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Hybrid exosomes: a rising horizon for precision cancer therapy

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Extracellular vesicles (EVs) are nanoscale vesicles, which show significant promise as biomarkers for cancer diagnosis and prognosis, by providing valuable information about cancer progression and treatment response. Their therapeutic potential (including their popular subset: exosomes) is significant, but challenges remain. These limitations with natural exosomes, necessitate innovative engineering strategies. However, current methods for engineering exosomes, such as chimeric and surface modifications, still need to be improved. A prominent issue is drug off-targeting, leading to ineffective treatment and side effects. To address these challenges, "hybrid exosomes" have been engineered by combining the inherent biocompatibility of natural exosomes with the versatility of synthetic nanoparticles. Cutting-edge design strategies for hybrid exosomes, such as bio-hybrid approaches, emphasize their superior drug loading capacity, and targeted delivery to tumor sites, resulting in minimized toxicity profiles. Furthermore, we showcase recent breakthroughs in leveraging hybrid exosomes for the effective delivery and cellular uptake of chemotherapeutic agents and immunotherapies, which offer significantly enhanced therapeutic outcomes in preclinical cancer models, with emerging clinical relevance. This review explores the evolving field of hybrid exosomes, a novel approach to cancer therapeutics and highlights their potential to overcome existing limitations in cancer treatment. Hybrid exosomes offer a transformative approach to cancer treatment, promising affordable and effective precision therapy with a significant impact on cancer therapeutics.

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1 Introduction

The global shadow cast by cancer continues to lengthen, with the year 2020 alone witnessing a staggering 19.3 million new diagnoses and 10 million lives lost.[1] Projections paint an even grimmer picture, forecasting a surge to 28.4 million cases by 2040, driven by a confluence of population growth and aging.[2,3] These stark figures underscore the urgent imperative for a paradigm shift in our approach to cancer diagnosis and treatment.[1,3,4] At the heart of cancer's devastating progression lies a complex interplay between malignant cells and their surrounding microenvironment.[5,6] This intricate ecosystem, known as the tumor microenvironment,[7,8] comprises of cellular and non-cellular components, including immune cells, fibroblasts, blood vessels, and the extracellular matrix.[5,7] These components play a pivotal role to influence tumor growth, invasion, and metastasis.[6,8,9] Emerging from the depths of this intricate interplay are exosomes,[10] nanoscale vesicles secreted by cells, serving as key mediators of intercellular communication.[11–13] These nano vesicles carry a diverse cargo of biomolecules, including proteins, lipids, and nucleic acids, deeply influencing the behavior of recipient cells.[12,13] Tumor-derived exosomes, in particular, have revealed a darker side, implicated in promoting angiogenesis, suppressing immune surveillance, and facilitating metastasis[14,15] key processes that drive cancer advancement.[10] Yet, within these seemingly double-edged messengers lies a glimmer of hope. The unique molecular signatures of exosomes offer a source of potential biomarkers for early cancer diagnosis and prognosis[16–18] potentially empowering clinicians to intervene at a stage when treatment is most likely to be effective.[7,19] Recent advancements

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in exosome research have spurred the exploration and development of exosome-based therapeutics (a cell-free approach).[9,20–22] Their inherent qualities – nanoscale size, biocompatibility, an ability to evade immune detection, and a remarkable capacity to traverse biological barriers – position them as ideal candidates for targeted drug delivery.[23,24] However, natural exosomes are not without their limitations. Their drug-carrying capacity is often restricted, and off-target effects remain a concern, hindering their full therapeutic potential.[25] Undeterred, scientists embarked on a quest to engineer exosomes. Despite the initial efforts in exosome engineering, including surface modifications and the creation of chimeric exosomes, certain limitations persisted. This spurred the development of "hybrid exosomes," a new class of engineered exosomes designed to overcome these limitations and achieve enhanced therapeutic properties.[26] These engineered vesicles represent a bold leap forward, merging the innate advantages of natural exosomes with the precision engineering of synthetic nanoparticles. This fusion of nature's ingenuity and human innovation aims to amplify drug loading capacity, enhance targeted delivery to malignant cells, and minimize the unwanted side effects of toxicity and immunogenicity.[26] This ambitious endeavor is being pursued through a multifaceted approach. Top-down methods focus on meticulously sculpting nanovesicles, while bottom-up strategies center on designing hybrid exosomes with bespoke properties. Biohybrid techniques, a testament to the power of synergy, strive to seamlessly merge synthetic nanoparticles with natural exosomes, creating a new breed of therapeutic agents.[27] As the global cancer burden continues its relentless ascent, the development of novel diagnostic and therapeutic strategies is no longer a matter of scientific curiosity but a humanitarian imperative. Exosomes have taken center stage, their potential as both biomarkers and therapeutic agents undeniable. Yet, it is in the realm of hybrid exosomes, where nature's ingenuity meets human innovation, that the brightest hope for conquering cancer may lie. These engineered marvels hold the potential to overcome the limitations of their natural counterparts, ushering in a new era of precision medicine where cancer treatment is tailored to the individual, minimizing suffering and maximizing treatment outcomes. This review explore cancer and tumor development interlink, dynamic therapeutic exosomes sources, hybrid exosomes, clinical trials and its future orientation.

2 Exosome biogenesis

Exosome biogenesis (Fig. 1) is a meticulously regulated cascade of pathways, molecular components and associated events in the cellular endomembrane system, leading to the formation of these nanoscale extracellular vesicles (30–120 nm approx.). The Endosomal Sorting Complex Required for Transport (ESCRT) pathway is pivotal in this process, comprising of several complexes: ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, alongside proteins such as VPS4 (the disassembly engine) and ALIX (versatile adaptor protein).[28–30] Initially, ESCRT-0 identifies ubiquitinated cargo at the endosomal membrane, initiating the sorting process. This is followed by the sequential recruitment of ESCRT-I, -II, and -III, which facilitate membrane invagination and vesicle scission to produce intraluminal vesicles (ILVs).[31,32] VPS4, an ATPase, disassembles ESCRT components, enabling their reuse after the formation of multivesicular bodies (MVBs) containing ILVs.[28,31,33] Beyond the ESCRT pathway, ceramide-rich microdomains, potentially *via* nuclear envelope budding, contribute to the diverse routes of exosome biogenesis.[34] Ceramide-dependent mechanisms induce membrane curvature and budding, while tetraspanin-enriched microdomains assist in cargo sorting.[34–37] While membrane lipid microdomains, including specific components like SPFH proteins and caveolin, and heat shock proteins (HSP70/90) are implicated in exosome biogenesis, their precise roles require further investigation.[35,37,38] Ultimately, MVBs can either fuse with lysosomes for degradation or with the plasma membrane to release ILVs as exosomes into the extracellular milieu.

