Review

Senescent extracellular vesicles in therapy-induced senescence: Orchestrators of immune remodeling and mitochondrial stress in cancer

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Abstract

Using the framework of "cancer therapy → SASP escalation → extracellular vesicles (EVs) as a key controllable form of 'particulate SASP' → immune regulation and mitochondrial signaling as determinants of recurrence versus clearance," this review

systematically summarizes the biogenesis of senescent cell-derived extracellular vesicles (senEVs), their immune-remodeling effects, and the mitochondrial/mtDNA signaling axis. It also proposes potentially translatable intervention strategies and highlights major unresolved questions in the field. In particular, this review emphasizes the paradox that the same class of senEVs may exert either tumor-promoting or tumor-suppressive effects under different therapeutic contexts and microenvironmental conditions, and discusses the factors that determine this functional divergence.

Keyword: Therapy-induced senescence, senescent extracellular vesicles, senescence-associated secretory phenotype (SASP), immune remodeling, mitochondrial stress, tumor microenvironment

INTRODUCTION

The "Senescence-Associated Secretory System" under therapeutic stress and particulate SASP

Cancer therapies, including radiotherapy, chemotherapy, targeted therapy, and immunotherapy, not only eliminate tumor cells but also inevitably impose persistent cellular stress, such as DNA damage[1-3], oxidative stress[1,2,4], metabolic pressure[1,4,5] and disruption of organelle homeostasis[6-8]. These stresses can drive tumor cells or stromal cells into a long-term senescence-like reprogrammed state characterized by durable growth arrest[5,6,9-11]: in some contexts, they can enhance immune surveillance and promote clearance and tissue repair[5,12-15]; but in other contexts, they may generate a background of chronic inflammation, drive myeloid suppressive networks and immune evasion, thereby providing a niche for the regrowth of residual cells and ultimately leading to recurrence and resistance[16-20,21].

Traditionally, this secretory phenotype has been summarized as SASP[5,22-25]. However, increasing evidence suggests that SASP is not only a "liquid-phase signal" composed of soluble factors (cytokines, chemokines, proteases, *etc.*), but also includes "particulate

signals" represented by EVs[5,6,9-11,26]. Compared with soluble factors, EVs have stronger characteristics of structured packaging, biofluid stability, long-distance delivery, and selective cellular uptake[27-31], They are able to package and transport proteins, lipids, metabolites, and "danger-associated molecular patterns" such as DNA/mtDNA[16,27-31], thereby extending the influence of senescence-like cells from local paracrine signaling to transmissible systemic regulation[6,9-11]. On this basis, EVs may represent a structurally organized form of particulate SASP with greater signaling stability than soluble factors[6,9-11]: they may act as catalysts for the immune system to amplify clearance responses[12-20], but they may also act as suppressors that drive immune tolerance and therapeutic resistance[2-4,16-20,32-36].

This leads to the central question of this review: why do senescent cell-derived EVs (senEVs) show opposite effects under different treatment modalities, time windows, and immune niches? What are the upstream switches that determine their directionality? We will focus on an answer that is both more explanatory and more actionable for intervention: mitochondrial stress and the leakage of related nucleic acids (especially mtDNA), together with the DNA-sensing axis, may alter EV cargo loading and the innate immune threshold of recipient cells, thereby pushing the immune outcome toward either "clearance" or "escape"[7,8,34,37-41]. This review focuses on how therapy-induced senescence reshapes EV-mediated signaling, with particular attention to mitochondrial stress, immune remodeling, and translational implications[2-4,12-15,32-36,42]. It will further establish a causal chain from phenomenon to mechanism at the levels of immune regulation and mitochondrial signaling[7,8,14-20,37-41,43-50], and finally propose potentially translatable intervention strategies and biomarker routes[7,8,39-41,51-57][Figure 1].

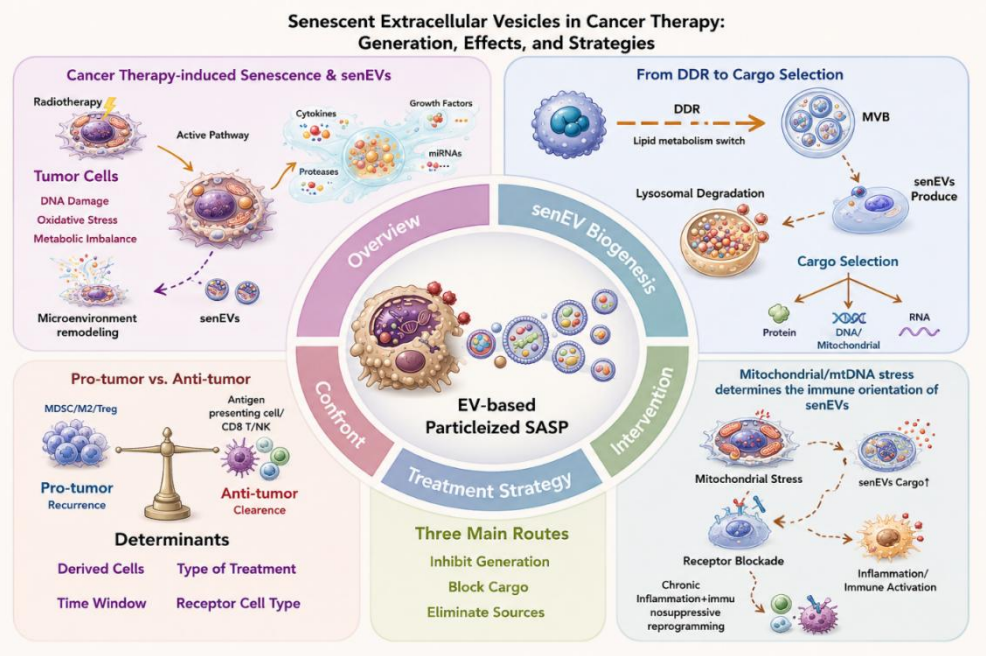

Figure 1. Overview of therapy-induced senescence and senEV-mediated immune remodeling in the tumor microenvironment. Cancer therapies such as chemotherapy, radiotherapy, targeted therapy, and immunotherapy can induce persistent cellular stress and therapy-induced senescence in tumor cells and stromal cells within the tumor microenvironment. Senescent cells subsequently develop a complex senescence-associated secretory phenotype (SASP), including the release of soluble inflammatory mediators and senescent cell-derived extracellular vesicles (senEVs). Compared with soluble SASP factors, senEVs provide a more stable and spatially transferable signaling platform capable of delivering proteins, nucleic acids, lipids, and mitochondrial components to surrounding recipient cells. Following uptake by immune and stromal cells, senEV-associated signals may influence dendritic-cell activation, macrophage polarization, T-cell responses, and myeloid-cell remodeling in a context-dependent manner. These dynamic interactions may either support transient antitumor immune surveillance and residual tumor-cell clearance or contribute to chronic immunosuppressive remodeling, immune escape, and recurrence following therapy. The figure summarizes the overall framework linking therapy-induced senescence, senEV signaling, and divergent immune outcomes discussed throughout this review.

CONCEPTUAL AND METHODOLOGICAL FOUNDATIONS

Terminology and classification: From "senescent cells/TIS" to the definition of "EVs/senEVs"

Cellular senescence generally refers to a state of long-term proliferative arrest that cells enter after persistent stress. Its core feature is not merely a "temporary cessation of division," but rather a state characterized by stable cell-cycle exit, sustained activation of stress pathways, and reprogramming of the secretory system[22-25]. Unlike quiescence, which is a reversible pause in proliferation, senescence places greater emphasis on persistence and phenotypic remodeling: while maintaining viability, cells undergo systematic changes in morphology, metabolism, chromatin state, and secretory profile (including SASP)[5,22-25]. It should be emphasized that senescence is not a single phenotype: different triggers (replicative senescence, oncogene-induced senescence, oxidative stress, radiotherapy/chemotherapy, *etc.*) and different cell types can generate marked heterogeneity. Therefore, in EV research, "senescence" must first be a clearly validated and traceable experimental state, rather than an inference based on a single marker[5,22-25].

Therapy-induced senescence (TIS) is the most common senescence-like program in the context of cancer therapy, and also the one that requires the strictest definition[1-4,32-36]. DNA damage, replication stress, and metabolic/oxidative stress caused by radiotherapy, chemotherapy, targeted therapy, and other treatments can drive tumor cells or stromal cells into TIS.The key significance of TIS is that it often occurs in cell populations that have "not been killed," and it has a time window-dependent double-edged effect: in the acute phase, it may favor immune clearance, whereas in the chronic residual phase, it may promote recurrence and resistance. Therefore, when discussing "senEVs" in this review, we assume by default that their sources may include not only senescent cells in the traditional sense, but also TIS cell populations that have entered senescence-like reprogramming under therapeutic stress.

Extracellular vesicles (EVs) are particulate structures actively released by cells and

enclosed by a lipid bilayer; they carry diverse cargos such as proteins, lipids, and nucleic acids, and participate in intercellular communication[27-31]. A consensus in the EV field is that, under most experimental conditions, it is difficult to "prove their biogenetic origin" solely on the basis of isolated products (for example, to strictly distinguish exosomes from microvesicles). Therefore, the International Society for Extracellular Vesicles (ISEV) advocates more operational and reproducible modes of nomenclature and classification, and the MISEV guidelines specify the minimum information that should be reported in EV studies[27-31,58]. Specifically, terms based on physical properties (mainly size range), such as small EV (sEV) and medium/large EV (m/lEV), are recommended unless sufficient evidence can be provided to demonstrate origin from a specific biogenetic pathway[27-31,58].

Scope of inclusion and levels of evidence in this review: how the "evidence chain" is organized from basic mechanisms to translational strategies

Centered on the main line of "cancer therapy-induced stress → senescence-like secretory system → particulate SASP (EVs) → immune outcomes/recurrence," this review follows a three-level progression in evidence inclusion—mechanism, context, and translation—and classifies the literature according to interpretability and extrapolatability, so as to avoid conflating conclusions derived from different levels of evidence[1-5,27-36,58,59].

