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Metabolic Reprogramming and Tumor Microenvironment: Emerging Mechanisms of Cancer Development and Therapeutic Resistance

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Abstract

Cancer progression is driven by complex interactions between metabolic reprogramming, mitochondrial dysfunction, and the tumor microenvironment (TME). Cancer cells undergo profound metabolic adaptations that enable them to sustain rapid proliferation, maintain redox balance, and survive under conditions of hypoxia, nutrient deprivation, and cellular stress.

Although aerobic glycolysis is a recognized feature of many tumors, mitochondrial oxidative phosphorylation (OXPHOS) remains essential in numerous cancer types, contributing to ATP production, biosynthetic pathways, and metabolic flexibility. These complementary energy-generating mechanisms allow malignant cells to adapt to changing microenvironmental conditions and maintain tumor growth.The tumor microenvironment further shapes cancer metabolism through interactions among malignant cells, cancer-associated fibroblasts, immune cells, endothelial cells, and extracellular matrix components. Hypoxia-inducible signaling, nutrient competition, lactate accumulation, and altered lipid and amino acid metabolism can promote angiogenesis, immune evasion, invasion, and metastatic dissemination. In addition, metabolic communication between tumor cells and surrounding stromal cells can influence mitochondrial activity, inflammatory signaling, and resistance to anticancer treatments. These interactions create a dynamic ecosystem in which metabolic adaptation and microenvironmental signaling reinforce tumor survival and progression.

Mitochondrial reactive oxygen species (ROS) and redox-regulatory pathways contribute to this process by acting as signaling mediators that influence genomic stability, cellular proliferation, apoptosis, and therapeutic responses. Cancer cells may also modify mitochondrial dynamics, mitophagy, and antioxidant defenses to preserve cellular fitness under stress. Meanwhile, metabolic alterations within the TME can impair antitumor immune responses and support the persistence of treatment-resistant cancer cell populations. Understanding the relationship between mitochondrial function, metabolic plasticity, and microenvironmental regulation is therefore essential for identifying the mechanisms underlying cancer progression and therapeutic resistance.Emerging technologies, including single-cell transcriptomics, spatial multi-omics, metabolomics, and advanced mitochondrial profiling, provide opportunities to characterize metabolic heterogeneity across tumor regions and cellular populations. Integrating these approaches can reveal how specific metabolic states are associated with tumor evolution, immune suppression, metastatic potential, and treatment response. Ultimately, identifying the metabolic dependencies and signaling pathways that connect cancer cells with their microenvironment may support the development of targeted therapeutic strategies, improve patient stratification, and provide new opportunities to overcome resistance to conventional cancer treatments.

Keywords: Mitochondrial TRACER, mtDNA Editing, Cancer Progression, Heteroplasmy Tracking, Single-cell Multi-omics

Introduction

Mitochondria are classically described as the “powerhouses” of the cell, generating ATP through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) [1]. However, they function as metabolic hubs, integrating glucose, fatty acid β-oxidation, and amino acid metabolism to supply biosynthetic precursors for nucleotides, lipids, and non-essential amino acids that fuel rapid tumor growth [2]. Mitochondria also regulate apoptosis, in part through mitochondrial outer membrane permeabilization and cytochrome c release, positioning them as gatekeepers of cell fate in cancer [3]. In parallel, they are major sources of reactive oxygen species (ROS), particularly from

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complexes I and III of the electron transport chain, where ROS serve as both signalling mediators of proliferation, survival, and immune modulation and, when excessive, drivers of DNA damage and oncogenic transformation [4]. Beyond energy and signalling, mitochondria support biosynthesis (e.g., heme and amino acids), redox and calcium homeostasis, and can be exchanged between cells, thereby contributing to tumor progression, metastasis, and therapy resistance [3].

