

Dual targeting of mitochondrial metabolism and Rho GTPase signaling to suppress cancer metastasis (Review)

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Abstract. Despite the numerous advances in cancer therapy, disseminated neoplastic disease remains largely incurable and the primary cause of cancer-related deaths. This process demands substantial bioenergetic and mechanical adaptability, orchestrated by two core cellular systems: Mitochondrial metabolism and Rho GTPase-driven cytoskeletal dynamics. Traditionally studied independently, these systems form a tightly integrated, bidirectional network. Mitochondria supply ATP and reactive oxygen species (ROS) that fuel and signal through Rho GTPases to drive invasion, while cytoskeletal remodeling and cell polarity direct mitochondrial positioning to meet local energy demands. The present review synthesizes the molecular mechanisms underlying this metabolic-mechanical crosstalk and highlights a feedforward loop in which mitochondrial oxidative phosphorylation-derived ATP and ROS activate Rho GTPase signaling, while Rho-driven cytoskeletal remodeling increases energy demand and promotes mitochondrial redistribution, thereby reinforcing metastatic progression. Critically, this interdependence represents a therapeutic vulnerability. A dual-targeting strategy was discussed, combining Rho GTPase silencing (via small interfering RNA) with mitochondrial inhibition (via repurposed antibiotics) to simultaneously disrupt the cytoskeletal ‘engine’ and its metabolic ‘fuel’. Such approaches may overcome compensatory adaptive resistance that limits single-target therapies. By framing mitochondrial and Rho GTPase signaling as an integrated functional axis, the present review provides a

mechanistic and translational framework for the development of next-generation, combination-based anti-metastatic therapies.

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1. Introduction

Cancer is a leading cause of death worldwide, and metastasis accounts for most cancer-related fatalities due to the limited efficacy of current therapies against disseminated disease (1-3). This multistep cascade, from local invasion to colonization, challenges cancer cells to overcome profound metabolic, mechanical and signaling stresses. While numerous oncogenic pathways contribute to dissemination, including Phosphoinositide 3-kinase/Protein kinase B (PI3K/AKT), mitogen-activated protein kinase (MAPK) signaling, and immune evasion mechanisms, the present review focuses on two core, interdependent systems that are fundamental to the metastatic process which are the cytoskeletal machinery and mitochondrial bioenergetics (4,5).

A defining feature of metastatic cells is their capacity for dynamic cytoskeletal remodeling. This process is driven by actin polymerization, which forms lamellipodia and filopodia for protrusion. Simultaneously, actomyosin contractility generates the force required for invasion. Microtubules and intermediate filaments further coordinate cell polarity and vesicle transport (6). These processes are precisely governed by Rho family GTPases (RhoA, Rac1 and Cdc42), which act as molecular switches that spatially and temporally regulate cytoskeletal dynamics through effectors such as Rho-associated coiled-coil containing protein kinase (ROCK) and WAVE/Arp2/3 (6-8). Consequently, elevated Rho GTPase activity is a recurrent feature across cancers and is strongly

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associated with aggressive invasion, epithelial-to-mesenchymal transition (EMT), and poor patient outcomes (9,10).

Simultaneously, to power these demanding mechanical processes, metastatic cells undergo profound mitochondrial reprogramming. Beyond their standard role in ATP synthesis, mitochondria are dynamically trafficked to the cell's leading edge. This positioning supplies local ATP for actin turnover and focal adhesion dynamics. Additionally, mitochondria facilitate the production of reactive oxygen species (ROS), which function as essential second messengers in pro-migratory signaling pathways (11-13). The upregulation of oxidative phosphorylation (OXPHOS) and mitochondrial biogenesis, fine-tuned by regulators such as peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1 α), supports invasion and distant colonization (12,14). Critically, these adaptations are also a feature of therapy-resistant, cancer stem-like cells (CSCs), a population responsible for seeding metastases and driving relapse (15).

Rather than operating independently, these two aforementioned systems engage in a tightly coordinated bidirectional crosstalk that forms a feedforward loop of metabolic-mechanical coupling. In this loop, mitochondrial metabolism directly supports cytoskeletal remodeling by providing ATP for actin dynamics and generating ROS that modulate Rho GTPase signaling through redox-sensitive pathways, including Sarcoma (Src) family kinases and Rho regulatory proteins (11,16-19). In parallel, activation of Rho GTPases drives cytoskeletal reorganization and cell polarity, which in turn regulates mitochondrial trafficking, positioning, and dynamics through effectors such as Dynamin-related protein 1 (DRP1) and mitochondrial Rho GTPase (MIRO) proteins (6-8,16,20). This reciprocal interaction spatially couples energy production to mechanical demand while reinforcing pro-migratory signaling. Consequently, this self-sustaining loop integrates metabolic adaptation with force generation to promote invasion, survival under stress, and metastatic dissemination (18-21).

This integrated framework provides a mechanistic explanation for the limited efficacy of single-target therapies, since inhibition of either metabolic or cytoskeletal pathways alone can be compensated by adaptive rewiring of the other. Furthermore, it motivates a therapeutic strategy of dual targeting (18,22) through two translationally tractable modalities: Small interfering RNA (siRNA) based silencing of Rho GTPases to overcome the historical intractability of inhibiting these protein-protein interaction-dependent switches, and repurposing mitochondria-targeting antibiotics (doxycycline and tigecycline) to suppress OXPHOS and pro-tumorigenic ROS signaling (7,23,24). Moreover, numerous advances in biological delivery platforms, such as lipid nanoparticles, will be discussed addressing limitations including off-target effects and poor bioavailability (25,26). By synthesizing mechanistic insights into mitochondrial-Rho GTPase crosstalk and integrating them with emerging therapeutic strategies, the present review aims to establish a dual-target framework for disrupting this feedforward loop and suppressing metastatic progression (Fig. 1).

2. Mitochondrial metabolism and cancer metastasis

Energy demands for metastatic cells. The metastatic cascade imposes exceptional bioenergetic demands on disseminating

tumor cells, requiring ATP for motility, invasion and adaptation to hostile microenvironments. While numerous primary tumors exhibit a glycolytic phenotype (Warburg effect), metastatic cells often show a reliance on mitochondrial OXPHOS to meet the sustained energy requirements for migration and colonization (14,27,28). It is important to note that metastatic cells are metabolically plastic, often retaining the capacity to utilize glycolysis in hypoxic niches while upregulating OXPHOS to fuel invasion (29).

The invasive process necessitates dynamic cytoskeletal remodeling to form protrusions such as lamellipodia and invadopodia which degrade the extracellular matrix (ECM). These energy-intensive processes require a high, localized supply of ATP at the leading edge. Mitochondria are actively redistributed to these subcellular locations through DRP1-mediated mitochondrial fission and trafficking along microtubules, where they provide energy for actin polymerization and focal adhesion turnover (11,30). Beyond ATP, mitochondria generate ROS at controlled levels, which function as essential signaling molecules. Mitochondrial ROS can oxidize cysteine residues in key regulatory proteins, thereby directly activating pro-migratory signaling cascades, including those involving Rho GTPases, focal adhesion kinase (FAK), and Src kinase (31-34).

The secretion of proteolytic enzymes, such as matrix metalloproteinases (MMPs), for ECM remodeling is another process heavily dependent on OXPHOS-derived ATP, underscoring the critical role of mitochondria in invasion (12,35). Upon intravasation, tumor cells endure extreme stresses, including shear stress, anoikis and immune surveillance. Mitochondria contribute to survival during this phase. Moderately elevated ROS levels can stabilize hypoxia-inducible factor-1 α (HIF-1 α) through the inhibition of prolyl hydroxylases (PHDs), promoting EMT and anoikis resistance (33,36,37). Concurrently, mitochondrial metabolism supports antioxidant defense systems, such as generating nicotinamide adenine dinucleotide phosphate (NADPH), allowing circulating tumor cells to mitigate oxidative damage (14,38).

The last step of colonization requires adaptation to the stringent and often nutrient-poor, microenvironment of distant organs. Through the tricarboxylic acid (TCA) cycle and fatty acid oxidation (FAO), mitochondria allow metastatic cells to utilize available nutrients, such as lipids and glutamine. For instance, in breast cancer models, enhanced FAO is critical for metastatic outgrowth in the lipid-rich environment of the liver and lung (39,40). Similarly, OXPHOS dependency has been demonstrated in metastatic melanoma and pancreatic cancer (41,42).

In addition to bioenergetic and biosynthetic functions, other mitochondrial processes support metastasis. Mitophagy maintains a healthy pool of mitochondria to sustain energy production during stress, and mitochondrial-ER contacts facilitate lipid transfer and calcium signaling, both of which can influence cell migration (43,44).

In summary, mitochondria are central, dynamic regulators of metastatic competence, far exceeding their role as mere powerhouses. They integrate energy production (OXPHOS, FAO), redox signaling (ROS), and metabolic plasticity to enable every step of dissemination. This foundational role establishes them as a compelling therapeutic target and provides the

