# Mitochondria-associated small RNAs in breast cancer: An asymmetric evidence landscape from metabolic rewiring to immune selection (Review)

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**Abstract.** Mitochondria enable breast cancers (BC) to switch among metabolic, redox and stress-adaptive states, but the associated small-RNA literature often merges molecules with different origins, itineraries and sites of action. The present critical narrative Review separates bona fide nuclear-encoded mitochondrial microRNAs (mitomiRs), candidate mitochondrial miRNAs, mtDNA-derived miRNA-like RNAs, other mitochondrial small RNAs, cytosolic mitochondrial regulators, extracellular RNA cargo and engineered systems. Their reported functions are organized within a 3R Mito-Ecosystem: Rewiring of cell-autonomous metabolism, mitochondrial-origin RNA-dependent growth signaling, organelle quality and ferroptosis competence; directional Relay of RNA cargo or intact mitochondria; and Release of mitochondrial signals and their immune-sensing consequences. LIFT records Localization, Interaction, Function and Translation separately, while context tiers distinguish BC RNA perturbation from non-RNA enabling biology, cross-system support and hypotheses. A revision-stage PubMed search informed an illustrative mechanistic comparison, with source and selection limits stated explicitly. Rewiring includes directly perturbed metabolic and ferroptotic relationships and reported mitochondrial-origin tiRNA-Met activity. Relay includes causal extracellular RNA effects and organelle-transfer studies. Release includes miR-25/93-dependent NCOA3-cGAS immune sensing, without demonstrated control over mtDNA-release quantity. A causal chain connecting an endogenous mitochondria-resident or mitochondria-derived small RNA, altered release of a defined mitochondrial signal and a lineage-resolved immune outcome remains unestablished in the evaluated material. Ferroptosis provides a testable interface rather than a universally immune-activating endpoint. Target binding was distinguished from functional rescue and propose source-, time- and lineage-resolved experiments and operational immune-activating or suppressive Release states. These criteria require validation and are not patient classifiers or treatment rules.

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## 1. Introduction: An asymmetric mitochondrial small-RNA evidence landscape

Breast cancer (BC) is not metabolically fixed. Primary, residual and metastatic cells can redistribute flux among glycolysis, oxidative phosphorylation (OXPHOS), fatty-acid oxidation (FAO), lactate oxidation and anaplerosis as oxygen, nutrients and therapy change. PGC-1α-driven mitochondrial biogenesis

can support dissemination; MYC-high triple-negative BC (TNBC) can depend on FAO; FAO inhibition can enhance HER2-targeted therapy; and endocrine-therapy-tolerant ER-positive cells can acquire an OXPHOS dependency (1-4). Reversible drug-tolerant TNBC states, metastatic stem-like populations, SLC6A14-linked fusion, mitochondrial lactate oxidation, DDX3-DRP1 plasticity, MNK1 signaling and mitochondrial elongation further show that organelle use is state- and context-dependent rather than a fixed subtype property (5-11). The relevant question is therefore not whether a BC is glycolytic or respiratory, but how mitochondrial state changes under selective pressure.

Small RNAs are plausible controllers of such switching because one RNA can tune a distributed transcript set and several RNAs can converge on the same metabolic module. Canonical miRNAs guide Argonaute-containing complexes to partially complementary targets (12). Mitochondrial fractionation and sequencing have recovered miRNAs or miRNA-like species in mitochondrial preparations, while the mitochondrial transcriptome can also generate tRNA- and rRNA-derived fragments (13-15). These observations define a heterogeneous small-RNA landscape rather than one mechanistic class (16-18). The six provenance categories and their minimum evidentiary requirements are summarized in Table I. Studies of mitochondrial Argonaute and PNPASE-dependent import do not establish a universal miRNA import route, and the compact mitochondrial transcriptome makes sequence-only target prediction especially uncertain (13,15,19,20). Localization, topology and target-site dependence therefore have to be demonstrated rather than inferred from an organelle phenotype.

BC studies implicate claimed mitochondrial small RNAs and mitochondria-regulatory cytosolic miRNAs in respiratory-chain control, transcription, fission-fusion, mitophagy, therapy tolerance and ferroptosis (21,22). Yet a miRNA enriched in a crude mitochondrial pellet, a cytosolic miRNA targeting TFAM, an mt-tRF detected in bulk tumor RNA and an engineered mimic routed to mitochondria are still sometimes discussed as equivalent mitomiRs.

Existing BC reviews have catalogued candidates and translational opportunities, and broader syntheses have surveyed mitochondrial-miRNA biology across cancer and other diseases (23-29). The present review instead evaluates how far individual claims connect molecular provenance, organelle function and ecosystem consequences. The 3R framework distinguishes directly perturbed RNA relationships from enabling biological mechanisms in BC that lack an RNA-specific causal link, as well as from cross-system precedents. Its contribution is not to assume that the three arms form a continuous pathway, but to identify which links are supported in the evaluated material and which remain testable. The comparison is qualitative and claim-specific, not a systematic estimate of the prevalence or quality of evidence across the entire field.

The present review addresses three questions: Which small RNAs have credible mitochondrial provenance; which mechanistic edges have been demonstrated directly in BC; and which links to immune selection remain supported only by enabling or cross-system evidence. The 3R Mito-Ecosystem asks what a small-RNA-associated mitochondrial state does: Rewiring denotes cell-autonomous metabolic, growth-signaling, quality-control and regulated-cell-death adaptation linked to mitochondrial RNA origin or function; Relay denotes directed transfer of small RNAs, mitochondrial components or intact organelles; and Release denotes exposure of mitochondrial stress signals and regulation of their sensing in tumor or immune cells (Fig. 1). LIFT asks how each edge has been shown through Localization, direct Interaction, causal Function and Translation. A separate four-tier label distinguishes direct BC evidence, small-RNA-independent enabling evidence, cross-system support and hypothesis. Neither framework is a total score; both are designed to identify the demonstrated and missing links in a proposed mitochondrial-small-RNA-to-immune-selection chain.

*Literature search and evidence adjudication*. The present critical narrative review combines a previously assembled literature collection with a new revision-stage PubMed search and claim-specific source assessment. On 18 October 2026, six searches were executed through NCBI E-utilities (ESearch and EFetch), covering publication dates from 1 January 1900 through 18 October 2026 without language or publication-type restrictions at retrieval. The queries combined BC terms with small RNAs and mitochondrial function, ferroptosis, extracellular-vesicle communication, mitochondrial-nucleic-acid immune sensing, or intercellular mitochondrial transfer. Exact queries, PubMed translations and per-query yields are provided in Data S1. The six searches returned 801 query memberships corresponding to 734 unique PMIDs; Data S2 records query membership, bibliographic triage, material examined and scope decisions for every record. A separate DOI-based query verified the 35 publication sources in the prior claim panel and is reported as ID01, outside the thematic-search yields. This new search does not reconstruct the original collection process or represent a systematic review.

Selection for detailed appraisal prioritized distinct RNA-target-condition-endpoint or donor-cargo-recipient relationships that clarify mitochondrial RNA provenance, metabolic or death mechanisms, transfer routes, or immune sensing in BC. Association-only studies, whole-organelle/protein interventions, engineered systems and cross-system precedents were retained as separately identified comparators. All retrieved titles and metadata underwent bibliographic screening, with targeted abstract review and primary-text checks for consequential candidates. The search and selection process was not an independent duplicate screening procedure. Reviews, correction notices and preprints were not counted as independent primary observations, and retracted publications were not used as positive mechanistic evidence. The search identified a BC miR-25/93-NCOA3-cGAS immune-sensing mechanism and a mitochondrial tRNA-derived tiRNA-Met mechanism, which were added to the claim panel. Data S3 explains the source route and biological role of each selected example. Additional relevant records remain visible in the search inventory. The 38-record panel provides an illustrative mechanistic comparison; it does not include all eligible studies and should not be treated as a field-wide denominator. PubMed-only, terminology-dependent retrieval and purposive detailed appraisal introduce selection bias; retrieval alone does not establish mitochondrial residency, direct interaction or causal function.

Table I. Provenance-aware taxonomy of mitochondrial-small RNA systems.

| Category | Definition | Minimum evidence | Causal question | Required terminology |
|---|---|---|---|---|
| 1. Bona fide nuclear-encoded mitomiR | Canonically processed, nuclear-encoded mature miRNA resident inside a defined mitochondrial compartment. | Organelle integrity and contamination controls; RNase protection plus mitoplast analysis; orthogonal spatial evidence; absolute copies. | Does endogenous mitochondrial residency enable direct target engagement and function? | 'MitomiR', with compartment and LIFT axes stated. |
| 2. mtDNA-derived miRNA-like RNA | Precisely processed small RNA arising from an mtDNA transcript and proposed to act sequence-specifically. | Exact ends and precursor; exclusion of NUMTs, mt-tRF/mt-rRF and degradation products; direct target and perturbation-rescue evidence. | Is the endogenous mtDNA-derived species necessary, and is its action miRNA-like? | 'mtDNA-derived miRNA-like RNA'; do not imply canonical miRNA biogenesis. |
| 3. Other mitochondrial small RNA | mt-tRF, mt-rRF or another small RNA generated from a mitochondrial transcript. | Origin-resolving sequencing; precise species quantification; biogenesis test; direct target or functional rescue where mechanism is claimed. | Functional regulator, stress product or biomarker? | Name the species class; do not relabel as a miRNA. |
| 4. Mitochondria-regulatory cytosolic miRNA | Cytosolic miRNA targeting nuclear-encoded regulators of mitochondrial function, cell death or mitochondrial-signal sensing. | Target validation, functional rescue and quantitative mitochondrial or signal-sensing endpoints. | Does cytosolic target regulation alter mitochondrial function or mitochondrial-signal sensing? | 'Mitochondria-regulatory miRNA'; mitochondrial localization is not implied. |
| 5. Extracellular small-RNA system | Naturally released RNA transmitted between a defined donor and recipient, often in an EV. | Current EV characterization; cargo copies; donor depletion; recipient uptake; physiologically plausible rescue; lineage-resolved endpoint. | Is the RNA cargo necessary and sufficient for a directed Relay edge? | 'EV-associated' or 'extracellular small-RNA Relay'; do not imply organelle entry. |
| 6. Engineered system | Mimic, inhibitor, nanoparticle, vesicle or organelle-targeting device that produces a mitochondrial phenotype. | Dose, biodistribution, recipient and organelle exposure, target engagement, safety and manufacturing controls. | What can designed delivery achieve, and at what exposure and risk? | 'Engineered' or 'mitochondria-directed delivery'; do not infer endogenous trafficking. |

The six categories are related by mitochondrial phenotype, but they make different claims about origin, localization and endogenous trafficking.

