Review

Cancer Research

Understanding Mitochondrial Polymorphisms in Cancer

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Abstract

Alterations in mitochondrial DNA (mtDNA) were once thought to be predominantly innocuous to cell growth. Recent evidence suggests that mtDNA undergo naturally occurring alterations, including mutations and polymorphisms, which profoundly affect the cells in which they appear and contribute to a variety of diseases, including cardiovascular disease, diabetes, and cancer. Furthermore, interplay between mtDNA and nuclear DNA has been found in cancer cells, necessitating consideration of these complex interactions for future studies of cancer mutations and polymorphisms. In this issue of *Cancer Research*, Vivian and colleagues utilize a unique mouse model, called Mitochondrial Nuclear eXchange mice, that contain the nuclear DNA from one inbred mouse strain, and the mtDNA from a different inbred mouse strain to examine the genome-wide nuclear DNA methylation and gene expression patterns of brain tissue. Results demonstrated there were alterations in nuclear DNA expression and DNA methylation driven by mtDNA. These alterations may impact disease pathogenesis. In light of these results, in this review, we highlight alterations in mtDNA, with a specific focus on polymorphisms associated with cancer susceptibility and/or prognosis, mtDNA as cancer biomarkers, and considerations for investigating the role of mtDNA in cancer progression for future studies. *Cancer Res; 77(22); 6051–9. ©2017 AACR.*

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Mitochondrial DNA and Cancer

Despite substantial progress in understanding cancer etiology, improved methods of detection, prevention strategies, and treatment modalities, cancer remains a leading cause of death, second only to heart disease (1). This is due, in part, to the complexity of genomic changes within cancer cells. In addition to the nuclear genome (nDNA), each human cell contains a mitochondrial genome composed of thousands to tens of thousands of copies of mitochondrial DNA (mtDNA) that are capable of replicating independently of nDNA (2). As early as 1956, Otto Warburg suggested that defects in mitochondrial function may contribute to the development and progression of cancer (3). Since then, alterations in mtDNA content, copy number, mutations, and polymorphisms involved in cancer have been identified and characterized.

mtDNA mutations and polymorphisms have been increasingly reported in a wide variety of cancers, including breast, prostate, and colorectal cancers (4, 5). These data have been supported by epidemiologic studies indicating that alterations in mtDNA copy number correlate with increased cancer risk (6). Although it is evident that these alterations exist within the human population, methods to study their biology are limited, technically challenging, and difficult to interpret at best. Furthermore, there is no model available that permits the study of a "pure" mitochondrial genome uncontaminated by fragments of the nuclear genome to study the specificity and pathogenesis of mtDNA involvement in cancer. Thus, few resources are available to investigate important questions pertaining to the impact of mtDNA alterations in cancer initiation, growth, and progression.

Nevertheless, some recent advances have made progress toward achieving this goal. To that end, we discuss current approaches to study the role of mtDNA in cancer, with a specific focus on understanding mtDNA polymorphisms present in different populations (as opposed to somatic mutations that have been recently reviewed in refs. 7, 8). Furthermore, we highlight mouse models for studying mtDNA in cancer, including a relatively new mouse model as described by Vivian and colleagues in this issue of *Cancer Research* (9).

Mitochondrial Structure and Function in the Human Genome

Mitochondrial structure

To understand the impact of mtDNA in cancer, one must consider mitochondrial structure and function. The mitochondrial chromosome is a closed circular, double-stranded DNA molecule. At 16,569 base pairs in length, the human mitochondrial genome is 1.8×10^{-5} times smaller than the nuclear genome. As such, there is little room for nonfunctional DNA, and the vast majority of the human mitochondrial genome encodes 13 protein subunits of the electron transport chain, two rRNA genes, and 22 tRNAs genes (10). The only segment of mtDNA that is mostly noncoding is the displacement loop (D-loop) region. The two DNA strands comprising mtDNA differ in their nucleotide content: the heavy (H) strand contains more guanines and the light (L) strand contains more cytosines. Most mtDNA transcription occurs off the H strand, because the L strand

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encodes only 8 tRNA genes and one protein-coding gene (11). However, the vast majority (1,500+) of proteins within mitochondria are encoded by the nuclear genome and imported into the mitochondria.