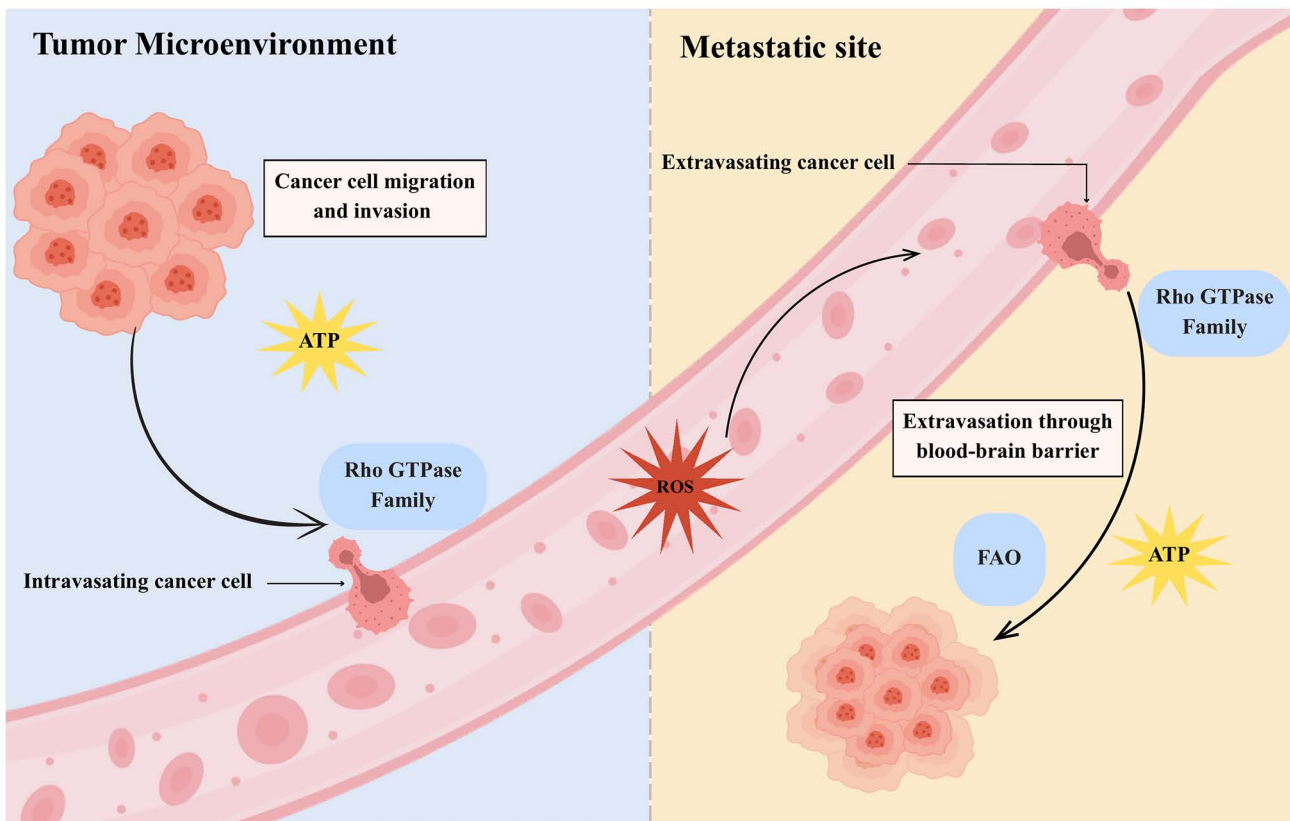


Figure 1. Metastatic cascade with energetic and mechanical demands. This schematic illustrates the sequential steps of metastasis from the primary tumor microenvironment to the metastatic site, highlighting specific energy and signaling requirements. At the invasion and intravasation steps, cancer cells rely on local ATP production and Rho GTPase signaling to drive actin remodeling and cytoskeletal contractility required for matrix degradation and endothelial penetration. During circulation, mitochondrial-derived ROS act as adaptive signals that promote cell survival in the bloodstream. At extravasation, Rho GTPases again support cytoskeletal reorganization, while mitochondrial metabolism provides bioenergetic support for barrier crossing. Finally, at colonization in the distant site, cells depend on ATP production and FAO to adapt to nutrient limitations and establish proliferative growth. Overall, this process reflects a bidirectional metabolic-mechanical feedback loop, in which mitochondrial metabolism and Rho GTPase-driven cytoskeletal dynamics reciprocally reinforce metastatic progression. ROS, reactive oxygen species; FAO, fatty acid oxidation.

rationale for exploring their inhibition in combination with strategies targeting the cytoskeletal machinery they fuel, as discussed in the following sections.

Mitochondrial pathways in cancer cells. Building upon the exceptional bioenergetic demands of metastasis, this section delves into the specific mitochondrial pathways that are co-opted to fuel dissemination. Mitochondria occupy a central position in bioenergetic and biosynthetic pathways, and their reprogramming is a hallmark of metastatic efficiency. The core metabolic axes, OXPHOS, TCA cycle and FAO are systematically rewired to maximize adaptability to the fluctuating nutrient and oxygen conditions met during dissemination (45).

Metastatic cells exhibit pronounced metabolic plasticity, often retaining glycolytic capacity and switching between energy sources based on microenvironmental cues (29). While the classical Warburg effect emphasizes glycolysis, accumulating evidence indicates that metastatic cells frequently upregulate OXPHOS to meet the high ATP demands of invasion and colonization. Enhanced OXPHOS supports the intense energy requirements of actin cytoskeletal remodeling and membrane trafficking (14,28). A key byproduct of electron transport is the generation of ROS. While excessive ROS induces cell death, controlled levels

serve as critical signaling molecules. Mitochondrial ROS can oxidize key cysteine residues in phosphatases, such as PTEN, and activate kinases within the Src/PI3K pathway, thereby promoting the activation of Rho GTPases and FAK to drive motility (16,32,33).

The TCA cycle functions as a crucial biosynthetic hub. Metastatic cells divert intermediates to support anabolic processes. For example, citrate is exported for lipid synthesis and histone acetylation, while α -ketoglutarate (α -KG) serves as a cofactor for α -KG-dependent dioxygenases, including histone and DNA demethylases. This reprogramming reinforces pro-metastatic phenotypes, including EMT and stemness (41,46). Furthermore, the oncometabolites succinate and fumarate, when accumulated, inhibit PHDs, leading to HIF-1 α stabilization and the expression of pro-angiogenic and pro-invasive genes even under normoxia (36,47).

FAO represents another critical adaptive pathway. In nutrient-deprived niches, metastatic cells oxidize fatty acids to generate NADH and FADH₂ to fuel OXPHOS. This pathway is particularly important for colonization of lipid-rich environments; for example, FAO is essential for breast cancer metastasis to the bone marrow and for ovarian cancer metastasis to the adipose-rich omentum (14,39,48). Additionally, FAO generates NADPH, which is essential for maintaining

redox homeostasis and mitigating oxidative stress during circulation (38).

In addition to metabolic reprogramming, the physical dynamics of mitochondria (fission and fusion) are precisely regulated. DRP1-mediated fission generates fragmented mitochondria that can be trafficked to the cell's leading edge to supply localized ATP for protrusions and invasion. Conversely, Mitofusin 1 and Mitofusin 2 (MFN1/2)-mediated fusion maintains an interconnected network that supports efficient OXPHOS and biomass synthesis. The balance between these states precisely regulates energy allocation to support migratory demands (16,30).

The coordination of these functions is managed by key upstream regulators. The transcriptional coactivator PGC-1 α , which is upregulated in specific aggressive subtypes (triple-negative breast cancer, BRAF-resistant melanoma), drives mitochondrial biogenesis and enhances oxidative capacity (14,49). While HIF-1 α typically promotes glycolysis, it can also support metastasis by maintaining pro-migratory ROS signaling (36). The nutrient sensors adenosine monophosphate activated protein kinase (AMPK) and mechanistic target of rapamycin (mTOR) integrate energy availability with anabolic processes, critically influencing metastatic fitness (45,50). Other regulators such as MYC and Nuclear Factor Erythroid 2-Related Factor 2 (NRF2) further contribute to this coordinated mitochondrial reprogramming, promoting biogenesis and antioxidant responses, respectively (51,52).

Collectively, metastatic cells exploit OXPHOS, the TCA cycle, and FAO in a coordinated manner, supported by dynamic organelle remodeling and primary transcriptional regulators. This integrated rewiring ensures that tumor cells can generate energy, biomass, and signaling molecules to complete the metastatic cascade. The centrality of these mitochondrial functions establishes them as a compelling therapeutic vulnerability, a premise that will be explored in the following sections on targeting strategies (Fig. 2).

Mitochondria as therapeutic targets. Mitochondrial metabolism plays a leading role in powering metastasis. Consequently, numerous strategies have been developed to target mitochondrial function, ranging from direct inhibition of respiration to interference with biogenesis and dynamics.

Direct pharmacological inhibition of OXPHOS impairs ATP production and attenuates essential ROS signaling, disrupting the cytoskeletal dynamics required for invasion. Small-molecule inhibitors targeting electron transport chain complexes, such as complex I (IACS-010759) and complex III, have proven efficacy in preclinical models by reducing metastatic burden (24). For instance, the complex I inhibitor IACS-010759 has demonstrated potent antitumor activity in acute myeloid leukemia (AML) and solid tumor xenograft models, achieving significant tumor growth inhibition at doses of 5-10 mg/kg (53). The complex I inhibitor IACS-010759 has progressed to early-phase clinical evaluation in advanced solid tumors and hematological malignancies. Notably, the Phase I clinical trial NCT03291938 (2017-2020; n=29) assessed the safety and feasibility of this approach (54). However, peer-reviewed results from this trial have not yet been formally published, and currently available data are limited to clinical trial registry reports. Despite initial promise, dose-limiting

toxicities, including cardiac and neurological adverse effects, as well as metabolic adaptation through glycolytic compensation, have limited its clinical efficacy (53,55). These limitations highlight the current gap between promising preclinical findings and clinically validated mitochondrial-targeted therapies.

Targeting the upstream drivers of mitochondrial mass represents another potential strategy. Metastatic and therapy-resistant subpopulations often depend on PGC-1 α -driven mitochondrial biogenesis. While directly inhibiting PGC-1 α remains challenging, targeting its upstream regulators or synthetic lethal partners shows preclinical promise in suppressing oxidative capacity and metastasis (14,56).

A particularly tractable approach is the repurposing of antibiotics that inhibit mitochondrial translation, exploiting the bacterial ancestry of mitochondrial ribosomes. Tigecycline and doxycycline collapse OXPHOS capacity and have shown selective efficacy against metastatic and cancer stem cells in models of breast, ovarian and blood cancers (23,46). For example, tigecycline has been shown to significantly reduce leukemia burden and target leukemia stem cells in AML xenograft models (57). Similarly, doxycycline reduced metastatic tumor burden by ~70% in an intracardiac MDA-MB-231 breast cancer model in nude mice (58). This selectivity arises from the heightened mitochondrial dependency of these aggressive cells. Unfortunately, this therapeutic window remains context-dependent, as normal tissues with high mitochondrial activity may also be affected, limiting clinical applicability. Moreover, the clinical translation of long-term, high-dose antibiotic regimens is complicated by systemic toxicity, microbiome disruption, and the emergence of drug-resistant bacterial strains (57,59).

Beyond metabolism, targeting the machinery that controls mitochondrial dynamics is gaining interest. Inhibiting DRP1-mediated fission (with mdivi-1) prevents the mitochondrial fragmentation and redistribution needed for invasion, thereby impairing cell motility. Consistently, inhibition of DRP1 has been reported to reduce migration and invasion phenotypes in cancer cell models, supporting its role in metastatic progression (60). It is important to note that compounds such as mdivi-1 may have off-target effects, underscoring the need for more specific inhibitors (61,62). Conversely, disrupting fusion through MFN inhibition compromises metabolic efficiency and sensitizes cells to stress.

ROS-modulating therapies present a complex, but context-dependent, therapeutic opportunity due to the dual role of ROS in both pro-migratory signaling and oxidative cell death. The strategy involves either elevating ROS to toxic levels to induce apoptosis or scavenging mitochondrial ROS to blunt pro-invasive signaling. However, the clinical application of ROS modulators has been challenging due to their context-dependent effects and the risk of harming normal tissues (36,63).

A major limitation across these strategies is the inherent metabolic plasticity of tumor cells. Inhibition of OXPHOS or mitochondrial function often triggers adaptive responses, including a shift to glycolysis, increased glutaminolysis, or upregulation of alternative nutrient salvage pathways, leading to therapeutic resistance (29,55). In addition, toxicity to normal tissues with high metabolic demands (cardiac muscle, neurons) remains a primary barrier.