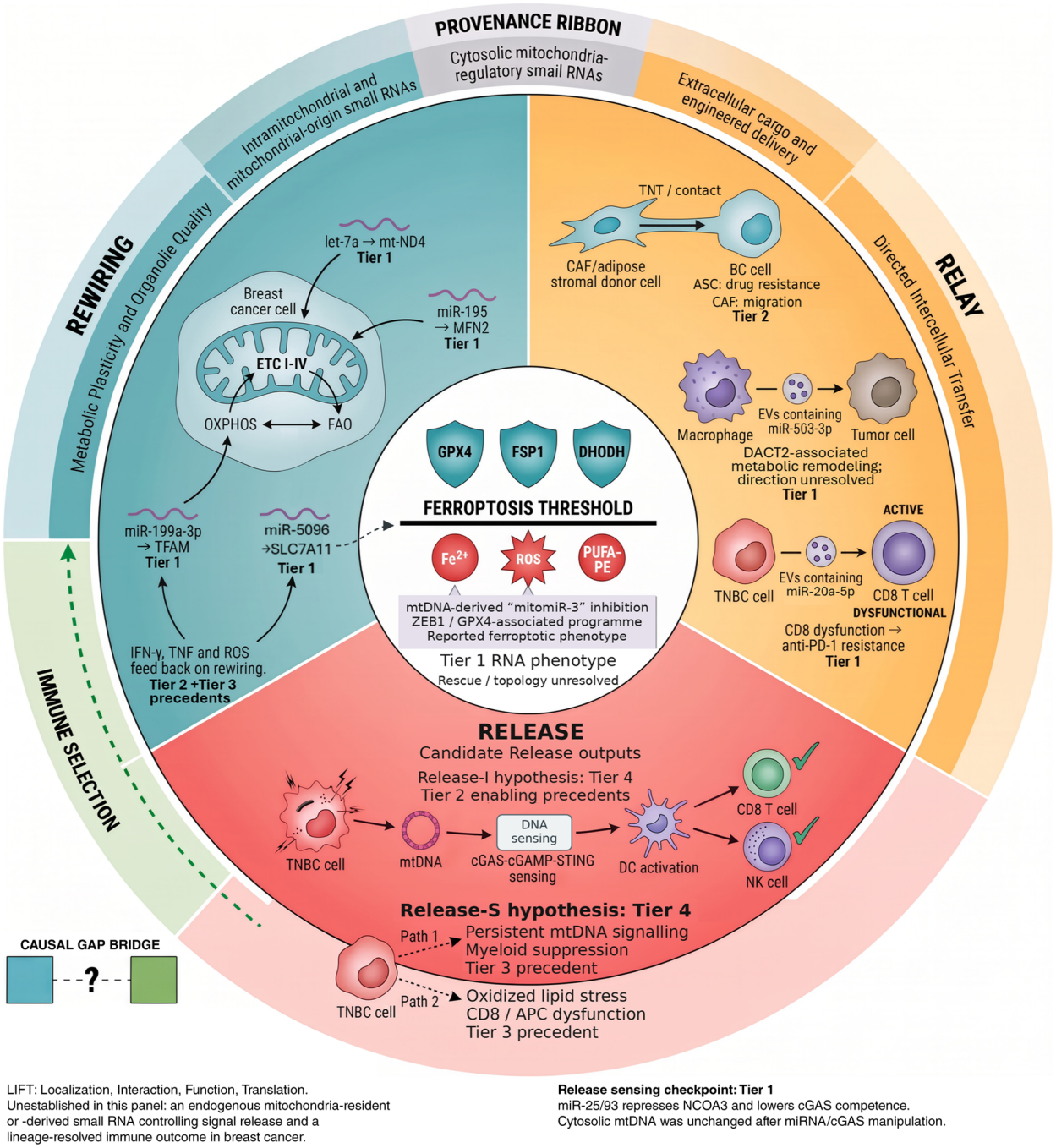



Figure 1. A provenance-aware 3R model of the asymmetric mitochondrial small-RNA evidence landscape in breast cancer. The outer ribbon groups the systems considered in this Review into three visual umbrella domains: intramitochondrial and mitochondrial-origin small RNAs, cytosolic mitochondria-regulatory small RNAs, and extracellular cargo or engineered systems. These domains are not interchangeable molecular classes; the six provenance categories and their minimum evidentiary requirements are defined in Table I. Rewiring encompasses cell-autonomous metabolism, growth signaling, organelle quality and ferroptosis defense; the illustrated examples concern OXPHOS, FAO, morphology and death control. The miR-5096-SLC7A11 relationship points to ferroptosis competence through a dashed convergence connector; it is not a direct SLC7A11-to-OXPHOS relationship. Solid example connectors summarize the stated RNA/target or transfer relationship and do not confer a High LIFT grade. Relay denotes directed transfer of EV RNA cargo or intact mitochondria among stromal, tumor and immune cells. The combined stromal-donor illustration distinguishes adipose stromal-cell (ASC)-associated drug resistance from cancer-associated fibroblast (CAF)-associated migration. TNT denotes tunnelling nanotubes, one reported route; transfer can also be contact-dependent without uniform nanotube dependence. Release denotes exposure of mitochondrial material and its immune-sensing consequences. The DNA-sensing box denotes cGAS-cGAMP-STING signaling, not a mitochondrial organelle. The separate Tier 1 sensing-checkpoint note identifies miR-25/93-NCOA3-dependent control of cGAS competence; the reported cytosolic mtDNA amount was unchanged after miRNA or cGAS manipulation (135). Release-I and Release-S are Tier 4 operational hypotheses defined jointly by exposure kinetics and immune function, not by duration alone; neither is a validated patient class. The ferroptosis threshold integrates GPX4-, FSP1- and DHODH-dependent defense against iron, reactive oxygen species and peroxidizable phospholipids. Inhibition of the mtDNA-derived RNA reported as mitomiR-3 is linked to a ZEB1-associated program, GPX4 downregulation and a ferroptotic phenotype, but Localization, direct Interaction and Translation remain incomplete. Claim tiers denote direct breast-cancer evidence, enabling breast-cancer biology, cross-system support or hypothesis, whereas LIFT records Localization, Interaction, Function and Translation independently. 'Tier 2 + Tier 3 precedents' distinguishes disease-specific enabling observations from cross-system support. Proposed Release-I and Release-S states themselves remain Tier 4; the tier of a supporting precedent is not the tier of the proposed classifier. The miR-503-3p-DACT2 relation is shown direction-neutral because the cited article contains an unresolved internal inconsistency in the reported metabolic direction. The evaluated material does not establish a continuous endogenous mitochondrial-small-RNA-controlled Release chain with a lineage-resolved breast-cancer immune endpoint. Engineered delivery and non-RNA enabling studies are distinct categories.

The appraisal unit is an RNA-target-condition-endpoint or donor-cargo-recipient relationship. Each LIFT axis was assessed separately using the source material available for that claim. NR denotes insufficient source detail for grading, distinct from Limited (L), relevant but unestablished evidence (U), and non-applicability (NA). R1, R7b and R20 are retained descriptively and excluded from graded-Function comparisons. Source availability and purposive selection remain limitations of this synthesis.

During this revision, HYL and PYC independently evaluated the original publications for all 25 core LIFT profiles and separately submitted initial judgments before comparing their assessments. Both also reviewed the context tier and inclusion status of the full 38-record claim panel. This claim-level assessment is distinct from the bibliographic search and title/metadata screening, for which independent duplicate screening is not claimed. Following comparison and source-based discussion, the final LIFT grades, context classifications and inclusion decisions were unchanged from the revised claim panel and were approved by all authors. HWHo was the designated third-author adjudicator for any disagreements remaining after discussion.

Each assessment addressed the specified model, intervention condition, target/pathway and quantitative endpoint using the prespecified criteria in Table II-A. Any discordant judgments were considered against the original experiment, source location, claim scope and necessary LIFT conditions; remaining disagreements were subject to third-author adjudication. The highest category meeting all criteria was selected rather than averaging ratings or voting. NR remained appropriate when available source detail was insufficient (R1, R7b and R20), and these records were excluded from graded-Function comparisons without treating unavailable experiments as negative evidence. Author-approved ratings and the unchanged 3R counts are reported in Tables II and SI. The assessment and resolution procedure is described in Table SI. No inter-rater agreement statistic is reported.

## 2. Provenance taxonomy and claim-level evidence adjudication

*A location- and provenance-aware taxonomy.* A defensible taxonomy begins with molecular origin, cellular itinerary and site of action, not phenotype. Category 1 comprises bona fide nuclear-encoded mitomiRs: canonical miRNAs for which intramitochondrial residency is supported by the combined purity, protection, compartment and abundance requirements in Table I. Fractionation studies and direct BC investigations provide candidate examples, but incomplete controls do not confer bona fide Category 1 status (14,16,30). Category 2 comprises mtDNA-derived miRNA-like RNAs. Because the mammalian mitochondrial genome is densely transcribed and is not known to encode canonical Drosha-dependent miRNA hairpins, these candidates require especially stringent proof of precise ends, biogenesis and target engagement. Reports of mitochondrial-genome-derived small RNAs therefore require independent confirmation of their processing and targets (31). Category 3 comprises other mitochondrial small RNAs, notably mitochondrial tRNA-derived fragments (mt-tRFs) and mitochondrial rRNA-derived fragments (mt-rRFs). Some members have experimentally supported functions, whereas others show disease-associated expression patterns and potential biomarker value; neither class should be designated as miRNAs without evidence of miRNA-type biogenesis (32-36). Category 4 comprises cytosolic miRNAs that regulate mitochondrial functions or sensing of mitochondrial danger signals. Examples target nuclear-encoded mitochondrial regulators such as TFAM and MFN2 (37-39). Other Category 4 examples target VDAC1, PCK2 or PDHX (40-42). The reported mitochondrial miRNA miR-4485 illustrates why binding and functional evidence still require a separate assessment of compartment topology (43). Category 5 comprises naturally transferred extracellular small RNAs; claims of endogenous transfer should establish selective donor loading or release, uptake by defined recipient cells, and delivery at a functionally sufficient dose (44-49). Category 6 comprises experimentally introduced mimics, inhibitors and engineered vesicular or organelle-targeted delivery systems. Such interventions can establish molecular sufficiency or therapeutic feasibility under imposed delivery conditions but cannot by themselves establish endogenous trafficking (50-54). 'MitomiR' is reserved here for Category 1. Category 2 is named explicitly by origin, and the remaining categories retain their own labels (Table I).

Accordingly, let-7a and miR-4485 are described here as candidate Category 1 RNAs in BC models, and miR-2392 as a candidate Category 1 RNA studied in tongue squamous carcinoma (30,43,55). The qualifier concerns whether the full residency standard has been met; it does not negate the reported RNA perturbations, binding assays or mitochondrial phenotypes. A mimic used to test an endogenous candidate is an experimental intervention, not proof that the endogenous RNA follows the same itinerary.

This vocabulary changes both inference and therapeutic design. If miR-199a-3p suppresses nuclear-encoded TFAM in the cytosol, mitochondrial entry is unnecessary and a conventional cytosolic delivery system may suffice (37,56). If let-7a directly engages mtDNA-encoded ND4, organelle access and mitochondrial target-site occupancy are essential (30). If mitochondrial calcium uptake alters selective vesicle loading, EV miRNA abundance may report mitochondrial state rather than mediate it (57). If an engineered mimic reaches the matrix, that experiment demonstrates what targeted delivery can cause, not where the endogenous RNA resides. The same discipline is important for other small RNAs: mt-tRF-Tyr-GTA-001 is associated with BC outcomes, whereas a nuclear tRNA-derived tRF-33 can perturb mitochondrial homeostasis through an IGF1-linked mechanism; neither observation justifies a mitomiR label (36,58).

*Criteria for establishing the validity of mitochondrial small-RNA claims.* The itinerary of a nuclear-encoded miRNA to a defined mitochondrial compartment remains unresolved. PNPASE can import selected structured RNAs, and AGO2 or other RNA-interference components have been recovered in mitochondrial fractions, but neither observation establishes a general BC miRNA-import pathway (13,19). Candidate routes involving RNA-binding chaperones, protein-import machinery, organelle contact sites or stress-dependent permeability are mechanistically distinct and remain largely cross-system proposals (16-18). A mitochondrial claim should therefore

Table II. Operational LIFT criteria and claim-level context atlas.

A, Operational criteria for LIFT ratings

| Axis | High (H) | Moderate (M) | Limited (L) |
|---|---|---|---|
| Localization | Protected intramitochondrial localization in a defined compartment supported by at least two orthogonal methods, with organelle-integrity and contamination controls and compatible absolute abundance. | Mitochondrial fractionation plus one limited topology or orthogonal localization control, but with compartment, protection or stoichiometry unresolved. | Crude mitochondrial-pellet enrichment, colocalization, mitochondrial-transcript origin, sequence inference or prediction alone. |
| Interaction | Direct endogenous binding together with target-site disruption and mechanism-specific rescue at the relevant target. | Reporter, pulldown, ligation or RNA-binding-protein occupancy and/or downstream target restoration, without endogenous target-site disruption. | Sequence prediction, expression anticorrelation or indirect pathway inference only. |
| Function | Bidirectional RNA perturbation and mechanism-specific rescue addressing the same stated quantitative disease-relevant phenotype. | Quantitative disease-relevant endpoint plus reciprocal RNA perturbation or functional reversal after target/cargo intervention; at least one High criterion is unmet. Partial rescue remains M. | Association, one-direction RNA perturbation without functional reversal, or a nonspecific growth/survival endpoint. Model number alone does not confer M. |
| Translation | Human evidence together with disease-relevant *in vivo* target engagement and a linked pharmacodynamic or treatment-response relationship; independent validation strengthens the assignment. | Empirical human association and/or a disease-relevant *in vivo* or physiologically faithful three-dimensional model linked to a mechanistic or therapeutic endpoint. Specify which route is supported. | Cell-line-only evidence, including unqualified cell-line spheroids, or computational inference without empirical human or disease-faithful model support. |

Apply H first only when all criteria are met; otherwise assess the stated M minimum, then L. An unresolved directional contradiction prevents H even when the M functional minimum is met. A limited model set, reporter response or target-expression change alone does not confer Function M. NA denotes non-applicability, U a relevant but unestablished axis, and NR insufficient source detail for grading. NR is not a strength grade or an experimental negative. Translation records human evidence, model, engagement, pharmacodynamic and response components separately; neither M nor H denotes clinical readiness.

Table II. Continued.