As mitochondria are maternally inherited (solely from the oocyte), most cells have identical copies of mtDNA (termed homoplasmy; ref. 11). However, if there is a change in the sequence of mtDNA within a cell (such as from deficiencies in DNA replication and repair), and if those changes are propagated within the same cell, the cell now contains more than one type of mtDNA (termed heteroplasmy; ref. 11). For any given disease caused by a mutation in mtDNA, the ratio of normal to mutant mtDNAs within the cells of the body can substantially impact the clinical presentation, penetrance, and severity of the disease phenotype.

Mitochondrial function

Mitochondria have numerous functions within the cell, most notably to provide energy by producing ATP for respiration, through the oxidative phosphorylation system (OXPHOS; ref. 11). In addition, mitochondria have roles in controlling redox homeostasis, cellular metabolism, cell signaling, innate immunity, survival, and apoptosis (12). Thus, mitochondria are critical for health due to their essential bioenergetic and biosynthetic functions.

It is currently unclear whether cancer cells possess a normal OXPHOS system. Although it is evident that many cancer cells undergo aerobic glycolysis (the "Warburg effect"), not all cells have defects in their mitochondrial respiration (3). In fact, in 1957, Aisenberg and colleagues showed that mitochondria are capable of inhibiting fermentation (13). In this way, mitochondria are capable of regulating oxidative phosphorylation versus aerobic glycolysis, and thus may be key players in tumor progression. As examples, both LeBleu and colleagues (14) and Liu and colleagues (15) demonstrated that cancer cells are capable of regulating the transcription coactivator peroxisome proliferator-activated receptor gamma, coactivator 1α (PPARGC1A, or PGC-1α), which supports increased OXPHOS, oxygen consumption, and mitochondrial mass. However, interestingly, Liu and colleagues discovered that cells expressing the metastasis-suppressor KISS1 had 30% to 50% more mitochondrial mass, accompanied by increased PGC-1α expression, and reduced invasion and migration (15). On the contrary, LeBleu and colleagues demonstrated that increased expression of PGC-1α lead to increased cancer cell migration, invasion, and metastasis (14). Suppression of PGC-1α did not impact primary tumor cell growth (14), however, suggesting that mtDNA content may vary between primary tumor and subsequent metastases.

Mitochondrial Polymorphisms Implicated in Cancer

It is becoming increasingly evident that mtDNA content can be a predictor of cancer risk and/or prognosis. Table 1 summarizes the results of studies designed to determine whether specific polymorphisms within mtDNA from different populations were associated more often with cancer patients than healthy controls. For clarity, mtDNA can be categorized into haplotype groups (called haplogroups) based on single nucleotide polymorphism (SNP) markers that collectively represent populations of different ancestral origin (www.mitomap.org). The haplogroups were named in order of their discovery, using the capital letters A to Z. Subgroups of a haplogroup (called subclades) are distinguished by numbers (and sometimes lowercase letters) following the original capital letter designation and indicate that they derive from the ancestral capital letter haplogroup (16).

As one example, Chen and colleagues used next-generation sequencing to examine the mitochondrial genome of 188 hepatocellular carcinoma (HCC) patients compared with 344 healthy patients to assess associations between mtDNA SNPs (or haplogroups) on patient prognosis (17). The authors identified that haplogroups M7 and M8 had relatively high odds ratios for patients to develop HCC. On the other hand, SNPs T15784C, C16185T, and A16399G were found to be associated with improved prognosis in patients with HCC (17). Another group determined that mtDNA SNP C150T was also important in the prognosis of HCC (18).

As another example, SNPs located in the D-loop region of mtDNA have been associated as risk factors for cancer. In particular, there was a statistically significant increase in the frequency of specific alleles (see Table 1) at SNPs A73G, C150T, C151T, T492C, C16257A, C16261T, and A16399G in patients with gastroenteropancreatic neuroendocrine cancers (GEP-NEN) compared with healthy volunteers (19). On the other hand, there was a decreased association in patients with GEP-NEN and SNPs T489C and T16519C (19). Guo and colleagues also found that the presence of specific alleles (see Table 1) at mtDNA polymorphic loci T146C, C324G, G73A, T195C, T16304C, and C16261T in the D-loop region were risk factors for colon cancer (20).