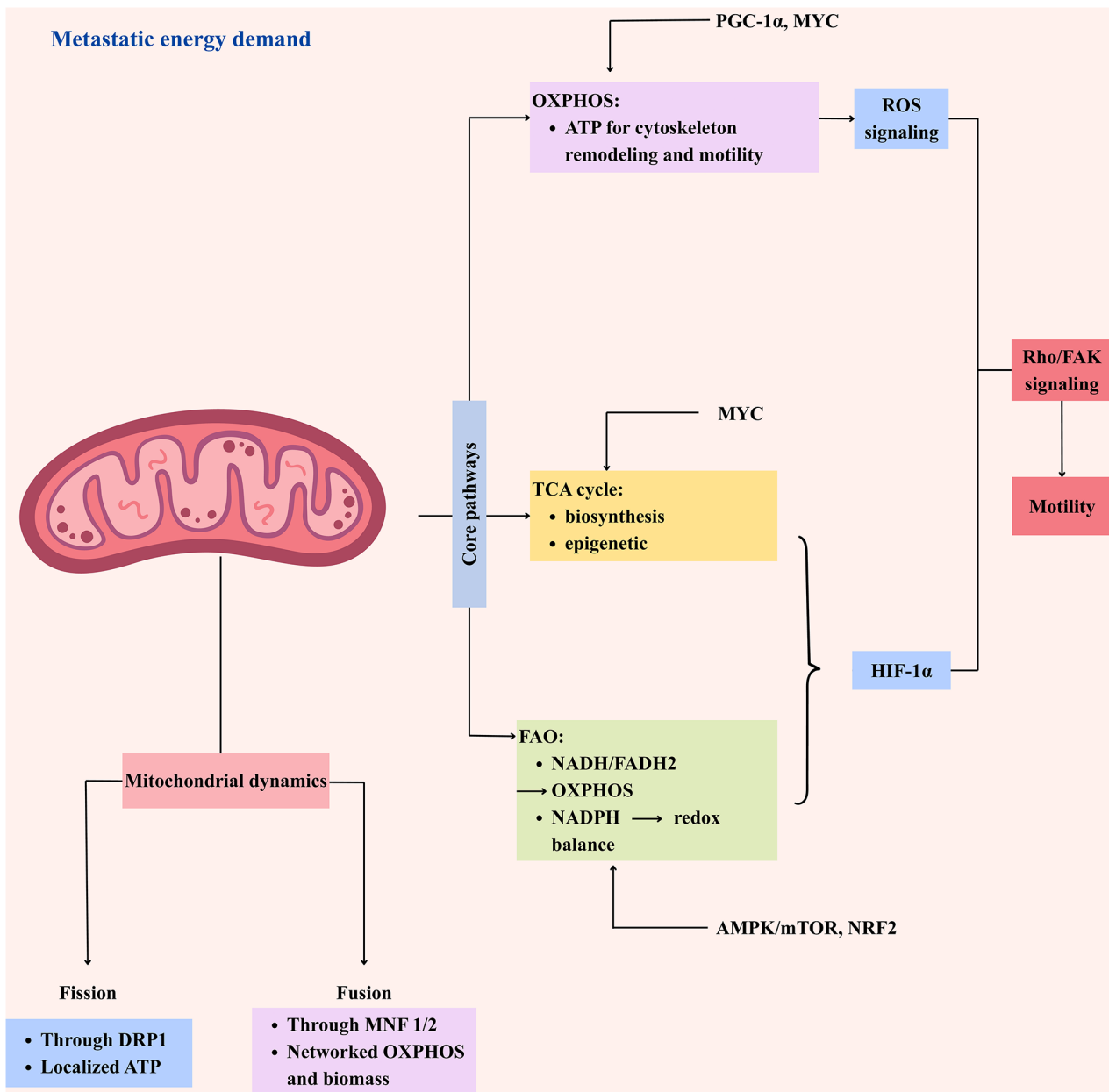


Figure 2. Mitochondrial coordination of metastatic energy metabolism and motility. Metastatic cancer cells rewire core mitochondrial pathways (OXPHOS, TCA, FAO) to produce ATP, biosynthetic precursors, and redox signals that support motility. ROS and HIF-1 α activate Rho/FAK signaling, driving cytoskeletal remodeling. Mitochondrial dynamics (DRP1-mediated fission and MFN1/2-mediated fusion) distribute ATP and maintain networked metabolism to meet energetic and biosynthetic demands. Key upstream regulators (PGC-1 α , MYC, AMPK/mTOR, NRF2) orchestrate metabolic rewiring, dynamics, and redox balance. The integrated output enhances energy availability, biomass synthesis, ROS signaling, and promotes cancer cell migration, invasion, and colonization. OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid; FAO, fatty acid oxidation; ROS, reactive oxygen species.

In summary, targeting mitochondrial metabolism offers a rational, multi-pronged approach to disrupt the energetic and signaling foundations of metastasis. The combinatory use of these strategies, or their integration with agents targeting compensatory pathways (glycolysis inhibitors), may help overcome the limitations of monotherapy. The translational feasibility of these approaches, particularly with advanced delivery systems to improve selectivity, will be explored in the context of targeting the cytoskeletal machinery in Section 3 (Table I).

Advantages and limitations of targeting mitochondria. While the preceding section outlined various strategies to target mitochondrial function, a critical assessment of their

advantages and limitations is essential for evaluating their therapeutic potential. The approach offers distinct benefits rooted in the biology of metastasis but also faces significant challenges due to the indispensable role of mitochondria in normal physiology.

A key advantage is the disproportionate dependence of metastatic and stem-like tumor cells on OXPHOS compared with bulk tumor populations. This dependency can create a therapeutic window, allowing mitochondrial inhibition to selectively impair metastatic competence while sparing more glycolytic cells (14,64). Furthermore, mitochondrial inhibitors exert a dual impact; they deprive cells of ATP while simultaneously suppressing ROS-driven signaling cascades that

Table I. Experimental evidence supporting mitochondrial-targeting agents in metastatic cancer models.

| First author/s, year | Drug | Mechanism | Model system | Dose | Duration | Quantitative outcome | Statistical analysis | (Refs.) |
|---|-------------|---|---|--|------------|---|--------------------------|---------|
| Duivenvoorden <i>et al</i> , 2002 | Doxycycline | Mitochondrial translation inhibitor | Female nude mice (BALB/c nu/nu, ~5 weeks), intracardiac injection of MDA-MB-231 breast cancer cells; n=9-14/group | 10 mg slow-release pellet (equivalent systemic exposure) | 21 days | ~70% reduction in bone metastatic tumor burden (histomorphometric analysis) | Student's t-test, P<0.01 | (58) |
| Škrtić <i>et al</i> , 2011 | Tigecycline | Mitochondrial translation inhibitor | NOD/SCID mice xenografted with human AML (OCI-AML2 cells); n ≈ 8-10/group | 50 mg/kg (intraperitoneal injection) | ~14 days | ~70-77% reduction in leukemia burden and impaired leukemia stem cell function | Student's t-test, P<0.05 | (57) |
| Molina <i>et al</i> , 2018; Yap <i>et al</i> , 2023 | IACS-010759 | Complex I (OXPHOS) inhibitor | NSG mice xenografted with human AML and brain tumor cells; n ≈ 8-10/group | 5-10 mg/kg (oral administration) | 14-21 days | 50-70% reduction in tumor growth and evidence of OXPHOS-dependent tumors | ANOVA, P<0.01 | (53,54) |
| Kashatus <i>et al</i> , 2015 | Midvi-1 | DRP1-mediated mitochondrial fission inhibitor | Breast cancer cell lines (MDA-MB-231) migration/invasion assays (<i>in vitro</i>); n≥3 independent experiments | 25-50 μM | 24-72 h | ~40-60% reduction in migration and invasion capacity | Student's t-test, P<0.05 | (60) |

Data are derived from representative primary preclinical studies; experimental parameters may vary across studies. BALB/c nu/nu, athymic nude mice; MDA-MB-231, human triple-negative breast cancer cell line; NOD/SCID, non-obese diabetic/severe combined immunodeficient mice; NSG, NOD/SCID gamma mice; OXPHOS: oxidative phosphorylation; CSCs, cancer stem cells; IACS-010759, inhibitor of mitochondrial complex I; AML, acute myeloid leukemia; OCI-AML2, human acute myeloid leukemia cell line; Midvi-1, mitochondrial division inhibitor-1; ANOVA, analysis of variance; DRP-1, dynamin-related protein 1.

activate critical drivers of invasion, such as Rho GTPases and focal adhesion kinases (63). Another promising advantage is the opportunity to repurpose antibiotics including doxycycline and tigecycline, which have known pharmacokinetic and safety profiles from decades of clinical use (23). However, long-term high-dose regimens required for antitumor efficacy can lead to off-target effects, including microbiome disruption and cumulative toxicity in normal tissues. Mitochondrial inhibition also shows efficacy against numerous therapy-resistant CSCs, potentially reducing long-term recurrence. However, it is crucial to acknowledge that not all CSCs rely on OXPHOS; some utilize glycolysis, highlighting the need for patient stratification based on metabolic phenotypes (36,65).

Despite these strengths, some limitations complicate clinical translation. These limitations reflect the same adaptive features that enable metastatic success, underscoring the challenge of targeting mitochondrial metabolism without affecting normal cellular function. The indispensability of mitochondria for high-demand normal tissues (heart, brain, nerves) poses a risk of dose-limiting toxicities, such as cardiomyopathy and neuropathy (66). Moreover, tumor cells exhibit pronounced metabolic plasticity as a key resistance mechanism. When OXPHOS is suppressed, cells can rapidly switch to glycolysis (often driven by HIF-1 α stabilization), glutaminolysis (via MYC upregulation), or fatty acid metabolism, thereby maintaining energy production and viability (12,29). Both inter- and intra-tumoral heterogeneity further complicate treatment. For example, hypoxic cells within a tumor or in certain metastatic niches, such as the bone marrow may be inherently glycolytic and thus resistant to OXPHOS inhibition alone (67). Finally, sustained inhibition can select for clones with adaptive resistance mechanisms, including altered mitochondrial dynamics or amplified compensatory glycolytic flux (10).

Ultimately, mitochondria represent a metabolic vulnerability that can be exploited for therapy, but they also confer resilience through their plasticity and role in stress adaptation. Overcoming the challenges of toxicity, heterogeneity, and resistance will require innovative strategies. These include developing targeted delivery systems (nanoparticles) to improve selectivity, combining mitochondrial inhibitors with agents that block compensatory pathways (glycolysis inhibitors), and basing therapeutic decisions on metabolic biomarkers. The potential of such rational combination strategies, particularly in the context of disrupting metabolic-mechanical crosstalk, will be explored in the closing section of this review.

3. Rho GTPase signaling and siRNA in cancer metastasis

Why cancer cells depend on Rho GTPase signaling. Having established mitochondria as the metabolic engine of metastasis, the cytoskeletal machinery that this engine powers is then discussed. The metastatic cascade demands that tumor cells dynamically alter their shape and adhesion to detach, invade the ECM, intravasate, survive circulation, and colonize distant sites. Rho family GTPases serve as master molecular switches that regulate these essential processes by cycling between active GTP-bound and inactive GDP-bound states (7,9,68).