Mechanistic core

This review first includes studies that address the following questions: why senescent cells increase EV release, how cargo is selectively loaded, and which upstream signals can serve as switches controlling EV biogenesis. The focus includes: (a)DDR-related pathways and the lipid metabolism axis: in particular, how the DDR–ceramide–nSMase2 pathway drives sEV release and functions in inflammation control/cellular homeostasis[51-54]; (b)The innate immune nucleic acid-sensing axis: for example, how coupling between cGAS–STING and abnormal cytosolic DNA/mtDNA affects the inflammatory

background of senescence and EV-related signal export[37,38]. These studies form the theoretical starting point for the "intervenable targets" discussed throughout the review.

Contextual oncology evidence

The second level places senEVs into real therapeutic settings: how EVs released by TIS tumor cells or stromal cells after radiotherapy, chemotherapy, or targeted therapy influence the ecology of residual lesions, immune surveillance, recurrence risk, and responses to immunotherapy[2,3,12,13,16-20,32-35]. The key point of this type of evidence is the annotation of time window and cellular source: EV profiles induced by the same treatment at different stages may be completely different, thereby determining whether they favor clearance or recurrence.

The mitochondria–mtDNA–immunity axis

The third level focuses on a candidate directional switch that may explain why "the same type of senEVs" can exhibit opposite functions: how mitochondrial stress, mtDNA leakage, and their carriage and transmission in EVs reset the innate immune threshold in recipient immune cells and shape immunosuppressive networks[7,8,34,37-41]. This level of evidence serves as a bridge connecting "mechanisms of biogenesis" with "immune outcomes".

Translational strategies

The final level addresses "how to intervene," including inhibition of EV biogenesis/release (such as nSMase2-related pathways), neutralization of key cargos, blockade of recipient uptake, and the potential and risks of combining these approaches with senotherapy or the "one-two punch" strategy[51-54]. This part places greater emphasis on the strategic logic of designing interventions around treatment windows, tissue specificity, and controllable side effects[51-54].

MECHANISMS OF SENEV GENERATION: FROM DDR TO LIPID METABOLISM, ENDOSOMAL SYSTEM, AND DETOX-LIKE SECRETION

The biogenesis of senEVs is a multi-pathway regulated process that is closely associated with cellular stress response, lipid metabolism remodeling and organelle quality control in senescent cells. The core generation pathways include the DDR–ceramide–nSMase2 axis, endosome-MVB pathway, autophagy-mitochondrial quality control coupling, and plasma membrane budding, with key regulatory molecules and cargo loading mechanisms labeled in detail in Figure 2.

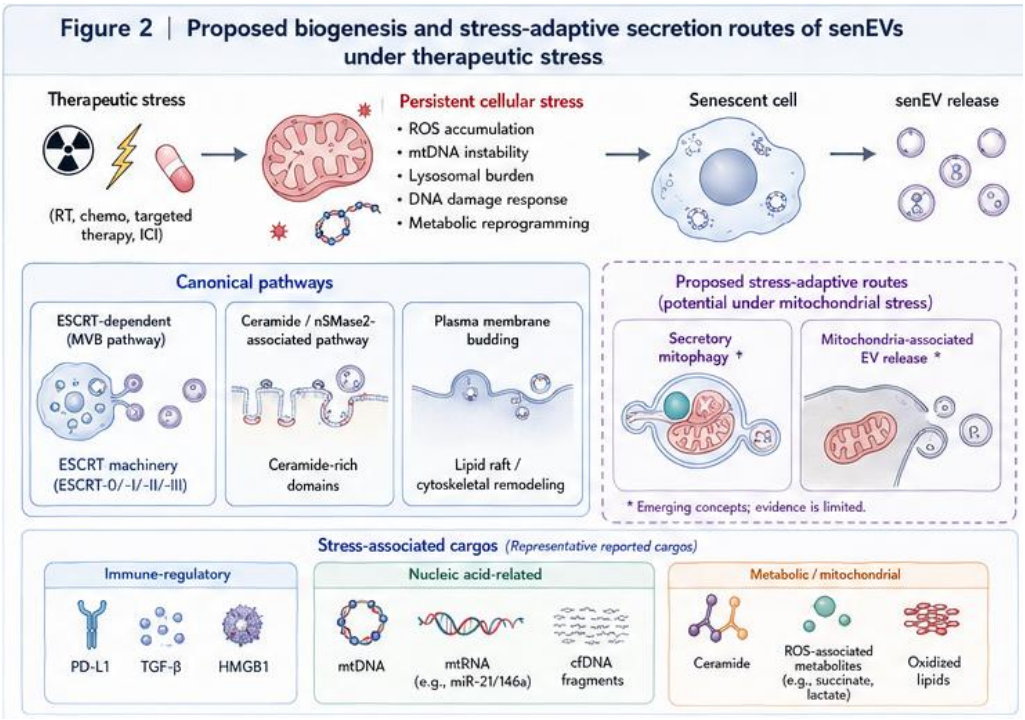

Figure 2. Peoposed biogenesis and stress-adaptive secretion routes of senEVs under therapeutic stress. Persistent therapeutic stress induces cellular senescence accompanied by DNA damage, oxidative stress, mitochondrial dysfunction, lysosomal burden, and metabolic remodeling. Under these conditions, senescent cells increase extracellular vesicle (senEV) release through multiple interconnected pathways, including ESCRT-dependent/endosomal multivesicular body (MVB) biogenesis, ceramide/nSMase2-associated secretion, and plasma membrane budding. In parallel, stress-adaptive routes such as secretory mitophagy and mitochondria-associated EV release may contribute to

the export of mitochondrial components under conditions of impaired degradative capacity. The figure also summarizes representative stress-associated cargos enriched in senEVs, including immune-regulatory proteins, nucleic acids, mitochondrial components, and metabolic mediators, which together may influence microenvironmental remodeling and immune responses.

Why is EV secretion markedly increased in senescent cells? Viewing EV release as a "homeostatic disposal channel"

Multiple studies have reported increased EV secretion across different senescence models, including replicative senescence, stress-induced senescence, and therapy-induced senescence (TIS). Rather than representing a passive by-product of SASP activation, this phenomenon may reflect a broader adaptive response to persistent intracellular stress. Senescent cells experience sustained burdens related to DNA damage, oxidative stress, organelle dysfunction, and impaired degradative capacity. Under these conditions, EV release may partially function as an alternative route for stress disposal and homeostatic adaptation [Figure 2]. Senescent cells face multiple burdens—persistent DNA damage, accumulation of oxidized proteins/lipids and metabolic by-products, and increased pressure on lysosomal and mitochondrial quality control. When conventional degradative pathways (such as the autophagy–lysosome system) are constrained or reprogrammed, cells may divert a portion of molecular or organellar components that are "difficult to degrade or unsuitable to retain" into secretory pathways, thereby achieving particulate export through EVs[8,40,51].This export may be regarded as a form of detox-like secretion: at the intracellular level, it helps maintain survival and homeostasis, but at the tissue level, it externalizes stress signals and thereby reshapes the microenvironment and immune responses[6,10,11]. This also provides a starting explanation for why senEVs display a double-edged effect in different contexts[2,3,12,13,32].

From a systems perspective, the secretory system of senescent cells contains at least two parallel channels:(a)Liquid-phase SASP: soluble cytokines/chemokines/proteases and related factors, emphasizing diffusion and receptor binding[22-25]; (b)Particulate SASP

(EVs): emphasizing membrane encapsulation, long-distance delivery, and information integration enabled by "cargo combinations"[27-31].

Under persistent damage, the advantage of particulate SASP is that it allows cells to dispose of and propagate signals in a more selective manner, outputting not only immune-alerting cues but also immune-suppressive or tolerance-promoting cues[6,10,11,16]. Understanding this point is critical for the later discussion of which upstream signals determine EV abundance and cargo loading[27-31].

The DDR→ceramide/nSMase2 axis: One of the most translatable switches of biogenesis

Among the upstream regulatory mechanisms proposed so far, the coupling between the DNA damage response (DDR) and the sphingolipid metabolism/sphingomyelinase axis provides one of the clearest and most druggable explanations for why senescence increases sEV release[51-54]. The core logic may be summarized as follows: DNA damage does not only trigger cell-cycle arrest and transcriptional remodeling in the nucleus; through membrane lipid metabolic reprogramming, it also alters endosomal membrane dynamics and the probability of vesicle budding, thereby increasing EV release[51-54]. This is consistent with the 2020 study on senescence-associated EV release: DNA damage can downregulate SMS2 and upregulate nSMase2, activate the ceramide pathway, increase SA-EV biogenesis, and participate in the removal of cytoplasmic DNA fragments while limiting excessive inflammatory responses.

At the mechanistic level, ceramide is considered an important lipid molecule that promotes membrane curvature changes and microdomain formation. It can drive inward budding of endosomal membranes to form ILVs (intraluminal vesicles), thereby providing a structural basis for the generation of exosome-like sEVs[52,60]. neutral sphingomyelinase 2(nSMase2)Neutral sphingomyelinase 2 (nSMase2), by hydrolyzing sphingomyelin to generate ceramide, is regarded as a key node linking DDR to sEV

release[51-54,60]. Further studies have indicated that nSMase2 not only promotes membrane budding by providing ceramide, but may also influence the fate choice of multivesicular bodies (MVBs) by regulating endosomal acidification: when MVBs are more inclined to fuse with the plasma membrane rather than enter the lysosomal degradation pathway, secretory output is enhanced and sEV release correspondingly increases[52]. This is consistent with the 2022 *Journal of Cell Science* study: nSMase2 counteracts V-ATPase-mediated luminal acidification of MVBs and thereby shifts MVB fate toward sEV secretion.

