Understanding the impact of mitochondrial DNA mutations on aging and carcinogenesis (Review)

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Abstract. Mitochondria and mitochondrial DNA (mtDNA) are crucial for cellular energy metabolism and the adaptive response to environmental changes. mtDNA collaborates with the nuclear genome to regulate mitochondrial function. Dysfunctional mitochondria and mutations in mtDNA are implicated in a wide range of diseases, including mitochondrial disorders, neurodegenerative conditions, age-associated pathologies and cancer. While the nuclear genome has been extensively studied for its role in driving the clonal expansion of oncogenes and other aging-related genetic alterations, knowledge regarding mtDNA remains comparatively limited. However, advances in quantitative analysis have provided information regarding the complex patterns of mtDNA mutations. The present review offers a detailed examination of mtDNA mutations and their classifications in the contexts of aging and cancer, and elucidates the role of mtDNA mutations in these processes. Mutations in mtDNA can be detected as early as the neonatal stage, yet most transition mutations retain a normal cellular phenotype. In contrast to mutations in oncogenes and tumor suppressor genes within the nuclear genome, mtDNA exhibits conserved mutational signatures, irrespective of cancer tissue origin. To adapt to the aging process, mitochondria undergo clonal expansion of advantageous mtDNA mutations, maintaining a dynamic equilibrium among various mitochondrial clones. Over time, however, the loss of strand bias can disrupt this equilibrium, diminishing the pool of adaptive clones. This breakdown in mitochondrial homeostasis may contribute to tumorigenesis. In conclusion, the heterogeneity of mtDNA mutations and the collapse of its homeostasis are pivotal in the progression of age-related diseases, including cancer, underscoring the importance of mtDNA mutations in health and disease.

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1. Introduction

Enhancing mitochondrial function has been shown to positively influence cellular and organismal longevity (1). However, the progressive dysregulation of mitochondrial function over time constitutes a hallmark of aging and contributes to the onset of age-related diseases. Consequently, the increased risk of cancer development with advancing age is closely associated with mitochondrial dysfunction, which is driven by alterations in both mitochondrial DNA (mtDNA) and nuclear-encoded mitochondrial genes (2). Aging and cancer share overlapping mitochondrial pathways, including reactive oxygen species (ROS) production, changes in mitochondrial biogenesis and mitophagy, and the accumulation of mtDNA mutations (2). These alterations create a biological environment conducive to both aging and oncogenesis. While aging is typified by a gradual decline in mitochondrial function, cancer cells frequently reprogram mitochondrial activity to optimize survival and proliferative potential, highlighting the paradoxical relationship between these two processes, which remains a complex and unresolved scientific question.

Although mitochondrial dysfunction is intuitively linked to the accumulation of mtDNA mutations, advances in quantitative mtDNA mutation analysis have revealed that such mutations are detectable across all age groups in healthy tissues (3). This raises critical questions, such as how young

individuals maintain normal phenotypes despite the presence of mtDNA mutations, and which mechanisms drive the phenotypic shifts associated with aging and cancer. Moreover, mitochondria engage in intricate interactions with nuclear genes to regulate functionality. While the nuclear genome exerts regulatory control over mitochondria, retrograde signaling from mitochondria to the nucleus can either promote cellular adaptation to environmental changes or, conversely, contribute to aging and cancer progression by influencing nuclear gene expression (4). The mitochondrial genome accumulates specific mutation patterns in various tissues during aging and cancer (5). Ongoing research aims to determine the molecular mechanisms underlying the dynamic changes in mtDNA mutation patterns during aging and oncogenesis.

The present review offers a detailed examination of mtDNA mutations and their classifications in the contexts of aging and cancer, and elucidates the role of mtDNA mutations in these processes.

2. Mitochondrial function and structure

Cells house 100-20,000 mitochondria depending on energy needs, each typically containing 2-10 circular mtDNA molecules. Mitochondrial quantity and mtDNA content vary according to cell type, energy demand and physiological condition (6). Essential for cellular metabolism and homeostasis, mitochondria support ATP production, calcium regulation, iron-sulfur cluster biosynthesis, apoptosis, metabolic precursor synthesis and ROS generation (7). They proliferate and replicate independently of the cell cycle to enable growth, division and organelle repair (6,8,9). Compared with nuclear DNA, mtDNA replicates faster, lacks histones, has limited repair mechanisms and is 10-1,000-fold more prone to mutations (10,11). Its coding density (~93%) far exceeds that of nuclear DNA (1-2%), reflecting a streamlined structure for energy production (12). This density heightens the impact of mutations, leading to mitochondrial dysfunction, including membrane potential loss (8,13). To preserve mitochondrial integrity, cells use quality control mechanisms, such as mitophagy and fusion/fission pathways (14), preventing mtDNA mutations from exceeding thresholds that disrupt homeostasis (15). Unlike nuclear DNA, mtDNA lacks robust repair systems.

Mitochondria contain double-stranded circular DNA of bacterial origin, consisting of heavy and light chains (6,12). mtDNA spans 16.569 kb and encodes 37 genes, including 13 electron transport chain (ETC) polypeptides, 2 ribosomal RNA (rRNA) genes and 22 transfer RNAs (tRNAs) (12) (Fig. 1). Complex I of the ETC is composed of 45 subunits in humans, including mtDNA-encoded [mitochondrial-nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit (ND)1, ND2, ND3, ND4, ND4L, ND5 and ND6] and nuclear DNA-encoded genes. Complex V is composed of two main parts: F1 (catalytic) and FO (membrane-bound); it has 16 subunits in humans, encoded by mtDNA (mitochondrial-ATP6 and ATP8) and nuclear DNA. Unlike nuclear DNA, mtDNA is maternally inherited. It encodes oxidative phosphorylation (OXPHOS)-related protein subunits (coding regions) and respiratory control machinery (non-coding D-loops) (6,16,17). The D-loop contains replication origins and transcription promoters but does not code for proteins or functional RNA. Heavy chains, which are rich in guanine, house most protein-coding genes, whereas cytosine-rich light chains, encode fewer genes. mtDNA, organized into nucleoid structures, is anchored to the inner mitochondrial membrane (IMM) via proteins such as mitochondrial transcription factor A (TFAM) and ATPase family AAA domain-containing 3A (12). This tethering aids mtDNA transcription and replication. Proximity to the IMM ensures coordination of mtDNA-encoded protein integration and mitochondrial function. Key replication proteins, such as polymerase γ (POLG) and Twinkle helicase (TWINKLE), are IMM-anchored, enabling efficient mtDNA replication, transcription and inheritance during mitochondrial division (18,19).

