Mitochondrial Haplotype Alters Mammary Cancer Tumorigenicity and Metastasis in an Oncogenic Driver–Dependent Manner

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Abstract

Using a novel mouse model, a mitochondrial–nuclear exchange model termed MNX, we tested the hypothesis that inherited mitochondrial haplotypes alter primary tumor latency and metastatic efficiency. Male FVB/N-Tg(MMTVneu)202Mul/J (Her2) transgenic mice were bred to female MNX mice having FVB/NJ nuclear DNA with either FVB/NJ, C57BL/6J, or BALB/cJ mtDNA. Pups receiving the C57BL/6J or BALB/cJ mitochondrial genome (i.e., females crossed with Her2 males) showed significantly (P < 0.001) longer tumor latency (262 vs. 293 vs. 225 days), fewer pulmonary metastases (5 vs. 7 vs. 15), and differences in size of lung metastases (1.2 vs. 1.4 vs. 1.0 mm diameter) compared with FVB/NJ mtDNA. Although polyoma virus middle T–driven tumors showed altered primary and metastatic profiles in previous studies, depending upon nuclear and mtDNA haplotype, the magnitude and direction of changes were not the same in the HER2-driven mammary carcinomas. Collectively, these results establish mitochondrial polymorphisms as quantitative trait loci in mammary carcinogenesis, and they implicate distinct interactions between tumor drivers and mitochondria as critical modifiers of tumorigenicity and metastasis.

Introduction

Establishment of metastases, not the primary tumor, is responsible for the overwhelming majority of cancer-related morbidities and mortality (1, 2). The pathobiology of metastatic disease remains relatively poorly understood because intrinsic (i.e., genetic) and extrinsic (i.e., tumor–microenvironment interactions) are involved (1). A growing body of data indicates discrete underlying genetic components to the processes involved in cancer spread and colonization (3).

The inbred mouse has long been used to model human cancers. Just as humans have unique genetic subpopulations with distinct cancer incidence, mice have unique strains that show differences in susceptibility to cancers (4–7). For example, offspring of C57BL/6J and BALB/cJ female mice mated with male transgenic mice carrying the polyoma virus middle T (PyMT) antigen have altered tumor latency and decreased metastatic burden compared with offspring of similarly mated FVB/NJ mice (8). Hunter and colleagues showed nuclear quantitative trait loci that affected the efficiency of mammary carcinoma metastasis (9–12), establishing that an individual’s underlying genetic background could affect metastasis efficiency. They have since identified several metastasis efficiency modifier loci within the nuclear genome for breast and prostate cancers (13–15).

In the same vein, Ishikawa and colleagues showed metastatic characteristics followed the mitochondria in cytoplasmic hybrid (cybrid) cells constructed from combinations of high and low metastatic nuclei and mitochondria (16, 17). Using pharmacologic inhibitors, Felding-Habermann and colleagues established NAD/NADH ratios as critical determinants of metastatic efficiency (18). Still others have compared mitochondrial profiles and demonstrated mitochondrial mass (19, 20), metabolic profiles (21–23), and reactive oxygen species (24–26) as contributors to processes critical to invasion and metastasis.

Recently, we determined that the mitochondrial genome influenced the process of metastasis in addition to nuclear genetic contributors (27). To determine whether mitochondrial genetics could be playing a role in metastasis, mitochondrial–nuclear exchange (MNX) mice were generated that have the same nuclear background with different mitochondrial backgrounds (28, 29). These mice were made by enucleating a fertilized oocyte of one strain of mouse, leaving the cytoplasm and mitochondria, and then transferring a karyoplast from another mouse strain (27–29). These mouse strains are designated as nuclear background–mtMNX (mtDNA Background) (e.g., the MNX strain FVB/NJ-mtMNX(C57BL/6J) has the FVB/NJ nucleus and C57BL/6J mitochondrial DNA). Using FVB/NJ-mtMNX(C57BL/6J) and FVB/NJ-mtMNX(BALB/cJ) MNX strains crossed with the FVB/N-Tg(MMTV-PyVT)634Mul/J (hereafter PyMT), we...
showed tumor latency and metastasis tracked with mtDNA (27). These data established that mtDNA, in addition to the nuclear DNA, plays a role in both tumor onset and metastasis. Several studies have described changes in transgene behavior on different mouse strains (4, 15, 30). Nuclear modifiers appear to be combination-dependent, i.e., nuclear modifiers are driver-dependent or vice versa. To our knowledge, no such study has yet been performed to determine whether mitochondrial DNA effects on metastasis are oncogene specific. Given the abundance of data showing retrograde communication between the mitochondria and nucleus (31–36), we hypothesized that the effects of mtDNA on tumor latency and metastasis would vary depending on the nuclear-encoded oncogenic driver.