Overall, the reports shown in Table 1 highlight several key facts, namely that (i) SNPs in both transcribed as well as nontranscribed regions of mtDNA have been associated with increased cancer risk and/or prognosis; (ii) different nucleotides corresponding to the same SNP can have opposite effects in different ethnic populations (i.e., G10398A); and (iii) some SNPs are associated with several different cancers (i.e., T195C), whereas others appear to be cancer-type specific (i.e., T16189C). Furthermore, these data suggest that polymorphisms in mtDNA warrant further study not just for their predictive potential, but also to understand the mechanism(s) by which they influence cancer pathways.

mtDNA as Biomarkers for Cancer

Numerous studies have shown the potential for mtDNA as biomarkers for certain cancers due to alterations in their structure.

D-loop region

Kodron and colleagues found that there were common polymorphisms in pediatric acute lymphoblastic leukemia patients (21). Nearly all of the alterations in mtDNA were microsatellite variations that were common population polymorphisms as identified by the MITOMAP database (www.mitomap.org). The highest level of polymorphisms was found in the D-loop region as well as in the mitochondrial *CYTB* gene (21). These alterations varied during therapeutic treatment and thus have the potential to function as both biomarkers and indicators of successful cancer treatment. Furthermore, Cai and colleagues sequenced two hypervariable regions (HVR1 and HVR2) within the D-loop region from the tissue of 10 breast cancer patients. The results showed that 85 mutations of germline mtDNA were found, specifically located in the D-loop region (22).

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Table 1. Correlations frequently found between specific polymorphisms in mtDNA, ethnicity, and cancer type[a]

		Polymorphisms[b]			
Type of cancer	**Ethnicity**	**Gene/region**	**Polymorphism**	**Allele[c]**	**Reference**
Breast cancer	European Caucasian	Haplogroup U	A12308G	A	52
Breast cancer	Chinese	D-loop region	(CA)n repeat	Heteroplasmy associated with poor prognosis	53
Breast cancer	Malaysian		G10398A	G	54
Breast cancer	Chinese	Haplogroups M and N	Sub-haplogroup D5		55
Breast cancer	Polish		G10398A	G	56
Breast cancer	North Indian	Haplogroup N	G10398A	A	57
Breast cancer	South Indian	D-loop region	310(ins)C	(ins)C	58
			T16189C	C	
Breast cancer - metastatic	African-American		G10398A	A	59
Cervical cancer and HPV risk	Amerindian	Haplogroup B2	(MT-TD)[d]		60
			(MT-TK)[d]		
Cervical cancer and HPV risk	Chinese	D-loop region	C150T	T	61
Colon cancer	Chinese	D-loop region	G73A	G	20
			T146C	T	
			T195C	T	
			C324G	C	
			C16261T	T	
			T16304C	T	
			C309(ins)C	(ins)C	
Colorectal cancer	European Americans	MT-ND2			62
		Haplogroup T			
Colorectal cancer	Latino	Haplogroup L			62
Epithelial ovarian cancer	Caucasian	MT-CO1	T6777C		63
Esophageal cancer	Chinese	Haplogroup D	Sub-haplogroups D4a and D5		64
Esophageal cancer	North Indian	Haplogroup N	G10398A	A	57
Gastric cancer	Chinese	D-loop region	D310 (mononucleotide repeat)	(ins)C	65
			T16521C	C	
GEP-NEN (gastroenteropancreatic-neuroendocrine neoplasms)	Chinese	D-loop region	A73G	G	19
			C150T	T	
			C151T	T	
			T489C	T	
			T492C	C	
			C16257A	A	
			C16261T	T	
			A16399G	G	
			T16519C	T	
Hepatocellular carcinoma	Chinese		mtDNA 9-bp deletion (seed region for hsa-miR-519-5p and hsa-miR-526a)	del	66
Hepatocellular carcinoma	Chinese	Haplogroup M7	T152C		17
			T199C		
			G4048A		
			T9824C		
			T15784C		
			C16185T		
			A16399G		
Hepatocellular carcinoma	Chinese	D-loop region	C150T	C	18
			T16263C	C	
			N315(ins)C	N	
Malignant Melanoma	Chinese Han	D-loop region	T195C	C	67
			T16362C	C	
			A16399G	G	
Non-small cell lung cancer	Chinese	Haplogroup N	G10398A	A	68
Oral cancer	India	Haplogroup M	G10398A	G	69
			T10400C	T	
			A11467G	A	
			A12308G	A	
Thyroid cancer	Chinese	Haplogroup D4a	Sub-haplogroup D4a		55

