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REVIEW

Polarization-Specific Macrophage-Derived Extracellular Vesicles: Molecular Cargo, Tumor Microenvironment Remodeling, and Therapeutic Opportunities

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Video abstract

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Abstract: Extracellular vesicles (EVs) derived from macrophages have emerged as critical regulators of tumor progression by functioning as polarization-dependent carriers of bioactive molecular information. Rather than acting as passive byproducts, macrophage-derived EVs reflect the activation state of their parent cells and actively reprogram tumor behavior and the tumor microenvironment. In this review, we propose a conceptual framework in which macrophage-derived EVs serve as information hubs that link macrophage polarization, selective cargo loading, and coordinated modulation of tumor and immune cell phenotypes. EVs released from classically activated (M1) macrophages predominantly convey tumor-suppressive signals, including specific noncoding RNAs and immunomodulatory proteins, thereby inhibiting tumor proliferation, invasion, immune evasion, and therapeutic resistance while reinforcing anti-tumor immunity. In contrast, EVs derived from alternatively activated (M2) macrophages deliver a coherent pro-tumor program that integrates epithelial–mesenchymal transition, metabolic reprogramming, stemness maintenance, ferroptosis resistance, immune suppression, and therapy tolerance across multiple cancer types. We systematically summarize the emerging mechanisms governing polarization-dependent cargo selection, including RNA-binding protein–mediated sorting, metabolic and signaling pathway control, and EV biogenesis regulation. In addition, this review highlights the translational implications of macrophage-derived EVs as engineering-ready platforms. We discuss strategies to enhance the therapeutic utility of M1 EVs through cargo engineering and surface functionalization, as well as approaches to disrupt, reprogram, or selectively block M2 EV–mediated oncogenic information flow. Collectively, this work advances a unifying molecular and translational perspective, positioning macrophage-derived EVs as actionable targets and tools for precision modulation of the tumor microenvironment in cancer diagnosis and therapy.

Keywords: extracellular vesicles, exosomes, macrophages, TAM, tumor

Introduction

EVs are membrane-bound nanoparticles released by virtually all cell types and function as essential mediators of intercellular communication in both physiological and pathological contexts. Within tumors, EVs participate in the bidirectional exchange of proteins, nucleic acids, lipids, and metabolites, thereby shaping cancer cell behavior, immune responses, and stromal remodeling. As the complexity of EV biogenesis, cargo selection, and functional heterogeneity has become increasingly apparent, EVs are now recognized not merely as passive byproducts of cellular activity, but as dynamic conveyors of context-specific biological information. Macrophages represent one of the most abundant and plastic immune cell populations within the tumor microenvironment (TME). Rather than existing as discrete activation

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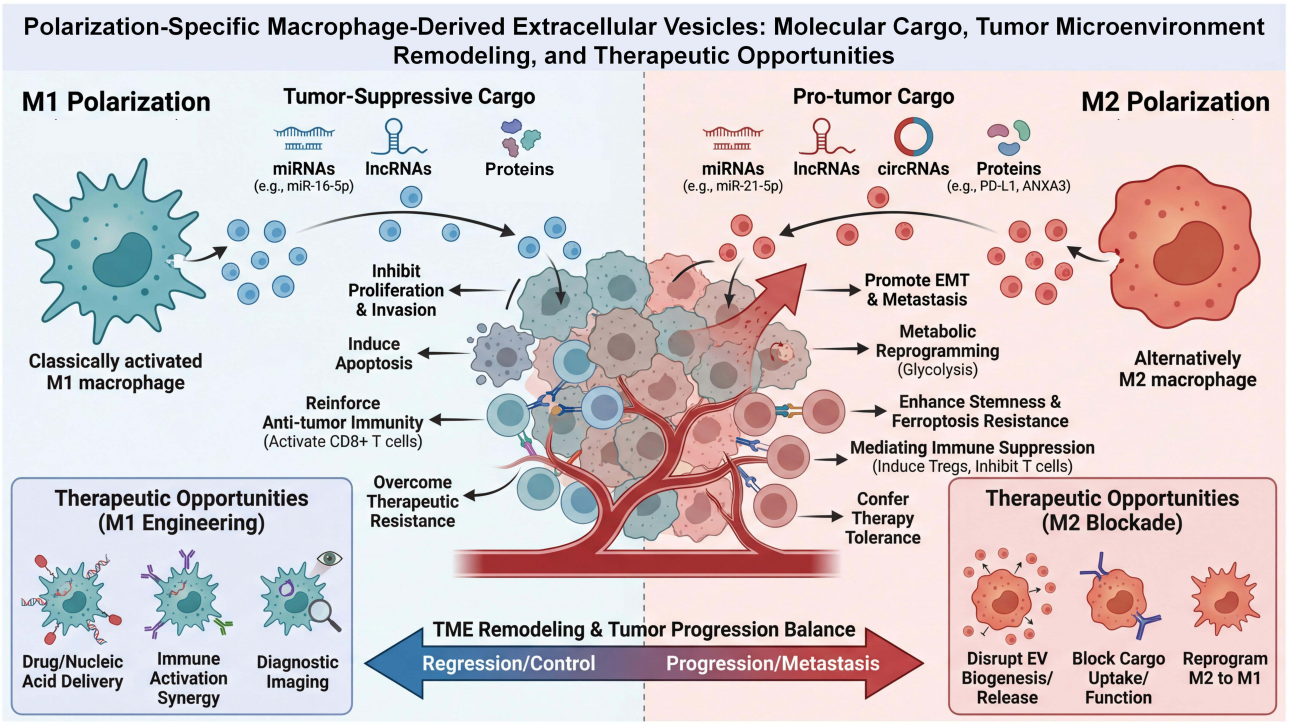
Graphical Abstract



states, tumor-associated macrophages (TAMs) occupy a continuous spectrum of functional phenotypes shaped by cytokine gradients, metabolic constraints, hypoxia, and cell–cell interactions. While the classical M1/M2 framework has provided a useful conceptual entry point, it incompletely captures the spatial, temporal, and functional heterogeneity of macrophages in vivo. Importantly, this plasticity raises a fundamental question: how are diverse macrophage activation states translated into stable, transferable signals capable of coordinating multicellular tumor ecosystems? Accumulating evidence suggests that macrophage-derived EVs may serve as a critical molecular interface between macrophage functional states and tumor progression. EV cargo profiles partially reflect the activation status of donor macrophages, yet remain sufficiently stable to exert sustained effects on recipient tumor, immune, and stromal cells. However, key knowledge gaps remain unresolved. It is unclear how macrophage functional diversity is encoded into EV cargo composition, whether distinct EV populations represent discrete or overlapping macrophage states, and how these vesicular signals integrate across multiple cellular compartments to drive tumor evolution.

In this review, we synthesize recent advances in the biology of macrophage-derived EVs with a focus on cargo selection mechanisms, functional consequences, and translational opportunities. While M1- and M2-like states are used as operational reference points for clarity, we emphasize their dynamic and context-dependent nature. We further propose a unifying framework in which macrophage-derived EVs act as information hubs that transmit, amplify, or reprogram immune and tumor signals within the TME. By highlighting both mechanistic insights and unresolved questions, this review aims to move beyond descriptive classification and provide a conceptual basis for precision modulation of EV-mediated communication in cancer.

Classification and Biogenesis of Extracellular Vesicles

EVs are nanoscale entities enveloped by lipid bilayer membranes, secreted by cells, and actively engaged in diverse modes of intercellular communication as well as a broad spectrum of pathophysiological processes. Owing to their pronounced heterogeneity and the technical challenges surrounding their isolation and characterization, the International

Society for Extracellular Vesicles (ISEV), in its MISEV2018 and latest MISEV2023 guidelines, underscores the importance of employing operational and feature-based descriptions rather than relying solely on nomenclature such as "exosome" or "microvesicle". The guidelines further recommend a multifactorial profiling approach to elucidate the origin and properties of distinct EV subtypes.[1,2]

Classification and Nomenclature Paradigm

Operational Classification: The guidelines advocate descriptions based on physical and biochemical parameters, including size and density (eg, small EVs, sEVs; medium/large EVs, m/lEVs), isolation methods (ultracentrifugation, density gradient, SEC, etc)., biochemical markers (transmembrane/membrane-associated proteins, cytosolic proteins), and inferred biogenetic pathways (endosomal/multivesicular body-derived versus plasma membrane budding versus apoptosis-associated). Such composite labeling should be favored over simplistic size-based definitions or reliance on a single marker to designate "exosomes" or "microvesicles".[1,2]

Conventional Terms and Cautionary Use: Classically, vesicles formed within multivesicular bodies (MVBs) and released upon fusion with the plasma membrane have been termed "exosomes", whereas those arising directly from plasma membrane budding are designated "microvesicles/microparticles". Apoptotic bodies generated during programmed cell death also belong to the EV family. However, numerous reviews have highlighted the limitations in separation purity and marker specificity, which preclude definitive functional attribution to a single named subtype. Hence, more prudent terminology, such as "sEVs" or "plasma membrane-origin EVs", is advised.[1,3,4]

Biogenesis Mechanisms

Endosomal Pathway-Derived Small EVs (Commonly Overlapping with Exosome-Enriched Populations)

Small EVs originate from intraluminal vesicle (ILV) formation within late endosomes and the subsequent maturation of MVBs. The canonical mechanism encompasses the ESCRT cascade (ESCRT-0/I/II/III) and associated adaptor proteins such as TSG101 and ALIX, which govern membrane invagination and cargo sorting. Parallel ESCRT-independent routes have been described, notably those relying on ceramide and membrane microdomains enriched in lipid rafts/tetraspanins (CD9, CD63, CD81), which promote ILV assembly and cargo incorporation.[3–5] Protein and nucleic acid sorting is intricately linked to endosomal dynamics, involving ubiquitination, ligand–receptor internalization, and motif recognition within cytosolic tails of transmembrane proteins. Fusion of MVBs with the plasma membrane to release ILVs as small EVs constitutes a pivotal event, orchestrated by Rab family small GTPases and the SNARE machinery—for instance, the Rab27 subfamily mediates MVB docking and secretion, while other members such as Rab11 and Rab35 contribute to endosomal trafficking in cell-type- and pathway-specific contexts. The fate determination between lysosomal degradation of MVBs and their secretion at the plasma membrane ultimately shapes both the quantity and molecular composition of released EVs (Figure 1).[3–5]

EVs Budding from the Plasma Membrane (Microvesicles/Ectosomes, Often Classified as m/lEVs)

These vesicles bud directly from the plasma membrane and are released. This process is often associated with changes in membrane lipid asymmetry (phospholipid flipping/scrambling), local membrane curvature, and cortical cytoskeleton remodeling. Ca2+ signaling, actin-myosin tension, and small GTPase pathways are involved in regulation across different cell types, determining the budding site and the loaded cargo. Their membrane protein profile is closer to that of the plasma membrane, and the vesicles contain richer cytoplasmic components.[3,4] Microvesicles have a wider size range and diverse composition. Their functions involve coagulation, immune regulation, and tumor microenvironment remodeling. However, due to difficulties in isolation purity and characterization, functional attribution must be cautious and adhere to the evidence requirements set by MISEV (Minimal Information for Studies of Extracellular Vesicles).[1,3]

Apoptosis-Related EVs (Apoptotic Bodies, Etc)

During programmed cell death, membrane blebbing occurs, and the cytoplasm and nuclear contents fragment, forming larger apoptotic bodies and other apoptosis-related EVs, which often contain organelle fragments and nucleic acids. They