3. mtDNA variations

mtDNA variations are classified into haplotypes (ancient lineages and adaptive polymorphisms), germline mutations (heritable alterations within families) and somatic mutations (arising in individual cells), each with unique traits and implications (20-22). Haplotypes, maternally inherited combinations of single nucleotide polymorphisms (SNPs) and genetic markers, provide insights into ancestry, population genetics and evolution. They represent normal genetic diversity and may influence disease susceptibility without being inherently pathological. Germline mutations occur in reproductive cells, primarily oocytes, and are transmitted across generations (20-22). Even monozygotic twins may differ in mtDNA variant proportions due to mutations during early embryonic development, resulting in heteroplasmy. Heteroplasmy in the context of mtDNA mutations describes the coexistence of multiple mtDNA sequence variants; specifically, the presence of both mutant and wild-type mtDNA molecules, within a single cell, tissue or organism (10). Somatic mutations arise from environmental factors, cellular processes or mtDNA replication errors, accumulating with age and contributing to disease. High mtDNA replication rates in energy-demanding cells increase error risk. In addition, ROS generated during OXPHOS damage mtDNA, causing mutations that coexist with normal mtDNA, often as low-level heteroplasmy in healthy individuals (21). Heteroplasmy levels fluctuate due to genetic drift and selective replication, which shape mtDNA mutation patterns over time (23,24).

4. Crosstalk between nuclear and mitochondrial genomes

Impact of nuclear DNA on mitochondrial function. Nuclear genes regulate mtDNA because most mitochondrial functions depend on nuclear-encoded proteins. Key roles of nuclear genes include replication and repair, copy number regulation, biogenesis, fusion/fission and antioxidant defense (25). Firstly, nuclear-encoded enzymes (such as POLG, TWINKLE and mitochondrial single stranded DNA-binding protein) are essential for mtDNA replication (26) (Fig. 2). Repair enzymes address damage caused by ROS and replication errors. Secondly, nuclear proteins regulate mtDNA copy number through transcriptional and epigenetic mechanisms, POLG, TFAM and TWINKLE genes, and pathways such as peroxisome proliferator-activated receptor γ, coactivator 1α

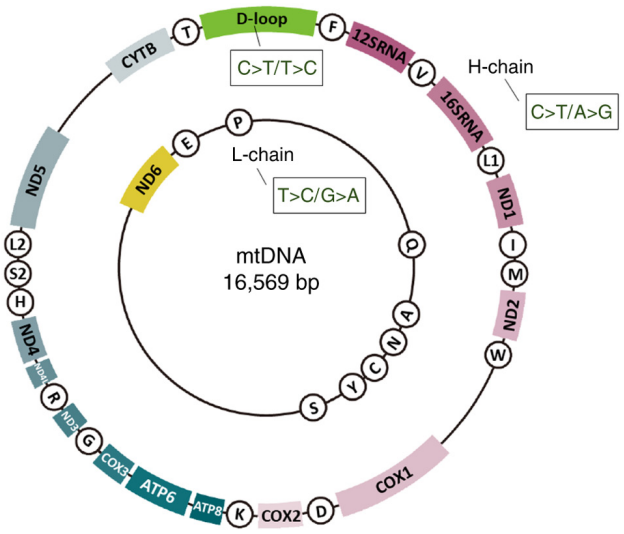

Figure 1. mtDNA structure and strand bias. This figure is taken from Fig. 2 of reference (19), with minor modifications limited to the placement of the 'H-chain' label and the depiction of strand bias; reprinted under license (https://creativecommons.org/licenses/by/4.0/). In this version, only the strand-specific bias has been incorporated. This dual-ring diagram depicts the organization of the mitochondrial genome, with the outer ring representing the heavy strand and the inner ring denoting the light strand. The 22 circular elements correspond to tRNA genes. The two labeled boxes, 12SRNA (small subunit) and 16SRNA (large subunit), represent rRNA genes. The remaining boxes illustrate the 13 protein-coding genes involved in the electron transport chain and the one D-loop region. The 'C>T' notation within the boxes indicates a strand-specific mutational bias. COX2, cytochrome *c* oxidase 2; mtDNA, mitochondrial DNA; ND, nicotinamide adenine dinucleotide dehydrogenase subunit; rRNA, ribosomal RNA; tRNA, transfer RNA.

(PGC-1α; a master regulator of mitochondrial biogenesis) and AMP-activated protein kinase (AMPK) (27). PGC-1α activates TFAM via nuclear respiratory factors 1 and 2 (28-32); this process supports rapid energy generation, for example in cancer cells, which reprograms metabolism from OXPHOS to glycolysis (Warburg effect) (33,34). Thirdly, oncogenes (MYC, RAS) and loss of tumor suppressor genes [TP53, phosphatase and tensin homolog (PTEN)] enhance glycolysis (34,35) and glutaminolysis (36). Hypoxia-inducible factor 1α (HIF-1α) promotes glycolysis and inhibits mitochondrial activity in hypoxic tumors (34). Mutations in nuclear genes encoding components of the ETC, such as succinate dehydrogenase (SDH) and fumarate hydratase (FH), can arise through mutations in oncogenes or tumor suppressor genes (37). Loss-of-function mutations in SDH or FH lead to the accumulation of succinate and fumarate, respectively, which can stabilize HIF-1α and promote the Warburg effect, forcing reliance on hexokinase 2-mediated glycolysis (35,37-39). Fourthly, nuclear genes control mitochondrial fusion/fission through mitofusin (MFN)1, MFN2 and dynamin-related protein 1, ensuring mtDNA distribution and removing defective mtDNA (40). Mitophagy, mediated by PTEN-induced kinase 1 and Parkin, maintains mitochondrial health (41). Finally, nuclear genes encode antioxidants such as superoxide dismutase 2 (SOD2) and glutathione peroxidase to minimize oxidative mtDNA damage (42). This nuclear-mitochondrial coordination supports cellular energy needs and prevents mitochondrial dysfunction.

