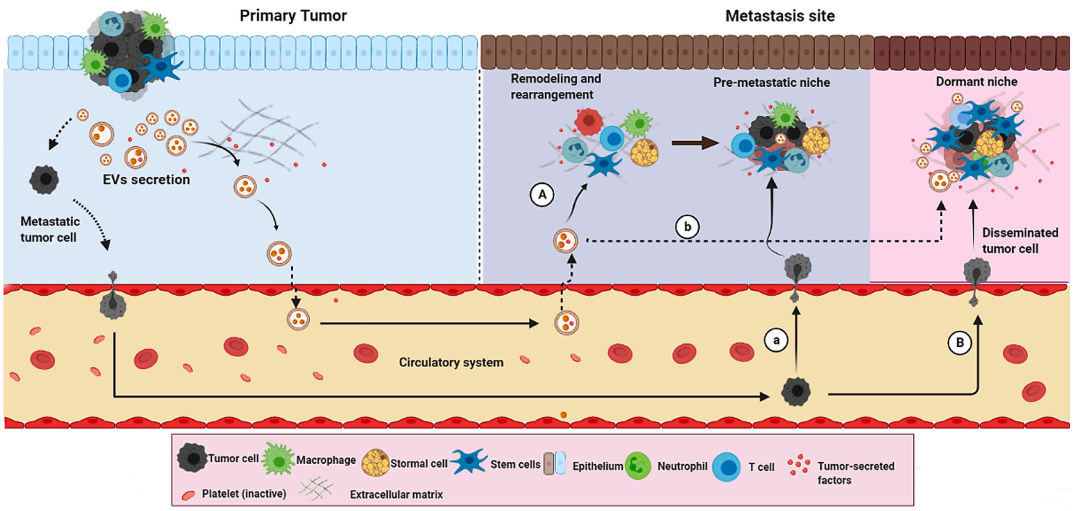

Fig. 2. Schematic illustration of the key function of extracellular vesicles (EVs) in tumor metastasis. Tumor cells release EVs into the extracellular matrix, transferring different oncogenic molecules to target organs. EVs carry proteins, RNAs, and bioactive cargos through the bloodstream and also several bio-fluids, protecting them from enzymatic degradation. A growing body of evidence suggests that primary tumor derived EVs contribute to the formation of the pre-metastatic niche (A). In this model, once reach metastatic sites, EVs interact with the microenvironment and drive remodeling and rearrangement forces to the host extracellular matrix and resident cells' function (A). This action facilitates enrollment of primary tumor cells to pre-metastatic niche (a). The process of niche formation and propagation of tumor cells is not random, and tumor cells will favorably search for particular organs under the management of EVs and form nests there and tumor progresses (a). This finding a proper destination may resemble the communication of seeds and soil. Seeds can fertilize the soil by EVs before tumor cells reach the soil, therefore formulating for tumor colonization and metastasis. However, in recent years, investigations on organs metastasis showed that metastatic tumor cells from the primary tumor may reside in organ sites as disseminated tumor cells (DTCs) (B). These DTCs are quiescent and persist long term essentially in a single-cell state within organs, known probably as dormant niche (B). Extrinsic and intrinsic signals may contribute to DTCs dormancy or reawakening. Besides other factors, there is an evidence that EVs from tumor cells contribute to the reawakening of DTCs (b). It is not clear that whether EVs mediate both the formation and reactivation of niches or not? The molecular mechanisms of the niches that endorse DTC dormancy/reawakening are now being studied intensively. Regarding EVs function, many questions should be considered in the field. Whether EVs from tumor cells exactly promote the outgrowth of DTCs or induce dormancy? May similar signalings be involved in both processes? Do DTCs produce EVs favoring their microenvironment? Whether the function of different EVs from the different tumor is differing or not? What is the exact role of EVs from stromal cells on DTCs dormancy?

reference the recent progress in the field of EVs that resulted in the establishment of the hypothesis, which indicates EVs participate in tumor cells dormancy. It was proposed that DTCs can spread from the primary tumor [22,49]. Once they reach distal organ sites, DTCs may continue in a dormant state for long periods until their development is reactivated and they form metastatic lesions [22,49]. The kinetics of specific tumors reveals that, rather than founding PMNs that promote the efficacy of metastasis, particular microenvironments where DTCs can survive in a dormant state could happen. Possibly, these niches as silent PMNs that might result in a prevalent delay to the progress of metastasis, as observed in some individuals with cancer [22,49]. Intensive scrutiny on silent and active PMNs can support understanding the various progressions of metastasis. The underlying mechanisms that the niches induce DTCs dormancy are currently being described. Studies by Peinado and his team [21,22,50] discussed that EVs from various stromal cells may induce DTCs dormancy in different tumor types (Figs. 2 and 3B). For instance, in the bone marrow, EVs of MSCs contain several miRNAs that can mediate many pro-dormant features like reduced proliferation and quiescence in breast cancer cells, whereas these EVs inhibit the development of bladder cancer. Bliss et al. showed that MDA-MB-231 and T47D BC cells induce MSCs to produce EVs loaded with miRNAs like miR-222/223 that may induce dormancy of a subset of tumor cells and confers drug resistance in bone marrow niches [51] (Fig. 3B). In the liver, several EVs miRNAs including miR186, miR23a, and miR205 can decrease BC cell proliferation in the hepatic niche [52] (Fig. 3B). Similarly, miRNAs of fibroblasts-derived EVs promote a stem cell-like phenotype and drug-resistance in colorectal and BC cells [53,54]. In many breast cancer (BC) patients, distant metastases develop after years or even periods of latency. For example, Ghajar and co-workers reported that quiescent disseminated tumor cells (DTCs) reside on the microvasculature of bone marrow, lung, and brain [55]. In detail, using *in vitro* organotypic microvascular niche models, they found that thrombospondin-1 secreted by endothelial cells induces continuous quiescence in BC cells, however, these findings did not observe in sprouting neovasculature. In keeping, in the zebrafish model, endothelial tip cells produce tumor-promoting mediators like active TGF-β1 and periostin. The authors concluded that constant microvasculature establishes a quiescent niche, although sprouting neovasculature triggers metastatic progress [55].