NOTE: Here, predominant mtDNA polymorphisms are listed, along with the corresponding ethnicity and cancer type they are frequently found in. Citations detailing the specific mtDNA polymorphisms and their associated ethnic groups are located in the column on the far right. The gray box indicates that we were unable to find additional information for the indicated column. Hypervariable regions within the D-loop are HVR1 (positions 16024-16383), HVR2 (positions 57-372), and HVR3 (positions 438-574; www.mitomap.org).

[a]Mitochondrial polymorphisms reported in the past decade. Additional references are described in refs. 12, 70.

[b]Polymorphisms defined as those present in the population at an allele frequency >1%.

[c]The alleles listed are susceptibility alleles.

[d]Suggested genes based on RNA transcript levels.

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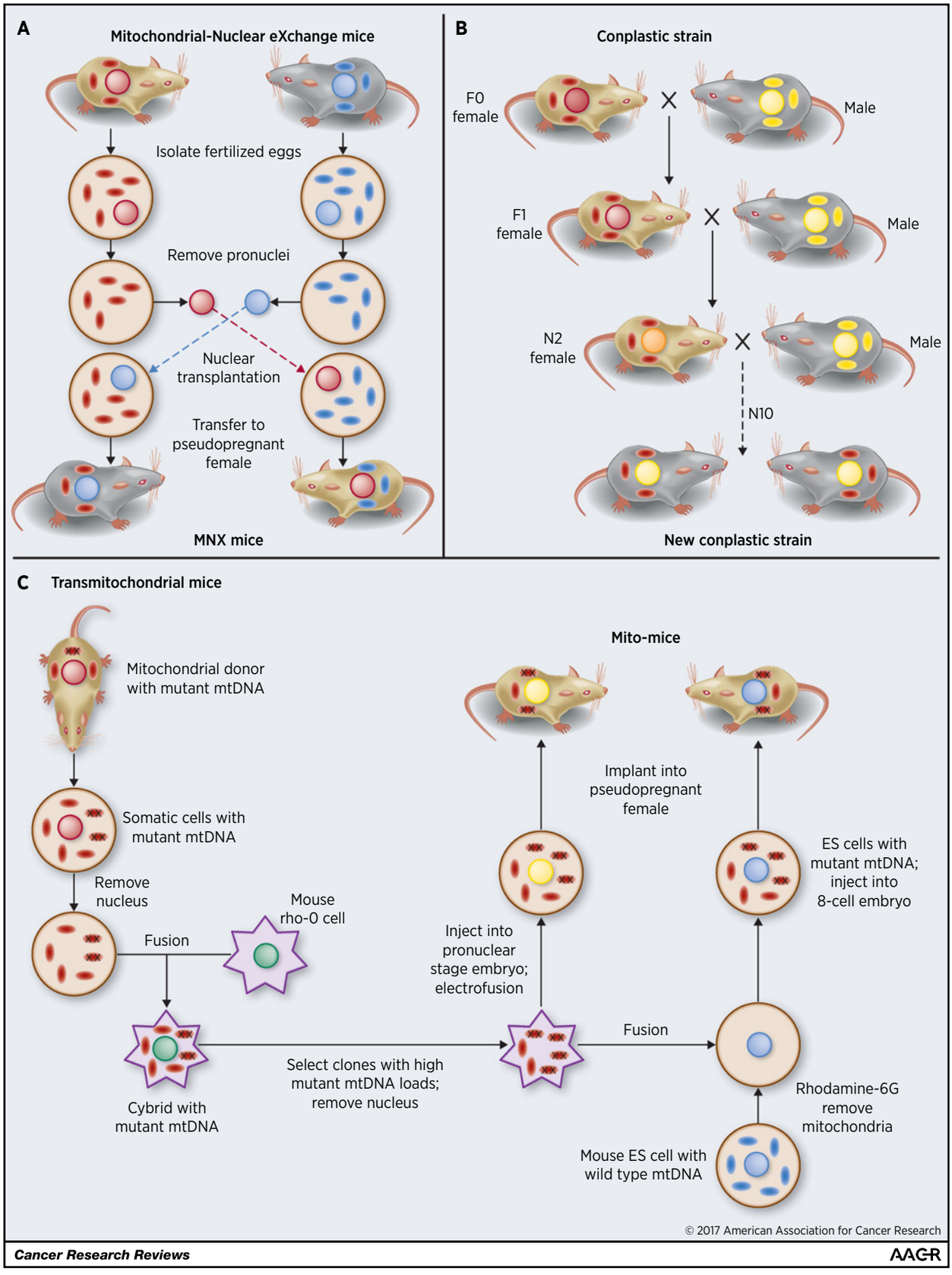