B, Applied claim-level LIFT profiles and context tiers (25 records)

| Record/3R arm | Mechanistic edge/provenance | Specific claim assessed | L/I/F/T | Context tier | Evidence boundary and source |
|---|---|---|---|---|---|
| R1/Rewiring | mitomiR-3 inhibition → ZEB1/GPX4-related ferroptosis Category 2; breast-cancer/TNBC models | Endogenous small-RNA perturbation changes a ZEB1-GPX4-associated pro-ferroptotic program. | L/M/NR/M | 1 | Tier 1 reported breast-cancer RNA/ferroptosis phenotype. F=NR: source-limited and excluded from graded-Function comparisons; not an experimental negative. Protected topology, target dependence and immune linkage are separate questions (22). |
| R2/Rewiring | let-7a → mt-ND4/respiratory phenotype Candidate Category 1; breast-cancer cells | Let-7a perturbation alters ND4 and respiratory phenotypes in a cell-line-dependent manner; repression is not universal across breast-cancer cells. | M/M/M/L | 1 | Tier 1. Reciprocal respiratory effects support M; ND4 repression is MCF-7-specific in the tested comparison. Candidate residency is not complete topology or target-rescue evidence (30). |
| R3/Rewiring | miR-4485 → 16S-rRNA-associated mitochondrial function/ tumorigenicity Candidate Category 1; breast-cancer models | Reported binding to mitochondrial 16S rRNA is linked to RNA processing, mitochondrial function and tumorigenicity. | M/M/M/M | 1 | Tier 1. Reported 16S-rRNA binding supports I=M. Reciprocal RNA effects support F=M; protected topology and target-specific phenotype rescue remain separate questions (43). |
| R4/Rewiring | miR-2392 → mitochondrial transcription/chemoresistance Candidate Category 1; tongue squamous carcinoma | Mitochondrial miR-2392 represses selected mtDNA transcription, shifts OXPHOS/glycolysis and promotes cisplatin resistance. | M/M/M/M | 3 | Tier 3 tongue-carcinoma evidence. Reciprocal RNA effects support F=M; compensatory reporter rescue is not complete chemoresistance phenotype rescue (55). |
| R5/Rewiring | mt-tRF-Tyr-GTA-001 → clinical outcome Category 3; breast-cancer cohorts | Mitochondrial-transcript-derived tRF abundance is associated with survival and inferred immune features. | L/L/L/M | 2 | Tier 2 association. Mitochondrial transcript origin is not protected localization; neither computational targets nor survival association establishes an RNA perturbation mechanism (36). |
| R6/Rewiring | tRF-33 → IGF1/mitochondrial homeostasis non-mitochondrial tRF; HER2-negative breast cancer | tRF-33 binds the IGF1 3'-UTR and is linked to mitochondrial-homeostasis and tumor-progression phenotypes. | NA/M/M/M | 1 | Tier 1 non-mitochondrial tRF. Reciprocal RNA phenotypes support F=M; the inspected main text does not establish IGF1-specific phenotype restoration (58). |
| R7a/Rewiring | miR-199a-3p → TFAM/cisplatin sensitivity Category 4; breast cancer | miR-199a-3p changes cisplatin sensitivity in parental and resistant MDA-MB-231 cells through a partially rescued TFAM-associated mechanism. | NA/M/M/L | 1 | Tier 1 cisplatin-specific claim. Partial TFAM rescue supports F=M; cell-line translation is T=L. The hypoxia/SNHG1 relationship is evaluated separately (37). |
| R7b/Rewiring | HIF-1/SNHG1/miR-199a-3p/ TFAM → hypoxic phenotype Category 4; breast cancer | The hypoxia-associated HIF-1/ SNHG1/miR-199a-3p/TFAM study reports angiogenesis and metastatic phenotypes. | NA/M/NR/M | 1 | Tier 1 reported hypoxia-associated RNA relationship. F=NR: descriptive source-limited use only; excluded from graded-Function comparisons. No rescue inference is transferred from R7a (56). |
| R8/Rewiring | miR-27b → PDHX/metabolism Category 4; breast cancer | Cytosolic miR-27b regulates PDHX-linked pyruvate metabolism and growth. | NA/M/M/M | 1 | Tier 1. Reciprocal miR-27b/metabolic effects support F=M; PDHX knockdown is a phenocopy, not target-restoration rescue. Human association supports T=M (41). |

Table II. Continued.

| Record/3R arm | Mechanistic edge/provenance | Specific claim assessed | L/I/F/T | Context tier | Evidence boundary and source |
|---|---|---|---|---|---|
| R9/Rewiring | miR-218-5p → Parkin-dependent mitophagy Category 4; breast-cancer cells | miR-218-5p combined with doxorubicin alters Parkin-dependent mitophagy and treatment response. | NA/L/L/L | 1 | Tier 1 for the breast-cell RNA-perturbation phenotype, not for complete direct target causality. I=L and F=L under the same rubric; Parkin phenocopy is not target-specific rescue (80). |
| R10/Rewiring | miR-5096 → SLC7A11/ferroptosis Category 4; breast cancer | Cytosolic miR-5096 represses SLC7A11; target restoration partially reverses ferroptosis-related phenotypes. | NA/M/M/M | 1 | Tier 1. Partial SLC7A11 rescue supports M, not H. Mitochondrial localization is not required by this cytosolic claim (96) |
| R16/Rewiring | SLC25A13 → OXPHOS/ ferroptosis/CD8/anti-PD-1 phenotype Small-RNA-independent Rewiring; TNBC | A mitochondrial metabolic program couples ferroptosis defense to immune resistance. | NA/NA/NA/H | 2 | Tier 2 protein/metabolic-to-immune bridge. RNA L/I/F=NA; T=H is preclinical evidence integration, not clinical readiness or proof of released-cargo/RNA causality (100). |
| R18/Rewiring | miR-195 → MFN2-dependent mitochondrial morphology Category 4; breast-cancer cells | miR-195 controls MFN2-dependent mitochondrial fragmentation; the assessed endpoint is morphology, not ferroptosis. | NA/M/M/L | 1 | Tier 1. F=M: quantitative morphology rescue; reciprocal morphology perturbation is not established. No ferroptosis mechanism is inferred (38). |
| R19/Rewiring | miR-325-3p → GSTP1/ferroptosis sensitization Category 4; paclitaxel-resistant TNBC | GSTP1-associated ferroptosis and paclitaxel sensitization are partially reversed by target restoration. | NA/M/M/M | 1 | Tier 1. Partial rescue supports M; no protected mitochondrial residency or complete shared defense-module mechanism is established (79). |
| R11/Relay | Macrophage EV miR-503-3p → DACT2-associated metabolic remodeling Category 5; macrophage-to-breast-cancer Relay | Macrophage EV cargo is linked to DACT2-dependent changes in recipient-cell glycolysis and OXPHOS. | NA/M/M/M | 1 | Tier 1. DACT2-associated metabolic remodeling; direction unresolved. Physiological cargo dose and recipient mitochondrial mediation require separate tests (104). |
| R12/Relay | tumor EV miR-20a-5p → CD8 dysfunction/anti-PD-1 resistance Category 5; TNBC-to-CD8 Relay | TNBC-derived EV miR-20a-5p impairs CD8 T-cell function and treatment response. | NA/M/M/M | 1 | Tier 1 immune Relay. F=M on reviewed main-article evidence, with a stated SI limit; neither mitochondrial entry nor mitochondrial mediation in CD8 cells is established (105). |
| R13/Relay | EV/cytosolic miR-181a → STING/PARP-inhibitor resistance Categories 4-5; BRCA-mutated TNBC | miR-181a suppresses STING output and transfers PARP-inhibitor resistance between cells. | NA/M/M/M | 1 | Tier 1 TNBC gain/loss and EV-associated resistance evidence. F=M is supported by breast-cancer perturbations; ovarian knockout evidence and complete EV-cargo rescue are not assumed (144). |
| R14a/Relay | Adipose stromal mitochondria → drug resistance Small-RNA-independent Relay; breast cancer | Adipose stromal donors transfer mitochondria to breast-cancer recipients, with metabolic and multidrug-resistance effects. | NA/NA/NA/M | 2 | Tier 2 enabling Relay. Transfer route is model-dependent; persistence or necessity of a small-RNA cargo is untested (121). |

Table II. Continued.

| Record/3R arm | Mechanistic edge/provenance | Specific claim assessed | L/I/F/T | Context tier | Evidence boundary and source |
|---|---|---|---|---|---|
| R14b/Relay | CAF mitochondria → breast-cancer migration Small-RNA-independent Relay; breast cancer | CAF-to-breast-cancer mitochondrial transfer is associated with increased migration in aggressive breast-cancer models. | NA/NA/NA/M | 2 | Tier 2 enabling Relay. Donor, endpoint and source remain separate from R14a; detailed rescue appraisal is source-limited (122) |
| R15/Relay | Transferred mitochondria carry a functional small-RNA program Proposed Relay mechanism | Donor mitochondrial RNA persists in the recipient and is necessary for the rescue phenotype. | U/U/U/U | 4 | Tier 4. RNA necessity after whole-organelle transfer remains unestablished in the evaluated material; hypothesis is excluded from observed-record counts. Experimental priority |
| R20/Relay | MCU-dependent miR-4488 sorting → angiogenesis Category 5; breast-cancer EV-to-endothelium | MCU reduces EV miR-4488 loading; reduced loading of this inhibitory cargo favors angiogenesis and metastatic colonization. | NA/M/NR/M | 1 | Tier 1 reported EV/RNA-associated angiogenesis relationship. Reduced inhibitory miR-4488 loading favors angiogenesis. Integrated cargo-specific Function is NR and excluded from grade comparisons (57). |
| R17a/Release/ immune bridge | CD36-associated CD8 ferroptosis → impaired function Immune-cell ferroptosis; cross-cancer | CD36-linked lipid uptake and ferroptotic injury can impair intratumoral CD8 effector function. | NA/NA/NA/M | 3 | Tier 3 immune-consequence precedent. Ferroptotic death is specific to this assessed source; no breast-cancer small-RNA Release chain is inferred (148). |
| R17b /Release/ immune bridge | CD36/oxidized lipids → CD8 dysfunction Immune-cell ferroptosis; cross-cancer | Oxidized-lipid uptake and lipid peroxidation impair tumor-infiltrating CD8 T-cell function. | NA/NA/NA/M | 3 | Tier 3 immune-consequence precedent. Keep dysfunction distinct from demonstrated ferroptotic cell death and from a specific mitochondrial Release route (149). |
| R34/Release | miR-25/93 → NCOA3/cGAS-dependent immune suppression Category 4; breast-cancer immune sensing | RNA gain/loss and cGAS rescue establish immune consequences of reduced DNA sensing; cGAS is an indirect target, and mtDNA release is not shown to be controlled by these RNAs. | NA/M/H/M | 1 | Tier 1. H concerns cGAS-dependent immune/tumor endpoints; no endogenous mitomiR or RNA-controlled mtDNA-release claim (135). |
| R35/Rewiring | tiRNA-Met → SNRPA/RANBP3L-dependent TNBC phenotypes Category 3; reported mitochondrial tRNA origin | Predominantly cytoplasmic fragment regulates SNRPA/RANBP3L and mTORC1-associated malignant phenotypes; mitochondrial residency and export are not established. | L/M/M/M | 1 | Tier 1. F=M from reciprocal RNA phenotypes and partial functional reversal; no direct mitochondrial-respiration or immune endpoint. (99). |

Claim IDs and grades match Table SI. Tier 1 uses the same breast-cancer RNA-perturbation/arm-endpoint criterion throughout; target binding, functional rescue and mitochondrial itinerary are evaluated separately. NA is non-applicability, U relevant but unestablished evidence, and NR insufficient accessible detail for grading. R1/R7b/R20 remain descriptive records and are excluded from graded-Function comparisons. Split records preserve distinct conditions, donors or endpoints. MiR-503-3p is direction-neutral; R18 Function M reflects morphology rescue without verified bidirectional morphology perturbation; R16 Translation H denotes advanced preclinical evidence, not patient benefit.

Table II. Continued.

C, Number and type of records in the selected expanded 3R register

| Primary arm | BC RNA Tier 1 | BC association Tier 2 | BC non-RNA Tier 2 | BC engineered Tier 2 | Cross-system Tier 3 | Hypothesis Tier 4 | Total |
|---|---|---|---|---|---|---|---|
| Rewiring | 12 | 1 | 1 | 0 | 1 | 0 | 15 |
| Relay | 4 | 0 | 4 | 0 | 1 | 1 | 10 |
| Release | 1 | 0 | 2 | 1 | 8 | 0 | 12 |
| TOTAL | 17 | 1 | 7 | 1 | 10 | 1 | 37 |

Panel A defines axis-specific criteria for high (H), moderate (M) and limited (L) evidence across Localization, Interaction, Function and Translation. Panel B presents selected Rewiring, Relay and Release RNA claims together with explicitly labeled non-RNA immune bridges. The miR-25/93 claim concerns RNA-dependent immune sensing; it does not establish control of mitochondrial release. Non-RNA bridges remain separate causal units. LIFT records evidentiary completeness within the stated edge, whereas context tier records how directly that edge addresses breast-cancer small-RNA biology. The two records are orthogonal and are not combined into a composite score. Panel C provides the bounded numerical comparison; the expanded source-context records are in Table SI. BC, breast cancer. The 37 classified records comprise 36 observed relationships and one hypothesis; R27 is an additional unclassified background record excluded from these totals. Counts describe this selected panel, not papers, unique RNAs, independent replications or field-wide evidence. Tier 1 requires a breast-cancer RNA perturbation and the arm-specific endpoint, consistently across arms. R34 is a Release immune-sensing claim, not evidence of RNA control over mtDNA-release quantity. The complete endogenous mitochondrial-RNA-released-signal-immune chain is a separate test. Excluding F=NR records gives Tier 1 counts of 10/3/1 (Rewiring/Relay/Release); requiring F=M or H gives 9/3/1. These are sensitivity subsets, not additional records or proof of exhaustive absence. Selection and source boundaries are in Table SI.

specify the compartment reached, exclude outer-membrane or co-purifying material and show that the endogenous RNA can access the proposed site of action.

Target recognition is likewise non-canonical. Mitochondrial mRNAs have minimal untranslated regions and are embedded in ribonucleoprotein assemblies, giving sequence-only prediction a high false-positive burden. Direct evidence should combine RNA-RNA ligation or pulldown, occupancy by the relevant RNA-binding complex, site-directed disruption of complementarity and rescue with a target-refractory construct. CLASH and eCLIP illustrate interaction- and RNA-binding-protein mapping strategies (20,59). APEX-seq provides orthogonal spatial evidence for RNAs near defined subcellular compartments (60,61), but proximity labeling alone does not establish direct target recognition. BC work on NRAD1 shows that altered RNA localization can change miRNA biology without proving a mitochondrial interaction (62). Export is even less resolved: Stress-dependent release of mitochondrial double-stranded RNA can activate cytosolic sensing in human cells, whereas vesicular packaging and loss during mitophagy remain separate hypotheses (63). EV studies must also test whether recipient cells receive a functionally plausible number of RNA molecules (64).

*LIFT and claim-level tiers: Orthogonal evidence records.* Localization (L) requires more than enrichment in a pellet. Minimum practice should include markers for cytosol, nucleus, endoplasmic reticulum and endosomes; assessment of mitochondrial integrity; RNase treatment of intact mitochondria followed by mitoplast analysis; and an orthogonal spatial assay. Absolute copy number is essential because a large fold enrichment can still represent fewer than one molecule per organelle. The complexity of the mitochondrial transcriptome and mitochondrial variants recovered in single-cell data reinforces the need to exclude NUMTs and abundant tRNA/rRNA-derived fragments (15,65). Proximity labeling such as APEX-seq can complement biochemical purification by capturing RNAs near defined mitochondrial compartments in living cells, although labeling radius and expression of the enzyme remain interpretive constraints (60,61).