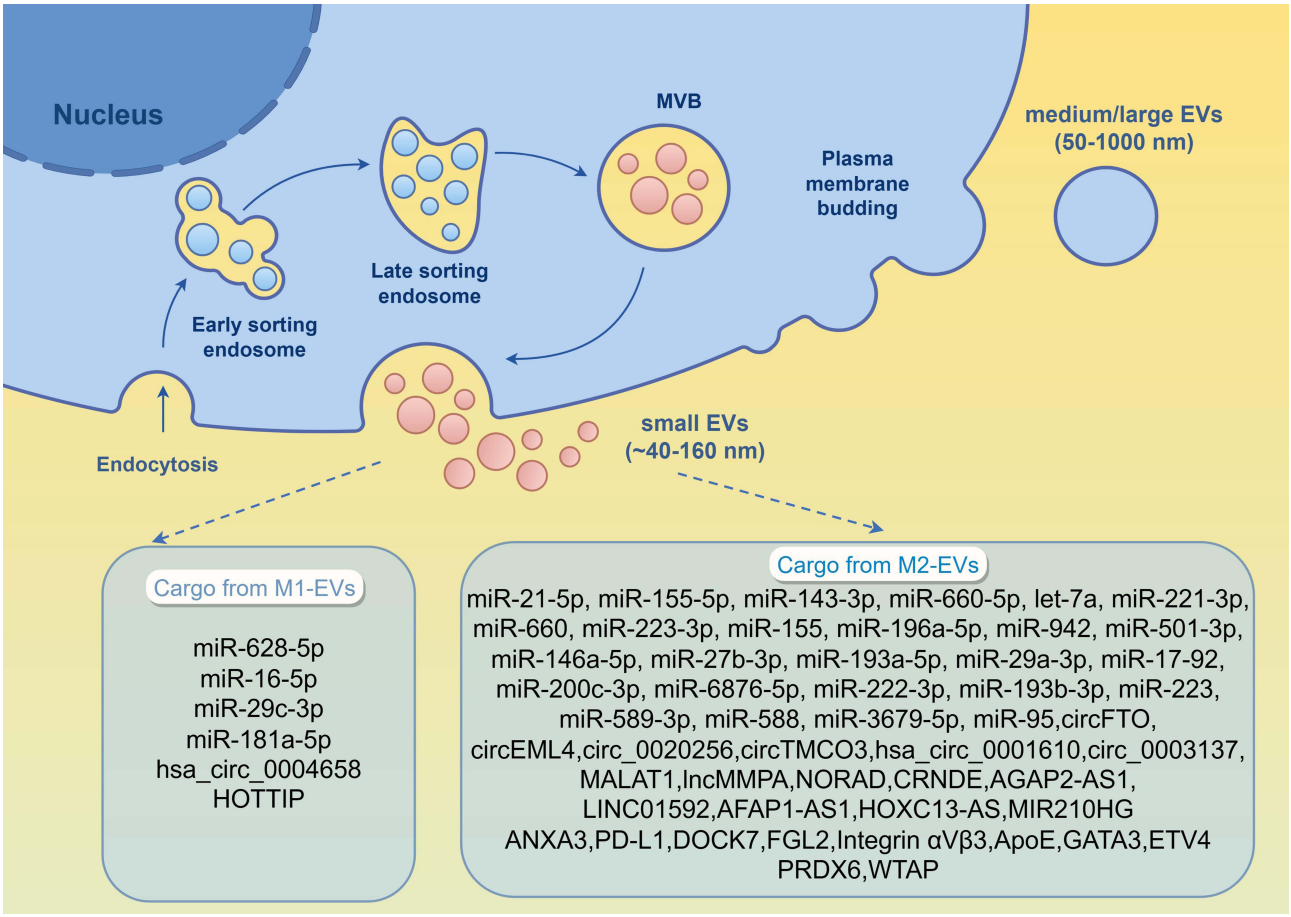


Figure 1 Generation Pathways and Molecular Cargo of Macrophage-Derived Extracellular Vesicles.

play roles in clearing cellular debris, immune tolerance, and tissue homeostasis, but they differ significantly from sEVs/mEVs in terms of composition and function.[6]

Cargo Loading Mechanism of Extracellular Vesicles from Macrophages

Cargo Loading Clues for EVs Derived from M1 Macrophages

The mechanism by which M1 macrophage-derived EVs load anti-tumor cargo (such as specific miRNAs and lncRNAs) is not yet fully elucidated regarding direct sorting by the ESCRT complex or RNA-binding proteins (RBPs). However, several studies offer crucial clues, mainly suggesting that the cargo expression level within the donor cell directly drives EV loading, and that the M1 polarization state itself confers an inherent enrichment preference for specific anti-tumor molecules.

Direct Driving Force of Donor Cell Expression Levels

Studies have shown that the expression level of non-coding RNA within donor M1 macrophages directly affects the amount loaded into their EVs. For example, in research concerning head and neck squamous cell carcinoma, lncRNA HOTTIP is significantly upregulated in M1 macrophages. When HOTTIP is overexpressed or knocked down in M1 macrophages, the content of HOTTIP in their EVs increases or decreases proportionally. This suggests that the EV loading of HOTTIP is largely driven by its expression level within the donor cell.[7] This indicates that EV cargo loading is not entirely random but is closely related to the abundance of endogenous molecules within the cell.

Selective Enrichment of Specific miRNAs by the M1 Polarization State

Multiple studies have observed that EVs from M1 polarized macrophages specifically enrich certain anti-tumor miRNAs compared to M0 or M2 macrophages. For example, M1 macrophage-derived EVs are rich in miR-16-5p, a miRNA that can inhibit PD-L1 expression in gastric cancer cells.[8] Similarly, M1 macrophage EVs have been found to enrich miR-181a-5p in lung adenocarcinoma[9] and miR-29c-3p in melanoma.[10] Although these studies did not directly elucidate how miRNAs like miR-16-5p are loaded, the upregulation of their expression during M1 polarization and their high abundance in EVs indirectly suggest the existence of a polarization-state-dependent selective packaging mechanism. This mechanism might involve the expression or activation of specific RNA-binding proteins (RBPs) during M1 polarization. These RBPs recognize specific miRNA sequences and guide them into intraluminal vesicles (ILVs) within multivesicular bodies (MVBs).

Upstream Regulation Enhancing Cargo Loading

In studies of hepatocellular carcinoma (HCC), driving macrophage M1-like activation by upregulating RBPJ resulted in a significant increase in hsa_circ_0004658 levels in their EVs. Furthermore, the enrichment level in the EVs was approximately 3.8 times higher than in the producing cells.[11] This provides evidence that M1 polarization, mediated by an upstream transcription factor (RBPJ), affects intracellular miRNA/circRNA expression, which subsequently influences the selective loading of EV cargo. This indicates that the EV cargo profile is plastic and can be "customized" by regulating the state of the donor cell.

Cargo Loading Mechanisms in M2 Macrophage-Derived EVs

Research into the loading mechanisms of pro-tumorigenic cargo (miRNA, circRNA, lncRNA, and various proteins) carried by M2 macrophage EVs is more advanced, and has begun to touch upon RNA-binding protein (RBP) sorting, post-translational modifications, and the direct influence of donor cell metabolism/signaling pathways on cargo packaging.

RNA-Binding Protein (RBP)-Mediated miRNA Sorting

In studies concerning HCC,[12] miR-23a-3p within M2 macrophage-derived EVs was confirmed to be loaded into the EVs via a selective sorting mechanism mediated by the RNA-binding protein (RBP) hnRNPA2B1. Specifically, the expression of hnRNPA2B1 is upregulated in M2 macrophages. By recognizing a specific motif on miR-23a-3p, hnRNPA2B1 efficiently sorts miR-23a-3p into the EVs. The elucidation of this sorting mechanism represents a significant advance in the field of M2-EV cargo sorting, defining hnRNPA2B1's central role as a miRNA sorter in the pro-oncogenic actions of M2-EVs.

M2 Polarization and the Driving of Loading by Metabolic/Signaling Pathways

The polarization state of M2 macrophages itself, along with the resulting changes in intracellular metabolism and signaling pathways, can directly influence EV cargo loading. For instance, in laryngeal cancer research, the enrichment of the WTAP protein in tumor-associated macrophage (TAM)-derived EVs is associated with the reprogramming of aerobic glycolysis in tumor cells.[13] As an important component of the m6A methylation complex, the high level of WTAP in EVs may suggest the preferential packaging of m6A-related proteins by M2 macrophages during polarization, thereby affecting the stability or translation of mRNA in recipient cells.

Impact of Post-Translational Modifications on Protein Cargo Loading

In studies concerning protein cargo within M2 macrophage-derived EVs, evidence suggests a potential role for post-translational modifications (PTMs) in cargo loading. For instance, in laryngeal squamous cell carcinoma, ANXA3 activates the AKT–GSK3β–β-catenin pathway within TAMs, driving M2 polarization. The high level of ANXA3 found in EVs suggests that its loading may be influenced by its intracellular localization or modification status.[14] In fibrosarcoma, ceruloplasmin mRNA is transferred to tumor cells via EVs and translated into protein, conferring resistance to ferroptosis.[15] Although this example focuses on the transport of mRNA cargo, the functional activity of

the resulting protein and whether its packaging into EVs is regulated by specific protein modifications during EV biogenesis are areas that warrant further investigation.

Specific Regulation of EV Secretion Pathways

EV secretion is inherently a complex, multi-step process involving the ESCRT complex, small G proteins (such as the Rab family), and the ceramide pathway. The regulation of these secretion pathways by M2 macrophages indirectly influences EV cargo loading. For example, TAM reprogramming can significantly increase EV secretion, mechanistically driven by AKT phosphorylation of MADD, which subsequently activates Rab27a to enhance exosome release.[16] The activation of this secretion pathway may promote the loading and release of specific cargo (such as PD-L1), thereby enhancing immunosuppression.

In summary, cargo loading in M1 and M2 macrophage-derived EVs is a highly dynamic and precisely regulated process. Although we have preliminarily identified several key RBPs and signaling pathways, many details remain to be elucidated. Future research should focus on: 1) Constructing detailed cargo profiles of M1 and M2 EVs by combining multi-omics approaches (proteomics, transcriptomics, lipidomics) with advanced EV isolation techniques; 2) Identifying novel loading mechanisms using CRISPR/Cas9 editing, overexpression or knockdown of RBPs and EV biogenesis-related factors, combined with high-throughput screening; 3) Exploring how post-translational modifications (eg, ubiquitination, phosphorylation, methylation) influence the loading fate of proteins and non-coding RNAs; 4) Developing advanced imaging and biophysical methods to track the dynamic behavior of cargo during EV formation and loading in real-time. A deeper understanding of these mechanisms will provide a more solid theoretical foundation and more effective engineering strategies for the precision diagnosis and treatment of tumors based on macrophage EVs.