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Copy number variation

Alterations in mtDNA copy number have been discovered in a variety of cancers. In particular, mtDNA copy number may be a predictor of cancer in renal cell carcinoma. Elsayed and colleagues utilized quantitative PCR to measure the peripheral blood of 57 Egyptian patients with newly diagnosed early-stage renal cell carcinoma (23). The authors discovered that median mtDNA copy number was significantly higher in patients with renal cell carcinoma than healthy patients. As a predictor of renal cell carcinoma, mtDNA copy number had 86% sensitivity, 80% specificity, and 80.3% predictive value compared with healthy controls (23). On the other hand, Bao and colleagues examined the peripheral blood leukocytes of 250 hepatocellular carcinoma patients and found that those with a lower mtDNA copy number and longer leukocyte telomere length had a reduced overall time of survival (24). These data also showed that increased mtDNA content was associated with increased survival time, compared to patients with less mtDNA content (24). Thus, these results suggest that correlations between the number of mtDNA copies and patient prognosis may be cancer-type dependent.

mtDNA and Metastasis

Emerging evidence has suggested that alterations in mtDNA can lead to increased metastasis. Kenny and colleagues discovered that the activation of SIRT/FOXO/SOD2 axis of the mitochondrial unfolded protein response (UPR^{mt}) is a common feature of the mtDNA of metastatic cells (25). In particular, super-oxide dismutase 2 (SOD2) was found to be substantially increased in more highly metastatic human breast cancer cell lines (MDA-MB-231, MDA-MB-361, and MDA-MB-157) compared with less metastatic cell lines MCF-7 and ZR-75-1. Interestingly, Kenny and colleagues found that there was no one specific mtDNA mutation that drove metastasis in the cancer cells, but rather the composition of the mtDNA genome [i.e., presence or absence of reactive oxygen species (ROS); activation of the SIRT/FOXO/SOD2 axis of the UPR^{mt}] dictated either promotion or repression of cancer cell metastasis (25). In comparison with Kenny and colleagues, Arnold and colleagues sequenced the mitochondrial genome of 10 prostate cancer patients with bone metastases (26). They found that there was a single recurring mtDNA mutation in 77% of the patients' bone metastases. This mutation was identified as a missense mutation within the mitochondrial *ND3* gene at nucleotide position 10398 (26). Arnold and colleagues found evidence for mutations at other sites; however, these mutations were not exclusively restricted to bone metastases and were found in a smaller number of patients (30%). On the other hand, Kleist and colleagues determined that microsatellite instability in hypervariable regions (HVR1, HVR2, and HVR3) within the D-loop region, specifically at positions D310 in HVR2, D514 in HVR3, and D16184 in HVR1, was more frequently associated with lymph node metastases than primary tumors in colorectal cancer (27). In another study, genetic modulation (KO mouse model) of the human DNA repair enzyme 8-oxoguanine DNA glycosylase isoform 1-α (OGG1) protein in mitochondria was protective against increased mtDNA damage and dysfunction (28). Reduced mtDNA damage led to suppressed mitochondrial ROS production and reduced ROS-dependent metastases in a polyoma virus middle T antigen (PyMT) model of breast cancer (28). Thus, these data collectively suggest that alterations in mtDNA that drive cancer metastases may be cancer-type dependent, with specific mutations driving metastasis in one cancer type, and the collective mitochondrial genome dictating metastatic susceptibility in another.

Mouse Models for Studying mtDNA in Cancer

The large numbers of polymorphisms within mtDNA that have been reported as being associated with cancer to date (Table 1) are most likely the tip of the iceberg. Although these analyses of mtDNA polymorphisms and their cooccurrence with specific

Figure 1.

