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Messaging malignancy: Tumour-derived exosomes at the nexus of immune escape, vascular remodelling and metastatic competence

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Abstract

Exosomes, nano-sized extracellular vesicles secreted by all varieties of living cells, have emerged as pivotal mediators of intercellular communication within the tumor microenvironment. While exosomes significantly contribute to tumor progression, metastasis, immune modulation, and resistance to therapy, the mechanisms of cargo selection and clinical translation remain controversial and insufficiently resolved. Recent high-throughput technologies have enabled detailed profiling of exosomal cargo; however, substantial challenges persist in their clinical application due to issues in isolation and standardization. This review systematically dissects these molecular biogenesis controversies, the roles of tumor-derived exosomes in modulating angiogenesis, immune escape, metastasis, and therapy resistance, and critically evaluates barriers hindering their clinical adoption.

Keywords: Exosomes, Oncology, Precision Medicine, Tumor niche, molecular communication

Introduction

As Traditional paradigms in tumor biology have long focused on cell-autonomous genetic and epigenetic alterations as the primary drivers of cancer progression. However, the discovery that tumor cells actively secrete exosomes, nano-sized vesicles carrying proteins, lipids, and nucleic acids, has reframed our understanding of intercellular communication in cancer. Exosome-mediated signaling orchestrates dynamic crosstalk between malignant cells and stromal, endothelial, or immune components of the tumor microenvironment (TME), and even primes distant pre-metastatic niches [1]. Despite recognition as mediators of dynamic crosstalk within the TME, debates persist regarding specific roles, cargo selection, and exosome heterogeneity across various cancers. Resolving these controversies is crucial for their successful clinical integration.

To fully appreciate this paradigm shift, it is essential first to delineate the complexity of the tumor microenvironment itself. The tumor microenvironment comprises a heterogeneous and dynamic network of tumor cells, stromal cells, immune infiltrates, extracellular matrix (ECM) components, and soluble factors [2,3]. Rather than being a passive bystander, the TME actively contributes to cancer initiation, progression, metastasis, and therapeutic resistance. Central to this interaction is the ability of cells within the TME to communicate via various mechanisms, including direct cell-cell contact, cytokines, and extracellular vesicles (EVs). Among EVs, exosomes, typically 30-150 nm in diameter (average 100 nm), have gained increasing attention due to their capacity to modulate local and systemic recipient cell behavior [4].

Exosomes originate from the endosomal system, specifically through the inward budding of multivesicular bodies (MVBs), which are subsequently released upon fusion with the plasma membrane [5]. These vesicles carry a molecular signature reflective of their

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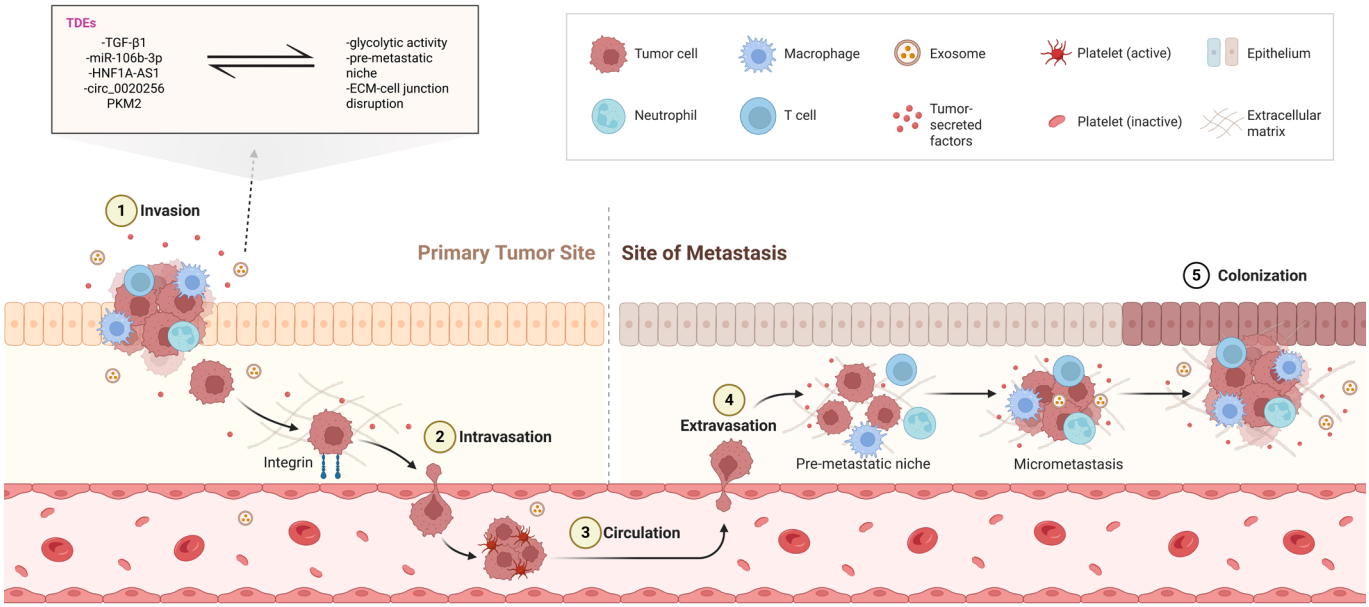

Figure 1. The roles of TDEs and various cellular components involved in TME. Initially, at the primary tumor site, tumor cells invade adjacent tissues by disrupting ECM structures and cellular junctions, a process facilitated by the secretion of exosomes and other tumor-secreted factors (**Step 1: Invasion**). Subsequently, invasive tumor cells intravasate into the bloodstream, aided by integrin-mediated interactions with ECM components and endothelial cells (**Step 2: Intravasation**). Once in circulation, these disseminated tumor cells interact closely with platelets, immune cells, and exosomes, which collectively protect them from immune detection and physical stress (**Step 3: Circulation**). At distant sites, exosomes help establish a pre-metastatic niche by conditioning the microenvironment, remodeling ECM components, and recruiting immune and stromal cells, thereby facilitating tumor cell extravasation from circulation into the tissue (**Step 4: Extravasation**). This step is crucial for the initial colonization of tumor cells and formation of micrometastases. Finally, micrometastases progress through extensive proliferation, stromal remodeling, and continuous interactions with recruited immune and stromal cells, resulting in the formation of fully established metastatic lesions at secondary sites (**Step 5: Colonization**).

cell of origin, encompassing proteins, lipids, and various nucleic acids. In cancer, tumor-derived exosomes (TDEs) play a crucial role in remodeling the TME, facilitating immune escape, enhancing angiogenesis, and preparing pre-metastatic niches (Figure 1) [6].

The recognition of exosomes as central communicators in the TME has spurred intense research into their potential as both biomarkers and therapeutic tools. While the field has advanced rapidly, challenges remain in terms of isolation techniques, functional characterization, and clinical translation.

Here, we discuss how exosomal signaling reshapes immune landscapes, vascular remodeling, and metastatic niches, and explore its translation into precision-oncology interventions.

1. Exosomes as Key Mediators in the Tumor Microenvironment

1.1. Biogenesis and Secretion of Exosomes

Exosomes originate from the endosomal compartment of cells via a tightly regulated multistep process. Initially, early endosomes mature into late endosomes, during which intraluminal vesicles (ILVs) are formed by inward budding of the endosomal membrane. These late endosomes, or MVBs, can either fuse with lysosomes for degradation or with the plasma membrane to release ILVs as exosomes into the extracellular space (Figure 2)[5].

The biogenesis of exosomes involves ESCRT-dependent and ESCRT-independent pathways. Despite substantial research, the coexistence of these pathways within individual MVBs and their functional implications remain poorly understood. Recent studies highlight significant variations in exosome biogenesis across tumor types, underscoring the necessity for targeted mechanistic research [7]. The Endosomal Sorting Complex Required for Transport (ESCRT) machinery-comprising ESCRT-0, I, II, and III plays a critical role in the sorting of ubiquitinated cargo into ILVs [8]. Alternatively, ceramide-driven membrane curvature, tetraspanin-enriched domains, and lip-