Inhibitory Effects of M1 Macrophage-Derived Extracellular Vesicles on Tumors

As a classic pro-inflammatory phenotype, M1 macrophage-derived EVs exert a dual effect— "direct tumor suppression + immune remodeling"—in various tumors by delivering specific non-coding RNAs and immunoregulatory molecules, demonstrating broad mechanistic diversity and translational potential. Notably, although M1-derived EVs are predominantly tumor-suppressive, accumulating evidence suggests that their biological effects are highly context-dependent and influenced by tumor type, inflammatory duration, and microenvironmental status. Taking HCC as an example, EVs from M1-polarized macrophages carry miR-628-5p, which blocks the m6A-related modification and function of circFUT8, thereby weakening its pro-oncogenic activity and inhibiting tumor progression. This reveals the unique advantage of cross-level regulation involving "miRNA—circRNA—epitranscriptomic modification".[17] Furthermore, from the perspective of upstream activation, pro-inflammatory macrophages driven by RBPJ overexpression produce EVs containing hsa_circ_0004658, which sponges miR-499b-5p to upregulate JAM3, significantly inhibiting HCC malignant behavior. This suggests that the tumor-suppressive efficacy of EVs can be further amplified by enhancing macrophage M1 polarization and targeted cargo loading.[11] In HNSCC, M1-derived EVs are enriched with lncRNA HOTTIP, which competitively binds to miR-19a-3p/miR-19b-3p and upregulates the TLR5/NF-κB signaling pathway. This inhibits proliferation, migration, and invasion in vitro and in vivo and induces apoptosis. Moreover, it can "re-educate" circulating monocytes and local tumor-associated macrophages toward the M1 phenotype, thereby breaking down the immunosuppressive microenvironment.[7] In gastric cancer, miR-16-5p carried by EVs from M1-polarized macrophages downregulates PD-L1 expression in tumor cells, enhancing T cell-dependent anti-tumor immunity. This indicates complementary and synergistic potential when combined with immune checkpoint inhibition strategies.[8] In melanoma, which is highly involved in immune responses, M1-derived EV miR-29c-3p targets and inhibits ENPP2, concurrently altering cholesterol metabolism and extracellular matrix remodeling. This weakens the migration and invasion capabilities of tumor cells at the metabolic-matrix level.[10] However, recent evidence indicates that in melanoma, prolonged exposure to pro-inflammatory M1-derived EVs may paradoxically induce an inflammatory and invasive tumor phenotype by activating NF-κB–dependent signaling pathways and enhancing cytokine-driven tumor cell plasticity, underscoring the dualistic role of M1-EVs in inflammation-associated cancers.[18] In lung adenocarcinoma, the key cargo miR-181a-5p from M1 macrophage EVs synergistically targets ETS1 and STK16, blocking the AKT1 survival pathway, inhibiting cell

viability, and promoting apoptosis.[9] Collectively, these findings highlight that the anti-tumor activity of M1-derived EVs is predominantly mediated by precise delivery of functional ncRNAs; nevertheless, excessive or chronic inflammatory signaling conveyed by M1-EVs may, in specific tumor contexts, favor tumor invasion and progression rather than suppression.

In summary, M1-derived EVs utilize key cargo such as miR-628-5p, miR-16-5p, miR-29c-3p, miR-181a-5p, hsa_circ_0004658, and lncRNA HOTTIP to precisely target nodes like PD-L1, ENPP2, ETS1, STK16, TLR5/NF-κB, and the m6A-related functions of circFUT8. They synergistically inhibit proliferation, migration, and invasion, induce apoptosis, and simultaneously reprogram the tumor microenvironment to enhance anti-tumor immunity. At the same time, these observations emphasize the importance of fine-tuning EV dosage, exposure duration, and cargo composition to avoid inflammation-driven tumor adaptation. These mechanisms provide a solid basis for their combined application with immunotherapy, molecular targeting, and even chemotherapy, highlighting the need for standardization and clinical validation regarding isolation and purification, cargo consistency, in vivo distribution, and safety assessment (Table 1 and Figure 2).

Tumor-Promoting Effects of Non-Coding RNAs Carried by M2 Macrophage-Derived Extracellular Vesicles

miRNAs Shape the Pro-Tumor Ecosystem via Multi-Level Signaling Axes and Intercellular Networks

Among EVs derived from M2 polarized macrophages (M2-EVs), miRNAs are the most efficient and functionally defined pro-oncogenic information carriers. Their pro-oncogenic mechanisms can be summarized as multi-level reprogramming

Table 1 Core Anti-Tumor Cargo and Mechanisms of M1 Macrophage-Derived EVs

Cargo Type	Cancer Type	Key EV Cargo from M1 Macrophages	Molecular Targets	Main Mechanism of Action	References
miRNA	HCC	miR-628-5p	circFUT8 – m6A-related modification	Blocks m6A modification and oncogenic function of circFUT8; cross-level regulation involving miRNA–circRNA–epitranscriptomic modification; inhibits tumor progression	[17]
miRNA	HCC	hsa_circ_0004658	miR-499b-5p / JAM3	Sponges miR-499b-5p to upregulate JAM3; markedly suppresses malignant behavior; RBPJ overexpression enhances M1 polarization and cargo loading	[11]
miRNA	HNSCC	lncRNA HOTTIP	miR-19a-3p / miR-19b-3p → TLR5/NF-κB pathway	Competitively binds miR-19a/b, activating TLR5/NF-κB signaling; inhibits proliferation, migration, invasion; induces apoptosis; re-educates TAMs toward M1 phenotype and disrupts immunosuppressive TME	[7]
miRNA	Gastric cancer	miR-16-5p	PD-L1	Downregulates PD-L1 in tumor cells, enhancing T cell–dependent anti-tumor immunity; potential synergy with immune checkpoint inhibitors	[8]
circRNA	Melanoma	miR-29c-3p	ENPP2 → cholesterol metabolism / ECM remodeling	Targets ENPP2, alters cholesterol metabolism and ECM remodeling; reduces migration and invasion	[10]
lncRNA	Lung adenocarcinoma	miR-181a-5p	ETS1 / STK16 → AKT1 pathway	Simultaneously targets ETS1 and STK16, blocking AKT1 survival pathway; inhibits viability and promotes apoptosis	[9]

Notes: ↑ indicates upregulation or increase; ↓ indicates downregulation or decrease; → indicates downstream functional regulation or signaling effects between the listed molecules and pathways.

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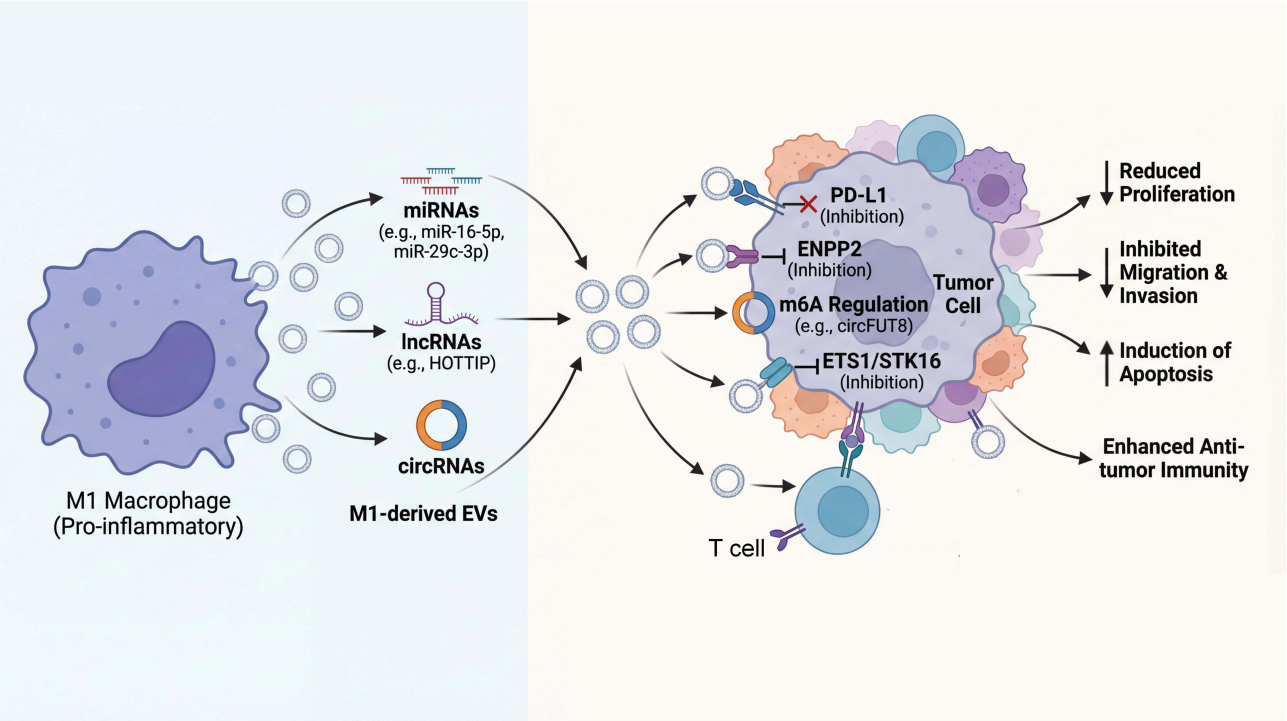

Figure 2 Functional Roles of M1 Macrophage-Derived EVs in Tumor and Immune Regulation.

revolving around four main axes: the migration, invasion, and epithelial-mesenchymal transition axis; the immunosuppression and immune evasion axis; the therapeutic resistance and drug resistance axis; and are often accompanied by the metabolic reprogramming and tumor stemness maintenance axis.

Migration, Invasion, and Epithelial-Mesenchymal Transition (EMT) Axis

Firstly, M2 macrophage-derived EVs "pave the way" for the dissemination and distant colonization of tumor cells by remodeling the vasculature and the microenvironment. In HCC, M2 macrophage-secreted EVs directly increase vascular permeability and promote metastasis, revealing the early disruption of the vascular barrier and the establishment of a pre-metastatic niche by the vesicles.[12] In models of HNSCC and LUAD, M2-EVs deliver miR-21-5p and miR-942, respectively, to endothelial cells. These miRNAs either inhibit LATS1/VHL and upregulate YAP1/HIF-1α, or target FOXO1 to relieve the suppression of β-catenin, thereby significantly enhancing angiogenesis and lumen perfusion capacity, directly boosting migration and invasion fitness.[19,20] Besides classical angiogenesis, in RCC, M2-EVs carrying miR-193a-5p downregulate TIMP2, inducing vasculogenic mimicry (VM) and enhancing invasion, suggesting that vesicles can also support metastasis through "atypical vascularization" pathways.[21] This "microenvironment and vasculature—metastatic channel" module forms the basis for subsequent reinforcement of EMT and motility programming.