Diagram of the creation of MNX, conplastic mice, and transmitochondrial mice. All three mouse models highlighted in Fig. 1 have advantages and disadvantages. However, sequencing of mtDNA from individual mice or from cells provides a known starting point for all of these models. Thus, mixing and matching of selected mtDNA content with specific nuclear genomes can be achieved with each of these methods. In addition, these models can be designed to generate mice that are either homoplastic or heteroplastic for mtDNAs. **A,** MNX mice are established by first isolating fertilized eggs from one inbred strain (gold mouse) and removing both the female and male pronuclei (red circle). The same process is repeated with a second inbred strain (gray mouse) and removing both the female and male pronuclei (blue circle). The female and male pronuclei (red circle) are "eXchanged" and are transplanted into the enucleated egg of the other strain. The resulting one-celled zygotes are transferred to the oviduct of pseudopregnant females. Live born pups have their mitochondria from one inbred strain (blue ovals) and their nuclear genome from a different inbred strain (red and blue circles), or vice versa. **B,** Conplastic strains are established by a series of backcrosses involving two inbred strains. The nuclear genome from one inbred strain is essentially backcrossed into the cytoplasm of another inbred strain. This special breeding scheme involves selecting only females for subsequent generations. F1 females carry mitochondria (red ovals) inherited solely from their mother (gold mouse), whereas 50% of their nuclear genome is inherited from their father and 50% of their nuclear genome is inherited from their mother (faded red circle). F1 females are then bred to males from the original progenitor strain (gray males), generating N2 females that still have all of their mitochondria (red ovals) from the maternal progenitor strain (gold mouse), whereas 75% of their nuclear genome is from their father and only 25% of their nuclear genome is from their mother (orange circle). After 10 backcross generations (always selecting N# females for mating), the nuclear genome (yellow circle) is essentially identical to the nuclear genome of the original male progenitor strain (gray mouse), whereas the mitochondria (red ovals) are solely from the original female progenitor strain (gold mouse). A minimum of 10 backcross generations are required to produce a conplastic strain. **C,** The derivation of transmitochondrial (mito-mice) mice. The first step involves identifying mice that carry mtDNA mutations and isolating somatic cells. Removal of the nuclear genome creates cytoplasts, which are then fused to mtDNA-negative rho-0 cells to create cytoplasmic hybrids (cybrids). Cloning and selection of cybrid lines with a high load of mutant mtDNA is possible. The selected cybrids undergo removal of their nuclear genome. Next, either of two paths is possible: (i) cybrids are microinjected into pronuclear stage embryos and subjected to electrofusion to fuse their membrane with that of the embryo. The embryos are then implanted into pseudopregnant hosts to obtain live born heteroplasmic mito-mice carrying both wild-type and mutant mtDNA. Alternatively, (ii) cybrids are fused with mouse embryonic stem (ES) cells that have been depleted of mitochondria via treatment with rhodamine-6G. The resulting embryonic stem cells are injected into 8-cell embryos and implanted into pseudopregnant hosts to obtain live born chimeric mice. Mating of the chimeras to wild-type mice results in the production of heteroplasmic mito-mice carrying both wild-type and mutant mtDNA. Mutant mtDNA is represented by colored ovals with "XX."

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cancers has provided statistical justifications for significant relationships, further experimentation is needed to define the role of specific mtDNA polymorphisms in cancer. Model systems that can replicate different combinations of mitochondrial and nuclear genomes *in vivo* would provide a means to investigate the underlying mechanisms responsible for these genetic relationships. One way to demonstrate how changes in mtDNA sequence are translated into effects on cancer initiation, growth, progression, and/or metastases is via the use of suitable mouse models. We highlight below three methods that result in designer combinations of mtDNA and nuclear DNA in live mice and indicate the advantages and applications of each method for further investigations.

MNX mice

The ability to generate live born mice from fertilized eggs whose nuclear genomes had been removed and replaced with ones from other embryos was elegantly demonstrated in the 1980s (29, 30); these embryonic nuclear transplant experiments proved that contributions from the maternal and the paternal genomes were both required for an embryo to develop to term. This research also demonstrated the nonequivalence of the maternal and paternal genomes, ushering in the concept of imprinting. Since that time, successful manipulation of both the nuclear as well as the mitochondrial content of mouse cells has opened the door to studying questions regarding mitochondrial biology, and the interplay between the mitochondrial and nuclear genomes.

