Understanding Mitochondrial Polymorphisms in Cancer
Karen M. Bussard and Linda D. Siracusa

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 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.

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.

Mitochondrial Structure and Function

Mitochondrial DNA transcription occurs off the H strand, because the L strand contains more cytosines. Most 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...
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 LeBlu 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, LeBlu 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, 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.
Table 1. Correlations frequently found between specific polymorphisms in mtDNA, ethnicity, and cancer type

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Ethnicity</th>
<th>Gene/region</th>
<th>Polymorphism</th>
<th>Allele</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>European Caucasian</td>
<td>Haplogroup U</td>
<td>A12308G</td>
<td>A</td>
<td>52</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Chinese</td>
<td>D-loop region</td>
<td>G10398A</td>
<td>G</td>
<td>53</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Malaysian</td>
<td>Haplogroups M and N</td>
<td>Sub-haplogroup D5</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Polish</td>
<td></td>
<td>G10398A</td>
<td>G</td>
<td>55</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>North Indian</td>
<td>Haplogroup N</td>
<td>310(ins)C</td>
<td>(ins)C</td>
<td>56</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>South Indian</td>
<td>D-loop region</td>
<td>T16189C</td>
<td>C</td>
<td>57</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Chinese - metastatic</td>
<td>American</td>
<td>G10398A</td>
<td>A</td>
<td>58</td>
</tr>
<tr>
<td>Cervical cancer and HPV risk</td>
<td>Amerindian</td>
<td>Haplogroup B2</td>
<td>(MT-TD) (MT-TK)</td>
<td></td>
<td>59</td>
</tr>
<tr>
<td>Cervical cancer and HPV risk</td>
<td>Chinese</td>
<td>D-loop region</td>
<td>C150T</td>
<td>T</td>
<td>60</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>European Americans</td>
<td>MT-ND2</td>
<td></td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>Latino</td>
<td>Haplogroup L</td>
<td>T1677C</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>Epithelial ovarian cancer</td>
<td>Caucasian</td>
<td>MT-COI</td>
<td></td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>Chinese</td>
<td>Haplogroup D</td>
<td>G10398A</td>
<td>A</td>
<td>57</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>North Indian</td>
<td>Haplogroup N</td>
<td>D310 (mononucleotide repeat)</td>
<td>(ins)C</td>
<td>65</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Chinese</td>
<td>D-loop region</td>
<td>T16519C</td>
<td>T</td>
<td>66</td>
</tr>
<tr>
<td>GEP-NEN (gastroenteropancreatic-neuroendocrine neoplasms)</td>
<td>Chinese</td>
<td>D-loop region</td>
<td>A73G, T16519C</td>
<td>G</td>
<td>19</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>Chinese</td>
<td>mtDNA 9-bp deletion (seed region for hsa-miR-519-5p and hsa-miR-526a)</td>
<td>del</td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>Malignant Melanoma</td>
<td>Chinese Han</td>
<td>D-loop region</td>
<td>T195C, T16362C, A16399G</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>Chinese</td>
<td>Haplogroup N</td>
<td>G10398A</td>
<td>A</td>
<td>71</td>
</tr>
<tr>
<td>Oral cancer</td>
<td>India</td>
<td>Haplogroup M</td>
<td>G10398A, T10400C, A11467G, T16304C</td>
<td>A</td>
<td>72</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>Chinese</td>
<td>Haplogroup D4a</td>
<td>G10398A</td>
<td>A</td>
<td>73</td>
</tr>
</tbody>
</table>

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).

aMitochondrial polymorphisms reported in the past decade. Additional references are described in refs. 12, 70.
bPolymorphisms defined as those present in the population at an allele frequency >1%.
cThe alleles listed are susceptibility alleles.
dSuggested genes based on RNA transcript levels.
Mitochondrial-Nuclear eXchange mice

Isolate fertilized eggs

Remove pronuclei

Nuclear transplantation

Transfer to pseudopregnant female

MNX mice

Transmitochondrial mice

Mitochondrial donor with mutant mtDNA

Somatic cells with mutant mtDNA

Remove nucleus

Mouse rho-0 cell

Fusion

Cybrid with mutant mtDNA

Select clones with high mutant mtDNA loads; remove nucleus

Mito-mice

Implant into pseudopregnant female

ES cells with mutant mtDNA; inject into 8-cell embryo

Mouse ES cell with wild type mtDNA

Rhodamine-6G remove mitochondria

Conplastic strain

F0 female × Male

F1 female × Male

N2 female × Male

N10

New conplastic strain

Male

F0 female

F1 female

N2 female

N10

MNX mice

Conplastic strain

AAGR © 2017 American Association for Cancer Research

Cancer Research Reviews

OF4 Cancer Res: 77(22) November 15, 2017

Downloaded from cancerres.aacrjournals.org on November 5, 2017. © 2017 American Association for Cancer Research.
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 hepato-cellular 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 (UPRmt) 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 UPRmt] 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 hyper-variable regions (HRV1, HRV2, and HRV3) within the D-loop region, specifically at positions D310 in HRV2, D514 in HRV3, and D16184 in HRV1, 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 electrosuction 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 B-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”.

www.aacnjournals.org

Cancer Res; 77(22) November 15, 2017

OF5

Downloaded from cancerres.aacnjournals.org on November 5, 2017. © 2017 American Association for Cancer Research.
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 mitochondrial (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 FVBn mice with mtDNA from the C57BL/6J (BL/6mt) strain had an increased metastatic burden when compared with FVBn mice containing mtDNA from the C57BL/6J (BL/6mt) 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 heteroplasmacy 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 mtND6 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.
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 5-adenosylmethionine, that can transfer a methyl group (-CH3) 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 increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-renewal, but halting cancer cell differentiation. For example, Lee and colleagues discovered that increased proliferation and self-regulation of mitochondrial gene expression. Cancer Res 2017;77:6202–14.

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.

Acknowledgments

We thank Dr. Arthur M. Buchberg for reviewing the manuscript and for assistance with Table 1 and Figure 1. We thank Dr. Jan B. Hoek for consultation on mitochondrial biology. We would like to acknowledge all authors whose outstanding work we were unable to report on.

Grant Support

K.M. Bussard and L.D. Siracusa are supported by grants from the NCI.

Received June 28, 2017; revised July 25, 2017; accepted September 13, 2017; published OnlineFirst November 2, 2017.

References


Understanding Mitochondrial Polymorphisms in Cancer

Karen M. Bussard and Linda D. Siracusa

Cancer Res  Published OnlineFirst November 2, 2017.

Updated version  Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-17-1939

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.