Materials and Methods

Mouse lines

Stable MNX mouse strains were created as previously reported (27, 29). Briefly, pronuclei were isolated from fertilized oocytes of FVB/NJ mice and transferred into enucleated fertilized oocytes of either C57BL/6J or BALB/c origin. Wild-type FVB/NJ, C57BL/6J, BALB/c, and FVB-N-Tg(MMTVneu) 202MUL/J (Her2) mice were purchased from Jackson Labs. MNX colonies were maintained by breeding MNX females with nuclear genome–matched male mice. All animal studies were approved by the Institutional Animal Care and Use Committee at the University of Kansas Medical Center (IACUC #2014-2215; #2017-2408).

Genotyping

Tail clips (<3 mm) were collected from all breeding and experimental mice at weaning. DNA extraction was performed using the REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich: XNAT-100). Restriction fragment length polymorphism was used to ensure homoplasmic mitochondrial background as described (27, 28). Briefly, primers were designed to span regions of SNP (listed below), which distinguish C57BL/6J (C9461T) and BALB/c (A9348G) mtDNA from FVB/NJ (37). The mutation at 9461 present in C57BL/6J mtDNA will not allow a restriction digest site for BclI to incorporate. Upon incubation with BclI (New England Biolabs, R0160S), cleavage will only occur in animals with FVB/NJ mitochondria (Supplementary Fig. S1A). The mutation at 9348, present in BALB/c mtDNA, results in the incorporation of a restriction digest site for PplfI (New England Biolabs: R0595S). Upon digest with PplfI, cleavage will occur in animals with BALB/c mtDNA, but not in those with FVB/NJ mtDNA (Supplementary Fig. S1B). Presence of the transgene was determined using the strain-specific standard PCR genotyping protocol from the product page of Jackson Labs. Briefly, total DNA isolated from tail clips was utilized in a multiplexed PCR reaction using primers for the transgene as well as an internal reference site (Supplementary Table S1).

Determining the cytoplasmic and mtDNA effects on tumorigenesis and metastasis

Her2 female mice were bred with eighth-generation FVB/NJ–mtMNX(C57BL/6J) or FVB/NJ–mtMNX(BALB/c) males. Offspring were genotyped to ensure mitochondrial homoplasy and presence of the transgene. N1 males were bred back to Her2 female mice. Female second-generation offspring were genotyped to ensure homoplasy and presence of transgene. Because mtDNAs are inherited maternally, offspring had FVB/NJ nuclear and mtDNA. This breeding scheme allowed assessment of long-lived cytoplasmic factors (i.e., not mtDNA) on tumor behavior.

To determine mtDNA effects on Her2 tumor development and progression to metastasis, male mice expressing Her2 were bred with eighth-generation FVB/NJ–mtMNX(C57BL/6J) or FVB/NJ–mtMNX(BALB/c) females. For all experiments, offspring were genotyped to ensure mitochondrial homoplasy and presence of transgene. These females were backcrossed to parental Her2 male mice. Female second-generation offspring were genotyped to ensure homoplasy and presence of transgene. In this breeding scheme, Her2 mice would inherit mtDNA from the female MNX, thereby allowing assessment of mtDNA contributions to tumor development, latency, and metastasis.

Tumorigenicity

Beginning at 8 weeks, all mammary glands were palpated daily, and the date of tumor onset was noted. Time from birth to tumor onset was recorded. Significance of tumor latency differences between groups was determined using the Kruskal–Wallis one-way ANOVA on Ranks test followed by the Dunn’s Method, a pairwise multiple comparison procedure.

