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Review Article

Decoding the secrets of small extracellular vesicle communications: exploring the inhibition of vesicle-associated pathways and interception strategies for cancer treatment

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Abstract: Cancer disease is the second leading cause of death worldwide. In 2023, about 2 million new cancer cases and 609,820 cancer deaths are projected to occur in the United States. The driving forces of cancer progression and metastasis are widely varied and comprise multifactorial events. Although there is significant success in treating cancer, patients still present with tumors at advanced stages. Therefore, the discovery of novel oncologic pathways has been widely developed. Tumor cells communicate with each other through small extracellular vesicles (sEVs), which contribute to tumor-stromal interaction and promote tumor growth and metastasis. sEV-specific inhibitors are being investigated as a next-generation cancer therapy. A literature search was conducted to discuss different options for targeting sEV pathways in cancer cells. However, there are some challenges that need to be addressed in targeting sEVs: i) specificity and toxicity of sEV inhibitor, ii) targeted delivery of sEV inhibitors, iii) combination of sEV inhibitors with current standard chemotherapy to improve patients' clinical outcomes, and iv) data reproducibility and applicability at distinct levels of the disease. Despite these challenges, sEV inhibitors have immense potential for effectively treating cancer patients.

Keywords: Small extracellular vesicles, cancer treatment, sEV inhibitors, clinical applications

Introduction

Recent advancements in research of Small Extracellular Vesicles (sEVs), also called exosomes, have sparked excitement in the scientific community as these tiny extracellular vesicles have emerged as powerful mediators of intercellular communications. Once dismissed as cellular cargo, sEVs are recognized as critical players in various physiological and pathological processes. Their ability to shuttle proteins, nucleic acids, and lipids between cells has captivated researchers, driving the rapid expansion of the field of sEV research [1-3] in different directions.

Understanding the intricate functions of sEVs in normal physiology and disease is of paramount importance. Researchers have turned their attention to blocking different sEVs pathways to unravel the complex mechanisms underlying sEVs biology. These inhibitors have proven invaluable in deciphering the intricate processes involved in sEV biogenesis, cargo selection, release, and uptake [4, 5]. Furthermore, they hold tremendous potential as therapeutic agents to modulate disease-associated sEV-mediated communication, opening new avenues for novel therapeutic interventions [6, 7]. Recently, sEVs have been modified and used as vehicles for drug delivery [8].

The primary objective of this review is to provide an extensive overview of the current landscape of sEV inhibitors. We aim to investigate the

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diverse classes of sEV inhibitors and explore their mechanisms of action. Additionally, we will examine the implications of sEV inhibition for understanding sEV biology and its potential applications in therapeutic interventions. By discussing recent advancements, addressing challenges, and outlining future directions, this review consolidates existing knowledge and presents a comprehensive perspective on targeting sEV-associated pathways.

Small extracellular vesicles structure and function

sEVs are double membrane nanobodies, their size are smaller than 200 nm in diameter, released by cells at normal physiological and pathophysiological conditions [9]. sEVs encapsulate active biomolecules, including various proteins, nucleic acids, and lipids. The cargo composition of sEVs is distinct from that of their parental cells, indicating cargo selectively during their formation [10]. The number and composition of sEVs depend on the physiological status of the cells. For example, tumor cells release more sEVs compared to normal cells [11]. Understanding the composition of sEVs is essential for deciphering their functional roles and exploring their clinical applications. Once synthesized by cells, sEVs, derived from a double invagination of the cell plasma membrane to form multivesicular bodies (MVBs), will either fuse with lysosomes to degrade their cargo contents or fuse with the plasma membrane to be released out of the cell. MVBs formation is either endosomal sorting complexes required for transport (ESCRT)-dependent or ESCRT-independent process [12]. The main components of the ESCRT machinery are ESCRT-0, I, II, and III, along with a few auxiliary proteins such as vacuolar protein sorting 32 (VPS32) and apoptosis linked gene 2 (ALG2)-interacting protein X (ALIX) [13]. In the microdomains of the limiting membrane of MVBs, ubiquitylated transmembrane cargo are gathered by ESCRT-0 and ESCRT-I subunits and subsequently recruited by ESCRT-II and ESCRT-III. This process causes the microdomain budding and fission, forming intraluminal vesicles (ILVs) in the lumen of MVBs [13]. sEVs, however, can also be produced by ESCRT-independent mechanisms. Numerous mechanisms, including ceramide, Ras-related protein Rab 27A (RAB27a), RAB27b, and tetraspanin proteins, have been discussed. However, other pathways seem to be involved in synthesizing sEVs [14, 15]. Most of the sEV cargo protein is yet unknown. sEVs are endosomal in origin. As such, they contain a variety of proteins: lipid-related proteins, phospholipases, membrane transport and fusion proteins (GTPases, Annexins, and flotillin), tetraspanins (clusters of differentiation (CD)9, CD63, CD81, and CD82), and heat shock proteins (Hsp90, Hsp60, and Hsp20) [16, 17]. sEVs released by antigen presenting cells are abundant in antigen-presenting proteins, including MHC class I and class II. Dendritic cell (DC)-derived sEVs contain CD86, a crucial T-cell co-stimulatory molecule. sEVs also contain distinct α- and β-chains of integrins, ICAM1/CD54, A33 antigen and P-selectin, and cell-surface peptidases (CD26 and CD13) [18]. Significant amounts of mRNA, microRNA (miR), and other non-coding RNAs are present in sEVs. These RNAs can be transmitted across cells and alter the expression of specific genes in recipient cells [19]. For example, miR-126 is a crucial regulator of angiogenesis and vascular integrity since it is upregulated in different sEVs and encourages re-endothelialization *in vivo* [20].

Cancer progression requires direct interaction between tumor cells and other cells in the tumor microenvironment (TME). sEVs derived from cancer cells are known to transport oncogenic proteins and nucleic acids that alter recipient cells' activities and are essential for carcinogenesis, cell proliferation, metastasis, and drug resistance [21]. sEVs generated from prostate cancer cells carry in their cargo oncogenic proteins (GTPases from the Ras superfamily), mRNA (H-Ras and K-Ras), and miRs (miR-125b, miR-130b, and miR-155) and have the ability to induce neoplastic transformation in human adipose-derived stem cells [5, 22]. Hypoxic glioblastoma cells produce sEVs, which can stimulate angiogenesis [5]. Exosomal miR-92a, generated from K562 leukemia cells, binds to integrin α5 to promote endothelial cell migration and tube formation [23]. sEVs enriched with miR-210 released by hypoxic K562 cells can induce angiogenesis in endothelial cells [24]. In addition to inducing apoptosis and impairing the function of effector T cells and natural killer cells (NKs), sEVs also expand myeloid-derived suppressor cells (MDSCs), in-