3 Exosome isolation and characterization

Exosome isolation (Fig. 2) hinges on exploiting the biophysical properties of these nanovesicles, leading to a diverse array of techniques.[39–41] Differential ultracentrifugation, a cornerstone method, leverages sequential centrifugation steps at increasing gravitational forces (g) to pellet exosomes based on their sedimentation coefficient, effectively separating them from larger vesicles and cellular debris.[15,41] Density gradient centrifugation refines this by layering a sucrose or iodixanol gradient, allowing exosomes to band at their buoyant density (1.13–1.18 g mL^{-1}) for enhanced purity.[42] Size-exclusion chromatography, on the other hand, separates based on the hydrodynamic radius, with smaller exosomes eluting later due to their restricted access to the porous stationary phase.[43,44] Tangential flow filtration, scalable for large volumes, utilizes a membrane with defined pore sizes (typically 50–100 nm) to selectively retain exosomes while smaller molecules pass through.[45,46] Ultrafiltration operates similarly but relies on centrifugal force to drive the sample through the membrane, making it ideal for small volumes.[40,47] Precipitation-based methods, often commercially available kits, exploit the altered solubility of exosomes in the presence of water-excluding polymers like polyethylene glycol, leading to their precipitation.[40,48] Immunoaffinity capture offers high specificity by employing antibodies immobilized on beads or surfaces to selectively bind exosomes expressing specific surface markers.[49,50] Microfluidic isolation leverages miniaturized devices integrating various separation mechanisms, including filtration, affinity capture, and acoustic trapping, for high-throughput and automated isolation.[47,51,52] Acoustic fluid handling manipulates exosomes based on their acoustic properties, using sound waves to focus and separate them.[53] Magnetophoresis utilizes magnetic beads coated with antibodies against exosomal markers, allowing for efficient capture and release of exosomes through magnetic fields.[54] Deterministic lateral displacement (DLD) exploits the size and deformability of exosomes, separating them based on their trajectory in

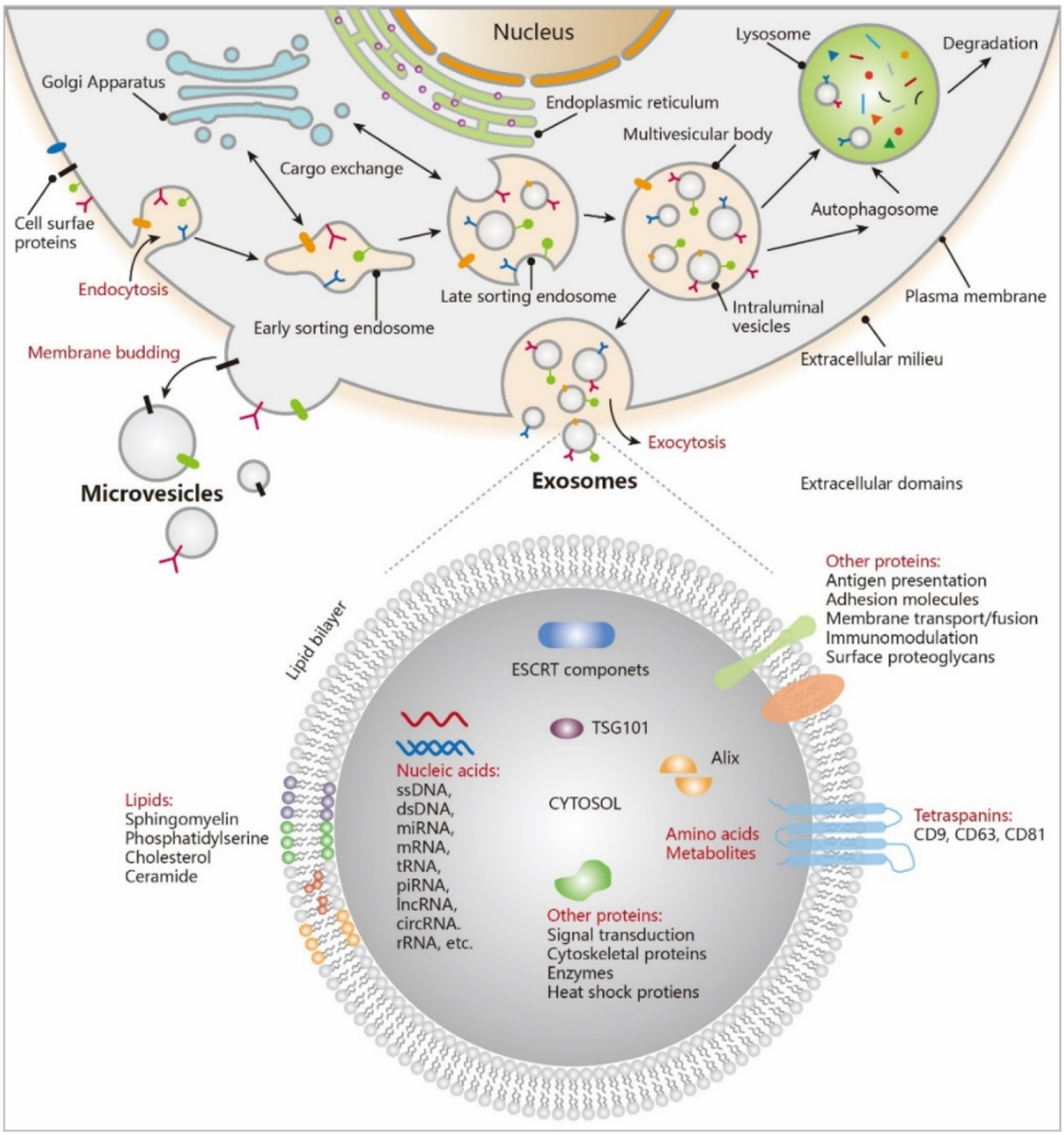

Fig. 1 Exosome biogenesis and its molecular cargos. (Reproduced with permission from ref. 167 Copyright @ 2021 American Chemical Society).

microfluidic channels with asymmetrically arranged obstacles.[55,56] Field-flow fractionation separates particles in a thin channel under an external field, such as a flow field or electric field, based on their differential migration.[57–60] Exosome characterization relies on a diverse toolkit of techniques to elucidate their biophysical and biochemical properties.[61] Nanoparticle tracking analysis visualizes and tracks the Brownian motion of individual exosomes, using light scattering to determine their size distribution and concentration.[62,63] Dynamic light scattering also exploits light scattering but measures the fluctuations in scattered intensity due to Brownian motion, providing information on hydrodynamic size and polydispersity.[60,64] Electron microscopy, encompassing transmission EM and scanning EM, offers high-resolution visualization of exosome morphology, size, and structure.[61] Atomic force microscopy provides nanoscale topographical information by scanning a sharp tip over the exosome surface, revealing details about size, shape, and surface features.[61,65] Western blotting, a staple technique, detects the presence and relative abundance of specific proteins in exosome lysates, confirming the presence of exosomal markers and target proteins.[15,66] Flow cytometry, though challenging due to exosome size, can analyze individual exosomes for size, granularity, and surface marker expression using fluorescently labeled antibodies.[48,67] Enzyme-linked immunosorbent assay quantifies specific proteins or other molecules in exosome samples using antibody-based detection, offering high sensitivity and specificity.[41,61] Raman spectroscopy analyzes the inelastic scattering of light by molecules in