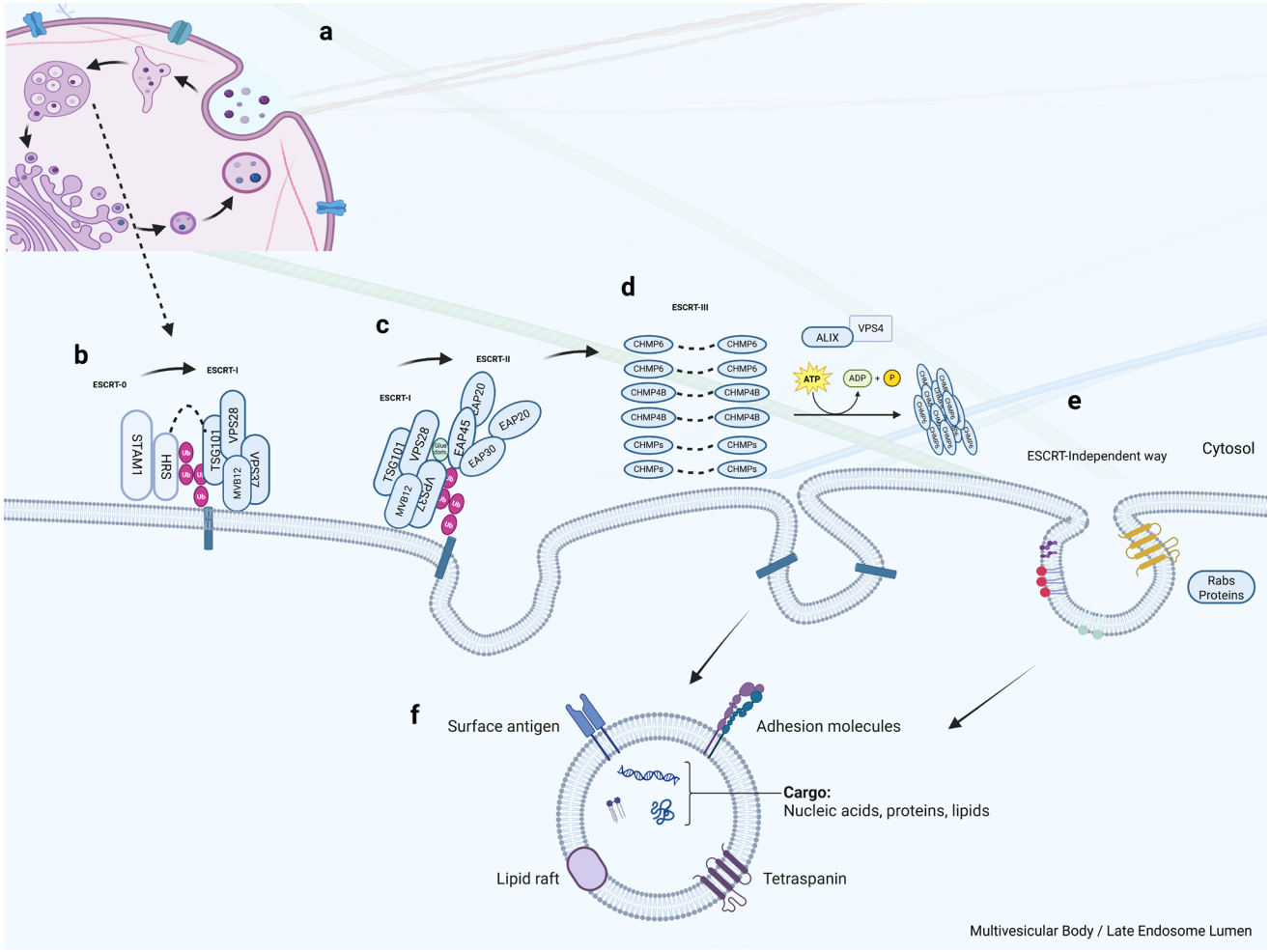

Figure 2. Molecular mechanisms by which MVBs are released as exosomes, ESCRT-dependent and independent pathways involved in this process, and exosome structure: (**a**) Schematic representation of the overall process of exosome formation within the cell. In this process, early endosomes differentiate into late endosomes, which in turn differentiate into MVBs that fuse with the plasma membrane and release exosomes into the extracellular environment. In sections (**b-e**) the ESCRT mechanism is detailed: (**b**) The ESCRT-0 complex, composed of HRS and STAM1 proteins, recognizes and binds ubiquitin-bound cargo proteins; (**c**) This cargo then allows ILVs to bud into the membrane through ESCRT-I complexes consisting of TSG101, VPS28, VPS37, MVB12 and ESCRT-II complexes consisting of EAP45, EAP20, EAP30, VPS22, VPS25 and VPS36; (**d**) The ESCRT-III complex, represented by members of the CHMP protein family, completes ILV formation by regulating the final folding of the membrane and membrane rupture of ILVs. This process leads to the recycling of the ESCRT-III complex with the participation of ALIX and VPS4 proteins in an ATP-dependent process; (**e**) The ESCRT-independent mechanism is mediated through membrane microdomains such as lipid raft domains and tetraspanin proteins. Rab GTPaz ailesine ait proteinler ise MVB'lerin plazma membranına füzyonunu ve eksozomların ekstrasellüler ortama salınmasını düzenler; (**f**) Cargo molecules (nucleic acids, proteins and lipids) and surface proteins (adhesion molecules, surface antigens, lipid rafts and tetraspanins) inside the exosome are shown.

id rafts also contribute to ESCRT-independent biogenesis [9]. Rab GTPases regulate MVB trafficking and exosome secretion, highlighting the complexity and specificity of exosomal release [10].

Components of the ESCRT machinery, including tumour susceptibility gene 101 protein (TSG101), syntenin-1, and ALG-2 interacting protein X (ALIX), are essential for ILV formation. The incorporation of proteins into ILVs is primarily governed by ubiquitination [11]. When ESCRT components such as hepatocyte growth factor-regulated tyrosine kinase substrate, TSG101, or vacuolar protein-sorting 28 (VPS28) are either depleted or overproduced, ubiquitinated proteins tend to build up within endosomal compartments [12]. ALIX, in particular, directs ESCRT-III components to endosomes and mediates cargo sorting via interactions with tetraspanins [13]. Syntenin-1 has been shown to influence the Focal adhesion kinase-steroid receptor coactivator (FAK-Src) signaling cascade, an essential cellular adhesion and growth regulator, by interacting with integrins [14,15]. It also modulates the mTOR pathway, a central regulator of cell proliferation that may simultaneously impact the production of exosomes [16].

Recent studies further illustrate that these pathways are not

strictly compartmentalized. For instance, in multiple myeloma and breast cancer, silencing of Rab27a significantly impairs exosomal release and alters the composition of secreted vesicles, suggesting cargo-selective regulation by Rab GTPases [17–19]. Moreover, inhibition of ceramide synthesis using GW4869, a non-competitive neutral sphingomyelinase inhibitor, not only reduces overall exosome yield but also decreases the release of pro-angiogenic miRNAs such as miR-210, which are critical for hypoxia-driven communication in the tumor microenvironment [20,21]. Importantly, evidence from glioblastoma models indicates that ESCRT-dependent and -independent pathways may coexist within a single MVB, giving rise to heterogeneous ILV populations with differential oncogenic potential [22,23]. This convergence may reflect context-specific regulatory demands, influenced by tumor type, metabolic status, and the dynamic cellular milieu.

Deciphering the molecular switches of exosome biogenesis will be pivotal for translating vesicle biology into precision-oncology modalities that can selectively disrupt tumor-promoting communication.

1.2. Molecular Composition of Exosomes

Exosomes encapsulate a diverse array of bioactive molecules that mirror their cell of origin and reflect the physiological or pathological status of the producing cell. Among protein cargos, tetraspanins, CD9, CD63, CD81, and CD82, are commonly enriched and serve as canonical exosomal markers [24,25]. Notably, CD63 facilitates the sorting of Epstein-Barr virus-encoded Epstein–Barr virus latent membrane protein 1 (LMP1) into exosomes, aiding its escape from lysosomal degradation [26]. Furthermore, expression of CD9 on pre-B cells triggers the release of CD10 from exosomes into the ECM, which alters the composition of the extracellular matrix and affects the migration of pre-B cells to different hematopoietic sites [27].

Heat shock proteins, HSP70 and HSP90, frequently identified in exosomal preparations, play roles in protein folding and stress responses, and are implicated in modulating immune and inflammatory signaling upon delivery to recipient cells [28]. Tumor cells exhibit elevated expression of HSP70 on their plasma membranes and actively secrete exosomes enriched with surface-bound HSP70 [29]. These exosomes activate natural killer (NK) cells, enhancing their cytolytic activity against HSP70-positive tumor targets [29]. Clinically, two distinct forms of circulating HSP70 are observed in cancer patients: one associated with exosomes released by viable tumor cells, and another as a soluble form liberated from dying tumor cells, functioning as damage-associated molecular patterns (DAMPs) that modulate immune responses [30,31]. The concurrent induction of immunogenic cell death and apoptosis-associated DAMP release by specific anticancer modalities, such as chemotherapeutic agents and ionizing radiation, has been shown to elicit a robust antitumor immune response [32–34]. This synergistic activation of innate and adaptive immunity enhances the immunogenicity of dying tumor cells, thereby augmenting the therapeutic potential of these treatments in oncological settings.

On the lipid front, exosomes are highly enriched in sphingomyelin and ceramides, the latter of which are crucial for ESCRT-independent biogenesis by promoting negative membrane curvature [35]. Cholesterol further supports membrane rigidity and fusogenicity, enhancing exosome stability in extracellular environments [36]. Experimental analyses demonstrated that enrichment of cholesterol within the exosomal membrane significantly enhances its biophysical properties, facilitating more efficient interaction and direct fusion with the plasma membrane of target cells [37]. This fusion-based entry mechanism not only circumvents endocytic internalization and subsequent lysosomal degradation but also enables the direct cytoplasmic delivery of siRNA [37]. The molecular modeling further revealed that cholesterol-rich exosomes exhibit increased membrane deformability and a larger interfacial contact area with recipient cells, thereby improving the functional efficacy of exosome-mediated therapeutic cargo delivery. Importantly, phosphatidylserine (PS), typically restricted to the inner leaflet of plasma membranes, is found on the outer surface of exosomes, thereby mediating recognition by phagocytes and possibly facilitating immune evasion [38]. PS, initially recognized for mediating the silent clearance of apoptotic cells via macrophage recognition [39], has since been implicated in active immunosuppression, particularly through its presence on tumor-derived exosomes, inhibiting T-cell activation. Targeting PS has emerged as a potential therapeutic strategy to reverse exosome-mediated immune suppression in cancer and inflammatory diseases. Although early interventions using PS-binding antibodies or annexin V showed limited efficacy, the development of ExoBlock, a hexameric molecule with enhanced PS-binding avidity, offers a promising alternative with superior neutralizing potential [40]. These molecular constituents not only ensure the structural and functional integrity of exosomes but also play vital roles in mediating their biological effects in health and disease.

One of the most significant aspects of exosomes is their nucleic acid content, particularly regulatory RNAs. These molecules are not merely passive biomarkers but function as active mediators of intercellular communication within the TME [41,42]. They can modulate gene expression in recipient cells by altering transcriptional programs, silencing tumor suppressor genes, or activating oncogenic pathways [43,44].

Tumor-derived exosomal miR-21, one of the most abundantly expressed oncomiRs, has been shown to promote angiogenesis by targeting PTEN and activating the AKT/ERK signaling cascade in endothelial cells, thereby enhancing vascularization in glioblastoma and breast cancer models [45,46]. Similarly, exosomal miR-210, often induced under hypoxic conditions, facilitates angiogenesis and cell survival by repressing Ephrin-A3 and PTP1B in endothelial cells, promoting tumor adaptation to low-oxygen environments [47]. Exosome-derived miRNAs exhibit a multifaceted role in colorectal cancer (CRC). Transfection of miR-21-5p and miR-155-5p into CRC cells resulted in the downregulation of BRG1, a process that complicates tumor

progression [48]. In contrast, introducing miR-25-3p, miR-130b-3p, and miR-425-5p into CRC cells induced metastasis [49–51]. Notably, miR-130b-3p, which is generally recognized as a tumor suppressor miRNA, demonstrates a paradoxical function in this context, further highlighting the complexity of miRNA-mediated regulation in CRC metastasis [52].

Exosomal lncRNAs also exert profound effects. For example, lncRNA HOTAIR, transferred via breast cancer-derived exosomes, can reprogram recipient stromal fibroblasts into cancer-associated fibroblasts (CAFs), enhancing matrix remodeling and metastasis [53]. Likewise, exosomal lncRNA PVT1 in gastric cancer has been associated with chemoresistance through upregulation of Bcl-2 and suppression of apoptosis pathways [54].