Interaction (I) concerns recognition of the stated target; a reporter or pulldown does not establish target-dependent phenotype rescue. High Function (F) requires bidirectional RNA perturbation and mechanism-specific rescue of the stated quantitative disease-relevant phenotype. Bidirectionality and rescue cannot be assembled from different endpoints. Moderate requires that endpoint plus either reciprocal RNA perturbation or a functional reversal after target/cargo intervention but lacks at least one High criterion. Partial target restoration is Moderate under this rubric. One-direction RNA perturbation without functional reversal, association alone or nonspecific growth effects are Limited. Model number alone does not determine a grade. Reporter rescue, pathway-inhibitor rescue and target-restoration rescue are distinguished; endogenous target-site editing is not an additional mandatory Function criterion. Whole-organelle/protein effects are described separately rather than scored as RNA Function (Table II-A).

Translation (T) records the evidence route rather than clinical readiness. Moderate may reflect an empirical human association or a disease-relevant *in vivo* or physiologically

faithful three-dimensional model linked to a mechanistic or therapeutic endpoint. These routes are not equivalent: The human cohort, model type, target engagement, pharmacodynamic change and treatment-response result must be specified separately. High requires human evidence together with *in vivo* target engagement and a linked pharmacodynamic or treatment-response relationship; independent validation strengthens this assignment. Neither Moderate nor High is synonymous with a validated predictive biomarker, demonstrated patient benefit, regulatory approval or a treatment recommendation. Cell-line-only and computational evidence remains Limited; cell-line spheroids do not automatically qualify as physiologically faithful human models.

Context tier records the disease and causal unit, not overall study quality. Tier 1 denotes BC RNA-perturbation evidence for the stated arm-specific endpoint. The same rule applies across Rewiring, Relay and Release; it does not require every LIFT axis to be High. Tier 2 includes BC associations and non-RNA enabling or separately identified engineered interventions. Tier 3 denotes another cancer or biological system, and Tier 4 a hypothesis. The additional question of a complete endogenous mitochondrial-RNA-released-signal-immune chain is assessed separately from these context labels. Thus, a strong cross-system experiment remains Tier 3, and a BC RNA phenotype can retain an ungraded or Limited mechanistic axis (panels A-C of Table II).

Table II-C summarizes 38 selected records: 37 context-classified records comprising 36 observed relationships and one hypothesis, plus one unclassified pharmacological record (R27). Each has one primary arm; different treatment conditions or donor-recipient endpoints are separated. The BC RNA-perturbation counts are 12 for Rewiring, four for Relay and one for Release. The remaining records distinguish association, non-RNA interventions, engineered systems and cross-system precedents. These are counts of selected claims, not all studies in the field, not counts of distinct RNAs and not a quality ranking. Excluding the three ungraded Function records leaves Tier 1 counts of 10/3/1 (Rewiring/Relay/Release); requiring Function M or H leaves 9/3/1. R34 contributes direct RNA-dependent control of immune sensing, without evidence that the RNAs control the amount of released mtDNA. These sensitivity descriptions do not remove selection bias or establish field-wide absence. Separately, no record in this panel establishes the complete endogenous mitochondrial-small-RNA-released-signal-lineage-resolved immune chain (Table SI).

## 3. Rewiring: Metabolic plasticity and ferroptosis competence

*Mitochondrial-nuclear regulatory circuits*. A proposed model of mitochondrial small-RNA regulation is bidirectional rather than exclusively intramitochondrial. Nuclear miRNAs can directly tune mtDNA-encoded respiratory transcripts, while cytosolic miRNAs regulate nuclear genes that determine mtDNA copy number, substrate entry, respiratory-chain assembly and mitochondrial dynamics. Mitochondrial dysfunction then generates retrograde signals, ROS, $NAD^+$/NADH imbalance, calcium, integrated stress responses and chromatin-active metabolites, that reshape nuclear transcription and miRNA expression. This bidirectional model is supported conceptually by mitochondrial miRNA localization studies but does not imply that every component has been demonstrated in BC (16). In tongue squamous carcinoma, the reported mitochondrial miR-2392 program reprogrammed metabolism and chemoresistance through mitochondrial transcription, providing cross-cancer evidence (55). Let-7a altered ND4 and respiratory readouts in a cell-line-dependent manner: ND4 decreased in MCF-7 but increased in MDA-MB-231 cells after overexpression. Mimic/antisense functional data support M, not a universal inhibitory direction or complete target-rescue chain (30). MiR-4485 was reported to bind mitochondrial 16S rRNA and alter mitochondrial function (43). The reported binding supports Moderate Interaction, while compartment-resolved topology and a complete target-specific rescue chain remain separate questions. Reports of mitochondrial-genome-derived miRNAs add a distinct candidate class but still require independent biogenesis and target validation (31). Independent replication with target-specific rescue and compartment-resolved perturbation remains important.

Cytosolic regulation provides additional, experimentally separable relationships. miR-200a and miR-199a-3p regulate TFAM-linked phenotypes (37,39,56). The cisplatin study reports partial TFAM reversal (Function M); the separate hypoxia/SNHG1 relationship is described without a Function grade because its rescue detail was not accessible (37,56). miR-485-3p/5p suppresses PGC-1α, whereas miR-195 targets MFN2 (38,66). Separate BC studies link miR-874-3p to VDAC1, miR-200c to the mutant-p53-PCK2 axis, and miR-27b to PDHX-dependent pyruvate metabolism (40-42). Epigenetic control of miR-663 has also been connected to mitochondria-to-nucleus retrograde signaling (67). These are mitochondria-regulatory miRNAs rather than automatically bona fide mitomiRs. Together with the reported let-7a-mt-ND4 edge, these studies identify separate RNA-target relationships affecting respiration, metabolic flux or organelle state. They do not demonstrate that each target connects to the complete GPX4-FSP1-DHODH defense network in BC. The shared ferroptosis framework in Fig. 2 is therefore a proposed convergence model; support for each RNA-target edge must be distinguished from support for its downstream connection to ferroptosis.

*Metabolic switching in treatment-relevant states*. Metabolic plasticity should be measured as flux and state transitions, not inferred from one enzyme or a static transcriptome. BC models demonstrate mitochondrial-biogenesis-, FAO-, OXPHOS-, lactate-oxidation- and fusion-dependent states across dissemination, targeted-therapy persistence and stemness (1-9). Small-RNA studies add miR-342-3p-MCT1, miR-526b-COX-2/EP4 and miR-6126-GRP78-associated programs, but these do not define a universal mitochondrial direction (68-71). The inference is therefore contextual: the dominant flux, its switching capacity and the treatment-relevant state must be measured directly.

Suppressing respiratory capacity may impair dissemination but may also force glycolysis, elevate lactate and favor an immunosuppressive niche. Conversely, increasing OXPHOS can improve energetic fitness while increasing electron-transport-derived ROS and ferroptotic pressure. Studies of

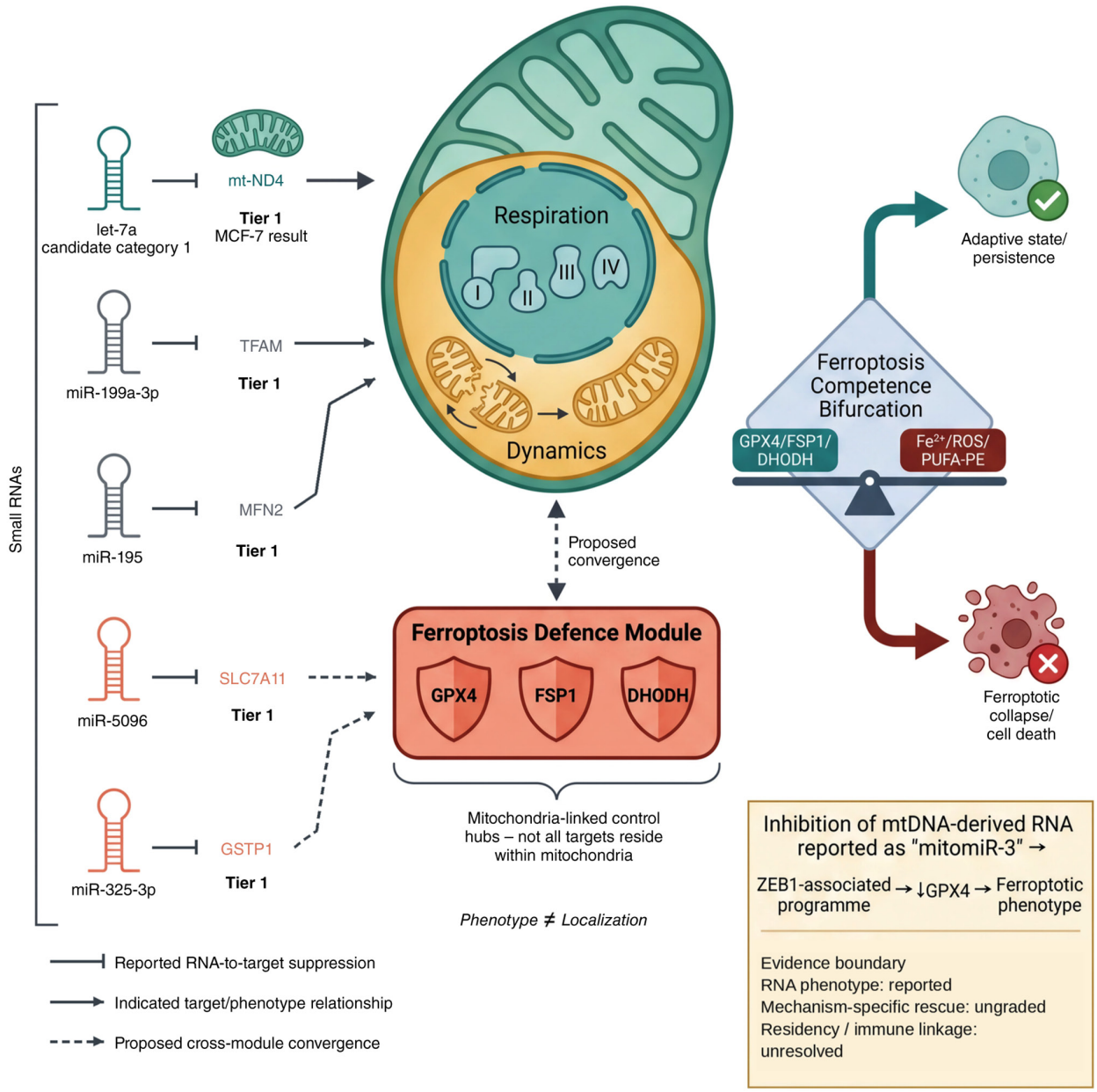



Figure 2. Reported RNA-target relationships and proposed convergence on ferroptosis competence. Solid inhibitory bars indicate reported RNA-to-target suppression; these are not universal High LIFT assignments. Candidate Category 1 let-7a is linked to mt-ND4, and cytosolic miR-199a-3p, miR-195, miR-5096 and miR-325-3p are linked to TFAM, MFN2, SLC7A11 and GSTP1, respectively. Solid target-to-phenotype links denote the indicated respiratory or morphology relationship. Dashed connectors denote proposed cross-module convergence, not an experimentally completed route from every RNA target to GPX4, FSP1 or DHODH. In particular, MFN2 morphology rescue supports Function M because reciprocal RNA effects and rescue are not established for the same morphology endpoint; it does not prove ferroptosis mediation. 'Mitochondria-linked' does not place all targets or defense systems inside mitochondria. The mitomiR-3 inset records a reported RNA-perturbation/ferroptosis phenotype, while complete rescue appraisal, protected residency and immune linkage remain unresolved. Evidence ratings and their source limits are specified by claim in Table II-B and Table SI. The let-7a inhibitory symbol depicts the reported MCF-7 result; the tested MDA-MB-231 cells showed an opposite ND4 response. The mitomiR-3 rescue axis remains ungraded because of source-access limits, not because an experiment was shown to be negative.

mitochondrial elongation, MNK1, α-ketoglutarate dehydrogenase, serine availability, phytosesquiterpene lactones and LINC00667 illustrate this conditionality without defining a single pro-tumor mitochondrial state (10,11,72-76). Future small-RNA studies should report media composition, oxygen tension, organ-specific niche and treatment history, and should pair OCR/ECAR with isotope tracing and lipidomics.

*Dynamics, biogenesis and mitophagy as state stabilizers.* A metabolic state persists only if the mitochondrial network can be remodeled. Fusion can distribute metabolites and complement damaged organelles; fission can support migration and segregate damage; biogenesis expands capacity; and mitophagy constrains ROS and release of mitochondrial nucleic acids. BC studies link miR-195-MFN2 to mitochondrial function and SLC6A14 to fusion and stemness (7,38). OPA1 supports tumor growth, whereas mitochondrial elongation can reduce metastatic competence (10,77). These observations caution against labeling fusion or fission as uniformly oncogenic. Network architecture is a conditional fitness program

whose consequence depends on cell cycle, migration, substrate availability and the need to eliminate damage. BET-inhibitor effects on OXPHOS and mitochondrial dynamics in TNBC further illustrate this context dependence (78).