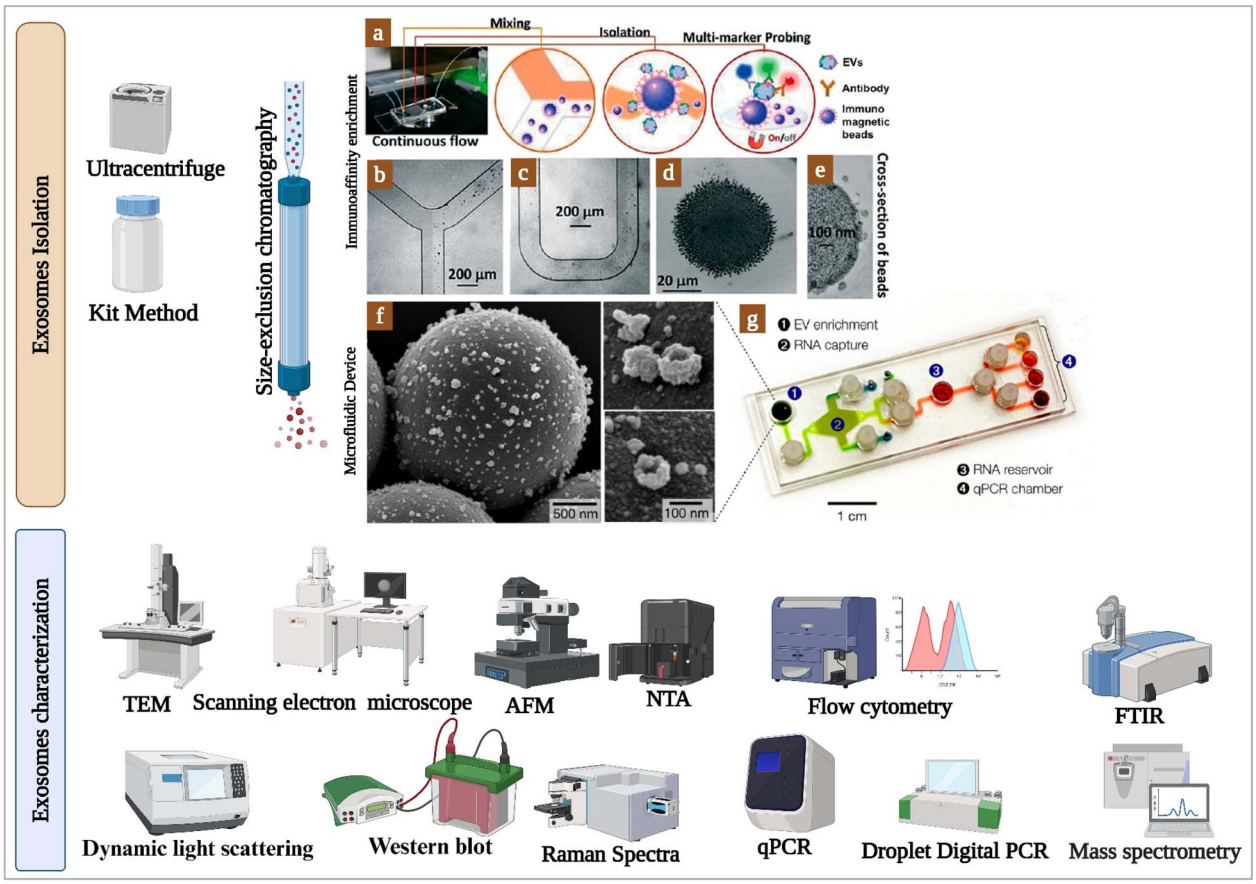

Fig. 2 Exosomes isolation and characterization method. Immunoaffinity enrichment. (a) Schematics of a microfluidic chip, microscopic view of the device: (b) Y-shaped injector, (c) serpentine fluidic mixer for immunomagnetic binding, (d) magnetic aggregates, and (e) bound EVs on immunomagnetic beads. Microfluidic device (f) scanning electron micrographs of magnetic microbeads after immunoaffinity capture, (g) image of the microfluidic iMER (immuno-magnetic exosome RNA analysis) prototype (for (a–g) reproduced with permission from ref. 61 Copyright @ 2018 American Chemical Society).

exosomes, providing information about their biochemical composition and structure.[68] Mass spectrometry identifies and quantifies proteins, lipids, and metabolites in exosome samples, offering a comprehensive molecular profile.[57,69,70] RNA sequencing analyzes the RNA content of exosomes, revealing their mRNA, miRNA, and other RNA species, providing insights into their function and origin.[61] Lipidomics focuses on characterizing the lipid composition of exosomes, providing insights into their membrane structure and function.[69,71] Finally, Integrated Magnetic-Electrochemical Exosome (iMEX) sensor can be used for exosome characterization, specifically for quantifying exosome surface markers. This method is a rapid and sensitive approach that emphasizes the iMEX's ability to detect varying numbers of EVs spiked into human plasma, demonstrating its effectiveness in quantifying exosomes.[61]

4 Role of exosomes in cancer

The tumor microenvironment (TME) encompasses an intricate network of cellular and non-cellular components that collectively foster cancer progression and metastasis.[11,72,73] Hypoxic, or oxygen-deprived, conditions within the tumor niche are a hallmark feature, characterized by the insufficient blood supply and inadequate oxygen availability.[74,75] In response to this hostile, nutrient-depleted environment, cancer cells exhibit remarkable adaptability. Activating various survival pathways and oncogenic signaling cascades, primarily mediated by hypoxia-inducible factors (HIFs).[76–78] This allows the malignant cells to proliferate uncontrollably, evade cell death, and divert essential resources, such as oxygen and nutrients, for their rapid growth and expansion.[77,79,80] Through this process, the cancer cells effectively hijack the body's natural homeostatic mechanisms, disrupting the delicate balance of the surrounding normal tissue and creating a permissive microenvironment that supports their malignant transformation and metastasis.[74,81] Researchers also indicate that hypoxia drives the secretion of exosomes from tumor cells.[82,83] These nanoscale vesicles, secreted by tumor cells, act as critical mediators of intercellular communication, which carry molecular signals that further alter the TME, promoting angiogenesis[84] and tumorigenesis (Fig. 3).[82,83,85]

One crucial aspect of the TME is the process of angiogenesis, where new blood vessels are formed to support the rapidly dividing cancer cells and facilitate metastasis by creating

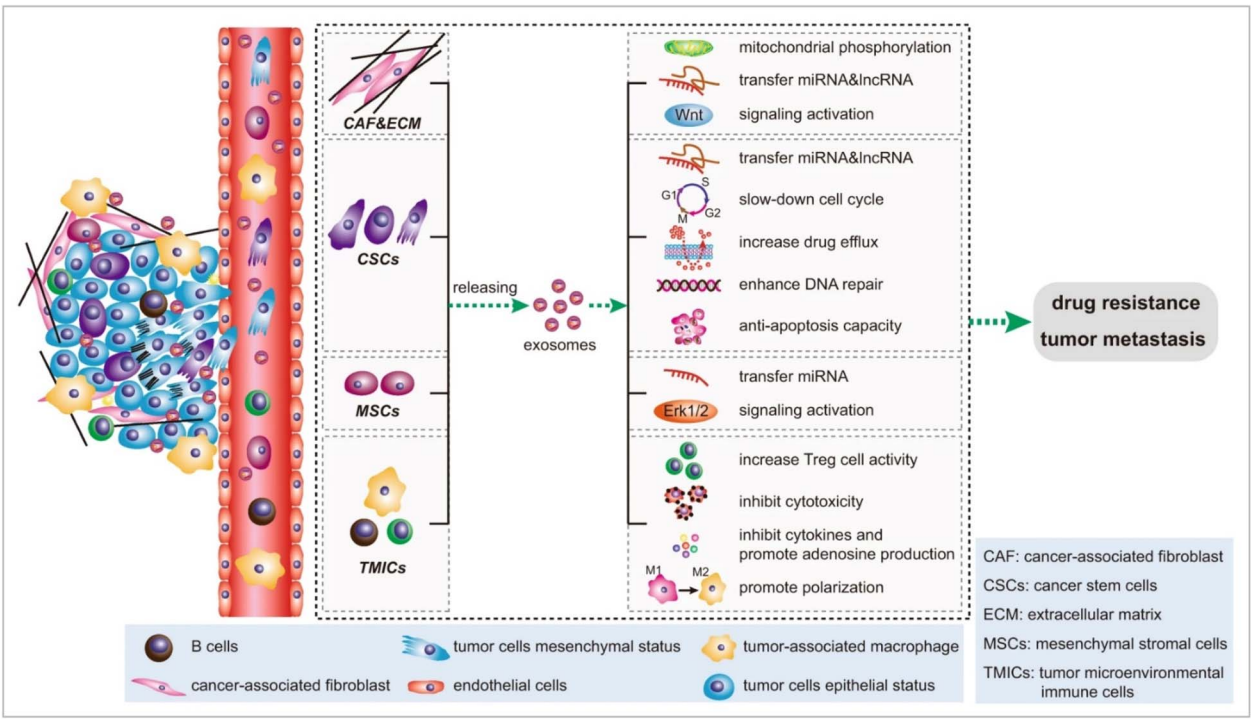

Fig. 3 Role of tumor-derived exosomes in TME (reproduced with permission under Creative Commons CC BY 4.0 license from ref. 21 Copyright @ 2020 The Authors).