In addition, circRNAs transported via exosomes, such as circRNA_100284 in hepatocellular carcinoma, act as sponges for tumor-suppressive miRNAs like miR-217, leading to upregulation of target oncogenes like *EZH2* and enhanced proliferation [55].

2. Interactions with Tumor and Stromal Cells

Within the TME, exosomes serve as critical vehicles for horizontal transfer of oncogenic information between tumor cells and various stromal components, including CAFs, endothelial cells, tumor-associated macrophages (TAMs), dendritic cells, and T cells (Figure 3).

2.1. Interaction with immune cells

TDEs play a pivotal role in sculpting an immunosuppressive tumor microenvironment by interacting with various immune cell populations. A primary mechanism involves the exosomal delivery of programmed death-ligand 1 (PD-L1), which binds to PD-1 receptors on activated T cells, leading to the inhibition of T cell receptor (TCR)-mediated signaling, T cell exhaustion, and eventual immune evasion (Figure 4) [56]. Exosomal PD-L1 derived from metastatic melanoma cells has been shown to effectively reduce anti-tumor immunity by directly inhibiting $CD8^+$ T cell proliferation and cytokine secretion [57]. The molecular pathways through which exosome-derived PD-L1 exerts its inhibitory effects on T cells remain incompletely understood [58].

In addition to PD-L1, tumor exosomes also carry Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), which can induce apoptosis in activated T cells, further contributing to immune escape [59]. FasL-positive exosomes released by ovarian cancer cells have been reported to mediate apoptosis of effector T lymphocytes via engagement of the Fas/FasL axis [60]. A similar mechanism appears to contribute to protecting the fetus from maternal immune responses. The human placenta has been demonstrated to secrete functional exosomes containing FasL and TRAIL, which can induce apoptosis in activated immune cells [61]. This exosome-mediated immunomodulation supports the establishment of fetal immune privilege and suggests that tumors may exploit analogous pathways to evade immune surveillance.

Exosomal immunosuppressive miRNAs represent another key mechanism. miR-24, miR-21, miR-146a, and miR-212 found in TDEs have been shown to modulate T cell differentiation, macrophage polarization, and dendritic cell function [62–65]. For example, miR-21 and miR-29a in lung cancer-derived exosomes activate TLR8 signaling in macrophages, promoting NF-κB–dependent secretion of proinflammatory cytokines that paradoxically foster a tumor-supportive inflammatory environment [66,67]. Moreover, miR-146a delivered via breast cancer exosomes can reprogram tumor-associated macrophages toward an M2-like phenotype, characterized by increased IL-10 and TGF-β secretion and suppression of cytotoxic responses [68].

TDEs also target NK cells. Exosomes from leukemia and breast cancer cells have been reported to carry TGF-β1, which downregulates natural killer group 2 member D (NKG2D) expression on NK cells, a critical activating receptor for NK-mediated cytotoxicity, thereby impairing NK cell recognition and lysis of tumor cells [69]. Exosomes derived from acute myeloid leukemia (AML), pancreatic cancer, and multiple myeloma have all been shown to markedly suppress NK cell-mediated cytotoxicity, particularly against canonical target cells such as K562 [70]. Specifically, pre-exposure of NK-92 cells to AML-derived exosomes significantly reduces their lytic activity, mirroring similar impairments observed upon treatment with pancreatic cancer-derived EVs, which diminish NK cytotoxicity toward pancreatic cancer stem cells [71]. Likewise, exosomes isolated from multiple myeloma cells effectively attenuate NK cell function, further supporting the conserved immunoevasive strategies employed by hematologic malignancies [72]. These findings underscore the capacity of tumor-derived EVs to act as potent modulators of NK cell surveillance and suggest a tumor-selective mechanism for exosome-mediated immune suppression.

Notably, a key way TDEs impair NK cell function is by downregulating activating receptors, including the previously discussed NKG2D and NKp30, on the NK cell surface [69,73]. NKG2D is a C-type lectin-like activating receptor that recognizes stress-induced ligands, MICA, MICB, and ULBP1-6, enabling NK cell-mediated cytotoxicity against transformed or infected cells [74]. NKp30 is another critical immunoreceptor that recognizes B7-H6, a tumor-expressed ligand, and is essential for immunological synapse formation and perforin/granzyme release [75]. Similarly, NKp30 expression can be downregulated via exosomal delivery of TGF-β1 or tumor-derived ligands that disrupt receptor recycling [76]. Textural changes in NKp30 have been reported following exposure to TDEs in patients with gastrointestinal tumors, correlating with immune evasion and disease progression [77].

Furthermore, tumor exosomes can modulate antigen-presenting cells such as dendritic cells. TDEs containing HSP72 or galectin-9 can inhibit dendritic cell maturation or induce tolerogenic dendritic cell phenotypes, reducing their capacity to stimulate T cells effectively [78]. Additionally, exosomal miR-212-3p has been shown to suppress MHC class II expres-

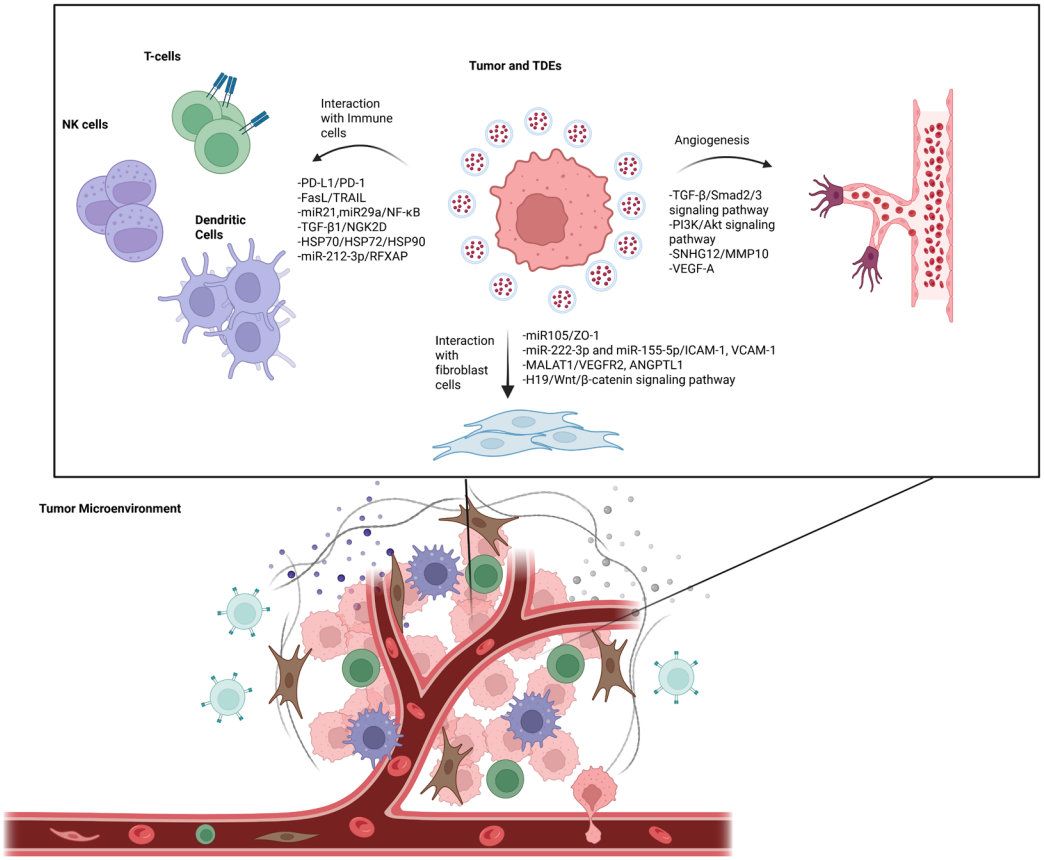

Figure 3. Interactions of TDEs in the tumor microenvironment with immune cells, fibroblasts and their role in angiogenesis. Tumor cells secrete exosomes that significantly influence immune surveillance mechanisms by modulating fundamental interactions with T cells, NK cells and dendritic cells through various molecules, collectively facilitating immune escape and promoting immune suppression. These molecular exchanges significantly contribute to fibroblast activation and subsequent remodeling of the tumor stroma.

sion in dendritic cells by targeting RFXAP, thereby impairing antigen presentation and T cell priming [79].

These converging lines of evidence elevate tumour-derived exosomes from mere biomarkers to actionable therapeutic nodes. Intercepting their immunosuppressive circuitry will likely require a multi-pronged strategy that (i) neutralises exosomal immune checkpoints such as PD-L1, either with decoy nanoparticles or monoclonal antibodies engineered for vesicle affinity [80], (ii) pharmacologically throttles vesicle biogenesis/secretion (nSMase2 or Rab27 blockade), thereby synergising with first-line chemotherapy and PD-1/PD-L1 inhibitors now entering early-phase trials [81], and (iii) re-purposes immunogenic exosomes as cell-free vaccines to restimulate exhausted T and NK compartments [82]. Initial clinical signals, for example, GW4869 analogues enhancing chemotherapy responses in small-cell lung cancer [81] and mRNA-loaded exosome vaccines advancing toward first-in-human evaluation [82], underline the tractability of this approach. The next frontier will be to embed high-dimensional exosomal cargo profiling into patient-selection algorithms, enabling precision deployment of vesicle-targeted agents and converting a once passive conduit of immune escape into a therapeutic liability for the tumour. Such an integrated roadmap holds promise to recalibrate the tumour–immune dialogue in favour of durable antitumour immunity.