Two points need to be emphasized. First, this axis is not a pathway that "only increases secretion"; it may also be embedded in inflammatory negative feedback (preventing excessive inflammation or cell death), suggesting that EV release in some contexts functions as a homeostatic protective mechanism[51]. Second, DDR does not regulate EVs only through nSMase2: the p53 axis has also been shown to regulate exosome secretion under stress through its target gene TSAP6/Steap3, constituting another interpretable "DDR–p53–secretory pathway" chain[61-65]. This is consistent with the results reported in *Cancer Research* (2006) and *Cell Death & Differentiation* (2008): the p53 response can enhance exosome production, and the DNA damage-induced p53-dependent secretory pathway is markedly impaired when TSAP6/Steap3 is absent. This means that, even when EV increase is similarly "driven by DNA damage," the upstream modules may differ according to cell type and damage spectrum, thereby influencing cargo profiles and immune effects[51-54,61,62,65-67].

The endosome–multivesicular body–exosome biogenesis framework: How senescence alters "endocytic–secretory flux" and cargo loading

From the perspective of cell biology, the core biogenesis pathway of exosome-like sEVs is as follows: plasma membrane internalization forms early endosomes → endosomes mature and form MVBs → MVBs fuse with the plasma membrane and release ILVs extracellularly (at which point ILVs are referred to as exosomes / part of sEVs)[37-39,64-69].

Two types of key decisions exist in this process: (a)how ILVs are formed and which cargos are loaded (membrane budding and cargo sorting); (b)whether MVBs ultimately proceed toward secretion or degradation (fusion with the plasma membrane vs fusion with lysosomes).

Classic EV reviews have summarized multiple modules involved in ILV formation and cargo sorting, including ESCRT-dependent and ESCRT-independent mechanisms, lipid raft/sphingolipid-related mechanisms, and sorting mechanisms involving membrane protein microdomains such as tetraspanins[64,68-72]; At the level of MVB transport and fusion, Rab family small GTPases, the cytoskeleton, and membrane fusion modules together determine the efficiency of vesicle trafficking and membrane fusion. Among these, Rab27a/Rab27b have been shown to function at different steps of the exosome secretion pathway, and the syndecan–syntenin–ALIX axis has been shown to participate in exosome formation and endosomal intraluminal budding.

When this “general framework” is placed into the senescent state, a key inference emerges: senescence does not merely alter one sorting protein, but may systemically rewrite endocytic–secretory flux[6,10,11,29-31,51-54]. For example, senescent cells are often accompanied by increased cell size, elevated lysosomal/endosomal burden, and changes in acidification/degradative capacity. In this context, MVBs that would originally be more likely to be sent to lysosomes for degradation may instead be more frequently redirected toward secretory release, thereby increasing total sEV output[51-54]. In addition, senescence-associated transcriptional programs (including p53-related modules) may indirectly alter the probability of MVB secretion and cargo-loading preference by affecting key nodes in membrane trafficking[61-63,65,67]. In other words, the increase in EVs in the senescent state may result both from increased production and from pathway rewiring from degradation to secretion[51-54].

Therefore, when discussing the mechanisms of senEV biogenesis, a more operational research question is: (a)in a given senescence model, does EV increase mainly result from enhanced ILV formation, or from MVB fate being biased toward secretion? (b)do cargo changes mainly result from altered sorting mechanisms, or from changes in the intracellular pool of materials awaiting disposal (for example, accumulation of damaged DNA or mitochondrial components)?

These questions directly determine the choice of later intervention points: if the major driver is the nSMase2/ceramide-dependent membrane budding process, lipid-metabolism targeting is more relevant; if the major driver is altered endosomal trafficking/lysosomal fate, then Rab/acidification/fusion-related modules as well as lysosomal functional status need closer attention[51-54,60,64,68-72].

Coupling with autophagy/mitochondrial quality control: A detox-like secretion model from "degradation" to "secretory clearance"

Under senescent conditions, homeostatic pressure is manifested not only at the levels of DNA and membrane trafficking, but also, more centrally, in the autophagy–lysosome system and mitochondrial quality control[8,40,41,51]. Autophagy normally undertakes the degradation of cytoplasmic protein aggregates and damaged organelles; however, under prolonged stress and functional reprogramming, autophagic pathways may exhibit "insufficient capacity or altered flux," leading to easier accumulation of danger signals such as damaged mitochondria, oxidatively damaged components, and cytoplasmic DNA fragments[7,8,37-41,51]. At this point, cells may adopt an alternative strategy: diverting some contents that would otherwise be degraded into secretory routes and achieving secretory clearance through EVs[8,40,51].

From this perspective, "detox-like secretion" has two levels of meaning: (a)intracellular homeostasis level: EV-mediated export reduces the retention of damaged mitochondria/oxidative products/nucleic acid fragments in the cytoplasm, thereby

relieving stress and maintaining survival[8,40]; (b)microenvironmental signaling level: exported mitochondrial components or DNA/mtDNA may be read by neighboring cells or immune cells as DAMP-like signals, thereby translating an “intracellular crisis” into a “tissue-level immune/inflammatory program”[7,8,37-41].

Recent studies on secretory mitophagy and mitochondria-encapsulating EVs provide more concrete biological carriers for this model: damaged mitochondria may not only be degraded through classical mitophagy, but may also leave the cell in a “vesicularized and secreted” form[8,40]. The 2025 *Frontiers* study directly proposed that, under oxidative stress, tumor cells can bypass lysosomes and export damaged mitochondrial fragments via EVs, a process termed secretory mitophagy; meanwhile, a 2024 review systematically discussed the types, features, and potential biogenetic mechanisms of mitochondria-encapsulating EVs. For tumor or stromal cells under senescence or therapeutic stress, such secretory clearance may represent both a stress adaptation (helping cells survive under oxidative pressure) and an important upstream factor directing immune outcomes[7,8,34,39-41].

The possible contribution of plasma membrane-budding EVs (microvesicles/ectosomes) in senescence: Membrane tension, Rho-GTPases, and ESCRT-III

In addition to endosome-derived sEVs, senescent cells may also increase the direct plasma membrane budding and release of microvesicles/ectosomes[73-76]. This type of EV relies more heavily on plasma membrane–cortical cytoskeleton remodeling and changes in membrane lipid asymmetry, and its formation commonly involves small GTPase signaling, membrane lipid metabolism, myosin light-chain phosphorylation, and relaxation of the membrane skeleton[74-76]. Among the classic studies, work on ARF6-regulated shedding showed that the ARF6 GTP/GDP cycle can regulate plasma membrane-derived microvesicle release through the phospholipase D–ERK–MLCK axis. Under the senescent background, increased cell size, altered membrane tension and

cytoskeletal homeostasis, and persistent chronic inflammatory kinase signaling may all increase the probability of plasma membrane budding, thereby contributing to the overall rise in EVs[6,10,11,73-76].

It is worth noting that ESCRT does not function only in inward budding within endosomes. In plasma membrane-budding microvesicles, ARRDC1 can recruit ESCRT-I components such as TSG101 through its PSAP motif, thereby driving budding and scission at the plasma membrane and forming ARRDC1-mediated microvesicles. This suggests that senescence-related changes in ESCRT availability/localization may simultaneously affect both the "endosomal budding" and "plasma membrane budding" pathways.

The "selective rules" of cargo loading: Ubiquitination–ESCRT, the glycocalyx–syntenin axis, and RNA-binding proteins–sequence motifs

The reason senEVs can exert strong paracrine effects lies not only in increased quantity, but also in the systematic shift of cargo loading[6,10,11,16]. For protein cargo, the classic model is that membrane proteins enter ILVs through ESCRT-related sorting, whereas the syndecan–syntenin–ALIX pathway is more inclined to selectively load particular signaling complexes and membrane proteins[29-31,69,70,77]. Under senescence/therapeutic stress, changes in membrane protein glycosylation/shedding and endocytic recycling may systemically drive specific "pro-inflammatory/pro-survival/immune-regulatory" cargos into EVs through these sorting axes.

With regard to RNA cargo, growing evidence suggests that loading is not "random": specific RNA-binding proteins (RBPs) can recognize RNA sequence or structural motifs and mediate their entry into exosomes. For example, hnRNPA2B1 can recognize specific motifs in miRNAs, and its SUMOylation status affects miRNA loading efficiency. Combined with the LDELS model, a more senescence-related inference can be proposed: stress translation and RNA metabolic reprogramming in senescent cells may, by altering

RBP modification states and autophagy–secretion coupling, selectively enrich a set of miRNAs and RBP complexes that "maintain senescence/educate immunity," thereby amplifying paracrine senescence and immune remodeling.

It should be pointed out that the above "selective loading" determines not only which molecules senEVs carry, but also, at a deeper level, what kind of stress information is ultimately read by the immune system. Especially under therapeutic stress where mitochondrial dysfunction, elevated ROS, and nucleic acid leakage coexist, whether mitochondrial-related components and mtDNA are preferentially loaded into EVs, and how they subsequently trigger different innate immune programs in recipient cells, may be precisely the critical dividing line explaining the dual effects of senEVs. On this basis, the next section will specifically discuss the mechanistic links among mitochondrial stress, mtDNA export, and EV-mediated immune remodeling.

MITOCHONDRIAL SIGNALING: FROM "MITOCHONDRIAL SENESCENCE" TO "MITOCHONDRIAL EV CARGO" AND THEN IMMUNE OUTCOMES

Mitochondrial stress and the subsequent release of mtDNA-containing senEVs are the core upstream switches determining the immune directionality of senEVs, and the underlying bidirectional immune regulatory pathway is illustrated in detail in Figure 3.