Retrograde signaling from mitochondria to the nucleus. Retrograde signaling enables mitochondria to influence nuclear gene expression in response to mitochondrial function or stress (38,43). This pathway maintains cellular homeostasis by aligning nuclear transcription with mitochondrial states. Triggers include mitochondrial dysfunction, ROS, calcium dysregulation, metabolic shifts and mtDNA mutations (38,43). Impaired OXPHOS and ATP production activate AMPK, which restores energy balance by promoting catabolic pathways and influencing nuclear transcription factors such as PGC-1α to enhance mitochondrial biogenesis (44). Dysfunction mimics hypoxia, stabilizing HIF-1α, which activates glycolysis and survival genes (39). Mitochondrial stress, particularly the increase in ROS and mutations in mtDNA, leads to the activation of NF-κB as part of the cellular adaptive response (45). ROS can activate the NF-κB signaling pathway through the phosphorylation and subsequent degradation of IκB, leading to the nuclear translocation of NF-κB. The regulation of nuclear responses by NF-κB includes the upregulation of antioxidant genes (such as SOD2 and catalase), the induction of pro-inflammatory cytokines (including TNF-α and IL-6), and the modulation of apoptosis and cell survival pathways (43). Moderate concentrations of ROS serve as pivotal signaling entities in a range of physiological processes, encompassing cellular survival, inflammatory regulation and immune system modulation (46). ROS also contribute to genomic instability via inflammation, oxidative stress and DNA repair suppression (43). Excess ROS cause oxidative damage to nuclear DNA, impair repair mechanisms due to ATP reduction, and disrupt deoxynucleotide triphosphate pools, causing replication stress (38,47). Mitochondrial dysfunction alters NAD^+/NADH ratios and tricarboxylic acid cycle intermediates, such as fumarate and succinate, which affect DNA and histone methylation (48). These metabolites inhibit α-ketoglutarate-dependent enzymes, causing hypermethylation and stabilizing HIF-1α, thus promoting tumorigenesis (34,38). Altered mitochondrial calcium buffering affects nuclear calcium pathways, influencing factors such as nuclear factor of activated T cells and cyclic adenosine monophosphate response element binding protein, which are critical for chromatin remodeling, DNA repair and cell survival (49). Taken together, retrograde signaling enables mitochondria to adapt nuclear responses to stress, metabolic shifts and damage, preserving cellular function.

5. mtDNA replication and repair errors

Advances in the quantitative analysis of mtDNA mutations. The frequency of mtDNA mutations varies widely across individuals and studies due to differences in mutations analyzed, detection methods and populations (50). Advances in DNA mutation detection now provide notable sensitivity and accuracy (6,50-52). The field has transitioned from low-throughput methods, such as Sanger sequencing, to high-throughput, ultra-sensitive technologies capable of identifying rare mutations (6,8,53-55). Techniques such as duplex sequencing, digital PCR and single-molecule sequencing detect mutations with unprecedented precision. Duplex sequencing reads both DNA strands independently, confirming mutations only when complementary changes occur in both strands, reducing the

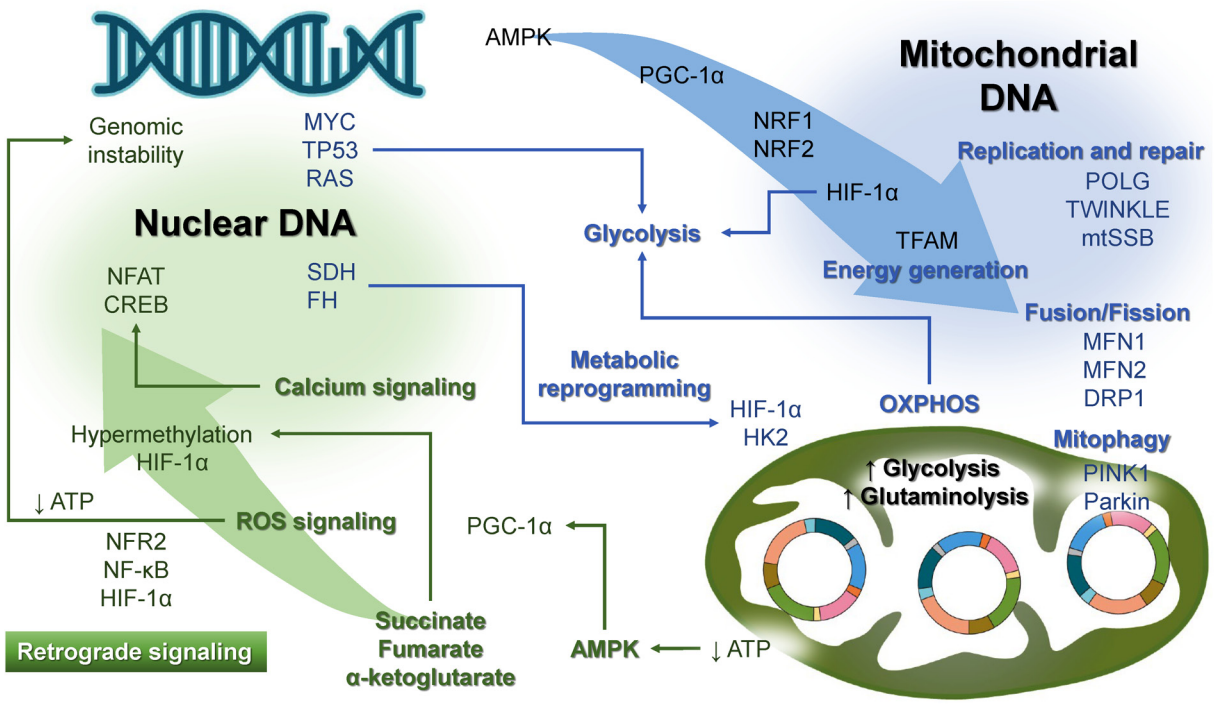

Figure 2. Crosstalk between nuclear and mitochondrial genomes. The left portion of the figure represents nuclear genes, whereas the right portion depicts mtDNA. Blue arrows denote anterograde signaling from nuclear genes to mitochondria, whereas green arrows illustrate retrograde signaling from mitochondria to the nucleus. AMPK, AMP-activated protein kinase; CREB, cyclic adenosine monophosphate response element binding protein; DRP1, dynamin-related protein 1; FH, fumarate hydratase; HIF-1α, hypoxia-inducible factor 1α; HK2, hexokinase 2; mtDNA, mitochondrial DNA; MFN, mitofusin; mtSSB, mitochondrial single stranded DNA-binding protein; NFAT, nuclear factor of activated T cells; NRF, nuclear respiratory factor; OXPHOS, oxidative phosphorylation; PGC-1α, peroxisome proliferator-activated receptor γ, coactivator 1α; PINK1, PTEN-induced kinase 1; POLG, polymerase γ; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TFAM, mitochondrial transcription factor A; TWINKLE, Twinkle helicase.

rate of false positives (53). This type of sequencing detects mutations with variant allele frequencies (VAFs) as low as 1 in 10 million bases, aiding research on rare mutations in cancer, aging and genetic disorders. Advances have shed light on mtDNA mutation mechanisms in normal tissues (6,8,51,56). In 2023, double-stranded sequencing revealed >89,000 somatic mtDNA mutations across eight aged mouse tissues, uncovering tissue-specific mutational patterns linked to aging (8). In humans, similar mutational patterns accumulate over time, including in cancer-affected tissues (5).