The miR-195-MFN2 study illustrates why Function must be judged at the stated endpoint. RNA gain and loss changed MFN2 abundance, and respiration was assessed under reciprocal RNA perturbation. Quantified morphology experiments showed miR-195 overexpression and rescue by MFN2 lacking its 3'-UTR; a reciprocal anti-miR morphology arm was not identified in the assessed figures (38). R18 is therefore graded Function M, retaining the morphology rescue without treating separate protein or respiratory readouts as bidirectional morphology evidence. MFN2 restoration did not reverse the reported apoptotic response, and these findings do not establish ferroptosis mediation. A partial GSTP1 rescue in the miR-325-3p study likewise supports Function M for ferroptosis sensitization (79).

Mitophagy also connects treatment injury with mitochondrial quality control. In BC cell models, miR-218-5p combined with doxorubicin was linked to reduced Parkin-dependent mitophagy and enhanced anticancer activity (80,81). These findings should be separated from prior target-validation experiments in other cellular systems, and Parkin-knockdown phenocopy should not be described as target-restoration rescue of the miRNA mechanism. HMGB3 inhibition by miR-142-3p was linked to autophagy, ROS accumulation and mitochondrial apoptosis (82). BC radiation studies show that autophagy can restrain mtDNA-associated immune signaling, while vaccination studies provide additional cross-system precedent (83,84). Neither establishes that miRNA-mediated mitophagy inhibition controls a mitochondrial-nucleic-acid-to-immune-cell pathway. Testing that possibility requires measurements of organelle turnover, cell-death mode, released material and recipient immune function in the same perturbation-rescue experiment.

*Ferroptosis as the redox hinge*. Ferroptosis is driven by iron-dependent phospholipid peroxidation when antioxidant defense cannot contain lipid radicals (85,86). The core landscape includes cystine import through the cystine/glutamate antiporter system $xc^-$, glutathione and GPX4, as summarized in mechanistic reviews (87,88). ACSL4-dependent incorporation of polyunsaturated fatty acids shapes ferroptosis sensitivity (89). FSP1 provides a CoQ-dependent defense at membranes (90,91). Mitochondrial DHODH constitutes a distinct ferroptosis-defense system, whereas TCA-cycle and electron-transport contributions depend on context (92,93). Ether-lipid remodeling further creates plasticity in ferroptosis susceptibility (94). Mitochondria are therefore neither obligatory for every ferroptotic trigger nor dispensable from the clinically relevant state; their role depends on substrate metabolism, antioxidant architecture and the initiating lesion.

The mtDNA-derived miRNA-like RNA reported as mitomiR-3 provides a directly perturbed BC ferroptosis example. Kandettu and colleagues linked its inhibition to a ZEB1-associated program, GPX4 downregulation and pro-ferroptotic lipid metabolism (22). The reported perturbation and phenotype support a Tier 1 relationship in the tested models, but do not by themselves verify canonical miRNA biogenesis, protected intramitochondrial action, endogenous target-site dependence or an immune endpoint. The accessible source supports the reported phenotype but not a criterion-level rescue judgment; Function is therefore ungraded (NR), not High. This source limit is distinct from evidence that a rescue experiment was absent. Complementary studies link miR-128-3p to CD98hc-associated ferroptosis in anoikis-resistant BC cells and identify SLC7A11 as a miR-5096 target (95,96). For miR-5096, SLC7A11 restoration was explicitly partial, supporting Moderate rather than High Function while retaining Tier 1 context. The zebrafish-larva model contributes preclinical evidence, not mammalian immune validation. LINC00152-linked cAMP/Ca2+ signaling and the miR-325-3p-GSTP1 axis provide treatment-resistance examples (79,97). Clinical-transcriptomic ferroptosis associations do not establish RNA causality (98). Cytosolic death-control miRNAs remain Category 4 unless mitochondrial residency is separately demonstrated (Table II-B; Fig. 2).

Other small-RNA classes broaden the Rewiring layer without collapsing its taxonomy. A mitochondrial tRNA fragment, mt-tRF-Tyr-GTA-001, was reduced in breast tumors and associated with outcome in two cohorts (36), but its proposed regulatory targets and immune associations remain inferred rather than causally perturbed. For the nuclear tRNA-derived tRF-33-MEF91SS2PMFI0Q, reporter-supported IGF1 regulation and reciprocal RNA-perturbation phenotypes support Function M; IGF1-specific phenotype restoration was not demonstrated in the inspected main text (58). These studies justify inclusion of other small RNAs in the framework while illustrating why origin, localization and direct interaction must remain separate axes.

A distinct mitochondrial-origin example is the reported 29-nucleotide tiRNA-Met derived from mitochondrial tRNA-Met-CAT. In TNBC cells, RNA gain/loss altered proliferation, migration and invasion; RNA pull-down, SNRPA immunoprecipitation and domain mapping supported an SNRPA interaction, while SNRPA and RANBP3L perturbations provided functional reversal. RANBP3L depletion only partially reversed the RNA-associated proliferation, colony and mTORC1/RPS6 phenotypes, supporting Function M. The RNA was predominantly cytoplasmic: sequence origin does not establish protected mitochondrial residency, intramitochondrial processing or export. Paired TNBC tissues and nude-mouse xenografts support a preclinical Translation M assignment, without demonstrating mitochondrial-respiration control, an intact adaptive-immune mechanism or clinical treatment benefit (99). This Category 3 example therefore expands the functional mitochondrial-origin RNA evidence while retaining a separate localization limit (Table II-B, R35). R35 is assigned to Rewiring for cell-autonomous mTORC1-linked growth regulation by a reported mitochondrial-origin RNA; mitochondrial bioenergetic control was not demonstrated.

Recent work makes the immune relevance of the ferroptosis threshold concrete but does not make it small-RNA dependent. In TNBC, SLC25A13 was reported to promote complex-I-linked OXPHOS, suppress mitochondrial ROS and induce a STAT3-IFI6 program that limits lipid peroxidation, weakens CD8 T-cell cytotoxicity and reduces anti-PD-1 efficacy; a small-molecule degrader restored ferroptosis and immune function in syngeneic models (100). This is Tier 2

enabling BC evidence for a metabolic-to-immune edge. A large multi-omics analysis of ferroptosis heterogeneity in TNBC similarly identified context-specific GPX4 dependence and an immunotherapy combination rationale without establishing a small-RNA driver (101). Separately, CD8-derived IFN-γ can repress SLC7A11 and promote tumor ferroptosis, while radiotherapy and immunotherapy cooperate through lipid oxidation (102,103). Ferroptosis is therefore a bidirectional interface: small-RNA-associated mitochondrial states may set tumor susceptibility, and immune pressure can move that threshold.

## 4. Directional relay: Donor, cargo and recipient determine function

*Extracellular small-RNA relay*. The unit of adaptation is not always the tumor cell. Small extracellular vesicles (sEVs), microvesicles and other particles carry miRNAs, proteins, metabolites and nucleic acids among malignant, stromal and immune cells. Together with whole-organelle transfer, these exchanges form a directed Relay network in which donor identity, transmitted material, recipient lineage and spatial proximity determine phenotype (Fig. 3A). Functional proof requires quantitative cargo loading, uptake by the relevant recipient, loss of phenotype after donor-cargo depletion and rescue at a physiologically plausible copy number under current EV identity and purity standards (44,48,64). Natural Category 5 Relay and engineered Category 6 delivery are therefore not interchangeable.

BC studies support several directional RNA-cargo relationships, but their causal scope differs. Macrophage-derived EV miR-503-3p has been linked to DACT2-associated metabolic remodeling in recipient BC cells. The metabolic direction remains unresolved because the article's title, abstract and results are inconsistent; Function remains Moderate, while the metabolic direction is unresolved (Tables II-B and III) (104). TNBC-derived EV miR-20a-5p was linked to CD8 T-cell dysfunction and resistance to PD-1 blockade (105). Donor-cargo perturbation and NPAT-target assays support an RNA-Relay effect, but target-reporter specificity must be distinguished from NPAT-specific rescue of the recipient phenotype. The treatment-response experiments used NSG mice reconstituted with human peripheral blood mononuclear cells (PBMCs), an immune context distinct from an intact syngeneic model. Neither this model nor the extracellular phenotype establishes mitochondrial entry or mitochondrial-state mediation in recipient T cells. Additional EV or macrophage-directed miRNA studies concern invasion, myeloid-suppressor development, PD-L1 regulation and polarization; not every study demonstrates natural EV-mediated transfer (106-113). Thus, Tier 1 immune Relay denotes a directly perturbed RNA relationship, not a mitochondrial mechanism.

Relay also extends to organotropism, angiogenesis and state reporting. Tumor EV miR-200b-3p can alter lung epithelial CCL2, MCU-dependent reduction of EV miR-4488 loading can relieve its inhibitory effect on angiogenesis and favor metastatic colonization, and estrogen or endoplasmic-reticulum stress can alter vesicle release and cargo selection (57,108,114,115). In the miR-4488 study, published figure captions describe endothelial gain-of-function, CX3CL1 reporter assays and CX3CL1 knockdown phenotypes; these are not interchangeable with restoration of a complete donor-EV-recipient mechanism (57). Tumor sEVs can also induce PD-L1-positive macrophage states (116). These observations suggest that an extracellular miRNA profile may report a changing intracellular state rather than a stable tumor genotype. Exosome-associated mtDNA transfer in endocrine-resistant BC provides a separate example in which extracellular cargo reflects organelle adaptation without constituting an EV-miRNA mechanism (117).

*Whole-mitochondrion transfer as enabling Relay biology*. Whole-organelle transfer is a distinct causal unit from RNA transfer. Tunnelling nanotubes and other contact- or vesicle-associated routes have been implicated in intercellular mitochondrial exchange (118,119). The broader cancer-transfer literature has been reviewed separately (120). In BC systems, adipose-derived stromal-cell mitochondrial transfer was linked to multidrug resistance, whereas fibroblast-to-cancer transfer increased migration (121,122). These are separate donor-recipient-endpoint relationships, not interchangeable demonstrations of one rescue mechanism. The route is also model-dependent: nanotube blockade did not eliminate transfer in the hybrid organoid setting examined by Del Vecchio and colleagues (121). Organelle tracing, metabolic effects and transfer perturbation constitute enabling functional evidence; RNA Function is not applicable unless a particular RNA is depleted and its contribution restored. Persistence, recipient targeting and necessity of a transferred mitochondrial small-RNA program remain separate hypotheses.

Direction and disease context must be assigned to the experiment rather than to the article as a whole. Saha and colleagues reported immune-to-cancer mitochondrial trafficking and included an immunocompetent BC treatment model (118). Hoover and colleagues demonstrated nerve-to-cancer transfer in BC models, including 4T1 and human BC systems; the BC experiments provide disease-specific enabling evidence (123). Their BC organelle-transfer claims are Tier 2 enabling biology, not Tier 1 RNA mechanisms. Other studies concern MIRO2-mediated cancer-to-fibroblast transfer and immune-to-tumor transfer during lymph-node metastasis, with their respective model contexts retained (124,125). In separate systems, experimentally enhanced transfer into T cells improved metabolic fitness and antitumor efficacy (126), whereas acquisition of tumor mitochondria carrying mtDNA mutations was linked to immune evasion (127). These contrasting findings motivate donor-, genotype- and recipient-specific experiments; they do not establish small-RNA dependence or make every transfer edge a demonstrated BC mechanism.

*Donor-recipient specificity and therapy-induced transitions*. Relay should be mapped as a directed graph rather than a pool of extracellular material. Donor identity determines cargo composition; recipient lineage determines uptake, metabolic integration and immune consequence; and spatial adjacency determines exposure. Fibroblast- or adipocyte-to-tumor transfer may rescue malignant-cell fitness, macrophage-to-tumor vesicles may alter metabolism, and tumor-to-myeloid or tumor-to-lymphocyte vesicles may impose suppressive states (104,105,107,121,122). Binary