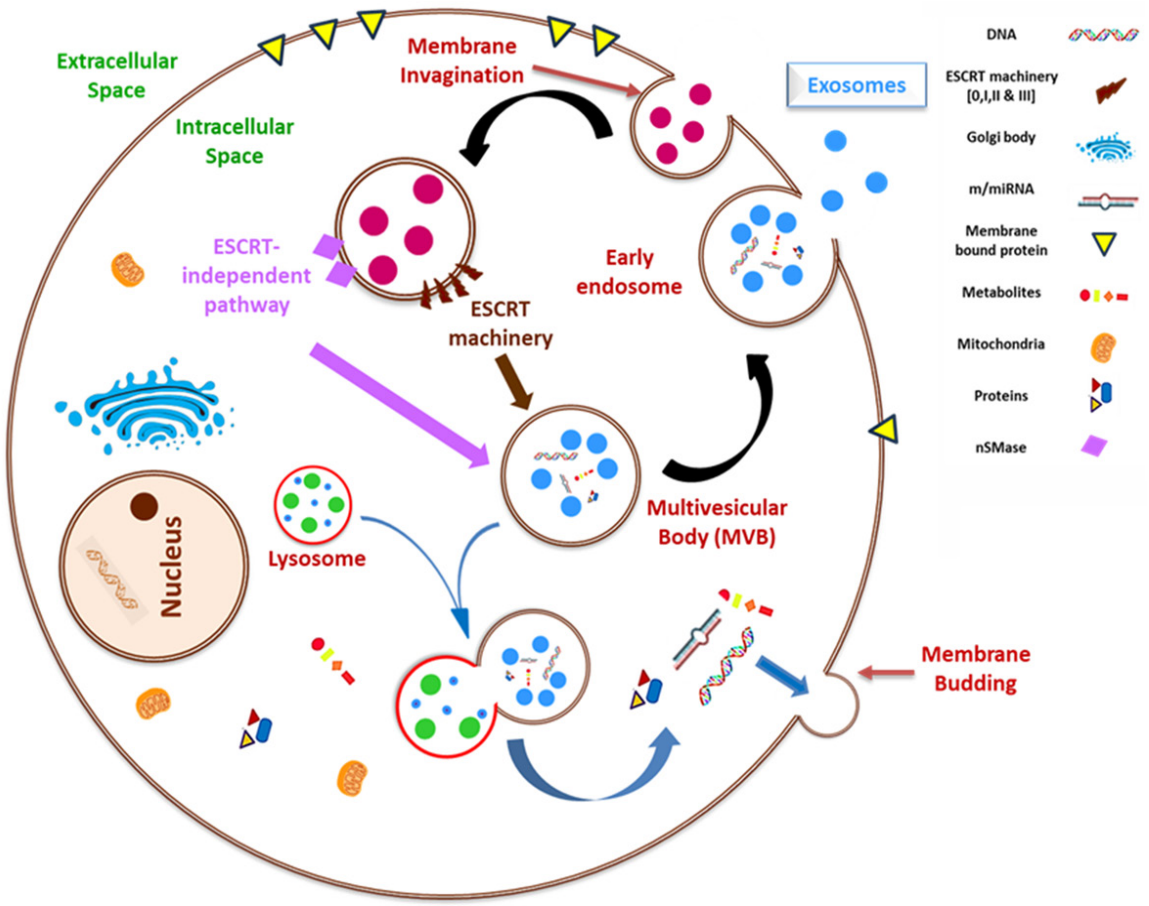

Figure 1. Biogenesis of small extracellular vesicles (sEVs). The biogenesis of sEVs includes an endosomal sorting complex required for transport (ESCRT)-dependent and ESCRT-independent pathways.

hibit DC differentiation, and promote regulatory T cell (Treg) activity in an immunosuppressive microenvironment [25].

Tumor angiogenesis and metastasis can be aided by sEVs produced from tumor cells, which can also transform fibroblasts and mesenchymal stromal cells into myofibroblasts. sEVs released by tumor cells can activate neutrophils and skew macrophage M2 polarization, therefore accelerating the growth of tumors [26]. Furthermore, by exporting anti-cancer medications, neutralizing antibody-based medications, and transferring multidrug-resistant proteins and miRs, tumor-derived sEVs can promote tumor cells in acquiring drug resistance. sEVs derived from stromal cells, macrophages, and activated T cells can also encourage treatment resistance and promote tumor metastasis [5].

sEV biogenesis and cargo loading

Overview of sEV biogenesis, including ESCRT-dependent and ESCRT-independent pathways

sEVs, intriguing mediators of intercellular communication, are formed through intricate biogenesis pathways that involve both ESCRT-dependent and ESCRT-independent mechanisms (**Figure 1**). The ESCRT pathway, recognized as a significant pathway for sEV biogenesis, sequentially recruits ESCRT complexes, including ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, facilitating the inward budding of the endosomal membrane. This process leads to the formation of intraluminal vesicles (ILVs) within MVBs, which eventually fuse with the plasma membrane, releasing ILVs as sEVs into the extracellular environment [2, 4]. These complexes are critical for recognizing and

Table 1. Surface membrane proteins for sEV characterization and their molecular functions

Protein Name	Characteristics
CD63	Member of transmembrane 4 superfamily Cell-surface glycoprotein Exosomal membrane tetraspanin
CD9	Member of transmembrane 4 superfamily Cell-surface glycoprotein Exosomal membrane tetraspanin
CD81	Member of transmembrane 4 superfamily Cell-surface glycoprotein Exosomal membrane tetraspanin
TSG101	Inactive homolog of ubiquitin-conjugating enzymes Component of ESCRT-I subunit
ALIX	Protein product of Programmed Cell Death 6 Interacting Protein (PDCD6IP) gene Associated with ESCRT-III subunit
Caveolin-1	Hairpin-like surface membrane protein Component of lipid rafts Component of ESCRT-independent pathway
Flotillin-1	Membrane scaffolding protein Component of lipid rafts Component of ESCRT-independent pathway

transporting sEVs [27]. The ESCRT-0 complex identifies the ubiquitinated cytoplasmic domains of transmembrane proteins and then further sorts them into the endosomal membrane [28]. The ESCRT-I and II complexes bind to the outside of the endosomal membrane, inducing the luminal vesicles of MVBs. The ESCRT-III complex assembles on the outer surface of the endosomal membrane during the generation of MVBs, promoting their formation in the nucleus [29]. Moreover, alternative pathways independent of ESCRT have been identified, involving lipid raft microdomains, ceramide-dependent sorting, and tetraspanin-enriched microdomains, providing additional routes for sEV generation [30, 31].

Key molecules involved in cargo sorting and loading into sEVs

The precise sorting and loading of cargo molecules into sEVs rely on a complex interplay of various molecules. Tetraspanins, including CD63, CD81, and CD9, serve as sEV markers and play a crucial role in the biogenesis and cargo sorting of sEVs. They interact with other proteins, lipids, and RNA molecules, forming tetraspanin-enriched microdomains that act as platforms for cargo selection and loading [32]. Additionally, RNA-binding proteins such as Alix and tumor susceptibility gene 101 protein (TSG101), as components of the ESCRT machinery, contribute to the recruitment of cargo molecules into forming sEVs. Heat shock proteins, lipids such as ceramide, and nucleic acids (RNAs and DNAs) also influence the sorting and loading of cargo into sEVs, as shown in **Table 1**.

Role of sEV inhibitors in elucidating the regulatory mechanisms of cargo selection and packaging

sEV inhibitors are pivotal in unraveling the intricate regulatory mechanisms underlying cargo selection and packaging into sEVs. By specifically targeting key molecules involved in sEV biogenesis, such as components of the ESCRT machinery or tetraspanins, sEV inhibitors can disrupt or modulate the sorting and loading of cargo molecules. Using these types of inhibitors, researchers have identified critical players and pathways involved in cargo selection and packaging, shedding light on the functional significance of specific proteins, lipids, and nucleic acids in determining the cargo composition of sEVs. Furthermore, sEV inhibitors have contributed to unraveling the interplay between different cellular processes and signaling pathways that influence cargo loading [30, 32, 33].

By elucidating the regulatory mechanisms of cargo selection and packaging, sEV inhibitors

Table 2. Techniques used for studying sEV inhibitors

Technique	Application	References
Ultracentrifugation Polymer Precipitation Immunoaffinity Chromatography Size-Exclusion Chromatography	sEV isolation sEV inhibitor identification Measures serum sEV content after treatment with potential inhibitor	[35]
Mass-Spectrometry Proteomics		[2, 37]
ExoScreen Assay & Antibody-Mediated Detection of Atypical Lipid (LBPA)	sEV inhibitor identification Provides intracellular and extracellular data of sEV changes after treatment with potential inhibitor	
Artificial Intelligence	Prediction of sEV structures Drug delivery assessment for potential sEV inhibitors	[135, 142]
Electron Microscopy	Visualization of: sEV morphology intracellular trafficking cell-cell interactions	[32]
Flow Cytometry	Phenotypic analysis of sEVs including: cargo content size distribution surface markers	[143]

will significantly enhance our understanding of the cargo-specific functions of sEVs. They pave the way for developing strategies to manipulate sEV cargo for therapeutic purposes, opening new avenues for targeted therapeutics and advancing the field of sEV-based therapies.