Characteristics of mtDNA mutation patterns. Replication of mtDNA is initiated at the origin of the heavy strand and proceeds through a strand-displacement mechanism, wherein the parental heavy strand remains in a single-stranded state until replication of the light strand is subsequently initiated. The prolonged exposure of the heavy strand in its single-stranded form renders it particularly susceptible to spontaneous deamination events, notably the conversion of cytosine to uracil, resulting in C-to-T transition mutations (57). This reflects the inherently asymmetric nature of mtDNA replication, wherein the temporal delay between heavy strand and light strand synthesis predisposes the heavy strand to increased accumulation of base damage and transition-type mutations. Strand bias in mtDNA mutations refers to the asymmetric distribution of mutation frequency and types between the heavy and light strands. The non-random distribution of mutations observed during mtDNA replication, termed 'replication bias', contributes to the emergence of distinct mutational accumulation patterns across the mitochondrial genome. mtDNA replication errors occur when polymerases incorporate incorrect nucleotides or during slippage, causing small insertions or deletions, especially in repetitive sequences (12,58). Repair errors happen when the repair machinery incorrectly restores DNA damaged by internal factors (including ROS) or external factors (such as ultraviolet light). If uncorrected, replication errors lead to mutations in daughter strands, causing genetic changes in the cell lineage.

Transition mutations (the replacement of one purine with another purine, or one pyrimidine with another pyrimidine) are the most common in mtDNA and result from replication errors or spontaneous deamination, such as cytosine to uracil. Heavy chains are linked to C>T and A>G transitions, whereas light chains are associated with T>C and G>A transitions (54,59). These patterns are observed in human tissues, tumors and model organisms such as *Drosophila* (54,60,61). Transversions (the replacement of purines with pyrimidines) are less common, and are often caused by ROS or environmental mutagens (54,55,62). Transitions more frequently result in synonymous changes, whereas transversions are more likely to cause nonsynonymous mutations that impair protein function. The majority of mtDNA mutations that accumulate during aging and tumorigenesis are transition mutations, whereas transversion mutations are relatively infrequent. This skewed mutation spectrum can be attributed not only to the molecular mechanisms underlying mutagenesis, but also to selective pressures at both the mitochondrial quality control level and the cellular level, which preferentially eliminate functionally

deleterious mutations (51,56,63). Notably, the subunits of the OXPHOS system encoded by mtDNA are subject to stringent structural and functional constraints, and transversion mutations are more likely to induce non-conservative amino acid substitutions, severely compromising protein conformation and enzymatic activity. Consequently, such mutations can impair mitochondrial function by reducing ATP production and increasing ROS generation, thereby promoting the selective elimination of dysfunctional mitochondria via quality control mechanisms such as mitophagy (51,56,63). Therefore, highly pathogenic mutations, including transversions, are less likely to undergo clonal expansion due to purifying selection, and their prevalence remains low even in the context of aging and cancer progression. Thus, mutation fate depends on genetic drift and natural selection, influencing whether mutations are fixed, remain heteroplasmic or are eliminated (56,64). Strand bias in mtDNA mutations reflects asymmetric replication dynamics, oxidative stress exposure, limited repair efficiency and selective pressures acting on mutational events. Studying transition and transversion mutations offers insights into mitochondrial dysfunction, mutagenesis factors, and their potential as diagnostic or therapeutic tools. Furthermore, the non-D-loop region exhibits high mutation rates, primarily C>T and T>C transitions (65). Mutations in coding regions, such as ND or cytochrome *c* oxidase genes, may be synonymous or pathogenic, whereas mutations in tRNA genes can disrupt protein synthesis (66). Common mtDNA mutations in healthy individuals, such as C>T, T>C and A>G, depend on factors including heteroplasmy and affected genes. Additional influences on mtDNA mutations include bottleneck effects, genetic drift, selection, mitophagy and mitochondrial dynamics. The bottleneck theory explains rapid shifts in mtDNA mutation prevalence during development due to reduced mtDNA copy numbers (67). Genetic drift in small mitochondrial populations can fix or eliminate mutations regardless of selection. Selection acts on mutations based on cellular fitness (68), with mitophagy and mitochondrial dynamics further shaping mtDNA integrity in health, aging and disease.

6. Characteristics of mtDNA mutations in aging and cancer

Effect of aging on mtDNA mutations. mtDNA mutations are present in all human body fluids and tissues, with frequencies influenced by age, environmental exposure, oxidative stress, genetic factors and health status (3,69). A 2008 study of umbilical cord blood revealed that 0.5% of healthy newborns had pathogenic mtDNA mutations, with the A3243G mutation in the $tRNA^{Leu(UUR)}$ gene being common. This mutation is associated with several mitochondrial diseases, including mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome; maternally inherited diabetes and deafness; and chronic progressive external ophthalmoplegia (3). However, the frequency of these mutations does not exceed the disease threshold, likely due to their lack of health impact. Recently, Hong *et al* (70) analyzed mtDNA mutations from the UK Biobank, involving 500,000 participants aged 40-69 years. The study revealed that 30.5% of 194,871 participants had heteroplasmic single nucleotide variants with a VAF of 5%. Nonsynonymous mutations in complexes I and V of the ETC occurred at frequencies of 46.5 and 65.8%, respectively. Despite the high mutation rate in complex V, the low VAF suggested no notable adverse effects on the ETC. Additionally, gene mutations have been validated with single-cell sequencing by targeted amplification of multiplex probes, revealing an average of 0.7 mutations per cell in blood cells obtained from a 76-year-old woman (23). The same study found that >60% of these mutations were in protein-coding genes, with >70% classified as nonsynonymous, and over half of these predicted to be highly pathogenic (23). In addition, despite being derived from clinically healthy elderly individuals, 20% of detected mutations exhibited a VAF exceeding 90% (23), suggesting that high-VAF clones may proliferate due to less efficient mtDNA quality control with age (71,72). Notably, these pathogenic mtDNAs may be particularly well-adapted to the aging cellular environment (73).

Clonal expansion of mtDNA mutations denotes the process by which a particular mtDNA mutation, initially confined to a limited number of mitochondrial genomes, progressively becomes predominant within a cell or tissue over time. This clonal expansion could increase genetic diversity under selective pressure. Cote-L'Heureux *et al* (6) studied mtDNA mutations in tissues from young (4.5 months) and old (26 months) mice, with human equivalents being ~10 and 65 years old, respectively. Different tissues showed distinct mtDNA mutation profiles, with the kidney accumulating the most mutations. This study confirmed that mutated mtDNA molecules may propagate through stochastic processes, indicating clonal proliferation with age. Finally, a report investigated mtDNA mutations in germ cells; in mice, mtDNA mutations in oocytes increased with age (74). However, in macaques, mutations in the liver and muscle have been reported to increase with age, whereas oocyte mutations may plateau after 9 years of age (27 years in humans), suggesting protection against the accumulation of age-related mutations in oocytes (53).