Secondly, the miRNA cargo delivered by the vesicles promotes EMT, migration, and invasion through layered activation around central pathways such as PTEN/Akt, PI3K/AKT, Wnt/β-catenin, and TGF-β/BMP. Along the PTEN/Akt axis, miR-21-5p from M2-EVs in RCC targets PTEN to activate Akt, directly enhancing migration and invasion.[22] Under hypoxic conditions, miR-155-5p from M2-EVs binds to HuR, increasing IGF1R mRNA stability and activating the PI3K/AKT cascade, further exacerbating progression.[23] Along the Wnt/β-catenin axis, miR-942 from M2-EVs in LUAD targets FOXO1 to relieve β-catenin suppression, promoting migration, invasion, and angiogenesis.[20] In CRC, miR-186-5p from M2-EVs inhibits DLC1, amplifying the β-catenin signal, and enhancing proliferation and motility.[24] Along the TGF-β/BMP axis, the miR-17-92 cluster from M2-EVs in HCC strongly promotes EMT and invasion by inhibiting TGFBR2 and affecting Smurf1/ACVR1, causing an imbalance between TGF-β1/BMP-7 and the upregulation

of ID1.[25] Furthermore, androgen and adhesion/chromatin programs are also remodeled in parallel by the vesicles: In HCC, miR-92a-2-5p in EVs downregulates AR, enhancing invasion via the PHLPP/p-AKT/β-catenin axis.[26] In CRC, miR-21-5p and miR-155-5p from M2-EVs jointly inhibit BRG1, leading to a significant increase in migration and invasion,[27] while exosomal miR-183-5p from M2-EVs further reinforces the overall progressive phenotype.[28]

Thirdly, the vesicles consolidate EMT and metastatic programming through transcriptional/epigenetic and inflammatory signaling networks, forming a cross-cancer "pro-migration—pro-EMT—pro-colonization" common blueprint. In HCC, M2-EVs carrying miR-660-5p regulate KLF3 to promote tumor development,[29] while miR-6876-5p from CD63 highly expressed macrophage EVs inhibits PTEN and enhances invasion and stemness via the Akt-EMT axis, constituting a driving force for EMT—stemness interaction.[30] In breast cancer, M2-EVs' miR-660 directly enhances metastatic fitness,[31] and M2-EVs' miR-223-3p targets Cbx5 to significantly promote lung metastasis, exhibiting organ-specific dissemination.[32] In NSCLC models, M2-EVs' miR-155 and miR-196a-5p jointly enhance metastasis,[33] and M2-EVs' miR-501-3p also accelerates progression.[34] In prostate cancer, M2-EVs' miR-95 targets JunB to promote proliferation, invasion, and EMT.[35] In osteosarcoma, M2-EVs' miR-221-3p accelerates growth and metastasis via the SOCS3/JAK2/STAT3 axis,[36] and EVs-let-7a targets C15orf41 to promote metastasis, suggesting the phenomenon of "contextual pro-metastatic function of classic miRNA families".[37] In ovarian cancer, M2-EVs' miR-29a-3p and miR-589-3p accelerate overall progression through the FOXO3/PD-L1 and BCL2L13 pathways, respectively, possessing both pro-proliferative and enhanced motility effects.[38,39] Regarding pancreatic cancer, M2-EVs' miRNAs generally promote distant metastasis,[40] and M2-EVs' miR-501-3p intervenes in TGF-β signaling via TGFBR3, further driving progression and invasion.[41] It is noteworthy that in gastric cancer, M2-EVs' miR-513b-5p has been proven to be a druggable target, and traditional Chinese medicine formulas can suppress tumors by inhibiting its vesicular pathway, indirectly reflecting the critical role of this vesicular axis in promoting migration and EMT.[42]

Finally, neurological tumors provide unique evidence of EMT and its lineage transition, completing the EV-driven "invasion-shaping" map. In glioma, the loss of M2-EVs' miR-146a-5p relieves the inhibition of the TRAF6/IRAK1 complex, activating IKK/NF-κB and enhancing EMT, invasion, and migration;[43] M2-EVs' miR-27b-3p maintains stem-like properties and tumorigenicity via the MLL4/PRDM1/IL-33 axis, functionally supporting stronger invasion and colonization.[44] More broadly, M2-EVs trigger the proneural-to-mesenchymal transition (PMT) in glioma stem cells, paralleling high invasiveness and therapeutic resistance, suggesting that EMT-like lineage transition occupies a central role in EV-driven progression.[45]

Overall, the miRNA-dominated cargo of M2-derived extracellular vesicles cooperates across multiple central pathways: on one hand, they open metastatic channels through vascular permeability, angiogenesis, and VM; on the other hand, they systematically activate pathways such as PTEN/Akt, PI3K/AKT, Wnt/β-catenin, and TGF-β/BMP within tumor cells, linking transcriptional/epigenetic and inflammatory signals to drive proliferation, migration, EMT, and distant metastasis. This consistent cross-cancer blueprint suggests that blocking vesicle generation/uptake, antagonizing key miRNA cargo, or inhibiting downstream central pathways may be effective strategies to interrupt the "microenvironment paving—signaling programming—metastatic colonization" continuum.

Immunosuppression and Immune Evasion Axis

In CRC, M2-EVs are enriched with miR-155-5p. After transfer to tumor cells, this miRNA targets and downregulates the deubiquitinating factor ZC3H12B, accompanied by IL-6 upregulation and enhanced tumor cell proliferation/anti-apoptosis. This forms an inflammatory, immunosuppressive local ecosystem, thereby weakening anti-tumor immunity and promoting immune evasion.[46] This chain of "EVs-miRNA → tumor cell signaling and inflammatory factors → immunosuppressive phenotype" suggests that the intrinsic changes in tumor cells and the enhancement of immune evasion capacity are directly driven by the cargo of M2-EVs. At the level of T cell lineage, M2-EVs achieve deeper immune evasion by shaping the fate of CD4+ T cells. In epithelial ovarian cancer, EVs released by M2 macrophages transport miRNAs, leading to a significant imbalance in the Treg/Th17 ratio (Treg increase, relative Th17 decrease). This directly increases the tension of the Treg inhibitory network, thereby systematically reducing anti-tumor immune effects and promoting tumor progression.[47] This exosome-mediated T cell lineage reprogramming reflects an immune evasion pathway "beyond immune checkpoints"—by weighting the quantity and function of immunosuppressive Tregs, thereby

weakening the effector immune response. In the metastasis-associated microenvironment, macrophage EVs further consolidate the immunosuppressive landscape. Malignant pleural effusion, acting as a specialized metastatic niche, has been studied, showing that macrophage-derived EVs can directly promote the differentiation of Tregs, amplifying immunosuppressive signals and supporting tumor survival and dissemination.[48] Furthermore, studies on intracranial aneurysms provide collateral evidence for the general mechanism of "macrophage EVs miRNA remodeling the inflammatory/immune microenvironment": M2-derived EVs carry miR-155-5p, which promotes smooth muscle cell proliferation and migration by targeting the antagonistic factor GREM1, while simultaneously enhancing macrophage activation and infiltration.[49] Overall, M2-derived EVs drive immune evasion through two complementary main axes: first, "intrinsic tumor cell pathways and inflammatory factor upregulation" reinforce the tumor's resistance to immune attack; and second, "shaping the fate of immune cells", systematically increasing the breadth and intensity of immunosuppression.

Therapeutic Resistance and Survival Signaling Axis

Multiple studies have shown that M2-EVs establish a "high survival threshold" via the PI3K/AKT axis, counteracting drug-induced stress and apoptosis. In HCC, M2 macrophage EVs carrying miR-200c-3p confer primary resistance to Sorafenib, suggesting that EV cargo can directly penetrate the killing threshold of multi-target TKIs.[50] In pancreatic cancer, M2-EVs' miR-222-3p targets TSC1 and upregulates the PI3K/AKT/mTOR pathway, significantly reducing sensitivity to Gemcitabine and inhibiting drug-induced apoptosis, validated both in vitro and in vivo.[51] In gastric cancer, the transfer of M2-EVs' miR-21 allows recipient cells to acquire resistance to Cisplatin, accompanied by the activation of survival pathways and enhanced anti-apoptosis, establishing an early evidence chain for "EVs-miRNA—PI3K/AKT—Cisplatin resistance".[52] Subsequent studies in the same cancer type further indicate that miR-588 in M2 polarized EVs also contributes to Cisplatin resistance.[53] Tumor adaptation to chemotherapy is also reflected in the programmed enhancement of glucose metabolism. In lung cancer, M2-EVs enriched with miR-3679-5p inhibit the E3 ligase NEDD4L, stabilize c-Myc, and significantly increase glycolytic flux, thereby maintaining energy supply under drug pressure and driving Cisplatin resistance; this axis is tightly linked from the molecular level, metabolism, to the drug sensitivity phenotype.[54] In CRC, GRP78-induced macrophages polarize towards an M2-like phenotype, and the secreted EVs significantly enhance stemness and induce chemoresistance, with miR-769-5p identified as the core cargo; this miRNA directly targets MAPK1 and rearranges cell cycle proteins (RB1, Cyclin D1, Cyclin E1), causing tumor cells to enter a low-proliferation/quiescent state, thereby escaping chemotherapy killing targeting proliferation, also suggesting a resonant relationship between stemness and resistance.[55] In pancreatic cancer, after selective uptake by tumor cells, the transported miRNAs in M2-EVs can reprogram drug metabolism and stress response, systematically reducing the efficacy of Gemcitabine, confirmed by multiple methods including genetically engineered mouse models.[56] In ovarian cancer, macrophage-EVs delivering miR-223 can confer a chemoresistant phenotype to tumor cells, which is particularly evident in the hypoxic microenvironment. This study constructed a complete evidence chain from vesicular transport and drug sensitivity phenotype to in vivo validation.[57] Overall, M2-EVs form a cross-cancer resistance network by inhibiting upstream brake factors (such as TSC1), activating PI3K/AKT/mTOR and stabilizing c-Myc, and adapting to drug pressure through quiescence/stemness and glycolysis.

Tumor Stemness and Metabolic Reprogramming Axis

Regarding the shaping of stemness, multiple pathways characterized by "EVs-miRNA → inactivation of tumor suppressor nodes/transcriptional network reconstruction → EMT—CSC enhancement" have been confirmed. In HCC, CD63 highly expressed macrophage EVs deliver miR-6876-5p, which inhibits PTEN, activates Akt, and links with EMT, significantly enhancing tumor stemness;[30] M2-EVs' miR-27a-3p enhances CSC characteristics by inhibiting TXNIP, forming a critical link in the interaction between anti-oxidation and stemness.[58] In CRC, GRP78-induced macrophage EVs are enriched with miR-769-5p, which targets MAPK1 and rearranges RB1/cyclin proteins, causing cells to enter a quiescent state, simultaneously increasing stemness and resonating with drug resistance.[55] In pancreatic cancer, M2-EVs' miR-21-5p promotes cancer stem cell differentiation and activity via KLF3, directly expanding the CSC pool.[59] Central nervous system tumors also provide strong evidence: M2-EVs' miR-27b-3p maintains glioma stem-like properties via the

MLL4/PRDM1/IL-33 axis, revealing the core role of the "epigenetic—cytokine—stemness" tri-level network.[44] Regarding metabolic reprogramming, M2-EVs provide metabolic support for stemness and tolerance through "glycolysis ↑ / OXPHOS ↓ and amino acid uptake ↑". In breast cancer, macrophage-EVs' miR-503-3p downregulates DACT2, activates Wnt/β-catenin, enhances glucose uptake and glycolysis, and inhibits oxidative phosphorylation (OXPHOS) and ATP production, thereby facilitating the malignant phenotype (conversely, inhibiting miR-503-3p restores DACT2 and increases OXPHOS).[60] In lung cancer, M2-EVs' miR-3679-5p inhibits NEDD4L to stabilize c-Myc, significantly enhancing glycolysis and coupling it with chemoresistance.[54] In pancreatic cancer, M2-EVs' miR-193b-3p targets TRIM62 to enhance glutamine uptake, providing nitrogen sources and reducing power for rapid growth and stress adaptation.[61]

The cross-amplification effect of stemness and metabolism is reflected in the fact that metabolic pathway reprogramming provides the energy and biosynthetic basis for CSC maintenance, while the stemness program, in turn, reduces drug accessibility through EMT and quiescence, forming a "stemness—metabolism—tolerance" closed loop (Figure 3 and Table 2).