In this issue of *Cancer Research*, Vivian and colleagues (9) utilized the novel Mitochondrial-Nuclear eXchange (MNX) mouse model (31) to examine the effects of the mitochondrial genome on the nuclear genome (32). Previously, Ballinger and Welch had pioneered the use of nuclear transplantation technology to selectively develop mice that had their nuclear genome from one inbred strain and their mitochondria (mtDNA) from a different inbred strain (originally described in refs. 33, 34; see Fig. 1A and legend for details). The resulting MNX mice allowed these investigators to evaluate changes in nuclear DNA methylation and gene expression patterns that could only be due to differences in mitochondrial content (as the nuclear genomes were the same). The key finding is that different mitochondrial genomes, in combination with the same nuclear genome, result in differential methylation of specific sites in the nuclear genomes as well as differential expression of genes encoded by the nuclear genome (32).

Previously, Ballinger and Welch had utilized MNX mice to study how mtDNA impacts cancer progression (35). Strains examined include C57BL/6J, FVB/NJ, and BALB/cJ. To investigate the role of mtDNA in breast cancer, female MNX mice were crossed with males carrying a PyMT transgene to produce mice hemizygous for PyMT with mtDNA from different genetic backgrounds (35). The authors discovered that FVB^n mice with mtDNA from the C57BL/6J ($BL/6^{mt}$) strain had an increased primary PyMT tumor latency (~65 days) when compared with FVB^n mice with mtDNA from the FVB/NJ (FVB^{mt}) or BALB/cJ ($BALB/c^{mt}$) strain (~59 and ~52 days, respectively). Furthermore, FVB^n mice with mtDNA from the BALB/cJ ($BALB/c^{mt}$) strain developed larger individual lung metastases (in cm^2), indicating an increased metastatic burden when compared with FVB^n mice containing mtDNA from the C57BL/6J ($BL/6^{mt}$) strain (35). Thus, these results suggest that the mitochondrial genome of an individual may influence disease progression. These results also suggest that polymorphisms in mtDNA directly affect breast cancer progression. Furthermore, these results show the utility of the MNX mice as a model system to examine the impact of differences in the mitochondrial genome versus the nuclear genome.

Conplastic mice

Another type of inbred strain that is useful for understanding the interplay between the mitochondrial and nuclear genomes is conplastic strains. Conplastic strains are mice that have been bred via a series of backcrosses to have their nuclear genome from one inbred strain and their mtDNA from a different inbred strain (see Fig. 1B and legend for details), thus essentially establishing new inbred strains similar to MNX mice, although the time to establish conplastic strains can take 4 to 5 years (36). Yu and colleagues demonstrated how a panel of conplastic strains carrying different mtDNA mutations can be generated and used to study disease (36). They sequenced the mtDNA of 27 inbred strains and compared their results with published mtDNA sequences of 29 inbred mouse strains. They identified mutations in mtDNA and established a panel of conplastic strains that each carried a different mtDNA in combination with the C57BL/6J nuclear genome. Evaluation of these conplastic strains demonstrated that mtDNA variations affected susceptibility to complex traits (namely autoimmune encephalomyelitis and anxiety-related behavior; ref. 36). This research represents one of several reports that demonstrate the utility of conplastic strains to study the effects of mtDNA polymorphisms and mutations on a variety of phenotypes.

Mito-Mice

The technology to establish transmitochondrial mice was originally designed to introduce mutant mtDNA into wild-type mice (see Fig. 1C and legend for details). Alternatively called "mito-mice," different lines have been established to study a variety of phenotypes. Although technically challenging, mito-mice have been selected for differing levels of heteroplasmy to assess threshold effects for polymorphic (or mutant) mtDNAs on the same nuclear genetic background (37).