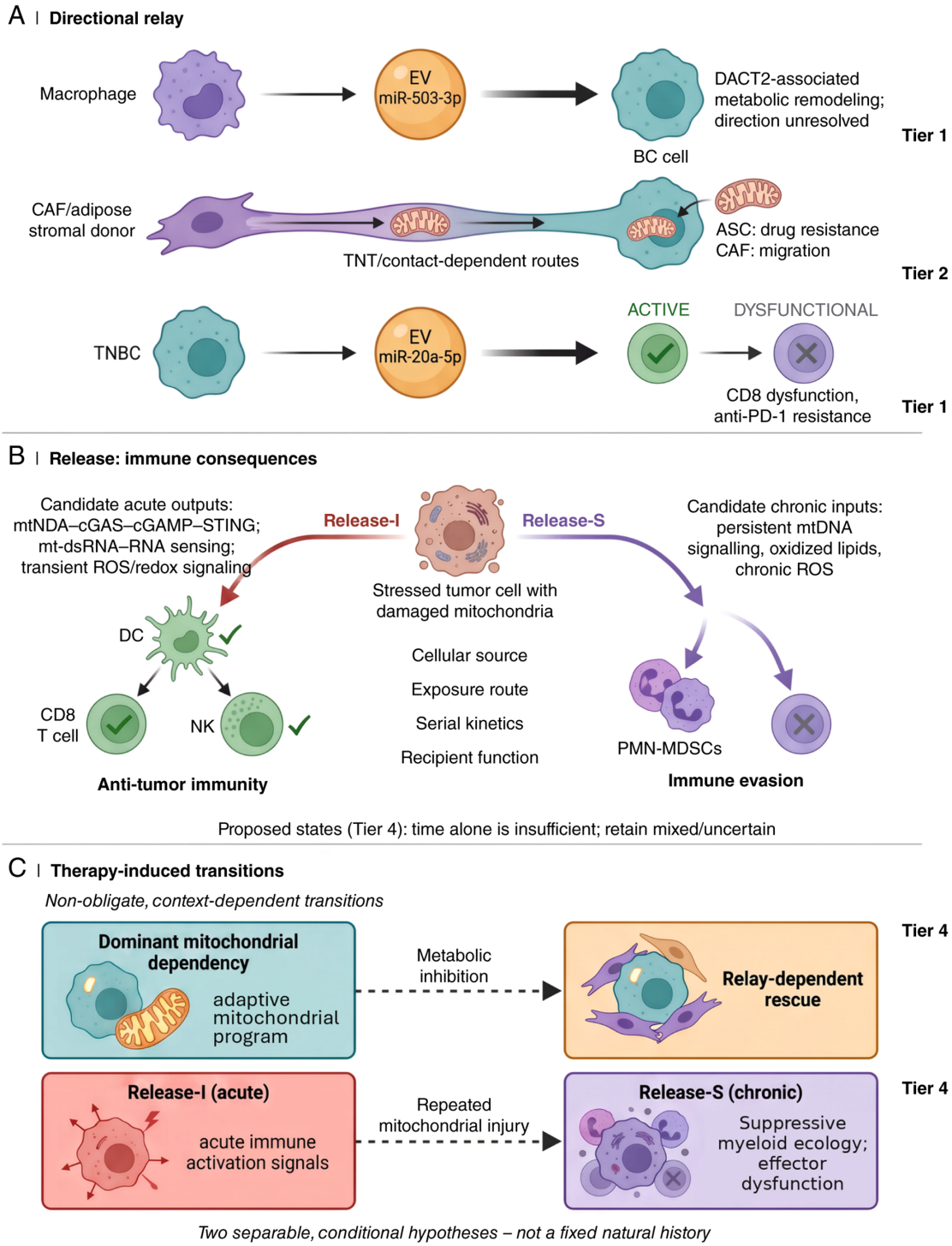



Figure 3. Direction, cargo, recipient lineage and timing determine the ecosystem consequences of Relay and Release. (A) Directional Relay. Three representative donor-cargo-recipient relationships illustrate how adaptive capacity is redistributed within the breast-tumor ecosystem. Macrophage-derived extracellular vesicles carrying miR-503-3p have been linked to DACT2-associated metabolic remodeling in breast-cancer cells; the direction of the glycolysis/OXPHOS effect is not assigned here because the cited article is internally inconsistent. Adipose stromal-cell transfer has been linked to drug resistance and fibroblast-to-cancer transfer to migration. Transfer routes depend on the model and are not uniformly nanotube-dependent. TNBC-derived extracellular vesicles carrying miR-20a-5p impair CD8 T-cell function and promote resistance to PD-1 blockade. The indicated claim tiers distinguish direct small-RNA evidence from small-RNA-independent enabling mitochondrial-transfer biology. (B) Release: immune consequences. Mitochondrial DNA exposure can engage cGAS-cGAMP-STING signaling, whereas mitochondrial double-stranded RNA requires a separately established RNA-sensing route. ROS and oxidized lipids are redox-associated outputs, not direct substitutes for DNA- or RNA-sensing evidence. The proposed Release-I state combines source-resolved transient exposure with improved antigen presentation or cytotoxic-cell function. The proposed Release-S state combines persistent or recurrent exposure with measured myeloid suppression or effector dysfunction. Cellular source, exposure route, time course and recipient lineage determine interpretation; acute and persistent signals are not intrinsically beneficial and harmful, respectively. Discordant lineage effects remain mixed and incomplete observations uncertain. Both states are Tier 4 hypotheses requiring the prospective tests specified in Section 6.2, not validated patient classes. (C) Therapy-induced transitions. Therapy may reconfigure the dominant communication mode through two separable and non-obligate transitions. Metabolic inhibition may shift a Rewiring-dominant tumor towards Relay-dependent stromal or organelle-mediated rescue. Independently, repeated mitochondrial injury may convert an acute Release-I state into persistent Release-S. Dashed arrows indicate context-dependent Tier 4 hypotheses that require prospective temporal, donor-recipient and lineage-resolved validation; they should not be interpreted as a fixed natural history.

Table III. Directional Relay and Release evidence matrix.

| Donor or source | Cargo/stressor | Recipient or sensor | Reported outcome | Tier | Unresolved test | (Refs.) |
|---|---|---|---|---|---|---|
| Macrophage | EV miR-503-3p | Breast cancer cell | DACT2-associated metabolic remodeling; direction unresolved. | 1 | Reconcile the primary metabolic direction; quantify physiological cargo copies and recipient itinerary. | (104) |
| TNBC cell | EV miR-20a-5p | CD8 T cell | Dysfunction and anti-PD-1 resistance. | 1 | Whether immune-cell mitochondrial state mediates the phenotype. | (105) |
| PARP-inhibitor-resistant TNBC cell | EV miR-181a | Sensitive TNBC cell | STING suppression and transferred drug resistance. | 1 | Separate EV necessity from other co-cargo; test immune recipient effects. | (144) |
| Breast-cancer cell | MCU-dependent reduction of EV miR-4488 loading | Endothelial/metastatic niche | Reduced loading of inhibitory cargo favors angiogenesis and metastatic colonization. | 1 | Distinguish mitochondrial-state reporter from causal cargo across recipients. | (57) |
| Adipose stromal cell | Intact mitochondria | Breast-cancer cell | Multidrug resistance and metabolic rescue. | 2 | Identify persistent causal cargo; test RNA-independent and RNA-dependent components. | (121) |
| Cancer-associated fibroblast | Intact mitochondria | Aggressive breast cancer cell | Increased migration. | 2 | Generalize across subtypes and distinguish transfer from cell-contact signaling | (122) |
| Tumor cell | Mutant mitochondria | Tumor-infiltrating immune cell | Immune evasion in a cross-cancer. model | 3 | Test the directional edge and organelle genotype in breast cancer. | (127) |
| Irradiated breast-cancer cells | mtDNA | cGAS-STING-competent tumor/immune cells | Radiation-associated mtDNA signaling supports antitumor responses and is restrained by autophagy. | 2 | Resolve source, kinetics and responding lineage; test small-RNA dependence. | (84) |
| Oxidatively stressed tumor cells | Oxidized mtDNA | STING-competent immune cells | Anti-tumor vaccination or immune activation in cross-system models. | 3 | Demonstrate the corresponding source-recipient edge and kinetics in breast. cancer | (83) |
| Injured mitochondria | mt-dsRNA | Cytosolic antiviral sensors | Antiviral signaling in human cells. | 3 | Establish a small-RNA-regulated mt-dsRNA Release edge in breast cancer. | (63) |
| Ferroptotic tumor cell | Oxidized lipids/ damage signals | Dendritic cell | Ferroptotic material can impair antitumor priming. | 3 | Define exposure window, lipid species and tumor-selective dosing in breast cancer. | (147) |
| Repeated or senescent tumor stress | Persistent mtDNA/ STING-associated signals | PMN-MDSC/myeloid compartment | Suppressive myeloid ecology. | 3 | Prospectively define and validate the proposed Release-S state. | (142) |
| Immune cell | Intact mitochondria | Breast-cancer cell | Mitochondrial acquisition/metabolic advantage in the tested breast-cancer context. | 2 | Separate organelle effects from RNA necessity. | (118) |
| Neuron | Intact mitochondria | 4T1/MDA-MB-231 recipient | Breast-cancer adaptation/metastatic phenotypes. | 2 | Resolve donor cargo and endpoint by model; keep the human prostate findings separate from the breast-cancer models. | (123) |

Table III. Continued.

| Donor or source | Cargo/stressor | Recipient or sensor | Reported outcome | Tier | Unresolved test | (Refs.) |
|---|---|---|---|---|---|---|
| Engineered plant-vesicle platform | MicroRNAs plus sorafenib and targeting components | TNBC model/immune ecosystem | Ferroptosis sensitization and immunoactivation in a composite delivery system. | 2 | Establish component-specific RNA necessity; no endogenous mitochondrial-RNA chain inferred. | (146) |
| Serine-restricted colorectal cell | Cytosolic mtDNA | cGAS-STING/immune ecosystem | Antitumor immunity in colorectal models; 4T1 was a heterologous rechallenge control. | 3 | Reproduce the actual mechanism in breast. cancer | (73) |
| Raddeanin A-treated B16/MC38 cell | TDP-43-associated mtDNA exposure | cGAS-STING/immune cells | Immune activation in melanoma/colorectal models. | 3 | No breast-cancer mechanism is supplied by these model results. | (137) |

Each row specifies donor, cargo or stressor, recipient and outcome so that intercellular causality is not inferred from a bulk sample.

M1/M2 labels are inadequate because macrophage respiratory-chain remodeling can amplify interferon signaling, and macrophage-derived vesicles can have context-dependent antitumor effects (128,129). The same mitochondrial intervention may therefore produce opposite outcomes in tumor and immune cells.

Adipokines provide additional stromal context but should not be conflated with vesicular RNA or organelle transfer. Tissue studies of chemerin and panels including visfatin, lipocalin-2 and adiponectin report associations with receptor status, proliferation or lymphovascular features, with results depending on whether staining is evaluated in cancer cells, fibroblasts or adipocytes (130,131). The broader adipokine literature is heterogeneous (132). These observational findings justify compartment-resolved covariates when testing donor-recipient biology; they do not establish a uniform chemerin-driven aggressive state, a mitochondrial mechanism or an RNA-Relay pathway. Overlapping investigators or patient sources should not be treated as independent validation without confirming cohort independence.

Therapy is likely to perturb the graph. Hypoxia, nutrient deprivation, chemotherapy and ferroptotic stress can increase vesicle release, alter cargo sorting or select donor populations that restore a damaged recipient. A tumor that initially appears Rewiring-dominant may therefore become Relay-dependent after metabolic inhibition. The critical measurements are longitudinal: labeled donor mitochondria or vesicles, donor-specific mtDNA variants, single-cell metabolic phenotypes and lineage-aware imaging before and after treatment. Without temporal resolution, relay-mediated rescue can be mistaken for selection of a genetically resistant clone. This therapy-induced Relay transition is a Tier 4 prediction until prospective perturbation distinguishes increased transfer, state selection and simple survivor outgrowth.

## 5. Release: Immune selection and the missing causal chain

*Mitochondrial danger signals: source, route and duration*. Mitochondrial injury can expose mtDNA, oxidized mtDNA, mt-dsRNA, ROS and oxidized lipid signals to engage different sensors in tumor cells, dendritic cells, macrophages and myeloid suppressor cells. The route matters: transient cytosolic leakage differs from vesicle-protected extracellular cargo, and immunogenic death differs from chronic sublethal stress. In BC, radiation-induced mtDNA signaling is restrained by autophagy (84). Oxidized mtDNA can promote STING-dependent antitumor vaccination, whereas mitochondrial double-stranded RNA can trigger antiviral signaling; both observations derive from non-BC systems (63,83). Mitochondrial-DNA-enhanced dendritic-cell vaccination provides another cross-cancer example of productive Release (133). Cytosolic DNA composition can also determine dendritic-cell-mediated antitumor immunity (134). Mitophagy, autophagy and membrane permeabilization therefore determine which signal is exposed and for how long.

BC RNA perturbations also establish a direct immune-sensing checkpoint. Under hypoxia, miR-25/93 directly represses NCOA3 and thereby lowers cGAS expression; cGAS is an indirect target. In E0771 tumors, RNA gain/loss altered tumor growth and effector CD8/Treg endpoints, and cGAS

restoration rescued the immune-cell and tumor-growth phenotypes. cGAS knockdown also reversed the IFN-β response to miRNA inhibition (135). These experiments support Tier 1 Release and Function H for the stated cGAS-dependent immune consequences (Table II-B, R34). The main text reports similar cytosolic mtDNA amounts after miRNA or cGAS manipulation. The established edge therefore concerns competence to sense exposed mitochondrial DNA, not RNA control of mtDNA-release quantity. Mitochondrial residency or origin of these cytosolic miRNAs is not established or required for the NCOA3 mechanism.

The miR-25/93 result establishes RNA-dependent immune sensing but leaves the upstream mitochondrial-small-RNA Release question open. The full-chain claim assessed here requires a defined endogenous mitochondria-resident or mitochondria-derived small-RNA perturbation, a consequent change in an identified mitochondrial output or exposure route, sensing in a specified cell lineage, and a rescued immune-function endpoint. That chain remains unestablished in this panel. RNA-dependent changes in respiration, mitophagy or ferroptosis could alter mtDNA exposure or oxidized-lipid output, but a mitochondrial phenotype alone does not demonstrate that route. The distinction concerns release control vs. sensing competence, rather than absence of RNA-related immune effects or engineered RNA activity.

*Acute vs. chronic cGAS-STING signaling.* cGAS detects cytosolic DNA and generates cGAMP, activating STING-TBK1-IRF3 and type I interferon programs. Radiation-associated mtDNA responses restrained by autophagy provide a BC enabling precedent (84). Ultrasound-responsive low-dose doxorubicin delivery also showed therapeutic and immune-related activity in 4T1 models, although mechanistic perturbations performed in other tumor models must not automatically be attributed to the BC arm (136). By contrast, the serine-restriction mechanism was established principally in colorectal cancer; the use of 4T1 as a heterologous rechallenge control does not demonstrate the serine-mtDNA-STING mechanism in BC (73). Raddeanin A/TDP-43 experiments in B16 melanoma and MC38 colorectal models likewise provide cross-system rather than BC evidence (137). Low-dose prexasertib offers another non-RNA mtDNA-STING precedent, but the inspected primary record is insufficient for disease-context assignment; it is retained as unclassified background and excluded from Table II-C (138). A mitochondria-targeted nanoplatform sensitized TNBC to anti-PD-L1 therapy, illustrating engineered, not endogenous small-RNA, intervention (139). VDAC2 loss, broader immunometabolic studies and oxidized-mtDNA vaccination further motivate productive Release experiments without supplying a mitochondrial-small-RNA causal link (83,140,141).