mtDNA mutations in cancer. Studies on mtDNA mutations in cancer cells have been ongoing since 1985, with notable discoveries made by the late 2000s (38,75). Mutations at nucleotides 10398 and 16189 have been linked to breast and endometrial cancer (35). In early-stage breast cancer, two tumor-specific heteroplasmic transitions (T2275C and A8601G) have been identified (76). Earlier studies identified less frequent detections, possibly due to the use of less sensitive detection methods (35,76) or the relatively higher prevalence of neutral SNPs (77,78). Systematic investigations of mtDNA in various types of cancer have become increasingly prevalent, with Lu *et al* reviewing 101 studies published between 1998 and 2008 (38). mtDNA mutations, including point mutations, deletions and copy number changes, are common in various types of cancer, such as gastric cancer, where 65% of patients have been reported to have at least one mutation (30,79). Most gastric mutations occur in the D-loop region, although complex I genes also exhibit mutations (38). In endometrial and pancreatic cancer, mutations have also been found in complex I and other regions (22,80). Mutations in complex I can impair the ETC and increase ROS production, leading to a higher risk of B-cell lymphoma (81). Mutations in complex V, especially ATP8, may hinder cancer cell proliferation (82). Additionally, tRNA and rRNA mutations have been observed in several types of cancer (35,38,66,76,83), and the 4,977-bp

deletion mutation in mtDNA is common in gastric (84), lung (85) and liver cancer (29,38). Overall, the occurrence of mtDNA mutations is not the primary cause of cancer development, but similar mutations are commonly observed across different cancer types (30,86).

Notably, advances in mtDNA mutation detection have allowed for detailed analysis of mutation types, VAFs and heteroplasmic variants. In 2021, whole-exome sequencing revealed that pathogenic mtDNA mutations occur at frequencies similar to cancer driver mutations in nuclear genes (17). Nonsynonymous mutations have been shown to be common in complex I, whereas synonymous mutations are frequent in complex V (17,22). A 2023 study using droplet digital PCR showed that tissue-derived mtDNA has more heteroplasmic mutations than whole blood mtDNA, suggesting that heteroplasmic mutations contribute to carcinogenesis (87). Also in 2023, research on mtDNA in extracellular vesicles in patients with colorectal cancer showed higher mutation rates, and more missense and nonsense mutations compared with whole blood mtDNA, highlighting the inadequacy of whole blood mtDNA for cancer detection (88). Overall, unlike nuclear DNA mutations that exhibit cancer type-specific signatures, mtDNA mutations are largely consistent across various tumor types (22,86), with no distinct mtDNA corresponding to oncogenes or tumor suppressor genes. Carcinogenesis likely involves the transition from non-clonal mutations to clonal mutations with high VAFs.

7. Changes in mtDNA mutation patterns and dynamics of clonal expansion

Age-related changes. This subsection explores how mtDNA mutation types and clonal expansion dynamics evolve with age. Recent findings have highlighted that aging impacts mtDNA mutations, with data from the UK Biobank (2023) showing a transition-to-transversion mutation ratio of 28.7 among individuals aged 40-69 years, suggesting most mtDNA mutations result from DNA POLG errors rather than oxidative stress (70,89). Oxidative stress-induced 8-hydroxydeoxyguanosine causes G>T/C>A translocations; however, studies in humans (89-91), mice (51,92) and *Drosophila* (93,94) have found limited evidence of such mutations, indicating oxidative damage is not the primary cause of age-related mtDNA mutations (8). Even in patients with polycystic ovary syndrome, a condition linked to oxidative stress, transition mutations dominate over translocations (82.35 vs. 17.64%) (95). Additionally, a 2023 study in a 76-year-old woman revealed >95% of mtDNA mutations were heteroplasmic transitions, predominantly G>A and T>C (24). While these increase with age, G>T and C>A translocations do not accumulate (56,57,65,90,96), suggesting they are either eliminated or fail to persist (6,8,54). Although translocations are rare in *Drosophila* (90), high-resolution analyses in *Caenorhabditis elegans* (97) have linked them to oxidative damage, and higher translocation frequencies in metabolically active tissues indicate organ-specific mutation patterns (8). Furthermore, clonal expansion mutations in mitotic tissues also increase with age (9). In healthy elderly individuals, clonal mtDNA mutations are present in colonic epithelial crypts (98), with expanding OXPHOS-deficient mtDNA-mutant cells found in prostate epithelial stem cells (99). In mice, clonal expansion mutations are predominantly transitions, whereas translocations fail to form clones (8). These findings suggest that transition mutations proliferate with age, whereas oxidative damage-related translocations, often nonsynonymous, are eliminated. Transition mutations are thus more likely to undergo clonal expansion as aging progresses.

Cancer-related changes. This subsection examines mtDNA mutation signatures and clonal proliferation profiles in carcinogenesis. Since 2005, progress has elucidated how mtDNA mutations influence tumor initiation and progression (100). mtDNA mutation types in cancer cells may vary by histological subtype. While translocation mutations are present in some cancers, transition mutations predominate (22,63). For example, 80% of medullary thyroid carcinoma samples have been reported to display nonsynonymous mutations, including both transition and translocation mutations (101). Rectal cancer shows relatively high translocation mutation prevalence (102), whereas gastric cancer exhibits T>C or G>A transitions and indels associated with nucleotide repeat instability (30,79). Diffuse large B-cell lymphoma displays random strand bias with increased C>T and A>G transitions on the heavy strand (103). In endometrial cancer, most G>A and T>C transition mutations occurred on the mtDNA light strand, whereas C>T and A>G transitions have been predominantly observed on the heavy strand, with transition mutations occurring 24.4 times more frequently than translocation mutations (22). A large-scale analysis conducted by the International Cancer Genome Consortium revealed that mtDNA mutation signatures are largely consistent across tumor types, with transition mutations dominating (86). Further analysis of 1,675 human cancers has demonstrated strand bias favoring the heavy chain, primarily C>T and A>G transitions (63). Among 625 cancers, transition mutations have been shown to be significantly more frequent than translocation mutations, with most exhibiting homoplasmic characteristics (66). Furthermore, studies have linked mtDNA mutations to mitochondrial dysfunction in cancer (65,103). Mutations in genes encoding complex I components impair mitochondrial function, as seen in thyroid tumors (100) and triple-negative breast cancer (104). Approximately 12% of cancers harbor truncating mtDNA mutations, primarily in ETC genes, reducing OXPHOS (17). Conversely, large mtDNA deletions, common in aging tissues (4), are less frequent in gastric cancer, suggesting selective elimination during tumorigenesis (105). Heteroplasmic mtDNA mutations, combining normal and mutated mtDNA, may mitigate deleterious effects, preserving ATP synthesis and mitochondrial integrity (22,106). This resilience likely enables cancer cells to maintain ATP synthesis capacity and mitochondrial integrity despite impairments in specific complexes (17,107). Colorectal cancer tissues show fewer random mtDNA mutations than non-cancerous tissues, reflecting a metabolic shift from OXPHOS to glycolysis (108). Similar shifts in mtDNA mutational landscapes occur in head and neck squamous cell carcinoma during cancer progression (109).