Two signaling may involve, the first is niche-based signals that are tissue-specific [56]. For instance, factors that support and endure dormancy of DTCs from prostate cancer, breast cancer, and head and neck squamous cell carcinoma performance exclusively in the lung such as bone morphogenetic protein 4 (BMP4)[57] or in bone marrow like transforming growth factor-β2 (TGFβ2) and BMP7 regardless of their existence in other tissues, proposing that they corporate with further factors [58,59]. But thrombospondin 1 (TSP1) induce dormancy in DTCs of both bone marrow and lung, neither BMP4, TSP1, TGFβ2 nor BMP7 impacts DTCs dormancy in brain tissue [60]. The second signal is that conservation of cellular homeostasis is vital. Most dormant BC DTCs are positioned on the abluminal surface of the vascular bed of distant organ sites [60]. Ghajar et al. showed that stable endothelium induces DTCs quiescence, and perivascular TSP1 form endothelium inhibits DTCs development [60]. But, sprouting vessels cannot express significant levels of TSP1, and endothelial tip cells produce factors found in PMNs such as TGFβ1, fibronectin, periostin, versican and tenascin C proteins [60]. Whereas some processes may disrupt endothelial dormancy including, wounding, inflammation, and ageing. Primary tumor-derived factors released into the circulation may likely induce the perivascular niche [61,62].

Overall, many of the alterations derived by EVs might also impact DTCs awakening, including (i) DTCs survival and angiogenesis; (ii) Induction in metastatic activities, (iii) motivating ECM remodeling; (iv) the enrollment of bone marrow-derived cells and local inflammation.

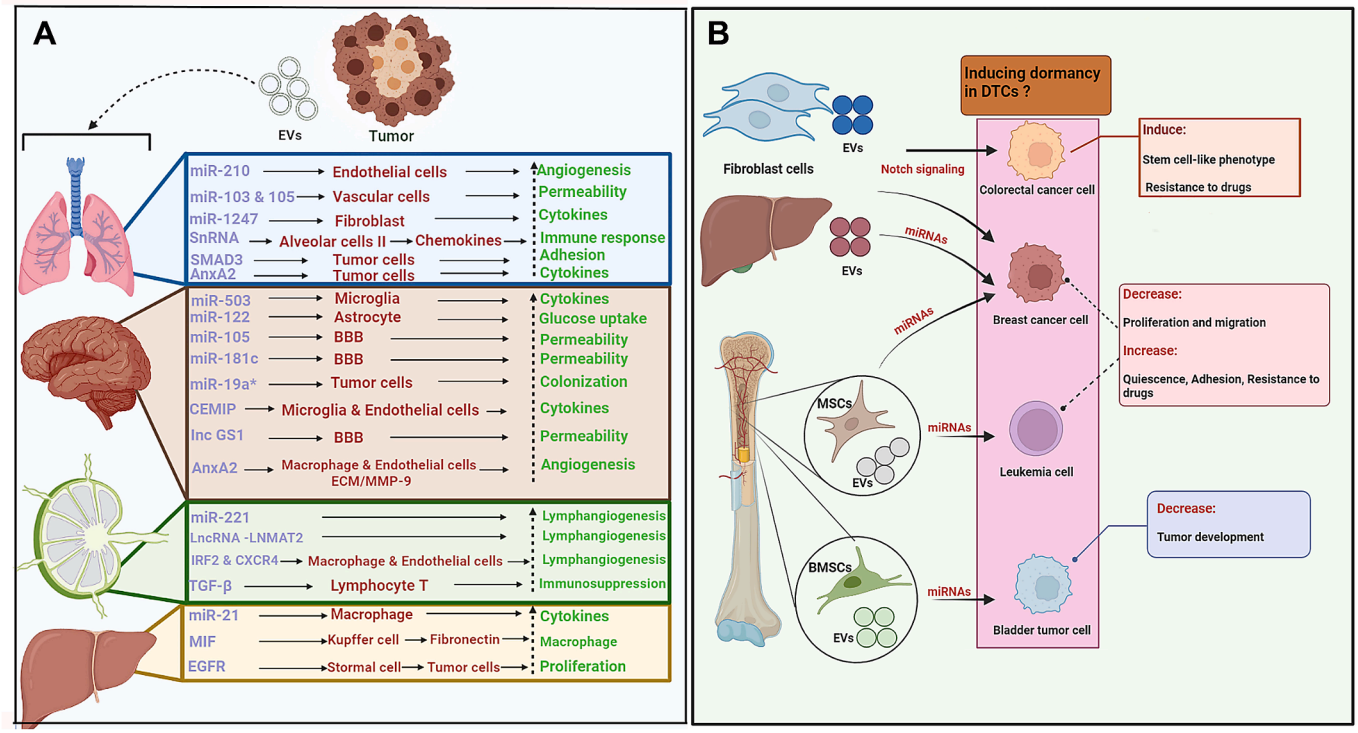

Fig. 3. Schematic illustration of the key role of extracellular vesicles (EVs) in organs metastasis (A) and inducing dormancy in disseminated tumor cells (DTCs) (B). EVs have been suggested to drive visceral metastasis through different pathways, they may participate in pre-metastatic formation and/or reawakening DTCs located at silent niches. The remodeling and pre-metastatic sites formation are requirements for effective homing and proliferation of tumor cells in the brain, lungs, lymph node, and liver. *: miR-19a is derived from astrocytes and target tumor cells. DTCs from different tumor types interact with the bone marrow, lung, brain, and liver microenvironment at distant metastatic sites. In recent years, it was demonstrated that EVs from stromal cells and/or resident cells contain many types of miRNAs that can mediate dormancy phenotype in DTCs (B). Further researches are needed to uncover the exact mechanisms involved in DTCs dormancy.