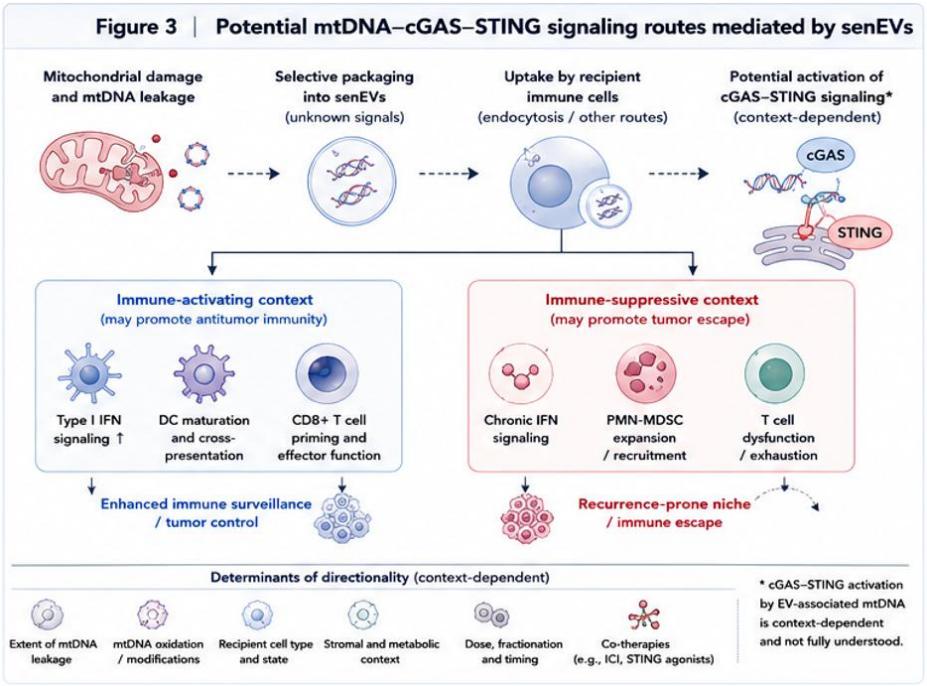

Figure 3. Potential mtDNA-cGAS-STING signaling routes mediated by senEVs. Therapeutic stress and mitochondrial dysfunction may promote mtDNA leakage and the selective incorporation of mitochondrial components into senEVs. Following uptake by recipient immune cells, EV-associated mtDNA may contribute to context-dependent activation of innate immune signaling pathways, including cGAS–STING-related responses. Depending on recipient cell type, treatment context, and signaling duration, these pathways may either favor transient immune activation characterized by dendritic-cell maturation, type I interferon signaling, and effector T-cell priming, or promote chronic immunosuppressive remodeling associated with PMN-MDSC expansion, persistent inflammatory signaling, and T-cell dysfunction. The figure highlights the proposed role of mitochondrial stress signaling as a potential directional regulator linking senEV biogenesis to divergent immune outcomes.

Why is mitochondrial/nucleic acid stress more likely to determine the immune directionality of senEVs?

Building on the previous discussion of senEV biogenesis and cargo loading, a more integrative question is this: why do EVs produced under the same therapeutic or senescent state sometimes promote immune clearance, yet at other times drive immunosuppression

and recurrence[7,8,34,37-41]? The key may not lie in whether EVs are present, but rather in what type and what intensity of stress information they carry. Under conditions of aggravated mitochondrial stress, cells are more prone to elevated ROS, altered membrane permeability, abnormal exposure of mitochondrial components, and nucleic acid leakage, and can package these "danger/stress cues" into EVs for delivery to immune cells as well as tumor–stromal cells, thereby resetting the innate immune threshold and inflammatory response mode of recipient cells[7,8,34,37-41]. These observations raise the possibility that mitochondrial stress may influence the functional polarization of senEV signaling[7,8,34,37-41].

Within this framework, mitochondria should not be understood merely as metabolic organelles, but rather as a source of immune information. When mitochondrial stress is relatively low and the system is still within the acute post-treatment clearance window, the associated nucleic acids and stress signals are more likely to be interpreted as "alarms," thereby enhancing immune surveillance. By contrast, when mitochondrial stress persists over time and is compounded by post-treatment residual cells, myeloid expansion, and a background of chronic inflammation, similar signals are more likely to be translated into immunosuppression and tolerance, ultimately promoting recurrence and poor responses to immunotherapy. In other words, the real significance of mitochondria-related EV cargo lies in its ability to externalize an intracellular quality-control crisis and convert it into changes in the immune niche at the tissue scale.

Mitochondrial quality control and the generation of "mitoEVs/mtDNA-EVs": From secretory mitophagy to adaptive extrusion

Senescence and therapeutic stress are usually accompanied by mitochondrial dysfunction, ROS accumulation, and disruption of membrane homeostasis, thereby increasing the risk of mtDNA leakage and leading to the accumulation of mitochondrial fragments. Recent studies on mitoEVs, mitochondria-encapsulating EVs, and secretory mitophagy suggest that when the pressure on mitochondrial quality control becomes too high, or when the

classical lysosomal degradation pathway is constrained, cells may expel mitochondrial components in the form of EVs as an adaptive strategy of "secretory clearance"[8,40]. Although contamination remains a technical concern, several studies support the idea that mitochondrial cargo loading can occur in an organized manner under stress conditions.

The key significance of this process is that it "packages and externalizes" intracellular nucleic acid and mitochondrial stress signals[7,8,37-41]. When EVs containing mtDNA or other mitochondria-related molecules are taken up by immune cells, DNA-sensing pathways such as cGAS–STING may be reactivated in recipient cells, thereby extending what was originally a stress response confined to donor cells into immune remodeling at the tissue level[37,38]. An unresolved question is whether impaired mitochondrial quality control preferentially drives degradation or extracellular extrusion pathways.

cGAS–STING and "abnormal nuclear/cytoplasmic DNA" as the core of senescence-associated inflammation

The chronic inflammatory background of senescent cells does not arise out of nowhere. One repeatedly emphasized central axis is that abnormal cytoplasmic DNA is recognized by the DNA-sensing system and drives inflammatory transcriptional programs and paracrine senescence through cGAS–STING[37,38]. Classic studies have shown that cytoplasmic chromatin fragments (CCFs) can form during senescence and, once recognized by cGAS, trigger STING-related signaling, thereby amplifying inflammation and SASP output[37,38]. This indicates that DNA sensing is not merely a downstream accompanying phenomenon of senescence, but rather an important mechanistic support for maintaining its inflammatory phenotype.

When this axis is placed in the EV context, its significance expands further. DNA sensing need not occur only within donor cells; if senescent cells export DNA/mtDNA and other nucleic acids as EV cargo, then the DNA-sensing axis may itself become "externalized"—that is, reactivated in recipient immune cells—thereby escalating single-

cell stress into remodeling of the immune niche at the tissue scale. Therefore, senEVs are not only a particulate form of SASP, but may also serve as carriers for the propagation of DNA-sensing signals. The key question is no longer simply whether they carry nucleic acids, but rather who takes up those nucleic acids, when and where they are recognized, and what kind of immune program is ultimately induced.

Secretory mitophagy: A key bridge from quality control to the externalization of immune signals

In the classical view, damaged mitochondria are mainly degraded through mitophagy after delivery to lysosomes. However, secretory mitophagy proposes a supplementary model that is better suited to explaining conditions of sustained therapeutic stress: when the mitochondrial damage burden is too high, or when autophagy–lysosome flux is insufficient, cells may carry out "secretory clearance" by exporting some damaged mitochondria in EV form, thereby relieving intracellular stress and maintaining survival[8,40]. This means that "mitochondrial quality control" and "EV biogenesis" are no longer two independent pathways, but may instead become deeply coupled in the senescent state.

When this concept is brought back into the TIS/senescence context, its theoretical importance lies in providing an interpretable upstream mechanism for why senescent cells release more mitochondria-related cargo[8,40]. More importantly, it links mitochondrial quality control to immune regulation: (a)For donor cells, this is a form of "detox-like secretion" that favors homeostasis and survival[8]; (b)For tissues/the immune system, it amounts to releasing damaged mitochondria and nucleic acids as potential DAMP-like signals into the extracellular space, making them available as sources of stimulation—or tolerance—for immune cells to interpret[7,8,37-41].

Accordingly, secretory mitophagy may be a key bridge for understanding how mitochondrial stress is translated into immune outcomes.

mtDNA/cytoplasmic DNA → cGAS–STING: EVs "externalize" the DNA-sensing axis, but the direction depends on the recipient and the time window

As discussed in Section 4.3, cGAS–STING is an important endogenous axis of senescence-associated inflammation; when EVs carry DNA/mtDNA and are taken up by immune cells, this axis may be re-triggered in recipient cells[37,38][Figure 3].The key issue here is not simply whether activation can occur, but in which cells it occurs, and with what intensity and duration: (a)If it occurs in cell populations capable of effectively initiating antitumor immunity (for example, nodes that promote antigen presentation and effector priming), it may bias the system toward immune activation[12,13,37,38]; (b)If it occurs in myeloid populations that are more prone to forming immunosuppressive networks, or if it occurs as chronic low-intensity stimulation, then it may instead be translated into immunosuppression and tolerance, ultimately promoting immune escape[16-20,34].

Therefore, EV-associated mtDNA is neither an inherently antitumor label nor an inherently protumor one; it is better understood as a directionally variable regulator of the immune threshold[7,8,37-41].

A key closed loop of evidence: mtDNA signals released by senescent tumor cells enhance PMN-MDSC-mediated immunosuppression

Recent work provides a strong mechanistic closed loop for the "pro-suppressive" direction: mtDNA signals released by senescent tumor cells can enhance PMN-MDSC-driven immunosuppression through a cGAS–STING-related mechanism, thereby promoting immune escape[34]. As explicitly stated in the source text, the abstract and supplementary materials of the 2025 *Immunity* paper indicate that senescent tumor cells release mtDNA into the extracellular space, and that internalized mtDNA activates the cGAS–STING–NF-κB pathway in PMN-MDSCs, thereby amplifying tumor immunosuppression.

This chain is important because it connects "senescence → mtDNA → myeloid suppression → immune escape" into a causal pathway that can be experimentally tested, and it naturally fits the real post-treatment scenario in cancer: senescence-like residual cells persist after therapy, myeloid networks are readily reprogrammed, and responses to immunotherapy are constrained by the surrounding niche[1-4,16-20,32-36].