The Enigmatic mtDNA and Cancer

Human mitochondrial DNA (mtDNA) is a small, 16.5-kb circular genome encoding 13 core OXPHOS subunits, 22 tRNAs, and 2 rRNAs [5]. mtDNA experiences a high mutation rate due to its proximity to ROS, limited repair capacity, and absence of histones, leading to point mutations, insertions, deletions, and copy-number variation in many tumors [6]. These variants frequently exist in a heteroplasmic state coexistence of mutant and wild-type genomes within the same cell where the proportion of mutant mtDNA critically shapes mitochondrial function and disease phenotypes [7]. Large-scale sequencing and functional studies show that mtDNA mutations and heteroplasmy shifts are common across cancer types and associate with tumor initiation, metabolic reprogramming, progression, metastasis, immune remodeling, and responses to therapies including chemotherapy, anti-VEGF agents, and immune checkpoint blockade [8]. Yet, despite strong correlations, a central challenge has been distinguishing causality from correlation, because observational genomics and indirect models (e.g., transmitochondrial cybrids) often cannot definitively prove that specific mtDNA variants drive, rather than merely accompany, malignant phenotypes [9].

The Technological Gap

The modern understanding of nuclear oncogenes has been transformed by precise gene-editing tools such as CRISPR–Cas9, base editors, and prime editors [10]. However, these platforms largely rely on guide RNAs and DNA double-strand break repair, which are poorly compatible with mitochondria. Efficient RNA import into the mitochondrial matrix has not been established, and canonical CRISPR systems have not been used reliably for mtDNA engineering [10, 11]. Earlier approaches using mitochondria-targeted nucleases (ZF- or TALE-nucleases) could selectively cleave mutant mtDNA but only decreased mutant load rather than install defined sequence changes because mitochondria lack robust double-strand break repair [12]. Only recently have CRISPR-free, all-protein base editors (e.g., DdCBE and ZF-DdCBE) enabled targeted C→T editing at selected mtDNA sites, yet their sequence scope, efficiency, and off-target profiles remain constrained [12, 13]. This technological gap has limited the field's ability to perform systematic, causal tests of mtDNA mutations in cancer.

Introducing the TRACER Platform

The TRACER (Transfer CRISPR Editing Reporters) conceptually addresses this challenge by coupling hard-to-measure mitochondrial edits to an easy-to-monitor nuclear signal. At its core, TRACER links the successful introduction of a desired mtDNA edit to a simple, selectable or reportable nuclear readout such as fluorescence, surface markers, or drug resistance so that cells harbouring specific mitochondrial genotypes can be prospectively isolated, quantified, and phenotyped at scale. A TRACER-style system mapping of mtDNA variants to functional consequences in (cancer cells, tumour microenvironments, and therapeutic responses, in a way analogous to pooled, CRISPR screens in the nucleus) [9, 10].

This review will examine how the Mitochondrial TRACER platform is revolutionizing the causal interrogation of mtDNA in cancer, bridging the longstanding gap between association and mechanism, and how this capability is poised to unlock new diagnostic biomarkers, response predictors, and mitochondria-targeted therapeutic strategies across oncology.

The Technical Foundation of Mitochondrial TRACER

Deconstructing the System

The mtDNA editor

At the core of Mitochondrial TRACER lies a programmable mtDNA base editor that can introduce precise point mutations into the mitochondrial genome without double-strand breaks as (figure1). Contemporary implementations primarily exploit DddA-derived cytosine base editors (DdCBEs) and TALE-linked deaminases (TALEDs) [12, 14].

DdCBEs are all-Protein Constructs in which:

- A double-stranded DNA–specific cytidine deaminase (DddAtox) is split into two inactive halves to mitigate toxicity.
- Each DddA half is fused to a custom-designed transcription activator–like effector (TALE) that binds a defined mtDNA sequence.
- A uracil glycosylase inhibitor (UGI) is included to channel deamination products into C•G→T•A conversions [14, 15].