Techniques used for studying sEV inhibition

Experimental approaches and methodologies used to investigate sEV inhibitors

A diverse array of experimental approaches and methodologies have been employed to investigate sEV inhibitors and unravel their effects on sEV biology. These investigations encompass *in vitro* and *in vivo* studies, utilizing cell culture models, animal models, and patient samples, as shown in **Table 2**. *In vitro* studies involve treating cells or isolating sEVs with specific inhibitors, followed by comprehensive characterization of the resulting alterations in sEV release, cargo composition, and biological functions [34]. *In vivo* studies employ appropriate animal models to assess the impact of sEV inhibitors on disease progression, intercellular communication, and therapeutic outcomes [30]. sEV isolation kits are commercially available tools currently used to isolate and measure serum sEV content [35]. This technique allows researchers to analyze the effect of sEV inhibitors on the biogenesis and stability of sEVs in the serum of animal models [36]. These kits are based on traditional isolation techniques, including ultracentrifugation, polymer precipitation, immunoaffinity chromatography, and size-based isolation techniques [35]. In addition to these kits, traditional methods such as Western blots and SDS-PAGE can illuminate the mechanism of sEV inhibitors on cancer cell status [37]. With the rapid emergence of sEV exploration, an efficient technique is needed to improve the identification of sEV inhibitors. A group of researchers proposed a strategy that utilizes an ExoScreen assay with an antibody-mediated detection of an atypical lipid (LBPA) technique to collect information on sEV alteration after treatment with potential sEV inhibitors [37]. These few techniques present a preview of the advancements in sEV inhibitor research.

Advancements in imaging, flow cytometry, and proteomics for assessing sEV inhibition

Significant advancements in imaging, flow cytometry, and proteomic technologies have revolutionized the assessment of sEV inhibition. Cutting-edge imaging techniques, including electron and super-resolution microscopy, offer exceptional visualization of sEV morphology, intracellular trafficking, and interactions with recipient cells, unraveling the intricate details of sEV dynamics [32]. Flow cytometry

enables precise quantification and phenotypic analysis of sEVs, providing invaluable insights into their size distribution, surface markers, and cargo content, which indicate sEV inhibitory effects [38]. Furthermore, proteomic approaches, such as mass spectrometry-based proteomics, allow for comprehensive profiling of sEV proteomes, facilitating the identification of specific proteins affected by sEV inhibition [2].

Challenges and limitations in studying sEV inhibition and potential solutions

While significant progress has been made in the study of sEV inhibition, several challenges and limitations persist. A notable challenge is the inherent heterogeneity of sEVs, originating from diverse cell types and exhibiting distinct cargo compositions. This heterogeneity poses difficulties in identifying specific targets for inhibition and deciphering the functional consequences of sEV inhibition. The lack of standardized methodologies for sEV isolation, purification, and characterization also presents challenges in comparing and interpreting results across different studies [30]. A number of other factors, including age, health, and even the time-of-day blood is obtained, can affect sEV levels [39]. Accordingly, it is difficult to define a single reference range due to the inherent biological heterogeneity.

On the other hand, preclinical research is beginning to reveal some possible medical disorders as a result of using sEV inhibitors. For example, a systemic review on sEVs indicates that Alzheimer's and Parkinson's diseases may be associated with low levels of sEVs in the cerebrospinal fluid [40]. Although the role of sEVs in the disease can be complicated, some research indicates that low concentrations of particular sEVs may be linked to a worse prognosis in some types of cancer [41]. To ascertain whether low sEV levels are a direct cause of any medical disorders, more research is required and a special focus on having a universal reference range is crucial.

To address these challenges, researchers have proposed potential solutions. One solution involves developing novel isolation and purification techniques, such as microfluidics-based platforms and immunoaffinity-based approaches, which facilitate the acquisition of homogeneous sEV populations for more reliable and reproducible analyses. In addition, standardizing procedures for sEV isolation, characterization, and quantification are the main emphasis of sEV research towards the development of a universal reference range. Moreover, the integration of multiomics approaches, encompassing transcriptomics and lipidomics, can provide a comprehensive understanding of the effects of sEV inhibitors on various molecular levels, shedding light on complicated regulatory networks and signaling pathways [2, 42]. By leveraging these innovative techniques and addressing the challenges and limitations, researchers are poised to unravel the complexities of sEV inhibition further, expanding our knowledge of their underlying mechanisms and paving the way for developing innovative therapeutic strategies harnessing the power of sEVs.

Inhibition of sEV release

Small molecule inhibitors for targeting sEV release

The inhibition of sEV release holds great promise for modulating intercellular communication and potentially intervening in disease progression. Small molecule inhibitors (SMIs) have been designed to target specific pathways involved in sEV biogenesis and release (**Figure 2**). The list of sEV biogenesis, release, and uptake is provided in **Tables 3** and **4**. Notably, compounds like GW4869 and manumycin A have demonstrated the ability to inhibit neutral sphingomyelinase 2 (nSMase2) [43, 44], a key enzyme in the ceramide-dependent pathway of sEV release [15]. Other SMIs such as nSMase 2 inhibitor 2,6-dimethoxy-4-(5-phenyl-4-thiophen-2-yl-1H-imidazole-2-yl)-phenol (DPTIP), antidiabetic medication glibenclamide, antidepressant imipramine, hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor simvastatin, insulin secretion inducer dimethyl amiloride (DMA), antifungal agent ketoconazole, proton-pump inhibitor omeprazole and cannabis-derived compound cannabidiol are examples of sEV release inhibitors (reviewed in [45]). The list of these inhibitors is growing as researchers are discovering new compounds which have shown an inhibitory activity on sEV release. Through their action, these inhibitors have been proven effective in reducing sEV secretion across various cell types, serving as valuable tools for studying the functional roles

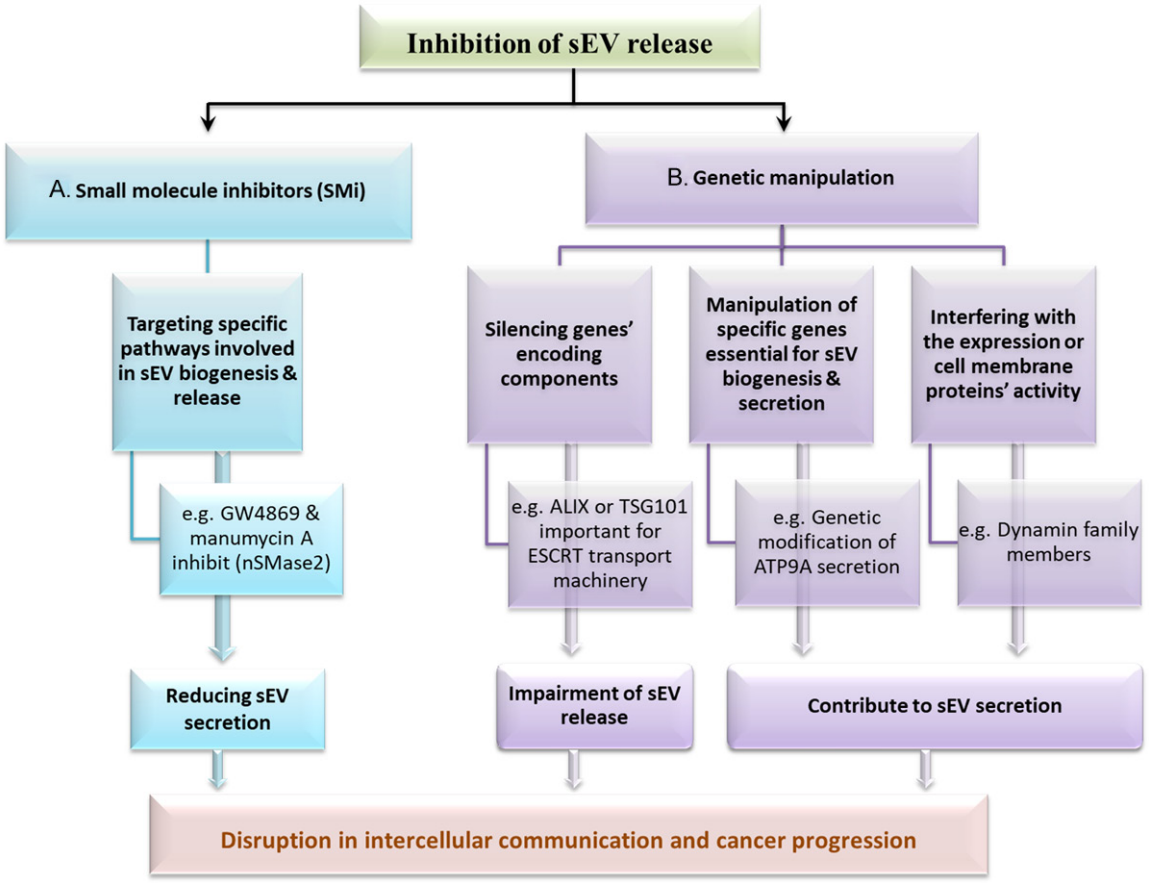

Figure 2. Interruption of cancer cell signaling via inhibiting sEV release. The release of sEVs can be inhibited by either small molecule inhibitors (A) or genetic manipulations (B) which can lead to impeding cell communications and therefore reducing cancer progression and metastasis.

of sEVs in different physiological and pathological processes.