2.2. Interaction with endothelial cells

TDEs profoundly affect endothelial cells, critically contributing to tumor angiogenesis, vascular permeability, and metastatic dissemination (Figure 5) [83]. Exosomes derived from hypoxic glioma cells are enriched with VEGF-A and miR-210, both of which promote endothelial cell proliferation and tubulogenesis by activating VEGFR2 signaling and suppressing EFNA3 expression, respectively [84]. Similarly, breast cancer cell–derived exosomal miR-105 has been shown to disrupt endothelial tight junctions by downregulating ZO-1, leading to increased vascular permeability and facilitating metastatic spread [85]. In hepatocellular carcinoma (HCC), exosomal circRNA-100338 promotes angiogenesis by sponging miR-141-3p, thereby upregulating the expression of VEGF-A and FGF9 in endothelial cells [86]. Furthermore, melanoma-derived exosomes containing PD-L1 not only contribute to immune evasion but

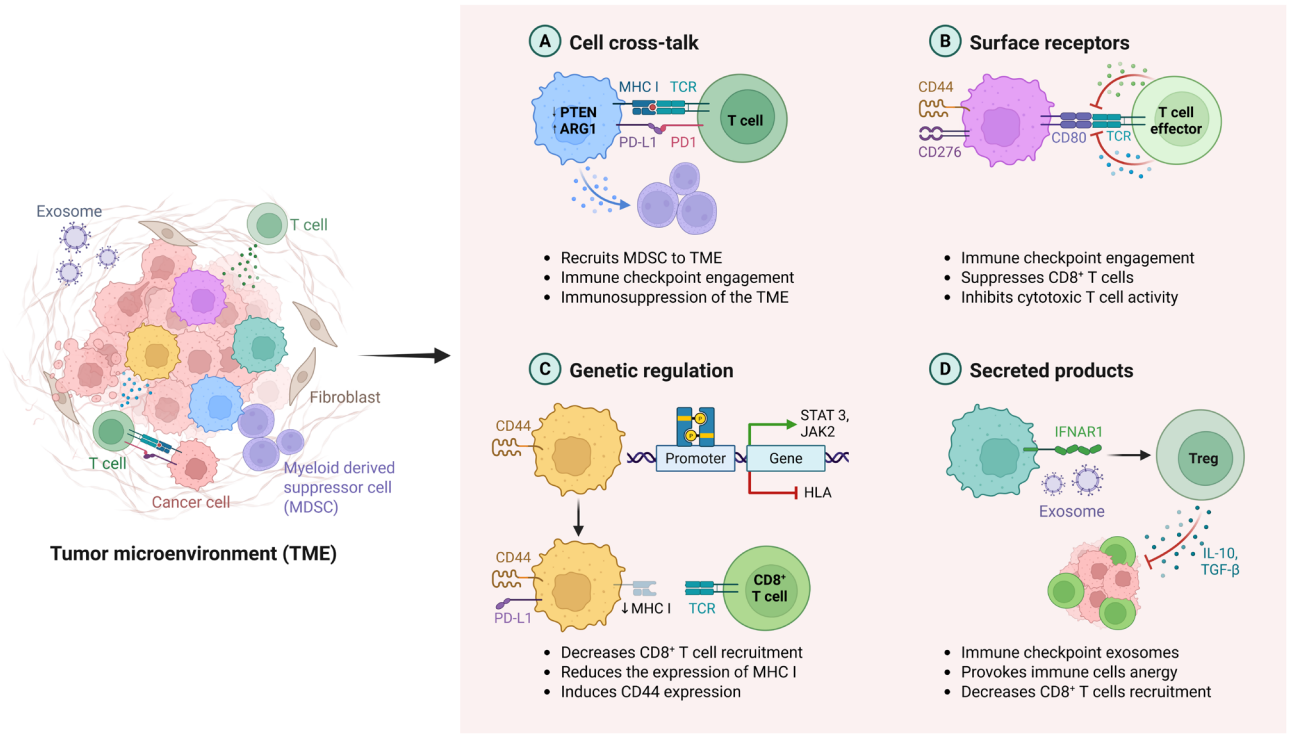

Figure 4. Multifaceted mechanisms by which the TME orchestrates immune evasion, with a particular emphasis on T cell suppression. A highlights direct cellular interactions within the TME, where tumor or MDSCs upregulate immunosuppressive mediators such as PTEN and ARG1. These cells engage immune checkpoints, most notably via PD-L1/PD-1 interactions with T cells, facilitating recruitment of MDSCs and promoting an immunosuppressive milieu. B demonstrates the role of altered surface molecules, such as CD44 and CD276, on tumor or stromal cells. These interact with effector T cells via immune checkpoint proteins like CD80 and TCR, leading to suppression of CD8+ T cell function and inhibition of cytotoxic activity. C depicts how the TME mediates transcriptional changes through signaling pathways (e.g., STAT3, JAK2), resulting in decreased MHC I expression and increased CD44 on tumor cells. This impairs CD8+ T cell recruitment and antigen presentation, further enabling immune evasion. D focuses on soluble and vesicular mediators, including exosomes and cytokines (IL-10, TGF-β), which promote Treg induction and T cell anergy. Exosomes also carry immune checkpoint molecules, contributing to decreased CD8+ T cell recruitment and reinforcing immunosuppression within the TME.

also induce a pro-inflammatory phenotype in endothelial cells, enhancing adhesion molecule expression such as ICAM-1 and VCAM-1, thus supporting extravasation of circulating tumor cells [87]. In particular, exosomes released from oral squamous cell carcinoma cells carry miR-222-3p and miR-155-5p [88]. In a similar way, when transferred to endothelial cells, these miRNAs facilitate tumor cell adhesion to the endothelium by increasing ICAM-1 and VCAM-1 expression [89].

TDEs deploy a remarkably diverse cargo repertoire to reprogramme endothelial cells toward a pro-angiogenic and pro-permeability phenotype. Beyond the hypoxic-glioma miR-210/VEGF-A axis and breast-cancer miR-105-mediated ZO-1 loss already discussed, colorectal-cancer exosomes rich in miR-25-3p downregulate the transcription factors KLF2 and KLF4, thereby suppressing occludin and claudin-5, loosening endothelial junctions and activating VEGFR2 signalling to seed pre-metastatic niches in liver and lung [90]. Long non-coding RNA MALAT1, shuttled by epithelial-ovarian-cancer exosomes, similarly enhances endothelial migration and vessel maturation by up-regulating VEGFR2 and ANGPT1 [91]. Conversely, not all cargo is pro-angiogenic: glioblastoma-derived exosomes enriched in the secreted glycoprotein ANGPTL1 dampen VEGFR2 phosphorylation and curtail micro-vessel density, illustrating that TDEs-Endothelial cells crosstalk can also restrain vascular expansion when tumour-suppressive signals predominate [92].

These mechanistic vignettes reveal at least three actionable principles. First, the angiogenic output of TDEs is tumour-type specific and cargo-defined, arguing for exosomal "angiogenic signatures" as companion diagnostics to guide anti-vascular therapy. Second, cargo-selective blockade, such as antisense oligonucleotides that neutralise miR-23a or miR-25-3p, or recombinant ANGPTL1-loaded decoy vesicles, could complement VEGF/VEGFR inhibitors and overcome resistance rooted in non-canonical pathways. Third, the existence of anti-angiogenic TDEs cargo suggests that re-engineering or enriching

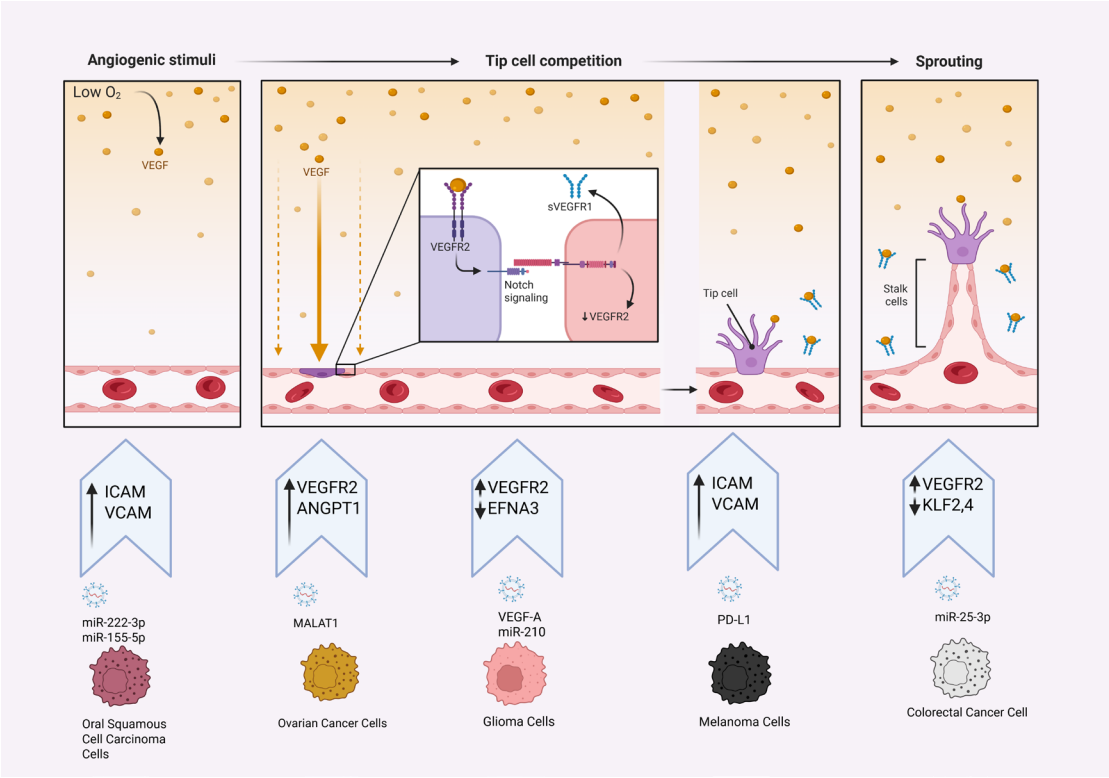

Figure 5. During the process of tip cell competition, VEGF/VEGFR2 signaling triggers Notch pathway activation in neighboring endothelial cells. This Notch signaling suppresses VEGFR2 expression in adjacent cells, ensuring that only selected cells adopt the tip cell phenotype, while others differentiate into stalk cells. Tip cells lead the new vessel sprout in response to VEGF gradients, while stalk cells proliferate and stabilize the growing vessel structure. The lower section of the figure highlights how tumor-derived exosomal miRNAs and proteins modulate the expression of critical angiogenic regulators such as VEGFR2, EFNA3, ICAM, VCAM, and KLF2/4. These signals, secreted by various cancer cell types (e.g., oral squamous cell carcinoma, ovarian cancer, glioma, melanoma, colorectal cancer), fine-tune endothelial cell behavior, enhance aberrant vascular remodeling, and promote tumor progression.

endogenous exosomes might convert them from vascular accomplices into vascular antagonists. Integrating high-dimensional exosome profiling with single-cell endothelial atlases should therefore be prioritised to stratify patients and to design multiplexed interventions that recalibrate tumour vasculature rather than merely pruning it, a shift that could amplify immunotherapy and drug-delivery efficacy in the next generation of precision oncology trials.