Regarding the similarities between the formation of PMNs and DTCs re-awakening, some questions remain unanswered, including; could similar mechanisms be involved in both processes? Are other factors associated with EVs in DTC re-awakening? Whether the function of different EVs from the different tumor is differing or not? What is the exact role of EVs from stromal cells on DTCs dormancy? Furthermore, it is not clear that whether EVs mediate both the formation and reactivation of niches or not? [21,22,50].

4. EVs in organs metastasis

4.1. Lung metastasis

Lung metastasis often occurs in several cancers like renal carcinomas, BC, malignant melanoma, and gastrointestinal tumors [63]. For metastasis into lung tissue, tumor cells must cross through the lung-blood barrier, which structure is made of lined ECs, the basement membrane, and lung-associated epithelial cells [37] (Fig. 3A). To infiltrate this barrier, metastatic tumor cells may express particular markers that induce flexibility in the tight junction between ECs and help cancer cell penetration into the lung [64,65]. Following invasion into the lung tissue, the majority of tumor cells pass out by tumor-stroma interface, however, few tumor cells survive for wherever from months to years and form metastatic tumors. Recent evidence suggests that EVs from metastatic tumor cells support the formation of metastasis niche in lungs through three ways, including remodeling local vascular bed, enrolment of immune cells, and rearrangement of stromal. EVs from metastatic melanoma cells have been shown to transfer activated c-MET that induce the formation of pre-metastatic niche in the lung through enrolment and regulating the bone marrow-derived cells [66]. Liu and co-workers reported that melanoma derived EVs contain many copies of small nuclear RNAs (snRNAs), up-regulating the expression of TLR3 in alveolar type II epithelial cells. In turn, it recruits neutrophils and induces immunosuppression status and formation of the pre-metastatic niche [67] (Fig. 3A). In this line, it was demonstrated that Anxa2-bearing EVs from metastatic BC cells activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), p38, MAPK, and signal transducer and activator of transcription 3 (STAT3) signaling in macrophages, which induce the production of pro-inflammatory cytokines and the formation of the pre-metastatic niche in the lung [68]. EVs can directly mediate vascular bed remodeling. For instance, Feng et al. declared that EVs derived from hepatocellular carcinoma (HCC) cells transfer miR-103 that can stimulate tumor cells motility by targeting ECs-related connection proteins and promoting vessel permeability *in vitro*. In keeping, using mice model with xenografts of miR-103-expressing HCC cells, they indicated that EVs were enriched with miR-103, vascular were highly permeable, and lung metastasis was increased [69]. Angiogenesis is an essential tool for lung metastasis. A study conducted by Kosaka et al. confirmed that the neutral sphingomyelinase 2 (nSMase2) mediate the secretion of EVs loaded with miR-210 that impact the capability for lung metastasis. These EVs transfer miR-210 to ECs, promoting angiogenesis and metastatic establishment efficacy in the lungs [70]. Modification of stromal cells completed by metastatic tumor cell-derived EVs plays a central role in forming the pre-metastatic niche. In this context, Fang and colleagues showed that EVs from HCC cells transfer miR-1247-3p into fibroblasts resident in tumor mass that target B4GALT3, resulting in the stimulation of the β1-integrin-NF-κB pathway. Subsequently, these activated cells release pro-inflammatory cytokines like IL-8 and IL-6 that increase lung metastasis [69] (Fig. 3A). Furthermore, there was evidence that HCC cells release EVs associated with both mRNAs and proteins of SMAD Family Member 3 (SMAD3) that were uptaken by recipient circulating HCC cells, which promote their proliferation and adhesion, and therefore support lung metastasis through the SMAD3-reactive oxygen species

pathway [71].

4.2. Liver metastasis

The liver is frequently targeted by tumor cells for metastasis originating from BC, gastrointestinal cancers, and malignant melanoma [72]. Drainage of intestinal mesenteric into the hepatic portal venous system makes liver parenchyma more accessible for tumor cells colonization with gastrointestinal cancer origin [64]. In contrast, the liver is partially inaccessible for BC and malignant melanoma cells for metastasis, because of their anatomical location. Thus, it is reasonable to mention that beyond the direct circulatory pathway, tumor cells-to- liver cell's communication may orchestrate liver-specific metastasis. The hepatic vasculature structure is slightly different as compared to those of the brain or the lung. The hepatic vasculature does not contain a basement membrane, as a result, metastatic cells can easily penetrate the liver as compared to the lungs and the brain [73]. In addition, different cells are resident in the liver tissue, including hepatic stellate cells, hepatocytes, sinusoidal ECs, fibroblasts, cholangiocytes, Kupffer cells, dendritic cells (DCs), and lymphocytes. Besides these cells, bone marrow-derived and circulating immune cells can infiltrate into the liver parenchyma. These immune cells are problems for tumor cells to progress to metastasis in the liver [74]. Tumor EVs contribute to the formation of a pre-metastatic niche in the liver. For instance, it was reported that EVs from pancreatic ductal adenocarcinoma (PDAC) regulate transforming growth factor (TGF)-β secretion by Kupffer cells, resulting in an augmented fibronectin release by hepatic stellate cells and enrolment of macrophages derived from bone marrow. Further scrutiny showed that the macrophage migration inhibitory factor was the vital mediator for the pre-metastatic niche formation in the liver. Authors declared that exosomal MIF reorganizes the liver tissue for metastasis and this factor may serve as a prognostic marker for the progress of liver metastasis [48] (Fig. 3A). Another study showed that EVs from pancreatic cancer cells revealing are taken by Kupffer cells and by the perivascular macrophages that induce pre-metastatic niche formation [75]. The key role of EVs miRNAs in liver metastasis has been documented. EVs enriched with miR-21-5p are released from colorectal cancer (CRC) cells can regulate the polarization of macrophages through TLR7, therefore activated macrophages release inflammatory cytokines like IL-6, inducing pre-metastatic niche formation in the liver [76]. Along with activating pro-inflammatory pathways, EVs regulate immune cell function in liver metastasis. Previous studies indicated that EVs from tumor cells interact with NK cells, leading to tumor immune evasion from NK reconnaissance [77]. These findings support the immunosuppression role of EVs in the formation of metastatic niches in the liver. Stromal cell modification can also facilitate the progress of liver metastasis. Liver metastasis-directed CRC cells release EVs that underling C-X-C chemokine receptor type 4 (CXCR4)-expressing stromal cells to progress to a metastatic microenvironment [78]. Through *in vitro* and *in vivo* experiments, Zhang et al. showed that epidermal growth factor receptor (EGFR) transferred by EVs of gastric cancer cells can be uptaken by liver stromal cells. EGFR inhibits miR-26a/b expression in the liver-associated stromal cells and induces the release of hepatocyte growth factor (HGF), which eventually interacts with the c-MET receptor on the tumor cells and support tumor cells proliferation [79].