This chain also points to an important translational proposition: in certain tumors and treatment combinations, inhibiting the extrusion/propagation of mitochondrial nucleic acid signals may be more selective and controllable than broadly suppressing all EVs[7,8,34,39-41].

Mitochondria-enriched EVs and the PD-L1/ROS axis: Immune escape and druggable nodes

In addition to the "nucleic acid–cGAS–STING–myeloid suppression" axis, another direction deserves emphasis: the coupling of mitochondria-enriched EVs with ROS status and checkpoint-related immunosuppressive pathways may constitute a new level of immune escape[16-20,34,35]. At the same time, studies showing that "super mitochondria-enriched EVs" promote mitochondrial transfer further suggest that mitochondria-enriched EVs are not only signal carriers, but may also function as transport tools for functional mitochondria and metabolic capacity[35]. The source text notes that the 2025 *Nature Communications* study showed that MSCs can release EV-Mito, and that this release is regulated by the CD38/IP3R/Ca2+ pathway.

From a translational perspective, the value of such mechanisms lies in the fact that they naturally contain druggable nodes: (a)EV biogenesis/loading nodes: limiting the entry of mitochondrial cargo into EVs or restricting the release of particular EV subtypes[7,8,39-41]; (b)ROS and metabolic nodes: reducing mitochondrial stress and ROS-driven signaling at the source, thereby decreasing the output of "immunosuppressive mitochondrial

information"[7,8,39-41]; (c)Checkpoint nodes: combining these approaches with PD-1/PD-L1 blockade in immunotherapy to test whether mitochondria-enriched EVs are upstream drivers of resistance[16-20].

It should be emphasized that the source text recommends a more "node-based" and stratified intervention strategy here: first identify which class of mitochondrial cargo (mtDNA? mitochondrial proteins/metabolites? structural transfer?) is driving immunosuppression, and then select the corresponding minimal intervention set, so as to avoid suppressing physiological EV functions at the same time[7,8,39-41].

Cancer-cell metabolic reprogramming: EV-mediated mitochondrial transfer/functional remodeling and drug resistance

From a broader tumor-biology perspective, the significance of mitochondrial EV cargo is not limited to immunity; it also extends to post-treatment adaptation and the evolution of drug resistance. EVs carrying mitochondria-related components may help recipient cells undergo metabolic reprogramming, enhance stress tolerance, and alter treatment sensitivity, thereby making it easier for residual cells to cross the post-treatment "bottleneck period"[35].

What is being emphasized here is not the classical proliferative signaling pathway, but rather the transferability of more fundamental capacities related to energy metabolism and stress tolerance[34,35]. Therefore, future studies of mitoEVs cannot stop at the descriptive level of merely detecting mtDNA or mitochondrial markers. They must go on to determine whether these cargos are membrane-protected, whether they truly enter specific recipient cells, whether they remodel the metabolic state of those cells, and whether they ultimately alter therapeutic responses. Only by linking "detection–localization–function–outcome" into a complete chain can mitochondrial EV cargo move from being an interesting phenomenon to becoming a usable mechanistic explanation.

Summary: Why mitochondrial signaling constitutes an upstream switch for senEV directionality

In summary, mitochondrial signaling deserves to be separated from the original chapter on biogenesis mechanisms because it is not merely one subcategory within the senEV cargo spectrum. Rather, it forms a longitudinal main line connecting "intracellular homeostatic crisis," "selective EV cargo loading," "innate immune sensing," "formation of myeloid suppressive networks," and the "evolution of post-treatment resistance/recurrence"[7,8,37-41]. From imbalance in mitochondrial quality control, to secretory mitophagy and mitoEV formation; from mtDNA extrusion, to reactivation of the cGAS–STING axis in recipient cells; and then to PMN-MDSC-driven immunosuppression and post-treatment niche remodeling, this series of events collectively indicates that what determines the functional direction of senEVs is often not the EVs themselves, but rather how the mitochondrial/nucleic acid stress information they carry is decoded by specific recipient cells within specific time windows.

For precisely this reason, mitochondrial signaling provides a more mechanistically explanatory intermediate layer for the later discussion of the "double-edged sword" effects of senEVs in cancer therapy: it explains why EVs of similar origin and similar abundance may lead to different outcomes, and it also suggests that future translational interventions should not stop at "suppressing the total amount of EVs," but should instead move toward "identifying and intercepting high-risk mitochondrial information packages." On this basis, the next section returns this mechanistic framework to the context of cancer therapy and specifically discusses how senEVs can exhibit both tumor-promoting and tumor-suppressive effects after treatment.

THE DOUBLE-EDGED ROLE OF SENEVS IN CANCER THERAPY: TUMOR-PROMOTING AND TUMOR-SUPPRESSIVE MECHANISMS

Cancer therapies (radiotherapy, chemotherapy, targeted therapy, *etc.*), while inducing tumor cell death, also generate a population of cells in both tumor and stromal

compartments that are not killed but instead enter therapy-induced senescence (TIS) / senescence-like reprogramming[1-4,32-36]. These cells exert persistent effects on the microenvironment with SASP as the core, and EVs, as a form of "particulate SASP," are able to package and deliver complex cargos over long distances, making post-therapy signal export no longer merely "diffusion of soluble factors," but rather a more structured and targetable network of particulate signaling[5,6,10,11,16]. The key paradox is that particles collectively referred to as senEVs may either amplify immune clearance and suppress recurrence, or provide a niche for residual cells that supports proliferation/invasion and immune escape[4,10,11,16,36]. Accordingly, this section presents the evidence chains for both tumor-promoting and tumor-suppressive effects within the same logical framework, and then summarizes the key variables that determine directionality[Figure 4].

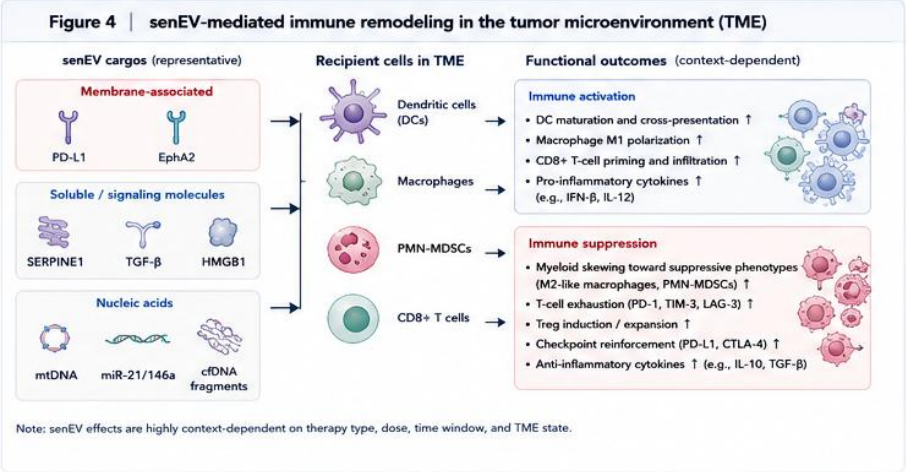

Figure 4. SenEV-mediated immune remodeling in the tumor microenvironment. senEVs released from therapy-induced senescent cells carry diverse membrane-associated proteins, soluble signaling molecules, nucleic acids, and mitochondrial components that collectively influence immune-cell behavior within the tumor microenvironment. Representative cargos such as PD-L1, EphA2, SERPINE1, TGF-β, mtDNA, and regulatory miRNAs may modulate dendritic cells, macrophages, PMN-MDSCs, and effector T cells through multiple signaling pathways. Depending on the immune context and treatment stage, senEV signaling may either support antitumor immune activation and immune surveillance or reinforce immunosuppressive remodeling

characterized by myeloid-cell reprogramming, checkpoint reinforcement, extracellular matrix remodeling, and T-cell dysfunction.

Tumor-promoting example 1: senEVs drive regrowth of residual cells through pro-proliferative signals such as EphA2

In the tumor-promoting direction, the most intuitive mechanism is that senEVs directly deliver pro-proliferative / pro-survival membrane proteins or signaling complexes to recipient tumor cells, thereby re-igniting growth pathways in cell populations that, after treatment, "should have remained suppressed"[42]. Classic work has suggested that small EVs secreted by senescent cells can be enriched in EphA2, and through membrane receptor-ligand interactions and downstream signaling cascades, activate proliferative programs in recipient cells, ultimately manifesting as enhanced tumor cell proliferation and the establishment of a growth advantage[42].

This type of mechanism deserves particular attention in the therapeutic context: after treatment, the microenvironment often simultaneously contains DNA damage stress, chronic inflammatory background noise, and fluctuations in nutrient and oxidative stress, making tumor cells more sensitive to exogenous signals.If senEVs provide a "sufficiently strong growth drive" at this point, then even if the overall tumor burden decreases, a small number of residual cells may still be "restarted" and become seeds of recurrence[42]. From the perspective of mechanistic intervention, the key nodes in this kind of "receptor-ligand / membrane protein-driven" tumor-promoting chain include the selective loading of specific membrane proteins after donor cells enter senescence, the stable persistence of EVs in the microenvironment, and the efficiency of EV uptake / membrane fusion in recipient cells[29-31,42,77]; These nodes also provide clear target concepts for subsequent strategies aimed at "blocking key cargos" or "blocking uptake"[42].

Tumor-promoting example 2: EVs from TIS tumor cells promote CRC progression—the SERPINE1-NF-kB axis as closed-loop evidence for "post-treatment progression"

Compared with the direct delivery of pro-proliferative signals, another tumor-promoting chain that is closer to clinical reality arises from the senescence-like tumor cells that remain after treatment: these cells are both products of therapeutic stress and participants in the evolution of recurrence. A colorectal cancer (CRC) example provides a more complete closed loop: EVs released by therapy-induced senescence-like tumor cells undergo changes in both quantity and cargo profile, and EVs enriched in SERPINE1 can promote key transcriptional events such as NF-kB p65 nuclear translocation in recipient cells or microenvironmental cells, thereby driving phenotypes associated with tumor progression[32].