pathways for cancer cells to enter the bloodstream. Interestingly, transformed cancer cells can exhibit characteristics of pericytes, cells that typically surround and support blood vessels. These cancer cells can cluster around blood vessels, influencing tumor development and metastasis. This intricate interplay between angiogenesis, pericyte-like cancer cells, and the TME highlights the complexity of tumor progression.[86] Exosomes, nanoscale vesicles, play a pivotal role in mediating intercellular communication within the TME.[87] Tumor-derived exosomes (TEXs) can promote angiogenesis by transferring pro-angiogenic factors and microRNAs to endothelial cells, enhancing their proliferation and migration.[72,87,88] For instance, exosomes from hypoxic tumor cells have been shown to contain elevated levels of miR-210, which promotes angiogenesis by targeting genes involved in endothelial cell function.[89,90] TEXs exhibit a multifaceted influence on the tumor microenvironment, profoundly impacting immune cell behavior and ultimately facilitating immune evasion.[91–93] These nanoscale vesicles, orchestrate a complex interplay between tumor cells and various immune cell populations. One of the key mechanisms by which exosomes contribute to tumor progression is by inducing a shift in the balance of immune cells towards an immunosuppressive state.[91,92,94] TEXs have been shown to reprogram macrophages to promote cancer development.[95,96] For instance, exosomal miR-934 has been shown to induce a shift in macrophages towards the pro-tumorigenic M2 phenotype, contributing to a tumor-supportive microenvironment that facilitates metastasis to the liver.[96] This polarization is associated with enhanced angiogenesis, moreover, M2 macrophages suppress anti-tumor immune responses, creating a permissive environment for tumor progression.[95,97] Beyond their influence on macrophages, TEXs can also promote the differentiation of monocytes into immunosuppressive myeloid-derived suppressor cells (MDSCs), further inhibiting T cell responses.[98] TEXs achieve this by transferring signaling molecules, including proteins like prostaglandin E2 (PGE2) and transforming growth factor beta (TGF-β), and miRNAs such as miR-21, miR-10a, miR-494-3p, and miR-1260a, to recipient monocytes. These molecules activate downstream signaling pathways, including STAT3 and MyD88, leading to the differentiation of monocytes into MDSCs.[98–101] Consequently, MDSCs expressing high levels of immunosuppressive molecules like Arg1, IL-6, VEGF, and Cox2 accumulate within the tumor microenvironment.[98,99] Tumor-derived exosomes directly suppress the cytotoxic activity of T cells and natural killer cells, contributing to immune evasion. This is achieved through exosomal cargo such as TGF-β, a potent immunosuppressive cytokine, which inhibits T cell activation and promotes regulatory T cell differentiation, and programmed death-ligand 1 (PD-L1), which engages with programmed cell death protein 1 (PD-1) on T cells, leading to T cell exhaustion and suppression of anti-tumor immunity.[102] Dendritic cells are pivotal in initiating immune responses. However, exosomes derived from tumors can convert DCs into tolerogenic cells, which fail to activate T cells effectively. This conversion is often mediated by exosomal TGF-β, which enhances the expression of inhibitory receptors on DCs, leading to a diminished anti-tumor response.[102] This intricate interplay between tumor cells,

exosomes, and immune cells highlights the key mechanisms by which tumors evade immune surveillance, ultimately promoting their growth and facilitating metastasis.[91,95,98,102] Research suggests exosomes to be critically involved in the cascade of metastasis, a hallmark of cancer progression, by facilitating epithelial-mesenchymal transition (EMT), a process where epithelial cells lose their polarity and cell-to-cell adhesion, acquiring mesenchymal properties that enhance their migratory and invasive capabilities. TEXs can transfer bioactive molecules, including microRNAs (miRNAs), to recipient cells within the TME, promoting EMT and thereby facilitating metastasis.[103] For instance, exosomes released from tumor cells can facilitate colorectal cancer metastasis by influencing the interaction between cancer cells undergoing epithelial-mesenchymal transition and M2-subtype tumor-associated macrophages (TAMs). Specifically, exosomal microRNA-106b-5p has been implicated in activating this cross-talk, promoting a tumor-supportive microenvironment that enhances the metastatic potential of colorectal cancer cells.[104] Furthermore, exosomes contribute to organ-specific metastasis, a phenomenon where tumor cells exhibit a predilection for metastasizing to specific organs.[105,106] Exosomal surface proteins like tetraspanins and integrins play a crucial role in cancer progression *via* metastasis and organotropism.[105,107] These surface proteins, including integrins α6β4 and αvβ5, acting as adhesion molecules, are selectively packaged into exosomes and mediate the interaction between circulating tumor cells and their target microenvironments.[107] This interaction is based on the specific binding affinity of these integrins to ligands expressed on the target cells. For instance, exosomes from lung-tropic tumor cells, enriched with specific integrins, preferentially bind to and fuse with lung fibroblasts and epithelial cells. Similarly, liver-tropic exosomes, carrying a different set of integrins, interact with Kupffer cells in the liver.[107] This selective adhesion, guided by the integrin expression patterns on exosomes, not only facilitates the anchoring of tumor cells to the new site but also triggers intracellular signaling cascades.[105,107] One such pathway involves the activation of Src kinase, a key regulator of cell survival, proliferation, and migration. The phosphorylation of Src, initiated by integrin engagement, promotes the survival and proliferation of tumor cells within the new environment.[105,107,108] This mechanism highlights the crucial role of exosomal integrins in orchestrating the metastatic process and underscores their potential as biomarkers for predicting organ-specific metastasis. Adding to the complexity of cancer progression, exosomes also contribute to therapeutic resistance (Fig. 4), either before treatment begins or emerging over time, a major obstacle in cancer treatment.[109,110] This resistance to therapy is a major contributor to the global cancer burden, contributing to staggering numbers of deaths.[1,111] Tumor-derived exosomes can transfer drug resistance genes and proteins, such as those conferring resistance to paclitaxel, enabling recipient cells to evade the effects of chemotherapy or targeted therapies. For example, exosomes from cisplatin-resistant lung cancer cells can transfer miRNA-100-5p, which alters mTOR signaling and enhances survival under chemotherapy.[110,112] Additionally, exosomal proteins like P-glycoprotein 1 (P-gp), a permeability glycoprotein, contribute to drug efflux mechanisms, where the uptake of P-gp-containing exosomes from resistant cells can induce resistance in sensitive cells.[113,114] Exosomes released from breast cancer cells can contribute to resistance against HER2-targeted therapies through two primary mechanisms. First, exosomes overexpressing HER2 can directly bind to and sequester targeted drugs, reducing their efficacy. Second, these exosomes can reprogram the gene expression of recipient HER2-positive breast cancer cells, promoting a shift towards a HER2-independent phenotype that renders them less susceptible to HER2-targeted treatments.[114,115] In conclusion, exosomes demonstrate a dual nature in the context of cancer, acting as both drivers of disease progression and potential sources of innovative therapeutic strategies.