4.3. Brain metastasis

Brain metastasis is more frequently occurred in all cancers and present nearly 10%–30% of all cancer individuals [80]. The advanced metastasis in the brain is frequently identified in the progressive stage of cancer and most patients with brain metastasis existent several tumors at the point of diagnosis. Among cancers, BC, lung cancer, and malignant melanoma are the most common primary tumors that metastasize to the brain [81]. Tumor cells reach the brain parenchyma by crossing the blood-brain barrier (BBB) [82], which is made up of the endothelium, basement membrane, and surrounding glia cells [83]. The BBB is the main barricade for different molecules and circulating tumor cells, therefore brain metastasis is dependent on the impairment of the BBB [84]. Previous studies have confirmed the association between EVs-derived noncoding RNAs and augmented the ECs penetrability in the BBB structure and brain metastasis in BC. For example, Zhou and coworkers showed that BC cells release EVs containing miR-105 that suppress ZO-1, the tight junction protein, prompting ECs permeability in the BBB and brain metastasis [85] (Fig. 3A). Tominaga et al. reported that miR-181c-5p cargo of EVs from BC cells disrupts the BBB by targeting 3-phosphoinositide-dependent protein kinase-1 (PDPK1), resulting in the deactivation of cofilin and separating of actin filaments [86]. EVs derived from brain metastatic breast tumor cells can destroy the BBB by transporting long noncoding RNA (lncRNA) GS1-600G8.5 into brain-related ECs that support the invasion of tumor cells through the BBB [87]. EVs can prime and rearrange the brain microenvironment cells for tumor cells adaptation that contribute to the development of brain metastatic tumors. EVs from brain metastatic cells can prime the brain microenvironment for boosting up tumor cell extension. Rodrigues et al. have revealed that EVs from brain metastatic cells contain hyaluronan-binding and cell migration-related proteins that generate a pro-metastatic niche, consequently supporting tumor cell colonization and brain metastasis. In addition, brain ECs and microglial cells can uptake these EVs that stimulates the secretion of chemokines and cytokines, therefore these events promote remodeling of vessels, inducing angiogenesis, and the formation of a pro-inflammatory vascular niche for metastasis [88] (Fig. 3A). Tumor cells can manipulate numerous central nervous system (CNS) protecting mechanisms and engage macrophages and microglia to colonize in CNS [89]. EVs can manage interaction among infiltrating macrophages, resident microglia, and brain resident metastatic tumor cells, which in turn rearrange the brain environment to form a pre-metastatic niche and eventually induce brain metastasis. A study by Xing et al. showed that the suppression of X-inactive-specific transcript (XIST) in brain metastatic tumors causes brain metastasis in BC. They declared that loss of lncRNA XIST induces metastatic development of BC through different mechanisms such as increasing self-renewal and aggressiveness of tumor cells, activation of c-MET, induction of EMT, and also the production of EVs bearing miR-503. Further scrutiny showed that these EVs contain abundantly miR-503 that reprogram microglia from a M1 to a M2 phenotype by regulating NF-κB and STAT3 signaling, which up-regulate the expression of programmed cell death ligand 1 (PD-L1), inhibiting immune system response [90]. In addition, EVs can interact with Toll-like receptor (TLR) 2 on macrophages and activate the NF-κB signaling pathway in macrophages that lead to the secretion of pro-inflammatory molecules like tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, C-C motif chemokine ligand 2 (CCL2), and granulocyte colony-stimulating factor (G-CSF) [91]. This TLR-dependent stimulatory signaling in macrophages may induce by multiple palmitoylated proteins ligands located on EVs membrane [91]. These results indicate that EVs derived from metastatic brain tumors have the immunomodulatory potential on infiltrating macrophages and resident microglial cells. The pivotal role of brain metastasis-associated astrocytes in the development of brain metastasis has been confirmed. Astrocytes, a glial cell, play complex roles in brain metastasis as they mediate different mechanisms of interactions with primary and secondary metastatic tumor cells [92]. The detailed mechanisms and interactions are still unknown, however recent studies showed that EVs may mediate interactions between metastatic tumor cells and astrocytes. For example, EVs from astrocytes enriched with phosphatase and tensin homolog (PTEN)-targeting miR-19a that target metastatic BC cells and inhibit PTEN in brain metastatic tumor cells. Inhibition of PTEN results in abundant secretion of CCL2 and the employment of IBA1+ myeloid cells that promote cell proliferation and inhibit apoptosis, increasing the development of brain metastatic tumor cells [93]. Several preclinical brain metastasis models have further confirmed the key role of PTEN-targeting miRNAs from EVs of breast