The importance of this evidence chain is that it links "therapy - senescent residual cells - EVs - progression / recurrence risk" into an interpretable causal pathway[2,32]: (a)Upstream trigger: therapeutic stress drives a fraction of tumor cells into a TIS / senescence-like state; (b)Intermediate carrier: the senEVs released by these cells undergo a shift in cargo loading; (c)Downstream readout: key inflammatory / survival pathways in recipient cells (such as NF-kB) are re-ignited, forming transcriptional programs that promote invasion, survival, and microenvironmental remodeling.

From the perspective of why this is tumor-promoting, pathways such as NF-kB sit precisely at the nodes most easily amplified in the post-treatment microenvironment: they can drive intrinsic tumor-cell survival and invasion, while also further shaping myeloid suppression and chronic inflammatory background noise through cytokine networks, thereby extending tumor-promoting signals from an "intracellular event in tumor cells" to an "event at the niche level"[1,5]. Therefore, the SERPINE1-NF-kB chain is not only one tumor-promoting example, but also suggests that EVs from therapy-induced

senescent cells may be a key mediator that translates “treatment-induced damage” into “chronic tumor-promoting inflammation and tissue remodeling”[2-4,32-36].

Tumor-suppressive example: senEVs coordinate immune surveillance and suppress recurrence—“particulate SASP” can cooperate with immune clearance

Alongside the tumor-promoting chains described above, recent studies have also reached the opposite conclusion: senEVs can suppress cancer recurrence by coordinating immune surveillance[14,15]. A 2025 *Cancer Research* study positioned senEVs as signal carriers capable of “coordinating” immune clearance at the tissue level: under specific contexts and within specific time windows, senEVs may promote immune-system recognition and sustained surveillance of residual lesions, thereby reducing the probability of recurrence[12].

It should be emphasized that this tumor-suppressive direction does not mean that senEVs are intrinsically antitumor, but more likely reflects a condition-dependent combination of mechanisms[12,13]: (a)Within the acute post-treatment window, EVs released by senescence-like cells may be enriched in danger signals or stress cues that can be read by innate immunity, thereby promoting the maturation of antigen-presenting cells, enhancing immune-cell recruitment, and forming more effective cooperation among effector cells[5,12,13]; (b)Provided that the immune niche permits clearance to occur (for example, when the MDSC burden is not high and immunosuppressive axes have not been fully established), this particulate signaling is more likely to be “read as an alarm” rather than “read as tolerance”[12,13].

A commentary in the same journal further highlighted several key unresolved questions: exactly which EV cargos, acting on which immune-cell subsets, and under what therapeutic and tissue backgrounds, cause senEVs to shift from “tumor-promoting signal packages” to “coordinators of immune surveillance”[13]. The existence of these questions itself indicates that research on senEVs must move toward a refined correspondence of

"subtype - cargo - recipient cell - outcome," rather than remaining at the coarse-grained level of simply describing "more EVs / fewer EVs"[6,10-13,16].

Determinants: Source cells, treatment type, time window, and immune background noise—a four-variable framework for explaining the "double-edged sword"

Taken together, the tumor-promoting and tumor-suppressive evidence supports a "four-variable framework" for explaining directional differences: source cells, treatment type, time window, and immune niche (background noise)[4,36].

Source cells: Tumor cells vs. stromal cells (such as CAFs) vs. immune cells

Even when all are called senEVs, if they originate from tumor cells, their cargos are more likely to directly serve tumor adaptation and evolution (such as survival, invasion, and metabolic reprogramming); by contrast, those derived from stromal cells are more likely to promote or suppress tumors indirectly through ECM remodeling, paracrine circuits, and immune-cell education. Differences in source determine the starting point of the "cargo pool," and also determine the spectrum of recipient cells and spatial distribution[6,10,11,16].

Treatment type: Different stress spectra induced by radiotherapy / chemotherapy / targeted therapy

Different treatments induce different types and intensities of damage: the relative contributions of DNA double-strand breaks, replication stress, ROS, and mitochondrial dysfunction differ, and this changes the molecular background of the senescence program, thereby altering the logic of EV loading[51-54]. This also means that, even within the same tumor type, senEVs may move in entirely different functional directions under different treatment strategies.

Time window: Acute "clearance window" vs. chronic "residual niche"

The effects of senEVs are highly time-dependent: in the acute phase they are more likely

to promote immune recruitment and clearance (consistent with "senescence surveillance"), whereas in the chronic residual phase they are more likely to drive consolidation of immunosuppressive networks, tissue remodeling, and the formation of a soil favorable for recurrence[14,15]. In other words, EVs released by the same population of cells may be decoded differently by the immune system at different stages[12,13].

Immune niche / background noise: Immune-hot vs. immune-cold status, MDSC burden, and the strength of immunosuppressive axes

When the tumor is in an "immune-hot" state, with effective antigen presentation and good effector-cell infiltration, senEVs are more likely to be translated into immune-clearance signals; conversely, when MDSCs are abundant or immunosuppressive axes dominate, the same danger signals may instead be reshaped into "chronic inflammation + tolerance," ultimately promoting recurrence and therapeutic resistance[34].

IMMUNE REGULATION: HOW senEVs SHAPE ANTITUMOR IMMUNITY AND RESPONSES TO IMMUNOTHERAPY

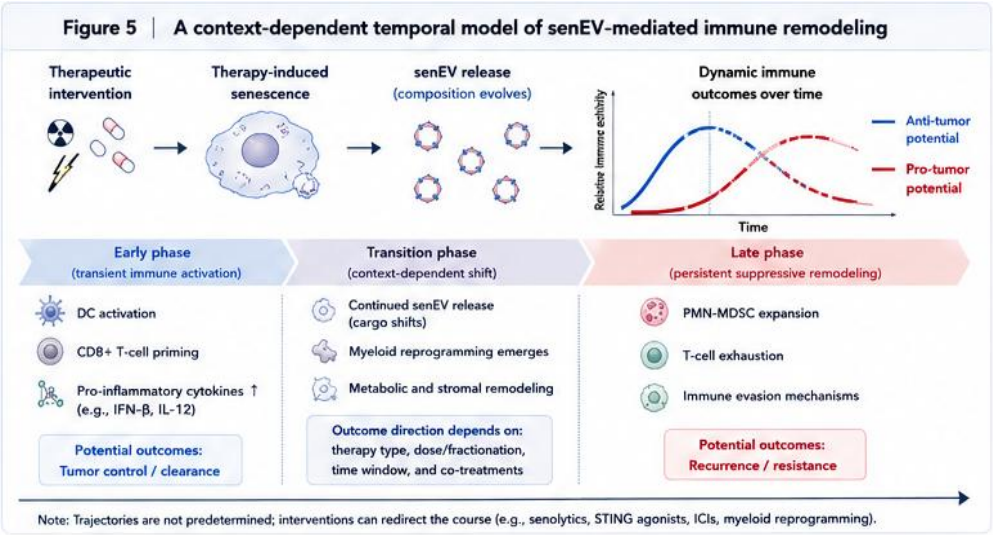

Figure 5. A context-dependent temporal model of senEV-mediated immune remodeling. The immunological effects of senEVs may vary dynamically across different therapeutic stages. During the early post-treatment phase, senEVs may function as transient immune-alert signals by delivering antigens, danger-associated molecular patterns, and inflammatory cues that support dendritic-cell activation, antigen cross-

presentation, and effector immune-cell recruitment, thereby favoring immune surveillance and tumor control. In contrast, persistent low-intensity senEV signaling during the chronic residual phase may promote progressive immunosuppressive remodeling through PMN-MDSC expansion, chronic inflammatory signaling, metabolic stress, and checkpoint-associated T-cell dysfunction, ultimately contributing to recurrence-prone niches and therapy resistance. The figure illustrates a proposed context-dependent transition from transient immune activation to persistent suppressive remodeling.

If Chapter 5 emphasizes that "tumor outcomes can be either promoted or suppressed," then one of the most central levels determining the direction of those outcomes is the immune system: the immune system may either clear senescent/residual cells, or be reprogrammed under chronic stress into immunosuppression and tolerance[12-15]. As stably transportable particulate signals, EVs are naturally suited to mediate immune regulation across cells, across space, and across therapeutic time windows[29,31]. Accordingly, this chapter adopts the framework of "the time dimension + the cancer-immunity cycle" to systematically discuss the bidirectional roles of senEVs in innate and adaptive immunity, as well as their potential effects on responses to immunotherapy[Figure 5].

The physiological/pathological duality of SASP: Immune recruitment and tissue repair vs chronic inflammation and immunosuppression

SASP has a typical biphasic nature: in physiological settings or acute injury-repair settings, SASP can promote immune-cell recruitment and tissue regeneration; under chronic, persistent stress, however, SASP may generate a low-grade but long-lasting inflammatory background, drive tissue remodeling, and promote the establishment of immunosuppressive networks, thereby facilitating tumor progression. When this framework is mapped onto senEVs, one key conclusion emerges: senEVs are the "particulate implementation" of the biphasic nature of SASP—they may either carry

immune-stimulatory cues that assist immune clearance, or carry suppressive signals or metabolic stress cues that promote immune escape[5,12,13].

More importantly, compared with soluble factors, EVs have greater stability and a stronger capacity for combinatorial encoding, making them more likely to sustain low-intensity but long-lasting regulatory effects during the chronic phase[16,27-31][Figure 5]. This may explain why, even when post-treatment residual senescence-like cells are limited in number, they can still exert profound effects on the immune niche through continuous EV release. In other words, the temporal dimension of SASP may be amplified at the EV level into a "memory effect"—after repeated education by EVs, the thresholds and functional states of immune cells may remain shifted for a prolonged period.

"Senescence surveillance" and tumor suppression: How the immune system clears senescent/premalignant cells

The concept of senescence surveillance holds that the immune system is not a passive bystander, but is capable of recognizing and clearing senescent or premalignant cells, thereby limiting tumor initiation and progression[14,15]. Classic studies in liver tumor and premalignant models demonstrated that the immune system participates in the clearance of senescent cells and is associated with tumor-suppressive outcomes[14,15].