Genetic manipulation strategies to block sEV release

Genetic manipulation strategies have also been used to identify proteins involved in sEV release. These strategies involve the manipulation of specific genes essential for sEV biogenesis and secretion (**Figure 2**). For example, the downregulation of ATPase phospholipid transporting 9A (ATP9A) expression in human hepatocellular carcinoma cells increased sEV secretion [46]. Using a pharmacological sEV release inhibitor in ATP9A-depleted cells, Naik and coworkers determined that ATP9A regulates sEV release [46]. With further research, genetic modification of ATP9A may be a promising therapeutic target for the treatment of different types of cancers. Silencing genes encoding components of the ESCRT required for its transport machinery, such as ALIX or TSG101, has been shown to impair sEV release [2]. Similarly, interfering with the expression or activity of proteins involved in membrane fission processes, such as the dynamin family members, can also decrease sEV secretion.

Effects of sEV release inhibition on intercellular communication and disease progression

Inhibiting the exosomal release has provided valuable insights into the roles of sEVs in intercellular communication and disease progression. By blocking sEV release, researchers have observed disruptions in transferring biomolecules, including proteins, nucleic acids, and lipids, between cells. These perturbations in intercellular communication mediated by sEVs have been implicated in various physiological and pathophysiological processes, such as immune

Table 3. sEVs release inhibitors

Name	Associated Disease/Cancer	Target	Therapeutic Effect
Biscurcumin	HER-2 Positive MDA-MB-43 and luminal A MCF7 Breast Cancer	Protein Kinase C	- Inhibits phorbol ester-induced PKC activity
Dinaciclib	Luminal A MCF7 Breast Cancer	Microtubule Network	- Inhibits cyclin-dependent kinases (CDK1, CDK2, CDK5, CDK9) - Tumor apoptosis and cell cycle repression
Docetaxel	HER-2 Positive MDA-MB-43 breast cancer Luminal A MCF7 breast cancer Lung cancer	Cytoskeletal Protein Network	- Inhibits cellular mitoses *via* binding to beta-tubulin subunits of microtubules producing atypical structures
Doxorubicin	HER-2 Positive MDA-MB-43 and luminal A MCF7 Breast Cancer	Ca^{2+} regulation/Proton Pumps	- Interrupts function of vital proton pumps (Na^+/K^+, H^+, Na^+/H^+) - Interrupts calcium regulation (Na^+/Ca^{2+})
Primaquine	HER-2 Positive MDA-MB-43 and luminal A MCF7 Breast Cancer	Lipid Rafts	- Alters structure of lipid membranes
Tipifarnib	Prostate Cancer (C4-2B & PC-3) cells	Rab27a	- Inhibits expression of Rab27a, ALIX, and nSMase2 selectively reducing sEV release and production in cancer cells - Does not affect normal prostate RWPE-1 cells
Ketoconazole	Prostate Cancer (C4-2B & PC-3) cells	Rab27a	- Inhibits expression of Rab27a, ALIX, and nSMase2 reducing sEV release and production in cancer cells - Affects RWPE-1 normal prostate cells
GW4869	Melanoma cells	Lipid Rafts	- Blocking the enzyme nSMase
Chloramidine and bisindolylmaleimide	PC-3 (prostate cancer) and MCF-7 breast cancer cells	sEV and MV (EMV) biogenesis inhibitors	- Bisindolylmaleimide-I is a protein kinase C (PKC) inhibitor that prevents release of phosphatidyl-serine (PS) - Chloramidine affects peptidylarginine deiminase activation, which causes post-translational protein deimination
Lansoprazole	Melanoma cells	(H^+, K^+)-ATPase enzyme	- Binds to the to the H^+, K^+ ATPase enzyme in gastric parietal cells, preventing gastric production
Manumycin A	Prostate cancer cells	Ras FTase	- Blocks RAS FTase which prevents sEV release
Indomethacin plus Doxorubicin and Pixantrone	B cell Lymphoma	ABCA3 transporter	- Nonselectively inhibit COX1 and COX2 as well as downregulate the ABCA3 transporter - which participates in lipid transport - When used with Doxorubicin and Pixantrone, Indomethacin has increased

Table 4. sEVs biogenesis inhibitors

Name	Associated Disease/Cancer	Target	Therapeutic Effect
Azole Antifungals (Ketoconazole, Neticonazole, Climbazole)	Prostate Cancer (C4-2B) cells	Mammalian CYP51	- Inhibits cholesterol synthesis reducing sEV biogenesis
Tipifarnib	Prostate Cancer (C4-2B & PC-3) cells	Farnesyl Transferase (FT)	- Inhibits FT reducing sEV biogenesis, secretion, cell growth, and induces apoptosis
Manumycin A	Prostate Cancer (C4-2B) cells	Farnesyl Transferase (FT)	- Inhibits FT reducing sEV biogenesis, prevents Ras activation, cell growth, and induces apoptosis
Y27632	Prostate Cancer (PC-3) cells	Rho A Kinase (ROCK)	- Inhibits Rho kinase disrupting exosomal actin-cytoskeleton distributions
Asteltoxin	HT29/CD63-Nluc cells	mTORC	- Inhibits mitochondrial ATP synthase. Activates lysosome function through AMPK-mediated mTORC1 inactivation
Docetaxel, Biscurcumin, Primaquine, and Doxorubicin	HER-2 positive MDA-MB-453 cells and hormone-dependent luminal A MCF7 cells	sMase2 or ESCRT-dependent pathway	- Induced the modulation of CD9 tetraspanin expression and down-regulated the expression of Rab27