These GTPases govern distinct, well-characterized aspects of cytoskeletal dynamics. RhoA signals through its effector ROCK to promote actomyosin contractility and stress fiber

formation, facilitating the rounded, amoeboid migration mode that is effective in dense tissues. Rac1 activates the WAVE/Arp2/3 complex to drive the formation of broad, sheet-like lamellipodia for protrusive mesenchymal migration. Cdc42, via effectors like N-WASP, stimulates the formation of finger-like filopodia for environmental sensing and establishes front-rear cell polarity, guiding directional movement (6,69,70). Aberrant activation of these pathways, whether through overexpression of the GTPases themselves, amplification of upstream activators such as guanine nucleotide exchange factor (GEFs), or loss of inhibitors such as GTPase-activating protein (GAPs), confers a potent invasive advantage, identifying them as additional critical vulnerabilities in metastatic cancers (71).

The pathway hyperactivity is often driven by sustained upstream signaling from receptor tyrosine kinases, integrins and G-protein-coupled receptors within the tumor microenvironment (TME) (68). The resulting persistent Rho GTPase enhances metastatic fitness in two key ways. First, it enables dynamic plasticity, allowing cells to switch between mesenchymal and amoeboid migration in response to ECM density and confinement. Second, it promotes immune evasion; for instance, the rapid, rounded amoeboid movement minimizes cell surface exposure and adhesive interactions, potentially reducing recognition by immune cells (9,72,73). The clinical relevance is underscored by tumor-type-specific dependencies; specifically, RhoA is frequently amplified in melanoma and glioblastoma, while Cdc42 activity is critical for invasion and metastasis in breast and pancreatic cancers (70,74).

Basically, Rho GTPases function as central signaling hubs, integrating extrinsic cues from the TME with intrinsic oncogenic drives to orchestrate dissemination. However, their dependence on protein-protein interactions and the absence of deep catalytic pockets have rendered them notoriously difficult to target with conventional small molecules, leading to their classification as 'undruggable' (71). This fundamental challenge motivates the exploration of alternative therapeutic strategies, most notably RNA interference (RNAi) to achieve precise silencing, as discussed in the following subsection.

Rho GTPases as molecular switches and their regulators. Building on their established role as master regulators of metastasis, the precise molecular control of Rho GTPase activity is fundamental to their function. Rather than acting as simple binary switches, their activity is tightly coordinated by a regulatory network of guanine nucleotide exchange factors (GEFs: Vav, Tiam1 and Trio), GTPase-activating proteins (GAPs: p190RhoGAP) and guanine nucleotide dissociation inhibitors (GDIs), which collectively ensure the spatial and temporal activation required for efficient cell migration (7,9,68).

In cancer, direct mutations in Rho GTPases are rare. Instead, dysregulation most commonly occurs through overexpression or hyperactivation of oncogenic GEFs, or the loss of tumor-suppressive GAPs. This imbalance results in sustained and spatially deregulated Rho GTPase signaling, a key driver of metastatic progression across multiple cancer types (69,71).

This sustained signaling drives invasion through distinct downstream effector pathways. Active RhoA signals through ROCK, which phosphorylates LIM kinase and myosin light chain (MLC) to drive stress fiber formation and actomyosin

contractility, facilitating amoeboid movement. Rac1 stimulates lamellipodia formation via the WAVE/Arp2/3 complex and can also activate NADPH oxidase (NOX) complexes to generate localized ROS that amplify pro-migratory signals (8,33). Cdc42 coordinates filopodia formation and establishes cell polarity through effectors such as N-WASP. Furthermore, Cdc42 regulates vesicle trafficking, guiding the exocytosis of MMPs and the endocytosis of integrins, thereby directly facilitating ECM degradation and directional migration (6,70).

These pathways are not isolated but are highly interdependent, allowing for adaptive migration. A key regulatory node is the antagonism between RhoA and Rac1, which controls the switch between contractile amoeboid and protrusive mesenchymal migration in response to ECM physical constraints. Furthermore, Cdc42 often cooperates with Rac1 at the leading edge to reinforce protrusive activity while simultaneously inhibiting RhoA-mediated contractility at the cell rear. This dynamic, spatially-controlled balance enables tumor cells to efficiently navigate the complex and heterogeneous microenvironments encountered during dissemination (75).

Importantly, the tight spatial and temporal regulation of Rho GTPase signaling imposes significant energetic demands, particularly for actin remodeling, vesicle trafficking and contractility. This functional dependence on energy supply suggests a close integration with mitochondrial metabolism, providing a mechanistic basis for the metabolic-mechanical crosstalk explored in Section 4.

The precise and complex nature of this regulatory network is what makes Rho GTPases so difficult to target with conventional small-molecule inhibitors. The lack of deep catalytic pockets and their reliance on protein-protein interactions and they have made Rho GTPase challenging to pursue therapeutically (71). This challenge underscores the need for alternative intervention strategies, such as RNAi, which can selectively silence key nodes within this signaling network, as discussed in the following subsection (Fig. 3).

Post-transcriptional regulation by miRNA. Beyond the immediate protein-level regulation by GEFs and GAPs, Rho GTPase signaling is finely tuned at the post-transcriptional level by miRNA. These small non-coding RNAs typically bind to the 3' untranslated regions of target mRNAs, leading to transcript degradation or translational repression. By directly targeting the mRNAs encoding Rho GTPases themselves, as well as their activators (GEFs) and inhibitors (GAPs and GDIs), microRNAs (miRNAs or miRs) constitute a critical and dynamic regulatory layer that shapes the cytoskeletal dynamics essential for metastasis (70,76). This endogenous regulatory mechanism provides a conceptual basis for therapeutic RNA-based approaches, including siRNA-mediated silencing strategies.

In cancer, global dysregulation of miRNA expression, through genomic deletion, amplification, or altered processing, frequently disrupts this precise balance. The loss of tumor-suppressive miRNAs or the overexpression of oncogenic miRNAs (oncomiRs) leads to the hyperactivation of pro-invasive Rho GTPase pathways. Numerous miRNAs have been experimentally validated as key regulators of this axis. For instance, the miR-200 family is a well-established suppressor of EMT and invasion. It directly targets the ZEB1

and ZEB2 transcription factors, which themselves repress epithelial genes. The restoration of an epithelial phenotype by miR-200 is accompanied by indirect downregulation of RhoA activity, promoting a less motile state. Conversely, the loss of miR-200, common in advanced carcinomas, unleashes ZEB1/2, driving EMT and RhoA-mediated invasion (76-78).

Other miRNAs act more directly on the Rho GTPase machinery. For example, miR-34a functions as a tumor suppressor and regulates key oncogenic pathways, including MET and SIRT1, thereby modulating Rho GTPase signaling and inhibiting cancer cell migration and invasion (79). The metastasis suppressor miR-31 directly targets the mRNAs of several GEFs (RDX and RhoA), thereby reducing Rac1 and RhoA activation and impairing invasion in breast and colorectal cancer models (80). On the other hand, oncogenic miRNAs such as miR-21 and miR-155 are frequently overexpressed in cancers. miR-21 promotes migration and invasion by targeting tumor suppressors such as programmed cell death protein 4, which in turn leads to increased RhoB and Cdc42 activity (81). Similarly, miR-155 enhances metastatic potential by repressing RhoA, shifting the balance towards Rac1-driven mesenchymal migration (76,82). Additionally, certain miRNAs exhibit context-dependent roles. For example, miR-142 directly targets Rac1 and can either suppress or promote cancer cell invasion depending on tumor type and cellular context (83,84).

Therapeutically, this regulatory axis presents a unique opportunity. The development of synthetic miRNA mimics (to restore tumor-suppressive miRNAs such as miR-31 or miR-200) or antagomiRs (to inhibit oncomiRs such as miR-21 and miR-155) represents a promising strategy to indirectly normalize Rho GTPase signaling. This approach is particularly relevant for cancers resistant to conventional therapies, offering a way to rewire the underlying signaling networks that drive adaptive invasion and metastasis (85). However, the development of miRNA therapeutics faces significant challenges, including delivery efficiency, potential off-target effects, and the need for careful dosing due to the ability of a single miRNA to regulate hundreds of genes.

In the end, miRNA-mediated regulation adds a sophisticated layer of control over the Rho GTPase network. While therapeutically complex, the ability to manipulate these networks with RNA-based agents connects the fundamental biology of cytoskeletal regulation to the emerging field of RNA therapeutics. This paves the way for the discussion of a more direct and potent approach: the use of siRNA for the specific silencing of individual Rho GTPase components (Table II).

Therapeutic targeting: siRNA against Rho GTPases. Pharmacological inhibition of Rho GTPases is challenging due to their reliance on protein-protein interactions, motivating alternative strategies such as RNAi. siRNAs are incorporated into the RNA-induced silencing complex, which directs sequence-specific mRNA degradation, resulting in potent and selective knockdown of target proteins (8).

Preclinical studies demonstrate that silencing RhoA, Rac1, or Cdc42 disrupts cytoskeletal structures, including lamellipodia, filopodia and stress fibers, impairs focal adhesion turnover, and suppresses migration, invasion and metastatic colonization in various cancer models (7,8,71).