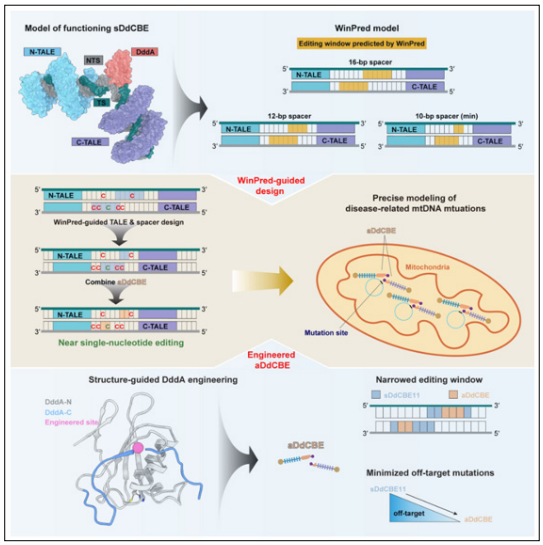


Figure 1: This schematic demonstrates a WinPred-guided strategy for engineering DddA-based mitochondrial cytosine base editors that achieve highly precise, near single-nucleotide mtDNA editing while minimizing off-target mutations [16].

Upon binding of both TALE–DddA halves to adjacent sites, the deaminase reconstitutes within a narrow "editing window" and catalyzes targeted C→T substitutions in mtDNA [12]. Deep engineering has subsequently yielded evolved variants (e.g., DddA6, DddA11; high-fidelity HiFi-DdCBEs; alternative homologs such as Ddd_Ss) with enhanced efficiency, expanded sequence compatibility, and reduced off-target activity [14]. TALEDs extend this logic to enable A→G editing in mtDNA by fusing TALE arrays to deoxyadenosine deaminases [17].

Compact zinc-finger–based DdCBEs (ZF-DdCBEs) provide additional, AAV-deliverable architectures for precise organellar editing [13].

Together, these platforms supply TRACER with a modular toolbox for installing defined oncogenic, neutral, or protective mtDNA alleles in cancer models with single-nucleotide resolution [17].

The Nuclear Reporter Construct

The second pillar of TRACER is a synthetic, nuclear-encoded reporter cassette that converts otherwise invisible mitochondrial editing events into a robust, selectable signal. Conceptually, the reporter is designed as a "silent" locus typically a disrupted or transcriptionally blocked fluorescent protein or selection marker that can only be activated when a predefined mtDNA editing–dependent event triggers DNA repair at this locus. In a TRACER-style design, successful mtDNA base editing is coupled to:

- Induction of a site-specific lesion (e.g., a double-strand break or nick) at the reporter locus through an engineered pathway that is activated only in cells harboring the desired mtDNA change.
- Template-directed repair (e.g., HDR or precise end joining) that reconstructs an in-frame fluorescent protein (such as GFP) or resistance gene.

As a result, only cells in which the targeted mtDNA edit has occurred undergo productive repair of the reporter and become fluorescent or selectable, creating a direct genetic link between mitochondrial genotype and nuclear readout. This design conceptually parallels pooled CRISPR reporter systems in the nucleus but is uniquely tailored to mtDNA base editing.

The Workflow: From Edit to Identification

Within the Mitochondrial TRACER framework, the experimental workflow can be understood as a four-step cascade that transforms rare mtDNA editing events into sortable cancer cell populations:

Step 1: Co-delivery of the mtDNA Editor and Reporter

Cancer cells are transfected, transduced, or electroporated with vectors encoding (i) the mitochondrial base editor (e.g., TALE-DdCBE, TALED, ZF-DdCBE) and (ii) the engineered nuclear reporter construct. Contemporary DdCBE and TALED systems have been optimized for robust mitochondrial targeting and expression in mammalian cells and embryos, enabling efficient installation of disease-relevant mtDNA mutations [18, 19].

Step 2: mtDNA Editing and Nuclear Reporter Activation

Following expression, the mtDNA editor localizes to mitochondria and installs the programmed base conversion at the target locus. The presence of this specific mtDNA edit, or its functional consequence, is wired to trigger a defined repair event at the reporter locus (such as a double-strand break processed by HDR using a supplied template). Only cells in which the mtDNA edit has been successfully introduced activate the relevant repair pathway at the reporter site.