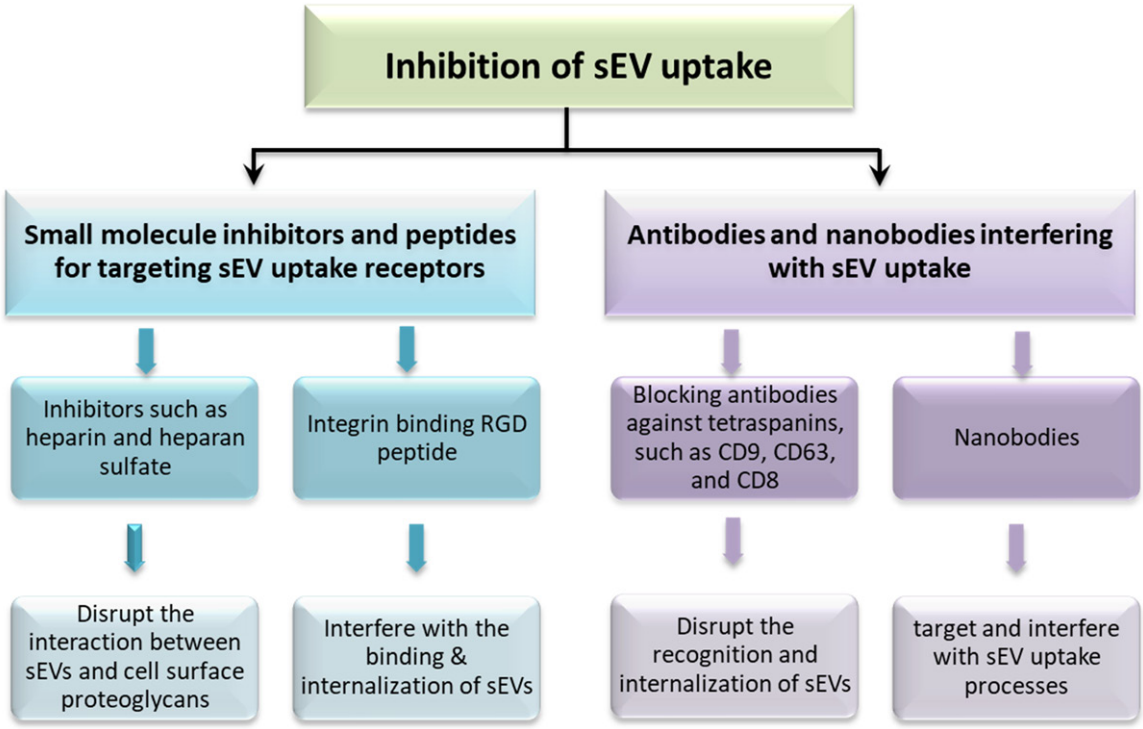

Figure 3. Targeting cancer cells by inhibiting sEV uptake by recipient cells. sEV uptake can be blocked by two main mechanisms: i) small molecule inhibitors and peptides for targeting sEV uptake receptors, and ii) antibodies and nanobodies interfering with sEV uptake.

response modulation, tumor progression, and neurodegenerative diseases [12]. Furthermore, studies investigating the effects of inhibiting sEV release in disease models have demonstrated promising therapeutic potential. Inhibition of sEV release has been shown to attenuate tumor growth, inhibit metastasis, and modulate immune responses in different cancer models [1, 47]. Additionally, in neurodegenerative diseases, inhibiting sEV release has been associated with a reduced spreading of pathological aggregates and improved neuronal survival [48]. These findings underscore the importance of comprehending the impact of sEV release inhibition on disease processes and suggest sEV release as a viable therapeutic target.

By employing SMIs and genetic manipulation strategies to block sEV release, researchers have made significant strides in understanding the mechanisms of sEV-mediated communication and its implications for various diseases. These advancements promote future investigations for the development of innovative therapeutic approaches aimed at modulating sEV release for improving clinical outcomes.

Inhibition of sEV uptake

Small molecule inhibitors and peptides for targeting sEV uptake receptors

The uptake of sEVs by recipient cells is a crucial step in their functional transfer of biomolecules as shown in **Figure 3**. SMIs and peptides have been developed to target specific receptors involved in sEV uptake, providing valuable tools to study the mechanisms underlying this process. For example, inhibitors such as heparin and heparan sulfate mimetics have been shown to disrupt the interaction between sEVs and cell surface proteoglycans, thereby inhibiting their uptake [49]. Additionally, peptides derived from the uptake receptor, such as the integrin binding arginylglycylaspartic acid (RGD) peptide, have been utilized to interfere with the binding and internalization of sEVs [50].

Antibodies and nanobodies interfering with sEV uptake

In addition to SMIs and peptides, antibodies, and nanobodies have emerged as effective tools to interfere with sEV uptake mechanisms.

By targeting specific surface proteins or receptors on sEVs or recipient cells, these antibodies and nanobodies can disrupt the recognition and internalization of sEVs. For instance, blocking antibodies against tetraspanins, such as CD9, CD63, and CD81, commonly enriched in sEVs, have been shown to inhibit their uptake by recipient cells [51]. Similarly, nanobodies, which are single-domain antibody fragments, have been developed to specifically target and interfere with sEV uptake processes [52].

Impact of sEV uptake inhibition on recipient cell's function and communication

The inhibition of sEV uptake has provided valuable insights into the functional consequences of intercellular communication mediated by sEVs. By blocking the uptake of sEVs, researchers have observed alterations in recipient cell function and communication. For example, inhibiting sEV uptake has been shown to affect cell signaling pathways, gene expression profiles, and cellular responses, such as proliferation, migration, and immune modulation [53]. Furthermore, other studies have demonstrated that inhibiting the uptake of sEV can influence disease processes, including tumor progression, immune responses, and neurodegenerative disorders [8, 54].

Modulation of sEV cargo content

Nucleic acid-based inhibitors for regulating sEV cargo composition

sEVs carry diverse biomolecules, including nucleic acids, contributing to their functional properties. Nucleic acid-based inhibitors have emerged as powerful tools for modulating sEV cargo composition. For example, small interfering RNA (siRNA) and antisense oligonucleotides (ASOs) can be employed to specifically target, and silence genes involved in the production or packaging of specific cargo molecules into sEVs [55-57]. By regulating the expression of these genes, researchers can manipulate the cargo content of sEVs and potentially control the functional outcomes associated with their transfer.

Small molecules and natural compounds influencing sEV cargo packaging

In addition to nucleic acid-based inhibitors, small molecules, and natural compounds have been investigated for their ability to influence sEV cargo packaging. These compounds can target specific pathways or molecular interactions involved in cargo sorting and packaging into sEVs. For example, small molecules that affect the activity of Rab GTPases or lipid metabolism have been shown to alter the cargo content of sEVs [58]. Natural compounds derived from plants or microorganisms have also demonstrated the ability to modulate sEV cargo, highlighting their potential as therapeutic agents or research tools for studying sEV biology [59].

Therapeutic potential of modulating sEVs cargo for targeted therapy

Modulating sEV cargo holds significant therapeutic potential, particularly in targeted therapy. By altering the cargo composition of sEVs, it is possible to selectively enrich or deplete specific molecules that can influence disease progression. This approach offers the opportunity to develop tailored sEV-based therapies for various health conditions, including cancer, neurological disorders, and inflammatory diseases. For example, engineering sEVs to carry therapeutic nucleic acids or proteins can enhance their delivery to target cells and facilitate precise modulation of disease-related pathways [60]. Furthermore, by modulating sEVs cargo, it may be possible to overcome therapeutic resistance or enhance the efficacy of existing treatments [61].

Application of sEV and their inhibitors in cancer therapeutics

sEVs play a critical role in cancer progression and metastasis by facilitating intercellular communication and modulating the TME. Consequently, targeting sEVs has emerged as a promising approach in cancer therapeutics. Various sEV inhibitors have been investigated for their potential to impede cancer progression. For instance, SMIs such as GW4869, have demonstrated the ability to block sEV release from cancer cells, thereby reducing their protumorigenic effects [62]. Additionally, targeting sEV biogenesis pathways, such as the ESCRT machinery, has shown promise in limiting the dissemination of cancer-derived sEVs [47]. The use of sEV inhibitors holds the potential for developing novel therapeutic strategies to disrupt tumor communication and suppress metastasis.

Lung cancer and sEV therapeutic targets

The primary cause of cancer death globally is lung cancer (LC), where 350 individuals die every day from this cancer type alone [63]. The primary cause of LC's low survival rate is due to late-stage diagnosis and treatment options are becoming limited. As a result, there is a rising interest in developing novel targeted medicines for the treatment of patients at late stages. Since LC-derived sEVs are essential in controlling the physiological processes of surrounding tissue cells and the TME, they may offer novel treatment perspectives (**Figure 4A**). A differential level of epidermal growth factor receptor (EGFR) was detected in sEVs collected from the plasma of LC patients compared to non-cancerous individuals. sEV-associated EGFR stimulates regulatory T cells specific to tumor antigens to block the activity of $CD8^+$ T cells specific to tumors, hence hastening LC initiation [64, 65] and blocking sEV release or uptake could impede this process. Specifically, sEVs isolated from plasma of non-small cell LC (NSCLC) are abundant in proteins linked to signal transduction, such as EGFR, proto-oncogene tyrosine kinase Src (Src), and growth factor receptor-bound protein 2 (GRB2). Thus, the proliferation of recipient cells can be actively controlled by these sEV-associated proteins [66]. Leucine-rich alpha-2 glycoprotein 1 (LRG1) also expressed itself at a higher level in NSCLC patients [67]. Tetraspanins are cell-specific markers with an endosomal origin abundant in sEVs. For example, tetraspanins CD151, CD171, and tetraspanin 8 (TSPAN8) were discovered in sEVs isolated from LC tissues. These tetraspanins were then used as LC biomarkers to differentiate the disease according to the pathological stages [68]. Exosomal CD151 and TSPAN8 can alter the extracellular matrix (ECM) *in vitro* and initiate the metastatic process [69]. Exosomal membrane protein CD91, released into the serum, has been employed as an LC detection index and is a valid biomarker for NSCLC diagnosis [70, 71].