5 Therapeutic exosomes

Exosomes, nanoscale vesicles secreted by cells majorly utilising the ESCRT pathway, are increasingly recognized for their superior therapeutic potential over conventional treatment modalities, demonstrating enhanced targeting efficacy and protected cargo delivery properties.[116,117] Exosome-based therapeutic (Fig. 5) strategies represent an effective cancer nanomedicine.[118] Naturally derived exosomes from various sources, such as milk, bacteria, mesenchymal stem cells, neurons, and even plants, have demonstrated remarkable potential in areas like cancer immunotherapy, regenerative medicine, and drug delivery.[116,119] Simultaneously, the field has witnessed the advent of synthetically engineered[173–175] or chimeric exosomes, which have been tailored to enhance targeting capabilities and improve cargo loading for gene therapy and other specialized applications.[118,120] Stem cell derived exosomes a promising anticancer therapeutic tool.[121] Another study demonstrated the potential of engineered macrophage-derived exosomes as a cancer immunotherapy.[176] By using gamma irradiation, they engineered these exosomes to carry pro-inflammatory cytokines and tumor antigens. These engineered exosomes effectively repolarized M2 macrophages into the anti-tumor M1 phenotype, resulting in increased inflammatory mediator production and enhanced T cell activation. *In vivo*, these exosomes significantly inhibited tumor growth and improved survival in a mouse model of colon cancer, highlighting the potential of engineering macrophage-derived exosomes to enhance antitumor immune responses.[122] Tumor-derived exosomes (TEXs) demonstrate a complex role in cancer immunology.[123] They can act as potent messengers, carrying tumor-associated antigens to dendritic cells, key orchestrators of the immune response. This interaction promotes dendritic cell maturation and enhances their ability to activate T cells, leading to a targeted anti-tumor immune response.[123] Pancreatic cancer-derived exosomes, abundant in tumor antigens, effectively activate CD8+ T cell responses *via* dendritic cell antigen presentation, highlighting their potential as an anti-tumor therapeutic strategy.[124,125] Red blood cell-derived exosomes are emerging as a promising drug delivery platform. Their high biocompatibility, coupled with the ability to be engineered for targeted delivery, makes them

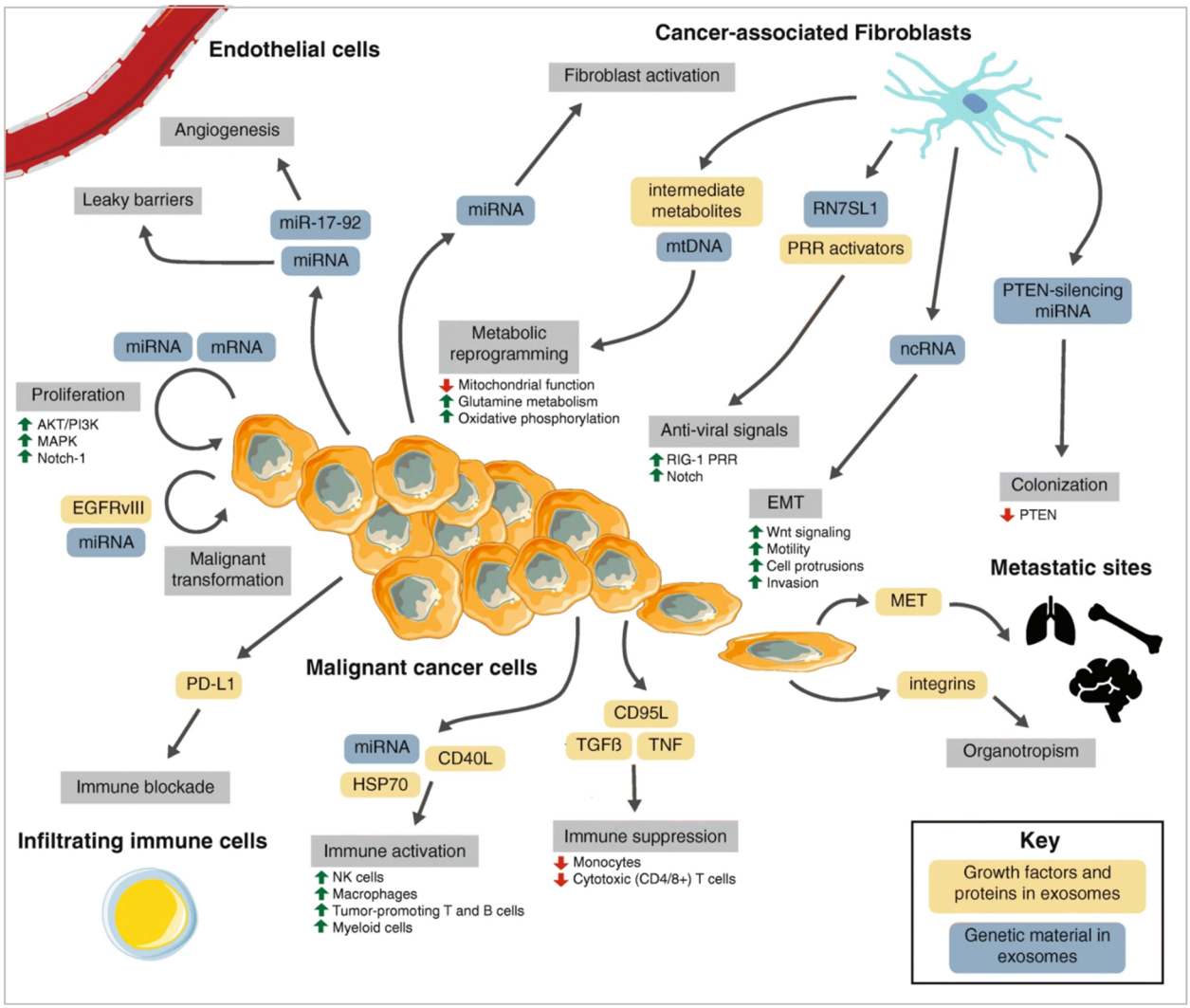

Fig. 4 Role of exosomes in therapeutic resistance (reproduced with permission under Creative Commons CC BY 4.0 license from ref. 169. Copyright @ 2019 The Authors).