tumor cells in PTEN down-regulation [94]. Deregulation of cellular metabolism is a fundamental property of cancer metastasis [95]. Tumor cells have an intense craving for glucose uptake with a reprogrammed metabolism and dependence on aerobic glycolysis, which property, providing tumor cells with a tool that expedite the growth and proliferation rate of cell. In the pre-metastatic niche, tumor-derived EVs can inhibit glucose uptake by non-tumor cells and change that glucose for their consumption [96]. For example, EVs from BC cells contain miR-122 molecules, which alter glucose metabolism by targeting astrocytes *via* down-regulation of the glucose transporter 1 (GLUT1) and glycolytic enzyme M2- pyruvate kinase (PKM2), resulting in promoted tumor cell proliferation, partly refereed by improved glucose accessibility to tumor cells [96]. Thus, EVs are loaded with miRNAs that reprogram total energy metabolism to mediate circulating cancer cell colonization and development in the brain. Another function of EVs released from brain metastatic tumors is the matrix modulatory role that consequently causes tumor progression in the brain microenvironment. EVs derived from the breast tumor cell line contain annexin A2 (AnxA2), which can up-regulate the expression of matrix metalloproteinase (MMP)-9 in the brain tissue and promote proteolysis of ECM [68]. AnxA2, expressed in many tumors, has been involved in several cancer-associated roles, comprising cytoskeletal reorganization, plasminogen activation, adhesion, migration, and growth [68,97]. In addition, AnxA2 is a receptor for and plasminogen tissue plasminogen activator (t-PA) that contributes to the formation of t-PA-dependent plasmin, a protein implicated in fibrinolysis and neovascularization [98,99]. When BC cells uptake AnxA2, it may make a network with t-PA, pro-cathepsin B, plasminogen, and light chain of AnxA2 (p11/S100A10), which subsequently promote ECM degradation, plasmin formation, and induce propagation and migration of the BC cells to both brain and lungs [68]. As well, AnxA2 can support angiogenesis through interaction with endothelial t-PA [68]. EVs from human BMEC can up-regulate the expression of S100A16 in lung cancer cells. Upon internalization to target cells, EVs from BMEC facilitate displacement of S100A16 from the cytoplasm to the nucleus, which activates the expression of prohibitin-1, therefore, suppresses apoptosis in lung cancer cells [100]. In summary, EVs play a vital role in the communication between tumor cells and the brain-associated cells, which synchronize colonization and adaptation of metastatic cells in the brain microenvironment (Fig. 3A). EVs-mediated tumor metastasis and development in the brain environment are far less understood and primarily focused on breast cancer cells metastasis into the brain. Further, studies using *in vitro* and *in vivo* models may improve our knowledge of EVs-mediated brain metastatic tendency of other cancers like melanoma and lung tumors, which helps us to progress anti-metastatic therapeutic implements.

4.4. Lymph node metastasis

Lymphatic dissemination is involved in the metastasis of several types of cancers. Several studies indicated that the process of rearrangement of sentinel lymph nodes by EVs is a requirement for the enrolment and development of tumor cells. EVs deliver various bioactive molecules to target cells, adjusting their activities by a process known as "EVs-driven education" [101]. These EVs induce metastasis to SLN and distal organs by supporting angiogenesis, lymphangiogenesis, and inflammation [101]. EVs secreted by melanoma target SLN and expedite pre-metastatic niche establishment *via* up-regulating the expression of genes implicated in cell recruitment, ECM removal, and angiogenesis in the LN [102]. Many molecules including proteins and IncRNAs cargo of EVs have been shown to induce lymphangiogenesis and participate in LN metastasis. EVs are mostly uptaken by the lymphatic ECs and stimulate lymphangiogenesis. For example, EVs miR-221-3p from cervical squamous cell carcinoma (CSCC) can reach LECs and support lymphangiogenesis and lymphatic metastasis by suppression of vasohibin1, an anti-lymphangiogenic factor [103,104] (Fig. 3A). lncRNA LN metastasis-associated transcript 2 (LNMAT2) associated with EVs can stimulate LEC tubulogenesis and migration *in vitro*, and also stimulate tumor lymphangiogenesis and LN metastasis *in vivo* through VEGF-C signaling [105]. Li et al. have shown that EVs from HCC cells promote the LEC growth rate and lymphatic tubulogenesis capability *via* the direction molecules like CXCR4 [106]. Besides, EVs can regulate macrophages function in LN and lymph metastasis. Piao et al. conducted an *in vivo* model, reporting EVs from BC cells stimulate primary tumor development and axillary LN metastasis by converting polarization of macrophage from 1 to type 2 macrophages [107]. Sun et al. found that CRC-derived EVs can deliver interferon regulatory factor 2 (IRF-2) to macrophages located at SLN, which induces VEGF-C release, lymphangiogenesis, and therefore lymphatic metastasis. Ablation of macrophages with clodrosome suppresses the influence of EVs on the lymphatic arrangement and SLN metastasis, proposing that these EVs typically target macrophages [108]. In the lymphatic environment, EVs can also disrupt anti-tumor immunity *via* engaging immunosuppressive regulatory T (Treg) cells. EVs from gastric cancer individuals induce the differentiation of CD25+/CTLA4+/FOXP3+ Treg cells from naïve T cells *via* TGF-β1 signaling [109] (Fig. 3A). Furthermore, the TGF-β1 cargo of EVs is related to cancer stages and LN metastasis, therefore, suggesting the significance of these EVs as a biomarker for LN metastasis in patients with gastric cancer [109].