circRNA Maintains Pro-Cancer "Memory" Through Stable ceRNA Platforms and Post-Transcriptional Regulation

EVs secreted by M2 macrophages can efficiently carry and transfer circRNA into tumor cells, remodeling cellular signaling, metabolism, and the epitranscriptome, thereby driving tumor proliferation, invasion, metastasis, and therapeutic resistance. Overall, M2 EV circRNAs primarily promote tumor progression by acting as competitive endogenous RNAs (ceRNAs) that sponge miRNAs, regulating the localization and function of epigenetic modification enzymes like m6A modifiers, and rewriting energy metabolism and cell cycle networks. In NSCLC, multiple clear M2-EV circRNA pro-cancer axes have been identified. First, circFTO from M2-derived sEVs acts as a sponge to adsorb miR-148a-3p, relieving its inhibition on Pyruvate Dehydrogenase Kinase 4 (PDK4), driving metabolic reprogramming and enhancing tumor malignancy (proliferation, migration, invasion).[62] Secondly, smoking-induced M2-EVs are rich in circEML4. After being transferred to NSCLC cells, this molecule directly binds to the m6A demethylase ALKBH5 and reduces its nuclear distribution, leading to an upregulation of global m6A levels; the enhanced m6A modification of the SOCS2

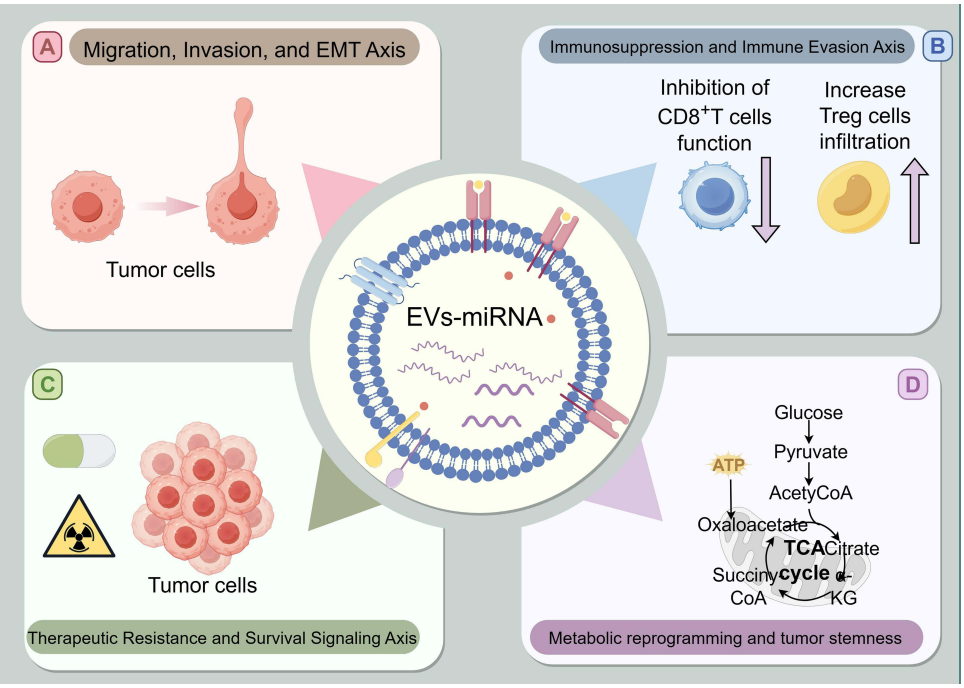

Figure 3 Functional Roles of Macrophage-Derived EVs-miRNA in Tumor.

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Table 2 miRNAs Carried by M2 Macrophage-Derived EVs and Their Pro-Tumorigenic Effects

Axis	Cancer Type	miRNA	Target	Activated/Inhibited Pathway or Mechanism	Biological Effect	References
Migration/Invasion & EMT Axis	HCC	Not specified (overall M2-EVs)	Not specified	Increase vascular permeability	Promote pre-metastatic niche and distant dissemination	[12]
	HNSCC	miR-21-5p	LATS1	VHL suppression → YAP1 upregulation	Increased angiogenesis, invasion ↑	[19]
	Lung adenocarcinoma	miR-942	FOXO1	β-catenin release + HIF-1α ↑	Angiogenesis ↑, migration/invasion ↑	[20]
	RCC	miR-193a-5p	TIMP2 down	Induce vasculogenic mimicry	Promote invasion	[21]
	RCC	miR-21-5p	PTEN down	Akt activation	Promote migration and invasion	[22]
	RCC(hypoxia)	miR-155-5p	Binds HuR → IGF1R mRNA stability ↑	PI3K/AKT activation	Increase progression ability	[23]
	Lung adenocarcinoma	miR-942	FOXO1 down	β-catenin signaling ↑	Migration/invasion ↑, angiogenesis ↑	[20]
	CRC	miR-186-5p	DLC1 down	β-catenin activation	Increased proliferation and motility	[24]
	HCC	miR-17-92 cluster	TGFBR2 down	TGF-β1/BMP-7 imbalance → ID1 ↑	EMT ↑, invasion ↑	[25]
	HCC	miR-92a-2-5p	AR down	PHLPP/p-AKT/β-catenin axis activated	Invasion fitness ↑	[26]
	CRC	miR-21-5p + miR-155-5p	BRG1 down	Chromatin remodeling	Migration ↑, invasion ↑	[27]
	CRC	miR-183-5p	Not specified	Not specified	Promote overall progression phenotype	[28]
	HCC	miR-660-5p	KLF3 down	Not specified	Tumor development ↑	[29]
	HCC	miR-6876-5p	PTEN down	Akt-EMT axis activation	Invasion ↑, stemness ↑	[30]
	Breast cancer	miR-660	Not specified	Not specified	Increase metastatic fitness	[31]
	Breast cancer	miR-223-3p	Cbx5 down	Not specified	Promote lung metastasis (organ-specific)	[32]
	NSCLC	miR-155 + miR-196a-5p	Not specified	Not specified	Synergistically enhance metastasis	[33]
	NSCLC	miR-501-3p	Not specified	Not specified	Accelerate progression	[34]
	Prostate cancer	miR-95	JunB down	Not specified	Proliferation ↑, invasion ↑, EMT ↑	[35]

	Osteosarcoma	miR-221-3p	SOCS3 down	JAK2/STAT3 activation	Growth ↑, metastasis ↑	[36]
	Osteosarcoma	let-7a	C15orf41 down	Not specified	Promote metastasis	[37]
	Ovarian cancer	miR-29a-3p	FOXO3 down	PD-L1 ↑	Proliferation ↑, motility ↑	[38]
	Ovarian cancer	miR-589-3p	BCL2L13 down	Not specified	Proliferation ↑, motility ↑	[39]
	Pancreatic cancer	Not specified (overall miRNA)	Not specified	Not specified	Promote distant metastasis	[40]
	Pancreatic cancer	miR-501-3p	TGFBR3 down	TGF-β activation	Progression ↑, invasion ↑	[41]
	Gastric cancer	miR-513b-5p	Not specified	Not specified	Druggable target—TCM inhibits its axis to suppress tumor	[42]
	Glioma	miR-146a-5p loss	Release TRAF6/IRAK1 complex	IKK/NF-κB activation	EMT ↑, invasion ↑, migration ↑	[43]
	Glioma	miR-27b-3p	MLL4/PRDM1/IL-33 activation	Maintain stem-like traits and tumorigenicity	Invasion ↑, colonization ↑	[44]
	Glioma stem cells	Not specified	Not specified	Proneural→mesenchymal transition	High invasiveness and therapy resistance	[45]
Immune Suppression & Immune Evasion Axis	CRC	miR-155-5p	ZC3H12B down	IL-6 ↑	Proliferation ↑, anti-apoptosis ↑, immune suppression ↑, escape ↑	[46]
	Epithelial ovarian cancer	Multiple miRNAs	Not specified	Treg ↑, Th17 ↓	Systemic immunosuppression, progression ↑	[47]
	Malignant pleural effusion	Not specified	Not specified	Promote Treg differentiation	Immune suppression ↑, metastasis niche support ↑	[48]
	Intracranial aneurysm (supportive evidence)	miR-155-5p	GREM1 down	Smooth muscle proliferation ↑, macrophage activation ↑	Inflammation/immune microenvironment remodeling	[49]

(*Continued*)

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Table 2 (Continued).

Axis	Cancer Type	miRNA	Target	Activated/Inhibited Pathway or Mechanism	Biological Effect	References
Therapy Resistance & Survival Axis	HCC	miR-200c-3p	Not specified	Not specified	Primary sorafenib resistance	[50]
	Pancreatic cancer	miR-222-3p	TSC1 down	PI3K/AKT/mTOR activation	Gemcitabine resistance, anti-apoptosis ↑	[51]
	Gastric cancer	miR-21	Not specified	PI3K/AKT activation	Cisplatin resistance	[52]
	Gastric cancer	miR-588	Not specified	Not specified	Cisplatin resistance	[53]
	Lung cancer	miR-3679-5p	NEDD4L down	c-Myc stabilization → glycolysis ↑	Cisplatin resistance	[54]
	CRC	miR-769-5p	MAPK1 down	RB1/Cyclin D1/E1 rearrangement → quiescence	Chemo resistance + stemness ↑	[55]
	Pancreatic cancer	Multiple miRNAs	Not specified	Reprogram drug metabolism and stress response	Reduced gemcitabine efficacy	[56]
	Ovarian cancer	miR-223	Not specified	Not specified	Chemo tolerance (exacerbated by hypoxia)	[57]
Stemness & Metabolic Reprogramming Axis	HCC	miR-6876-5p	PTEN down	Akt activation + EMT	CSC traits ↑	[30]
	HCC	miR-27a-3p	TXNIP down	Antioxidant capacity ↑	CSC ↑	[58]
	CRC	miR-769-5p	MAPK1 down	Quiescence + RB1/Cyclin rearrangement	Stemness ↑ + resistance ↑	[55]
	Pancreatic cancer	miR-21-5p	KLF3 down	Not specified	CSC differentiation ↑, activity ↑	[59]
	Glioma	miR-27b-3p	MLL4/PRDM1/IL-33 activation	Epigenetic-cytokine-stemness network	CSC maintenance ↑	[44]
	Breast cancer	miR-503-3p	DACT2 down	Wnt/β-catenin activation	Glycolysis ↑, OXPHOS ↓, ATP ↓	[60]
	Lung cancer	miR-3679-5p	NEDD4L down	c-Myc stabilization → glycolysis ↑	Coupled with drug resistance ↑	[54]
	Pancreatic cancer	miR-193b-3p	TRIM62 down	Glutamine uptake ↑	Rapid growth + stress adaptation	[61]

Notes: ↑ indicates upregulation or increase; ↓ indicates downregulation or decrease; → indicates downstream functional regulation or signaling effects between the listed molecules and pathways.

transcript further activates the JAK-STAT signaling pathway, significantly improving tumor formation and metastatic ability. Knockdown of circEML4 in M2 EVs can reverse the aforementioned pro-tumor effects; clinically, the increase of circEML4-positive M2-TAMs around tumors in smoking patients suggests its potential as both a pro-cancer factor and a diagnostic biomarker.[63] In other solid tumors, M2-EV circRNAs also exhibit powerful pro-cancer effects. Research in cholangiocarcinoma shows that M2-EVs are rich in circ_0020256, which sponges miR-432-5p, relieving the inhibition on the transcription factor E2F3, significantly promoting tumor cell proliferation, migration, and invasion; in vivo and in vitro intervention targeting circ_0020256 (siRNA) can effectively reverse these effects.[64] In ovarian cancer, M2-derived EVs circTMCO3 is upregulated and associated with poor survival. It acts as a ceRNA for miR-515-5p, increasing the expression of integrin ITGA8, thereby enhancing tumor malignancy; knockdown of circTMCO3 weakens the pro-proliferative and pro-metastatic effects of M2-EVs, an effect that can be "rescued" by inhibiting miR-515-5p or overexpressing ITGA8, and its pro-tumor capacity has been confirmed in mouse models.[65] Regarding therapeutic resistance, endometrial cancer research indicates that M2-like TAMs are enriched, and their EV-transferred hsa_circ_0001610 sponges miR-139-5p, upregulating the key cell cycle molecule Cyclin B1, thereby reducing radiosensitivity and promoting the growth of xenografts under irradiation.[66] In glioblastoma, hypoxia promotes macrophage polarization toward M2-type TAMs in a HIF1α-dependent manner. Subsequently, M2-type TAMs are able to transport circ_0003137-rich EVs to glioblastoma cells, leading to the upregulation of circ_0003137 expression in glioblastoma cells. Mechanistically, circ_0003137 physically binds to Polypyrimidine Tract Binding Protein 1 (PTBP1), enhancing the stability of Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 3 (PLOD3), thereby promoting the EMT of glioblastoma cells[67] (Table 3).