Profiling mtDNA mutations reveals distinct patterns in cancer compared with adjacent tissues. Colon cancer exhibits fewer non-clonal single base substitutions compared with adjacent tissues, indicating reduced non-clonal mutations

during cancer progression (82,108). In liver cancer with hepatitis B, mtDNA mutations in the D-loop region are reduced in tumor tissues (82). Studies on breast cancer progression have shown that specific transition mutations are prevalent in normal cells but absent in transformed cells (54,83,110). The predominant rare mutation types identified in normal stem cells are C>T/G>A and T>C/A>G transitions, whereas T>C/A>G transitions are notably absent during transformation of human breast stem cells into tumorigenic cells (54,110). Baker *et al* (65) provided a comprehensive analysis of mtDNA mutations during the progression from normal colonic epithelium to ulcerative colitis and colorectal cancer. This previous study reported that clonal and subclonal mutations account for 3.7% of all mutations but increase in frequency and pathogenicity during dysplasia, subsequently decreasing in cancer (65). These findings underscore the role of clonal expansion and the loss of strand bias in carcinogenesis. The loss of strand bias suggests a decline in the fidelity of mtDNA replication and repair, or a breakdown in regulatory mechanisms, potentially leading to genomic instability and mitochondrial dysfunction (8,60). Consequently, cellular energy metabolism and homeostasis may be compromised, thereby increasing the risk of age-related diseases and tumorigenesis (8). Unlike nuclear gene-driven carcinogenesis, mtDNA-driven processes emphasize loss of strand bias and clonal changes over specific mutations (110). Non-clonal mtDNA mutations decrease during carcinogenesis, whereas adaptive clonal mutations are selectively retained (108,111). Aging, immortalization and stem cell transformation favor the proliferation of environmentally adapted clones, reducing genetic diversity and driving carcinogenesis.

In summary, the mutational landscape of mtDNA within cells undergoes dynamic changes with aging. Initially, a diverse array of non-clonal mutations persists due to various factors, including environmental exposure and replication errors. However, as specific transition mutations confer a selective growth advantage, cells harboring these advantageous (adaptive) mutations undergo subclonal expansion, referred to as 'adapter clones', ultimately stabilizing the mutational balance. Conversely, during carcinogenesis, the prevalence of non-clonal mutations declines as clones carrying driver mutations proliferate. This process fosters tumor heterogeneity, characterized by dominant clones, termed 'inducer clones', with driver mutations against a backdrop of subclonal variations (adapter clones). A comprehensive understanding of these dynamics is crucial for elucidating the role of mtDNA mutations in aging and carcinogenesis.

8. Alterations in mtDNA copy number

Beyond mutations and deletions, variations in mtDNA copy number have been extensively studied across tumor types, and the regulation of mtDNA copy number is closely tied to nuclear gene activity (30). Nuclear-encoded genes, such as TFAM, which are crucial for mitochondrial biogenesis, maintain mtDNA replication and stability (29,30). Dysregulation or mutations in oncogenes (such as MYC) (112) and tumor suppressor genes (including TP53) (113) disrupt mitochondrial biogenesis and replication, altering mtDNA copy numbers. Hypoxia, nutrient deprivation and aberrant tumor microenvironment signaling also influence mitochondrial dynamics and mtDNA copy number (112,113).

The relationship between aging and mtDNA copy number is complex and varies across different tissues and individuals. In normal tissues, mtDNA copy number variations reflect energy needs, aging and stress, often serving adaptive functions. High-metabolic-demand tissues, such as the myocardium, have elevated mtDNA copy numbers, whereas low-demand tissues, including blood and skin cells, exhibit fewer mitochondria and lower copy numbers (114). Research has indicated that mtDNA copy number tends to decrease with age in certain tissues, such as blood and lymphocytes. For example, a study conducted on a subset of the Italian population demonstrated a modest but statistically significant age-associated decline in mtDNA content within lymphocytes (114). The observed decrease in mtDNA copy number in certain tissues has been associated with adverse health outcomes. In older populations, lower mtDNA levels in peripheral blood have been linked to higher mortality rates and diminished health, including declines in cognitive and physical performance (114). Conversely, other tissues do not exhibit a clear age-related decline in mtDNA copy number (114). Investigations into skeletal muscle and heart tissues have reported stable mtDNA levels across different age groups. While aging is often accompanied by a reduction in mtDNA copy number in specific tissues, this pattern is not universal. The decline in mtDNA levels in certain tissues may contribute to age-related health issues. In cancer, mtDNA copy number alterations show tissue-specific patterns (30,77). Reviews by Chatterjee *et al* (77) and Lee *et al* (30) have revealed elevated mtDNA copy numbers in head and neck squamous cell carcinoma, papillary thyroid carcinoma and lung cancer, but reduced levels (mtDNA depletion) in breast, kidney, liver, ovarian and gastric cancer (30,77), possibly due to D-loop region mutations (77). Reduced mtDNA copy numbers may limit OXPHOS, shifting metabolism to glycolysis and promoting tumor growth under hypoxia. Conversely, increased mtDNA copy numbers enhance mitochondrial function, ROS production, and signaling pathways supporting proliferation, survival and metastasis. mtDNA is highly vulnerable to damage, potentially triggering compensatory copy number changes (77). mtDNA copy number variations also affect cancer prognosis in a cancer type-specific manner. Elevated mtDNA copy numbers have been shown to be associated with lower tumor grades and better outcomes in patients with glioma (115). However, higher mtDNA copy numbers in peripheral blood predict poorer prognoses in hepatocellular carcinoma, glioma, colorectal cancer, and head and neck cancer (116). These findings highlight the complex, cancer-specific prognostic implications of mtDNA copy number variations.

9. Mechanisms of nuclear DNA underlying aging and carcinogenesis

The role of the nuclear genome in aging and cancer. Mutations in nuclear DNA contribute to both aging and tumorigenesis through distinct yet interconnected molecular pathways, including genomic instability, functional deterioration of cellular processes and dysregulation of cell proliferation control mechanisms (117). These mutations accumulate over

time as a result of DNA replication errors, spontaneous base lesions, and exposure to endogenous and exogenous stressors such as ultraviolet radiation and ROS, thereby compromising genomic integrity, and driving hallmark features of senescence and oncogenesis. Notably, certain mutations that confer selective advantages can lead to the clonal expansion of mutant cell populations (118). Furthermore, nuclear genomic aberrations exert direct influence on mtDNA mutational dynamics, an effect that becomes particularly pronounced in aged tissues and tumor microenvironments. These nuclear-mitochondrial interactions are mediated through nuclear-encoded genes involved in mtDNA replication and repair, mitochondrial quality control systems and OXPHOS functions (26,27,38,43). Consequently, elucidating the molecular mechanisms underlying nuclear DNA alterations in aging and cancer may provide critical insights into the etiology and propagation of mtDNA mutations. This subsection delineates the principal characteristics of the nuclear genome that influence aging and tumorigenesis.