4.5. Bone metastasis

Bone is another and frequent location for tumor metastasis [110] and usually predicts a poor short-range diagnosis in cancer patients. According to previous studies, bone is a mutual place for prostate cancer, BC, thyroid, bladder, lung, multiple myeloma (MM), and renal cancer [111] (Fig. 4). Communication among bone cells, tumor cells, and the

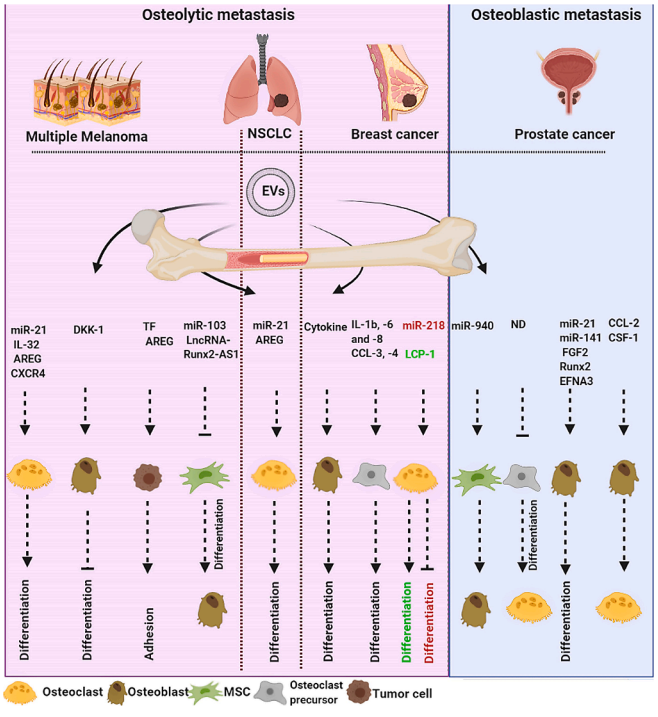

Fig. 4. Schematic representation of the role of extracellular vesicles in skeletal metastasis. EVs from MM, NSCLC, and breast cancer contain pro-osteoclastogenic molecules that induce osteoclastogenesis and osteolytic metastasis (left diagram). However, EVs from prostate cells transport various osteoblast-stimulating factors that mediate osteoblast differentiation and osteoblastic metastasis in the bone. EFNA3: fibroblast growth factor 2; PDGF-BB: platelet-derived growth factor BB; FGF2: ephrin A3; CCL4: chemokine (C-C motif) ligand 4; CCL3: chemokine (C-C motif) ligand 3; Runx2: Runt-related transcription factor 2; Dkk-1: Dickkopf-related protein 1; TF: transferrin.

bone matrix leads to the remodeling of the homeostatic bone process and inducing sclerotic and/or osteolytic injuries. MM derived EVs may contain a high level of miR-103a-3p that can impede osteogenesis in bone marrow-derived MSCs *in vitro* [112]. Nielsen et al. reported that EVs resealed by MM contain procoagulant phospholipid and tissue factor (TF) that facilitate cell invasion into distant skeletal places [113]. In the MM mice model, EVs from MM could inhibit osteoblast differentiation *via* the transmission of Dikkopf-1 (DKK-1) and down-regulation of osterix, runtrelated transcription factor 2 (Runx2), and collagen 1A1 in osteoblasts [114]. Li et al. found that lncRNA RUNX2-AS1 are packed into EVs from MM and transferred to MSCs, therefore suppressing the osteogenic potential of MSCs [115] (Fig. 4). EVs of MM can also upregulate the expression of CXCR4 and stimulate pre-osteoclast relocation. Incubation of MM derived EVs with pre-osteoclasts results in the expression of osteoclast markers like MMP-9, cathepsin K, and tartrate-resistant acid phosphatase (TRAcP), which in turn promotes differentiation of osteoclast into multinuclear osteoclasts with an ability to hollow out resorption lacunae [116]. Under hypoxic conditions, it was demonstrated that EVs from MM deliver IL-32 into cells and activate NF-kB signaling, which induce osteoclastic activation and differentiation [117,118]. Raimondo et al. reported that MM-derived EVs transfer the EGFR ligand Amphiregulin to MSCs and inhibit osteogenic differentiation *via* the secretion of IL-8 and inducing of the EGFR signaling [116]. EVs from non-small cell lung cancer (NSCLC) promote EGFR phosphorylation, which increases expression of receptor activator of nuclear factor-kappa B ligand (RANKL) and also MMP-9 and TRAcP, osteoclastogenic markers [119]. Amphiregulin cargo of EVs was found to be a significant molecule for inducing the EGFR signaling, making the distinctive type of mature osteoclasts [116,120]. Lung adenocarcinoma derived EVs-associated miR-21 may expedite osteoclastogenesis *via* targeting programmed cell death 4 (PDCD4), proposing a potential mechanism of bone metastasis [121]. BC-derived EVs shuttle active proteins that cause BC cells distribution toward particular metastatic homes [122] (Fig. 4). Metadherin and Ceruloplasmin, membrane proteins, are present on tumor EVs and are suggested to mediate the place-specific distribution of BC cells [122]. A growing body of evidence has revealed the aptitude of BC -derived EVs to affect the activities of resident cells of the bone microenvironment and induce bone osteotropism. These EVs bear the cytosolic protein L-plastin that interact with PRDX4, which promote osteoclastogenesis and therefore cause osteolysis. Hashimoto et al. declared that hsa-miR-940 carried by prostate cancer-derived EVs can stimulate differentiation of MSCs into the osteogenic