To determine tumor growth rates, the length (longest diameter) and width (diameter orthogonal to length) of the first tumor to arise were measured weekly for 75 days following initial palpation. Tumor growth rate was determined as previously described (38). Briefly, tumor volumes were calculated using the modified formula for an ellipsoid: \( V = \frac{4}{3} \pi \frac{d_1 d_2 d_3}{6} \). Since the rate-based model assumes exponential growth, these measurements were log transformed, low volumes (<50 mm\(^3\)) were truncated, and log volume was plotted versus time. Slopes were calculated and compared between groups using the Kruskal–Wallis test. Onset of additional tumors was noted and measured, but not utilized to determine growth rates.

Metastasis assays and analysis

Mice were euthanized 75 days after tumor onset. Lungs were harvested, stained for 1 hour in a diluted Bouin’s Solution (Sigma-Aldrich: HT10132 diluted into 5 parts 10% neutral-buffered formalin), and rinsed twice in PBS. Because 90% of lung metastases are visible on the lung surface (39), metastatic lesions were counted utilizing a dissecting microscope. Due to the spherical nature of pulmonary metastases (39), the diameter of each metastasis was measured using an ocular micrometer. Nonparametric Kruskal–Wallis tests, followed by the Dunn’s method, were used to assess the statistical significance of differences in the number and size of metastases between groups.

Histology

Upon euthanasia, primary tumors and lungs were harvested and placed into Bouin’s fixative. After quantifying metastases, lungs were rinsed 3 times in PBS and then placed into a neutral-buffered formalin solution. Primary tumors were fixed directly in 10% neutral-buffered formalin. Fixed tissues were processed, paraffin-embedded, and sectioned (5 μm) before staining with hematoxylin (Thermo Fisher Scientific, NC9964763) and eosin-Y (Thermo Scientific, 71304) using standard methods. Immunohistochemistry was performed on adjoining sections for the angiogenesis markers CD31 (Abcam, ab28364 at 1:50) and CD105 (Abcam, ab107595 at 1:100), and the
epithelial–mesenchymal transition (EMT) markers E-Cadherin (Cell Signaling Technology, 3195 at 1:400), Vimentin (Cell Signaling Technology, 5741 at 1:100), and SNAIL (Abcam, ab53519 at 1:75).

**Results**

Long-lived cytoplasmic components do not influence Her2 tumor latency or metastasis

Although mtDNA has been implicated in the regulation of tumor development and progression (26, 40–42), it has been challenging to dissect the precise contributions, largely because the models have been limited. Off-target effects of pharmacologic inhibitors, exposure to mutagenic agents, and challenges associated with cross-over between nuclear genomes have been raised as possible limitations for previously published studies (27, 28). The MNX strains overcome those limitations and take advantage of exclusive maternal inheritance of mtDNA. However, during the process of MNX generation, oocytes are enucleated prior to transfer of a new nucleus from a donor strain.

While mitochondria remain, so too do other components of the cytoplasm. Although unlikely that long-lived cytoplasmic factors could be responsible for changes in tumorigenicity or metastasis, especially since the studies reported here utilized F2 generation mice, male FVB/NJ-mtMNX(C57BL/6J) and FVB/NJ-mtMNX(BALB/cJ) MNX mice were crossed with Her2 mice (Fig. 1A and B). Male mice would, therefore, not pass on the C57BL/6J or BALB/cJ mtDNA, but would still contribute other cytoplasmic components.

No differences in tumor latency of second (N2) generation offspring from FVB/NJ-mt MNX(C57BL/6J) (n = 31) or FVB/NJ-mtMNX(BALB/cJ) (n = 31) founders compared with Her2 mice crossed to parental FVB/NJ males (Fig. 1C). Metastases also showed no significant differences between the N2 offspring and Her2 mice regarding mean number (Fig. 1D; FVB/NJ, n = 54, mean 15 ± 2; C57BL/6J, n = 23, mean 10 ± 2; BALB/cJ, n = 18, mean 8 ± 2); E, Lung metastases were measured using ocular micrometer (FVB/NJ, mean 1.00 ± 0.02 mm; C57BL/6J, mean 0.90 ± 0.03 mm; BALB/cJ, mean 0.88 ± 0.03 mm). Bottom of boxes are 25th percentile, top of boxes are 75th percentile, midline is median value, whiskers are maximum and minimum values of upper and lower fences, respectively, and dots represent statistically determined individual outliers.