2.3. Interaction with fibroblasts

By reprogramming fibroblasts into CAFs, exosomes significantly contribute to tumor progression via mechanisms such as ECM remodeling, neovascularization, immune modulation, and establishment of premetastatic niches (Figure 6) [93,94].

A striking example is seen in breast cancer, where exosomal miR-9 has been shown to activate normal fibroblasts into CAFs by downregulating EFEMP1 and promoting the JAK/STAT signaling pathway (Figure 7), leading to increased expression of α-SMA and FAP, classical CAF markers [95]. Similarly, in gastric cancer, exosomal miR-27a secreted by tumor cells induces CAF differentiation through suppression of BTG2, a known tumor suppressor, thereby enhancing the proliferative and migratory abilities of gastric cancer cells [96].

In pancreatic ductal adenocarcinoma (PDAC), tumor-derived exosomes enriched in miR-155 promote fibroblast activation and secretion of pro-inflammatory cytokines, IL-6 and CXCL8, which facilitate a pro-tumorigenic inflammatory milieu [97–99]. These CAFs, in turn, secrete exosomes containing lncRNA H19, which are internalized by tumor cells and promote epithelial–mesenchymal transition (EMT) via the Wnt/β-catenin signaling axis, highlighting a reciprocal feedback loop [100].

Importantly, exosomal TGF-β1 has been identified as a potent inducer of fibroblast-to-CAF transition across various tumor types, including colorectal and lung cancer. These activated CAFs secrete matrix metalloproteinases, fibronectin, and collagen I, contributing to ECM degradation and invasion-favorable remodeling [101,102]. Furthermore, exosomal Wnt10b derived from tumor cells in prostate cancer promotes CAF activation and enhances tumor cell invasiveness through the canonical Wnt signaling pathway [103].

Interestingly, CAF-derived exosomes also contribute to therapy resistance. For instance, in head and neck squamous cell carcinoma, CAF-derived exosomes containing miR-196a

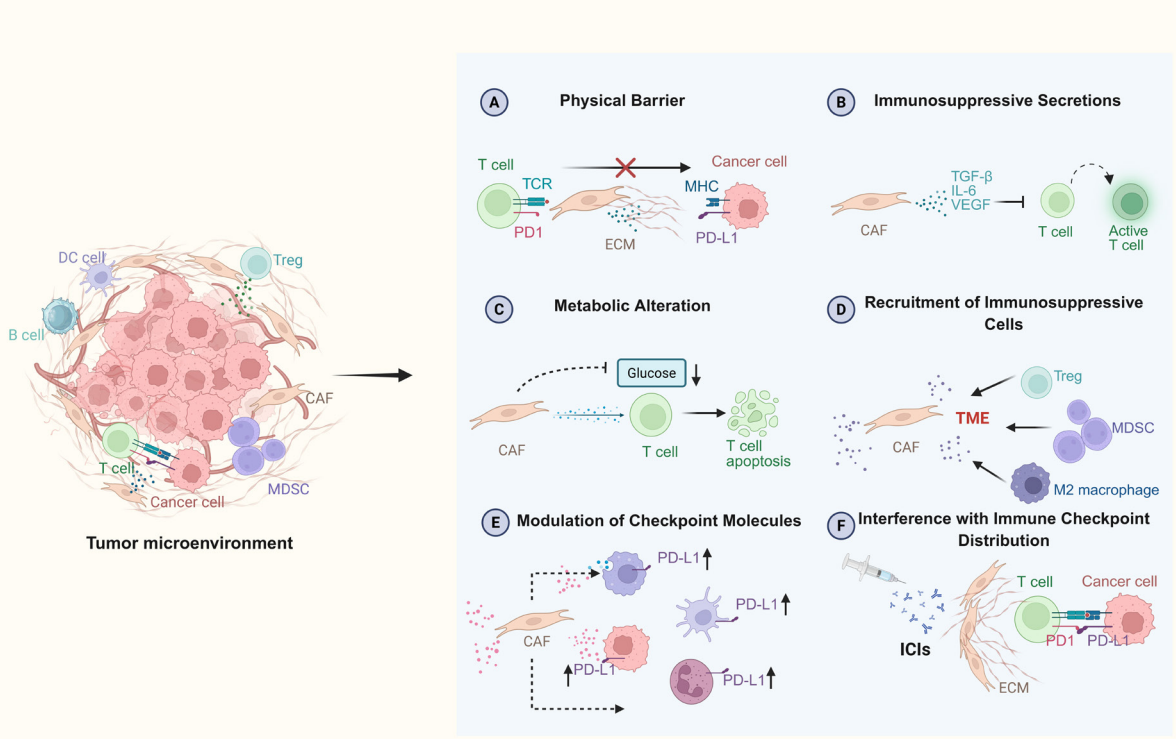

Figure 6. Mechanisms of Immune Evasion in the Tumor Microenvironment: A) Physical Barrier: CAFs and ECM components create a physical barrier, impeding T cell access to cancer cells and limiting effective immune surveillance; B) Immunosuppressive Secretions: CAFs secrete immunosuppressive cytokines (e.g., TGF-β, IL-6, VEGF), which inhibit T cell activation and function; C) Metabolic Alteration: CAFs alter the metabolic landscape by consuming nutrients like glucose, leading to T cell dysfunction and apoptosis due to nutrient deprivation; D) Recruitment of Immunosuppressive Cells: The TME facilitates the recruitment of regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC), and M2 macrophages, all of which suppress anti-tumor immunity; E) Modulation of Checkpoint Molecules: CAFs and other TME components upregulate immune checkpoint molecules (such as PD-L1) on various cells, further dampening T cell responses; F) Interference with Immune Checkpoint Distribution: Physical barriers and altered distribution of checkpoint molecules within the TME can limit the effectiveness of immune checkpoint inhibitors (ICIs), reducing therapeutic efficacy.

confer cisplatin resistance to tumor cells by targeting CDKN1B and ING5, two key regulators of cell cycle and apoptosis [104].

3. Exosome-Mediated Crosstalk and Tumor Progression

3.1. Immune evasion: Blocking the message

TDEs contribute significantly to immune evasion by modulating the phenotype and function of various immune cells through the delivery of immunosuppressive molecules. CRC-derived exosomes enriched in miR-372-5p have been shown to promote immune suppression by upregulating immune cells' exosomal PD-L1, Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL), which profoundly affect immune cell functions. However, the molecular pathways through which exosomes mediate these immune modulatory effects require deeper biochemical characterization, identifying actionable therapeutic targets. Nevertheless, recent work demonstrates that PD-L1 expression in macrophages via activation of the PTEN/AKT/NF-κB signaling axis, thereby reducing $CD8^+$ T cell activity [105]. In cervical cancer, exosomal miR-1468-5p enhances PD-L1 expression in lymphatic endothelial cells by activating the JAK2/STAT3 pathway, facilitating local immune escape [106]. Similarly, melanoma-derived exosomes carrying miR-3187-3p impair $CD8^+$ T cell cytotoxicity by attenuating T cell receptor signaling and reducing TNF-α secretion [107]. In the context of gastric cancer, exosomal miR-107 suppresses PTEN expression and promotes arginase-1 (ARG1) upregulation in myeloid-derived suppressor cells (MDSC), which in turn inhibit T cell responses [108–110]. Moreover, mutant p53-harboring colon cancer cells secrete exosomes containing miR-1246, which activate the TGF-β signaling pathway in macrophages, leading to an increase in regulatory T cells and the establishment of an immunosuppressive tumor microenvironment [111].

3.2. Angiogenesis and Vascular Remodeling

Angiogenesis, forming new blood vessels from pre-existing vasculature, is a critical process in tumor progression, facilitating nutrient delivery, waste removal, and metastatic dissemination [112].

In CRC, exosomal miR-1825 has been identified as a key pro-angiogenic factor. miR-1825 is transferred from CRC cells to human umbilical vein endothelial cells (HUVECs), where it

