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Review Article

Engineered exosomes-based theranostic strategy for tumor metastasis and recurrence

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ABSTRACT

Metastasis-associated processes are the predominant instigator of fatalities linked to cancer, wherein the pivotal role of circulating tumor cells lies in the resurgence of malignant growth. In recent epochs, exosomes, constituents of the extracellular vesicle cohort, have garnered attention within the field of tumor theranostics owing to their inherent attributes encompassing biocompatibility, modifiability, payload capacity, stability, and therapeutic suitability. Nonetheless, the rudimentary functionalities and limited efficacy of unmodified exosomes curtail their prospective utility. In an effort to surmount these shortcomings, intricate methodologies amalgamating nanotechnology with genetic manipulation, chemotherapy, immunotherapy, and optical intervention present themselves as enhanced avenues to surveil and intercede in tumor metastasis and relapse. This review delves into the manifold techniques currently employed to engineer exosomes, with a specific focus on elucidating the interplay between exosomes and the metastatic cascade, alongside the implementation of tailored exosomes in abating tumor metastasis and recurrence. This review not only advances comprehension of the evolving landscape within this domain but also steers the trajectory of forthcoming investigations.

1. Introduction

Metastasis stands as the paramount contributor to mortality in the oncological realm [1]. Despite advancements in multimodal therapeutic regimens, entailing a fusion of surgical, radiotherapeutic, chemotherapeutic, immunotherapeutic, and targeted therapeutic modalities, the overall prognosis for metastatic tumors remains adverse [2]. The constraints and adverse effects inherent

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in these therapeutic avenues have engendered an escalating imperative to explore novel treatment avenues. In recent years, the intricate role of extracellular vesicles (EVs) in processes encompassing tumorigenesis, proliferation, metastasis, programmed cell death, immune responses, and resistance to chemotherapy has gradually come to light [3–6], galvanizing researchers' focus on EVs. These EVs can be categorized into three distinct subclasses based on their genesis, composition, and dimensions: exosomes (30–120 nm), microvesicles (50 - 1000 nm), and apoptotic bodies (50–5000 nm). Exosomes originate from EVs formed by multivesicular bodies (MVBs) on the plasma membrane of cells. They release exosomes into the extracellular space through exocytosis, which can interact with the extracellular matrix or induce cellular reactions within or far from the microenvironment [7]. On the other hand, microvesicles are produced by the plasma membrane directly to the outgoing bud for EVs populations of varying sizes. Apoptotic bodies are mainly released by apoptotic cells during cell division [7,8]. Exosomes, uniquely distinguished from their EV counterparts, stem from a distinct biological origin and exhibit distinctive physical attributes [9]. Encased within lipid bilayers, they ferry an assortment of proteins, RNA, DNA, lipids, and other bioactive molecules, leveraging these cargoes to orchestrate intercellular communication and cellular reprogramming [10,11]. The application of unmodified exosomes in tumor therapeutics presents a range of inherent advantages, encompassing: (1) enduring stability during circulation, facilitating their long-range conveyance within the organism due to heightened bloodborne stability; (2) a hydrophilic core conducive to the encapsulation of both hydrophobic and hydrophilic pharmaceutical agents via exosomal lipid bilayers [12]; (3) diminished immunogenicity affording heightened biocompatibility and biodegradability relative to synthetic nanoparticles; (4) exploitation of the enhanced permeability and retention (EPR) effect to achieve targeted accumulation within tumor or inflammatory tissue locales [13], including traversing the blood-brain barrier [14]; (5) possession of tumor homing attributes, as exosomal surface antigens can selectively target primary tumors, metastatic foci, and even premetastatic niches (PMNs) [15].

Despite the advancement in utilizing natural exosomes for therapeutic purposes [16–19],our inability to fully control the characteristics of natural exosomes such as EV heterogeneity, size and charge range, characterization of intrinsic content, biological distribution studies, storage conditions, and more importantly, the inability to target them to specific regions as we want pose challenges that diminish therapeutic efficacy and potentially introduce safety concerns [20]. This drawback restricts their broader adoption and implementation within the context of tumor treatment. In light of evolving nanotechnology, nanoscale exosomes exhibit comparable size effects and biochemical attributes to nanoparticles. They offer considerable potential as versatile carriers for nucleic acids, proteins, drugs, and nanomaterials, boasting attributes such as enhanced circulatory stability, tissue penetrability, tumor selectivity, and biocompatibility [21,22]. Researchers are currently engaged in merging the distinctive merits of natural exosomes with the sophistication of nanotechnology to craft engineered exosomes endowed with both diagnostic and therapeutic functionalities. These promising prospects underlie the ongoing endeavors in this direction [23,24]. Notably, the dynamic advancement of nanotechnology has substantiated its prowess in tailored modifications [18,25–28]. In the future, the research emphasis on curtailing tumor metastasis will likely gravitate towards comprehensive multi-modal interventions grounded in nanotechnology.

While extant reviews have collated insights on engineered exosomal attributes and their progress within the oncological landscape [21,29], a comprehensive and forward-looking examination of their applicability to tumor metastasis remains conspicuously absent. This review, therefore, aspires to furnish a thorough exposition of the intricate interplay between exosomes and tumor metastatic processes. Moreover, it will expound upon diverse methodologies employed in the engineering of exosomes at their present juncture, as well as illuminate their practical utilization in mitigating tumor metastasis. Through this comprehensive undertaking, a more profound comprehension of the ongoing research strides within this domain will be achieved, thereby charting a course for future investigations.

2. The relationship between natural exosomes and tumor metastasis

Exosomes play a pivotal role in mediating intercellular communication during the progression of tumorigenesis [30]. These EVs function as key mediators within the complex network of communication between cancer cells and the microenvironment of the tumor. During the initial phases of metastasis initiation, exosomes initiate an elevation in proinflammatory moieties and provoke vascular permeability at metastatic loci, thereby establishing an environment conducive to niche formation. Subsequently, exosomes facilitate their transfer to remote organs by orchestrating the recruitment of bone marrow-derived cells (BMDCs), endothelial progenitor cells, and mesenchymal cells, effectively priming the PMNs [31]. The cargo of exosomes, encompassing diverse RNA and protein molecules, has been identified as a potent driver of metastatic processes. These encompass various facets such as the regulation of cell polarity and directional migration, modulation of immune responses, orchestration of the extracellular matrix (ECM), facilitation of intravasation and extravasation, as well as the determination of organotropism, as detailed in Table 1 [31,32]. As cancer cells intrude into lymphatic or microvascular networks and navigate the systemic circulation to reach remote anatomical destinations, a subset of disseminated tumor cells (DTCs) that manage to elude immune surveillance establish residence and enter a dormant state. These quiescent DTCs constitute the fundamental origin of both tumor metastasis and relapse. Exosomes are speculated to orchestrate modifications within dormant microenvironments, reactivating quiescent DTCs. Consequently, micrometastatic foci stemming from these reactivated DTCs progressively gain proficiency in establishing residence within host tissues, ultimately evolving into macrometastases [33]. Intriguingly, exosomes originating from distinct tumor types display preferential homing

Table 1 – Exosomes molecules that support the metastatic process.

Molecule	Mechanism	Exosomes cell origin	Refs
Controls cell polarity and direction of cell movement			
Tetrapeptin Cd81	Promoted cell motility and metastasis by activating autocrine WNT-PCP signaling	Breast cancer cell	[35]
RAB27A	Promoted the biogenesis of a pro-invasive exosome population	Melanoma cell	[36]
CircPACRGL	Through the miR-142–3p/miR-506–3p-TGF-1 axis, it helped CRC cells proliferation, migration and invasion, as well as differentiation of N1 to N2 neutrophils	Colorectal cancer cell	[37]
miR-146a, TXNIP	The activation of the WNT signaling pathway was enhanced through either overexpression of miR-146a or silencing of TXNIP	Breast cancer cell	[38]
miR-21–5p,miR-155–5p	Transferred through macrophage-derived exosomes, bonded to the BRG1 coding region, and down-regulated BRG1 expression in colorectal cancer cells	Colorectal cancer cell	[39]
Wnt11	Fibroblast exosomes mobilized autocrine Wnt-PCP signaling to drive breast cancer cell invasive behavior	Breast cancer cell	[35]
Intravasation			
miR-27b-3p	Exosomal miR-27b-3p produced by EMT-CRC cells promotes the formation of circulating tumor cells (CTCs) by increasing blood vessel permeability	Colorectal cancer cell	[40]
miR-939	Down-regulation of E-cadherin disrupted the endothelial barrier and promoted transendothelial migration of MDA-MB-231-GFP cells	Breast cancer cell	[41]
Matrix metalloproteinases, IL-8, PDGFs, and lysyl oxidase	Hypoxia can reprogramed the phenotype of endothelial cells, induce the secretion of growth factors and cytokines, and trigger the activation of PI3K/AKT signaling pathway and migration in pericytes	Glioblastoma multiforme cell	[42]
VEGF-A	Improved the permeability and angiogenesis potential of human endothelial cells	Glioblastoma multiforme cell	[43]
Extravasation			
miR-181c	By down-regulating PDPK1, induced abnormal localization of actin and promoted the destruction of the blood-brain barrier	Brain metastatic cancer cell	[44]
miR-105	By down-regulating tight junction protein ZO-1, induced metastasis and vascular permeability in distant organs	Breast cancer cell	[45]
Regulation of ECM and EMT			
CD44	The protease released by CD44 binds with hyaluronic acid can degrade collagen, lamin, lymph nodes and fibrin in the matrix	Pancreatic ductal adenocarcinoma cell	[46,47]
Integrin $\alpha 6\beta 4$	The protease released by $\alpha 6\beta 4$ binding with laminin 332 can degrade collagen, lamin, lymph nodes and fibrin in the matrix	Pancreatic ductal adenocarcinoma cell	[46]
MMP13	Promoted cancer metastasis by promoting angiogenesis and ECM degradation	Nasopharyngeal cancer cell	[48]
SNAI1	By transferring SNAI1 to recipient cancer cells through exosomes, CAFs triggered EMT in lung cancer cells	Lung cancer	[49]
LINC00963	Siah1 mRNA was degraded as a result of LINC00963's direct interaction with the HNRNPA2B1 protein, which also prevented Zeb1 from being ubiquitinated and degraded	Lung adenocarcinoma	[50]
Regulation of immunity			
FasL	Activated T cell exosomes increased MMP9 expression through Fas/FasL pathways and promoted the invasion of B16 and 3LL cancer cells	B16 and 3LL cancer cells	[51]
miR-29a-3p, miR-21–5p	miRNA induced an imbalance of Treg/Th17 cells in epithelial ovarian cancer, resulting in an immunosuppressive microenvironment that promotes EOC progression and metastasis	Epithelial ovarian cancer	[52]
Organotropism			
MET	Educated bone marrow progenitor cells through the receptor tyrosine kinase MET and reprogrammed bone marrow progenitor cells to an angiogenic phenotype	Melanoma cell	[53]
ENO1	The FAK/Src-p38MAPK pathway is activated by up-regulating the expression of integrin $\alpha 6\beta 4$, which promotes the growth and metastasis of HCC cells	Hepatocellular carcinoma cell	[54]
Hsa-miR-940	Induced a bone metastatic phenotype leading to extensive osteoblastic lesions by targeting ARHGAP1 and FAM134A	Prostate and breast cancer cell	[55]

to specific organs, a phenomenon guided by exosomal integrin content, which has demonstrated predictive value for metastatic destinations. Notably, exosomes showcasing distinctive integrin expression profiles are internalized by organ-specific cells, contributing to pre-metastatic niche preparation. This phenomenon results in organ-specific patterns of pre-metastatic niche characteristics, thereby influencing future organotropic metastatic preferences, including lungs, liver, bone, and lymph nodes [15,34].

Furthermore, the transfer of RNA molecules orchestrated by exosomes has been found to actively participate in driving cells towards a metastatic phenotype within an *in vivo* context. Emanating from highly metastatic MDA-MB-231 cells, exosomes ferrying migration and metastasis-associated mRNAs are internalized by low-metastatic T47D cells, both within the same tumor milieu and at distant tumor sites. Intriguingly, this uptake of exosomes from their highly metastatic counterparts results in enhanced migratory behavior and heightened metastatic capability of the recipient T47D cells [56]. Conversely, exosomes released by non-malignant cells can infiltrate metastatic niches, thereby exerting potential influences on the behavior of metastatic cancer cells. Notably, exosomes derived from astrocytes contain microRNAs with the capacity to suppress PTEN expression within pre-metastatic niche cells, thereby fostering the colonization and proliferation of brain-metastasizing tumor cells within the brain microenvironment [57]. Furthermore, an assortment of pro-epithelial mesenchymal transition (EMT) inducers borne by exosomes originating from tumor sources, encompassing factors such as TGFβ, HIF1α, and β-catenin, serve to not only heighten the migratory and invasive potential of recipient cells but also play a role in fostering the genesis of pre-metastatic microenvironments. These factors potentially underlie instances of tumor relapse subsequent to the surgical excision of primary tumors formed locally [56].

Nevertheless, it is imperative to note that not all endogenous exosomes inherently contribute to the initiation of tumor metastasis. Specific subcategories of tumor-derived exosomes (TEXs) exhibit the capacity for pronounced expression of major histocompatibility complex Class I molecules (MHC I), alongside heat shock proteins, notably HSP70 and HSP90. The pivotal roles of these molecules manifest in facilitating antigen presentation and the activation of T cells, thus inciting $CD8^+$ T cell-mediated anti-tumor responses that hold significant biological relevance [58]. In this context, activated $CD8^+$ T cells themselves release exosomes which serve to target and eliminate tumor cells, concurrently impeding tumor invasion and metastasis orchestrated by fibroblastic stroma [58,59]. Dendritic cell-derived exosomes (DEXs), arising from specialized antigen-presenting cells, are distinguished by their potential to bear membrane-associated MHC peptide complexes and an array of co-stimulatory molecules, including CD86 and HSP70–90 chaperones. These moieties effectively engage $CD4^+$ and $CD8^+$ T cell populations, thereby fostering immune cell-mediated repudiation of tumors [60].