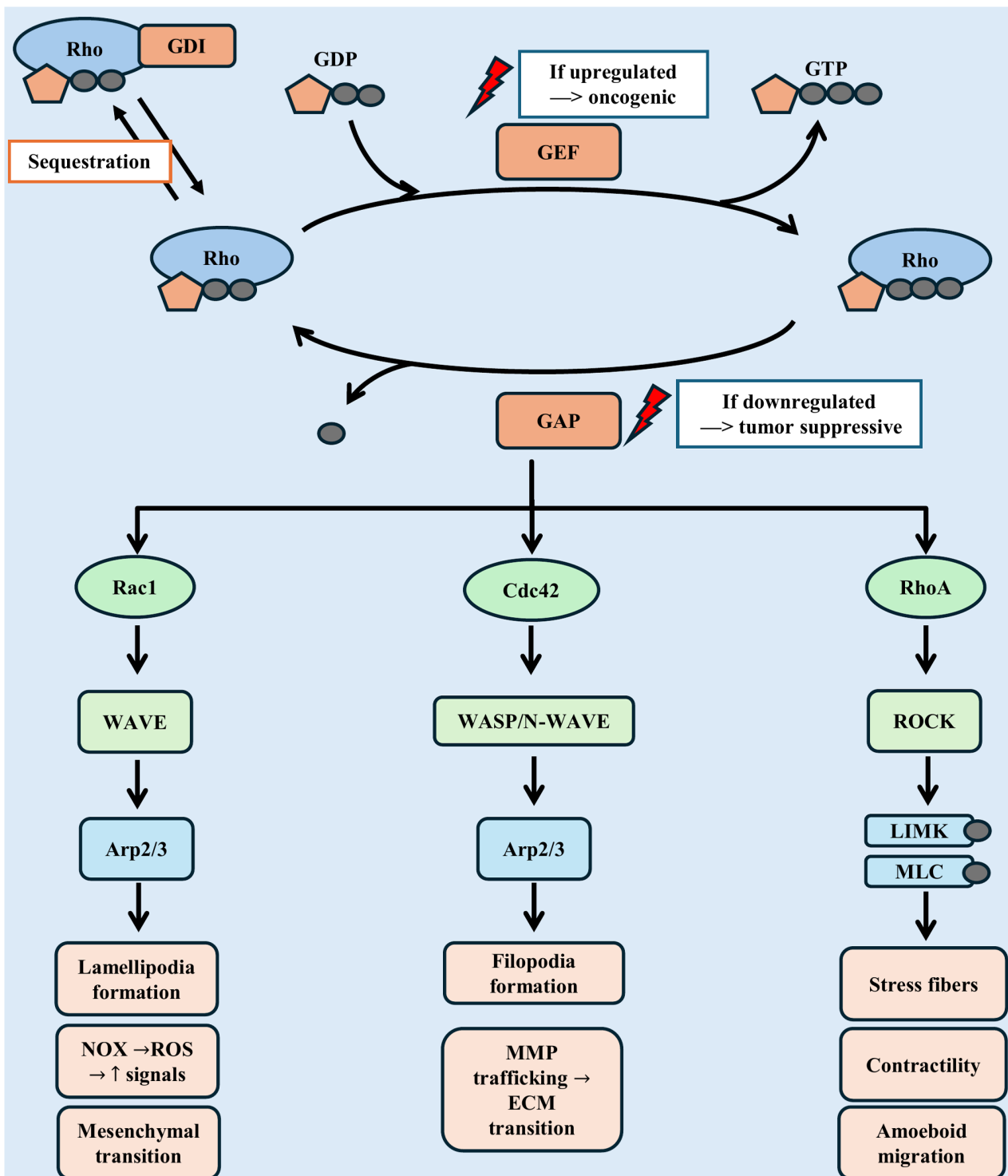


Figure 3. Regulation and downstream signaling of Rho GTPases in cancer metastasis. Extracellular stimuli, including growth factors, integrin-ECM interaction, and chemokines, activate Rho GTPases (RhoA, Rac1, Cdc42). These proteins function as molecular switches, cycling between inactive GDP-bound and active GTP-bound states. Their activity is tightly regulated by GEFs, GAPs and GDIs, where GEF hyperactivation or GAP loss leads to sustained Rho GTPase signaling. Activated RhoA drives ROCK-mediated stress fiber assembly and actomyosin contractility, facilitating amoeboid motility; Rac1 promotes lamellipodia formation via WAVE/Arp2/3 and generates localized ROS through NOX complexes, amplifying pro-migratory signals and mesenchymal motility; Cdc42 orchestrates filopodia formation, establishes polarity, and regulates MMP trafficking for ECM remodeling. Collectively, this coordinated signaling network drives cytoskeletal remodeling, directional migration, ECM degradation, and ultimately facilitates cancer cell invasion and metastatic dissemination. ECM, extracellular matrix; GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; ROS, reactive oxygen species; MMP, matrix metalloproteinase; NOX, NADPH oxidase.

Tumor-type-specific dependencies and intra-tumoral heterogeneity can influence efficacy, emphasizing the need for biomarker-guided patient selection.

The clinical potential of siRNA therapeutics is validated by FDA-approved agents such as patisiran, highlighting feasibility for systemic administration. However, oncology

Table II. Post-transcriptional regulation of Rho GTPases signaling by miRNAs in cancer metastasis.

| First author/s, year | miRNA | Role in cancer | Validated molecular targets | Effect on Rho GTPase signaling pathways | Functional outcome in metastasis | (Refs.) |
|---|----------------|-------------------|---------------------------------------|---|---|---------|
| Saliani <i>et al.</i> , 2021; Brabletz <i>et al.</i> , 2011; Hur <i>et al.</i> , 2013 | miR-200 family | Tumor suppressor | ZEB1, ZEB2 | Indirect downregulation of RhoA | Suppresses EMT, inhibits invasion | (76-78) |
| Augoff <i>et al.</i> , 2011 | miR-31 | Tumor suppressor | RDX, RhoA | Reduces Rac1 and RhoA | Impairs invasion and metastasis | (80) |
| Slabáková <i>et al.</i> , 2017 | miR-34a | Tumor suppressor | MET, SIRT1 | Modulates Rac1 and RhoA signaling | Inhibits cell migration and invasion | (79) |
| Gan <i>et al.</i> , 2024 | miR-21 | OncomiR | Programmed cell death protein 4, PTEN | Enhances RhoB and Cdc42 activity | Promotes migration, invasion, and EMT | (81) |
| Qian <i>et al.</i> , 2024 | miR-155 | OncomiR | RhoA, SOCS1 | Suppresses RhoA and enhances Rac1 signaling | Shifts balance to mesenchymal migration | (82) |
| Xie <i>et al.</i> , 2021; Pahlavan <i>et al.</i> , 2020 | miR-142 | Context-dependent | Rac1, ADCY9 | Directly targets Rac1 mRNA | Can suppress or promote invasion depending on context | (83,84) |

Tumor-suppressive miRNAs, including miR-200, miR-31, and miR-34a, inhibit key components of the Rho GTPase machinery, targeting RhoA, Rac1, Cdc42, or upstream regulators (GEFs, GAPs, GDIs) and repress EMT transcription factors (ZEB1/2), collectively reducing cancer cell motility and invasion. In contrast, oncogenic miRNAs, such as miR-21 and miR-155, repress tumor suppressors, leading to hyperactivation of RhoB, Cdc42, and Rac1, thereby promoting invasive phenotypes. Therapeutically, synthetic miRNA mimics can restore tumor-suppressive miRNAs, while antagomiRs can inhibit oncomiRs, providing a strategy to normalize Rho GTPase signaling. Certain miRNAs, including miR-142, exhibit context-dependent effects and may function as either tumor suppressors or promoters depending on cancer type and cellular context. miR-200, microRNA-200; ZEB1, zinc finger E-box-binding homeobox 1; ZEB2, zinc finger E-box-binding homeobox 2; RhoA, Ras homolog family member A; EMT, epithelial-to-mesenchymal transition; miR-31, microRNA-31; RDX, radixin; Rac1, Ras-related C3 botulinum toxin substrate 1; miR-34a, microRNA-34a; MET, mesenchymal-to-epithelial transition; SIRT1, sirtuin 1; miR-21, microRNA-21; oncomiR, oncogenic microRNA; SOCS1, suppressor of cytokine signaling 1; miR-142, microRNA-142; ADCY9, adenylate cyclase 9; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GDI, guanine nucleotide dissociation inhibitor.

applications face significant hurdles. Naked siRNAs are unstable in circulation, prone to nuclease degradation, and can elicit innate immune responses. Their negative charge limits passive cellular uptake, while non-specific gene silencing and unintended immune activation remain key concerns (85,86). These challenges are intensified in solid tumors due to dense extracellular matrix, irregular vasculature, and high interstitial pressure.

Consequently, advanced delivery platforms are essential to overcome these limitations. Lipid nanoparticles (LNPs) can encapsulate siRNA and facilitate cellular uptake via endocytosis, enabling efficient intracellular delivery and gene silencing in preclinical cancer models (86-88). Recent advances in LNP design, including lipoprotein-mimicking nanocarriers and ligand-targeted formulations, have further improved tumor specificity and delivery efficiency (89). Polymeric nanocarriers,

including cationic polymers like polyethyleneimine or biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA), form stable polyplexes and allow controlled release, while surface modifications enhance tumor specificity (87). Exosome-based systems leverage natural biocompatibility for improved tissue targeting and endosomal escape (90,91). Stimuli-responsive carriers release siRNA in response to TME cues, such as low pH or high ROS, enhancing localized silencing (87,88,92).

Each platform balances delivery efficiency, toxicity and manufacturability, requiring careful optimization for specific applications. The ultimate promise of siRNA may lie in combinatorial strategies. Co-delivering siRNAs against Rho GTPases with mitochondrial inhibitors, such as doxycycline, could synergistically disrupt both cytoskeletal dynamics and metabolic support, potentially

overcoming compensatory resistance mechanisms that limit monotherapies (Table III).

Limitations and future directions. Despite the compelling rationale for siRNA targeting of Rho GTPases outlined in the previous section, several significant limitations must be overcome for clinical success. Tumor heterogeneity and adaptive metabolic and cytoskeletal plasticity represent a central challenge. When a specific Rho GTPase such as RhoA is silenced, metastatic cells can activate compensatory pathways to maintain invasiveness. This includes upregulation of related family members (RhoC compensating for RhoA loss), activating parallel cytoskeletal regulators, or inducing EMT programs, effectively bypassing the therapeutic blockade (70). It is important to note that functional redundancy varies by context; for example, while RhoA and RhoC are often pro-metastatic, RhoB can exhibit tumor-suppressive functions, highlighting the need for precise target selection (68,93).

The second major barrier is delivery. The hostile physiology of solid tumors, characterized by a dense ECM, aberrant vasculature and hypoxic cores, severely restricts the penetration and uniform distribution of siRNA carriers. Furthermore, systemic administration faces obstacles such as rapid renal clearance, nuclease degradation, and potential unintended immune activation, all of which reduce bioavailability and raise safety concerns (94). These barriers are far more pronounced than in hematological malignancies, creating a uniquely difficult environment for siRNA-based therapies in solid cancers.

Future progress therefore hinges on innovative strategies that simultaneously address biological compensation and physical delivery barriers. To counteract adaptive resistance, rational combination therapies are essential. This includes the co-targeting of multiple Rho GTPases or, more powerfully, the integration of Rho pathway inhibition with agents targeting complementary vulnerabilities. As a core thesis of the present review, a promising strategy approach may be the combination of Rho GTPase siRNA with mitochondrial inhibitors (doxycycline). This strategy simultaneously disables the cytoskeletal 'engine' and its metabolic 'fuel', limiting the cancer's compensatory escape mechanisms that may occur when using either drug alone (23,93).

Concurrently, the next generation of delivery platforms must be engineered to address three critical objectives. First, enhanced specificity can be achieved through the incorporation of targeting ligands, such as Arginyl-glycyl-aspartic acid (RGD) peptides or antibodies, that selectively recognize receptors overexpressed on metastatic cells. Second, controlled activation is essential, with carriers designed to release their siRNA payload only in response to defined TME cues, including acidic pH or elevated protease activity. Third, improved penetration strategies are required to overcome the dense extracellular matrix and abnormal stromal architecture of solid tumors. Approaches such as incorporating ECM-degrading enzymes or developing size-tunable nanoparticles are being actively explored to ensure deeper intratumoral delivery and more effective silencing.

Platforms incorporating these features, such as the targeted and stimuli-responsive systems currently in preclinical development, hold immense promise for improving the therapeutic

index by maximizing on-target effects while minimizing systemic toxicity (87,88,92).

While the path to clinical application is fraught with challenges, the ability of siRNA to directly silence 'undruggable' primary regulators of metastasis remains a highly attractive and valid therapeutic goal. Overcoming the intertwined obstacles of tumor plasticity, delivery efficiency, and adaptive resistance through intelligent combinatorial and delivery strategies will be paramount. Success in this endeavor will not only yield new anti-metastatic agents but could also establish a novel treatment paradigm centered on disrupting the integrated metabolic and mechanical dependencies of cancer dissemination.