Introducing this concept into the EV context of the present review is meaningful because senEVs are likely to be one of the key signaling mediators that allow senescence surveillance to occur across space. Compared with senescence markers displayed "in place" on the cell surface, EVs can deliver senescence-stress information over long distances to antigen-presenting cells and effector cells, helping the immune system identify and lock onto targets that need to be cleared at the tissue scale[12,13,16-20]; This also theoretically echoes the line of evidence discussed in Chapter 5, namely that senEVs can coordinate immune surveillance and suppress recurrence[12,13].

Placing senEVs within the "cancer-immunity cycle": At which steps do they act?

To avoid reducing immune effects to a generalized "enhancement/suppression" framework, this section adopts the process-based framework of the cancer-immunity cycle (antigen release → presentation → T-cell priming → infiltration → killing) to locate the steps at which senEVs may intervene[17-21]: (a)Antigen and danger-signal release: senEVs may carry tumor-associated antigen fragments, stress proteins, or nucleic acid danger signals, thereby increasing the density of signals that can be read by the immune system[45]; (b)Antigen presentation and priming: after uptake of senEVs by DCs/macrophages, their maturation, costimulatory-molecule expression, and cross-presentation efficiency may be altered[46-48]; (c)Infiltration and effector execution: senEVs may indirectly change immune-cell migration through cargos related to chemotaxis/adhesion, and may also reduce effector-cell function through suppressive molecules or metabolic stress signals[49,50]; (d)Therapeutic response: the role of EVs in resistance to immunotherapy has been systematically summarized, and senEVs are therefore likely to be one of the key variables determining the divergence of post-treatment immune responses[16,20].

The value of this framework is that it allows us to decompose the phenomenon of "the same senEVs producing opposite outcomes" into multiple testable node-level questions—for example, whether in a given context senEVs mainly affect DC cross-presentation, the myeloid suppressive network, or the threshold for effector T-cell exhaustion. Differences at these nodes in turn determine whether intervention strategies should target biogenesis, cargo, or recipient cells[51-54].

Innate immunity: Bidirectional reprogramming of DCs, macrophages, neutrophils, and PMN-MDSCs

Innate immune cells are often the primary "readers" of EVs and are also key amplifiers of immune-outcome directionality. In this review, they are divided into four cell groups that merit parallel discussion: DCs, macrophages, neutrophils, and PMN-MDSCs.

Dendritic cells (DCs): Maturation/cross-presentation can be enhanced or suppressed

On the one hand, if senEVs are enriched in danger signals or maturation-promoting cues, DCs may mature more readily and improve cross-presentation efficiency, thereby enhancing subsequent T-cell priming[45,48]; On the other hand, if senEVs carry immunosuppressive cargos or tolerance-inducing metabolic/lipid signals, DCs may remain in a low-maturation state, resulting in failed T-cell priming. This is precisely the earliest branching point in the immunity cycle.

Macrophages: A shift from clearance/inflammation toward immunosuppressive phenotypes

Macrophages are highly plastic in post-treatment tissues. If senEVs continuously provide chronic inflammatory and tissue-remodeling signals, they may drive macrophages toward immunosuppressive/repair-like phenotypes, thereby suppressing effector immunity and promoting tumor regrowth. Conversely, within the acute clearance window, senEVs may also enhance phagocytosis and inflammatory clearance responses, helping senescence surveillance complete the removal of target cells.

Neutrophils and PMN-MDSCs: mtDNA/nucleic acid signals may push innate immunity toward the suppressive end

As noted above, mtDNA signals released by senescent tumor cells can enhance PMN-MDSC-driven immunosuppression through a cGAS–STING-related mechanism, thereby promoting immune escape[34]. This suggests that when senEVs/related EV cargos are dominated by nucleic acid and mitochondrial stress information, recipient myeloid cells do not necessarily move toward a pro-inflammatory antitumor state, but may instead shift toward suppressive tolerance, coupled to recurrence risk during the post-treatment phase[34].

Relevance to post-treatment recurrence: The myeloid network is the most likely amplifier of chronic-phase outcomes

From a systems perspective, myeloid cells—especially the MDSC network—function more like a chronic-phase set point regulator: once educated by EVs into a suppressive network over time, even if effector T cells are subsequently present, clearance may still fail because of insufficient antigen presentation, a suppressive metabolic environment, and strengthened checkpoint axes. This provides an immune-niche-level basis for understanding poor responses/acquired resistance to immunotherapy.

Adaptive immunity: The connection point between T cells/NK cells and senescence surveillance—enhancement or suppression?

Adaptive immunity is the final execution layer that determines whether clearance truly occurs[14,15]. Classical studies on senescence surveillance have shown that effector components such as T cells participate in the control and clearance of senescent/premalignant cells.

On this basis, the effects of senEVs on T cells/NK cells may be understood in two opposite modes: (a)Enhancement of transmissible immunity: when senEVs carry stress-related cues or cargos that promote antigen presentation, they may enhance T-cell priming, improve infiltration efficiency, or support effector maintenance, thereby promoting clearance and suppressing recurrence[45-49]; (b)Promotion of immunosuppression/metabolic suppression: when senEVs carry suppressive molecules, enhance checkpoint axes, or introduce metabolic/oxidative stress, they may reduce T-cell effector function, promote exhaustion, or reduce NK-cell cytotoxicity, thereby driving immune escape and resistance[16-20].

Accordingly, the net effect of senEVs on adaptive immunity should be understood more as the sum of multiple nodes (presentation, priming, infiltration, suppressive axes), rather than as the linear effect of a single molecule.

Immune escape and therapeutic resistance: The PD-L1 and oxidative stress/metabolic axes—why senEVs may become entangled with responses to immunotherapy

In the era of immunotherapy, EVs influence not only whether immune clearance occurs, but also whether immunotherapies such as checkpoint inhibitors can work effectively. Particular attention should be paid to the PD-L1 axis and the oxidative stress/metabolic axis, while also incorporating evidence that mitochondria-enriched EVs may promote immune escape through coupling with PD-L1/ROS-related pathways; the role of EVs in immunotherapy resistance has also been systematically summarized[35].

At the mechanistic level, this part may be summarized into three mutually reinforcing pathways: (a)Enhancement of checkpoint axes: EVs may directly carry checkpoint-related molecules or induce recipient cells to upregulate suppressive axes, thereby weakening effector T-cell killing; (b)Oxidative stress and metabolic resetting: post-treatment tumor microenvironments are often characterized by ROS fluctuations and nutrient redistribution. If EVs transmit mitochondria-related cargos or cues of metabolic reprogramming, they may increase stress tolerance in tumor cells and may also impair immune-cell function[7,8,34,35,39-41]; (c)Stabilization of myeloid suppressive networks: when EV signals chronically favor MDSCs/suppressive macrophage networks, immunotherapy may remain ineffective even if some checkpoints are relieved, because the upstream problems of presentation and niche organization have not been corrected.

Therefore, the relationship between senEVs and responses to immunotherapy is unlikely to be a simple matter of enhancing or reducing efficacy; rather, it depends on which nodes in the immunity cycle are primarily targeted by senEVs: targeting presentation/priming may amplify efficacy, whereas targeting suppressive axes/myeloid networks may promote resistance.

Summary: Where does the directionality of immune outcomes come from?—what exactly is the immune system "reading"?

Taken together, the directionality of immune outcomes may be condensed into three sequential questions: (a)At the phenomenological level: why can the same senEVs promote both clearance and immunosuppression[12,13,34]? (b)At the level of immune reading: which dimensions of information in senEVs is the immune system actually reading—danger signals, metabolic states, combinations of membrane-surface molecules, or nucleic acid/mitochondrial stress cues[45-50]? (c)At the level of switches: which upstream stress axes—especially mitochondrial and nucleic acid stress—push particulate SASP toward immune activation or immune tolerance[7,8,37-41]?

This naturally leads to the core of the next chapter: why the mitochondria/mtDNA and DNA-sensing axes may be the key switches that determine the immune directionality of senEVs.

TRANSLATIONAL AND THERAPEUTIC STRATEGIES

Strategic overview: Three main routes—suppress biogenesis, block cargo, and eliminate the source

To truly translate the above mechanisms into therapeutic strategies, the core task is not to propose more "possible targets," but rather to establish a clinically actionable roadmap: first stratify and identify the risk posed by tumor-promoting senEVs, and then apply the minimally sufficient intervention at key nodes. On this basis, three translatable routes can be proposed: (a)Suppress senEV biogenesis/release: preferentially target relatively concentrated upstream pathways such as DDR–ceramide/nSMase2, or key endosomal–secretory trafficking nodes, so as to reduce the overall output of "particulate SASP" after therapy[51-54]; (b)Neutralize/block key cargos: for cargos that have already been shown to exert tumor-promoting or immunosuppressive effects (for example, EphA2, SERPINE1, and mtDNA-related signals), adopt strategies such as neutralization, blockade of receptor binding, inhibition of loading, or blockade of uptake, thereby transforming the problem

of “EVs with different information despite belonging to the same class” into one of precisely intercepting high-risk information packages[32,34,42]; (c)Eliminate senescent cells themselves: use senotherapy and the “one-two punch” strategy to clear residual senescence-like cells within an appropriate window after TIS induction, thereby reducing long-term EV output and the chronic soil for immunosuppression at the source[1-4,32-36].

It must be emphasized in particular that these three routes are not mutually exclusive, but should instead be combined in a stratified manner around tumor-suppressive vs tumor-promoting senEVs. For example, if early senEVs are more inclined to cooperate with immune surveillance, then global suppression of biogenesis should not be initiated too early; if evidence suggests that mtDNA drives myeloid suppression, then it may be more appropriate to prioritize interception at the cargo level or source elimination within the therapeutic window. This logic of “stratification + timing” is, more precisely, a translational extrapolation based on the currently available mechanistic evidence.