Table 3 circRNAs Carried by M2 Macrophage-Derived EVs and Their Pro-Tumorigenic Effects

Cancer Type	Key EV circRNA Cargo	Targets/Pathways	Main Mechanism of Action	References
Non-small cell lung cancer (NSCLC)	circFTO	miR-148a-3p → PDK4	Acts as a sponge for miR-148a-3p → relieves inhibition on Pyruvate Dehydrogenase Kinase 4 → metabolic reprogramming → ↑ proliferation, migration, invasion	[62]
NSCLC (smoking-induced M2 TAMs)	circEML4	ALKBH5 (m6A demethylase) → SOCS2 m6A modification → JAK-STAT	Binds ALKBH5 → reduces nuclear distribution → ↑ global m6A levels → enhances SOCS2 m6A modification → activates JAK-STAT pathway → ↑ tumor formation and metastasis	[63]
Cholangiocarcinoma	circ_0020256	miR-432-5p → E2F3	Sponges miR-432-5p → releases inhibition on E2F3 → ↑ proliferation, migration, invasion	[64]
Ovarian cancer	circTMCO3	miR-515-5p → ITGA8	CeRNA for miR-515-5p → ↑ ITGA8 expression → ↑ proliferation and metastasis; knockdown weakens effects; rescue by miR-515-5p inhibition or ITGA8 overexpression	[65]
Endometrial cancer	hsa_circ_0001610	miR-139-5p → Cyclin B1	Sponges miR-139-5p → ↑ Cyclin B1 → reduces radiosensitivity; promotes xenograft growth under irradiation	[66]
Glioblastoma	circ_0003137	PTBP1 → PLOD3 stability	Hypoxia-driven M2 TAMs transport circ_0003137-rich EVs → circ_0003137 binds PTBP1 → enhances PLOD3 stability → promotes EMT	[67]

Notes: ↑ indicates upregulation or increase; ↓ indicates downregulation or decrease; → indicates downstream functional regulation or signaling effects between the listed molecules and pathways.

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lncRNA Coordinates Immunosuppression, EMT, and Drug Resistance Through Cross-Level Orchestration

Due to their length and structural complexity, lncRNAs possess cross-level organizational capabilities. They can serve as scaffolds for transcription and epigenetic modification complexes, integrating various molecules, or act as decoys or sponges to integrate broader networks at the post-transcriptional level. lncRNAs carried within M2 EVs are often coupled with immunosuppression, EMT, and therapeutic resistance, characterized by the pattern of "upstream regulation and multi-point effectiveness downstream".

First, from the perspective of metabolic reprogramming and proliferation/invasion, M2-derived EVs can directly drive glycolysis and malignant phenotypes by transferring non-coding RNAs. In gastric cancer, M2-EVs transfer MALAT1, which not only interacts with δ-catenin to inhibit its β-TRCP-mediated ubiquitination and degradation but also acts as a miR-217-5p sponge to upregulate HIF-1α. These dual pathways synergistically enhance aerobic glycolysis, promote proliferation and metastasis, and increase chemoresistance; dual inhibition of MALAT1 in both tumor cells and macrophages (via EV-delivered siRNA) significantly suppresses tumors and enhances sensitivity in mouse models.[68] Liver cancer research also points out that M2-EVs can transfer lncMMPA to promote glycolysis and malignant behavior, revealing the universality of the "macrophage-metabolism-tumor" interaction circuit.[69] In NSCLC, NORAD in M2-EVs promotes glycolysis and proliferation and drives xenograft growth by targeting SMIM22/GALE via the miR-520g-3p axis.[70] Secondly, therapeutic resistance is another important target and phenotypic output of M2-EVs non-coding RNAs. In cisplatin treatment for gastric cancer, M2-EVs transfer CRNDE, promoting NEDD4-1-mediated ubiquitination and degradation of PTEN, thereby inducing drug resistance and tumor growth; silencing CRNDE within M2-EVs can partially reverse this effect.[71] In the context of radiotherapy for lung cancer, M2-EVs AGAP2-AS1 reduces miR-296 and elevates NOTCH2, promoting the maintenance of malignant phenotypes and immune-related changes after irradiation.[72] Furthermore, concerning immune evasion and antigen presentation, esophageal cancer research found that M2-EVs LINC01592 can reduce the expression of MHC-I on the surface of tumor cells, promoting immune evasion;[73] M2-EVs AFAP1-AS1 promotes migration, invasion, and lung metastasis by downregulating miR-26a and upregulating ATF2.[74] In laryngeal cancer, M2-EVs HOXC13-AS regulates PD-L1 via the miR-485-5p/IGF2BP2 axis, directly driving immune evasion and malignant progression.[75] In the hypoxic breast cancer microenvironment, M2-EVs MIR210HG is upregulated. It both strengthens M2 markers and PI3K/Akt/mTOR signaling in macrophages and increases HIF-1, initiates RASSF7 transcription, and induces EMT, vasculogenic mimicry, and metastasis in tumor cells; dual inhibition of endogenous and EVs MIR210HG synergistically suppresses metastasis.[76]

Overall, a key feature of this network is the parallel synergy of the three types of non-coding RNAs. The same M2-EVs can simultaneously carry various miRNAs, circRNAs, and lncRNAs, which each execute their respective roles while mutually amplifying one another, leading to the formation of multi-point positive feedback in the recipient tumor cells across transcriptional, epigenetic, and signaling pathways. The non-coding RNA cargo of M2-EVs collectively reprograms the biological attributes of tumor cells from "controllable proliferation, limited infiltration, susceptible to immune surveillance, and drug killing" to "strong invasion, high immune evasion, and high drug resistance", providing energy and plasticity support for this phenotype in the metabolic and stemness dimensions. This mechanistic landscape not only explains the clinical commonalities across multiple cancer types but also provides a solid molecular basis for identifying intervenable "vulnerable loops" and formulating network-based blockade strategies (Table 4).

Pro-Cancer Effects and Pathway Integration of Proteins Carried by M2 Macrophage-Derived Extracellular Vesicles

The protein cargo of EVs derived from M2 macrophages serves as important "information carriers" promoting tumor progression. They can directly remodel metabolism, inhibit ferroptosis, activate migration and adhesion pathways in recipient tumor cells, and powerfully shape the immunosuppressive microenvironment. In laryngeal squamous cell carcinoma (LSCC), M2-EVs are rich in the adhesion protein ANXA3. On one hand, ANXA3 promotes M2-like polarization within macrophages via the AKT–GSK3β–β-catenin pathway. On the other hand, after being transported to tumor cells as EVs protein cargo, it inhibits the ubiquitination of transcription factor ATF2 and downregulates its target

Table 4 lncRNAs Carried by M2 Macrophage-Derived EVs and Their Pro-Tumorigenic Effects

Cancer Type	Key EV circRNA Cargo	Targets/Pathways	Main Mechanism of Action	References
Gastric cancer	MALAT1	δ-catenin (β-TRCP-mediated ubiquitination/degradation), miR-217-5p → HIF-1α	Interacts with δ-catenin to prevent degradation; sponges miR-217-5p to upregulate HIF-1α → synergistically enhances aerobic glycolysis, proliferation, metastasis, and chemoresistance	[68]
HCC	lncMMPA	— (metabolic regulation)	Promotes glycolysis and malignant behavior, supporting universality of macrophage-driven tumor metabolism	[69]
NSCLC	NORAD	miR-520g-3p → SMIM22 / GALE	Sponges miR-520g-3p to regulate SMIM22/GALE → promotes glycolysis, proliferation, and xenograft growth	[70]
Gastric cancer	CRNDE	PTEN (via NEDD4-1-mediated ubiquitination/degradation)	Induces PTEN degradation → drug resistance and tumor growth; silencing in M2 EVs partially reverses effect	[71]
Lung cancer	AGAP2-AS1	miR-296 → NOTCH2	Reduces miR-296 → increases NOTCH2 → maintains malignant phenotype and immune changes post-irradiation	[72]
Esophageal cancer	LINC01592	MHC-I	Downregulates MHC-I on tumor cells → promotes immune evasion	[73]
Esophageal cancer	AFAP1-AS1	miR-26a → ATF2	Downregulates miR-26a → upregulates ATF2 → promotes migration, invasion, and lung metastasis	[74]
Laryngeal cancer	HOXC13-AS	miR-485-5p → IGF2BP2 → PD-L1	Sponges miR-485-5p → upregulates IGF2BP2 → stabilizes PD-L1 → immune evasion and malignant progression	[75]
Breast cancer	MIR210HG	M2 macrophage polarization markers, PI3K/Akt/mTOR, HIF-1, RASSF7	Upregulates M2 markers and PI3K/Akt/mTOR in macrophages; increases HIF-1 and initiates RASSF7 transcription in tumor cells → induces EMT, vasculogenic mimicry, and metastasis	[76]

Notes: ↑ indicates upregulation or increase; ↓ indicates downregulation or decrease; → indicates downstream functional regulation or signaling effects between the listed molecules and pathways.