4. Crosstalk between mitochondria and Rho GTPases in cancer metastasis

Rationale for crosstalk discussion. The preceding sections have established both mitochondria and Rho GTPases as powerful, independent drivers of metastasis, governing bioenergetic adaptation and cytoskeletal remodeling, respectively. However, a more complete, systems-level understanding reveals that their functions are deeply intertwined, creating an integrated functional axis that is essential for metastatic competency (11,16). This extensive bidirectional crosstalk ensures that the energetic supply is precisely matched to mechanical demand.

The mechanistic basis for this interplay is well-documented. Mitochondria provide the ATP required to directly fuel actin polymerization and focal adhesion turnover, while mitochondrial-derived ROS act as crucial second messengers that oxidize cysteine residues in regulatory proteins, thereby activating key Rho GTPase signaling pathways such as Src and PI3K (95-98). More specifically, mitochondrial ROS, particularly hydrogen peroxide (H₂O₂), oxidize redox-sensitive cysteine residues in regulatory proteins, leading to activation of Src-family kinases and modulation of RhoGEFs and RhoGAPs. This process differentially regulates Rac1-driven protrusion and RhoA-mediated contractility in a context-dependent manner (17,99-102). Importantly, ROS signaling is highly dose-dependent: Low to moderate ROS levels promote pro-migratory signaling, whereas excessive ROS induces oxidative damage and suppresses metastatic potential (17,18,38). Conversely, Rho GTPase-driven cytoskeletal dynamics and the establishment of cell polarity govern the trafficking and positioning of mitochondria via proteins such as Miro and DRP1, ensuring that mitochondria are recruited to regions of high energy demand, such as the leading edge of migratory cells (11,30). This reciprocal reinforcement creates a feedforward loop that potentially optimizes the cell's migratory and invasive capacity.

Therefore, this section will dissect the molecular nodes of this metabolic-mechanical interplay, examining both signaling and bioenergetic integration. The academic exercise of understanding this crosstalk will provide the fundamental rationale for designing innovative combination therapies that simultaneously disrupt the energetic supply and the cytoskeletal machinery of metastasis. This synergistic strategy of targeting complementary vulnerabilities, represents a promising

Table III. Delivery platforms for siRNA-mediated targeting Rho GTPases in cancer metastasis.

| First author/s, year | Delivery platform | Composition | Delivery mechanism | Advantages | Limitations | Preclinical evidence (model and outcome) | (Refs.) |
|---|----------------------------|---|---|---|---------------------------------------|---|------------|
| Hou <i>et al</i> , 2024; Jin <i>et al</i> , 2024; Zeng <i>et al</i> , 2024 | LNPs | Ionizable or cationic lipids, PEG-lipid | Endocytosis and membrane fusion | Clinically validated, high loading capacity, scalable | Immunogenicity and liver tropism | Rac1 siRNA delivery in murine models of metastasis model ↓ metastatic burden | (86-88) |
| Jin <i>et al</i> , 2024 | Polymeric nanoparticle | PEI, PLGA, chitosan | Endocytosis, proton-sponge' effect | Tunable, controlled release | Cytotoxicity (PEI), aggregation | RhoA siRNA-loaded PLGA nanoparticles in <i>in vivo</i> cancer models ↓ tumor growth | (87) |
| Kamerkar <i>et al</i> , 2017; Ubanako <i>et al</i> , 2024 | Exosomes | Natural lipid bilayer with surface proteins | Membrane fusion, receptor-mediated uptake | Biocompatibility, low immunogenicity, inherent tissue tropism | Low yield, limited loading efficiency | Rac1 siRNA-loaded exosomes ↓ melanoma metastasis <i>in vivo</i> | (90,91) |
| Jin <i>et al</i> , 2024; Zeng <i>et al</i> , 2024; Thomas <i>et al</i> , 2020 | Stimuli-Responsive Systems | pH/ROS/enzyme-sensitive polymers | TME-triggered release | Enhanced specificity, reduced off-target toxicity | Complex design, stability concerns | RhoA siRNA released under acidic TME conditions improves localized gene silencing | (87,88,92) |

LNP, lipid nanoparticle; PEG, polyethylene glycol; Rac1, Ras-related C3 botulinum toxin substrate 1; siRNA, small interfering RNA; PLGA, poly(lactic-co-glycolic acid); RhoA, Ras homolog family member A; TME, tumor microenvironment; pH, potential of hydrogen; ROS, reactive oxygen species; ↓, decrease.

approach to overcome the adaptive resistance that frequently limits the efficacy of single-target interventions (23,103).

Functional convergence of mitochondria and Rho GTPases. Building on the rationale for their crosstalk, the functional integration of mitochondria and Rho GTPases occurs at several key nodes that directly control cell motility. This convergence ensures that bioenergetic supply is precisely coupled to mechanical demand during invasion.

At the leading edge of migratory cells, mitochondrial ATP is indispensable for fueling the actin polymerization that drives protrusion formation. This localized energy supply directly supports the extension of lamellipodia and filopodia, processes governed by Rac1 and Cdc42, respectively (11,104,105). Beyond ATP, mitochondria-derived ROS, particularly H₂O₂, function as spatially restricted signaling molecules under conditions of increased metabolic flux, hypoxia and oncogenic stimulation (17,24,99,106). At low to moderate concentrations, H₂O₂ oxidizes redox-sensitive cysteine residues within protein tyrosine phosphatases (PTPs), including PTP1B, leading to their transient inactivation (107). This oxidation-dependent inhibition shifts signaling toward kinase-dominant states, resulting in sustained phosphorylation and activation of Src-family kinases, which in turn enhances Rho GTPase signaling pathways, including phosphorylation-dependent activation of RhoGEFs (17,99,108). This, in turn, amplifies signaling through downstream effectors such as ROCK (promoting actomyosin contractility) and the WAVE/Arp2/3 complex (reinforcing lamellipodia assembly), thereby consolidating directional motility (104,109). Functionally, Rac1 preferentially promotes lamellipodia formation and mesenchymal migration via WAVE/Arp2/3 signaling, whereas RhoA drives stress fiber formation and ROCK-mediated actomyosin contractility, supporting contractile or amoeboid movement (68,69). In addition, Rac1-associated oxidant signaling can further reinforce pro-migratory pathways, contributing to a positive feedback between redox signaling and cytoskeletal remodeling (32,99).

Conversely, the cytoskeletal machinery exerts significant control over mitochondrial function and localization. Rho GTPase-mediated activation of ROCK generates actomyosin contractility, which influences mitochondrial dynamics by modulating the activity of fission factors such as DRP1. Furthermore, cellular polarity established by Cdc42 signaling guides the trafficking of mitochondria along microtubules via adaptor proteins such as Miro1-dependent adaptor complexes, which link mitochondria to kinesin motors, ensuring their strategic positioning at the cell's leading edge to sustain localized energy production (110,111). In addition to general mitochondrial trafficking mechanisms, mitochondrial Rho GTPases, particularly MIRO1 and MIRO2, have emerged as critical regulators of organelle positioning along the cytoskeleton. These proteins function as adaptor molecules linking mitochondria to microtubule-based motor complexes, including kinesin, thereby enabling directed transport toward regions of high energetic demand such as the leading edge of migrating cancer cells. This spatial positioning is essential for sustaining localized ATP production required for actin polymerization and focal adhesion dynamics. Importantly,

previous studies indicate that MIRO-dependent trafficking actively contributes to metastatic behavior by modulating cytoskeletal signaling and Rho GTPase activity, further reinforcing the metabolic-mechanical coupling underlying invasion (16,110,112). This spatial redistribution enables localized ATP delivery and ROS signaling at the leading edge, thereby reinforcing Rac1-dependent protrusive activity and directional migration (30,113). For instance, in breast cancer cells, mitochondrial translocation to invadopodia is a Rho GTPase-dependent process essential for invasive capacity.

Bidirectional interaction reinforces the coordination between metabolic output and cytoskeletal remodeling, which then recruits more mitochondria to sustain the invasive activity. Importantly, this metabolic-mechanical coupling is regulated in a dose-dependent manner, as low to moderate ROS levels preferentially sustain Rac1-driven protrusion and controlled RhoA activity, whereas excessive ROS disrupt cytoskeletal organization and suppress migration (17,18,38). The integration of this metabolic output with mechanical activity is not merely supportive but is a fundamental driver of efficient and sustained invasion.

Molecular nodes of intersection. The functional convergence of mitochondria and Rho GTPases is orchestrated by specific molecular nodes that serve as direct signaling conduits. Understanding the cellular energy sensors, redox-sensitive pathways, and the machinery controlling organelle dynamics is crucial for appreciating how metabolic status is translated into mechanical action.

A primary node of integration involves the energy-sensing kinases AMPK and mTOR. These sensors directly couple the cell's metabolic state to its migratory machinery. Under conditions of metabolic stress, including hypoxia, nutrient deprivation, or increased AMP/ATP ratio, AMPK is activated and directly suppresses RhoA-driven cytoskeletal contractility through phosphorylation-dependent mechanisms. Specifically, AMPK inhibits downstream effectors such as ROCK and MLC, thereby reducing actomyosin contractility and limiting energy-consuming processes such as cell contraction and migration (114-116). This establishes a metabolic checkpoint that restrains RhoA-mediated mechanical output under low-energy conditions.

Conversely, under nutrient-rich conditions and growth factor stimulation, mTORC1 becomes activated and promotes Rac1-dependent cytoskeletal remodeling. Mechanistically, mTORC1 enhances actin polymerization and lamellipodia formation by regulating cytoskeletal effectors such as the WAVE complex and Arp2/3, thereby facilitating Rac1-driven protrusive migration (117-119). In addition, mTOR signaling supports anabolic metabolism and protein synthesis required for sustained migratory activity.

Together, these pathways establish a context-dependent regulatory axis in which AMPK suppresses RhoA-mediated contractility under energy stress, whereas mTORC1 promotes Rac1-driven protrusion under nutrient-replete conditions, thereby coordinating metabolic status with distinct modes of cell migration.

A second critical node is the mitochondrial production of ROS. As described above, mitochondrial ROS act as redox signaling intermediates that sustain activation of Src and

FAK, thereby amplifying Rac1- and RhoA-dependent pathways (99-101).

Finally, the physical distribution of mitochondria, controlled by dynamics proteins, constitutes a third key node. The master fission GTPase DRP1 is activated by various cues, including phosphorylation at Ser616 (activation) and Ser637 (inhibition), and creates small, mobile organelles (120). These mitochondria are trafficked along microtubules to the leading edge by adaptor proteins such as Miro, ensuring a localized supply of ATP to power Rac1- and Cdc42-mediated actin polymerization (11,30,121). Beyond its role as a trafficking adaptor, emerging evidence indicates that mitochondrial Rho GTPases such as MIRO2 can directly modulate RhoA signaling pathways. Mechanistically, MIRO2-dependent mitochondrial positioning has been linked to regulation of cytoskeletal dynamics through MYO9B-mediated control of RhoA activity, thereby influencing cell migration and invasion. This reveals an additional layer of bidirectional crosstalk in which mitochondrial trafficking not only responds to cytoskeletal demands but also actively regulates Rho GTPase signaling (16).