Feasibility and risks of intervening in EV pathways

At the level of druggability, nSMase2 is often regarded as one of the nodes closest to translation: the pathway is relatively concentrated, it is directly associated with sEV release, and multiple inhibitory/interventional approaches are already available for mechanistic validation[55-58]. A 2020 study directly showed that DNA damage can activate the ceramide synthetic pathway by downregulating SMS2 and upregulating nSMase2, thereby increasing senescence-associated EV biogenesis; at the same time, this pathway is associated with the clearance of cytoplasmic DNA fragments and the limitation of excessive inflammation.

However, the risks are equally clear: EV release does not serve only tumors; it also participates in tissue homeostasis and immune regulation. In the senescent context, EVs may additionally perform a negative-feedback function of preventing excessive inflammatory responses / maintaining cell survival[51]. Therefore, the translational

strategy should not be long-term, systemic suppression of EVs, but rather intervention with clearly defined boundary conditions: (a)Window-based treatment: tie intervention in EV pathways to the post-treatment time window (for example, short-course intervention before the formation of suppressive networks), so as to avoid disrupting a potentially beneficial acute cooperative phase of immune clearance[12,13]; (b)Tissue-/tumor-specific delivery: whenever possible, prioritize local delivery or tissue-enriched strategies, in order to reduce the side effects caused by systemic suppression[51-54]; (c)Companion monitoring: use EV quantity/subtypes, mitochondrial cargo readouts, and myeloid suppression indicators as joint readouts of efficacy and safety, rather than focusing only on tumor volume[27,28].

In other words, the key translational question is not whether nSMase2 can be inhibited, but rather in whom, when, and at what intensity it should be inhibited, so that recurrence risk can be reduced without sacrificing immune clearance.

senEV liquid biopsy: Biomarker combinations rather than a single indicator—constructing a "senescence burden + immune risk" score

If the previous two subsections answer how to treat, this subsection answers how to select patients and how to monitor them. As candidates for liquid biopsy, the greatest misconception regarding senEVs is the pursuit of a single biomarker; a more reasonable direction is to construct multidimensional composite fingerprints[56,57]. The more representative dimensions at present include:

The surfaceome dimension: infer tissue distribution and target-cell spectra based on differences in the surfaceome of senescent EVs. A 2023 *PNAS* study showed that the surfaceome of senescent cell-derived EVs exhibits measurable differences and identified DPP4 as an uptake repressor, suggesting that the surface features of senEVs are directly related to their biological behavior[56];

The physical-properties dimension: use "label-free fingerprints" such as nanomechanical properties, electrical properties, and spectroscopy as supplementary dimensions, thereby improving the resolution for discriminating subtype differences. A 2022 *Nanoscale Horizons* study showed that senescent cell-derived sEVs differ from non-senescent sEVs in stiffness, surface potential, and Raman features[57];

The mitochondrial dimension: incorporate mitochondrial cargo/mtDNA readouts into risk assessment, especially to capture signals associated with a tendency toward myeloid suppression[34];

The methodological foundation: the entire workflow must follow MISEV and standardized pre-analytical reporting, in order to avoid mistaking contaminants such as cell debris, inflammatory particles, and lipoproteins for EV signals, which would otherwise lead to false positives and irreproducibility[27,28]. MISEV2023 continues to emphasize that EV studies should report the minimum information on terminology, isolation, characterization, and functional validation, and clearly defines EVs as particles enclosed by a lipid bilayer and incapable of self-replication

At the level of clinical implementation, it is more advisable to integrate these dimensions into a "senescence burden + immune risk" score, to be used for:(a)identifying patient subgroups that are more likely to progress toward chronic immunosuppression/recurrence after therapy; (b)serving as a companion decision-making index in combination therapy for determining when to initiate senotherapy or EV-pathway intervention; and(c)dynamically monitoring whether the intervention has truly reduced the output of high-risk EV information packages.

CONCLUSION AND FUTURE DIRECTIONS

Cancer therapy pushes the tumor niche into a highly unbalanced stress state: DNA damage, oxidative stress, metabolic pressure, and disruption of organelle homeostasis

occur in parallel, driving a fraction of tumor cells and stromal cells into therapy-induced senescence (TIS) / senescence-like reprogramming[1-4,32-36]. This review centered on the unified framework of "therapy → upgrading of the senescence-like secretory system → EVs becoming a key controllable form of particulate SASP → immune regulation and the mitochondrial/mtDNA axis determining clearance or escape → recurrence and differences in therapeutic efficacy" [Figure 1], this review has systematically discussed the conceptual and methodological foundations of senEVs[27-31,58,59], their mechanisms of generation (DDR–ceramide/nSMase2, the endosomal system, and detox-like secretion)[51-54][Figure 2], their double-edged effects in therapeutic settings[2,3,12,13,32][Figure 4], and the central role of immune remodeling and mitochondrial signaling in directional divergence[34][Figure 3, Figure 5].These major themes are consistent with recent studies showing that senEVs can suppress recurrence, that TIS can promote recurrence, and that mtDNA can drive myeloid immunosuppression.

The apparently contradictory roles of senEVs may instead reflect differences in therapeutic timing, immune context, and cargo composition. Rather, it reminds us that the net effect of senEVs is a condition-dependent systemic outcome. During the acute window, senEVs may function as "immune alert packages" that cooperate with senescence surveillance and suppress recurrence; during the chronic residual phase, however, they may promote immunosuppression and therapeutic resistance through consolidation of myeloid networks, checkpoint/metabolic axes, and the propagation of mitochondrial nucleic acid signals. Future studies will likely need to focus less on total EV abundance and more on functionally distinct EV subpopulations.

Looking ahead, there are at least five key questions in this field that most urgently need to be answered and are also the most likely to directly drive translation:

Can senEV subtypes be matched one-to-one with specific functions?

One root cause of the fact that "all are called senEVs, yet their functions are opposite" is

population heterogeneity[12,13].The next step is to move from describing the overall effect of EVs to a refined mapping of subtype–cargo–recipient cell–outcome: which surfaceome or physical fingerprints correspond to clearance-promoting effects, and which correspond to suppression-promoting effects? Which are the true high-risk information packages that should be prioritized for interception[56,57]? Existing studies on the DPP4 surfaceome and nanophysical properties have already shown that differences among senEVs can be detected, but they are still far from establishing a one-to-one correspondence between subtype and function.

What is the route of origin of mitochondrial cargo: Is secretory mitophagy the main pathway or only a special case?

Mitochondrial cargo may enter EVs through multiple biogenetic routes, including secretory mitophagy, rerouting through the endosomal system, and budding from membrane microdomains[7,8,40]. Clarifying which pathway predominates under which treatment conditions and in which cell types will determine whether intervention should target the level of biogenesis or the level of cargo[27,28,40]. In particular, more rigorous operational definitions and validation chains are needed to distinguish genuine loading from isolation-related contamination[29,35-36]. Current studies on secretory mitophagy and mitochondria-encapsulating EVs provide a mechanistic entry point to this question, but a unified model is still lacking.

What is the immune targeting spectrum and spatial distribution: Whom do senEVs mainly educate, and where does this occur?

At present, many conclusions still remain at the macroscopic level of “immune enhancement/immune suppression”. Future work needs to place senEVs into the frameworks of spatial immunology and the cancer-immunity cycle: what “reading role” is assumed by DCs, macrophages, PMN-MDSCs, and T cells, respectively? Does this reading occur within the tumor, in the draining lymph node, or in the periphery[45-50]? The answers in terms of space and time window will directly determine the route and timing

design of combination therapy[12,13]. At present, the strongest direct evidence is the closed loop of "senescent tumor cell-derived mtDNA → PMN-MDSC," but a more complete cellular atlas and spatial map still need to be established.

How can senEVs be reliably distinguished from tumor EVs and inflammatory particles in clinical samples?

Both senescence models and post-treatment samples are more prone to contamination by cell debris, lipoproteins, and inflammation-related particles, making apparent "EV differences" into methodological artifacts[27,28]. Therefore, MISEV standardization in this field is not a mere "formal requirement," but rather the bottom line that determines comparability and translatability; At the same time, there is also a need to develop composite fingerprints that are better aligned with clinical workflows—surfaceome + physical properties + mitochondrial cargo—in order to construct a "senescence burden + immune risk" score. MISEV2023 continues to emphasize the minimum information required for terminology, isolation, characterization, and functional validation, which is especially critical for senEV/mitoEV research.

What is the optimal combination strategy and time window for intervening in senEVs?

From a translational perspective, the most practical question is how to intervene more safely and more effectively: when should the nSMase2/ceramide pathway be inhibited for a short course[51-54]? When should mtDNA signal propagation be preferentially blocked to prevent the consolidation of myeloid suppression? When should senotherapy / the one-two punch strategy be initiated to eliminate residual senescence-like cells?These questions need to be linked to the specific treatment type, the immune niche (immune-hot/immune-cold status and MDSC burden), and dynamic monitoring indicators, rather than pursuing a one-size-fits-all global inhibition of EVs.At present, the most cautious conclusion is that temporal, stratified, and minimally sufficient intervention is more consistent with the available evidence than "earlier and stronger global suppression of EVs."

In summary, research on senEVs is moving from phenomenological association toward attributable mechanisms. Its true clinical value will depend on two points. First, whether directional switches such as mitochondrial/nucleic acid stress can be converted into measurable risk markers and stratification criteria. Second, whether combination strategies can be designed around the treatment window so that high-risk information packages that promote recurrence or suppression can be precisely intercepted without sacrificing the potential benefits of immune clearance. Along this path, senEVs are not only a new language for explaining post-treatment recurrence and differences in responses to immunotherapy, but may also become one of the most intervenable particulate nodes within the post-treatment residual niche.

DECLARATIONS

Authors' contributions

Conceived the review framework and drafted the manuscript: Y.Q;

Collected and organized the literature: Z.W and N.L;

Revised the manuscript and supervised the study: Y.Z and H.P;

All authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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