attractive candidates.[126] Drugs and therapeutic loaded and their surface markers facilitate binding to endothelial cells *via* mechanisms like receptor-mediated endocytosis, enhancing drug delivery efficiency.[127,128] Interestingly, plant-derived exosomes have demonstrated the ability to mimic the structure and function of mammalian exosomes, opening up new avenues for therapeutic delivery.[129] Plant-derived exosomes, specifically from ginger, exhibit anti-inflammatory properties.[130] These vesicles, structurally and functionally similar yet safer than mammalian exosomes,[131] effectively deliver bioactive compounds that modulate inflammatory pathways, including NF-κB and MAPK. Notably, GDENs inhibit NLRP3 inflammasome activation, highlighting their potential as therapeutic agents for inflammatory diseases.[130,132,133] Bacterial EV-based delivery systems are being actively explored for their therapeutic and diagnostic potential in a wide range of diseases. These applications span across various conditions, including hyperammonemia, infections, cancer, and kidney failure.[134] Current time milk-derived exosomes are an effective cancer therapeutic approach (due to it's dual nature, these sources require more research).[135] Bacterial outer membrane vesicles, particularly from *Akkermansia muciniphila*, demonstrated potent anti-tumor activity by activating dendritic cells and triggering an interferon-γ-mediated immune response. This results in enhanced T cell cytotoxicity and an altered tumor microenvironment that effectively targets and destroys tumor cells.[136] Bovine milk-derived exosomes are gaining recognition as a novel class of therapeutic agents, demonstrating efficacy as nanocarriers for enhanced drug delivery and immunotherapy, particularly in the context of cancer. These naturally secreted nanovesicles exhibit the capacity to encapsulate and stabilize cancer antigens,[137] leading to enhanced antigen presentation by dendritic cells and subsequent activation of a cytotoxic CD8+ T cell response against tumor cells.[137,138] The field of exosome-based therapeutics has also witnessed the emergence of synthetically engineered or chimeric exosomes. Genetically engineered exosomes, modified with specific ligands or genetic material, have shown enhanced targeting capabilities for cancer

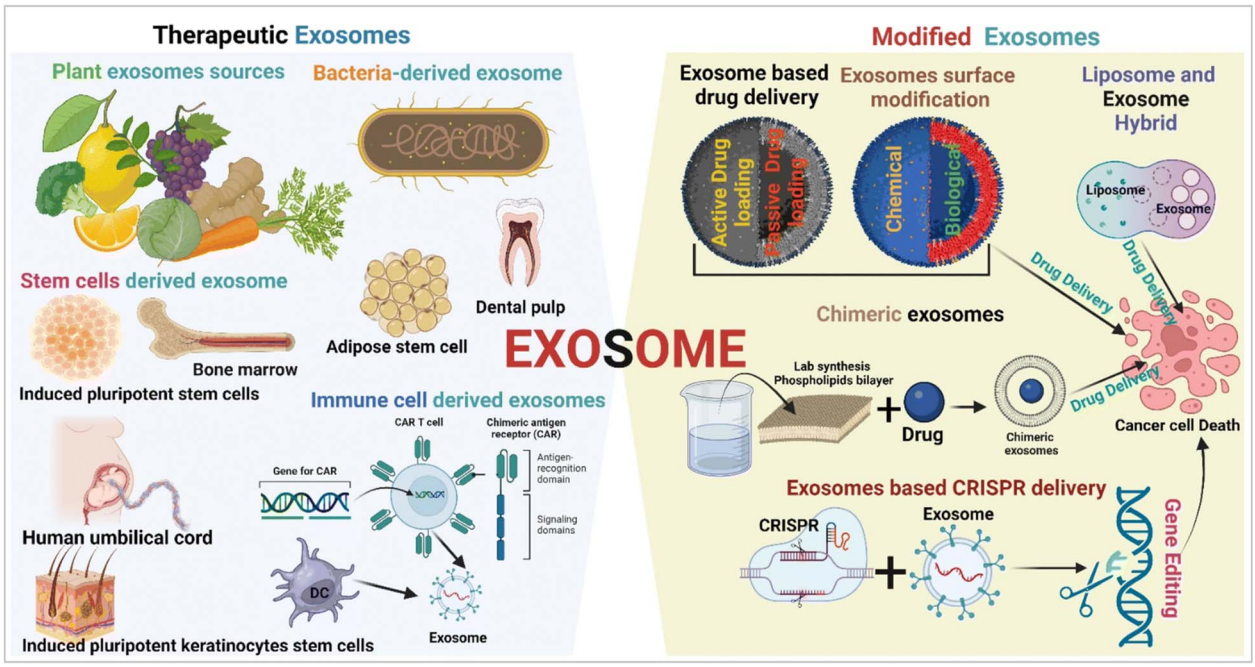

Fig. 5 Therapeutic exosomes. (Reproduced with permission under Creative Commons CC BY 4.0 license from ref. 177 Copyright @ 2024 The Authors).

therapies.[139] Chimeric exosomes, engineered to display both tumor-associated antigens and checkpoint inhibitors, offer a promising approach to enhancing cancer immunotherapy. These exosomes activate dendritic cells, leading to enhanced T cell activation and a more robust anti-tumor immune response. This approach promotes increased production of inflammatory mediators and cytotoxic T lymphocyte proliferation, ultimately boosting the immune system's ability to target and eliminate cancer cells.[140,141] Engineered exosomes show promise for efficient gene delivery. A study suggests that these synthetic exosomes effectively deliver plasmid DNA and siRNA into target cells *via* clathrin-mediated endocytosis. Once inside lung cancer cells (A549), the delivered genetic material successfully modulates gene expression, notably enhancing gene silencing effects, highlighting the potential of engineered exosomes as a valuable tool for gene therapy. Click chemistry is a promising approach for modifying exosomal surfaces, enabling robust functionalization with therapeutic molecules to enhance their therapeutic potential.[142] Exosomes can be engineered for enhanced tumor targeting by conjugating azide-modified exosomes with DBCO-modified antibodies, improving their potential for cancer immunotherapy.[140,143] Functionalization of exosomes with therapeutic ligands *via* click-chemistry enhances drug delivery and immune modulation within tumor microenvironments.[144] This approach also facilitates the attachment of fluorescent and imaging agents to exosomes, enabling their tracking and monitoring *in vivo*.[144] Hybrid exosomes represent a convergent evolution in drug delivery, combining the advantages of different exosome sources or incorporating synthetic components. Hybrid exosomes with their superior biocompatibility and therapeutic efficacy, minimize the toxicological concerns associated with some existing approaches, potentially surpassing them.

6 Hybrid therapeutic exosomes

After chimeric exosomes and surface-modified exosomes, hybrid exosomes were introduced to cancer therapy with numerous promising characteristics like a high drug loading capacity and targeted cellular uptake, along with low toxicity, high biocompatibility and low immunogenicity to reduce the limitations of exosome-based cancer therapeutics.[145,166] Hybrid exosomes (Fig. 6) are being designed by combining with various nanoparticles to increase the efficiency and resolve the challenge associated with exosome-based cancer therapeutics like drug off-targeting.[146,170] In recent research, two approaches have been highlighted for designing artificial exosomes, to be specific they are top-down (for developing nanovesicles or NVs) and bottom-up (for designing exosome-mimetic or EM). Additionally, bio-hybrid is another significant technique for combining synthetic nanoparticles (NPs) with natural extracellular vesicles, specially exosomes to design hybrid exosomes.[147] Numerous experiments have been conducted to combine synthetic nanoparticles (majorly liposomes) with various cell-derived exosomes with different bio-hybrid approaches to increase the efficacy of clinical applications (Table 1). Exosomes are being combined with liposomes through membrane fusion strategies to develop hybrid exosomes that are utilized for targeted drug delivery in chemotherapy. For instance, Lv *et al.*, have designed thermosensitive exosome–liposome hybrid nanoparticles and used them to co-deliver granulocyte-macrophage colony-stimulating factor (GM-CSF) and docetaxel