Dysregulation of any of these nodes can profoundly amplify metastatic potential. For instance, oncogenic signaling that constitutively activates mTORC1 can lead to hyperactive, Rac1-driven invasion (119,122,123). The understanding that these intersections are critical regulatory hubs underscores their high value as targets for multi-faceted therapeutic intervention (Fig. 4).

Complementarity in therapeutic targeting. The detailed molecular crosstalk between mitochondria and Rho GTPases, outlined in the preceding sections, reveals a fundamental vulnerability: Their interdependence. This relationship provides a powerful rationale for combinatorial therapeutic strategies designed to simultaneously disrupt both the metabolic and mechanical engines of metastasis, thereby overcoming the adaptive resistance that limits single-target therapies.

The logic of this approach is rooted in direct mechanistic complementarity. Mitochondria-targeting agents, such as the antibiotics tigecycline and doxycycline, inhibit OXPHOS. This action has a dual effect; first, it depletes the local ATP supply required for energy-intensive processes such as actin polymerization, and second, it reduces the generation of mitochondrial ROS, which function as essential second messengers for activating pro-migratory signaling pathways through Src kinase and Rho GTPases (23). Specifically, inhibition of mitochondrial ROS disrupts redox-dependent activation of Src and downstream RhoGEF signaling, while ATP depletion impairs Rac1-driven actin polymerization and RhoA-mediated contractility, effectively collapsing both protrusive and contractile components of migration.

When administered alone, cancer cells may compensate for mitochondrial inhibition by upregulating glycolysis. However, when combined with siRNA that directly silences key Rho GTPases (RhoA, Rac1, or Cdc42), the cytoskeletal machinery itself is dismantled. The cell is left with neither the efficient energy source (OXPHOS) nor the functional apparatus to utilize alternative energy for migration and invasion (8).

The potential efficacy of this combinatorial approach arises from the mechanistic interdependence between mitochondrial metabolism and Rho GTPase signaling. Mitochondrial inhibition limits ATP production and disrupts ROS-dependent activation of Src and RhoGEF-mediated signaling, thereby impairing both the energetic and signaling inputs required for cytoskeletal remodeling. Concurrently, direct silencing of Rho GTPases disables the downstream execution of migration by preventing Rac1-driven protrusion and RhoA-mediated contractility. Together, this dual targeting strategy restricts both the supply of metabolic resources and the cellular machinery required for invasion, thereby limiting the ability of cancer cells to engage compensatory adaptive responses (70,103).

Although the translational implementation of such combinations presents challenges, particularly the co-delivery of two therapeutic agents and managing potential toxicities, advances in nanomedicine, including multi-agent lipid nanoparticle systems, are actively addressing these hurdles. In conclusion, targeting the integrated mitochondria-Rho GTPase axis represents a mechanistically grounded strategy to exploit a central vulnerability of metastatic cells. By simultaneously disabling the 'fuel' and the 'engine', this combination therapy offers a promising path toward more durable and effective control of cancer dissemination (Fig. 5).

Overcoming limitations of single approaches. The rationale for dual targeting must be balanced against the intrinsic drawbacks of each monotherapy. Unless these challenges are addressed, promising strategies are unlikely to translate into clinical application.

Understanding these limitations highlights the distinct constraints associated with each therapeutic class. Mitochondrial inhibitors, such as OXPHOS disruptors, are effective at impairing tumor bioenergetics, but their clinical use is hindered by dose-limiting toxicities in energy-demanding tissues such as myocardium and neurons. In addition, tumor metabolic plasticity, including rapid shifts to glycolysis, further undermines durability of therapeutic response (55,124,125). By contrast, siRNA-based therapies face pharmacological barriers, including rapid renal clearance, nuclease-mediated degradation, inefficient cellular uptake and endosomal sequestration. These factors collectively compromise their therapeutic index, particularly in solid tumors characterized by dense stroma and abnormal vasculature (86,94).

These limitations directly impact the mitochondria-Rho GTPase axis, as insufficient delivery prevents effective disruption of both metabolic signaling (ROS and ATP) and cytoskeletal remodeling pathways (RhoA/ROCK and Rac1/WAVE), thereby allowing persistence of the metastatic feedback loop. Importantly, this bidirectional metabolic-mechanical loop provides a mechanistic basis for resistance to single-target therapies. Disruption of either mitochondrial function or Rho GTPase signaling alone is often insufficient, as cancer cells can compensate through the reciprocal pathway, maintaining invasive and metastatic potential.

Despite its strong mechanistic rationale, the dual-targeting strategy itself faces several translational challenges. A primary obstacle is the efficient co-delivery of two distinct

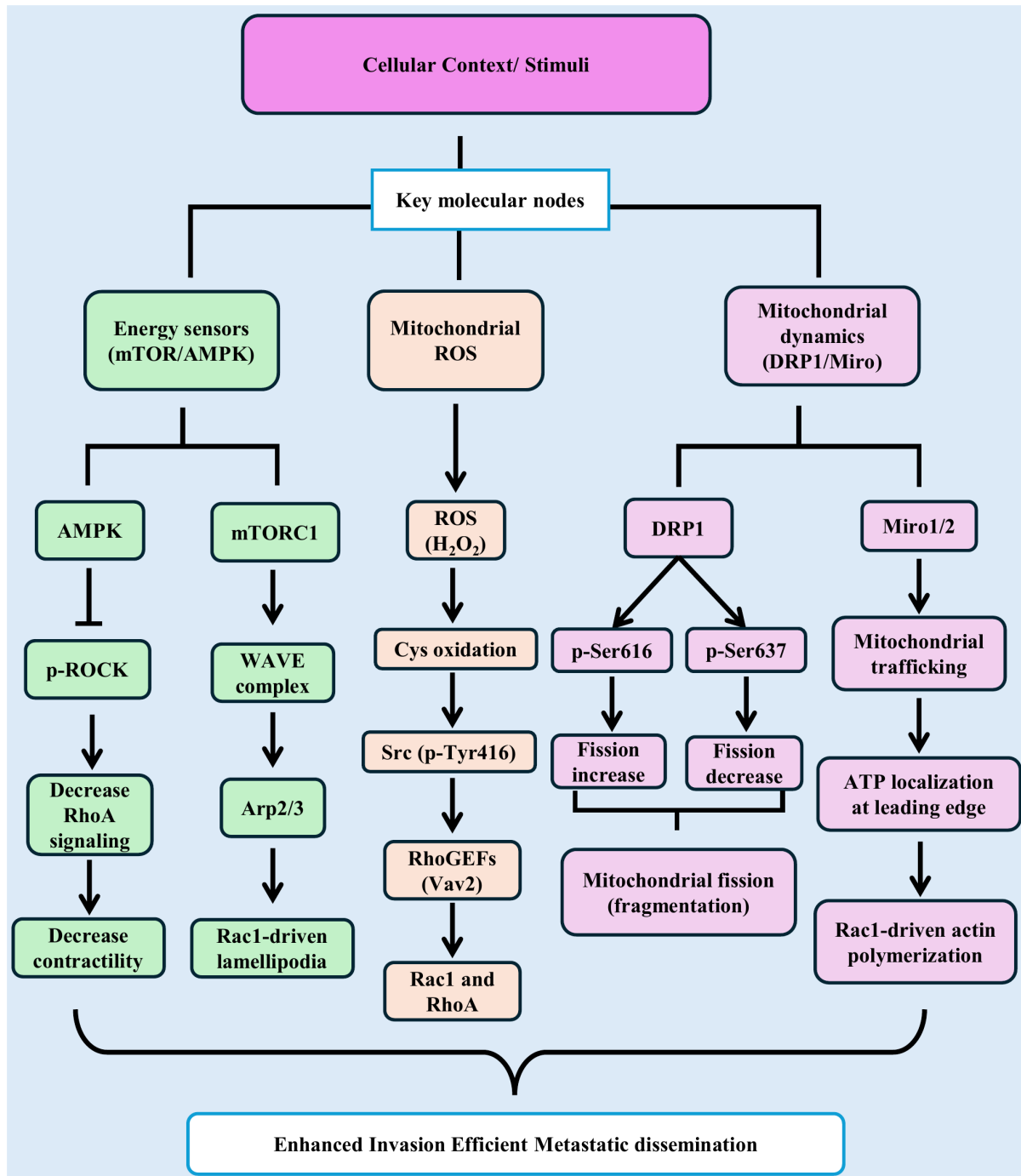


Figure 4. Molecular nodes integrating mitochondrial function with Rho GTPase-mediated cytoskeletal regulation in cancer metastasis. Metastatic signaling converges on three key molecular nodes linking mitochondrial function to Rho GTPase-mediated cytoskeletal remodeling. Energy-sensing kinases AMPK and mTORC1 translate nutrient and energy availability into distinct cytoskeletal outputs: AMPK inhibits RhoA-ROCK signaling via phosphorylation-dependent mechanisms, reducing actomyosin contractility, whereas mTORC1 promotes Rac1-driven protrusion through activation of the WAVE-Arp2/3 complex, leading to lamellipodia formation (68,75). Mitochondrial ROS, particularly H₂O₂, act as redox signaling molecules by oxidizing cysteine residues in regulatory proteins, leading to activation of Src (p-Tyr416) and downstream Rho-GEFs, thereby promoting Rac1 and RhoA signaling (17,99,100,102,119). In parallel, mitochondrial dynamics, are regulated by DRP1 phosphorylation (Ser616 promotes fission; Ser637 inhibits fission) and Miro1/2-mediated trafficking, which enables redistribution of mitochondria and localized ATP supply to support Rac1- and Cdc42-driven actin polymerization (67). These pathways form a bidirectional feedback loop in which mitochondrial metabolism (ATP and ROS) drives Rho GTPase activation, while Rho-dependent cytoskeletal remodeling regulates mitochondrial positioning. Together, these nodes converge to drive cytoskeletal remodeling, contractility, extracellular matrix degradation, and directional migration, enhancing invasive and metastatic potential. Therapeutically, this network highlights actionable targets, including AMPK activators (+), mTORC1 inhibitors (-), DRP1 or mitochondrial trafficking inhibitors (-), and context-dependent ROS modulators (±). ROS, reactive oxygen species; p-, phosphorylated; DRP1, dynamin-related protein 1.

therapeutic modalities, as siRNAs and small-molecule mitochondrial inhibitors differ in stability, pharmacokinetics, and cellular uptake pathways. Achieving synchronized delivery

and intracellular release remains technically challenging. In addition, simultaneous targeting of metabolic and cytoskeletal pathways may increase the risk of systemic toxicity, particularly