Figure 1. Cytoplasmic factors from parental nuclear transfer do not alter tumor latency or metastatic burden. A, Control mice were generated by breeding male and female Her2 transgenic mice. Female offspring were utilized in experiments. B, To generate mice without transfer of mitochondrial DNA, female Her2 mice were bred to male MNX with the same nuclear background (FVB) and C57BL/6J or BALB/cJ mitochondrial DNA. Male offspring were backcrossed to female Her2 mice, and second-generation female pups were utilized in further experiments. Importantly, all mice share FVB/NJ nuclear and mitochondrial DNA, but any putative long-lived cytoplasmic factors from parental C57BL/6J or BALB/cJ would be inherited from the father. C, N2 offspring from mice mated as shown in A and B were observed for tumor latency (i.e., time to first palpable mammary tumor). Each dot represents an individual mouse; black bars indicate mean (FVB/NJ, n = 57, 225 ± 5 days (mean ± SEM); C57BL/6J, n = 31, 219 ± 5 days; BALB/cJ, n = 31, mean 238 ± 6 days). D, To analyze metastasis, N2 offspring were aged 75 days past first tumor onset; visible lung metastases were counted (FVB/NJ, n = 54, mean 15 ± 2; C57BL/6J, n = 23, mean 10 ± 2; BALB/cJ, n = 18, mean 8 ± 2).
mtDNA influences Her2 tumor latency and metastasis

To determine whether mtDNA effects are driver dependent, FVB/NJ-m^MNX(C57BL/6J) and FVB/NJ-m^MNX(BALB/cJ) MNX strains were chosen to mimic previous studies with the transgenic polyoma middle-T oncogene (8, 27). MNX mice were crossed with transgenic mice sharing the same nuclear background (i.e., FVB/NJ) with the Her2 oncogenic driver. This strain was chosen because Her2 is overexpressed and not mutated. Although not amplified, this strain of mouse mimics overexpression that is often observed in human breast cancers of the HER2 subtype. Mammary gland overexpression of the proto-oncogene neu is driven by the mammary tumor virus promoter and forms focal tumors in approximately half of females with a relatively long latency. Of tumor-bearing female mice, 72% go on to form lung metastases (43). To generate homozygosity of the transgene with selective mtDNA, a two-generation breeding protocol was employed. N2 generation female offspring were followed for tumor latency and growth rate, as well as metastasis (Fig. 2A and B). Figure 2C showed that both FVB/NJ-m^MNX(C57BL/6J) and FVB/NJ-m^MNX(BALB/cJ) mice exhibited statistically longer tumor latency compared with FVB/NJ mice. FVB/NJ-m^MNX(BALB/cJ) took significantly greater time to form tumors than even FVB/NJ-m^MNX(C57BL/6J) mice, with an average difference of 31 days (Table 1).

Several different endpoints could have been employed for this metastasis study. Since mitochondria altered the time-to-tumor onset, choosing to euthanize at a set age could have biased metastasis results by giving some tumors more or less time to metastasize. In addition, the effects of mitochondrial haplotype on tumor growth rate were not yet known, so euthanasia at a set tumor diameter was also eliminated. To limit confounding effects, mice were euthanized at a set number of days past first tumor onset. Subsets of mice were euthanized at 30, 60, 90, and 120 days after tumor onset. It was determined that 75 days gave sufficient numbers of metastasis to observe variations while minimizing issues associated with morbidity. Both age and tumor volumes were recorded at euthanasia, however, upon review; neither factor altered the mitochondrial effects shown here.

To determine if mtDNA affected growth rates of Her2 tumors, subsets of mice were euthanized at 30, 60, 90, and 120 days after tumor onset. It was determined that 75 days gave sufficient numbers of metastasis to observe variations while minimizing issues associated with morbidity. Both age and tumor volumes were recorded at euthanasia, however, upon review; neither factor altered the mitochondrial effects shown here.

To explore whether mitochondrial haplotype affected metastasis, mice were euthanized 75 days after first tumor onset. Then, surface lung metastatic lesions were counted and measured. There are statistically fewer metastases (P < 0.001) in both the C57BL/6J and BALB/cJ mitochondrial mice than the FVB/NJ mitochondrial mice, with the fewest in C57BL/6J (Fig. 4A and Table 1). When diameters of metastatic lesions in Her2 mice were compared (Fig. 4B), both the C57BL/6J and BALB/cJ mitochondrial mice had larger metastases (mean diameter, 1.2 mm and 1.4 mm, respectively; Table 1) than the FVB/NJ mitochondrial mice (mean diameter, 1.0 mm). These data show that both overall number and the size of metastatic lesions were affected by mitochondrial DNA.