While mutations in oncogenes and tumor suppressor genes are closely linked to cancer initiation and progression, alterations in the nuclear genome also accumulate with aging, even in somatic cells devoid of disease (52). In the bone marrow of healthy elderly individuals, mutations in genes such as DNA methyltransferase 3 α (119), tet methylcytosine dioxygenase 2 (116) and ASXL transcriptional regulator 1 (120) can drive clonal hematopoiesis of indeterminate potential, a phenomenon associated with aging, and linked to elevated risks of hematological malignancies and cardiovascular diseases (121). Beyond the hematopoietic system, epithelial tissues also exemplify clonal expansion during aging. In aged skin and esophageal epithelium, clones bearing mutations in notch receptor 1 (122,123), TP53 (124) and cyclin-dependent kinase inhibitor 2A (124) are frequently observed. These mutations, common in non-cancerous aged bone marrow and skin, are not inherently indicative of malignancy. As aging diminishes cellular proliferative capacity in tissues such as the bone marrow (121) and skin (123), cells with driver gene mutations gain a competitive edge over non-mutated clones. Aging tissues often exhibit a mosaic of distinct clones, a reflection of the cumulative accrual and selective expansion of mutations, culminating in tissue mosaicism and clonal heterogeneity. Certain clones expand during aging due to selective advantages conferred by genetic or epigenetic modifications (72,125). These expansions may arise from mutations that enhance cellular survival or proliferation, competitive disadvantages of neighboring cells, or the tissue remodeling and turnover processes characteristic of aging.

Within dominant clones, subclones harboring mutations that confer pronounced selective advantages, such as increased proliferation, resistance to apoptosis or evasion of immune surveillance, may emerge and undergo further selection. Genomic instability, marked by the progressive accumulation of mutations and chromosomal aberrations, is a defining feature of both aging and cancer (72,125). As aging advances, hypoxic conditions, immune pressures and competition for limited nutrients create a highly selective microenvironment in which only clones with advantageous mutations can thrive. This selective proliferation often monopolizes resources such as nutrients and spatial niches, inhibiting the growth of competing clones (125). Consequently, only the most adaptive clones, often those with cancerous traits, dominate, outcompeting less viable clones and reducing clonal diversity. Although clonal proliferation may contribute to the maintenance of tissue homeostasis in certain contexts, it simultaneously elevates the risk of age-associated pathologies, including cancer and functional decline.

Role of nuclear-mitochondrial crosstalk in aging and cancer. Nuclear signaling pathways that directly modulate mitochondrial function, particularly PGC-1α and AMPK, serve pivotal roles in the pathophysiology of aging and cancer. PGC-1α is indispensable for mitochondrial biogenesis and functional maintenance, and its upregulation in mtDNA mutator mice, harboring mutations in mtDNA POLG, has been shown to enhance mitochondrial performance despite a modest elevation in mtDNA mutation load (126). This indicates that PGC-1α-induced mitochondrial biogenesis may mitigate the deleterious functional consequences of mtDNA mutations, and modulate clonal expansion and mutation selection. Furthermore, in rat myoblasts treated with the ATP synthase inhibitor oligomycin, AMPK is activated, subsequently promoting mtDNA replication (127), suggesting a role for AMPK in the regulation of mtDNA replication dynamics in response to mitochondrial stress.

With advancing age, PGC-1α expression declines across various tissues, including skeletal muscle, the brain and bone marrow, contributing to reduced mitochondrial biogenesis, diminished OXPHOS and impaired regenerative capacity (128). In aging bone tissue, decreased PGC-1α levels are associated with compromised osteoblast differentiation and enhanced adipogenic differentiation of mesenchymal stem cells, factors implicated in the pathogenesis of osteoporosis. In the central nervous system, diminished PGC-1α expression is associated with neurodegenerative disorders such as Parkinson's disease, exacerbating mitochondrial dysfunction and increasing neuronal susceptibility to stress. In cancer, the role of PGC-1α is highly context-dependent (128). In malignancies such as melanoma and invasive breast carcinoma, PGC-1α expression is upregulated, promoting mitochondrial biogenesis and OXPHOS, thereby facilitating metastatic potential (129). Conversely, in advanced thyroid cancer, particularly that harboring $BRAF^{V600E}$ mutations, PGC-1α expression is suppressed, contributing to mitochondrial dysfunction, heightened oxidative stress and a metabolic shift toward aerobic glycolysis (the Warburg effect), which collectively drive tumor progression (130). These dichotomous roles underscore the relevance of PGC-1α in mitochondrial regulation and highlight its potential as a therapeutic target.

AMPK, a master regulator of cellular energy homeostasis and mitochondrial function, also exhibits age-associated functional decline. In aged rat skeletal muscle, the acute activation of AMPK-α_2 by 5-aminoimidazole-4-carboxamide ribonucleotide (an AMPK activator) or exercise is notably reduced compared with in younger counterparts, leading to attenuated mitochondrial biogenesis (131). In cancer, the role of AMPK is likewise dualistic and context-specific. It can inhibit tumorigenesis by enhancing OXPHOS and fatty acid oxidation while suppressing glycolysis (132). However, under glucose-restricted conditions, AMPK sustains mitochondrial