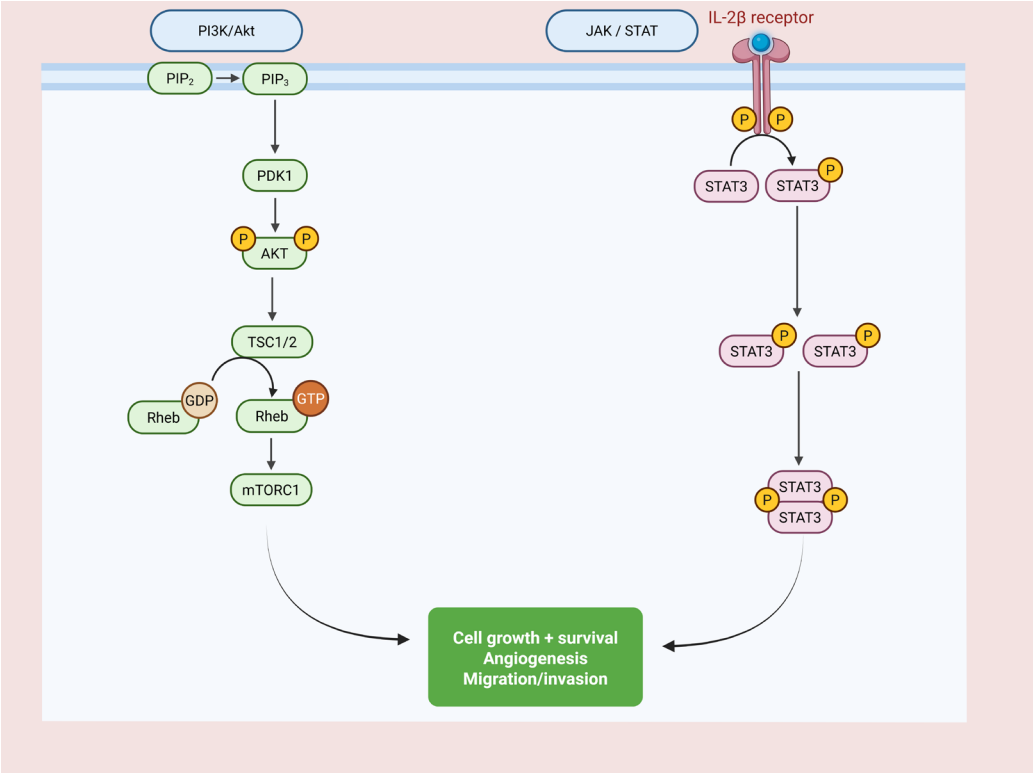

Figure 7. Mechanistic Crosstalk Between the PI3K/Akt/mTOR and JAK/STAT Pathways in Cellular Proliferation, Angiogenesis, and Invasion. PI3K/Akt/mTOR and JAK/STAT cascades, both of which are crucial mediators of cell growth, survival, angiogenesis, and cellular migration/invasion in various physiological and pathological contexts, notably cancer. PI3K/Akt/mTOR Pathway (Left Panel): Upon activation by upstream signals, PI3K catalyzes the phosphorylation of PIP2 to generate PIP3 at the plasma membrane. This lipid second messenger recruits PDK1 and AKT (protein kinase B), facilitating the phosphorylation and activation of AKT. Activated AKT subsequently phosphorylates the tuberous sclerosis complex (TSC1/2), leading to its inactivation. TSC1/2 inhibition enables Rheb, a small GTPase, to accumulate in its active GTP-bound form, which directly activates mTORC1. mTORC1 acts as a master regulator of protein synthesis, cell growth, and metabolism. Overall, this pathway integrates growth factor signals to promote cellular proliferation, survival, angiogenesis, and metastatic capacity. JAK/STAT Pathway (Right Panel): Canonical JAK/STAT signaling pathway, initiated by the binding of interleukin-2β (IL-2β) to its cell surface receptor. This interaction triggers receptor dimerization and autophosphorylation of associated JAKs, which in turn phosphorylate specific tyrosine residues on the receptor cytoplasmic domain. STAT3 is subsequently recruited, phosphorylated, and forms active dimers that translocate to the nucleus to drive transcriptional programs involved in cell proliferation, angiogenesis, and immune modulation.

downregulates ING1, leading to the activation of the TGF-β/Smad2/3 signaling pathway [113]. This activation enhances endothelial cell migration and tube formation and ultimately promotes angiogenesis and liver metastasis in CRC models.

In breast cancer, exosomal long non-coding RNA SNHG12 has been shown to facilitate angiogenesis. SNHG12 is enriched in breast cancer cell-derived exosomes and promotes proliferation and migration upon transfer to HUVECs. Mechanistically, SNHG12 binds to PBRM1, preventing its interaction with MMP10, thereby upregulating MMP10 expression, which is known to enhance angiogenic processes [114].

Gastric cancer-derived exosomes also contribute to angiogenesis through various mechanisms. Exosomal miR-21-5p targets LEMD3 in endothelial cells, leading to increased Smad1, Smad3, and TGF-β phosphorylation, and upregulation of VEGFA, collectively enhancing angiogenic signaling [115]. Additionally, exosomal circSHKBP1 has been reported to stabilize VEGF mRNA by decreasing miR-582-3p and increasing HUR expression, further promoting angiogenesis [116].

In glioma, exosomal long non-coding RNAs such as LINC-POU3F3 and LINC-CCAT2 are transferred to endothelial cells, where they upregulate VEGFA and TGF-β expression, while downregulating pro-apoptotic molecules like Bax and caspase-3, thereby promoting angiogenesis and inhibiting apoptosis [117].

Furthermore, exosomes derived from miR-126-overexpressing bone marrow mesenchymal stem cells have been shown to promote angiogenesis by targeting PIK3R2, leading to the activation of the PI3K/Akt signaling pathway (Figure 7) in endothelial cells [118].

The emerging evidences underline the mechanistic sophistication by which tumor-derived exosomes regulate angiogenesis, largely through RNA-mediated reprogramming of endothelial cells. Exosomes facilitate vascular plasticity and metastatic

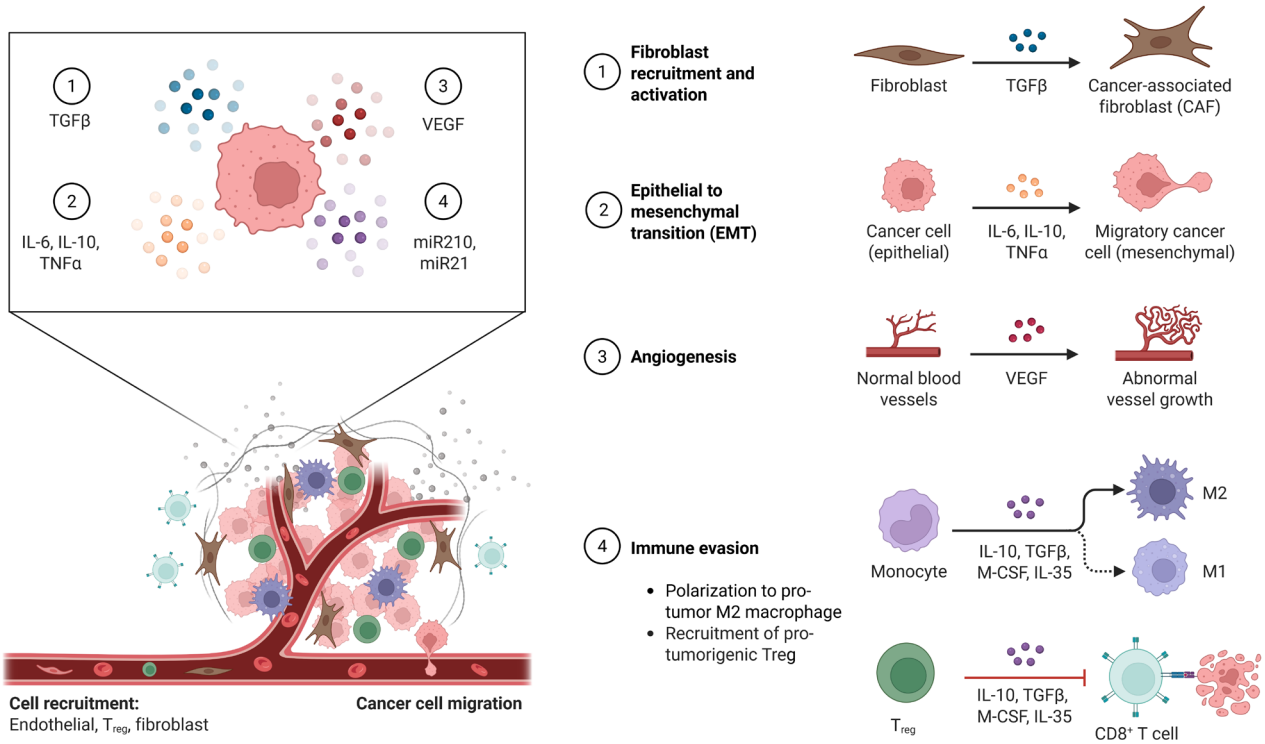

Figure 8. Tumor Microenvironment–Driven Cellular and Molecular Changes. Fibroblast Recruitment and Activation: Tumor-derived TGF-β induces the transformation of resident fibroblasts into cancer-associated fibroblasts (CAFs), which promote tumor progression. Epithelial to Mesenchymal Transition (EMT): Cytokines such as IL-6, IL-10, and TNF-α secreted by cancer cells drive EMT, resulting in migratory, invasive mesenchymal-like cancer cells; Angiogenesis: Tumor-secreted VEGF stimulates abnormal angiogenesis, leading to the formation of irregular and leaky blood vessels that support tumor growth; Immune Evasion: The TME promotes immune escape through polarization of macrophages toward an immunosuppressive M2 phenotype and recruitment of regulatory T cells (Treg), mediated by IL-10, TGF-β, M-CSF, and IL-35. These factors inhibit cytotoxic CD8 T cell responses and foster a pro-tumor immune landscape effectiveness of immune checkpoint inhibitors (ICIs), reducing therapeutic efficacy.

progression by activating key signaling cascades such as PI3K/Akt and TGF-β/Smad, and stabilizing angiogenic mRNAs. These insights point to exosomal cargo as both a biomarker source and a promising target for anti-angiogenic intervention.

3.3. Metastasis and Epithelial-Mesenchymal Transition (EMT)

EMT is a dynamic cellular program by which epithelial cells lose their polarity and cell–cell adhesion, gaining mesenchymal properties such as motility and invasiveness (Figure 8) [119]. This transition is a key facilitator of cancer metastasis, enabling tumor cells to dissociate from the primary site, invade surrounding tissues, and eventually colonize distant organs.

Recent studies have identified exosomal miR-106b-3p as a potent EMT inducer in HCC. miR-106b-3p, encapsulated within exosomes secreted by HCC cells, targets PTEN in neighboring epithelial cells, thereby activating the PI3K/Akt signaling cascade, a critical pathway promoting EMT. Functional consequences include downregulation of E-cadherin and upregulation of mesenchymal markers such as vimentin and N-cadherin, along with enhanced migratory potential and metastatic competence of recipient cells [120].

In CRC, tumor-secreted exosomes enriched with lncRNA HNF1A-AS1 have been shown to promote EMT by sponging miR-124, thereby de-repressing Slug, a key EMT transcription factor [121]. In vitro, HNF1A-AS1-containing exosomes were

sufficient to induce spindle-like morphology and enhanced invasion in epithelial cells [122,123]. In vivo, preconditioning with these exosomes significantly increased metastatic burden in xenograft models [124].

Similarly, exosomal circ_0020256 derived from cholangiocarcinoma cells was demonstrated to promote EMT via the KLF4/TGF-β1 axis [125]. In another study, exosomal circ_0084003, derived from PDAC cells, has been shown to facilitate epithelial-mesenchymal transition (EMT) and glycolytic reprogramming through modulation of the miR-143-3p/DNMT3A signaling axis [126]. Acting as a competing endogenous RNA, circ_0084003 sequesters miR-143-3p, thereby alleviating its suppressive effect on DNMT3A, a DNA methyltransferase implicated in epigenetic reprogramming and oncogenic progression [126]. This interaction promotes mesenchymal characteristics in recipient cells, evidenced by increased expression of EMT-related transcription factors and metabolic alterations favorable to tumor progression.