Notably, exosomes of natural killer cell origin manifest not only distinctive NK cell markers such as CD56, perforin, and granitase B cytotoxic molecules but also exhibit specific identifiers like tsg101, CD81, CD63, and CD9. This constellation of molecules confers the capability to elicit tumor apoptosis *in vivo*, thereby penetrating tumor tissues and executing cytolytic functions [34,56]. The transport of tumor suppressor miR-186 by these exosomes proves efficacious in dampening the tumorigenic potential and undermining immune evasion mechanisms [61]. Emanating from immune cells, these exosomes effectively evoke immune responses that culminate in the eradication of cancer cells in the preliminary stages preceding metastasis. However, it is imperative to acknowledge the limited corroboration substantiating the direct interference of these immune cell-derived exosomes in the intricate metastatic cascade.

3. Exosomes engineering strategies

Considering the discrete roles played by these intrinsic exosomes within the intricate framework of tumor metastasis, diverse engineering methodologies can be harnessed for their selective manipulation. A subset of exosomes participates in the multifaceted cascade of tumor metastasis in a complicit manner. Consequently, interventions may be directed towards the modulation of the secretion dynamics of these exosomes. Alternatively, this strategic utilization of implanting and concealment can safely deliver drugs to the target, similar to a "Trojan horse", which is expected to promote targeted contact with the metastatic site and promote cancer cell death. Conversely, another faction of exosomes contributes to fortifying the tumor's resistance against invasion and metastatic dissemination. In this context, augmentation of the exocytosis process from their cellular origins or potentiation of their inherent anti-tumor attributes through external molecular modification emerges as a viable avenue [26,62,63].

The pursuit of these objectives commonly involves the amalgamation of nanotechnology with complementary techniques, resulting in the generation of engineered exosomes distinguished by elevated purity and heightened specificity. In contrast to their natural counterparts, these tailored exosomes preserve the intrinsic functional traits synonymous with exosomal entities. Notably, the engineered exosomes exhibit superior colloidal stability, enhanced capacity for drug encapsulation, heightened amenability to molecular modifications, and refined aptitude for tumor-directed localization following purposeful adjustments. Consequently, they emerge as a propitious platform for the conveyance of therapeutic agents through the conduit of natural exosomes [64–67]. In the parlance of the scientific community, these bespoke constructs are referred to interchangeably as biomimetic exosomes or artificial exosomes. Delving into the realm of engineering strategies, a tripartite categorization can be delineated, encompassing bottom-up, top-down, and hybrid paradigms. The selection and implementation of these strategies are contingent upon the nature of the targeted modifications and the techniques enlisted (Fig. 1).

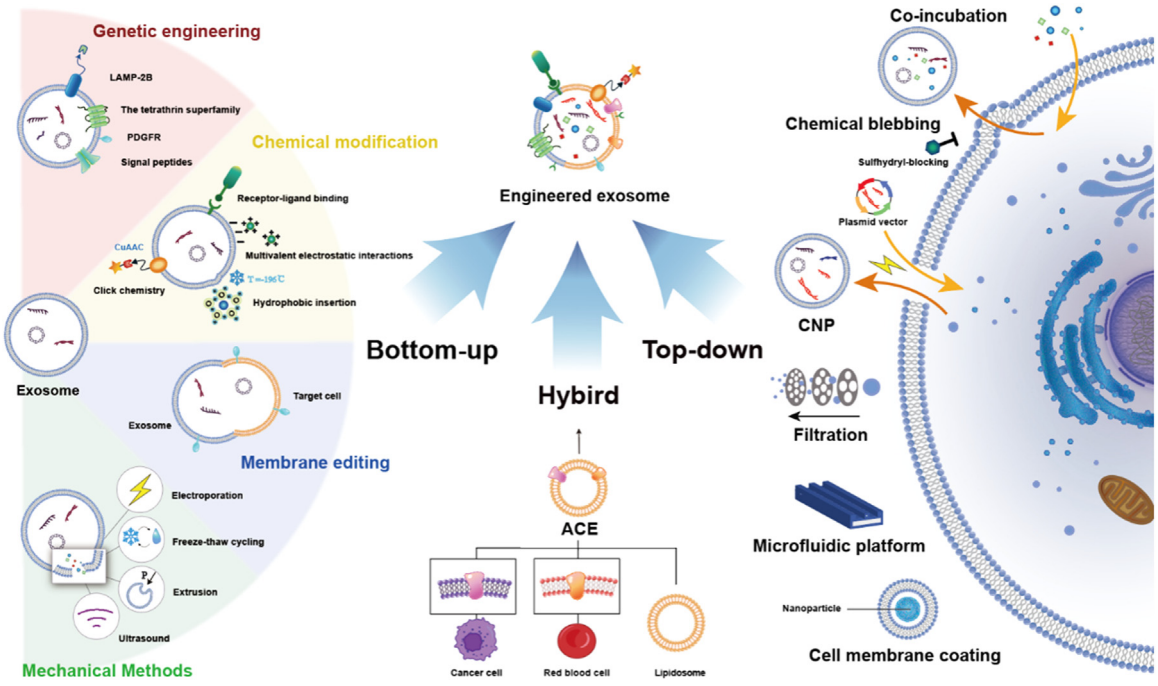

Fig. 1 – The engineering strategies of exosomes can be divided into bottom-up, top-down and hybrid biomimetic strategies. (A) Bottom-up strategies are exosome or single molecule-based assembly into larger and more complex engineered exosomes, including genetic engineering, chemical modification, membrane editing techniques, and mechanical methods. (B) Top-down strategies gradually disassembles cells into vesicular structures with specific functions, including co-incubation, chemical blebbing, CNP, filtration, microfluidic technology, and membrane package. (C) The hybrid biomimetic strategy combines cell membrane proteins and liposomes of various cell types to design artificial chimeric exosomes (ACEs), which have the characteristics of tumor targeting and multifunctional customization.

3.1. *Bottom-up approach*

The bottom-up paradigm delineates an approach characterized by the systematic construction of intricate and augmented architectures originating from individual molecules, accomplished through a progressive assembly methodology [23]. Tailored biomimetic exosomes can be realized via the integration of nano-scaled synthetic lipid bilayers with proteins. Techniques such as co-hatching, chemobiological conjugation, or de novo protein synthesis can be adeptly employed for this purpose [64]. This strategy conveys the advantage of mitigating uncertainties associated with cell cultivation and the subsequent isolation and purification of exosomes. Importantly, it enables the direct and streamlined generation of a substantial reservoir of standardized engineered exosomes, a pivotal advancement with profound implications for the prospective large-scale manufacturing of engineered exosomes, primed for imminent clinical applications.

3.1.1. *Genetic engineering*

The genetic engineering of exosomes finds its basis in the principles of gene editing, wherein a ligand or target peptide is integrated with the gene encoding the transmembrane protein expressed on the exosomal surface. This culminates in the synthesis of modified exosomes featuring the designated target ligand on their surface. The secretion of these engineered exosomes occurs subsequent to the transfection of donor cells with a plasmid harboring the fusion protein encoding sequence [62]. This innovative approach facilitates the transmission of the encapsulated plasmid DNA to recipient cancer cells, thereby instigating precise and targeted elimination of oncogenes implicated in metastatic processes [68]. Notably, LAMP-2B has emerged as a preeminent exosomal surface protein, frequently exploited for its distinctive targeting attributes. To realize genetic manipulations, the targeting peptide can be conjoined with the N-terminal extracellular domain of LAMP-2B to confer the desired targeting proficiency [69,70]. Beyond LAMP-2B, other proteins like PDGFR, members of the tetraspanin superfamily including CD63, CD9 and CD81, alongside signal peptides, constitute recurrent choices for membrane display [62].

It is imperative to acknowledge that the introduction of ligands might potentially perturb the normal functionality of exosomal membrane proteins. As such, refinement of purification methodologies is essential to effectively isolate surface-modified exosomes [71].

3.1.2. *Chemical modification*

Both covalent and noncovalent modification strategies stand as prevalent methodologies for effecting stable and controlled alterations to exosome surfaces. A distinctive advantage of exosomes, owing to their non-living nature, resides in their amenable susceptibility to modifications

with reagents and reaction conditions deemed incompatible with the functionalization of living cells [72]. A frequently employed covalent modification technique is grounded in the principles of click chemistry, particularly the copper-catalyzed azide-alkyne cycloaddition. This approach adeptly facilitates the bioconjugation of an assorted array of molecules, encompassing small and big molecules, onto the exosomal surface [73]. Noteworthy attributes of this reaction comprise its efficiency, mildness, and capacity for precise manipulation of the conjugation loci. Furthermore, covalent modifications confer robust stability without compromising the physicochemical attributes of exosomes, thereby circumventing potential risks linked to exosomal damage and unintended DNA transfer [74]. However, it is pertinent to acknowledge that non-specific binding of targeting moieties on exosomes may impose limitations on the applicability of click chemistry for surface modification [71].

In the realm of noncovalent modification strategies, encompassing multivalent electrostatic interactions, receptor-ligand bindings, and hydrophobic insertions, the latter holds significance. Notably, the creation of exosome-liposome hybrids through hydrophobic insertion has demonstrated remarkable targeting prowess while concurrently preserving the intrinsic functionality of exosomal membrane proteins. Despite its promise, this approach presents inherent challenges. Disruption of vesicle membranes for fusion necessitates repeated cycles of freeze-thaw (−196 °C) or elevated temperature (4.0 °C). However, by co-incubating minute lipophilic entities at ambient temperatures (25–37 °C), hydrophobic interactions catalyze their spontaneous integration into exosome membranes. The efficacy of this process positively correlates with the hydrophobicity of the introduced species [75]. The resultant exosome-liposome hybrids are characterized by potent targeting capabilities that remain unobtrusive to the exosomal membrane protein functionalities. This technique has already proven successful in the loading of small lipophilic therapeutic agents and a variety of commercially available EV membrane stains [76,77]. Its immense potential lies in the potential to load therapeutic agents tailored for targeted delivery to micro-metastatic sites, concurrently enabling real-time monitoring.

3.1.3. *Membrane editing technology*

Capitalizing on the shared compositional and functional attributes between exosomes and viruses, a pioneering biocompatible nanoplatform has been devised. This innovative approach involves the fusion and modification of exosomes with the plasma membrane, facilitated through the application of "membrane editing" technology. This strategic manipulation engenders the seamless integration of foreign proteins onto the target cell membrane. Leveraging the intrinsic biocompatibility and non-toxic nature of exosomes, this technique enables the natural localization and expression of bioactive membrane proteins upon the exosomal surface [78]. However, it is important to note that akin to exosomes, inherent heterogeneity can pose challenges in practical implementation, potentially leading to the generation of particles characterized by substantial size discrepancies and elevated polydispersion indices, undermining size homogeneity [65]. The membrane editing technology, however, holds promise in potentially attenuating the metastatic potential of cancer cells by selectively eliminating specific antigens implicated in metastasis, thereby potentially curbing their invasive and metastatic propensities.

3.1.4. *Physical methods*

An array of mechanical methodologies currently exists for the loading of cargo onto exosomes, encompassing electroporation, ultrasound, freeze-thaw cycling, and extrusion. Prevalently, electroporation stands as the most frequently employed physical technique. This approach entails the application of an external electric field surpassing the energy threshold of the exosomal phospholipid bilayer. This eventuates in the transient formation of nanopores upon the current's breach of the exosome membrane. Through these conduits, an assortment of exogenous substances, including miRNAs, siRNA, and small molecule drugs, can be internalized [79]. While electroporation offers facile parameter manipulation, concerns linger regarding the potential for EV or siRNA aggregation, which could undermine EV integrity or siRNA therapeutic efficacy. The introduction of chelating agents, such as trehalose pulse medium or EDTA, endeavors to mitigate siRNA aggregation; however, this may coincide with reduced siRNA retention levels (<0.05 %) [79].

Furthermore, the mechanical shear stress imparted by ultrasonic probes exhibits the potential to distort the exosomal membrane, thus facilitating the ingress of therapeutic agents [80]. Sonication has proven effective in the incorporation of therapeutic siRNA and miRNA, a strategy exploited to curtail the expression of target receptors [81]. Alternatively, it can serve to load chemical inducers within tumors, ultimately eliciting tumor demise [82]. Concomitantly, the freeze-thaw cycle strategy mandates successive incubation of drugs and exosomes at ambient temperatures, rapid freezing at −80 °C or in liquid nitrogen, and subsequent thawing at ambient temperatures. A minimum of three cycles ensures the drug's encapsulation [83]. Although the freeze-thaw method confers prolonged stability to exosomal products, its drug loading capacity typically falls short of ultrasound or extrusion techniques. Moreover, it bears the propensity to induce anomalous exosome aggregation, thereby giving rise to disparate product sizes.

The extrusion method, frequently executed in the creation of biomimetic nanovesicles, entails introducing the amalgamation into a lipid extruder equipped with a porous membrane (100–400 nm) under controlled temperature conditions [23]. The intricacies of the extrusion technique shall be expounded upon in subsequent elaboration concerning top-down strategies. However, it is pertinent to note that this method may expose exosomes to robust mechanical forces and extreme temperatures, thereby engendering membrane damage and surface protein denaturation. This approach also accentuates temporal and financial costs [72,84]. The employment of sonication is known to substantially modulate the physicochemical attributes of vesicles, influencing parameters such as size and zeta potential, albeit with concurrent effects on vesicle stability.