Table 1. Her2 tumor latency and lung metastatic burden are altered by mtDNA

<table>
<thead>
<tr>
<th>m^DNA</th>
<th>Latency (days)</th>
<th>N° mice with lung metastases/N° mice developing mammary tumors</th>
<th>N° pulmonary metastases per mouse</th>
<th>Metastasis diameter (mm)</th>
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<tr>
<td>FVB/NJ</td>
<td>225 ± 5</td>
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<td>15 ± 2</td>
<td>1.00 ± 0.02</td>
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<td>C57BL/6J</td>
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<td>26/78</td>
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<td>BALB/cJ</td>
<td>293 ± 6</td>
<td>29/119</td>
<td>7 ± 1</td>
<td>1.41 ± 0.04</td>
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*Time to palpable detection of the first mammary tumor. Tumor incidence was statistically indistinguishable between experimental groups.

Table 1 continued...

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*Mean ± SEM.
Hematoxylin and eosin sections of tumors arising in all MNX strains reveal densely packed, small cell tumors with relatively uniform morphologies, little residual normal gland, and some ischemic central necrosis. The borders of primary tumors are uniform and exhibit pushing, expansive growth with few areas of discrete invasion. The numbers of mitotic cells, while appreciable, are not particularly high. There are relatively few infiltrating immune cells noted, even in necrotic lesions (Fig. 5A–F). Morpologies of tumor cells are typical for HER2 human tumors. Similarly, lung metastases reveal a typical growth pattern for HER2 tumors, i.e., expansive growth prior to extravasation. There are multiple well-developed, but small (i.e., microscopic) metastases in all groups. Some larger metastases exhibit evidence of ischemic necrosis. The same patterns and histologic morphologies are present for all MNX strains in the lung metastases as well.

To evaluate whether differences in EMT might have been responsible for the observed changes in tumor latency or metastasis, sections of primary tumors and lung metastases were stained for E-cadherin (Fig. 5J–L), vimentin (Fig. 5M–O), and the EMT-associated transcription factor SNAIL. Although some differences in staining patterns were observed in individual sections, a consistent pattern was not noted between tumors or metastases arising in the MNX mice. As with the hematoxylin and eosin-stained sections, paired primary tumor and lung metastasis immunohistochemical staining patterns were similar in all groups. Both E-cadherin and vimentin staining were observed in clusters of cells in the lung metastases. Differences in angiogenesis were assessed by staining with CD105 or CD31 (Fig. 5P–R). No consistent differences in vessel density or staining pattern were consistently observed between experimental groups.

**Discussion**

Previous studies implicating mitochondrial genetics in tumorigenesis and metastasis were limited because of potential technical concerns related to off-target effects of drugs or mutagen activity. Our prior study demonstrating that MNX crosses with the PyMT mouse, while showing near identical results with whole genome (i.e., both nuclear and mtDNA) crosses, was limited in that only a single oncogenic driver was utilized (27). This study was undertaken to ask whether mammary tumorigenesis and metastasis are affected by mitochondrial haplotype in a driver-dependent manner. By utilizing MNX mice, contributions of mtDNA effects can be distinguished from phenotypes occurring due to nuclear admixing because the nuclear genomes are identical. As expected, tumor latency and metastasis were affected by mitochondrial haplotype in a driver-dependent manner. By utilizing MNX mice, contributions of mtDNA effects can be distinguished from phenotypes occurring due to nuclear admixing because the nuclear genomes are identical. As expected, tumor latency and metastasis were affected by mtDNA. However, the magnitude and direction of change were distinct from those observed in previous studies using different tumor types and experimental models.

To increase confidence that the observed phenotypic changes are due to mtDNA, and no other long-lived cytoplasmic factors retained in MNX mouse colonies, male MNX mice were crossed to...
Her2 females. Taking advantage of exclusive maternal inheritance of mtDNA, the progeny would inherit any hypothetical cytoplasmic factors, but would not transmit mtDNA, unless such factors are also exclusively maternally transmitted. As expected, neither changes in tumorigenesis nor metastasis were observed in this breeding scheme, supporting the roles of mtDNA in regulating both phenotypes. Furthermore, the data strongly suggest that nuclear genomes were not permanently altered in the parental generation.