The same signaling pathway can accompany tumor control or suppression, depending on the source and responding lineage. Senescent tumor-cell mtDNA has been linked to PMN-MDSC-mediated suppression, while PERK-dependent myeloid programs can inhibit STING and maintain suppressive function (142,143). Cytosolic DNA composition also affects dendritic-cell responses (134). Release-I and Release-S were therefore retained as Tier 4 operational hypotheses, not validated biomarkers or patient classes. Release-I requires a source-resolved, transient signal accompanied by improved antigen presentation or cytotoxic-cell function; Release-S requires persistent or recurrent exposure accompanied by demonstrated myeloid suppressor activity or loss of effector function. Acute signaling is not automatically beneficial, and persistent signaling is not automatically suppressive. A single STING/IFN measurement or an immune-cell abundance shift is insufficient for either assignment. An illustrative sampling scheme, functional readouts and decision rules are specified below. Fig. 3B and C represent these states and their possible transitions as conditional hypotheses rather than a fixed natural history.

Cytosolic miR-181a provides a boundary example rather than a mitochondrial RNA mechanism. In BRCA-mutated TNBC, overexpression promoted olaparib resistance, whereas inhibition in HCC1937-OlaR and HCC1395-OlaR cells increased drug sensitivity. EV-associated transfer and STING repression were also reported (144). These BC gain/loss results support M without importing ovarian knockout evidence; they do not establish a complete EV-cargo rescue or mitochondrial Release chain.

*Ferroptosis can activate or disable immunity.* Ferroptosis is often described as immunogenic because oxidized lipids and damage signals can expose tumor antigens and amplify inflammatory sensing. CD8 T cells can promote tumor ferroptosis during checkpoint therapy (103). Lipid peroxidation can cooperate with radiotherapy to promote tumor ferroptosis (102). Inhibition of ACLY increased polyunsaturated-fatty-acid peroxidation and overcame immunotherapy resistance through a cGAS-STING-linked mechanism (145). SLC25A13 degradation provides a protein-directed BC example linking ferroptosis modulation and immune function (100). Separately, Wang *et al* (146) reported coordinated sorafenib and microRNA delivery using a plant-vesicle-derived engineered nanoplatform with ferroptosis and immune-activation endpoints in TNBC. This Category 6 system must be considered in the RNA-immunity literature, but its component-specific effects cannot be equated with an endogenous human mitochondrial-small-RNA-controlled Release chain. These studies motivate combination testing under conditions that demonstrate productive immunity, not a general assumption that ferroptosis induction benefits checkpoint therapy.

Yet ferroptotic cells can also impair dendritic-cell-mediated antitumor immunity (147). Oxidized lipid cargo can paralyze antigen-presenting or effector cells. CD36-associated lipid uptake has been linked to ferroptotic loss of intratumoral CD8 T-cell function in one study (148) and to oxidized-lipid uptake, peroxidation and dysfunction in another (149). Lipid-peroxidation-associated dysfunction should not automatically be relabeled as demonstrated ferroptotic death. These Tier 3 studies demonstrate why a ferroptosis-promoting combination cannot be assumed to spare immune cells. Ferroptosis timing is likely decisive: early dying cells may not supply the same immunostimulatory repertoire as late ferroptotic material; high lipid-peroxide burden may exceed the oxidative tolerance of dendritic cells; and systemic induction may damage activated lymphocytes. Thus, tumor-selective delivery, kinetic sampling and

immune-cell redox measurements are required. A therapy should be judged not only by 4-HNE, malondialdehyde or lipid ROS in tumor cells, but by antigen presentation, T-cell clonality and the balance of cytotoxic vs. suppressive populations. Reviews of immunogenic ferroptosis and ferroptosis-associated immune evasion support this bidirectional interpretation but do not replace the direct cell-type-specific studies (87,88,150).

*Immune-cell mitochondrial fate closes the feedback loop.* Cytotoxic lymphocytes require mitochondrial fitness for persistence, memory formation and repeated killing, whereas suppressive myeloid and regulatory populations can exploit fatty acids, lactate and ROS-tolerant programs. STING signaling can maintain a TCF-1-positive NK-cell reservoir (151). Complex-IV remodeling in tumor-associated macrophages can amplify interferon signaling (128). Intercellular mitochondrial transfer can either damage T cells when tumor-derived organelles are acquired or improve their function when healthy mitochondria are delivered experimentally (126,127). Single-cell mapping independently shows that cancer-cell-T-cell mitochondrial transfer is directional rather than a generic exchange process (119). These apparently opposing observations are reconciled by donor-recipient specificity and organelle quality.

Immune outputs can feed back to tumor Rewiring. IFN-γ represses SLC7A11 in relevant models, whereas the SLC25A13-STAT3-IFI6 program links mitochondrial metabolism with ferroptosis resistance and immune outcomes (100,103). mtDNA mutations and chronic mtDNA exposure provide additional, context-specific connections between mitochondrial state and immune selection (142,152). Table III separates source, cargo or stressor, recipient and endpoint. Its non-RNA studies are not evidence that a mitochondrial small RNA controls Release. SLC25A13 is a BC protein-perturbation bridge, while the CD36 studies concern principally cross-system immune-cell lipid stress. Their functional evidence remains informative, but RNA-specific Function is NA. This distinction preserves organelle and protein causality without borrowing it to strengthen an RNA claim (Table II-B).

This asymmetry has practical consequences for interpretation. A direct BC small-RNA-to-ferroptosis result can support a Rewiring claim without establishing mitochondrial residency or immune causality, as illustrated by the mitomiR-3 and SLC7A11-related literature (22,96). A directional EV phenotype can support Relay even when the recipient mitochondrial compartment is not involved (104,105). The miR-25/93-NCOA3-cGAS mechanism provides direct RNA-dependent immune-sensing evidence in Release, while leaving the amount of released mtDNA unchanged in the described comparison (135). Other mtDNA-STING or oxidized-lipid studies supply source- and lineage-specific immune precedents (142,147). These relationships do not establish a continuous mitochondrial-small-RNA export or release-control chain. The framework asks whether the edges needed for a particular conclusion have been demonstrated; a candidate need not traverse all three arms. Missing and negative findings delimit the proposed mechanism without negating the supported edge.

## 6. From evidence gaps to decisive experiments

*Resolving provenance and direct interaction.* Resolving provenance begins with orthogonal localization rather than differential centrifugation alone. High-purity immunoisolation should be combined with organelle-integrity controls, RNase protection, mitoplast analysis, compartment-resolved imaging or proximity labeling and absolute molecule counting. NUMTs, abundant tRNA/rRNA fragments and co-purifying endomembranes must be excluded explicitly (15,60,61,65). A candidate should advance as a bona fide mitomiR only when its endogenous abundance and topology are compatible with the proposed function.

Direct interaction requires evidence that the RNA recognizes the proposed target at the relevant site. RNA-RNA ligation or pulldown, occupancy by the relevant RNA-binding complex, target-site disruption and rescue with a refractory target should be linked to quantitative respiratory, metabolic, mitophagic or ferroptotic phenotyping (20,59). Where an mtDNA target-site control is needed, an isogenic mitochondrial-genome perturbation should be used as a mechanistic control rather than presented as a direct mitomiR therapy. The decisive unit is a perturbation-rescue chain from localization to interaction to function.

Independent replication should be treated as a distinct evidentiary requirement rather than assumed from technical depth in one model. A candidate edge should be reproduced with an orthogonal perturbation, a second BC model representing a different subtype or treatment state, and a prespecified rescue criterion. Claimed mitochondrial actors such as let-7a, miR-4485 and the mtDNA-derived RNA reported as mitomiR-3 are particularly informative test cases because their current LIFT profiles are uneven across Localization, Interaction, Function and Translation (22,30,43). Negative localization, failure of target-site rescue or loss of effect at physiological RNA abundance should downgrade only the affected edge; it should not be obscured by retaining a molecule-wide label of mechanistic validity. Cross-laboratory concordance in RNA identity, compartment and phenotype would provide a stronger foundation than adding further candidates detected by a single platform. The accompanying claim-level ledger is organized around model system, evidence for each LIFT axis, context-tier rationale and unresolved tests (Table SI). Source limitations should remain distinguishable from experimental negative findings.

*Establishing directional and lineage-resolved causality.* Relay experiments should identify the donor, cargo, route and recipient rather than infer communication from bulk abundance. Donor-specific RNA depletion, donor-specific mtDNA variants, photoconvertible organelle labels, controls for dye leakage and recipient-specific rescue are required to distinguish active transfer from co-cargo effects or survivor selection. Current EV standards and stoichiometric cargo measurements remain essential (44,48,64). Spatial and single-cell analyses can then test whether the proposed cell pair is physically plausible and whether transfer precedes the adaptive phenotype (65,119,121,122).

Release studies should follow the same perturbation through source-cell mitochondrial integrity, intracellular leakage

or extracellular exposure, recipient sensing and immune function. For the DNA branch, compartment-controlled mtDNA measurements should be paired with cGAMP or STING-TBK1-IRF3 outputs; for mitochondrial dsRNA, RNA identity and the relevant RNA-sensing pathway must be tested separately rather than inferred from cGAS activation. Lipid peroxidation and ROS are additional outputs, not substitute evidence of DNA or RNA release (63,83,84,142,147-149). Dendritic-cell antigen presentation, antigen-specific T-cell activation, CD8/NK killing and myeloid-mediated suppression should be measured in the relevant recipient lineage. Tumor-cell and immune-cell lipid stress must be separated.

For prospective preclinical testing, an illustrative schedule is baseline, 6 and 24 h, 48-72 h, and day 7 after a defined intervention; repeated-dose studies should also sample before and after a later dose. These windows are proposed starting points, not validated biological thresholds, and must be adapted to the model's exposure and turnover kinetics before state assignment. RNA, organelle integrity, released material, recipient signaling and immune function should be paired within the same experimental series. A study-specific positivity threshold and minimum functional effect should be chosen from pilot assay precision and matched controls, fixed before validation, and reported with uncertainty rather than selected retrospectively to maximize class separation.

Under this proposed rule, Release-I requires an early source-resolved signal that declines toward the prespecified control range at later windows and a functional improvement in antigen presentation or cytotoxicity. Release-S requires persistence or recurrence across at least two separated post-acute windows, together with increased suppressor activity or impaired effector function. Increased myeloid-cell counts alone do not demonstrate suppression. Opposing lineage-specific effects are classified as mixed; insufficient temporal or source information is classified as uncertain. Even a reproducible state association is not causal proof: source/cargo depletion and recipient-sensor perturbation, followed by appropriate rescue, must disrupt the predicted link. A mitochondrial-small-RNA mechanism additionally requires RNA-specific necessity and rescue. Failure of temporal ordering, persistence after the relevant depletion, or an explanation based solely on changing cell composition falsifies the proposed transition.

*Translational boundaries and development criteria.* Translation should begin only after provenance, target engagement and mechanism-specific rescue are established. Direct mitochondrial-small-RNA intervention faces two independent barriers: delivery to the intended cell lineage and, when an intramitochondrial target is claimed, delivery to the relevant organelle compartment. Active RNA copies, recipient specificity, organelle exposure, biodistribution and innate-immune activation must therefore be quantified rather than inferred from a downstream mitochondrial phenotype (44,48,64). Normal-tissue and immune-cell mitochondrial fitness are co-primary safety endpoints, particularly when ferroptosis or redox stress is being manipulated (147-149).

Fig. 4 is interpreted in the present study as a prospective research-development and validation cycle, not as a validated clinical algorithm. Profile denotes multidimensional characterization of molecular provenance, mitochondrial state and ecosystem context. Assign denotes prospective testing of Rewiring-, Relay-, Release-I-, Release-S- or mixed/uncertain state hypotheses using the source-, time- and function-based definitions, without forcing incomplete or discordant observations into a binary class. Intervene denotes a mechanism-matched experimental intervention, and Monitor denotes serial measurement of target engagement, state kinetics and lineage-resolved immune consequences. These steps are developmental operations rather than patient classes or established treatment rules.

Advancement should require resolved provenance, reproducible state assignment, confirmed target engagement and adaptation detected early enough to be actionable. Failure of target-site or pathway rescue, unstable state assignment, nonspecific pharmacodynamic signals, absent lineage selectivity or unacceptable normal-tissue or immune toxicity should stop development (Fig. 4; Table IV). This evidence-gated approach preserves the translational ambition of the 3R framework without allowing platform feasibility to substitute for mechanism.

The priority is therefore not a longer catalogue of delivery platforms or mitochondrial editing tools, but a small number of independently replicated edges that connect molecular provenance to a quantitative phenotype and, ultimately, to a defined ecosystem consequence.

Biomarker development must retain biological provenance. Circulating and EV-associated miRNAs permit serial sampling, but hemolysis, platelet activation, isolation method, storage, normalization and changing cell composition can dominate the measured signal. A credible program should pair tumor tissue, plasma and purified vesicle fractions; quantify absolute abundance; assign a plausible cellular source; and demonstrate concordance with a measured mitochondrial or immune phenotype in the same experimental or patient sample. MCU-dependent and estrogen-regulated cargo sorting show that extracellular miRNA abundance can change with intracellular state, while systematic biomarker evidence does not establish mitochondrial origin or causal cargo function (57,114,153). A circulating signature should therefore be treated first as a state-associated observation, not as a mitomiR classifier.