Mito-mice have been used for a variety of studies, including the impact of respiration defects on disease. As an example of their utility for cancer studies, two lines of mito-mice were developed that have the same mutation (G13997A) in the mt-Nd6 gene, albeit in combination with the nuclear genomes of the C57BL/6 (B6) and A/J inbred strains. Studies demonstrated that mutant mtDNA enhanced the frequency of lymphoma development in the already predisposed B6 nuclear background, but did not enhance lymphoma development in the nonpredisposed A/J nuclear background (38). The differential effects noted above may be linked to respiration effects and/or ROS overproduction similar to what has been shown for mutant mtDNA in embryonic stem cells and mito-mice (38). Overall, these findings indicate that mitochondrial–nuclear interplay is important in the pathogenesis of disease states.

All of these technologies provide distinct models to investigate mtDNA, by comparing the same mtDNA albeit in combination with different nuclear genomes, or alternatively, by comparing different mtDNAs in combination with the same nuclear genome.

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Applications of the technology for studying effects of mtDNA alterations in cancer *in vivo*

As described in this issue of *Cancer Research* by Vivian and colleagues (9), the development of MNX mice via nuclear transplantation technology generated mice with the same nuclear genome, but different mitochondrial genomes (and vice versa; ref. 32). This model system has many applications. For example, MNX mice can be used to assess characteristics of pools of methyl donors. Methyl donors are substances, such as folate, methionine, or S-adenosylmethionine, that can transfer a methyl group ($-CH_3$) to another substance (40, 41). Both DNA and lipid metabolism rely on methylation, where the constant availability of a pool of methyl donors is necessary for consistent replication of a methylome (methylation pattern; refs. 41, 42). Methylation of DNA, in particular, impacts cancer initiation and progression (43, 44). In fact, DNA demethylation agents have been used as therapeutics for cancer patients to decrease cancer cell proliferation and induce terminal differentiation (45, 46). DNA demethylation agents allow cancer cells to function under aerobic glycolysis, enabling increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that cancer cell treatment (including breast, ovarian, glioblastoma multiforme, and myeloma) with the global demethylation agent (5-azaC), caused demethylation of the cancer cells within exon 2 of the *POLGA* gene and promoted cellular differentiation via increased mtDNA replication (47). In addition, as the MNX model system has been previously used to examine alterations in mtDNA, similar mouse models might be constructed to examine the impact of mtRNA, nuclear–mtDNA interplay, and noncoding RNA functions on disease progression.

At present, the significant limitations to creating designer alterations in mtDNA prohibit the recapitulation of polymorphisms (or mutations) found in the human population (48). However, the relatively high mutation rate of mtDNA, coupled with the ability to identify mice that carry polymorphic (or mutant) mtDNAs via sequencing, is unparalleled. The potent methods (Fig. 1) for establishing live mice with selected combinations of mitochondrial and nuclear genomes will enable answers to a vast array of questions, including those regarding the machinery responsible for retrograde and anterograde signaling between the mitochondrial and nuclear genomes (49) and the relationship of mitochondrial dysfunction to changes in the epigenetic landscape of the nuclear genome (50).

Considerations and unanswered questions

Many questions still remain about the role of mtDNA on the initiation, growth, and progression of cancer. One of the most critical questions is that although whole-genome sequencing of an individual's normal and tumor tissues can decipher what changes have occurred in both mtDNA and nuclear DNA, there is no model system currently available to evaluate the relevance of such changes. How mitochondrial function is altered in the presence of certain combinations of nuclear and mitochondrial genomes remains poorly understood and calls into question the safety of embryonic manipulations, including the three-parent baby (51). Furthermore, evolution may have preselected "compatible" mitochondrial and nuclear genomes to maximize function for survival (49); however, sequencing data argues that as mtDNA haplogroups coexist with divergent nuclear genomes in healthy individuals, "compatibility" is not an issue (16). The mouse models shown in Fig. 1 may be essential for testing hypotheses to resolve these controversies.

And, as highlighted by controversies in the field (14, 15), what impact does mtDNA content have on tumor progression? Does the mtDNA content of primary tumors differ from subsequent metastases? If so, what mechanisms do invasive cancer cells utilize to initiate these changes? Moreover, does mtDNA content, and subsequent regulation of mitochondrial respiration and/or other mitochondrial functions, influence disseminated cancer cell dormancy? Better understanding of the relevance and mechanisms behind these questions will aid in the development of therapeutics to manipulate mtDNA and suppress cancer initiation and progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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