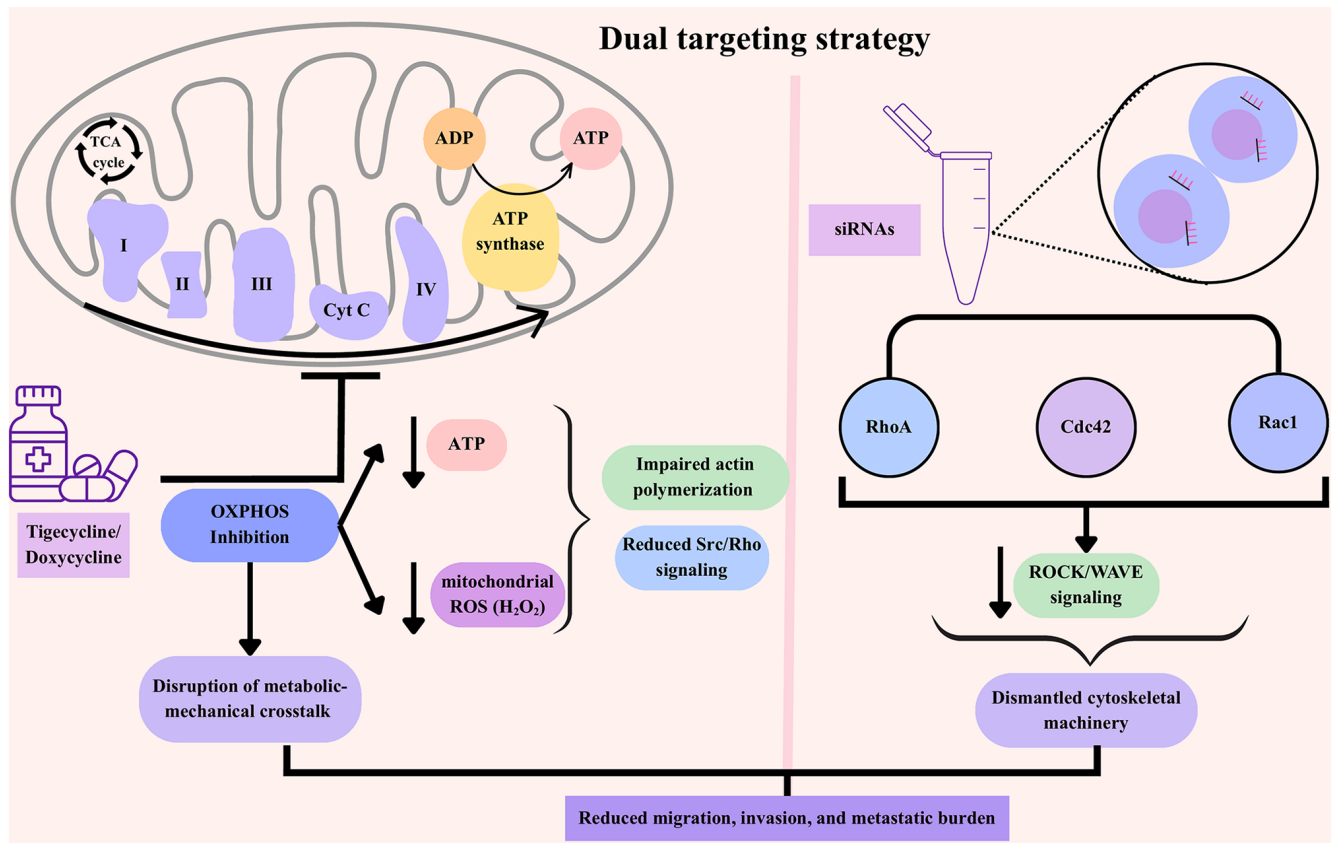


Figure 5. Mechanistically complementary dual targeting of mitochondria and Rho GTPases to inhibit cancer metastasis. Mitochondria-targeting agents, such as tigecycline or doxycycline, inhibit OXPHOS, leading to depletion of ATP and reduction of mitochondrial ROS. This limits the energy supply necessary for actin polymerization and dampens pro-migratory signaling via Src kinase and Rho GTPases. Simultaneously, siRNA-mediated silencing of Rho GTPases (RhoA, Rac1 and Cdc42) directly dismantles the cytoskeletal machinery required for migration and invasion. Individually, cells may compensate, by upregulating glycolysis or switching migration modes, but combined targeting creates a synergistic effect, blocking both the “fuel” and the “engine” of metastasis. Preclinical studies demonstrate that this dual approach is expected to reduce invasive capacity more effectively than monotherapies. OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; siRNAs, small interfering RNAs.

in highly energy-dependent normal tissues. Furthermore, tumor heterogeneity and pharmacodynamic variability may limit uniform therapeutic responses across patients.

To overcome these pharmacological and biological barriers, nanocarriers-based systems have emerged as promising platforms for co-delivery. LNPs, clinically validated for siRNA delivery, can be co-loaded with small-molecule inhibitors and further engineered with tumor-specific ligands to enhance selectivity and reduce systemic exposure (89,126). Biodegradable polymers such as PLGA enable co-encapsulation of hydrophilic siRNA and hydrophobic drugs, with tunable release kinetics and responsiveness to the TME (127). Hybrid lipid-polymer constructs further optimize stability, drug loading, and release dynamics.

Preclinical studies support the translational potential of such co-delivery systems, demonstrating enhanced therapeutic efficacy when metabolic and signaling pathways are simultaneously targeted (103). For instance, lipid nanoparticle-mediated delivery of siRNA targeting oncogenic drivers has been shown to suppress tumor progression and improve therapeutic outcomes *in vivo* (128). Mechanistically, dual targeting disrupts both bioenergetic adaptation and cytoskeletal reprogramming, thereby limiting the ability of tumor cells to engage compensatory escape mechanisms.

Taken together, these results underscore the necessity of rationally designed co-delivery systems to fully exploit the therapeutic potential of simultaneously targeting metabolic and cytoskeletal pathways.

Future perspectives. The synthesis of evidence presented herein establishes the mitochondria-Rho GTPase crosstalk as a critical, actionable axis in metastasis. Moving forward, the therapeutic potential of dual-targeting strategies must be systematically evaluated and advanced through a focused research agenda.

Future preclinical investigations must extend beyond conventional cell line models to systems that capture the complexity of metastatic disease. These include patient-derived xenografts, genetically engineered mouse models and 3D organoid cultures that recapitulate tumor heterogeneity, stromal interactions, and the stresses of the metastatic cascade. Within these systems, studies should precisely delineate the mechanistic consequences of dual inhibition, including its impact on mitochondrial ROS signaling, localized ATP availability, cytoskeletal dynamics, and the activation of compensatory pathways such as alternative GTPase expression or enhanced glycolytic flux. Particular emphasis should be placed on quantifying how dual targeting alters ROS-dependent Src activation,

RhoGEF/RhoGAP balance, and the spatial coordination of Rac1-driven protrusion versus RhoA-mediated contractility, as well as mitochondrial positioning at the leading edge.

Translational innovation will be essential. The development of advanced co-delivery platforms, such as tumor-targeted lipid nanoparticles or engineered exosomes capable of delivering both siRNA and mitochondrial inhibitors, is a critical prerequisite for clinical application. Exosome-based delivery systems, in particular, offer enhanced biocompatibility, reduced immunogenicity, and improved targeting efficiency, making them promising candidates for next-generation RNAi therapeutics (91).

Furthermore, the potential of integrating this dual-targeting approach with existing standard-of-care therapies, such as immune checkpoint inhibitors or targeted agents, should be explored to create multi-pronged treatment strategies. In parallel, the identification of predictive biomarkers, such as gene expression signatures reflecting mitochondrial dependency and Rho GTPase signaling activity, will be crucial for patient stratification and therapeutic optimization (14,68,70).

Beyond intracellular metabolic-mechanical crosstalk, emerging evidence highlights intercellular mitochondrial transfer as an additional layer contributing to tumor progression and metastasis. Cancer cells can exchange mitochondria with stromal and immune cells through mechanisms such as tunneling nanotubes and extracellular vesicles, enabling recipient cells to acquire functional mitochondria and enhance metabolic flexibility, resistance to oxidative stress, and survival under adverse microenvironmental conditions (129). This process is closely linked to cytoskeletal dynamics and depends on mitochondrial trafficking machinery, including proteins such as MIRO1 and MIRO2, further extending metabolic-mechanical crosstalk beyond single-cell systems (20,95). From a therapeutic perspective, targeting mitochondrial transfer and trafficking mechanisms may represent an additional strategy to disrupt metastatic progression. Combining mitochondrial inhibition with approaches that interfere with cytoskeletal regulation or mitochondrial transport, such as Rho GTPase silencing, could further enhance therapeutic efficacy by disrupting both intracellular energy distribution and intercellular metabolic support.

By addressing these challenges, future research can translate the compelling biology of the metabolic-mechanical interface into a new therapeutic paradigm. The goal is to develop next-generation interventions that simultaneously dismantle the energetic and mechanical underpinnings of cancer dissemination, offering a potent strategy to control the process responsible for most of the cancer-related mortality.

5. Conclusion

Metastasis remains the principal cause of cancer-related mortality, driven by the remarkable adaptability and plasticity of tumor cells (4,130). The present review has synthesized evidence establishing that mitochondrial metabolism and Rho GTPase signaling are not independent drivers of metastasis but rather functionally integrated into a coordinated metabolic-mechanical axis (11,16). This bidirectional crosstalk establishes a dynamic feedback loop in which mitochondria supply ATP and ROS to fuel cytoskeletal remodeling, while Rho GTPase-mediated cytoskeletal organization governs

mitochondrial positioning and function (30,102). This reciprocal interaction optimizes the spatial and energetic requirements of cell migration and represents a fundamental mechanism supporting metastatic efficiency (30,131).

Importantly, this interdependence provides a mechanistic explanation for the limited efficacy of single-target therapies (35). Disruption of either mitochondrial metabolism or Rho GTPase signaling alone is frequently insufficient, as tumor cells can compensate through the reciprocal pathway, maintaining invasive and metastatic potential. This highlights the need for therapeutic strategies that simultaneously target both metabolic and cytoskeletal components of this axis to overcome adaptive resistance.

To overcome this adaptive resistance, dual-targeting approaches combining siRNA-mediated silencing of Rho GTPases with mitochondrial inhibitors, such as doxycycline, represent a rational strategy to overcome compensatory resistance. By concurrently disrupting the cytoskeletal 'engine' and its metabolic 'fuel', this approach aims to achieve more effective suppression of metastatic progression (8,23,103). However, despite this strong mechanistic rationale, several translational challenges must be addressed. These include limited delivery to metastatic niches, efficient co-delivery of siRNA and small-molecule agents, potential systemic toxicity in energy-dependent tissues, and variability in therapeutic response due to tumor heterogeneity.

Emerging nanocarrier-based systems, including lipid nanoparticles and biodegradable polymer platforms, offer promising solutions by enabling co-encapsulation, targeted delivery, and controlled release of therapeutic agents (126,127). Furthermore, validating this approach requires advanced preclinical models that capture metastatic complexity and the identification of biomarkers to guide patient selection in future clinical trials (35).

Looking forward, integrating this dual-targeting paradigm into precision oncology frameworks offers a transformative opportunity. Targeting this integrated metabolic-mechanical network, rather than its individual components, provides a conceptual and therapeutic framework for overcoming resistance and achieving durable control of metastatic disease. Collectively, this work provides a mechanistic roadmap for the development of next-generation therapies aimed at addressing one of the most urgent unmet needs in oncology.

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Authors' contributions

AD drafted the initial manuscript. ZA restructured the manuscript's organization and ideas, created the figures, helped in

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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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