In the context of ovarian cancer, exosomal miR-99a-5p derived from epithelial ovarian cancer cells has been shown to promote peritoneal dissemination by modulating the tumor microenvironment. Upon transfer to human peritoneal mesothelial cells, miR-99a-5p upregulates the expression of extracellular matrix proteins fibronectin and vitronectin, facilitating cancer cell invasion [127]. These findings suggest a role for exosomal miR-99a-5p in enhancing the invasive potential of ovarian cancer cells through alterations in the peritoneal milieu.
In glioblastoma, TGF-β1 has been shown to induce the expression and secretion of miR-21 via the Smad3 signaling pathway in glioma cells [128]. This interaction suggests a regulatory mechanism where TGF-β1 influences miR-21 levels, potentially affecting the tumor microenvironment. Additionally, exosomes derived from glioblastoma-associated macrophages are enriched with miR-21, which can enhance the tumorigenic properties of GBM cells by targeting tumor suppressor genes such as PDCD4 [129]. These findings highlight the complex interplay between TGF-β1, miR-21, and the glioblastoma microenvironment

Emerging evidence also highlights the role of exosomal integrins in organ-specific metastasis. For instance, tumor-derived exosomes enriched in integrins α6β4 and α6β1 have been shown to preferentially home to the lungs, where they interact with resident cells such as fibroblasts and epithelial cells. This interaction activates Src phosphorylation and induces the expression of pro-inflammatory S100 genes, contributing to the formation of a pre-metastatic niche conducive to lung metastasis [130].

In melanoma, the protein TIPE (TNFAIP8) has been shown to interact with PKM2, promoting glycolysis and activating HIF-1α, which contributes to tumor progression and the enhancement of cancer stem cell-like properties [131]. Additionally, small EVs have been reported to carry glycolysis-related proteins such as PKM2 and GLUT1, which can be transferred to recipient cells, potentially enhancing glycolytic activity and contributing to tumor progression [132].

These studies emphasize that exosome-mediated EMT is not a monolithic process but a modular reprogramming network in which diverse cargoes, from miRNAs and lncRNAs to circRNAs, integrins, and metabolic enzymes converge on key EMT drivers (PTEN/PI3K-Akt, Slug/TGF-β, KLF4, DNMT3A) and on the tumour microenvironment itself (fibronectin, vitronectin) to orchestrate invasion and niche preparation. Therapeutically, this suggests two complementary strategies. First, cargo-selective blockade, for example, antisense oligonucleotides or CRISPR-based systems targeting miR-106b-3p, HNF1A-AS1, or circ_0084003, could intercept EMT initiation at the vesicular level. Second, vesicle-guided delivery of EMT-repressive factors, such as recombinant PTEN or miR-143-3p mimics packaged into engineered exosomes, could restore epithelial traits in disseminated cells. Moreover, the lung-tropic integrin signature (α6β4/α6β1) offers a biomarker-driven window for organ-specific interception of pre-metastatic niches. Integrating high-resolution exosome profiling with single-cell EMT atlases will be essential to stratify patients by their "EMT-exosome fingerprint" and to deploy these vesicle-centric interventions precisely. Such a precision-oncology roadmap holds promise not only to stall metastasis at its molecular roots but also to convert EMT's plasticity into a therapeutic vulnerability.

4. Therapeutic Implications

4.1. Exosomes as Diagnostic and Prognostic Biomarkers

Exosomes have emerged as powerful tools for non-invasive cancer diagnosis and prognosis due to their stability in biofluids, enrichment in disease-specific cargo, and accessibility via liquid biopsy [133]. In the oncologic context, TDEs serve as dynamic biomarkers that not only signal tumor presence but also offer prognostic insights into disease progression, treatment response, and metastatic potential [134–136].

In non-small cell lung cancer, exosomal miR-23a has been identified as a robust diagnostic biomarker. Elevated levels of miR-23a in patient plasma-derived exosomes are associated with early-stage disease and were shown to promote angiogenesis via downregulation of PHD1, PHD2, and ZO-1, stabilizing HIF-1α [137]. Importantly, miR-23a levels positively correlate with tumor vascularization and inversely correlate with patient survival, making it a dual-purpose diagnostic and prognostic marker [138].

In pancreatic cancer, recent proteomic profiling of circulating exosomes revealed that exosomal GPC1, a membrane-associated heparan sulfate proteoglycan, is selectively enriched in exosomes from early and late-stage patients but absent in healthy controls. GPC1+ exosomes not only distinguished malignant from benign breast disease with high specificity and sensitivity but also predicted residual disease and relapse after chemotherapy [139].

Another compelling example comes from glioblastoma, where exosomal lncRNA SBF2-AS1 has been shown to serve as a surrogate for tumor burden and aggressiveness. SBF2-AS1 facilitates tumor proliferation by acting as a competing en-

dogenous RNA (ceRNA) for miR-151a-3p, thereby upregulating XRCC4, a gene involved in DNA damage repair. Elevated serum exosomal SBF2-AS1 levels correlate with poor overall survival and resistance to temozolomide therapy, suggesting prognostic utility and potential predictive value for therapeutic response [140].

In PDAC, exosomal circular RNA circ-PDE8A has garnered attention as a novel prognostic biomarker. Circ-PDE8A promotes tumor invasion by acting through the miR-338/MACC1/MET pathway and is significantly elevated in metastatic PDAC compared to non-metastatic cases [141]. Notably, its expression in plasma exosomes correlated with lymph node metastasis, distant metastasis, and shortened disease-free survival.

Prostate cancer diagnostics also benefit from exosome-based biomarkers. The exosomal gene fusion transcript TMPRSS2:ERG, released into urine, has shown high specificity for prostate cancer detection, particularly in patients with PSA levels in the diagnostic grey zone (4–10 ng/mL). Moreover, exosomal expression levels of PCA3 and ERG are now used in risk calculators to guide biopsy decisions [142].

Beyond nucleic acids, protein-based exosomal biomarkers have shown promise as well. In CRC, plasma exosomes enriched with TSPAN8 and LGALS3BP have been shown to stratify patients by metastatic risk. Their presence correlated with liver metastasis and poor prognosis [143,144]. Mechanistically, TSPAN8 facilitates extracellular matrix degradation and migration, while LGALS3BP promotes immune evasion [145,146].

Recent advances in microfluidics and sequencing technologies have enabled the development of single-exosome RNA-sequencing approaches, allowing researchers to dissect the heterogeneity of exosomal cargo with unprecedented resolution [147–149]. One such approach is the ExoView platform, which enables multiplexed profiling of individual exosomes based on surface protein markers and nucleic acid content. This technology has revealed that tumor-derived exosomes exhibit marked cargo heterogeneity depending on their cellular origin, with selective enrichment of some molecules in specific vesicle subsets [150].

Taken together, Biomarkers Exosomes offer promise as biomarkers due to their stability in biofluids and disease-specific cargo enrichment. However, biomarker validation processes, such as sensitivity, specificity, and clinical applicability, are insufficiently addressed. Recent clinical trials (Table 1) demonstrate potential but highlight substantial translational hurdles [151].

4.2 Engineered Exosomes as Drug Delivery Vehicles, Current Challenges and Clinical Trials

Exosomes have emerged as a next-generation platform for targeted drug delivery, owing to their intrinsic biocompatibility, immune evasiveness, ability to cross biological barriers (including the blood–brain barrier), and natural tropism toward recipient cells [152]. Engineered exosomes, either modified at the parental cell level or functionalized post-isolation, are now being developed as precision tools for delivering chemotherapeutics, nucleic acids, proteins, and gene-editing molecules to specific tissues and tumor microenvironments [153].

One of the most promising strategies involves genetic engineering of donor cells to package therapeutic cargo into exosomes. For example, mesenchymal stem cells have been engineered to express miR-124, a tumor-suppressive microRNA, which is selectively packaged into exosomes [154]. When administered in glioblastoma models, these exosomes effectively suppressed tumor growth by targeting the STAT3 signaling pathway and reversing immunosuppressive cytokine profiles [155]. In another application, exosomes derived from HEK293 cells were engineered to load CRISPR-Cas9 components targeting $KRAS^{G12D}$. These exosomes, modified to display a CD47 "don't eat me" signal on their surface, demonstrated efficient tumor accumulation and KRAS knockdown in pancreatic cancer xenografts with minimal immune clearance [156].

Surface functionalization strategies have been pivotal in enhancing target specificity [157,158]. One widely adopted method includes conjugating ligands such as GE11 peptide (which binds to EGFR) onto the exosomal membrane [159]. In triple-negative breast cancer models, GE11-modified exosomes loaded with doxorubicin showed significantly higher tumor accumulation and cytotoxicity compared to non-modified exosomes, while sparing normal tissues [160]. Similarly, transferrin receptor-targeted exosomes have been developed to deliver siRNAs across the blood-brain barrier in glioma, demonstrating the feasibility of non-invasive central nervous system drug delivery [161].

Endogenous cargo loading via electroporation or sonication has also allowed for the successful encapsulation of hydrophobic chemotherapeutics such as paclitaxel and curcumin [162–164]. For instance, paclitaxel-loaded macrophage-derived exosomes demonstrated superior bioavailability and reduced systemic toxicity in lung metastasis models compared to free drug administration [165]. These exosomes also exhibited preferential accumulation in inflamed and metastatic tissues due to macrophage-derived homing signals.

Exosomes have also been adapted as vehicles for RNA interference-based therapies [166,167]. In HCC, exosomes modified to display ApoA1 peptide, targeting the SR-BI, successfully delivered siRNA against PLK1, a key mitotic kinase, resulting in robust tumor suppression and prolonged survival in orthotopic HCC models [168].

Recently, hybrid exosome systems that combine synthetic nanocarriers and exosomal membranes have been introduced [169–171]. These "exosome-mimetic" vesicles are created by fusing natural exosome membranes with liposomes or polymeric nanoparticles, offering the stability and high drug-loading capacity of synthetic systems while retaining the targeting capability and stealth properties of exosomes [172]. In melanoma models, engineered exosome systems loaded with STING agonists, such as 2',3'-cGAMP, have been shown to modulate the tumor microenvironment by promoting dendritic cell maturation and robust $CD8^+$ T cell activation, leading to sup-

Table 1. Leading clinical trials focusing on exosomes either as therapeutic targets or diagnostic/prognostic biomarkers.