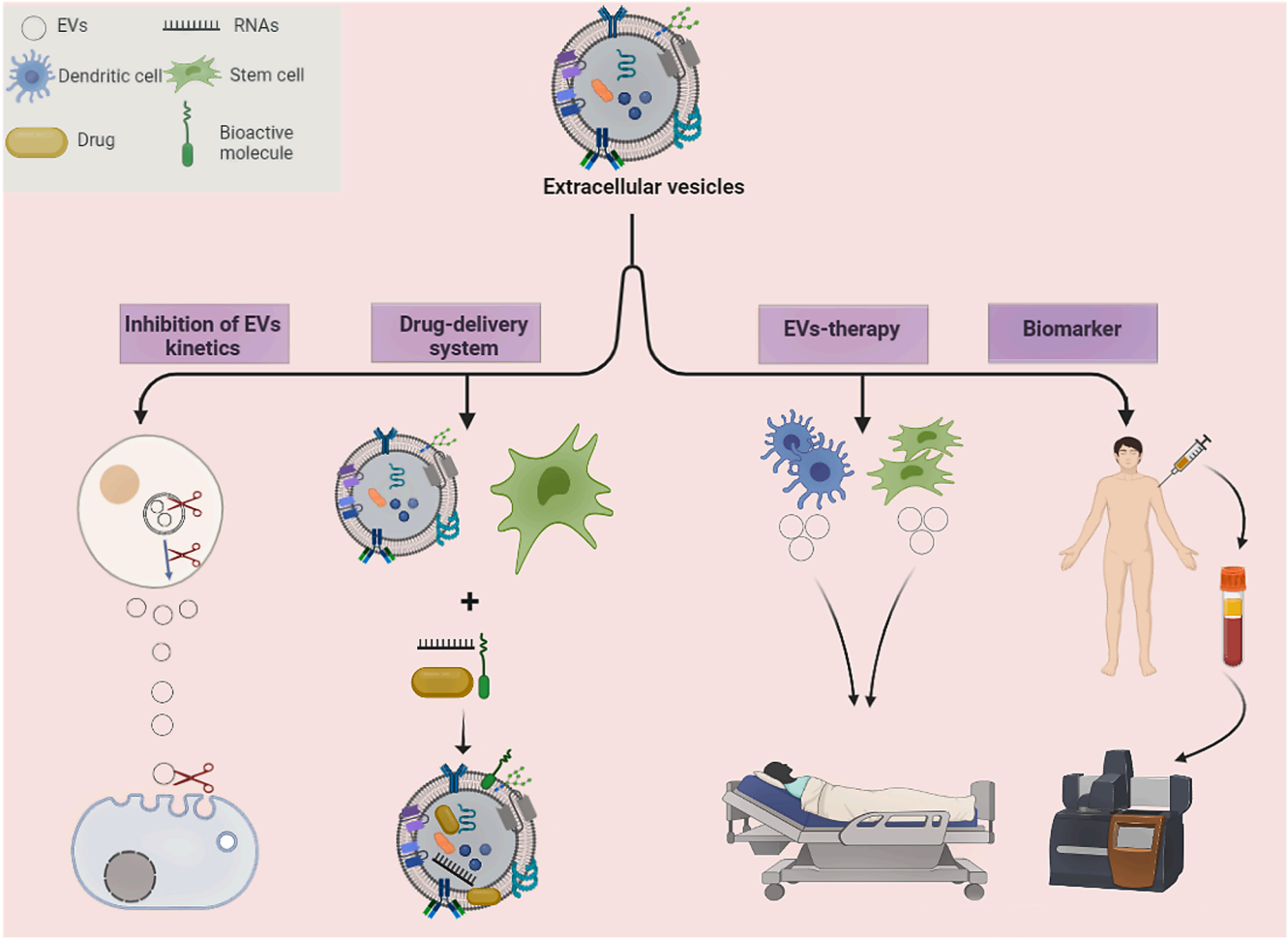

Fig. 5. Clinical application of EVs. Recently, EVs have progressively become multifunctional and promising therapeutic tools in multidisciplinary medicine. The panel illustrates for preventing tumorigenesis, inhibiting EVs biogenesis, secretion, and uptake may be a useful method. EVs can be used as a drug-delivery system to deliver optionally and loaded EVs to target organs/tumor by two methods in which isolated EVs or/and are manipulate to load therapeutic agents (drugs and biomolecules). Another potential is to use EVs as cell-free therapy for various diseases such as regeneration and cancers. A safe and confident source cell must be selected to purify EVs, for example, mesenchymal stem cells and dendritic cells exhibit regenerative and antitumor (EVs-based vaccines) properties against degenerative and cancer, respectively. Finally, as EVs distribute through body fluids, this makes them accessible by a simple liquid sampling. Isolated EVs maybe serve as a diagnostic biomarker for many cancers. Indeed, EVs have been considered as fingerprints, representing the status of cancer through analyzing their cargo. For example, different researches show that proteins and RNAs molecules of EVs are differently expressed in EVs derived from cancer cells compared to those derived from healthy cells or patients.

cells by inhibiting FAM134A and ARHGAP1 [123]. Another interesting feature of EVs is their ability to induce dormancy in BC cells in the bone marrow. *In vitro* study by Ono et al. indicated that EVs from BMSC can promote dormancy in MDA-MB-231 cells *via* miR-23b-facilitated inhibition of MARCKS, which codes a protein regulating cell cycling and migration [124]. Contrariwise, BC cells have been revealed to dictate MSCs to secret EVs inducing dormant phenotypes in tumor cells and promoting drug-resistance by specific miRNA cargo like miR-222/223 [51], authors proposed that these EVs could mediate DTCs dormancy. The evidence from this section suggests that EVs manage interactions between tumor cells and the host bone microenvironment *via* active cargo and inducing dormancy and tissue remodeling (Fig. 4).