3.2. *Top-down approach*

The top-down methodology entails the disintegration of larger and intricately structured cellular entities into smaller components, facilitating their assembly into diminutive vesicles characterized by compact dimensions [21]. The resultant nanovesicles, acquired through this procedure, closely resemble the architectures of proteins, nucleic acids, lipids, and biomolecules inherent to the source cells. This renders them akin to the intricate biological constructs comprising natural exosomes, allowing for their preparation without an exhaustive understanding of exosomal characterization.

3.2.1. *Co-incubation and chemical blebbing*

Co-incubation emerges as the simplest top-down modality, wherein the desired exosomes are acquired through the concurrent cultivation of drug and donor cells under varying conditions. While this approach circumvents the introduction of exogenous active agents, several challenges beset its implementation, encompassing low loading efficiency, non-uniformity, limited specificity, and potential cytotoxic ramifications [21,85].

Furthermore, drawing inspiration from bubble phenomena [86], the manipulation of sulfhydryl-blocking processes has facilitated the induction of nanovesicle liberation from cellular membranes, concurrently enabling the entrapment of chemotherapeutic agents. This avenue has been harnessed to craft therapeutic vectors [87]. In a bid to ameliorate the variations in vesicle size during formation, Ingato et al. adopted the supplementation of polyoformaldehyde and dithiothreitol during cell culture. This strategic augmentation facilitated the loading of the chemotherapeutic agent doxorubicin (DOX), culminating in targeted therapeutic interventions for tumors. The sulfhydryl-blocking technique confers advantages inclusive of facile scalability, robust drug loading capabilities, circumvention of non-specific drug distribution within vital organs, and efficient drug conveyance. This approach bears potential as a therapeutic carrier to enhance the efficacy of tumor treatment [87]. However, the applicability of sulfhydryl-blocking remains relatively unexplored. Questions surrounding its potential influence on exosome and loaded drug activity, as well as its plausible effects on tumor metastasis, necessitate further elucidation through rigorous investigation.

3.2.2. *Cellular nanoporation (CNP)*

The feasibility of producing therapeutic exosomes containing targeted peptides and therapeutic mRNAs via CNP has been empirically substantiated. In this context, researchers deployed approximately one million mesenchymal cells derived from human adipose tissue onto a nanoengineered silicon wafer. Subsequently, transient membrane pores were generated through electrical stimulation, followed by the introduction of plasmid DNA into the donor cells. This orchestrated sequence culminated in the extrusion of redundant materials, inclusive of DNA or mRNA fragments, via exosomes. Consequently, the liberation of exosomes encapsulating transcribed mRNAs and specific peptides was achieved [88].

An alternative avenue was pursued by Yang et al., involving the development of a nanofluidic apparatus termed "Exosome nanopore (ENP)". This innovative platform facilitated the creation of approximately 30,000 transient nanopores on the exosomal membrane, while preserving the structural integrity of the biofilm. Notably, ENP-treated exosomes demonstrated the capacity to convey therapeutic agents to human non-small cell lung cancer cells, thereby eliciting cellular apoptosis [89].

In contradistinction to exosome generation methods like electroporation, the potential for non-invasive exosome loading emerges as a prospect. Achieving this hinges on the manipulation of nanochannel dimensions and flow rates to facilitate the future loading of diverse cargo into exosomes. This anticipatory evolution holds the promise of standardizing production processes and evolving into an innovative platform tool [88,89].

3.2.3. *Microfluidic platform*

The microfluidic platform represents a cutting-edge exosome manipulation and separation system grounded in nanotechnology principles, characterized by attributes such as high precision, high-throughput capability, automation, and expeditious analytical capabilities [90]. In this context, living cells undergo elongation and disintegration into sheets under the imposition of shear stress within hydrophilic microchannels. Subsequently, nanovesicles are engendered within an aqueous milieu, capitalizing on the self-assembling nature of lipid bilayers [91]. The appeal of microfluidic platforms rests upon their inherent advantages encompassing automated workflows, precise flow regulation, and the potential for scalable production. This translates into a comparatively greater preservation of lipid membranes and membrane proteins during exosome generation as opposed to extrusion techniques.

Contemporary research predominantly converges on the efficacious isolation and purification of exosomes, non-invasive cancer diagnostics, and the prospect of engineering diverse lipid nanovesicles [92]. Yet, the translation of microfluidic technology research into clinical practice mandates the development of novel label-free microfluidic methods to avert buffer-induced perturbations in exosomal biological function. Moreover, the establishment of reproducible and standardized protocols for the isolation and purification of vesicles assumes paramount importance to meet the requisites of large-scale commercial production [93].

Novel approaches, such as the microfluidic ultrasonic assembly method, have emerged to fabricate exosome membrane-coated poly (lactic-co-glycolic acid) nanoparticles. These constructs exhibit reduced susceptibility to immune clearance, thereby augmenting biocompatibility and targeting capabilities [93].

3.2.4. *Extrusion and filtration*

Beyond nanoporation, an alternative avenue to obtain nanovesicles encompasses the continuous extrusion of donor cells through polycarbonate membrane filters with diverse pore sizes. The resultant nanovesicles garnered through this approach demonstrate concordance with

natural exosomes in terms of physical attributes, prominent protein markers, biological propensities, and therapeutic potential [94]. Presently, the widely employed extrusion modality leverages commercial liposome extruders for ease of implementation [23]. Furthermore, the construction of M1-type macrophage artificial vesicles, known for their selective tumor-killing efficacy, is feasible through the utilization of a pneumatic liposome extruder [95]. By resorting to filters to deconstruct monocytes or macrophages, Jiang et al. succeeded in yielding simulated nanovesicles, driving a 100-fold surge in exosome production. This innovation facilitated the targeted delivery of chemotherapy drugs encapsulated within nanovesicles to tumor tissues, circumventing the adverse reactions associated with equivalent free drugs [96].

Comparative evaluation between the extrusion and filtration methodologies underscores certain distinctions. While the extrusion technique necessitates manual intervention and entails less controlled procedural steps, the filtration approach boasts a more methodical protocol, demanding a reduced labor input. Despite the marked augmentation in nanovesicle yield engendered by extrusion and filtration, these methods warrant consideration for their potential to induce membrane property alterations. As such, supplementary purification steps might be warranted to ensure the attainment of requisite stabilization [94–96].

3.2.5. *Cell membrane coating nanotechnology*

An alternative method that has gained traction for the generation of exosome-mimicking constructs is membrane coating nanotechnology. This technique holds the potential to imbue intricate biological functionalities onto its surface, eliminating the reliance on synthetic methodologies [97]. This straightforward and controllable top-down approach further augments its effectiveness by curtailing the nonspecific uptake of nanoparticle particles prior to reaching the intended target locale. Presently, cell membrane coatings have found applications across an array of substrates, encompassing polymers, gels, inorganic materials, and metals. The core constituents can be tailored based on parameters such as hydrophobicity, charge, size, or structure. Moreover, the repertoire of membrane sources extends to diverse cell types, including immune cells, thereby accommodating an expansive array of cargoes [98].

Bolstered by biomimetic nanotechnology, the amalgamation of disparate cell membranes unfolds the possibility of hybrid membrane creation. This phenomenon fuses the functionalities of distinct cell types within a singular biomimetic nanoparticle, thereby conserving the inherent attributes of the original cells [99]. Self-assembled exosomes enveloped by nanoparticles have been demonstrated to exhibit heightened affinity for lung tissues, demonstrating potential for effective intervention in postoperative breast cancer metastasis [100]. Nonetheless, as membrane-encapsulated nanoparticles find increasing utilization within multidisciplinary cancer therapeutic strategies, consideration must extend to the integration of biologically active membrane components, encompassing entities such as ion channels and enzymes. Moreover, the presence of MHC on immune cell-derived membrane-coated constructs may engender immunogenicity concerns. Furthermore, optimization of membrane derivation processes is imperative to secure high-purity biofilm formulations.

3.3. *Hybrid biomimicry methodologies*

While both top-down and bottom-up strategies have been extensively developed and adopted, several constraints warrant consideration. In the case of bottom-up methodologies, there exists a notable potential for intricate and unfamiliar exosomal components, whose attributes can exhibit variance contingent upon the species, physiological status of the parental cells, and production procedures. This inherent variability complicates the characterization process, particularly concerning applications involving drug loading [101]. Additionally, the integration of multiple functional molecules necessitates elaborate synthesis and purification protocols. Deficiencies in these processes not only undermine protein expression but also render molecules or nanoparticles susceptible to immune system recognition, potentially eliciting immune responses leading to rejection [84].

Turning to the top-down paradigm, the inclusion of non-specialized components from source cells introduces inherent safety concerns. Furthermore, the incorporation of effective components such as non-coding RNA, lipids and proteins into cells mandates a prior screening process to ensure optimal efficacy and compatibility [101].

Furthermore, the drug encapsulation efficiency of top-down drug delivery systems is often modest, necessitating the introduction of supplementary materials to enhance encapsulation efficacy [102]. Equally significant is the fact that the existing platform exhibits suboptimal control over physical parameters, necessitating the formulation of standardized protocols governing production processes and storage conditions, particularly with a view to eventual market deployment [62,103].

To surmount the limitations inherent to bottom-up and top-down strategies and to achieve further refinement of engineered vesicle preparation, a hybrid approach fusing the strengths of both strategies has emerged. Nanovesicles generated through hybrid biomimetic techniques embody not only nano-synthetic constituents, with the attendant benefits of scalable production, controllable fabrication, and efficient drug conveyance characteristic of synthetic nano-capsules, but also inherit natural exosome-derived biomarkers, affording the biocompatibility, circulatory stability, and organ-targeting attributes innate to natural exosomes [21,66,84,104]. Currently, the application of hybrid strategies is pervasive in the design of hybrid exosomes, exemplified by constructs like exosome-liposome hybrid nanobubbles and exosome-coated nanoparticles [64,65]. Compared with liposomes, artificial exosomes have a longer retention time in tumor tissues while ensuring the effect of tumor inhibition *in vivo* and *in vitro*, which may be due to the use of tumor cell membrane protein camouflage [105]. An initial endeavor involved the amalgamation of membrane engineering techniques with genetic modification methodologies, resulting in the fusion of exosome membranes with synthetic liposomes via freeze-

Table 2 – Strategies for engineering exosomes.

Strategy	Methods	Advantages	Disadvantages	Refs
Bottom-up	Genetic engineering	Highly controlled	Inefficient transfection, need to construct a plasmid and overexpress the protein in donor cells	[62]
	Covalent modification	Stronger control over the conjugation site, stability, speed, and efficiency	May result in exosome aggregation or the inactivation of surface proteins	[73]
	Multivalent electrostatic interactions	Enhanced binding to recipient cells	Payloads that enter the cell may be degraded by lysosomes	[109]
	Receptor-ligand binding	Specificity, saturation, high affinity	May result in exosome aggregation or the inactivation of surface proteins	[110]
	Hydrophobic insertion	Require freeze-thaw or high temperature cycling conditions	May result in exosome aggregation or the inactivation of surface proteins	[78,70]
	Membrane editing technology	Membrane proteins are naturally localized, low toxicity and rejection	Heterogeneous particles produced by exosome heterogeneity	[78]
	Electroporation	Easy to control, loading with big molecules such as miRNA or siRNA	Compromise membrane integrity, siRNA aggregations	[79]
	Sonication	High rate of drug loading	Compromise membrane integrity	[80,81]
	Freeze and thaw cycles	Moderately efficient drug loading as well as liposome-exosome fusion	Exosome aggregations	[83]
Top-down	Co-incubation	Simple and mild reaction conditions	Low efficiency, low uniformity, low specificity, and cytotoxicity	[111]
	Chemical blebbing	High drug loading efficiency	Unknown toxicity	[81,82]
	Cellular nanorapidation	Efficient	Low voltage reduces the delivery efficiency, and high voltage promotes cell death	[83,84]
	Extrusion	High drug loading efficiency, few steps	Compromise membrane integrity	[21,90]
	Filtration	High drug loading efficiency, specific process	Compromise membrane integrity, time-consuming	[96]
	Microfluidic platform	High-throughput, automation and rapid analysis	Expensive	[85,86]
	Cell membrane coating	Improve uptake efficiency	The immunogenicity of biofilm components, membrane derivation techniques need to be optimized	[97]
Hybrid biomimicry methodologies	Hybrid biomimicry methodologies	Noninvasive exosome loading while producing large amounts of exosomes and mRNA transcripts, which is expected to standardize production and develop into a platform tool	Hybrid nanotechnology is complicated and immature, and potential toxicity of nanoparticles	[54,55,106,107]

thaw cycles. This approach offers the prospect of tailoring the properties or composition of exogenous lipids to modulate the features of blended exosomes, introducing an innovative avenue for advancing drug delivery systems [83]. Nonetheless, the freeze-thaw conditions bear the potential to compromise liposome encapsulation efficiency, potentially undermining the structure and function of exosome membrane proteins [97]. Fortunately, alternative strategies have emerged to mitigate the impact of freezing and thawing. Extruded membrane fusion methods meld recombinant immune cell exosomes with synthetic liposomes to engender refined biomimetic nanostructures, applicable in the delivery of DOX for breast cancer treatment [65]. Furthermore, recent investigations have spotlighted the utility of ACEs that adeptly circumvent the deleterious implications of freeze-thaw dynamics, concurrently manifesting tumor targeting prowess and multifunctional customization [106,107]. The incorporation of metastasis-related mRNA or integrin proteins into ACE constructs holds promise for precision drug delivery to metastatic sites. In addition, hydrogels constructed by genetically engineered artificial exosomes can be used to regulate the polarization, efferocytosis and phagocytosis of macrophages, connecting the innate effecting function of macrophages with its adaptive immune response to achieve effective ovarian cancer immunotherapy [105]. However, amid the burgeoning landscape of nanotechnology,

it remains pivotal to address the potential toxicity and safety considerations intrinsic to nanoparticles utilized in drug delivery [108]. Moreover, the mass synthesis of artificial exosomes lacks a strict quality control (QC) standard[66].