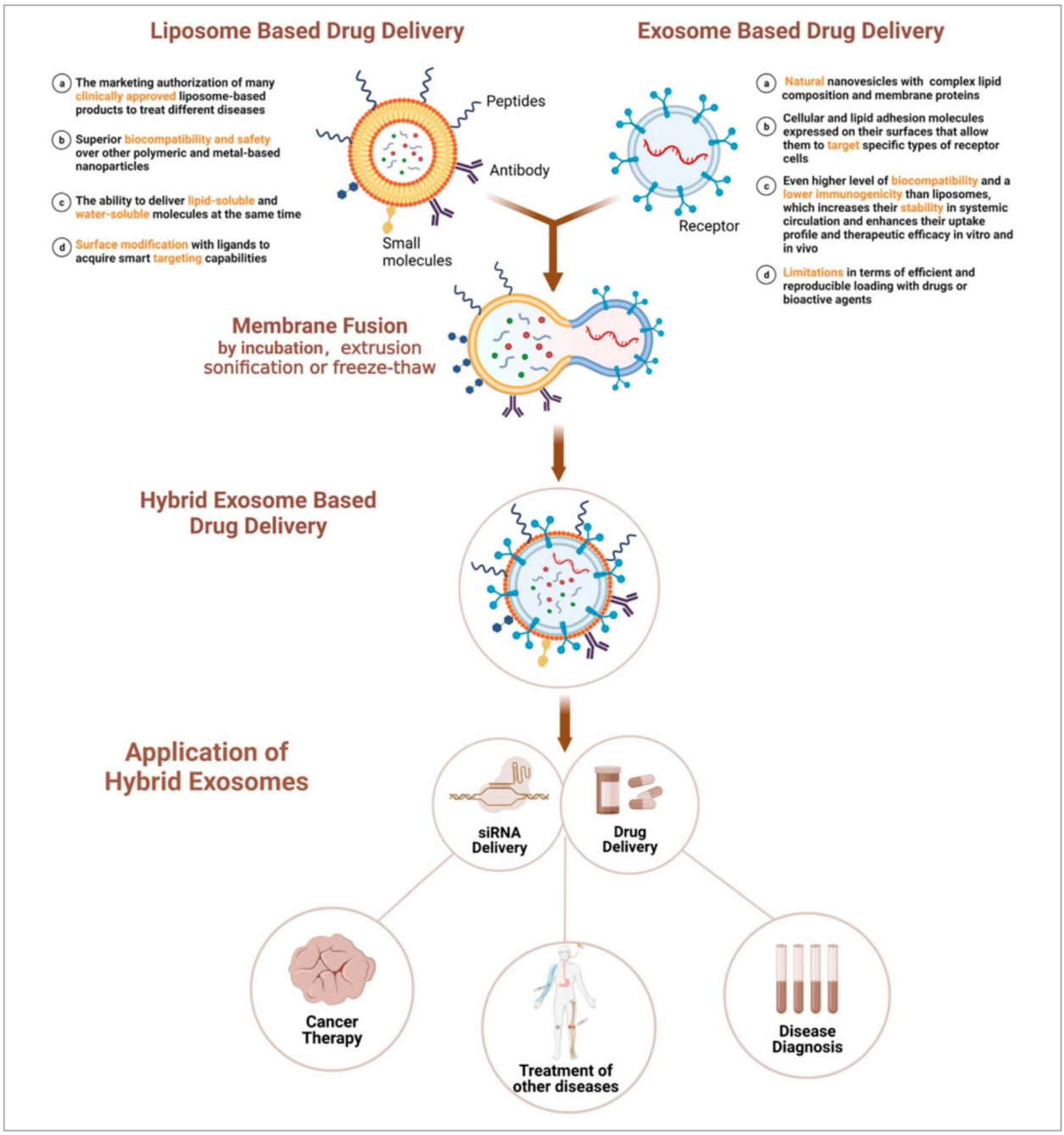

Fig. 6 Application of hybrid exosomes in the clinical field. (Reproduced with permission under Creative Commons CC BY 4.0 license from ref. 178 Copyright @ 2022 The Authors).

for treating metastatic peritoneal carcinoma. Their study found that these hybrid exosomes penetrated the tumor microenvironment effectively after intravenous injection, leading significant contribution to tumor suppression and improved chemotherapeutic efficacy.[148]

In a similar effort, Piffoux *et al.* also developed liposome–exosome hybrid nanoparticles with PEG to deliver meta-tetra (hydroxyphenyl)chlorin or mTHPC (an anti-tumor photosensitizer) to cancer cells. They found that the hybrid exosome achieved intracellular delivery that was 3 to 4 times greater than that of the free drug or the drug's liposomal formulation.[149] However, recent research suggests exosome–liposome hybrid nanoparticles can improve the carrier stability and drug-loading capability of paclitaxel (PTX) and showed this PTX-loaded nanodrug delivery system improves treatment efficacy in mice with colorectal tumors. Additionally, it alters the tumor immune microenvironment by boosting CD4+ and CD8+ T cell activation, enhancing M1 macrophage polarization, and reducing Treg cell levels. This hybrid system offers significant potential for advancements in exosome engineering and its

Table 1 Combination of synthetic nanoparticles with various cell-derived exosomes with different bio-hybrid approaches

Synthetic nanoparticles	Natural vesicles	Biohybrid approach	Comparison to natural exosomes	Specific advances	Significant application	Ref.
Liposomes (DOPC, DOTAP, DSPE-PEG2000)	Raw264.7 cell-derived exosomes	Freeze-thawing	Increased size and similar protein markers	Membrane surface engineering	Exosome modification	151
Liposomes (DSPE-PEG2000)	Genetically engineered fibroblast-derived exosomes	Freeze-thawing	Similar morphology and protein markers	Lipid engineering of exosomes	Thermo-sensitive chemoimmunotherapy	148
Liposomes (lipofectamine 2000)	HEK293FT cell-derived exosomes	Simple incubation	Increased size, but similar protein markers	Efficient encapsulation of large plasmids	CRISPR/Cas9 system transfer to MSCs	152
Liposomes (POPC, DOPE)	HUVEC-derived EVs	Incubation with PEG-mediated fusion	Increased size but similar morphology and protein markers	Efficient EV cargo loading and delivery	Drug loading and delivery	149
Liposomes (L-α-phosphatidylcholine and cholesterol)	Mouse macrophage J774A.1 cell-derived sEVs	Extruding (400 and 200 nm)	Increased size but similar protein markers	Colloidal stability drug loading and pH-sensitive sustained drug release	Tumor-targeted drug delivery	153
Lipids (DOTAP, POPC, DPPC or POPG)	EVs derived from fibroblast 3T3 cells or A549 lung cancer cells	Extruding (400, 200 and 100 nm)	Similar size and with native EV fractions	Mass production (6- to 43-fold vesicles)	Efficient siRNA delivery	154

future applications can significantly contribute to precision cancer therapeutics.[150]

7 Clinical trial

In recent years, extracellular vesicles (EVs), particularly exosomes, have a significant role in cancer theranostics research. Several preclinical studies have been conducted demonstrating the potential of exosomes in medical research.[13,155] Furthermore, numerous clinical trials have been conducted, showcasing their utility as diagnostic and prognostic biomarkers, drug delivery systems, and innovative therapeutic approaches.[156] Exosomes serve as robust messengers, carrying vital information from cells into various body fluids. When cells are affected by disease, they release exosomes with distinct molecular payloads (cargos).[157] By decoding these signatures, scientists can uncover potential biomarkers for diseases, enabling early detection and monitoring for improved disease management. For instance, the differential expression of several miRNAs is capable of providing essential information regarding cancer progression and validating treatment efficacy.[158,159] A huge number of exosome clinical trials (Fig. 7) focus on cancer markers detection compared to other domain.[160] On the other hand, exosome-based drug delivery is gaining significant attention in clinical trials as a promising strategy for addressing global health challenges, including cancer.[161] Exosomes derived from diverse sources are being explored for their potential to revolutionize cancer treatment. Furthermore, various cell-derived exosomes are emerging as a focal point of modern research in medicine and health sciences, due to their distinctive properties and capacity to augment therapeutic outcomes. Notably, several clinical trials are currently investigating exosome-based cancer vaccine approaches, aiming to enhance treatment efficacy and improve patient outcomes.[160,162]

Despite the promising potential of hybrid exosome approaches in cancer treatment, a significant gap exists in pre-clinical and clinical trials, hindering further advancements in this innovative field. As a novel and smart strategy, hybrid exosome approaches warrant more extensive investigation to fully realize their therapeutic potential. Therefore, it is imperative to conduct additional clinical trials that focus on bio-hybrid exosome approaches, harnessing their unique properties to develop more effective cancer treatments. By doing so, we can unlock new possibilities for cancer treatment.