Step 3: Reporter Expression (e.g., GFP)

Repair of the reporter cassette reconstitutes a functional coding sequence commonly a fluorescent protein such as GFP or mCherry which is placed under a constitutive or inducible promoter. This converts an otherwise cryptic mitochondrial genotype into a binary optical signal, enabling straightforward quantification and enrichment of edited cells. Analogous base-editing studies already use FACS enrichment of fluorescently labeled editor-expressing cells to enhance detection of mtDNA edits [12, 17].

Step 4: FACS Isolation of mtDNA-defined Populations

Cells are analyzed and sorted by fluorescence-activated cell sorting (FACS). Fluorescent cells represent a highly enriched, and ultimately clonable, population carrying the desired mtDNA mutation, while non-fluorescent cells serve as isogenic mtDNA-wild-type controls. Subsequent deep sequencing confirms mtDNA heteroplasmy levels and off-target profiles [14, 19]. This pipeline yields panels of isogenic cancer cell lines that differ only in a defined mtDNA edit, forming the basis for mechanistic and therapeutic studies.

Advantages Over Previous Methods

Historically, causal interrogation of mtDNA in cancer has relied on transmitochondrial cybrids or chemical perturbations. Cybrid generation fusion of enucleated donor cells bearing a given mitochondrial genome with a recipient nuclear background is technically demanding, low throughput, and often imperfectly isogenic, since nuclear and epigenetic states can drift during reconstitution [10, 20]. Cybrids also provide limited control over precise heteroplasmy levels and rarely allow systematic, single-nucleotide interrogation of patient-specific variants.

Chemical inhibitors of OXPHOS and mitochondrial metabolism, although powerful, act acutely and non-genetically, frequently affecting multiple complexes or pathways and failing to recapitulate the chronic, allele-specific effects of mtDNA mutations found in tumors [10, 21].

In Contrast, a TRACER-Style Platform Built on DdCBEs, TALEDs, and Related Tools Offers Several Decisive Advantages:

- Precision – single-base editing of endogenous mtDNA without double-strand breaks, including installation of clinically observed driver or disease-model mutations [17, 21].
- Isogenicity – generation of matched mtDNA-edited and mtDNA-wild-type cancer lines sharing the same nuclear background, enabling clean attribution of phenotypes to specific mitochondrial variants [14, 21].
- Chronic and allele-specific modeling – stable mtDNA edits support long-term studies of metabolic rewiring, ROS signaling, immune interactions, and therapy responses under conditions that mirror naturally occurring heteroplasmy and clonal selection [14, 21].
- Scalability and screening compatibility – reporter-coupled editing, combined with FACS and deep sequencing, aligns with pooled or arrayed screening strategies to systematically map mtDNA genotype–phenotype landscapes in cancer [22].
- By uniting "state-of-the-art" mtDNA base editors with a synthetic nuclear reporter architecture, Mitochondrial TRACER establishes a technically rigorous, scalable platform that overcomes long-standing methodological barriers and enables causal, high-resolution tracing of mitochondrial genetics in oncogenesis.

Applications in Cancer Research: Illuminating Oncogenic Mechanisms

Establishing Causality of mtDNA Mutations

A long-standing limitation in mitochondrial oncology has been the inability to rigorously test whether recurrent mtDNA mutations are drivers or merely passengers in tumorigenesis.

Mitochondrial TRACER directly addresses this gap by enabling the precise installation of defined mtDNA variants (for example, pathogenic substitutions in MT-ND1 or MT-ND6) into cancer cell lines, followed by reporter-based isolation of isogenic clones harboring the desired edit. These isogenic sub-lines differ exclusively at the engineered mitochondrial DNA locus, thereby establishing a highly controlled experimental framework to delineate the causal consequences of a single mitochondrial perturbation on tumor-cell behavior. Leveraging TRACER-derived models, investigators can systematically interrogate the effects of discrete mtDNA mutations on cellular proliferation, clonogenic capacity, and tumor-initiating potential in vitro, as well as on tumor growth dynamics and histopathological architecture in vivo. Collectively, this platform elevates mtDNA variants beyond associative biomarkers to experimentally substantiated determinants of oncogenic fitness.