gene CHAC1, thereby inhibiting ferroptosis and driving lymph node metastasis, forming a dual pro-cancer circuit of "immunosuppression + ferroptosis resistance".[14] Regarding immune evasion, TAM induction leads to increased EV secretion and significant enrichment of PD-L1 protein on the vesicle surface. These EVs effectively inhibit CD8+ T cell proliferation and effector function. This process is driven by Akt phosphorylation of MADD activating Rab27a, suggesting that "M2-EVs carrying high levels of PD-L1" are a crucial mechanism for tumors to acquire resistance to immunotherapy[16]) In CRC, DOCK7, highly expressed in TAM vesicles, acts as a Rho family regulator, triggering the RAC1/ABCA1 axis. This enhances membrane dynamics related to cytoskeleton rearrangement and cholesterol efflux, significantly increasing metastatic potential.[77] In the microenvironment of colorectal liver metastasis, the immunoglobulin-like protein FGL2 carried by M2-EVs binds to the tumor cell receptor FCGR2B, activating a p-STAT3/IL-1β positive feedback loop. This both enhances tumor stemness and induces neutrophils to form NETs (Neutrophil Extracellular Traps), thereby impairing the efficacy of anti-PD-1 therapy; blocking IL-1β synergizes with PD-1 blockade.[78] In NSCLC, M2-like macrophage EVs directly deliver Integrin αVβ3 to the tumor cell membrane, activating FAK adhesion signaling, which drives migration and invasion. High expression of αV and β3 in clinical samples correlates with metastasis and poor prognosis, suggesting EVs integrins are druggable targets for metastasis.[79] In gastric cancer, M2-EVs carry Apolipoprotein E (ApoE), which significantly promotes tumor cell migration. This pro-migratory effect disappears when ApoE is absent, demonstrating that ApoE is a key protein cargo in M2-EVs promoting infiltration.[80] In ovarian cancer, the transcription factor GATA3 encapsulated within M2-EVs enters tumor cells, upregulating the CD24/Siglec-10 axis. This enhances the "don't eat me" signal, promoting immune evasion and

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chemoresistance, revealing the oncogenic potential of transcription factors acting as vesicular protein cargo.[81] In liver cancer, M2 macrophage-derived EVs deliver the ETS family transcription factor ETV4, which increases the expression of cholesterol sulfotransferase SULT2B1, thereby simultaneously enhancing proliferation, glycolysis, and stemness, forming a pro-cancer link coupling "protein cargo–metabolism–stemness".[82] Ferroptosis regulation is another core output of M2 vesicular proteins: besides the ANXA3–ATF2–CHAC1 axis, macrophage-EVs are enriched with the glutathione metabolism-related protein PRDX6. Its glutathione peroxidase activity increases reduced glutathione in tumor cells, inhibits lipid peroxidation, and alleviates ferroptosis-induced mitochondrial autophagy, ultimately enhancing tumor cell survival and growth.[83] At the metabolic level, laryngeal cancer research suggests that M2-EVs can regulate glycolysis and chemosensitivity via WTAP/GLUT-1 (WTAP and GLUT-1 are protein nodes), revealing the coupling effect of EVs proteins on the "m6A–glucose transport–response" axis.[13] Regarding immune checkpoint resistance, M2-EVs can also upregulate PD-L1 via the MISP/IQGAP1 axis, leading to resistance to immunotherapy in liver cancer (MISP and IQGAP1 act as scaffold and signal integration proteins), suggesting that vesicular proteins can serve as plastic nodes upstream of PD-L1.[84] In the glioma microenvironment, M2-EVs induce Treg differentiation and immunosuppression, which is associated with the downregulation of the antibacterial permeability protein BPI, suggesting that regulating the expression of specific proteins or vesicular cargo can remodel the "macrophage-Treg" immune circuit to support tumor progression.[85]

Overall, M2 macrophage vesicular proteins synergistically drive tumor progression through three main axes: The Immune Axis (EVs PD-L1, CD24/Siglec-10, FGL2/FCGR2B, Treg polarization) shapes deep immunosuppression and reduces responsiveness to immunotherapy. The Migration/Metastasis Axis (Integrin αVβ3–FAK, DOCK7–RAC1/ABCA1, ApoE–membrane pathway) enhances adhesion, cytoskeleton rearrangement, and membrane dynamics. The Metabolism/Ferroptosis Axis (WTAP/GLUT-1, ETV4–SULT2B1, ANXA3–ATF2–CHAC1, PRDX6–Glutathione) boosts glycolysis and antioxidant capacity, conferring chemoresistance and survival advantages.

These mechanisms have been repeatedly validated across multiple cancer types and treatment settings, suggesting that "blocking M2 polarization and vesicle biogenesis/uptake, targeting key protein cargo or their receptors (e.g., αVβ3/FAK, FGL2/FCGR2B, PD-L1, CD24/Siglec-10), and concurrently targeting metabolism and ferroptosis pathways (GLUT-1, SULT2B1, PRDX6, ATF2/CHAC1)" holds promise for synergistically enhancing the efficacy and durability of radiotherapy, chemotherapy, and immunotherapy (Table 5).

Table 5 Proteins Carried by M2 Macrophage-Derived EVs and Their Pro-Tumorigenic Effects

Cancer Type	Key EV Proteins Cargo	Targets/Pathways	Main Mechanism of Action	References
Laryngeal squamous cell carcinoma	ANXA3	AKT–GSK3β–β-catenin (M2 polarization), ATF2 → CHAC1	Promotes M2 polarization; inhibits ubiquitination of ATF2 → downregulates CHAC1 → inhibits ferroptosis → drives lymph node metastasis	[14]
Multi-cancer / Immunotherapy	PD-L1	Akt–MADD → Rab27a	PD-L1 enrichment on EV surface → inhibits CD8+ T cell proliferation/function → immunotherapy resistance	[16]
CRC	DOCK7	RAC1 → ABCA1	Activates RAC1/ABCA1 axis → cytoskeleton rearrangement and cholesterol efflux → ↑ metastasis	[77]
Colorectal liver metastasis	FGL2	FCGR2B → p-STAT3 / IL-1β	Binds FCGR2B → activates p-STAT3/IL-1β loop → ↑ tumor stemness and NET formation → impairs anti-PD-1 efficacy; IL-1β blockade synergizes with PD-1 therapy	[78]
NSCLC	Integrin αVβ3	FAK signaling	Delivers αVβ3 to tumor cells → activates FAK adhesion signaling → ↑ migration and invasion; correlates with poor prognosis	[79]

(*Continued*)

Table 5 (Continued).

Cancer Type	Key EV Proteins Cargo	Targets/Pathways	Main Mechanism of Action	References
Gastric cancer	ApoE	Membrane dynamics pathway	Promotes tumor cell migration; pro-migratory effect lost if ApoE absent	[80]
Ovarian cancer	GATA3	CD24/Siglec-10	Upregulates CD24/Siglec-10 "don't eat me" signal → immune evasion and chemoresistance	[81]
HCC	ETV4	SULT2B1	Increases SULT2B1 → boosts proliferation, glycolysis, stemness	[82]
Multi-cancer / Ferroptosis	PRDX6	Glutathione metabolism	Increases reduced glutathione → inhibits lipid peroxidation → blocks ferroptosis-induced mitochondrial autophagy → ↑ survival	[83]
Laryngeal cancer	WTAP	m6A–glucose transport	Regulates glycolysis and chemosensitivity via m6A–GLUT-1 axis	[13]
HCC	Not mentioned	MISP/IQGAP1→ PD-L1 upregulation	Upregulates PD-L1 via scaffold/signal integration proteins → immunotherapy resistance	[84]
Glioma	Not mentioned	BPI	Downregulation → induces Treg differentiation → immunosuppression	[85]

Notes: ↑ indicates upregulation or increase; ↓ indicates downregulation or decrease; → indicates downstream functional regulation or signaling effects between the listed molecules and pathways.

Clinical Applications of Macrophage-Derived Extracellular Vesicles

Engineering of Macrophage-Derived EVs and Potential for Anti-Tumor Therapy

Macrophage-derived extracellular vesicles have attracted increasing attention in translational oncology due to their intrinsic biocompatibility, immune cell origin, and ability to transport complex molecular cargo within the tumor microenvironment. From a clinical perspective, extracellular vesicles derived from macrophages exhibit distinct and divergent translational trajectories depending on their polarization-associated functional states. M1-like macrophage-derived EVs are predominantly explored as active therapeutic platforms, owing to their immunostimulatory properties and amenability to engineering for drug, nucleic acid, and multimodal cargo delivery. In contrast, EVs released by M2-like macrophages are primarily relevant as mediators of immune suppression, therapeutic resistance, and metastatic progression, positioning them as clinically actionable targets for inhibition, reprogramming, or biomarker-based stratification rather than direct therapeutic carriers.

To facilitate a clearer comparison of their respective advantages, limitations, and translational roles, the key features of M1- and M2-derived macrophage extracellular vesicles are summarized in Table 6.

Table 6 Comparative Advantages, Limitations, and Translational Implications of Macrophage-Derived Extracellular Vesicles

Comparison Dimension	M1-Derived Extracellular Vesicles	M2-Derived Extracellular Vesicles
Dominant functional orientation	Immunostimulatory and tumor-suppressive	Immunosuppressive and tumor-promoting
Representative cargo features	Anti-tumor miRNAs, pro-inflammatory proteins, immune-activating molecules	Pro-oncogenic miRNAs, metabolic regulators, immune checkpoint–related proteins

(*Continued*)

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Table 6 (Continued).

Comparison Dimension	M1-Derived Extracellular Vesicles	M2-Derived Extracellular Vesicles
Primary effects on tumor cells	Inhibition of proliferation, invasion, stemness, and immune evasion	Promotion of EMT, metabolic reprogramming, stemness, and therapy resistance
Effects on immune microenvironment	Enhancement of antigen presentation, T-cell activation, and macrophage reprogramming toward inflammatory states	Induction of T-cell dysfunction, Treg expansion, and suppression of anti-tumor immunity
Therapeutic application mode	Active therapeutic carriers and immune-modulating delivery platforms	Targets for inhibition, reprogramming, or biomarker-based stratification
Key advantages	High engineering flexibility, immunological synergy, and delivery efficiency	High clinical relevance for predicting resistance and identifying intervention points
Major limitations	EV heterogeneity, targeting specificity, safety, and scalability	Intrinsic pro-tumorigenic properties limiting direct therapeutic use
Translational challenges	Standardized production, cargo consistency, biodistribution, and long-term safety	Selective blockade of EV biogenesis or uptake and avoidance of systemic immune disruption

Note: M1 and M2 are used as operational reference states to describe polarization-associated tendencies; macrophage phenotypes in vivo exist along a dynamic continuum.

Chemotherapeutic Drug Loading and Targeted Tumor Delivery

Chemotherapy platforms utilizing M1 macrophage-derived EVs as carriers have involved systematic practical efforts spanning from process optimization to efficacy assessment. Early studies demonstrated that macrophage EVs can efficiently load Paclitaxel or Doxorubicin, significantly inhibiting tumor growth in orthotopic and metastatic TNBC models. At the process level, regulating loading parameters such as pH, temperature, and sonication can simultaneously maintain vesicle integrity while enhancing the drug loading rate and in vivo accumulation.[86] Furthermore, Poly(lactic-co-glycolic acid) (PLGA) nanoplatforms coated with macrophage EV membranes or featuring a direct "EVs coating", combined with peptide ligands targeting c-Met or folic acid modification, significantly enhance uptake and anti-tumor effects in TNBC and solid tumors, demonstrating the combined advantage of "immunocyte biomimicry + receptor-mediated targeting".[87,88] Quality by Design (QbD)-driven Exo-DTX (Docetaxel EV formulation) achieved statistical optimization of formulation parameters, inhibiting migration and improving pharmacokinetics in the 4T1 metastasis model.[89] Meanwhile, a "Chemo + Immune" system loading Docetaxel into M1-EVs was shown to maintain M1 activation and infiltration long-term within the immunosuppressive Tumor Microenvironment (TME).[90] Notably, cord blood-derived M1-EVs loaded with Cisplatin show considerable tumor inhibition potential for ovarian cancer and platinum resistance.[91] As early as 2019, there was exploration into "macrophage EVs-liposome hybrid vesicles" for Doxorubicin delivery to enhance yield and stability.[92] Mechanistically, M1-EVs can also be used in combination with Paclitaxel to further amplify the chemotherapy effect by activating the macrophage-mediated inflammatory loop.[93] The evidence above collectively indicates that the membrane molecular fingerprint and immunoaffinity properties of macrophage EVs provide a "smarter coat" for chemotherapeutic drugs, offering advantages over traditional nanocarriers in terms of being "more precise, more stable, and more durable".