Clinical Study ID	Treatment	Condition	Phase
-NCT01294072	-Curcumin conjugated with plant exosomes, curcumin	-Colon Cancer, Colorectal Cancer	N/A
-NCT06654622	-Exosome-based liquid biopsy signature	-Stage II-III colorectal cancer	N/A
-NCT06342440	-DENEB (DEtection of colorectal NEoplasias in Blood), A panel of circulating microRNA, whose expression level is tested in cell-free and exosome-derived samples.	-Colon Cancer, Hemorrhoids, Adenocarcinoma, Colorectal Cancer, Colon Cancer; Rectal Cancer, Colon Polyps, Rectal Cancer, Neoplasms, Polyps, Cancer	N/A
-NCT06919380	-MSC-exos Nebulization Therapy	-Lupus, Collagen Vascular Diseases, Idiopathic Inflammatory Myopathies, Connective Tissue Diseases, Myositis	I
-NCT03608631	-Mesenchymal Stromal Cells-derived Exosomes with KRAS G12D siRNA	-Metastatic Pancreatic Adenocarcinoma, Pancreatic Ductal Adenocarcinoma, Stage IV Pancreatic Cancer AJCC v8	I
-NCT01159288	-Dex2	-Non Small Cell Lung Cancer	II
-NCT06536712	-Mesenchymal Stem Cells Derived Exosomes	-Rectal Cancer	I
-NCT01668849	-Grape exosomes	-Mucositis, Canker Sores, Human Papilloma Virus (Hpv), Head And Neck Cancer, Nasopharyngeal Cancer	I
-NCT06245746	-umbilical cord derived mesenchymal stem cells exosomes (UCMSC-Exo)	-Leukemia, Dysfunctional Uterine Bleeding, Anemia, Neutropenia, White Cell Disorders, Acute Myeloid Leukemia, Platelet Disorders, Thrombosis	I
-NCT05375604	-exoASO-STAT6	-Carcinoma, Neoplasms, Stomach Cancer, Gastric Cancer, Digestive System Neoplasms, Liver Cancer, Primary Biliary Cholangitis, Advanced Hepatocellular Carcinoma (HCC)	I
-NCT05286684	-Exo-LCR	-Breast Cancer, Meningitis, Cancer	N/A
-NCT05625529	-ExoVerita™	-Digestive System Neoplasms, Pancreatic Cancer	N/A
-NCT06116903	-ExoGLIE, Blood sampling	-Neurofibromatosis, Brain Cancer, Cancer/tumors, Brain Tumor, Astrocytoma, Cancer	N/A
-NCT02977468	-Merck 3475 Pembrolizumab	-Breast Cancer	I
-NCT02507583	-IGF-1R/AS ODN	-Neoplasms, Cancer, Brain Tumor, Neurofibromatosis, Gliomas, Brain Cancer, Glioblastoma Multiforme, Cancer/tumors, Astrocytoma	I
-NCT05563766	-Itraconazole	-Carcinoma, Squamous Cell Carcinoma, Esophageal Cancer, Esophageal Disorders, Digestive System Neoplasms, Adenocarcinoma	II
-NCT02535247	-MK-3475, Copanlisib	-Non-hodgkin's Lymphoma, Lymphoma	I/II
-NCT05698524	-PCI 24781, Temozolomide	-Neurofibromatosis, Cancer/tumors, Gliomas, Glioblastoma Multiforme, Astrocytoma, Brain Cancer, Oligodendroglioma, Brain Tumor, Cancer	I
-NCT02892734	-Ipilimumab, Nivolumab	-Carcinoma, Stage IV Inflammatory Breast Carcinoma	II
-NCT03228277	-Olmutinib	-Non Small Cell Lung Cancer	II
-NCT03824275	-18F- DCFPyL PET/CT	-Prostate Cancer, Early, Recurrent, Urologic Cancer, Prostate Disorders, Prostate Cancer	II/III
-NCT03927898	-Toripalimab	-Metastatic Cancer, Colorectal Cancer	II
-NCT05775146	-Stereotactic body radiation treatment (SBRT)	-Colorectal Cancer, Colon Cancer; Rectal Cancer, Colon Cancer, Rectal Cancer, Neoplasm Metastasis, Cancer	II
-NCT05864534	-Liposomal Doxorubicin, Balstilimab, Sonoclo-ud-9 (SC-9), Botensilimab	-Gliomas, Astrocytoma, Glioblastoma Multiforme	II
-NCT03537599	-Daratumumab	-Minimal Residual Disease, Leukemia	I/II
-NCT06894225	-ACT001	-Gliomas, Astrocytoma	II
-NCT02507583	IGF-1R/AS ODN	-Malignant Glioma, Neoplasms	I

pressed tumor growth and metastasis [173]. Similarly, cationic liposomes encapsulating STING agonists demonstrated synergistic effects when administered alongside anti-PD-1 antibodies, overcoming resistance in poorly immunogenic tumors by enhancing NK cell activation and boosting the overall immunogenicity of the tumor [174]. Additionally, novel nanotherapeutic platforms, including carbon-dot-based PROTACs, have been designed to induce proteasomal degradation of PD-L1 while simultaneously stimulating STING signaling, resulting in the near-complete ablation of PD-L1 expression and a marked increase in cytotoxic T lymphocyte infiltration [175].

Efforts to scale exosome production and ensure quality control have led to the development of bioreactor-based exosome manufacturing systems, alongside improved purification protocols such as tangential flow filtration and immunoaffinity capture [176,177]. Notably, several engineered exosome platforms, including exoIL-12, exoSTING™, and exoASO-STAT6, are currently undergoing early-phase clinical trials in solid tumors and hematologic malignancies [178].

Despite the remarkable progress in understanding the biological roles and therapeutic potential of exosomes, several critical challenges remain that hinder their full clinical translation. These limitations span across exosome isolation and characterization, large-scale production, heterogeneity in cargo content, targeting specificity, safety concerns, and regulatory standardization [179–181].

Although the International Society for Extracellular Vesicles has proposed a set of guidelines for the handling and analysis of exosome preparations, a universally accepted gold-standard method for their isolation and purification remains absent, thereby limiting reproducibility and comparability across studies [182]. Moreover, exosome populations are highly heterogeneous in size and composition, even within the same biological sample, posing difficulties in defining exosome subsets with consistent therapeutic or diagnostic utility [183].

Cargo heterogeneity and selective loading represent another unresolved issue. Although methods like electroporation, transfection, or endogenous engineering have shown promise, they often suffer from limited loading efficiency or cargo degradation [184]. In particular, delivering large molecules such as CRISPR-Cas9 or mRNA remains a major hurdle due to exosomal membrane rigidity and endosomal escape inefficiencies [185,186]. Moreover, unintended off-target effects and the potential for transferring oncogenic material raise significant biosafety concerns [187].

Targeting specificity of exosomes in vivo remains suboptimal. While ligand conjugation and membrane engineering have improved tropism toward certain tissues, most exosomes are still sequestered by the liver, spleen, and mononuclear phagocyte system upon systemic administration [188]. Strategies such as CD47 modification and membrane cloaking are being explored to prolong circulation time and enhance tumor-specific delivery, yet these remain largely experimental [189,190].

From a regulatory perspective, exosomes present a classification dilemma, straddling the boundaries of biologics, cell therapies, and drug delivery systems. The Food and Drug Administration (FDA) and European Medicines Agency (EMA) have yet to establish universal guidelines for exosome-based therapeutics, particularly regarding quality control, batch-to-batch consistency, and long-term safety assessments [191]. Additionally, exosomes derived from tumor cells or stem cells must undergo rigorous safety profiling to exclude risks of immunogenicity, mutagenicity, or unintentional tissue remodeling.

Conclusion

Despite the remarkable advances in understanding exosome biology within the TME, significant knowledge gaps persist that hinder complete clinical translation. A critical unmet need lies in standardizing exosome isolation, quantification, and cargo profiling methodologies. Current techniques such as ultracentrifugation, size-exclusion chromatography, and immunoaffinity capture vary in efficiency and yield, complicating cross-study comparisons and data reproducibility.

Furthermore, the mechanisms governing selective cargo loading into exosomes remain incompletely understood. Elucidating how specific RNAs or proteins are packaged, potentially through RNA-binding proteins, lipid rafts, or sorting motifs, will be essential for optimizing diagnostic and therapeutic applications. In parallel, the spatiotemporal dynamics of exosome-mediated signaling within the TME require further investigation, particularly in the context of immune evasion, stromal remodeling, and therapeutic resistance.

From a translational perspective, advancing exosome engineering techniques holds considerable promise. Synthetic biology approaches that enable the programmable design of exosome-producing cells, combined with targeted surface modifications and controlled cargo delivery systems, could redefine precision oncology paradigms. In addition, multi-omics integration may provide unprecedented resolution in exosome-based biomarker discovery.

Looking ahead, exosomes are poised to transition from biological curiosities to central elements in the next generation of cancer diagnostics and therapeutics. Their ability to bridge communication between tumor and host, coupled with their adaptability as nanoscale carriers, renders them uniquely powerful in the landscape of targeted therapy. However, collaborative efforts between molecular biologists, clinicians, bioengineers, and regulatory bodies are essential to unlock their full potential.

In conclusion, exosomal signaling constitutes not only a promising biomarker platform but also a biologically active conduit that plays a crucial role in modulating the tumour microenvironment. Far from being mere indicators of disease state, exosomes are increasingly recognised as functional mediators of intercellular communication, with the capacity to influence tumour progression, immune evasion, and therapeutic resistance. As our understanding of their multifaceted roles

deepens, exosomes are poised to become integral components of precision oncology strategies.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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Abbreviations:
ALIX: ALG-2 interacting protein X
AML: acute myeloid leukemia
CAFs: cancer-associated fibroblasts
CRC: colorectal cancer
DAMPs: damage-associated molecular patterns
ECM: extracellular matrix
EMA: European Medicines Agency
EMT: epithelial–mesenchymal transition
ESCRT: Endosomal Sorting Complex Required for Transport
EVs: extracellular vesicles
FAK-Src: Focal adhesion kinase-steroid receptor coactivator
FasL: Fas Ligand
FDA: Food and Drug Administration
HCC: hepatocellular carcinoma
HUVECs: human umbilical vein endothelial cells
ICD: immunogenic cell death
ILVs: intraluminal vesicles
LMP1: Epstein–Barr virus latent membrane protein 1
MDSC: myeloid-derived suppressor cells
MM: Multiple Myeloma
MVBs: multivesicular bodies
NK: Natural killer
NKG2D: natural killer group 2 member D
PDAC: pancreatic ductal adenocarcinoma
PD-L1: programmed death-ligand 1
PS:phosphatidylserine
TAMs: tumor-associated macrophages
TCR: T cell receptor
TDEs: tumor-derived exosomes
TME: Tumor microenvironment
TRAIL: TNF-related apoptosis-inducing ligand
TSG101: tumour susceptibility gene 101 protein
VPS28: vacuolar protein-sorting 28

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