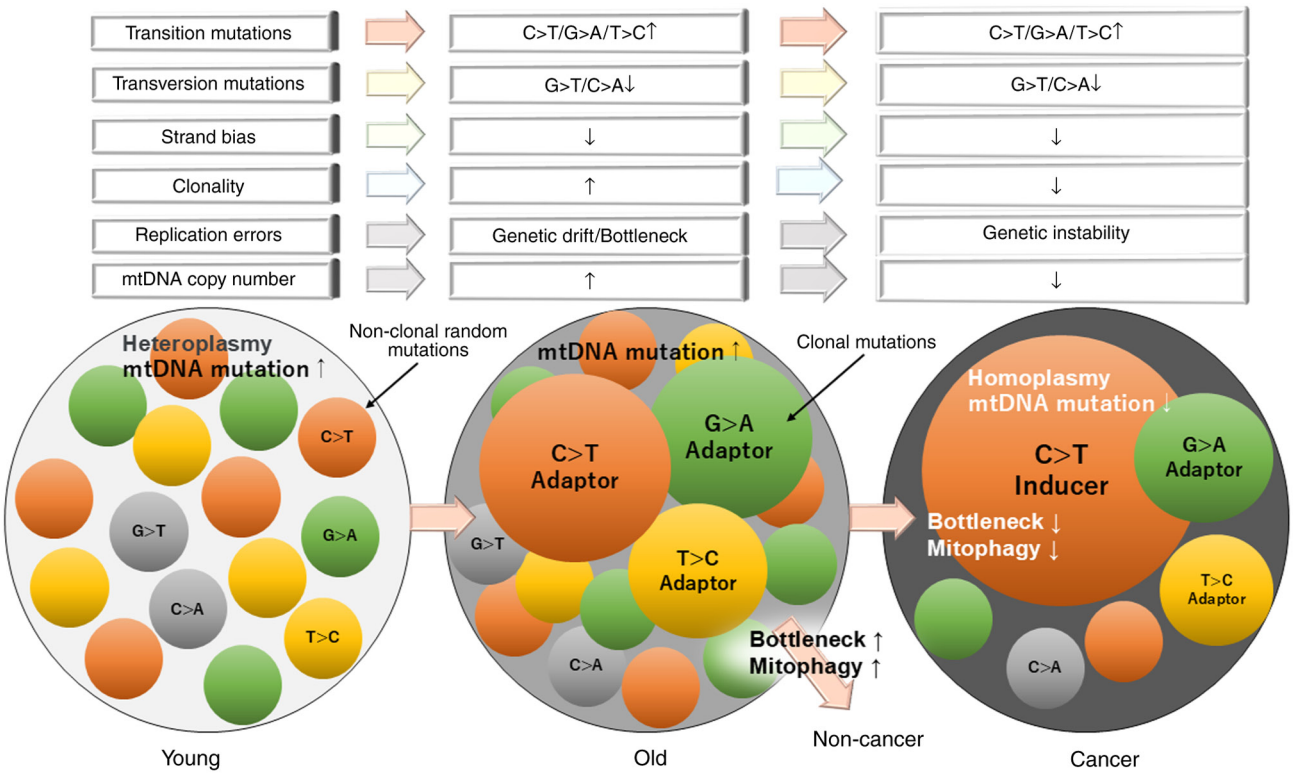

Figure 3. Mechanisms of mtDNA mutations underlying aging and carcinogenesis. The bottom row of the figure presents, from left to right, representations of mitochondria in youthful, senescent and cancerous states. Spherical structures symbolize mitochondria, with orange, green and yellow spheres indicating mitochondria harboring transition mutations, while gray spheres represent those with transversion mutations. An increase in sphere size signifies clonal proliferation or expansion. The top row of the figure delineates the distinct characteristics of mtDNA mutations associated with youthful, senescent and cancerous conditions. mtDNA, mitochondrial DNA.

biogenesis via the p38/PGC-1α axis, thus supporting cancer cell survival under metabolic duress (133). These findings highlight the integral function of AMPK in mediating cell survival during metabolic stress. Moreover, senescent cells in nutrient-poor environments can maintain a state of stable cell cycle arrest and reduced energy consumption via AMPK activation, contributing to homeostatic aging. By contrast, nutrient-rich microenvironments suppress AMPK activation, promoting cellular proliferation (132,133). These observations suggest that the fate of senescent cells, whether toward quiescence or malignant transformation, may be governed by the metabolic characteristics of their surrounding milieu.

10. Mechanisms of mtDNA mutations underlying aging and carcinogenesis

In this section, the aforementioned key findings are summarized, as illustrated in Fig. 3, and the mechanisms by which mtDNA mutations contribute to aging and carcinogenesis are investigated. mtDNA is continually influenced by endogenous and exogenous factors. Most mtDNA mutations are random alterations acquired early in life, typically without phenotypic effects. Even when mutations expand clonally, their impact on cell generations is minimized by genetic drift, bottleneck effects, mitochondrial dynamics and mitophagy. This layered defense contrasts with the reliance of the nuclear genome on robust repair mechanisms such as double-strand break repair. Transition mutations, caused by DNA POLG replication errors and base hydrolysis, are the primary source of mtDNA mutations in aging and cancer (59,86). While transition mutations increase with age, translocation mutations from oxidative stress are rapidly repaired, preventing accumulation or clonal expansion. The effects of carcinogens such as smoking (C:G>A:T), UV radiation (C:G>T:A) and ROS (G:C>T:A) are rare in mtDNA from lung and skin cancer (86), although smoking-related C>A mutations are prominent in the nuclear genome of patients with lung cancer (134). Unlike the nuclear genome, mitochondria emphasize quality control through mitophagy and dynamics over stringent repair. Advances in genetic analysis have revealed a more complex landscape, encompassing transition and translocation mutations. mtDNA displays conserved mutational signatures regardless of cancer tissue origin (22,110), unlike oncogene and tumor suppressor gene mutations in the nuclear genome.

Key distinctions between mtDNA in cancerous and normal cells include the loss of strand bias and mutation type variations, potentially driven by selective pressure, genetic drift (23,24) or chance (135). Some clones act as 'adapters', facilitating cellular adaptation to changing environments (21,22,35). Homoplasmic mtDNA mutations, typically synonymous, are considered adaptive rather than cancer-specific (77). Subclonal proliferation of dysfunctional mitochondria is counteracted by quality control mechanisms to preserve tissue function. Adaptive mtDNA mutations confer a competitive edge over wild-type mtDNA. When mutant clones meet equally fit other clones, a dynamic equilibrium forms, maintaining homeostasis

and reducing cancer risk. In aging tissues, adaptive clones gradually accumulate mutations with reduced strand bias. Among older individuals, mtDNA processivity declines, exposing it to selective pressures favoring mutations. This may disrupt equilibrium, selecting *de novo* oncogenic 'inducers' from pre-existing 'adapters' (21). As selection shifts toward 'inducer' clones, cells adapt to new conditions, thrive and gain advantages, potentially leading to tumorigenesis. Thus, the transition in mtDNA diversity, from non-clonal mutations in young tissues to clonal expansion of 'adapter' and 'inducer' mutations, illustrates the selective pressures driving cancer cell emergence with aging.

11. Conclusion

The present review highlights the relationship between mtDNA mutations and their role in aging and cancer, contrasting these with changes in the nuclear genome. Age-related mtDNA mutation increases are more complex than previously considered. Mitochondrial dysfunction results from reduced strand bias, clonal expansion and contraction, tissue-specific dynamics and regulatory mechanisms rather than a simple accumulation of mutations. These findings have implications for understanding mitochondrial biology and advancing treatments for age-related diseases.

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Authors' contributions

HK conceptualized the study, developed the methodology, administered the project and prepared the original draft of the manuscript. SI performed validation and provided the necessary resources. SI and HK curated the data, created the visualizations, and reviewed and edited the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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