Studying Metabolic Plasticity and Intratumoral Heterogeneity

Cancer cells exhibit definite metabolic plasticity, continuously recalibrating the interchange between glycolysis and oxidative phosphorylation (OXPHOS) to sustain spread and viability across fluctuating microenvironmental conditions. Given that many recurrent mtDNA mutations perturb the electron transport chain (ETC) complexes, these alterations are strategically positioned to reshape this metabolic balance. TRACER permits the generation of panels of isogenic sub-clones derived from a common parental tumour lineage, each harbouring discrete and precisely defined mtDNA alterations. Comparative analysis of these genetically matched derivatives allows agents to systematically delineate how specific mitochondrial genotypes influence glycolytic flux, OXPHOS performance, tricarboxylic acid (TCA) cycle activity, and ancillary pathways, including one-carbon metabolism and lipid biosynthesis.

Integration of TRACER with high-throughput metabolomics, stable isotope tracing, and single-cell profiling further simplifies the identification of metabolic dependencies and context-specific exposures associated with distinct mtDNA variants. Collectively, this framework establishes TRACER as a robust platform for interpreting how mitochondrial genetic heterogeneity drives metabolic adaptability and functional diversification among tumour subclonal populations.

Unravelling Machines of Therapy Battle

Resistance to chemotherapy/ radiotherapy/ and targeted therapies is often accompanied by profound mitochondrial modifications, about changes in respiratory chain activity, redox balance, and apoptotic priming. Nevertheless, explaining the precise role of mtDNA mutations in driving these resistance phenotypes has remained technically challenging. TRACER-engineered, mtDNA-defined isogenic cell lines provide a rigorous experimental framework to systematically dissect this relationship. Following the installation and enrichment of selected mtDNA variants, these models can be estimated using standardized dose–response assays and high-content showing platforms that encompass conventional cytotoxic agents, radiation mimetics, targeted inhibitors, and emerging mitochondria-directed therapeutics. Cross-comparative analyses of coordinated isogenic lines allow identification of mtDNA genotypes that intercede cross-resistance or heightened sensitivity, and can be integrated with transcriptomic, proteomic, and functional readouts—including apoptosis induction, reactive oxygen species (ROS) dynamics, and mitochondrial membrane potential—to elucidate mechanistic underpinnings. Together, these approaches not only clarify how mitochondrial genetics kerbs therapeutic response but also uncover genotype-specific drug vulnerabilities with potential clinical relevance.

Probing the Role of mtDNA in Metastasis

Metastatic dissemination is an energetically demanding, multi-step process that requires cancer cells to adapt to fluctuating nutrient and oxygen availability, survive oxidative stress, and rewire signaling pathways. Growing evidence suggests that mtDNA mutations can modulate these capacities, yet direct causal tests have been limited. TRACER enables the introduction of candidate metastasis-associated mtDNA variants into tumor cells, followed by rigorous functional assessment using migration and invasion assays in vitro, as well as experimental metastasis models in vivo (e.g., tail-vein injection, orthotopic implantation, or spontaneous metastasis from primary xenografts). By comparing metastatic seeding efficiency, colonization capacity, and organotropism between isogenic clones differing only in their mtDNA sequence, researchers can identify mitochondrial genotypes that enhance metastatic fitness. Integrating these phenotypic readouts with measurements of bioenergetics, ROS signalling, and epithelial–mesenchymal transition (EMT) programs further clarify how specific mitochondrial alterations enable successful dissemination and outgrowth at distant sites.

Modelling Tumour–Stroma and Microenvironmental Interactions

The influence of mitochondria on cancer progression extends beyond autonomous tumor-cell metabolism to encompass bidirectional communication with the tumor microenvironment (TME), including fibroblasts, immune cells, and endothelial cells. Mitochondrial retrograde signaling—where mitochondrial dysfunction feeds back to the nucleus to remodel transcriptional programs and secretory profiles—is thought to be a key mediator of these interactions. TRACER-sorted cancer cells, bearing defined mtDNA mutations on an isogenic nuclear background, provide an experimentally clean system to interrogate these processes.