In summary, the choice of strategy hinges on the cargo's inherent characteristics, encompassing its hydrophilicity, hydrophobicity, and molecular dimensions. Additionally, the decision is informed by a thorough assessment of the pros and cons of each method, harmonized with pragmatic requirements (Table 2).

4. Engineered exosomes for tumor metastasis and recurrence

Engineered exosomes enact multifaceted interventions in the context of tumor metastasis, as delineated in Fig. 2. These interventions encompass: (1) The incorporation of anticancer agents within exosomes, facilitating their conveyance to distant tumor sites via the circulatory system. (2) Loading exosomes with siRNA, thereby disrupting the expression of mRNA associated with metastasis-related genes within tumor cells. (3) Impeding the communication orchestrated by TEXs between cancer cells and the construction of PMNs. (4) Activating the host's immune system and curtailing the tumor's immunosuppressive milieu, thus indirectly restraining the metastatic process. The ensuing discourse will expound upon the diverse dimensions of employing engineered exosome-based therapies to combat tumor metastasis.

4.1. *Engineered modification of metastasis-related natural exosomes*

The distinct lipid and protein constitution of exosomes arising from diverse cellular origins engenders divergent biological functionalities, some of which may be intricately linked to the mechanisms underlying tumor metastasis [21]. Consequently, a logical approach to treatment involves direct modification or intervention in the operations of these naturally occurring exosomes implicated in metastatic processes. TEXs wield the ability to initiate the establishment of PMNs, enhance the colonization and proliferation of disseminated tumor cells, and actively partake in an array of tumorigenic facets encompassing vascular permeability, thrombotic events, immunosuppression, and extracellular matrix remodeling, both within primary tumor sites and distant organs [112,113]. This intricate interplay is significantly influenced by the myriad of tumor-specific information harbored upon TEXs' membranes, particularly the distinctive antigens that dictate tumor homing attributes and final metastatic destinations [3,15]. To counteract its propagation to adjacent or distant cells within the *in vivo* milieu and thereby activate the tumor microenvironment (TME), inhibiting tumor metastasis hinges on disrupting its signaling transduction. Conventional delivery systems often grapple with the challenge of concurrently targeting tumors and TEXs. In an innovative approach, He et al. efficiently and stably labeled tumor cells and TEXs by leveraging cellular metabolic glycoengineering methods and biological orthogonal click chemistry, thereby enabling monitoring and intervention in metastasis [114]. Ac4Mannaz-loaded nanoparticles (Ac4ManNAz-DP) were adeptly distributed to tumors, capitalizing on their sensitivity to tumor-specific metabolism, ultimately labeling both tumor cells and TEXs with azide functionalities. Subsequently, dibenzyl cyclooctyne-modified nanoparticles selectively engaged tumor cells and TEXs through a bioorthogonal click reaction. Noteworthy outcomes encompassed the repression of primary tumor growth and attenuation of TEX-mediated communication responsible for orchestrating liver PMN and subsequent metastasis, all achieved through the tracking and down-regulation of exosomal macrophage migration inhibitory factor (Figs. 2C and 3).

An additional focal point resides in macrophage-derived exosomes, which not only maintain the tumor-targeting prowess and pro-inflammatory capabilities inherent to macrophages but also serve as conveyors of miRNAs among tumor cells, thereby expediting cancer migration and invasion [39,115]. Moreover, they operate as vehicles for the exchange of immune cell constituents and actively contribute to the orchestration of the tumor's immune microenvironment dynamics [52,107]. Nonetheless, the elevation of distinct markers and cytokines characteristic of M1-type macrophages can facilitate the repolarization of M2-type macrophages toward an M1 phenotype, subsequently dampening the invasive potential of cancer cells [116]. Seeking to optimize the anti-tumor efficacy of classically activated M1 macrophages while ameliorating the immunosuppressive TME, Wang et al. co-cultured the isolated nuclei of tumor cells with activated M1-like macrophages to obtain macrophage-tumor hybrid cells (aMT), and isolated aMT-exos from the cell supernatant by gradient centrifugation and ultracentrifugation [107]. aMT-exos are outfitted with an array of immunomodulatory constituents, including MHC-I molecules, costimulatory molecules, and immune-activating cytokines. Leveraging their nanoscale dimensions and inherent tumor-tropic attributes, these aMT-exos simultaneously navigate to lymph nodes and tumor locales. This dual targeting engenders the activation of immune responses within lymph nodes, efficaciously curtailing primary tumor growth, tumor metastasis, and postoperative tumor recurrence.

Notably, exosomes exhibit the capability to surmount both the blood-brain barrier and blood-tumor barrier, a pivotal attribute that augments their significance in surmounting barriers associated with effective therapy for solid tumors and potential distant metastases [117]. Brain endothelium-derived exosomes carry specific homing biomarkers (such as CD63) that may provide their ability to transport across the blood-brain barrier, helping them target the delivery of DOX and PTX to brain tumor cells [118]. Modified exosomes can also impair EC-to-EC integrity, including but not limited to the following pathways: integrin-carrying exosomes activate tyrosine protein kinases Src and S100 to phosphorylate VE-Cadherin [119]; TEXs carrying miR-105 can target ZO-1, Claudin-5, Occludin, N-cadherin and actin filaments [120]; exosomes expressing ligands (such as LDLR-targeted apolipoprotein B) hijack receptor-mediated transcytosis [121]. Although there are many ways to help exosomes break through the blood-brain barrier, most exosomes are captured and neutralized by the reticuloendothelial system

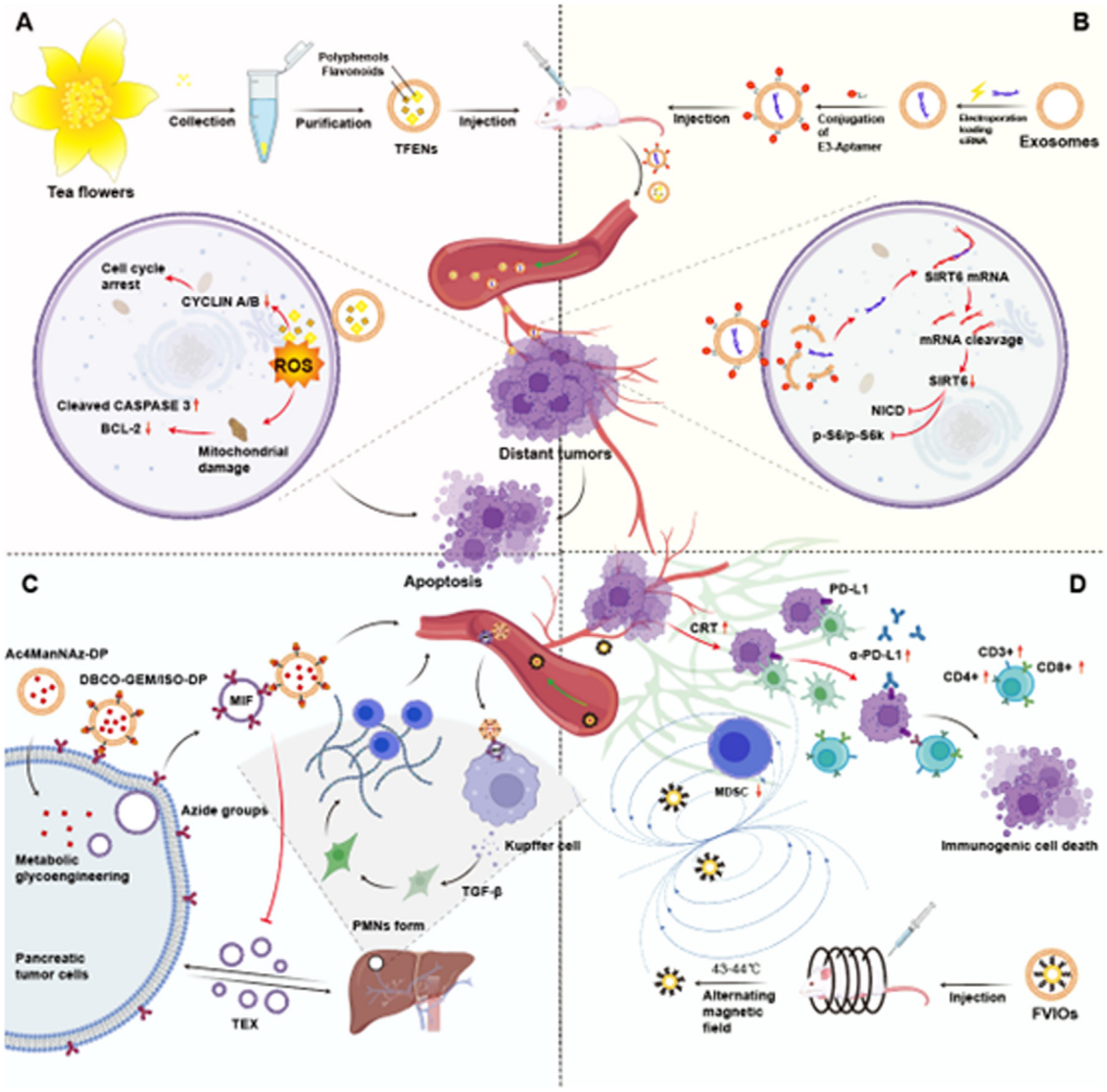

Fig. 2 – Typical ways of engineered exosomes to interfere with metastasis. (A) Loaded anti-cancer drugs and delivering them to distant tumors through the blood circulation. (B) Loaded with siRNA and delivered to tumor cells to interfere with the expression of mRNA related to metastasis genes. (C) Interference with TEXs-mediated communication between cancer cells and the formation of PMNs. (D) Activated the host immune system and suppressed the immunosuppressive response of the tumor, indirectly inhibiting metastasis.

during systemic administration [121], so achieving treatment delivery across the blood-brain barrier still needs to consider exosomes and maximize uptake and utilization.

Conventional engineering methodologies are progressively advancing in the realm of exosome isolation, purification, and fabrication. Notably, strategies such as genetic engineering, glucose deprivation, manipulation of acidic pH, and imposition of shear stress have emerged as means to bolster exosome secretion efficiency [122]. Furthermore, the realm of ACEs transcends the limitations posed by the modest yield of natural exosomes while preserving the inherent attributes of parent cells. Nevertheless, the realm of simple exosome modifications, whether encompassing cell engineering or exosome engineering, confronts a spectrum of issues encompassing biosafety concerns and suboptimal drug delivery efficiency. Necessitating innovative approaches to surmount these challenges is paramount.

4.2. *Nanotechnology combined with gene therapy*

The realm of nanotechnology holds immense potential in augmenting the intrinsic capabilities of engineered exosomes. Common traits exhibited by typical nanomaterials include an elevated surface-to-volume ratio, enhanced electrical conductivity, superparamagnetism, shifted optical absorption spectra, and distinctive fluorescence properties. These attributes confer natural advantages to nanomaterials in drug delivery and controlled release scenarios [123]. Additionally, nanomedicine demonstrates unique attributes like modifiability, exploitation of the EPR effect, intelligent

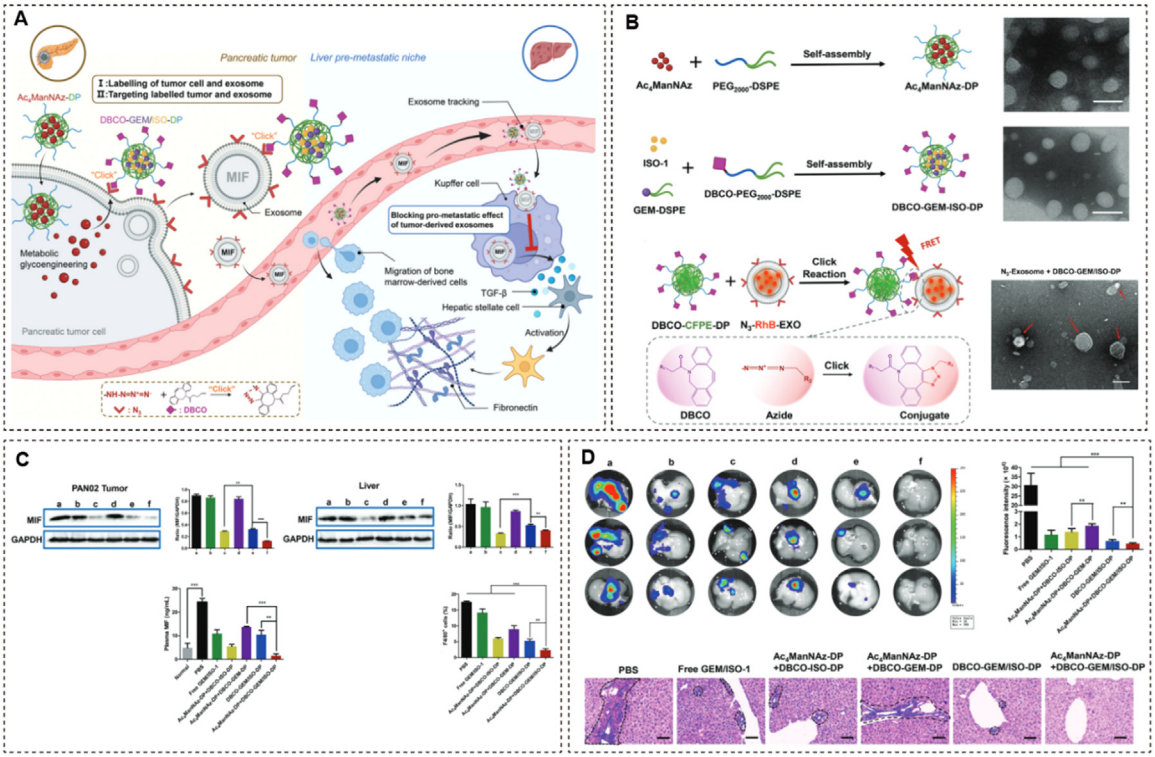

Fig. 3 – Engineered modification of metastasis-related TEXs with bio-orthogonal nanoparticles. (A) Bio-orthogonal nanoparticles targeting tumor and TEXs mechanism of action. (B) Construction and characterization of bio-orthogonal nanoparticles. (C) PMNs inhibition effect of bio-orthogonal nanoparticles on mice bearing orthotopic PDAC, among which AC4Manaz-DP +DBCO-GEM/ISO-DP had the strongest inhibitory effects on TGF-β expression, stectin activation, fibonectin deposition and bone marrow derived macrophages. (D) After bio-orthogonal nanoparticle treatment on mice bearing pancreatic cancer liver metastases, Bioluminescent images of mouse livers and H&E staining of livers. Reproduced with permission from [114]. Copyright 2023 Wiley-VCH GmbH.

responsiveness, and the capacity for co-delivery of multiple therapeutic agents. These features are pivotal in facilitating precise and collaborative tumor treatment while mitigating toxic and side effects [124]. However, the variances in surface markers among diverse tumor cells pose challenges to effective targeting, and the iterative use of conventional nanotherapies may yield severe adverse effects while offering marginal gains in overall survival [124,125]. Consequently, the evolution of nanotechnology-driven next-generation engineered exosome therapies emerges as a promising avenue, expected to yield advantages encompassing heightened safety, efficacy, metastasis prevention, cost-effectiveness, and streamlined mass production (Fig. 4).