Somewhat unexpectedly, extension or shortening of mammary tumor latency was not the same when tumors were initiated with Her2 and PyMT but under mitochondrial backgrounds (27). For example, mammary tumors in Her2 mice took 30% longer to develop their first tumor in the presence of BALB/cJ mtDNA, whereas PyMT-driven mammary tumors developed 7% faster than FVB mitochondrial mice (27). In contrast, mammary tumors developed later in FVB/NJ mice harboring C57BL/6J mtDNA in both the Her2 (16% longer) and PyMT (1.3% longer) transgenic mice. These data indicate that mtDNA is able to affect tumor latency, but it does so in an oncogene-dependent manner.

Once Her2 or PyMT tumors initiated, no differences in growth rates were observed between wild-type versus tumors growing in MNX mice. Thus, once formed, mitochondria did not appear to influence the rate of tumor growth under either oncogenic driver, indicating mtDNA's role in tumorigenesis may be limited to early stages. Further study into the effects of mtDNA on transformation and carcinogenic processes is needed.

Mitochondria from C57BL/6J mice had the fewest metastases, compared with both BALB/cJ and FVB/NJ mitochondrial mice. MNX mice with BALB/cJ mtDNA still had significantly fewer metastases than FVB/NJ mice. These results strikingly contrast to crosses with PyMT, which did not show significant differences in numbers of metastases between any of the groups. As observed with MNX crosses with the PyMT mice, lung metastasis size changed in Her2-driven tumor depending upon mitochondrial haplotype. Under conditions in which the Her2 proto-oncogene was over-expressed, the FVB/NJ mitochondrial group had the smallest metastases. While with the PyMT oncogene, the C57BL/6J
mitochondrial group had the smallest metastases. Again, metastatic seeding and colonization are influenced by mtDNA, and both processes are dependent upon the oncogenic driver.

The Her2 and PyMT oncogenes have very different defining characteristics. Although both tumors are driven by the MMTV promoter, Her2 mice have relatively long mammary tumor latency (~4 months) and inefficient progression to metastasis (~37% of females with metastasis at 8 months of age; ref. 25). In contrast, PyMT has rapid tumor onset (~30 days) and high penetrance of metastasis (~94% of females presenting with metastasis at 3 months; ref. 26). The former develops tumors mimicking the Her2, subtype of human breast cancers, while the latter more closely mimic luminal breast cancers. Thus, they represent distinct tumor types with different behaviors.

Differences in primary tumor growth rates or the number and size of metastases could have been, at least partly, explained by differences in angiogenic responses in various MNX strains. Preliminary immunohistochemical staining of primary tumors and lung metastases did not reveal differences in vessel density or staining patterns (Fig. 5P-R), suggesting that the differences in growth and metastasis are not explained by elicitation of angiogenesis. Other explanations for the differences in metastasis number and size are many, but include timing of earliest dissemination (i.e., cells from C57BL/6J or BALB/cJ MNX mice disseminate and seed the lung earlier), embolization when disseminating, interactions between tumor cells with different mitochondria responding differently to the metastatic microenvironment, and altered immune responses to tumors arising with different mitochondrial composition. Additional studies will be required to assess these alternatives.

The results presented here confirm previous studies implicating mitochondrial haplotypes as contributors to tumorigenicity and metastasis. They extend the previous reports by demonstrating that the mtDNA do so in an oncogenic driver-dependent manner (summarized in Fig. 6). Future studies exploring mtDNA modifications of triple-negative, basal, inflammatory, mucinous, or other breast cancer subtypes will be needed to refine what roles, if any, they play in those types of cancer. In addition, the roles of mtDNA in regulating behaviors of other tumor types remain an area of much needed investigation.

Tumor formation and progression involve differential regulation and/or mutation of multiple genes (1, 44). To date, there is no solid evidence implicating mutations in mtDNA as drivers of tumor formation; however, there are some nonrandom tumor progression-associated changes in mtDNA reported for some tumor types (26, 45, 46). Thus, mtDNA is more likely a contributor to controlling tumor latency or metastasis predicated on active mitochondrial-nuclear cross-talk. This interpretation is supported by our recent demonstration that mtDNA haplotypes selectively modify nuclear genomic DNA methylation (36). As mitochondria might regulate methylation substrates (hence global methylation), the mechanisms controlling specificity remain undetermined.