The two metabolic enzymes, exosomal fructose-bisphosphate aldolase (ALDOA) and aldehyde dehydrogenase 3-A1 (ALDH3A1) proteins are elevated in sEV derived from irradiated LC cells and function as key signaling regulators. This process accelerates the glycolytic process and controls the motility of recipient cells [72]. Furthermore, by carrying E-cadherin on their surface, sEVs from LC bronchoalveolar fluid stimulate the migration and invasion of A549 cells. Suggesting that E-cadherin may act through a vascular endothelial-cadherin dependent mechanism to promote LC metastasis [73]. These examples highlight the importance of sEV inhibition and disruption of cargo contents which act as a fuel for cancer cell survival and building its pre-metastatic niche.

Breast cancer and sEV therapeutic targets

In the United States alone, an estimated 300,000 women will be diagnosed with invasive breast cancer (BC), and among those, 43,170 will die each year [63]. The progression and metastasis of BC involves the transportation of diverse proteins, signaling molecules, and miRs through sEV. BC cells release sEVs, which transfer biological components in their cargo that regulate cytoskeleton remodeling, cell motility, and invasion [74, 75]. Comparative analysis of sEVs from metastatic BC cells reveals the presence of proteins associated with migration, invasion, and angiogenesis pathways, potentially directing primary tumor cells to specific metastatic sites [76]. The proteomic profile of sEVs from BC MDA-MB-231 cells indicates the enrichment of matrix-metalloproteinases (MMPs) associated with enhanced metastatic properties [74]. Therefore, targeting these signaling pathways could justify the need for specific inhibition of sEVs as a second generation of targeted therapy with a special consideration in those patients they failed the standard therapy.

Other potential targeted pathways associated with sEVs include specific signatures associated with clinicopathological parameters and tumor aggressiveness [77, 78]. For example, the transfer of exosomal miRs induces changes in recipient cells, promoting anchorage-independent growth and metastatic behavior [79]. sEVs also carry membrane proteins like Caveolin-1, promoting migration and invasion of BC cells [80]. Additionally, sEVs from HER2-overexpressing BC cells display immunosuppressive molecules and transfer the resistant phenotype to drug-sensitive cells [81, 82]. The communication between cancer and non-malignant cells involves sEVs acting as components of the TME signaling [83]. sEVs derived

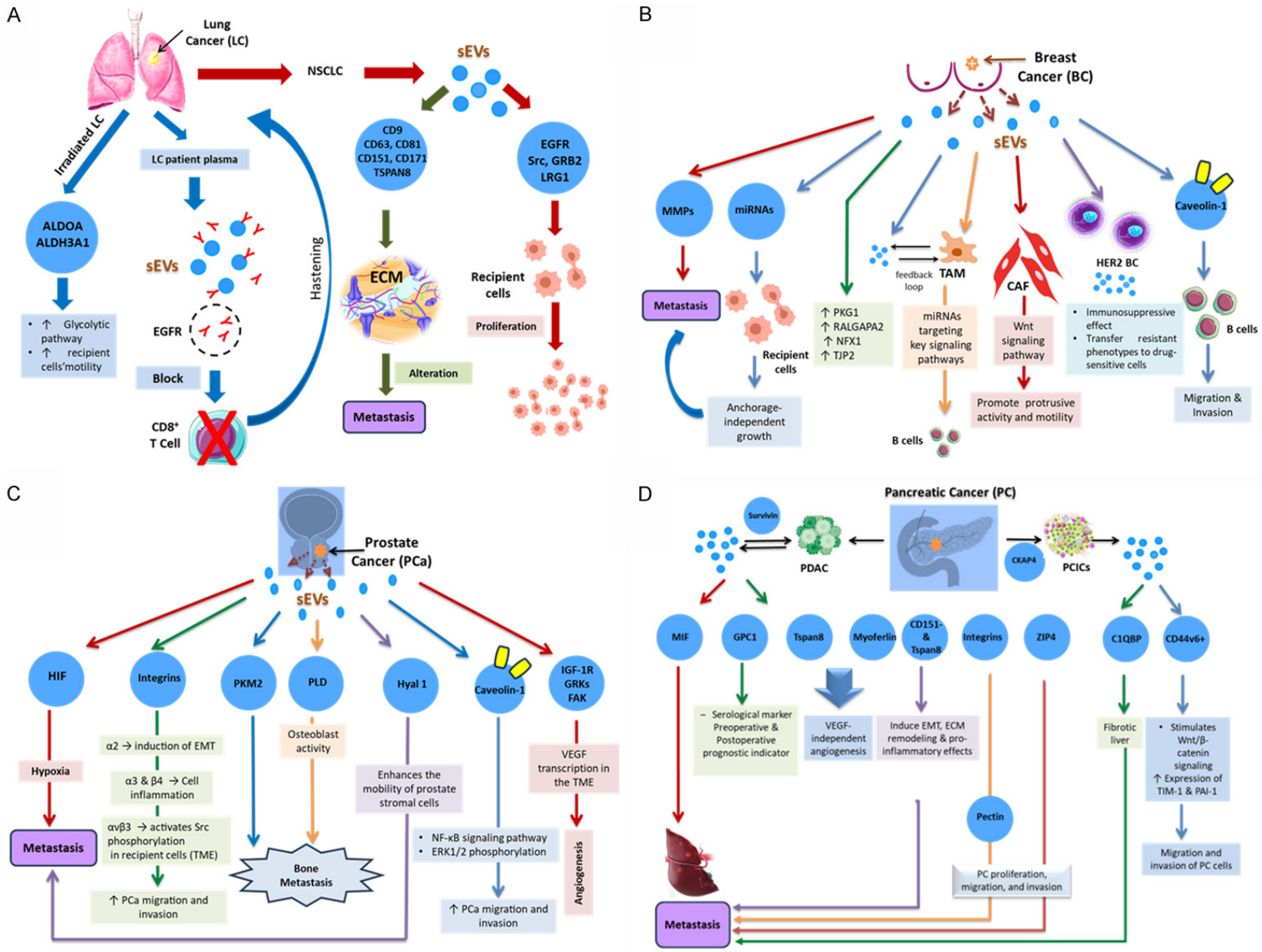
A
Lung Cancer (LC)
NSCLC
sEVs
Irradiated LC
LC patient plasma
CD9 CD63, CD81 CD151, CD171 TSPAN8
EGFR Src, GRB2 LRG1
ALDOA ALDH3A1
↑ Glycolytic pathway
↑ recipient cells'motility
sEVs
EGFR
Block
CD8+ T Cell
Hastening
ECM
Recipient cells
Proliferation
Alteration
Metastasis
B
Breast Cancer (BC)
sEVs
MMPs
miRNAs
Caveolin-1
Metastasis
Recipient cells
Anchorage-independent growth
↑ PKG1
↑ RALGAPA2
↑ NFX1
↑ TJP2
feedback loop
TAM
miRNAs targeting key signaling pathways
B cells
CAF
Wnt signaling pathway
Promote protrusive activity and motility
HER2 BC
Immunosuppressive effect
Transfer resistant phenotypes to drug-sensitive cells
B cells
Migration & Invasion
C
Prostate Cancer (PCa)
sEVs
HIF
Integrins
PKM2
PLD
Hyal 1
Caveolin-1
IGF-1R GRKs FAK
Hypoxia
Metastasis
α2 → induction of EMT
α3 & β4 → Cell inflammation
αvβ3 → activates Src phosphorylation in recipient cells (TME)
↑ PCa migration and invasion
Bone Metastasis
Osteoblast activity
Enhances the mobility of prostate stromal cells
NF-κB signaling pathway
ERK1/2 phosphorylation
↑ PCa migration and invasion
VEGF transcription in the TME
Angiogenesis
D
Pancreatic Cancer (PC)
Survivin
PDAC
CKAP4
PCICs
MIF
GPC1
Tspan8
Myoferlin
CD151- & Tspan8
Integrins
ZIP4
C1QBP
CD44v6+
Serological marker Preoperative & Postoperative prognostic indicator
VEGF-independent angiogenesis
Induce EMT, ECM remodeling & pro-inflammatory effects
Pectin
PC proliferation, migration, and invasion
Fibrotic liver
Stimulates Wnt/β-catenin signaling
↑ Expression of TIM-1 & PAI-1
Migration and invasion of PC cells
Metastasis