5. The therapeutic opportunity of EVs in cancer management

The interdisciplinary importance of EVs-based studies has fascinated emergent interests, and the EVs methodical platforms for their antitumor, drug-delivery and diagnostic prospects have significantly developed (Fig. 5). According to previous studies [18,125–127], we can categorize approaches using EVs as a novel avenue for cancer management as follows: (i) Inhibition of EVs biogenesis and secretion from tumor cells as well as blocking EVs uptake by other cells. In this opinion, called targeted therapy, such inhibitors or drugs may be useful for inhibiting or reducing EVs production to inhibit the formation of pre-metastatic niches. Although this method seems to be effective for inhibiting EVs, there are some limitations associated with selecting a specific inhibitor with low side effects on healthy cells and other organs. Another concern is that we exactly do not know that an inhibitor affects all the EVs or a distinct type of EVs such as exosomes. In addition, most studies on EVs inhibition are carried out *in vitro* with a focus on discovering EVs kinetics and biogenesis mechanisms. (ii) Using EVs as a drug-delivery system. A growing body of experiments has shown the unique properties of EVs that make them ideal for delivering anticancer agents (biological molecules and drugs) to cancer cells (Fig. 5). Therefore, researchers have attempted to load EVs with therapeutic agents by two methods well-known as (for further study, see review articles [128,129]): direct loading mechanism and indirect loading mechanism. In a direct method, EVs from a source are loaded with therapeutic agents on their lumen or surface. In an indirect method, source cells either are genetically manipulated to express active molecules on their own EVs or co-cultured with selective agents (usually drugs) to load agents into EVs by the endosomal pathway or the plasma membrane shedding. Therapeutically active agents can be packaged into EVs by either active or passive methods. This approach bears some concerns; first, the main challenge is selecting confident and safe source cells with a high level of EVs production potential, second selecting an operational and sensitive administration method for delivering EVs into the site of tumor cells, because EVs may be captured by the lungs and liver or deformed during loading processes, which can affect the efficiency of EVs. (iii) EVs-based therapy is another application of EVs in medicine [130,131] (Fig. 5). Many researchers reported that EVs from stem cells have cytotoxic and anti-proliferative effects on cancer cells, therefore they used cell-free therapy term against term cell therapy. For example, EVs from immune cells or MSCs have been shown to play pernicious roles against cancer cells in pre-clinical experiments. Personalized preparing EVs-based immunotherapy vaccines is a promising strategy to combat cancer. Many experiments confirmed that antigen-loaded EVs from DCs could induce effective anticancer immunity (see review articles [132,133]). EVs from DCs are immunotherapeutic agents and have been engaged as cell-free antitumor vaccines even in some clinical trials, such as one phase II [134] and two phases I [135,136] clinical trials in cancer patients. This approach also bears some limitations such as selecting a distinct subset of DCs, selecting donor DCs, and costly equipment. (iv) Using EVs as a biomarker for different cancers (Fig. 5). As EVs are present in different body fluids and have distinct cargo, they may be an ideal candidate for monitoring and diagnosis of cancers by a simple liquid sampling. Analyzing EVs proteins and RNAs is a novel tool for medications to predict the status of tumors in individuals with cancer. Many laboratories have examined the application of EVs for the diagnosis of cancers, in both *in vivo* and clinical trials (see references [101,137,138]). These EVs are present in the blood of patients with breast, glioblastoma, ovary, lung, prostate, melanoma, colorectal, and stomach cancer [101]. However, there is still concern regarding the application of EVs as biomarkers, for example, first, similarly to RNA and protein investigation, the EVs population isolated from body fluids is frequently heterogeneous. Thus, heterogeneous EVs may give various protein/RNA expression patterns and profiling, which mistakes in biomarkers annotation. The second issue is the absence of a common and confirmed biomarker for all cancers. Overall, by multidisciplinary associations in cell and molecular kinetics, inhibiting, engineering, analyzing, and medicine, we believe a hopeful future for clinical translation of EVs-based investigation for cancer treatment.

6. Conclusions

Advances in improvements of personalized and precision cancer medicine have increased the need for a detailed understanding of mechanisms implicated in metastasis. A strong relationship between EVs and metastasis has been reported in the literature. Before the arrival of tumor cells in target organs, EVs can mediate organ-specific metastasis and direct the organotropism in different metastatic tumors. A variety of molecules were present in EVs that drive organ-specific metastasis and are important regarding application for cancer management and clinical consequences. However, instead of pre-metastatic niche formation, there is evidence that these EVs may participate in reawaking of DTCs in dormant niches. Outlining the detailed mechanisms applied by EVs to support the formation of pro-metastatic niches at distant organs is vital for understanding metastatic development. Understanding the mechanisms governing EVs-related metastasis will inform innovative treatment opportunities, which comprise targeting EVs kinetics, using EVs targeted drug delivery, EVs-therapies, and using EVs as a biomarker to future antitumor treatments. The majority of researches have underlined the many roles and signaling driven by EVs in cancer progression and metastasis using isolated EVs from cell medium, which eventually was administrated into animal models. Thus, despite progress in the EVs field, future studies on the current topic are therefore recommended.

CRediT authorship contribution statement

Conception and manuscript design: J. R., and M. A. Collection of date: B. M, S. M., S. A., K.S., and N.J.B. Writing - Original Draft: T. E., and R.R. Writing - Review & Editing: J.R., and M.A. All authors read and approved the final manuscript.

Declaration of competing interest

The authors have no competing interests to declare.

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Availability of data and materials

Data and materials of this study are available upon request to the corresponding author.

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