Advancements *in vivo* gene delivery methodologies, encompassing viral vectors, liposomes, and synthetic nanocarriers, have positioned exosomes as a promising platform for loading drugs aimed at gene regulation mediated through the rich non-coding RNA sequences found in exosomes [126,127]. This exploits the inherent cell-binding propensity of vesicles and their capacity to safeguard RNA molecules from enzymatic degradation [72].

Engineered exosomes stand out as a promising vector for CRISPR/Cas9 gene therapy delivery, offering an alternative to address concerns associated with neutralizing antibodies, immunogenicity, and insertional mutations linked to adeno-associated virus capsids [128,129]. Moreover, these engineered exosomes surmount limitations linked to synthetic nanoparticles, such as immunogenicity, limited biocompatibility, and constrained drug release rates [130]. In practice, CRISPR/Cas9 plasmid DNA is encapsulated using transfection reagents and delivered to recipient cancer cells to target the deletion of the mutant Kras G12D oncogenic allele in pancreatic cancer cells. Notably, this approach significantly impedes tumor growth and metastasis in animal trials [68].

Furthermore, certain miRNAs conveyed by exosomes can amplify the proliferation and invasion of cancer cells, and modulating the expression levels of these miRNAs can orchestrate tumor progression and metastasis [131,132]. Engineered exosomes, as ideal gene delivery vectors, can also be laden with pivotal regulatory miRNAs, non-coding RNAs, or tumor suppressor factors for targeted delivery, thereby hampering pathways linked to cancer cell metastasis [133]. For example, SIRT6 activation influences diverse cancer-related signaling pathways, and its depletion markedly curbs the proliferation and metastasis of cancer cells, as observed in metastatic castrate-resistant prostate cancer both *in vitro* and *in vivo*. Addressing this, Han et al. engineered exosomes modified with a nucleic acid aptamer carrying siRNA to silence SIRT6, effectively restraining tumor growth and metastasis [134] (Fig. 2B).

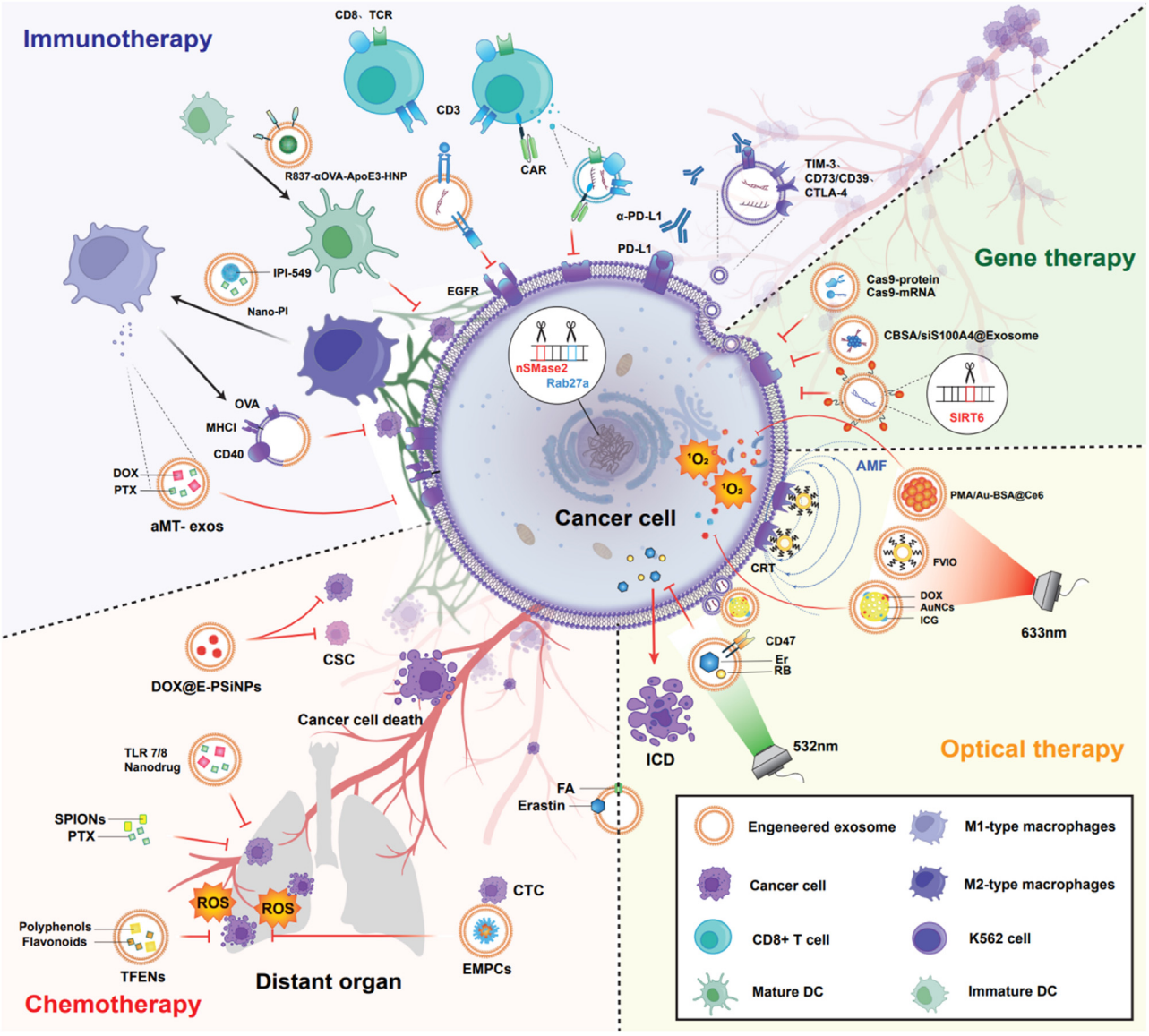

Fig. 4 – Multi-combination application of engineered exosomes in metastasis therapy. Engineered exosomes constructed by nanobiology can enhance immune effects and deliver drug molecules to tumor cells, and interfere with tumor metastasis and recurrence through multiple pathways such as combined immunotherapy, gene therapy, chemotherapy, and optical therapy.

The integration of nanotechnology can be further refined to enhance both biocompatibility and delivery efficiency. The efficacy of post-transcriptional gene silencing via siRNA is well-established in suppressing tumorigenesis [135]. Building upon this, Zhu and colleagues undertook the modification of bovine serum albumin through the introduction of cationic amino groups, yielding cationic bovine serum albumin (CBSA) featuring an optimized isoelectric point (pI) value while preserving the structural integrity of the protein. The resultant positive charge bestowed CBSA with the capacity to traverse the exosome membrane with ease, facilitating its biological binding interactions (Fig. 5). Through this approach, siRNA is effectively shielded from enzymatic degradation. Moreover, with the preservation of integrin on the exosome membrane, a robust affinity is established towards lung PMNs and lung tissue. Consequently, this attribute can be harnessed to specifically target the inhibition of lung metastasis following breast cancer surgery [100].

In spite of the numerous merits associated with exosomes, their application in gene delivery has been hindered by challenges related to their isolation, purification, and the limited efficiency of RNA packaging [136]. To address this, Yang et al. introduced a non-genetic approach that involves transfecting cells originating from diverse sources with plasmid DNA, followed by localized and brief electrical stimulation to induce the release of exosomes harboring specific peptides and mRNA transcripts. This method effectively enhances the incorporation of exogenous mRNAs into exosomes for targeted transcription manipulation and therapeutic purposes. In comparison to conventional

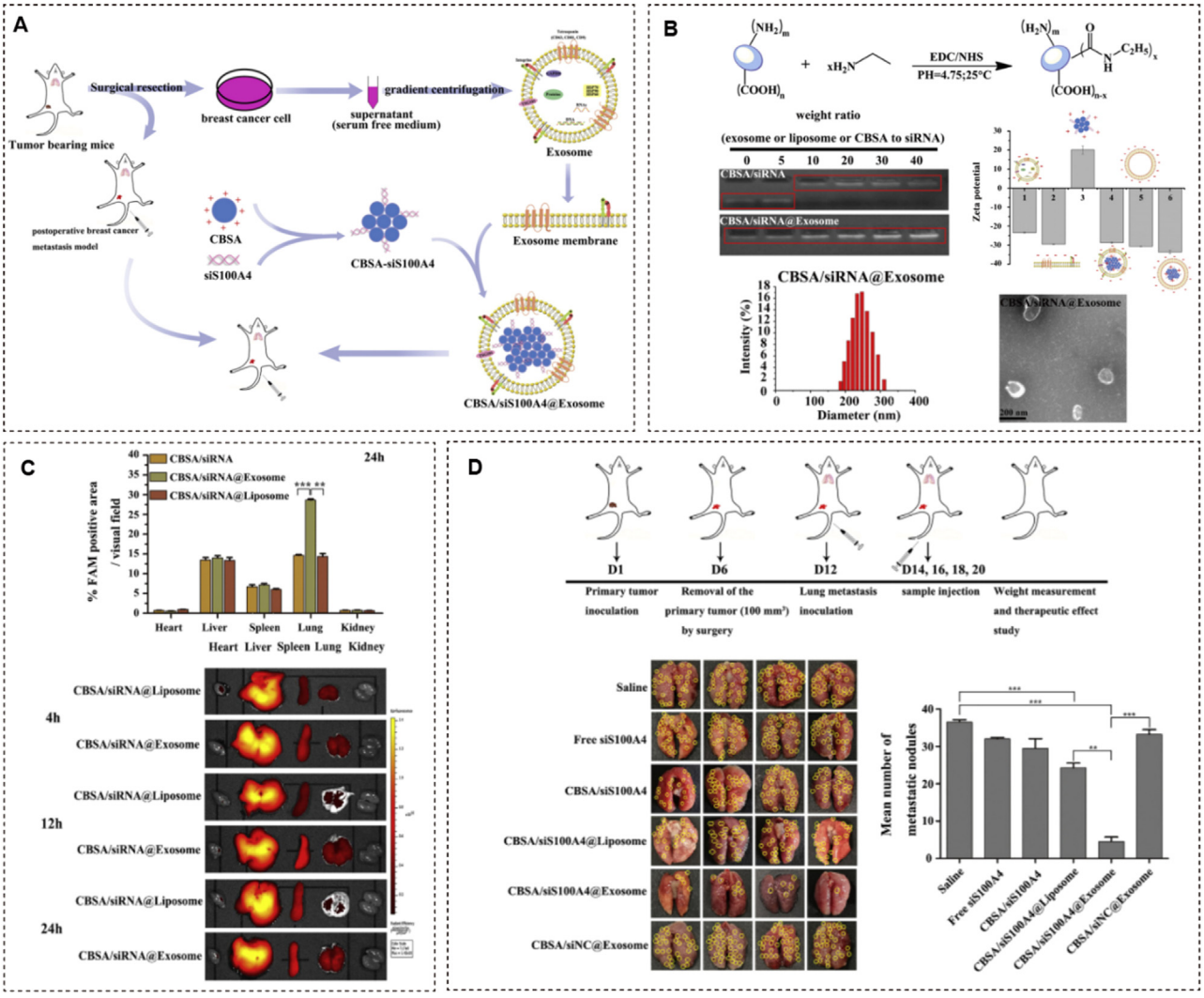

Fig. 5 – Nanotechnology-based engineered exosomes intervene in metastasis with gene therapy. (A) Schematic illustration of engineered exosome-mediated siRNA delivery for inhibition of postoperative metastasis in breast cancer. (B) Synthesis and characterization of CBSA. (C) The highest fluorescence expression was observed in the lung after intravenous injection of CBSA/siS100A4@Exosome24h. (D) CBSA/siS100A4@Exosome was used in the postoperative lung metastasis model, and the mean number of pulmonary metastatic nodules was decreased by gross eyes. Reproduced with permission from [100]. Copyright 2019 Elsevier B.V.

post-insertion electroporation techniques, this approach significantly enhances packaging and production efficiency, while also mitigating issues such as low EV loading efficiency and particle aggregation encountered during electroporation. Nonetheless, the findings unveil a novel mechanism wherein localized cell membrane disruption and heating during this approach result in the upregulation of heat shock protein signaling and increased intracellular calcium ion concentration ([Ca2+]), factors potentially correlated with multiple tumor metastases [88,137].