Model choice should match the causal edge. Purified organelles and isogenic cells are appropriate for Localization and Interaction; spatially organized co-cultures or organoid systems are needed for donor-recipient Relay; and immune-competent systems are required for Release and ferroptosis-immunity claims. Single-cell mitochondrial variants and lineage-aware imaging can help resolve donor and recipient populations, while BC stromal-transfer studies provide disease-specific starting edges (65,119,121,122). No single model can validate the complete 3R chain. A staged program should advance from molecular proof to cell-pair causality and only then to ecosystem-level function, with the required rescue and falsifier specified before each transition.

Histology is another boundary on model generalization. Invasive micropapillary carcinoma (IMPC) may occur as a rare pure tumor or as a component within carcinoma of no special type. Reports emphasize lymphovascular and nodal involvement, but component-percentage definitions and outcome comparisons vary (154). Recording the actual micropapillary proportion, stage and receptor profile is therefore preferable to

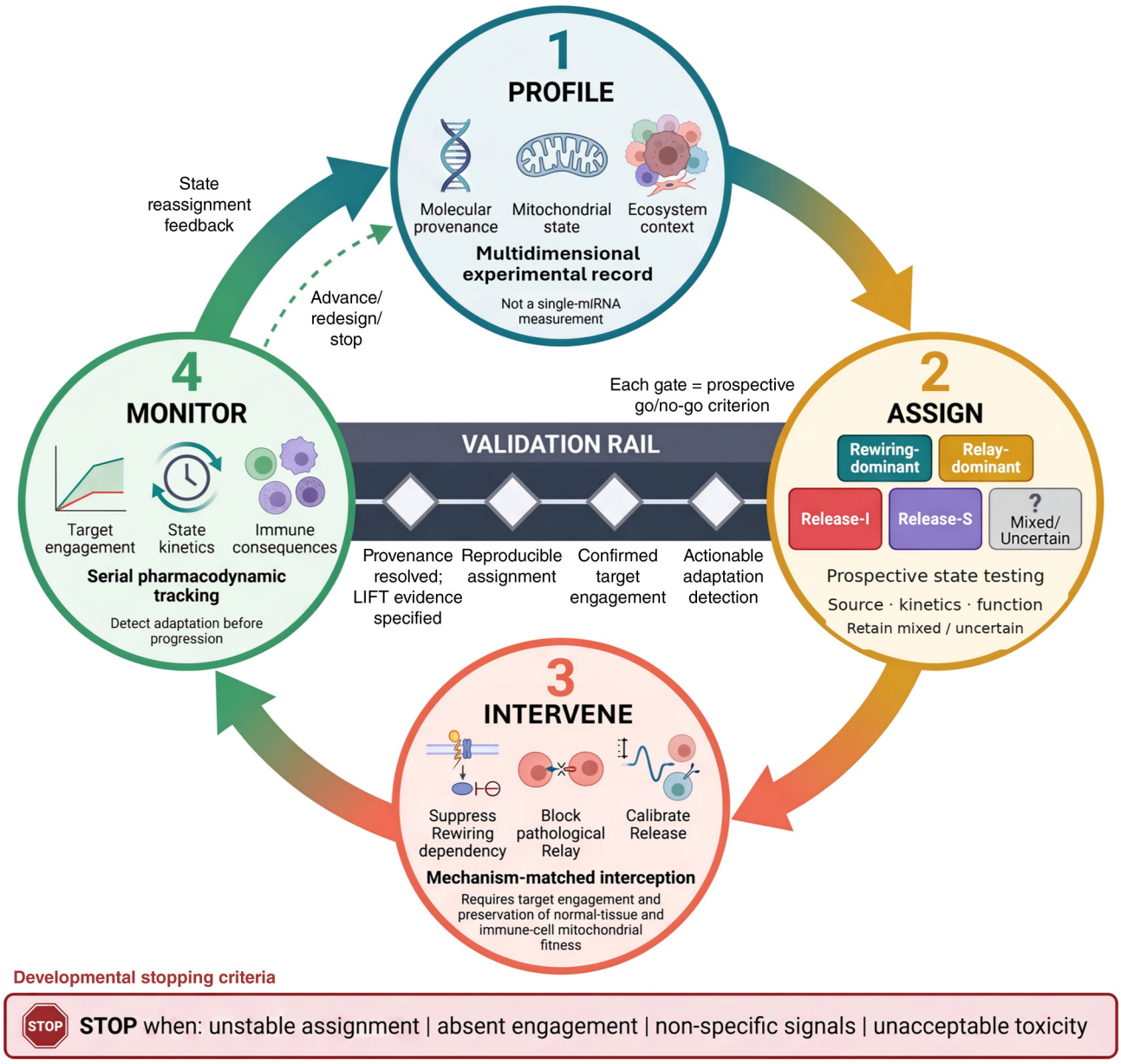



Figure 4. Prospective research-development and validation cycle for testing 3R states. This framework is intended for prospective validation and does not constitute a validated clinical treatment-selection algorithm. i) Profile integrates molecular provenance, mitochondrial state and ecosystem context into a multidimensional record; a single-miRNA measurement is insufficient because abundance alone does not establish cellular source, subcellular location or function. ii) Assign tests Rewiring-dominant, Relay-dominant, Release-I, Release-S and mixed/uncertain state hypotheses using the source-, time- and immune-function criteria in Sections 5.2 and 6.2, with mixed/uncertain outcomes retained. iii) Intervene denotes mechanism-matched experimental interception by suppressing an adaptive Rewiring dependency, blocking a pathological Relay edge or calibrating Release. iv) Monitor uses serial pharmacodynamic assessment of target engagement, state kinetics and lineage-resolved immune consequences. In the present Review, the visible Profile-Assign-Intervene-Monitor sequence is a development workflow rather than a patient classifier or established treatment rule. The validation rail summarizes the principal go/no-go requirements: resolved provenance with explicit LIFT evidence, reproducible state assignment, confirmed target engagement and actionable detection of adaptation. Development should stop when assignment is unstable, target engagement is absent, pharmacodynamic signals are nonspecific or toxicity is unacceptable.

treating a proposed percentage cutoff as a universal biological boundary. This clinical heterogeneity does not establish an IMPC-specific mitochondrial small-RNA program.

HER2 illustrates why invasive and in situ disease require separate interpretation. In a retrospective HER2-positive cohort treated with adjuvant trastuzumab and chemotherapy, an IMPC component was associated with shorter disease-free survival and strong MUC4 expression (155). Without an untreated comparator, this association does not prove that IMPC predicts absence of trastuzumab benefit. In DCIS, the relevant evidence concerns grade, proliferation and local recurrence; a large tissue study linked the combined HER2/Ki67 profile to recurrence, as discussed in the DCIS-focused review (156,157). Neither source provides a direct IMPC-DCIS treatment comparison or a mitochondrial-RNA explanation.

Indocyanine green (ICG) fluorescence has also been evaluated for sentinel-node localization, a clinically relevant sampling context for studying regional tumor-immune biology (158,159). In a randomized pilot study, adding ICG to technetium was feasible but did not establish superiority

Table IV. Prospective 3R research-development validation matrix.

| Step | Required evidence or action | Mechanistic validation | Prospective success criterion | Falsifier or stopping rule | Principal safety concern |
|---|---|---|---|---|---|
| PROFILE | Paired tumor, plasma/EV and spatial immune samples; quantitative mitochondrial, ferroptosis and mtDNA-*STING measurements. | Pre-analytic reproducibility; cell-source and compartment assignment; absolute abundance; longitudinal stability. | Prespecified features reproduce across models or cohorts and track measured biology. | Signal is dominated by hemolysis, batch, cell composition or unresolved provenance. | Sampling burden and false inference from non-specific circulating markers. |
| ASSIGN | Test Rewiring-, Relay-, Release-I-, Release-S- and mixed/uncertain states. For Release, use the source-time-function rules in Sections 5.2 and 6.2. | Prespecified windows and assay-based thresholds; paired source and recipient measurements; functional rather than abundance-only immune endpoints; blinded validation. | Release-I: transient source signal plus improved presentation/killing. Release-S: persistent/recurrent exposure plus suppression/effector loss. Confirm reproducibility in independent data. | No source or temporal resolution; discordant lineage effects; no functional change; unstable assignment. Retain mixed/uncertain rather than forcing a class. | Directionally incorrect intervention caused by unstable assignment. |
| INTERVENE | Evaluate mechanism-matched experimental interception of Rewiring dependencies, pathological Relay edges or Release states against appropriate controls and standard therapy. | Target engagement, target-site or pathway rescue, recipient specificity and schedule dependence. | Intervention improves tumor control while preserving immune-cell function in relevant models. | No target engagement, no mechanism-specific rescue, or benefit explained by non-specific toxicity. | Cardiac, hepatic, neurological, innate-immune and activated-lymphocyte injury. |
| MONITOR | Serial RNA/target, metabolic-flux, lipid-peroxidation, transfer and lineage-resolved immune measurements. | Assay precision and temporal relation between target engagement, state change and outcome. | Detects target engagement and cross-3R adaptation before overt resistance. | Marker changes only after progression or fails to distinguish mechanism from general tumor burden. | Escalation based on noisy markers or persistent inflammatory Release. |

The matrix operationalizes Fig. 4 as a preclinical and translational validation program; no row constitutes a validated patient classifier or treatment-selection rule.

for its primary detection endpoint (159). This is lymphatic mapping evidence, not evidence of EV-RNA trafficking, mitochondrial entry or a 3R-state classifier; molecular and lineage-resolved measurements would still be required in any linked translational study.

Future studies should report negative and contradictory findings alongside positive results. Failure to detect protected mitochondrial localization, insufficient RNA abundance, lack of recipient-specific rescue, discordant tumor and immune-cell responses, or irreproducible state assignments would delimit the proposed mechanism. A supported Rewiring relationship can remain informative when the corresponding Relay or Release hypothesis fails; an extracellular RNA effect can likewise be biologically important without mitochondrial mediation.

## 7. Outstanding questions and conclusions

Seven questions should determine whether this field becomes causal or remains descriptive: i) Which nuclear-encoded miRNAs reproducibly reside inside defined mitochondrial compartments in BC cells at a stoichiometry compatible with function? ii) How are mtDNA-derived miRNA-like RNAs, mt-tRFs and mt-rRFs processed, and which species have sequence-specific targets rather than stress-marker behavior? iii) Which direct RNA-target interactions survive target-site disruption, rescue and independent replication across BC subtypes and treatment states? iv) Do therapy-induced Rewiring states causally increase a defined EV or whole-mitochondrion Relay edge, rather than merely select pre-existing donor and recipient populations? v) Does an endogenous mitochondria-resident or mitochondria-derived small RNA control a defined released mitochondrial signal and its exposure route, with a rescued, lineage-resolved immune consequence in an immune-competent BC model? vi) Under which spatial and temporal conditions does tumor ferroptosis support antitumor immunity, and when does lipid-peroxidation stress disable antigen-presenting or effector cells? vii) Can a prespecified 3R state and LIFT-supported target be reproduced, engaged and monitored prospectively while preserving immune-cell and normal-tissue mitochondrial fitness?

Mitochondria-associated small RNAs connect distinct aspects of mitochondrial state, ferroptosis competence and immune selection in BC, but the demonstrated relationships remain uneven. Direct evidence supports several cytosolic mitochondria-regulatory RNA mechanisms, a reported mtDNA-derived mitomiR-3 ferroptosis phenotype and cytoplasmic tiRNA-Met activity from a reported mitochondrial tRNA origin. The miR-25/93-NCOA3-cGAS mechanism adds direct RNA-dependent immune sensing without showing control over mtDNA-release quantity. The broader literature establishes that BC mitochondria are metabolically plastic, can be transferred between cells and can expose signals associated with tumor rejection or immunosuppression. Within the evaluated material, a continuous causal chain joining an endogenous mitochondria-resident or mitochondria-derived small RNA to altered release of a defined mitochondrial signal and a lineage-resolved immune consequence remains unestablished. Each positive result and each unresolved link should therefore retain its own molecular, cellular and experimental scope.

The 3R Mito-Ecosystem represents this incomplete chain as testable relationships. Rewiring encompasses metabolic, mitochondrial-origin RNA growth-signaling and ferroptotic regulation; Relay encompasses directed RNA or organelle exchange; and Release concerns the immune consequences of exposed mitochondrial material. Feedback between these processes is a testable synthesis, not an obligatorily established sequence. LIFT prevents localization, interaction, function and translation from being treated as equivalent, while claim-level tiers prevent BC mechanism from being inferred from cross-system precedent. The field will advance less through the identification of additional mitochondrial small RNAs than through establishing where these RNAs reside, what they bind, which cellular interactions or immune outcomes they control, and which findings withstand independent perturbation-rescue testing. The proposed sampling and decision rules make this synthesis experimentally assessable. Fig. 4 remains a prospective development roadmap: state definitions, interventions, monitoring thresholds and go/no-go criteria require independent validation.

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## Authors' contributions

HYL and HWHo conceptualized the study. HWHs, YRS and HYL developed methodology, performed software analysis, visualized and curated data. PYC conducted data validation, provided resources, supervised the study, and wrote, reviewed and edited the manuscript. HYL performed formal analysis and investigation and wrote the original draft. HYL conducted project administration. HYL, HWH and PYC acquired funding. HYL and PYC performed independent claim-level source assessment. HWHo had third-author adjudication responsibility. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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