Co-culture models with cancer-associated fibroblasts, macrophages, T cells, or organotypic 3D matrices can be used to assess how mtDNA-driven mitochondrial stress reshapes cytokine secretion, extracellular matrix remodeling, immune evasion, and angiogenic signaling.

Additionally, TRACER-engineered cells can be deployed in in vivo models to study how mitochondrial genotypes influence TME composition, immune infiltration, and stromal metabolic coupling (e.g., lactate or metabolite shuttling). By decoupling nuclear from mitochondrial variation, TRACER thus offers a unique window into how mtDNA mutations orchestrate tumor–stroma crosstalk and sculpt the ecological landscape of cancer.

Diagnostic and Therapeutic Potential

Mitochondrial DNA (mtDNA) mutations are now recognized as a major class of cancer driver mutations, with clear implications for diagnostics and therapy design. Functional tools like TRACER can help connect specific mtDNA variants to disease behavior, then feed those insights into liquid biopsy biomarker development and synthetic-lethal drug strategies [7, 10].

TRACER as a Functional Screening Platform

Pooled, barcoded variant libraries—already used for high-throughput functional annotation of nuclear oncogenes such as PIK3CA—can distinguish rare driver vs. passenger mutations in growth and signaling assays [23]. TRACER could analogously pool many mtDNA variants and track their representation over time in competitive tumor models, directly identifying mtDNA alleles that enhance proliferation, metabolic fitness, or immune evasion [7, 10].

Informing Biomarker Discovery (cf-mtDNA)

Cell-free mtDNA in plasma and urine shows distinct fragmentation and mutation profiles with strong diagnostic potential [24, 25]. Functional TRACER data can indicate which mtDNA variants actually alter tumor behavior, helping to focus cf-mtDNA assays on truly actionable mutations rather than benign background variation [10, 26]. Such functionally curated panels could improve the specificity and interpretability of liquid biopsy tests that are already widely explored in clinical trials [27, 28].

Bridging to Therapeutics: Synthetic Lethality and Vulnerabilities

Synthetic lethality—where a mutation creates a non-oncogene dependency that can be drugged—is an established strategy in cancer therapy [29]. TRACER can:

- Introduce defined mtDNA mutations,
- Screen drug libraries or genetic perturbations,
- Map mtDNA–drug synthetic lethal pairs (e.g., dependence on specific TCA cycle or respiratory components, analogous to ARID1A–cuproptosis and IDH–partner interactions). Machine-learning and CRISPR-based SL platforms show how such interaction maps can systematically reveal new vulnerabilities for patient stratification [30].

Current Limitations and Future Perspectives

Technical Hurdles

Current mitochondrial editors (DdCBE, mitoBEs, mtABEs, mitoARCUS, ZF-DdCBEs, mitoTALENs/ZFNs) face several constraints:

Editing Efficiency and Sequence Scope

Early DdCBEs showed modest activity and strict 5'-TC-3′ context requirements, limiting the sites that can be edited [12, 31]. Engineered variants (DddA6, DddA11; mitoBEs; eTd-mtABEs) expand the sequence window and boost C→T and A→G efficiencies up to ~80% in cells, but performance remains highly context-dependent and variable across loci and models [12, 32].

Off-target Editing (mtDNA and nDNA)

Conventional DdCBEs induce hundreds of unintended C→T mutations across the mitochondrial genome; high-fidelity variants (HiFi-DdCBEs) reduce this but do not fully eliminate off-targets [15]. Nuclear mislocalization of base editors can also cause unwanted nuclear edits, prompting incorporation of high-fidelity DddA mutations and nuclear export signals to push activity toward mitochondria [13, 31].

Delivery Size and Complexity

TALE-based editors (DdCBE, mitoBEs) and dimeric nucleases (mitoTALENs, mitoZFNs) are large and often require two AAV vectors, complicating in vivo delivery [13, 33]. Compact systems such as mitoARCUS and ZF-DdCBEs fit within a single AAV, but still demand sophisticated engineering and raise their own specificity concerns [13, 34].