4.3. *Nanotechnology combined with chemotherapy*

Chemotherapy employing anticancer drugs stands as a prominent therapeutic approach to impede the advancement of cancer. Nonetheless, contemporary chemotherapy methodologies suffer from limitations encompassing inadequate specificity, cytotoxicity, limited half-life, poor solubility, multi-drug resistance, and the promotion of stem cell-like cell growth. In certain instances, cytotoxic agents can even trigger the release of EVs with pro-metastatic attributes [138,139], greatly affecting treatment efficacy and patient adherence. The utilization of engineered exosomes as drug delivery systems to transport chemotherapy agents to malignant cells holds the potential to alleviate these challenges.

Primarily, engineered exosome-mediated drug delivery exhibits the potential to mitigate the risks of drug resistance and toxicity while concurrently suppressing metastasis. The incorporation of polyethylene glycol (PEGylation) can ameliorate the *in vivo* circulation time and absorption efficiency of nanocarriers, diminishing drug clearance and

immune responses [140]. This technique can be harnessed to encapsulate therapeutic agents within exosomes, circumventing mononuclear phagocyte system clearance and enhancing targeted drug delivery efficiency. Researchers have developed macrophage-released exosomes with varied paclitaxel (PTX) loading (ExoPTX) achieved through methodologies such as sonication, yielding high drug loading efficiency and sustained drug release profiles. ExoPTX has effectively thwarted lung metastasis progression in a murine model and significantly amplified the cytotoxicity of drug-resistant MDCKMDR1 (Pgp+) cells by over 50-fold [80]. An alternative approach involves addressing the eradication of cancer stem cells (CSCs) in order to mitigate drug resistance and curtail tumor relapse subsequent to chemotherapy. Yong et al. constructed a cell-exocytosed exosome-sheathed PSiNPs (E-PSiNPs) that exocytosed DOX@E-PSiNPs after DOX-loaded exocytosis showed significant cross-reactive anticancer and CSCs killing activity in late-stage metastasis models [124].

Secondarily, inorganic nanoparticle-facilitated exosomal drug delivery can substantially elevate targeting precision and delivery efficiency. Exosomes laden with superparamagnetic iron oxide nanoparticles (SPIONs) and curcumin can adeptly target cancerous cells and adeptly traverse the blood-brain barrier under the influence of external magnetic fields, presenting a prospective strategy for targeted glioma brain metastasis imaging and treatment [77]. Further, an innovative nano-carrier predicated on macrophage-derived exosomes has emerged, designed to encapsulate the potent anticancer agent PTX, exhibiting superior anticancer efficacy relative to unmodified PTX within a murine lung metastasis model [140]. Novel strategies also encompass the loading of exosomal inhibitors to impede intercellular communication. This approach holds promise by impeding interactions among cancer cells and between cancer cells and other components within the TME, potentially sensitizing cancer cells to chemotherapy through negative feedback mechanisms [141].

Remarkably, CTCs, as the seed and carrier of metastasis, are critical for successful capture and elimination. However, the efficiency of small molecule chemotherapy drugs to eliminate CTCs is very limited [142]. Exosome-like sequential-bioactivating prodrug nanoplatform (EMPCs) can identify CTCs in blood very well, which present novel therapeutic prospects for metastasis intervention. Specifically, the thioether-linked paclitaxel-linoleic acid conjugate (PTX-S-LA) and cucurbitine B (CuB) of reactive oxygen species (ROS) were co-encapsulated in polymer micelles, and the nanoparticles were further modified with exosome membranes (EM). The results of *in vitro* and *in vivo* experiments showed that EMPCs could enhance predrug biological activity, prolong blood circulation, selectively target homologous tumor cells, and enhance tumor penetration ability. EMPCs could not only inhibit tumor metastasis by clearing CTCs and down-regulating FAK/ MMP signaling pathway, but also significantly increase intracellular ROS level and trigger cascade expansion of PTX chemotherapy [143] (**Fig. 6**).

In addition, certain biofunctional molecules within nanovesicles of plant origin present novel therapeutic prospects [144]. Researchers have extracted exosome-like nanovehicles from tea flowers (TFENs) and meticulously characterized their morphology, content, particle dimensions, zeta potential, and other physicochemical attributes. These entities house a range of bioactive compounds including polyphenols, flavonoids, functional proteins, and lipids, which have found applications in the management of inflammatory and malignant conditions [145]. The cumulative actions of these bioactive agents induce intracellular ROS accumulation, in turn provoking substantial oxidative stress, mitochondrial impairment, and cell cycle arrest within cancerous cells. Subsequent animal studies have unveiled the preferential accumulation of TFENs within breast tumor tissues and sites of lung metastasis, and the bioactive components inherent to TFENs appear to forestall breast tumor development and lung metastasis through ROS generation and microbial regulation [146] (Fig. 2A). This study contributes to the advancement of potential strategies for the development of efficacious nanotherapies intended for intravenous or oral administration in the context of metastatic breast cancer.

4.4. *Nanotechnology combined with optical therapy*

Photothermal therapy (PTT) and photodynamic therapy (PDT) are established hyperthermia methodologies that employ light-absorbing agents and laser irradiation to selectively eliminate cancer cells. The integration of nanotechnology with PDT introduces a novel avenue for comprehensive tumor treatment. Chlorin e6 (Ce6), a common photosensitizer essential for PDT, exhibits near-infrared fluorescence for clinical diagnostics and boasts substantial singlet oxygen ($^1O_2$) production capacity upon exposure to a 633 nm laser, thereby instigating cell death [147]. Yet, the clinical utility of Ce6 is limited by its hydrophobic properties, inadequate *in vivo* biocompatibility, and short half-life. To enhance effectiveness and biocompatibility, passionfruit-like exogenous polymeric micelle aggregates encapsulating albumin and Ce6 (PMA/Au-BSA@Ce6) nanoparticles were synthesized via transient electroporation, loading urine-derived exosomes with multifunctionalized nanoparticles. The inherent membrane structure and antigens of exosomes enabled efficient embedded into cancer cells while impeding macrophage endocytosis and prolonging blood circulation. Upon exposure to 633 nm laser and acidic conditions, the nanocarrier structure disintegrated, releasing numerous nanoparticles and generating copious amounts of intracellular singlet oxygen, thus restraining tumor cell growth [148]. Notably, exosomes derived from gastric cancer patients were explored for Au nanoparticle and Ce6 co-loading to enable real-time monitoring of Ce6 treatment, exemplifying a label-free tracking approach in exosome therapy. Furthermore, photothermal treatment fosters immunogenic cell death (ICD), releasing damage-associated molecular patterns (DAMPs), thereby inciting innate and adaptive immune responses to deter tumor metastasis [149].

Additionally, nanotechnology-based PDT intersects with innovative strategies for anti-metastatic intervention. Given the therapeutic role of ferroptosis in diverse tumor scenarios, its induction represents a promising avenue for cancer treatment [150,151]. In this context, an engineered exosome was devised with CD47 surface modification, incorporating the ferroptosis inducer Erastin and the photosensitizer RB as its core. CD47-engineered

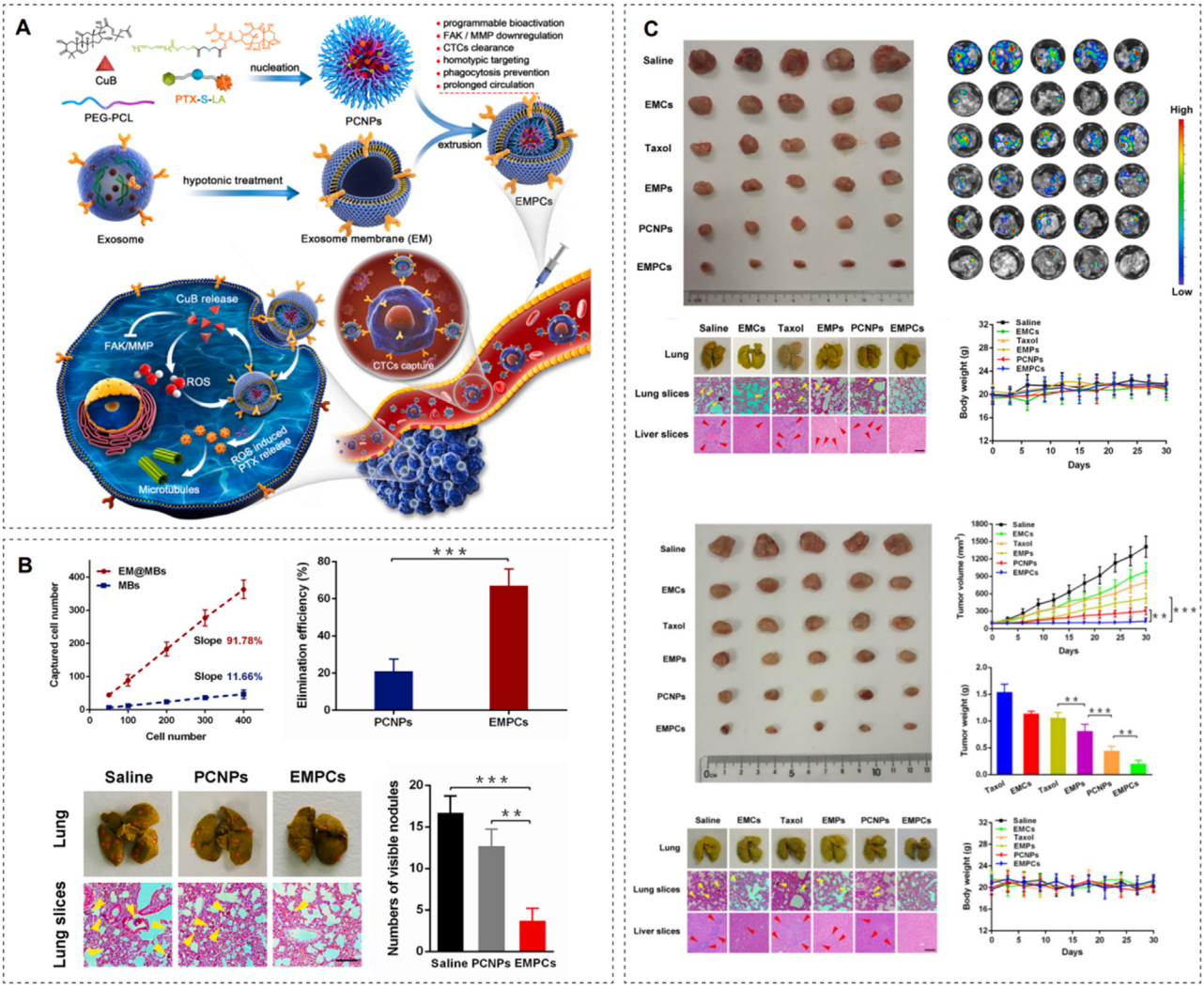

Fig. 6 – EMPCs based on multiple mechanisms are used to inhibit tumor metastasis. (A) Schematic representation of the EMPCs with CTCs clearance, CuB-mediated metastasis suppression, ROS enhancement, and cascade amplified PTX chemotherapy. (B) EMPCs showed excellent *in vitro* CTCs capture efficiency and *in vivo* CTCs elimination efficiency. (C) In both MDA-MB-231 xenograft and orthotopic tumor models, the tumor metastasis rate in EMPCs group was the lowest. Reproduced with permission from [143]. Copyright 2020 Elsevier Ltd.

exosomes circumvent macrophage phagocytosis, while Er and RB collaboratively provoke ferroptosis upon 532 nm laser exposure in the tumor region, offering a synergistic therapeutic approach [82]. The multifaceted impact of these three parts engineered exosomes extends beyond mere additive effects, showcasing heightened tumor delivery efficiency and providing innovative insights into metastasis prevention. Additionally, leveraging the participation of neutrophils and platelets in "early metastatic niches"[152], researchers formulated gold nanocages loaded with DOX and indocyanine green (ICG) into Au-DI complexes, subsequently enveloping these with a hybrid membrane composed of neutrophil and platelet constituents to create PNMAuDIs. Upon intravenous administration, this composite system holds promise for combatting metastasis by capitalizing on physiological interactions within the circulatory system (Fig. 7). The inherent affinity of neutrophil and platelet membranes to adhesion factors on the surface of CTCs has been harnessed to capture exosomes released by CTCs and breast cancer cells. This approach demonstrates a synergistic potential for combined chemotherapy and photothermal therapy, facilitated by light activation to eliminate cancer cells and EVs, thereby inhibiting tumor cell activity and metastasis. Additionally, the nanoparticle-based system exhibits the capability to modulate the TME and alleviate immunosuppression within the TME [153].