Figure 4. The role of sEVs in progression and metastasis of different cancer types. A schematic representative diagram showing the effect of sEVs on modulating different signaling pathways in lung (A), breast (B), prostate (C) and pancreatic (D) cancers to promote tumor progression and metastasis. Targeting these pathways can be used for future treatment of cancer patients.

from cancer-associated fibroblasts (CAFs) promote protrusive activity and motility in BC cells through the autocrine Wnt signaling pathway [84]. Macrophages, particularly tumor-associated macrophages (TAMs), influence BC cells through sEV-mediated delivery of miRs targeting key signaling pathways [85]. BC-derived sEVs also induce macrophages to release sEVs, creating a feedback loop [86].

Notably, circulating sEVs from healthy subjects affect tumor epithelial cell properties. sEVs from healthy donors stimulate adhesive, motile, and invasive properties of BC cells, both *in vitro* and *in vivo* [87]. In mouse model, serum-derived sEVs from highly metastatic BC tumors increased metastatic capacity to poorly metastatic tumors [88]. Protein phosphorylation events often offer insights into disease status [89]. While few phosphoproteins in biofluids have been reported as disease markers due to their dynamic nature and the presence of active phosphatases, several sEV-encapsulated phosphoproteins, including cGMP-dependent protein kinase 1 (PKG1), Ral GTPase-activating protein subunit alpha-2 (RALGAPA2), nuclear transcription factor, X-box-binding protein 1 (NFX1), and tight junction protein 2 (TJP2), are significantly upregulated in BC patients, suggesting their potential as novel biomarkers and/or therapeutic targets for the disease [90]. These findings underscore the multifaceted role of sEVs in BC progression and metastasis and their therapeutic potential as a new target for treating BC patients (**Figure 4B**).

Prostate cancer and sEV therapeutic targets

The most common solid tumor in older men is prostate cancer (PCa), and some of these patients present with more aggressive metastatic PCa. Patients with aggressive tumors have a relatively low survival rate compared to those with primary tumors [63]. As represented in **Figure 4C**, the proteins conveyed by sEVs released by cancer cells and transferred to less invasive cells are recognized for their pivotal role in promoting PCa progression and metastasis [91]. The direct involvement of sEVs in PCa pathogenesis could open new avenues for developing a novel and selective sEV-based therapy that target one of these bioactive molecules transferred by the vesicles to interfere with the PCa signaling pathways. Basically, PCa progression has been associated with hypoxia and the induction of hypoxia-inducible factor (HIF). Exosomal HIF-1α, in particular, fosters the onset and advancement of metastasis by suppressing E-cadherin [92]. On the surface of sEVs secreted by PCa cells, integrin α3, β1, αvβ6, and αvβ3, are able to induce cancer progression and invasion of integrin-negative cells or epithelial cells [93, 94]. In another study, exosome-mediated α2 integrin has been identified as a promoting agent in migration and invasion of PCa cells through the induction of epithelial-mesenchymal transition (EMT) [3]. In addition, integrins α3 and β4 have been linked to cell inflammation, migration, and invasion [95, 96]. Moreover, when PCa-derived exosomal integrin αvβ3 delivered to the TME, it activates Src phosphorylation in recipient cells and encourages the formation of a metastatic niche that alters angiogenesis and cell signaling pathways [97].

Exosomal pyruvate kinase M2 (Exo-PKM2) has been reported to instigate the development of a pre-metastatic niche, thereby promoting bone metastasis [98]. Likewise, phospholipase D (PLD) reported in PCa-derived sEVs stimulates osteoblast activity, serving as a potent regulator in establishing bone metastasis [99]. Another PCa exosomal protein, hyaluronidase 1 (Hyal1), enhances the mobility of prostate stromal cells, thereby augmenting their metastatic potential [100]. Exosomal caveolin-1 facilitates PCa invasion and metastasis through an endocrine mechanism involving the NF-κB signaling pathway and exosomal matrix metallopeptidase 9 (MMP-9) and MMP-14 *via* stimulating ERK1/2 phosphorylation [101, 102]. Additional sEVs-associated proteins, such as Src, insulin-like growth factor 1 receptor (IGF-1R), G-protein-coupled receptor kinases (GRKs), and focal adhesion kinase (FAK), induce PCa angiogenesis by stimulating vascular endothelial growth factor (VEGF) transcription in the TME [103].

A group of researchers recently engineered sEV to target a neuroendocrine PCa surface antigen called carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5). The results from the *in vitro* and *in vivo* studies suggest engineered sEVs carrying drugs as a future therapy for neuroendocrine PCa [104]. A research team demonstrated that inhibition of sEV release by treating PC-3 cells with chloramidine and bisindolylmaleimide-I increased 5-flurouracil sensitization and induced apoptosis in these cells [105]. High-throughput screening shows that the treatment of CD63-labelled C4-2B cells with Manumycin A and tipifarnib significantly reduced sEV by 50 to 70% compared to control cells [106].

Pancreatic cancer and sEV therapeutic targets

Exocrine pancreatic cancer (PC) includes pancreatic ductal adenocarcinoma (PDAC), which accounts for approximately 95% of all PCs [107]. PDAC is still an extremely dangerous gastrointestinal cancer type with a dismal prognosis and an 8%-9% 5-year overall survival rate [63]. Developing effective therapeutic options for PC is hampered by the lack of accurate diagnosis and the drawbacks of traditional treatments. sEVs released by PC cells are loaded with various protein molecules that stimulate neighboring stromal cells and promote ECM remodeling [62]. Consequently, these vesicles alter TME to favor tumor metastasis as shown in **Figure 4D**. Thus, targeting sEV release, uptake or other sEV-associated pathways in PC provide a strong rationale for their utilities in cancer treatment. Owing to their oncogenic role, sEVs secreted by PDAC cells exhibit a strong expression of the Macrophage Migration Inhibitory Factor (MIF), known to initiate the formation of a pre-metastatic niche in the liver [108]. Mechanistically, when PC-initiating cells (PCICs) release sEVs, they prominently express a transmembrane protein called CD44 variant isoform 6 (CD44v6). PCICs' CD44v6-positive sEVs stimulate Wnt/β-catenin signaling pathway, upregulating the expression of tissue inhibitor of metalloproteases 1 (TIM-1) and plasminogen activator inhibitor 1 (PAI-1), which in turn enhance PC cell migration and invasion [109, 110]. According to another study, the exosomal CD44v6/complement C1q binding protein (C1QBP) complex contributes to a fibrotic liver milieu, which induces PC liver metastasis [111]. PC cells stimulate VEGF-independent angiogenesis by producing sEVs enriched by TSPAN8 [112]. Furthermore, sEVs loaded with CD151 and TSPAN8 induce EMT, ECM remodeling, and pro-inflammatory effects, contributing to PC growth and metastasis [113, 114].

Other examples of sEV contributions to PC pathogenesis which can be targeted, claudin7 in tight junction triggers cell migration by altering the structure of sEV transporters and the functionality of sEVs generated from PCICs [115]. Myoferlin can mediate VEGF incorporation into sEVs, which promotes PC angiogenesis [116]. Integrins transferred by sEVs are contributed to the organotropic spread of cancer cells [47]. Pancreatic cell proliferation, migration, and invasion are facilitated by the transfer of plectin into sEVs, which is enabled by integrin β4 [117]. Highly PC metastatic cells release sEVs-associated zinc transporter protein ZIP4 to encourage PC growth, migration, and invasion of non-metastatic cells [118]. PDAC cell-derived sEVs overloaded with survivin have been shown to improve PDAC cell survival [119]. With regard to targeted PC therapy, sEVs isolated from mesenchymal stem cells can treat patients harboring KrasG12D mutation, through mutated gene-targeted siRNA-loaded sEVs [62].