Restricted Base-Change Chemistry

Most mtDNA base editors are limited to transition mutations (C→T/G→A or A→G/T→C), leaving many pathogenic variants and desired models inaccessible [34].

No robust programmable system yet supports mtDNA transversions or small indels.

Biological Complexity: Heteroplasmy and Threshold Effects

Pathogenic mtDNA mutations are typically heteroplasmic: mutant and wild-type genomes co-exist within a cell [35, 36]. Disease emerges only when mutant load surpasses tissue-specific thresholds, and partial shifts in heteroplasmy can yield meaningful phenotypic rescue [33]. However:

- Heteroplasmy levels vary across tissues, cell types, and over time, shaped by bottlenecks, selection, and mitophagy [35, 37].
- Single-cell and multi-omics studies show strong cell-state–specific selection and clonal dynamics, complicating predictions of how a given edit will behave in vivo [37, 38].
- These dynamics mean TRACER-style perturbations must be interpreted against a moving baseline of natural mtDNA evolution.

The Next Generation of TRACERs

Future TRACER iterations can leverage emerging tools:

More Versatile Editors and Broader Sequence Context

Evolved DdCBE variants (DddA6/11), mitoBEs, eTd-mtABEs, and high-fidelity architectures already expand editable motifs and reduce off-targets [15, 39]. TRACER could integrate panels of such editors to cover a larger fraction of cancer-relevant mtDNA sites.

Multiplexed TRACERs

Libraries like MitoKO, which ablate every protein-coding mtDNA gene with a defined base-editor set, demonstrate that systematic, parallel mtDNA perturbation is feasible with high on-target efficiency and controlled off-targeting [40]. Similar library designs could allow TRACER to interrogate multiple mutations or genes per tumor model, including combinatorial or epistatic effects.

In Vivo TRACER Models

AAV-delivered mitoARCUS, ZF-DdCBEs, and mtABEs have already achieved efficient mtDNA editing in mouse tissues and

embryos, creating disease models and shifting heteroplasmy in vivo [34, 38]. Coupling these delivery strategies with longitudinal mtDNA genotyping and single-cell multi-omics [38, 41] would enable real-time tracking of mtDNA evolution, clonal selection, and therapy response within tumors.

Concluding Vision

As editor precision, delivery, and coverage improve, mitochondrial TRACER can transition from a primarily mechanistic research tool to a platform technology for oncology: systematically mapping mtDNA driver mutations, modeling their effects in vivo, and identifying druggable vulnerabilities tied to specific heteroplasmy states. Integrated with single-cell and liquid-biopsy profiling, such TRACER systems could ultimately support precision medicine, where mtDNA genotype and clonal architecture inform patient stratification, biomarker design, and mtDNA-targeted or mtDNA-conditioned therapies.

Conclusion

the Mitochondrial TRACER platform represents a transformative advance in linking mitochondrial genetics to cancer biology. By enabling precise, traceable editing and functional assessment of mtDNA within an isogenic nuclear background, TRACER overcomes long-standing barriers that have limited our ability to distinguish causality from correlation in mtDNA–cancer associations. This technology allows systematic dissection of how specific mitochondrial variants shape metabolic rewiring, therapy resistance, metastatic potential, and tumor–microenvironment crosstalk, thereby illuminating mitochondrial contributions to cancer progression with unprecedented resolution. At the same time, the platform creates a powerful bridge from basic mechanistic insight to translational applications, including functional validation of mtDNA biomarkers, discovery of mitochondria-dependent vulnerabilities, and rational design of combination therapies that exploit mtDNA-encoded liabilities. Although technical challenges remain—such as editing efficiency, off-target risks, and the complexity of heteroplasmy—the continuing refinement of mitochondrial editors and reporter systems is likely to expand TRACER's scope and robustness. Ultimately, Mitochondrial TRACER is poised to evolve from an experimental research tool into an integral component of precision oncology, guiding both target validation and the development of mitochondria-informed diagnostic and therapeutic strategies.

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Competing interests

The authors declare no competing interests

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