Nucleic Acid Delivery and Tumor Immune Reprogramming

Nucleic acid drug delivery using M1-EVs as carriers represents the representative strategy of "integrating immunology and genetic intervention onto the same nanoparticle". One strategy directly targets TAMs as recipient cells for repolarization: M1-EVs are functionalized on the surface with the IL-4R ligand peptide (IL4RPep-1) for targeted entry into M2-TAMs, co-loading NF-κB p50 siRNA and miR-511-3p. This significantly suppresses M2 markers, upregulates M1 factors, alters the cellular composition of the TME, and inhibits tumor growth. In vivo tracing showed that these IL4R-targeted EVs exhibited tumor enrichment rather than preferential liver distribution.[94] Another strategy focuses on

the dual blockade of the "phagocytic brake and M2 signaling pathway": M1-EVs deliver siSIRPα to release the CD47 "don't eat me" signal, and combined with anti-PD-L1, achieves "innate + adaptive" dual immune checkpoint synergy. This significantly enhances macrophage phagocytosis, inhibits 4T1 migration/invasion, and promotes pro-inflammatory repolarization.[95] Furthermore, parallel delivery of siSIRPα + siSTAT6 into M2 macrophages combined with anti-PD-L1 can more powerfully remodel the TME and inhibit breast cancer progression.[96] Regarding tumor cell targeting, M1-EVs delivering siCX3CR1 can inhibit proliferation and migration mediated by the CX3CL1-CX3CR1 axis in a pancreatic cancer model, achieving precise targeting of the "chemokine receptor-ligand" signal.[97] Echoing this concept, SOCS3 endogenously secreted by EVs from resident alveolar macrophages in lung tissue can inhibit the STAT3 signal, restricting adenocarcinoma cell proliferation and survival. This pathway, which is broken in the tumor context, can be "artificially rescued" by "liposomal supplementation of SOCS3", providing an example of engineered "replacement/restoration" of endogenous anti-tumor braking mechanisms.[98] Overall, the M1-EVs nucleic acid platform packages multiple pathways—"disinhibition, pro-phagocytosis, M2-reversal, and reduced immune evasion"—into a single delivery vehicle, capable of precisely targeting TAMs/tumor cells and facilitating mechanistic complementarity with antibody drugs.

Multimodal Synergy of Photo/Sonodynamic, Ferroptosis, and Gas Therapy

Following the strategy of "enhanced immunity + physicochemical tumor ablation", M1-EVs are frequently used to carry photosensitizers, sonosensitizers, oxygen-releasing/delivering components, and ferroptosis triggering modules, creating multimodal synergistic platforms. When M1-EVs loaded with zinc phthalocyanine are used for Photodynamic Therapy (PDT), they not only outperform EVs from other sources in vitro and in vivo but also induce fully protective immune memory in cured mice.[99] By functionalizing the M1-EVs membrane with the chemiluminescent source CPPO and the photosensitizer Ce6, and loading the hypoxia-activated prodrug AQ4N into the lumen, the resulting "CCA-M1-EVs" can cross the blood-brain barrier, drive M2 → M1 repolarization, increase hydrogen peroxide and ROS generation, ultimately achieving strong synergistic therapeutic efficacy in Glioblastoma.[100] "Endogenous biosynthesis" loading of the photosensitizer PpIX and Doxorubicin into folic acid-modified EVs can enhance deep tumor parenchyma penetration and achieve more reliable photochemotherapy.[88] In CRC, M1-EVs coupled with upconversion nanomaterials are loaded with Ce6, while simultaneously utilizing nitric oxide synthase on the EV surface to produce NO, forming an "Immune + Gas + PDT" trimodal system, achieving 100% survival and zero recurrence in vivo.[101] Furthermore, acidic-activated nanoplatforms based on the M1-EV framework package PDT and ferroptosis, triggering the "warming up of immunologically cold tumors", significantly enhancing responsiveness to immunotherapy[102,103] Thermosensitive fusion "EVs-liposome" hybrid vesicles showed stronger in vivo targeting in a melanoma model,[104] while a degradable nanoplatform "camouflaged by macrophage EVs" introduced Sonodynamic Therapy (SDT) into GBM, solving the dual challenges of the BBB and hypoxia.[105] Targeting different tumor niches, recent research also integrated M1-EVs with MoS2 two-dimensional materials for synergistic ablation in bladder cancer, reflecting the material flexibility of the platform.[106] This body of work consistently reveals that a more robust positive feedback loop between "tumor inhibition, immune memory, and distant protection" can only be established when physicochemical killing resonates synchronously with M1 immune reprogramming.

Combination with Innate/Adaptive Immune Activation

The combination of M1-EVs as an "immune delivery base" with pattern recognition receptor agonists or small molecule chemotherapeutics can effectively break the immunosuppression within the TME. In a TNBC model, M1-EVs combined with TLR3 agonist (poly I:C) nanoparticles significantly downregulated AKT1 and PD-L1, enhanced CD8+ T cell and dendritic cell markers, inhibited metastasis, and prolonged survival, demonstrating a "vaccine-like immunomodulatory" effect.[107] The success of M1-EVs as an "immune adjuvant" in vaccine systems has also been validated in melanoma models.[108] Furthermore, M1-EVs can co-load combination drugs such as Oxaliplatin, all-trans retinoic acid, and plant active substances to comprehensively downregulate STAT3/NF-κB/AKT, reverse the M2-dominant TME, and reduce CRC peritoneal/liver/lung metastasis, showcasing the coupled regulation of "multi-drug—multi-target—multi-pathway" involving immunity, metabolism, and transcription.[109] In breast cancer, M1-EVs pre-treated with hyaluronic acid/β-receptor blocker (carvedilol) and then loaded with Doxorubicin not only inhibited primary and metastatic lesions and

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downregulated NF-κB and M2 markers but also validated the full-chain effect of "upregulating apoptosis—reducing migration—promoting M1 polarization" in vitro.[110] These results provide data support for the clinically translatable combination of "engineered M1-EVs + immune agonists/chemotherapy/antibodies".

Diagnostic and Imaging Capabilities of Macrophage-Derived EVs

Engineered M1-EVs are not only therapeutic carriers but are also gradually acquiring "diagnostic/monitoring" capabilities. By integrating a Gadolinium-based contrast agent (Gd) into macrophage EV-liposome hybrid vesicles (Gd-HEVs), the EV membrane proteins can be used as a "camouflage" to prolong in vivo retention, enhance tumor specificity, and increase the longitudinal relaxation rate in MRI. This allows for improved contrast while reducing the dose, mitigating the issues of rapid renal clearance and potential toxicity associated with traditional Gd agents.[111] In terms of therapy, most systems provide evidence of "tracing—accumulation—release" through in vivo live imaging: Thermosensitive M1-EVs-liposome hybrid vesicles showed superior real-time accumulation at mouse tumor sites compared to simple liposomes and native EVs.[104] Upconversion nanoprobe-M1-EVs generate a visible fluorescent signal under near-infrared excitation, which can indicate the activation of Ce6 to produce ROS and can also be used for dynamic monitoring of the delivery/therapeutic window.[101] For cell targeting, IL4R peptide-guided M1-EVs showed preferential tumor distribution in whole-body fluorescence imaging, providing a basis for in vivo safety and therapeutic window characterized by "low liver targeting/high tumor targeting".[94] These "theranostic" practices demonstrate that M1-EVs can carry both imaging and therapeutic functions without sacrificing biocompatibility, enabling a closed-loop system for diagnosis and treatment, and personalized dose regulation.

Challenges Facing Clinical Application

Although M1/M2 macrophage EVs show broad prospects in clinical application, they still face numerous challenges. First, standardized methods for EV isolation, identification, and quantification have not been fully unified, which limits the comparability of results across different studies. Second, the heterogeneity of EV source cells is complex. For example, TAMs are not purely M1 or M2 phenotypes but exist on a continuum between the two, which makes the interpretation of the EV cargo profile more challenging. Furthermore, the in vivo targeting specificity, immunogenicity, and long-term safety of EVs still require in-depth research, especially in the therapeutic application of engineered EVs. Future research requires more precise EV isolation techniques, more detailed donor cell phenotyping, more comprehensive cargo analysis, and validation through large-scale clinical trials to promote their practical application in tumor diagnosis and therapy.

Discussion

From a holistic perspective, M1 and M2 macrophage-derived EVs exhibit a significant "double-edged sword effect" in tumor progression, representing two extreme states of information flow within the TME. M1-type EVs are primarily anti-tumor oriented. By selectively carrying tumor-suppressive non-coding RNAs and protein cargo, they interrupt key oncogenic pathways such as PD-L1, ENPP2, and ETS1. Simultaneously, they drive immune cells toward a pro-inflammatory phenotype, reshaping the immune landscape, and achieving the dual effects of tumor suppression and immune activation. In contrast, M2-type EVs are the core drivers of multi-pathway pro-tumorigenesis. Their cargo establishes a closed-loop positive feedback mechanism involving ferroptosis inhibition, glucose metabolism reprogramming, cytoskeletal and adhesion structure remodeling, and immune evasion. This allows the tumor to establish high stability across three dimensions— "survival, motility, and evasion"—leading to clinically refractory phenotypes such as drug resistance and high metastatic potential.

Taken together, the available evidence indicates that macrophage-derived extracellular vesicles play a multifaceted and context-dependent role in tumor progression by coordinating molecular signaling across tumor, immune, and stromal compartments. Throughout this review, we have discussed how EV cargo composition reflects macrophage functional states and how these vesicles can either reinforce anti-tumor immunity or establish pro-tumor networks involving immune suppression, metabolic reprogramming, stemness maintenance, and therapeutic resistance. Importantly, these

effects do not arise from isolated molecular events but instead emerge from integrated signaling programs conveyed through EV-mediated information transfer.

At the same time, the current body of evidence also reveals substantial limitations that must temper overly optimistic interpretations. As emphasized in this review, macrophage activation in vivo exists along a dynamic continuum rather than discrete M1 or M2 states, complicating the attribution of EV origin, cargo specificity, and functional outcomes. Moreover, many mechanistic insights are derived from in vitro systems or animal models, and their relevance may vary across tumor types, anatomical sites, and experimental contexts. EV heterogeneity, together with the lack of standardized isolation, characterization, and quantification protocols, further limits cross-study comparability and the generalizability of reported findings.

These challenges are particularly relevant for translational applications. While engineered macrophage-derived EVs have shown promise as delivery vehicles and immunomodulatory platforms, critical issues—including in vivo targeting specificity, biodistribution, long-term safety, immunogenicity, and scalable manufacturing—remain insufficiently defined. Consequently, the diagnostic and therapeutic potential of macrophage-derived EVs should be viewed as conditional and highly context-dependent, rather than universally applicable solutions. Addressing these uncertainties will require rigorously designed studies that integrate physiologically relevant models, standardized EV methodologies, and clinically oriented validation strategies. Such efforts are essential to move the field beyond descriptive associations toward predictable and controllable EV-based interventions.

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Author Contributions

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