Magnetothermal therapy (MHT) represents a therapeutic strategy wherein magnetic nanoparticles are subjected to a robust alternating magnetic field (AMF), resulting in substantial heat generation and subsequent eradication of malignant tumors. Notably, AMF boasts superior tissue penetration compared to photothermal therapy, enabling the effective treatment of deeply located tumors in organs [154]. Notably, mild magnetic hyperthermia mediated by ferrimagnetic vortex-domain iron oxide nanoring (FVIO) has been observed to induce the expression of calreticulin on 4T1

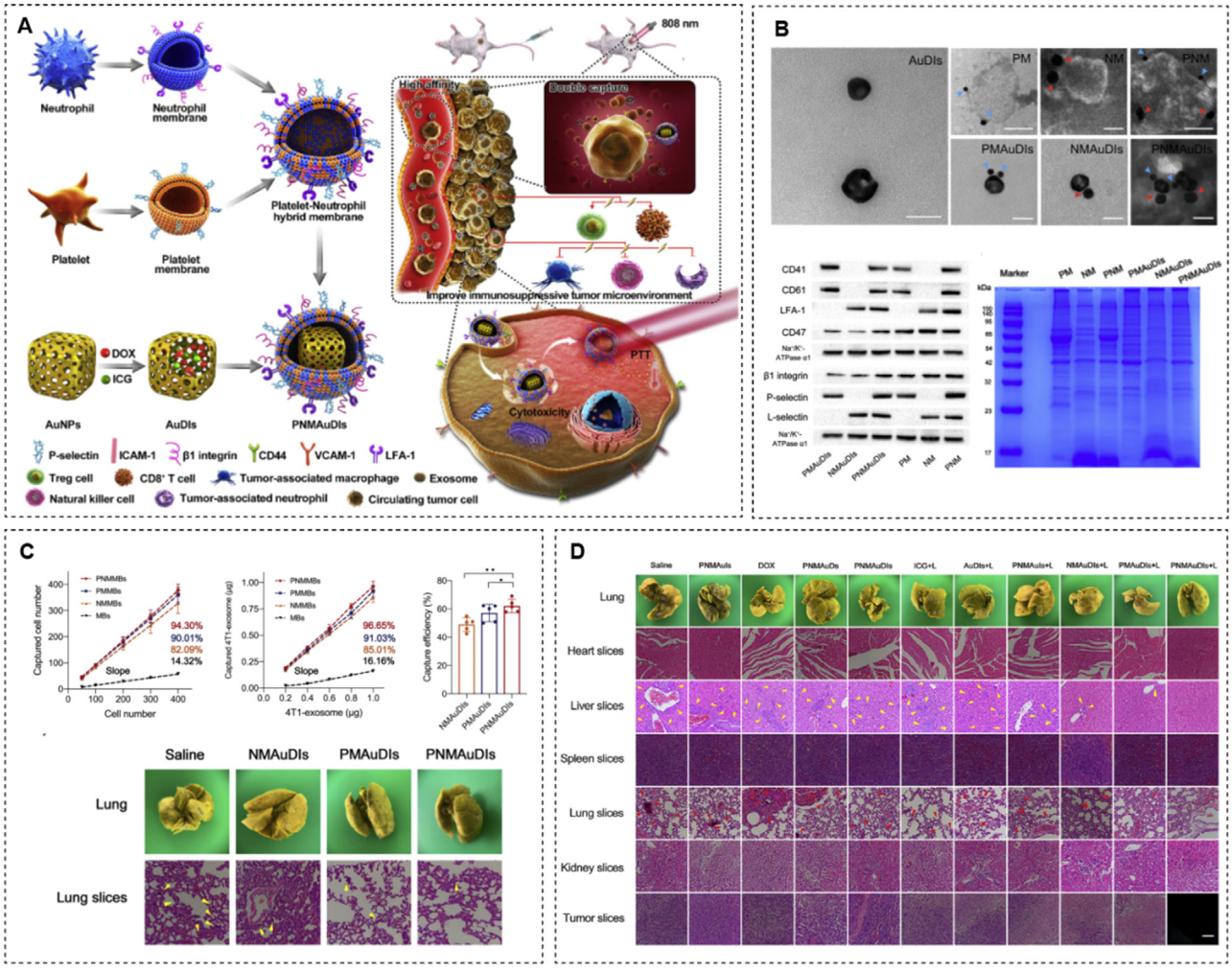

Fig. 7 – Nanosponges of circulating tumor-derived exosomes are used in combination treatment of chemotherapy and photothermal therapy for anti-metastasis. (A) Synthesis of nanosponges of circulating tumor-derived exosomes and mechanism of inhibiting breast cancer metastasis. (B) Protein analysis and FRET staining experiments on neutrophil membrane and platelet membrane confirmed the formation of hybrid membranes and the successful coating on the surface of gold nanoparticles. (C) After treatment with Exo-OVA-aCD3/aEGFR in metastatic and recurrent models, there was a large amount of $CD4^+$ and $CD8^+$ T cell infiltration in metastatic lung tissue, effectively preventing lung metastasis and recurrence of tumors. (D) In mice treated with PNMAuDIs *in situ* models, H&E staining showed almost no tumor metastasis in vital organs. Reproduced with permission from [153]. Copyright 2020 Elsevier Ltd.

breast cancer cells. This phenomenon triggers the emission of an "eat me" signal, facilitating the immune system's phagocytosis of cancer cells, consequently inducing effective ICD and promoting macrophage polarization. This mild MHT exhibits the ability to significantly enhance the infiltration of $CD8^+$ cytotoxic T cells within distant tumors. Furthermore, it effectively impedes the metastatic dissemination and growth of distant tumors through a synergistic interaction with PD-L1 blocking agents [155] (Fig. 2D).

4.5. *Nanotechnology combined with immunotherapy*

Cancer immunotherapy represents a therapeutic strategy aimed at modulating the immune system to effectively manage and eliminate tumors. This approach involves reactivating the immune response against cancer cells and disrupting the mechanisms that facilitate tumor evasion [156]. Currently, cancer immunotherapies (CIT) encompass several key modalities, including adoptive T-cell therapy (ACT), immune checkpoint blockade (ICB), and cancer vaccines. These approaches have demonstrated efficacy in clinical trials across various tumor types [157]. Nonetheless, the present landscape of CIT is characterized by challenges that impede the attainment of definitive cancer cures [158]. Moreover, the potential toxicities and adverse effects associated with these therapies present obstacles to their broader clinical implementation [156]. Given that exosomes play a pivotal role in intercellular substance transmission and immune signaling within tumor immunotherapy contexts [112,159], leveraging the distinct advantages of engineered

exosomes holds promise for applications in anti-metastasis therapy.

4.5.1. *Immune checkpoint inhibitors (ICIs)*

ICIs have demonstrated notable and sustained efficacy in cancer patients, including those with metastatic conditions [160]. Precise obstruction of the PD-1/PD-L1 axis can rescue T cells from exhaustion and restore immune responses against cancer cells. Regrettably, PD-1/PD-L1 inhibitors exhibit limited response rates and are effective in only a subset of cancer patients, typically ranging from 10 % to 30 % [161]. Investigations have revealed that PD-L1 can be transferred from tumor cells to neighboring cells via exosomes, thereby negatively impacting the tumor immune milieu [162,163]. Exosomes originating from melanoma cells have been identified as potent suppressors of tumor $CD8^+$ T cell activation, proliferation, and cytotoxic function, contingent upon PD-L1 expression, and have been implicated in the promotion of aggressive lung metastasis. Notably, these effects could be mitigated through genetic downregulation of nSMase2 and Rab27a, both pivotal enzymes in exosome secretion, as well as PD-L1 antibody pretreatment [163,164]. Furthermore, analogous immune checkpoint molecules such as TIM-3, CD73/CD39, and CTLA-4 have been identified on exosomes, and some of these molecules are associated with aggressive tumor characteristics, heightened tumor burden, and increased incidence of distant metastases [165]. Consequently, these molecules hold substantial potential as novel targets for engineered immunotherapy and as emergent biomarkers for liquid biopsy applications.

Moreover, the emerging liposome delivery system encapsulates ICIs within its core, markedly enhancing drug stability and packaging efficiency. Concurrently, this system effectively combines ICIs with chemotherapy, ROS, pH modulation, and enzymatic reactions, effectively mitigating the drug resistance commonly encountered with single ICI administration and ameliorating the attenuated synergistic effects attributed to pharmacokinetic disparities among distinct ICIs [166]. Researchers have synergistically combined dual PD-1 and TIM-3 blockade with PTT for MC38 tumors, and co-loaded anti-PD-L1 antibodies alongside a photothermal agent (IR820) within a lipid mixture for 4T1 tumors, both yielding promising outcomes in inhibiting distant metastasis [167,168].

4.5.2. *Engineered T-cell adoptive immunotherapy*

Adoptive cell immunotherapy stands as a promising approach in restraining cancer dissemination. Isolated tumor-specific T lymphocytes from cancer patients, upon activation and cultivation *in vitro*, exhibit potential to vigorously target secondary tumor sites [169]. Within the realm of personalized adoptive cell therapy, Chimeric Antigen Receptor T (CAR-T) cell therapy has garnered considerable attention within the landscape of tumor immunotherapy [170]. Nevertheless, traditional CAR-T cell therapy may incite cytokine release, giving rise to deleterious outcomes like cytokine release syndrome and off-target responses [171,172]. Furthermore, the limited efficacy of CAR-T cell therapy in solid tumors could be attributed to the paucity of significant CAR-T cell responses in solid tumors, a consequence of the profoundly immunosuppressive microenvironment characteristic of such tumors [173,174]. In response, CAR-T cells might generate exosomes bearing a complete spectrum of surface membrane constituents derived from the parental cells, encompassing CARs, CD3, CD8, and TCRs, but omitting PD1. This strategic omission of PD1 could profoundly curtail tumor progression and preclude the metastatic hazard prompted by PD1 interactions [175]. When juxtaposed with conventional CAR-T cell therapy, exosomes offer facile acquisition and storage, coupled with their potential to circumvent immunosuppressive mechanisms [175,176], thereby rendering them an optimal target for engineering enhancements. Inspired by the CAR-T treatment paradigm, Fan et al. pioneered an antibody-engineered exosome utilizing dendritic cells as the foundation. Through genetic engineering, this exosome was engineered to concurrently bind anti-CD3 and anti-EGFR, thereby enabling dual targeting of CD3 on T cells and EGFR on cancer cells [177] (Fig. 8). Exosomes originating from dendritic cells, following stimulation through antibody modifications and the incorporation of tumor antigen peptides, function as intermediary messengers between cancer cells and activated T cells, thereby potentiating the antitumor immune reactions of T cells [177]. This linkage facilitates the precise targeting of solid tumors cells by CAR-T cells, thereby proficiently curbing the reappearance and metastasis of solid tumors.

4.5.3. *Nano-immunomodulators*

Immunomodulatory agents present the potential to reshape the TME, thereby enhancing the effectiveness of α-PD1/α-PDL1 immunotherapy [178]. A strategic approach involving nanocardium-assisted immunometabolic therapy that centers on the ATP-adenosine axis aims to enact a metabolic reprogramming within the TME. Zhang and colleagues undertook the encapsulation of a CD39 antagonist and an AMPK agonist, metformin, within TEXs, which were subsequently directed towards tumor sites. This innovative methodology fosters the accrual of pro-inflammatory extracellular ATP (eATP), concurrently attenuating the levels of immunosuppressive adenosine. This orchestration elicits the maturation of dendritic cells, activation of tumor-specific $CD8^+$ T cells and NK cells, and the instigation of ATP-dependent anti-tumor immune responses. Notably, this strategy's efficacy has been validated within metastatic models, resulting in the inhibition of distant tumor metastasis, the induction of enduring immune memory for the prevention of tumor relapse, and an augmentation of tumor responsiveness to PD-1-based therapies. [179].The infiltration of M2 macrophages within lymph nodes has been linked to metastatic events and an unfavorable prognosis, as documented in several clinical investigations [180,181]. Consequently, the redirection of M2 macrophages towards the M1 phenotype has emerged as a pivotal research focus in the realm of tumor immune regulation [182]. Nonetheless, the limited accessibility of prevailing immunomodulators to tumor-associated macrophages within lymph nodes and tumor tissues has emerged as a significant constraint, potentially contributing to their suboptimal clinical outcomes [178]. Though engineered exosomes haven't been specifically engineered for this purpose, researchers have