Other disease contexts and their response to sEV inhibition

Beyond cancer and neurodegenerative diseases, sEV inhibition has also been explored in various other disease contexts. For example, in cardiovascular diseases, sEVs contribute to the progression of atherosclerosis and cardiac remodeling. Inhibition of sEV release or cargo loading mechanisms has shown the potential to attenuate the detrimental effects of these conditions [120, 121]. Furthermore, in inflammatory disorders, sEVs play a role in immune cell modulation and cytokine secretion. Targeting sEV release or uptake has been investigated to modulate inflammatory responses and potentially alleviate disease symptoms [122, 123]. These examples highlight the broad applicability of sEV inhibitors across various disease models, indicating their potential as therapeutic interventions in diverse pathological conditions.

Drug resistance and their response to sEV inhibition

The challenge of drug resistance in cancer treatment underscores the importance of innovative approaches to enhance therapeutic efficacy and reduce resistance. Recent cancer research highlights the potential of sEVs as promising nanocarriers for overcoming tumor drug resistance. For example, sEVs have been utilized to deliver anti-miR-214, which sensitizes cisplatin-resistant gastric cancer cells to the treatment [124]. Interventions that target sEVs release, such as rapamycin and U18666A, have demonstrated high efficacy in improving sensitivity to rituximab (anti-CD20 monoclonal antibody) in B lymphoma cells [125]. Similarly, β-elemene modulates the expression of resistance-related miRs in sEVs derived from BC cells, thereby reducing resistance transmission and enhancing chemotherapy sensitivity [126].

Other strategies have been explored to target sEVs for preventing and reversing chemoresistance in cancer cells. Inhibitors like GW4869 and compounds like ketotifen, cannabinol (CBD), and psoralen have sensitized cancer cells to chemotherapeutic drugs by reducing sEV secretion [127]. The therapeutic potential of sEVs derived from human umbilical cord mesenchymal cells was demonstrated in sensitizing myelogenous leukemia cells to a tyrosine kinase inhibitor, imatinib [128]. sEV-specific miR-770 was found to reverse doxorubicin resistance in triple negative BC cells and using Akt inhibitors proved effective in reversing chemoresistance induced by sEVs from cisplatin-resistant cells [129, 130]. In addition to their therapeutic applications, sEVs have emerged as valuable biomarkers for chemotherapy resistance. These small vesicles can be isolated from various biological fluids and serve as potential predictors for chemoresistance in non-sensitive patients. Exosomal miRs, proteins, and other biomolecules have been correlated with drug resistance and high recurrence rates in diverse types of cancers. In this context, a low serum level of sEV miR-146a-5p was linked to NSCLC recurrence and cisplatin resistance, while sEV miR-222-3p predicted gemcitabine sensitivity [131]. Circulating exosomal markers, including cirExo-TRPC5, GSTP1, and miR-151a, are promising predictors for the development of chemoresistance and response to chemotherapy in BC patients [132, 133].

Future directions and challenges

Emerging trends and technologies in sEV inhibitor research

The field of sEV research continues to evolve, driven by emerging trends and innovative technologies. One such trend is the development of more specific and targeted inhibitors that selectively interfere with sEV biogenesis, cargo loading, release, or uptake mechanisms. Advancements in nanotechnology, such as using nanocarriers for targeted delivery of sEV inhibitors, hold great promise for enhancing their efficacy and minimizing off-target effects [35]. Additionally, the integration of high-throughput screening approaches and multi-omics technologies enables the identification of novel sEV inhibitors and provides a systems-level understanding of their mechanisms of action [134]. These emerging trends and technologies bring more hope for discovering new sEV inhibitors and further elucidation of the complexity of sEV pathways in cancer disease.

Artificial intelligence and its use in sEV research

Artificial intelligence (AI) is an emerging tool utilized to analyze and predict sEV structures in hopes that they can be biomarkers for the early detection of several diseases [135]. sEVs are appealing targets for different disease-model research because they are abundant, actively secreted from donor cells, and stable for long-term storage [136]. In a retrospective study, sEV surface-enhanced Raman spectroscopy (SERS) and AI were used to detect simultaneous diagnoses of six types of cancers at early stages [136]. The results of this study support AI's use as an advantageous and efficient instrument capable of analyzing magnitudes of data in seconds to minutes. AI was recently used in different clinical settings such as tumor biomarkers, compared to available radiological and histological features of cancer patients [137, 138]. Another machine-learning algorithm identified a specific human cancer type based on the protein loaded in the cargo of sEVs [139]. In the same context, a recent interesting study used machine learning algorithm to elucidate the effects of tumor mutations on predicting drug resistance in patients receiving chemotherapy [140]. AI applications in cancer research are gaining momentum nowadays,

aiming to revisit unsolved problems and open new avenues for cancer treatment.

Translational potential and clinical considerations for sEV inhibitors

As the understanding of sEV biology expands, the translational potential of sEV inhibitors in clinical settings becomes increasingly apparent. sEV inhibitors hold great promise as therapeutic agents for a wide range of diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases. However, several clinical considerations need to be addressed for their successful translation. These include optimizing the delivery strategies of sEV inhibitors to target specific cell types or tissues, ensuring their stability and bioavailability in the physiological environment, and establishing effective dosage regimens [62, 141]. Furthermore, comprehensive preclinical and clinical studies are required to assess the safety and efficacy of sEV inhibitors, considering potential off-target effects and long-term follow-up. With the ubiquitous nature of sEVs in various cell types, it is crucial to consider the potential for unwanted effects of therapeutic sEV inhibitors on normal tissues. Further research is warranted to determine the safety and precision of sEV inhibitor delivery to the cell and tissue of interest. Using the advantage of new technological advancements can increase the use of sEVs in the diagnosis, prognosis, and treatment of cancer and reach prominent levels of specificity.

Unanswered questions and areas for future sEV investigation

As the scope of sEV research expands, continuous efforts to increase the efficiency of sEV isolation techniques and sEV inhibitor identification may propel this field of study even further. Many techniques are currently available; however, more studies are warranted to determine which methods are best suited to manage the vast workload. Despite considerable progress in the field, numerous unanswered questions and areas for future investigation remain. One key question pertains to the specificity of sEV inhibitors and their impact on normal physiological functions mediated by sEVs. Understanding the potential side effects and unintended consequences of modulating sEV biology is crucial.

Additionally, the mechanisms underlying the selectivity of sEV cargo sorting and the factors determining the packaging of specific molecules into sEVs require further exploration. Moreover, the dynamic interplay between sEVs and the immune system and their roles in tissue regeneration and repair represent intriguing areas for future investigation. Addressing these unanswered questions will provide valuable insights into the biology of sEVs and inform the development of effective sEV inhibitors with therapeutic potential.

Conclusions

In conclusion, the emerging field of sEV inhibitor research has provided valuable insights into the complex biology of sEVs and their role in intercellular communication. Through exploring various aspects, including sEV biogenesis, cargo loading, release, uptake, and modulation of cargo content, noteworthy progress has been made in understanding the regulatory mechanisms and functional implications of sEVs. sEV inhibitors have shown promise as potential therapeutic interventions in diverse disease settings, including cancer, neurodegenerative diseases, and cardiovascular disorders. They can potentially disrupt disease-associated communication networks and offer targeted therapeutic strategies. However, several challenges, such as optimizing delivery strategies, addressing potential off-target effects, and comprehensively assessing safety and efficacy, must be addressed for successful translation into clinical applications. Future exploration and collaborative efforts in the field are crucial to unraveling the intricate pathways of sEV biology further and unlocking the full therapeutic potential of sEV inhibitors. By continuously expanding our knowledge and understanding, sEV inhibitors may pave the way for innovative and effective therapeutic interventions in the future.

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Disclosure of conflict of interest

None.

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