8 Challenges and future perspective

Despite significant advances in exosome research, the field faces notable challenges, including gaps in understanding exosome biogenesis, isolation techniques, and their inherent heterogeneity. The concept of hybrid exosomes offers a promising approach for clinical therapeutics, particularly in oncology, but their practical use, especially in targeted drug delivery, encounters several difficulties. These include issues with large-scale production, purification, modification, drug loading, and storage, as well as the complexity introduced by the heterogeneity[168] of extracellular vesicle subpopulations.[163,164,171,172] Hybrid exosomes, produced through biohybrid methods, also face challenges such as low yields and the demanding process of combining synthetic liposomes with natural vesicles. Characterization is difficult due to their similarity to liposomes and natural exosomes, complicating purification. Major hurdles include refining preparation protocols, ensuring accurate characterization, and addressing biocompatibility.[147] Membrane fusion hybrid exosomes (MFHEs) integrate the benefits of both exosomes and liposomes, showing potential for improved targeted drug delivery. However, enhancing the fusion between liposomes and exosomes while

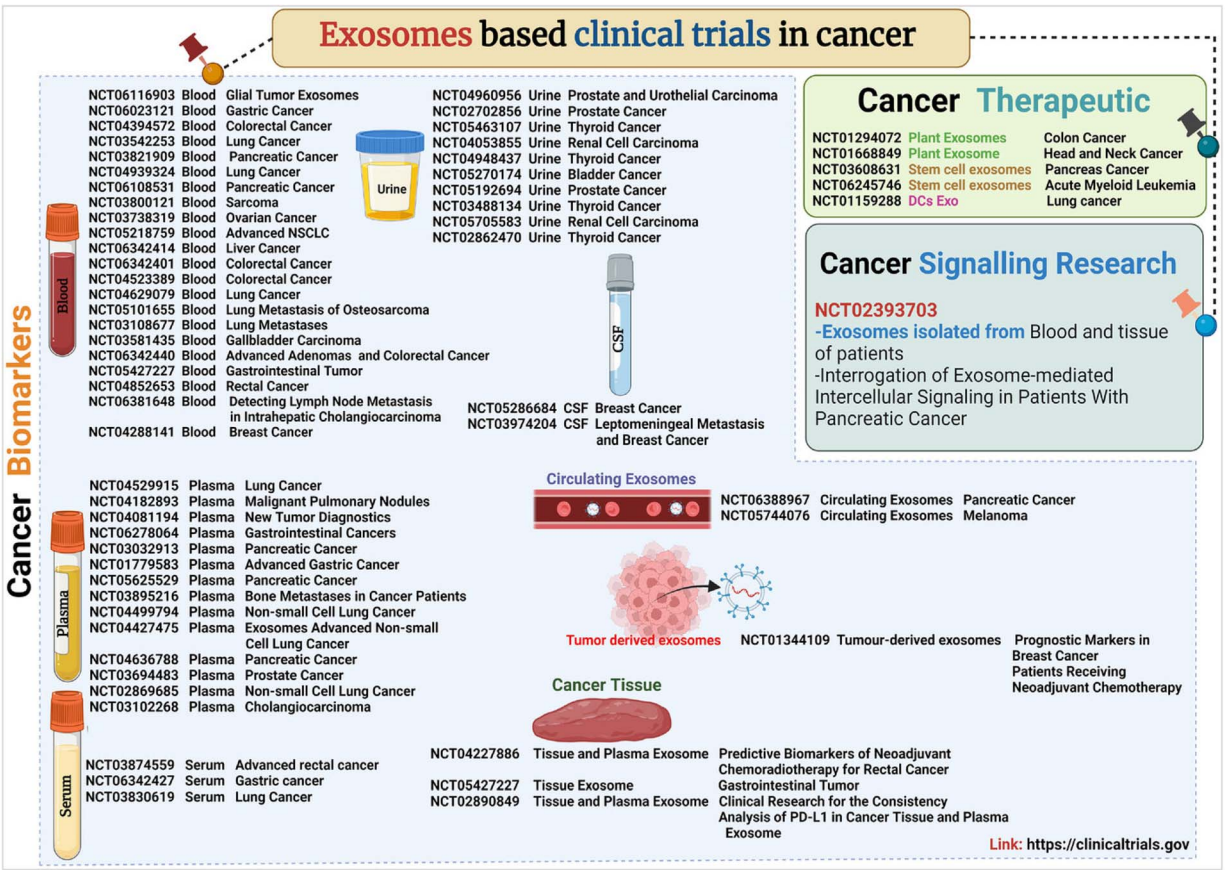

Fig. 7 Clinical trial of exosomes. (Reproduced with permission under Creative Commons CC BY 4.0 license from ref. 156 Copyright @ 2024 The Authors).

preventing unwanted liposome fusion remains a challenge. Recent use of single-stranded DNA to protect liposomes has improved their fusion with exosomes.[165] A combination of exosome biology and advanced nanotechnology becomes a solution for effective exosomes isolation.[40,164] Despite these issues, hybrid exosomes offer enhanced delivery efficiency and stability compared to liposomes or exosomes alone. The biohybrid approach offers a significant advantage by combining natural exosome components with other materials, leading to improved delivery efficiency compared to liposomes and enhanced stability compared to exosomes alone. This makes them highly versatile. Furthermore, the fusion technique used in these approaches allows for effective drug loading, accommodating both biological cargoes in liposomes and therapeutic agents in exosomes.[149] Additionally, a recent study has shown that genetically engineered exosomes-thermosensitive liposomes hybrid nanoparticles (gETL NPs) improve drug delivery to metastatic peritoneal carcinoma (mPC) tumors, effectively inhibit tumor growth.[148] Moreover, the exosome–liposome hybrid approach has shown higher efficacy in delivering CRISPR-Cas9 for cancer treatment. Considering all factors, it is evident that further research could advance hybrid exosome approaches into a cutting-edge therapeutic tool for cancer treatment.

9 Conclusion

In conclusion, engineered exosomes hold immense promise in cancer therapy as versatile vehicles for targeted drug delivery and gene regulation. Through precise targeting of specific genes or proteins, exosomes offer potent therapeutic effects with reduced off-target effects and toxicity. These engineered nanovesicles show potential in overcoming drug resistance, suppressing tumor growth, and enhancing the efficacy of conventional treatments. However, challenges such as standardization of isolation techniques, optimization of cargo loading, and elucidation of *in vivo* behavior remain to be addressed. With further research and development, engineered exosomes represent a promising avenue for personalized and effective cancer treatment strategies.

Conflicts of interest

The authors of this article declare no conflicts of interest.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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