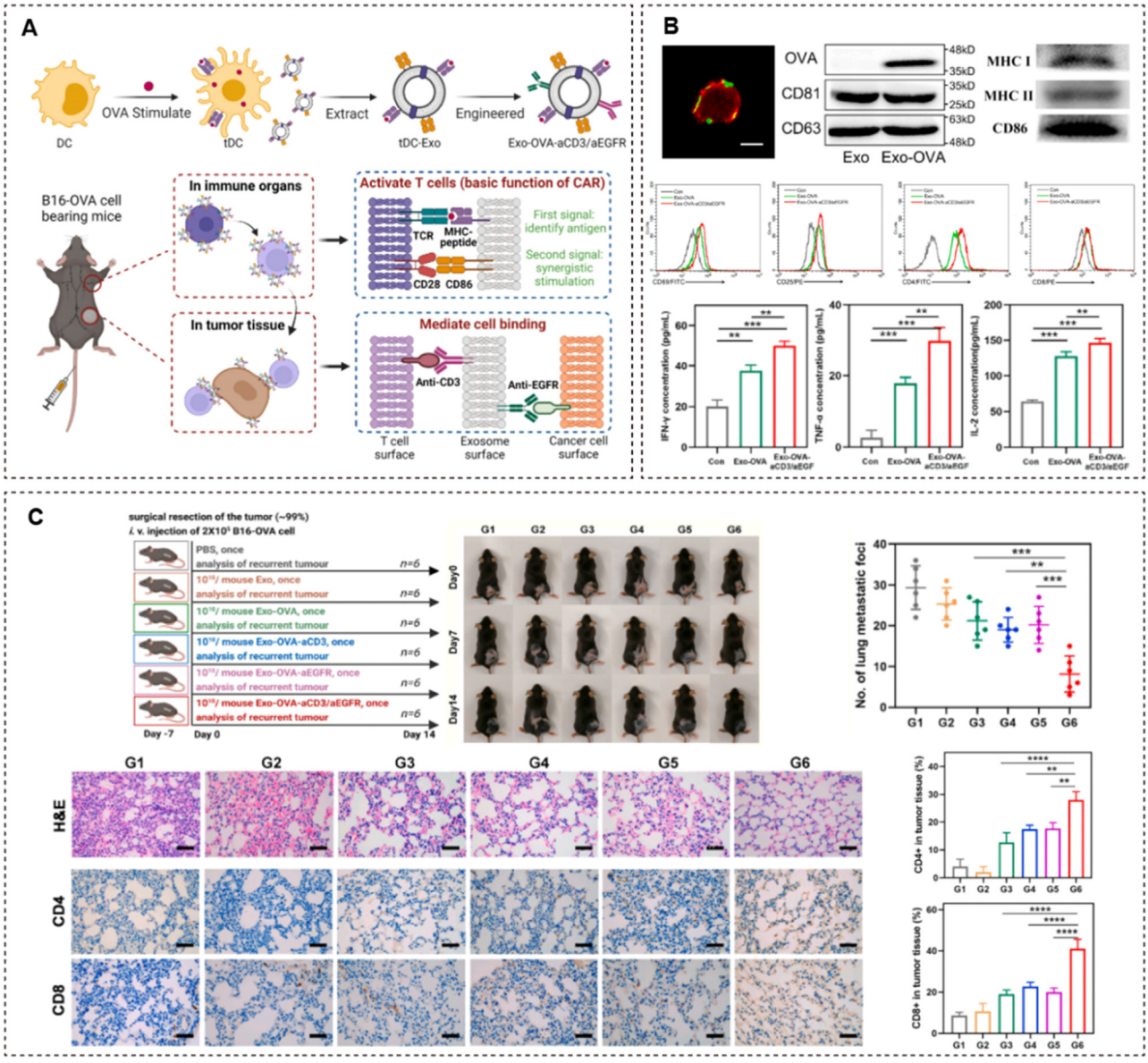

Fig. 8 – Car- T like therapy based on antibody-engineered exosomes from antigen-feeding dendritic cells can inhibit metastasis. (A) Construction and mechanism diagram of anti-CD3 and anti-EGFR antibody engineering tDC-Exo (Exo-OVA-aCD3/aEGFR). (B) Characterization of Exo-OVA-aCD3/aEGFR. (C) In the model of pulmonary metastatic melanoma, Exo-OVA-aCD3/aEGFR can significantly inhibit lung metastasis of the tumor, and there is a large number of $CD4^+$ and $CD8^+$ T cell infiltration in metastatic lung tissue. Reproduced with permission from [177]. Copyright 2022 Elsevier.

outlined a prototypical strategy using analogous inorganic nanoparticles. In a bid to enhance the intratumoral and lymphatic penetration of immune agents, Song et al. devised an albumin-based nanoparticle denoted as Nano-PI, designed to encapsulate a PI3Kγ inhibitor (IPI-549) and PTX as an immunomodulatory payload. The synergistic interplay between Nano-PI and α-PD1 instigates a remodeling of the immune milieu within both tumors and lymph nodes, inducing the shift of M2 to M1 macrophages and the elevation of $CD4^+$ and $CD8^+$ T cells, thereby significantly suppressing lymphatic metastasis-prone breast cancer [183]. In addition, the M1 macrophage-derived exosomes (M1Exos) designed by Liang et al., not only could polarize M2 macrophages into M1, but also could alleviate the immunosuppression of T cells and reshape the tumor suppressive microenvironment by the Anti-PD-L1 expression on their outer membranes. And no tumor metastasis or recurrence was observed within 3 months in animal experiments [184].

Moreover, immunomodulatory agents exhibit the capacity to overturn intrinsic immune suppression within tumors by invoking ICD in malignant cells. Due to various immune evasion mechanisms, the inner regions of solid tumors often lack infiltration by lymphocytes [185,186]. ICD offers a promising strategy to address this limitation by eliciting both adjuvant and antigenicity signals from dying cells [149]. For instance, through the loading of ICD inducers human

neutrophil elastase and TLR3 activators into breast cancer-derived exosomes modified with alpha-lactalbumin, Huang et al. achieved targeted modulation of the TME and induction of ICD in cancer cells. This process amplified the release of tumor antigens and damage-associated molecular patterns, thereby triggering a potent $CD8^+$ T cell response against the tumor [187]. The convergence of nanotechnology-assisted immunotherapy with PTT, MHT, and radiotherapy has yielded promising outcomes in promoting tumor ICD [149,155,188]. Collectively, these investigations underscore the considerable potential of amalgamating tumor ICD with nanotechnology-facilitated immunotherapy for mitigating tumor growth and suppressing metastasis.

4.6. *Noninvasive monitoring technology*

4.6.1. *Exosome-based liquid biopsy*

Exosome-based liquid biopsy presents compelling merits including reduced invasiveness, simplified sampling procedures, and sample stability. The cargo of exosomes encompasses a wealth of biologically significant information derived from their parent cells, rendering them potentially valuable for the identification of dormant and recurrent neoplasms [189,190]. Emerging insights reveal that the levels of miRNAs, circular RNAs, and lncRNAs encapsulated within serum exosomes correlate with distinct risks of metastasis and relapse. Consequently, these molecules can serve as promising biomarkers for the clinical diagnosis and prognosis of diverse cancer types, facilitating the prospective evaluation of treatment responses [191–193]. Notwithstanding these promising attributes, the realm of exosome-based liquid biopsies confronts challenges pertaining to limited sensitivity and the marked heterogeneity among distinct exosome subpopulations. Overcoming these limitations necessitates the development of novel detection platforms and separation techniques to enhance the precision of exosome isolation and purification [190].

4.6.2. *Noninvasive imaging technology*

Noninvasive imaging constitutes a pivotal methodology for investigating the intricate involvement of exosomes in the intricate process of tumor metastasis and recurrence. This approach facilitates the visualization and quantification of pivotal metastatic and recurrent events, thereby enabling the early detection of micro-metastases and the evaluation of therapeutic interventions. Contemporary non-invasive imaging techniques permit real-time, *in vivo* tracking of exosome activities while preserving their physicochemical attributes [194,195]. However, the advancement of integrated probes based on engineered exosomes intertwined with tumor-related therapeutics is hampered by the intricacies of labeling methodologies, undesired labeling byproducts, misplacement, indistinct imaging outcomes, and limited imaging duration [196,197].

Capitalizing on radiolabeling technologies, multimodal imaging through positron emission tomography (PET) facilitates the quantification of both PET and fluorescence images. This technique offers clear visualization of activities across diverse organs such as the lungs, liver, spleen, as well as blood and lymphatic vessels [189]. Integration with PET/MRI and near-infrared fluorescence imaging techniques enables the direct utilization of exosomes as imaging agents for tracking tumor metastasis or recurrence [190–192]. This amalgamation furnishes a more comprehensive and precise spatial depiction, coupled with molecular insights. Such an approach proves valuable for monitoring the biological dynamics of tumor cells and TEXs through distinct metastatic routes. Furthermore, it provides the technical support for probing the mechanisms underpinning tumor metastasis and relapse within living organisms and enables real-time tracking of therapeutic exosomes.

Another avenue for *in vivo* tracking and treatment lies in the utilization of exosomes loaded with inorganic nanoparticles as contrast agents or imaging probes[198]. Iron oxide nanoparticles, for instance, serve as effective magnetic resonance imaging (MRI) contrast agents, whereas gold (Au) nanoparticles function as contrast agents for computed tomography (CT) scans [193,199]. Moreover, silicon porous nanoparticles laden with DOX exhibit discernable anti-cancer capabilities within diverse advanced metastatic tumor models [124]. This stratagem facilitates prolonged tracking and multifunctional enhancement. It's important to note, however, that this approach, while efficacious for tracking, may exhibit high quenching coefficients that potentially compromise the structural integrity and physiological attributes of exosomes.

The pursuit of non-invasively monitoring exosome distribution during *in vivo* metastasis poses a significant challenge. This endeavor necessitates not only sophisticated, stable molecular imaging techniques, but also the requirement that the introduced markers minimally disrupt the intrinsic biological activity and tumor-targeting proficiency of exosomes themselves. A desirable non-invasive imaging methodology should encompass the grasp of exosome biological distribution, migratory potential, physiological function, and *in vivo* behavior during the intricate landscape of tumor metastasis. Furthermore, it should pave the way for optimizing exosome-mediated drug delivery to achieve the seamless integration of diagnostics and therapeutics. Regrettably, most of the existing labeling methods are built upon purified exosomes, and the existing purification technologies fall short of attaining sustained, high-quality monitoring. Furthermore, some non-targeted TEXs continue to accumulate within the liver, spleen, and kidney, introducing confounds in imaging outcomes [200].

5. Conclusion and prospective

With the advancement of exosome-related fundamental and clinical investigations, the domain of engineered exosomes is experiencing rapid advancement. Numerous enterprises have allocated resources to the development of therapies centered on engineered exosomes [201], and certain initiatives have commenced phase I/II clinical trials [202,203]. Nevertheless, most of these interventions target tumor cells themselves or augment immune responses to eliminate cancer cells, often overlooking the cascade process of tumor metastasis. Consequently, their efficacy in impeding tumor metastasis

might fall short of expectations. The ideal engineered exosomes should ideally possess the capacity to detect and hinder early-stage tumor progression or be administered prophylactically to post-tumor resection patients to mitigate the likelihood of postoperative metastasis. Despite their promising application potential, the further translation and advancement of engineered exosomes face multifaceted challenges.

Chief among these challenges is the translation of fundamental research into clinical applications. Despite substantial progress in exosome research over recent decades, the utilization of modified exosomes in tumor treatment remains in its nascent stages. Firstly, achieving feasible clinical translation demands robust technological foundations and well-established industrial support structures. Following preclinical research, clinical translation necessitates comprehensive considerations such as donor cell selection and characteristics, exosome composition characterization, isolation and purification, as well as product quality control encompassing effectiveness, storage stability, cost-effectiveness, reproducibility, and safety. Secondly, much of the current foundational research relies on preclinical cellular and animal xenograft models, and their actual implications for cancer patients and metastatic sites remain contentious [204,205]. Developing organoid model systems that emulate the conditions of nanocarriers in cancer patients offers a potential solution [206,207], albeit at an elevated cost for clinical translation. While certain xenogenic exosomes appear not to trigger immune rejection, their safety warrants further verification [208]. Exosomes could potentially mitigate this concern, yet their origin and transformation time warrant careful consideration.

Another quandary arises from the profusion of enigmatic mechanisms governing cellular interactions within the intricate landscape of the TME, accentuating the intricacy and ambiguity of therapeutic interventions. The precise functions and cooperative effects of molecules spanning across the spectrum of TME cells remain partially elucidated. Complications endure, not only in elucidating the effectiveness of engineered exosomes post their intricate voyage to metastatic loci, but also in appraising unforeseen prospective hazards linked to their integration. Furthermore, during the engineering procedures, it is paramount to ascertain whether the exosomes themselves might exert influence on the assimilated therapeutic constituents. Constrained by the present technological capabilities and the diversity in detection outcomes, there remains a notable absence of expansive, multicenter investigations into the applications of engineered exosomes in the context of tumor management. Consequently, comprehensive *in vivo* data encompassing considerations of safety, endurance, and effectiveness will prove pivotal in bolstering and propelling the clinical advancement of exosome-based therapeutic modalities. Such advancements will necessitate collaborative endeavors involving researchers, clinicians, pharmacists, and regulatory authorities [209].

It is worth mentioning that although the low immunogenicity of exosomes makes them relatively safer, the exogenous introduction of proteins or functional immune molecules in the engineering process may trigger a strong immune response from the host to some extent [210]. In addition, TEXs still has problems such as immune cell suppression, escape of immune surveillance, and formation of pre-metastasis niche, which should not be ignored before entering clinical research [211]. In the future, new methods need to be developed to characterize and screen exosomes with high immunogenicity and low tumorigenicity, or biomimetic exosome-based therapies to maintain their low immunogenicity biological characteristics as much as possible [84,211]. Interestingly, the immunogenicity of EVs can be utilized in some ingenious ways to exert anti-tumor effects. For example, enhancing the immunogenicity of EVs through reprogramming to synthesize antibodies targeting exosomes [212], or promoting tumor antigen delivery through radiation-induced sEV secreted by tumor cells [213].

Furthermore, diverse cellular origins yield exosomes that exert various anti-tumor responses and maintain homeostasis, as gleaned from the multifaceted roles exosomes play in cancer development [214]. Consequently, novel detection techniques are imperative to discriminate between "beneficial" and "detrimental" exosomes. With the evolution of liquid biopsy and non-invasive imaging technologies, it holds promise that tracking the treatment course of engineered exosomes *in vivo* in real-time will enable more precise early tumor diagnosis and prognosis assessment, thereby evaluating therapeutic efficacy. For exosomes exerting diverse effects, engineering design strategies can be bifurcated. On one hand, "beneficial" exosomes can be loaded with therapeutic molecules to actively target tumor cells; on the other hand, "detrimental" exosomes can be engineered to impede tumor progression.

In summary, engineered exosomes exhibit superior therapeutic efficacy and targeting capabilities compared to their natural counterparts. A comprehensive review of the application of engineered exosomes founded on nanotechnology in cancer metastasis treatment can furnish enhanced comprehension of the rationale, advantages, and constraints of various treatments. It is our belief that as these challenges are progressively addressed, novel strategies in exosome engineering will emerge and be refined, further catalyzing the clinical accomplishments of engineered exosomes.

Conflicts of interest

Ethics approval and consent to participate.

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