The penetrance of mtDNA influence on complex phenotypes, like tumor formation, progression, and metastasis, could be related to the kinetics of the oncogenic drivers, i.e., longer exposure of cells to mtDNA-mediated signals may influence cellular behavior. With the vastly different oncogenic phenotypes of the Her2 and PyMT models, it seems logical that mitochondrial effects on tumorigenesis and metastasis are dependent upon the driver(s) present. Recent studies from the Altieri lab implicate mitochondrial localization as a key regulator of the metastatic phenotype (40, 47, 48). Coordination of mitochondrial position requires interactions between these organelles and other cytoplasmic (nuclear-encoded) structures, further highlighting mitochondrial-nuclear cross-talk. In addition, it is intuitive that mitochondria sense energy sources and communicate to nuclei to assess and respond to energetic needs. Although numerous molecules have been implicated in this bidirectional communication (32, 49–51), the relative importance of each and context dependency have not yet been defined. Although topics of compelling interest, we have not yet defined parameters associated with mitochondrial localization, mitochondrial shape, or fusion/fission in the MNX models.

Although the genetic crosses presented here and elsewhere have interpreted the phenotypic changes as being intrinsic to the tumor cell population, all cells—both tumor and stroma—have the mtDNA manipulated in MNX mice. Therefore, readers are cautioned against assuming that the mtDNA effects are entirely due to interactions in tumor cells only.

Data presented here underscore the importance of mitochondrial genomic interactions with nuclear oncogenic drivers. Defining the signaling responsible requires development of methods to selectively manipulate the mitochondrial genome. While under development (52–56), there are currently no genome editing tools that can completely knock out or mutate all copies of a single mitochondrial gene due to the complexity and redundancy of the mitochondria genome. This complicates our ability to define exactly which variants or groups of variants in each mitochondrial haplotype are responsible for the different susceptibilities to tumorigenesis and metastasis. However, as these tools are refined, it is our hope that this research can pinpoint inherited mitochondrial variants, which, when combined with nuclear variants, would better predict patient outcomes and aid in treatment planning.

Disclosure of Potential Conflicts of Interest

D.R. Welch has ownership interest (including patents) in a patent for MNX mice. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: A.E. Brinker, R.A. Jensen, D.R. Welch
Development of methodology: A.E. Brinker, D.R. Welch
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.E. Brinker, C.J. Vivian, T.T. Tsue, R.A. Jensen, D.R. Welch

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Figure 6.
Mitochondrial DNA effects on tumor latency and metastasis are oncogenic driver-dependent. Male PyMT or Her2 mice on the FVB genetic background were crossed with female MNX mice with FVB nuclear background and either C57BL/6J or BALB/cJ mtDNA. Tumor latency, number of macroscopic lung metastases, and the size of surface lung metastases were recorded for each mouse. The direction of change is depicted by the arrows. Data for PyMT mice from ref. 27.
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.E. Brinker, C.J. Vivian, D.C. Koestler, R.A. Jensen, D.R. Welch

Writing, review, and/or revision of the manuscript: A.E. Brinker, C.J. Vivian, D.C. Koestler, D.R. Welch

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.E. Brinker, C.J. Vivian, D.R. Welch

Study supervision: D.R. Welch

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References


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19. Liao XS, Small WC, Sere PA, Butow RA. Intramitochondrial functions regulate nonmitochondrial citrate synthase (CIT2) expression in Saccha-
20. Luo Y, Bond JD, Ingram VM. Compromised mitochondrial function leads
to increased cytosolic calcium and to activation of MAP kinases. Proc Natl
21. Panikh VS, Morgan MM, Scott R, Clements LS, Butow RA. The mitochon-
drial genotype can influence nuclear gene expression in yeast. Science
Mitochondrial genomic backgrounds affect nuclear DNA methylation and
23. Bayona-Bafaluy MP, cin-Perez R, Mullikin JC, Park JS, Moreno-Loshuertos
rate analysis and efficient experimental design for tumor xenograft studies.
25. Welch DR, Neri A, Nicolson GL. Comparison of ‘spontaneous’ and
‘experimental’ metastasis using rat 13762 mammary adenocarcinoma cell
26. Hayashi JI, Hashizume O, Ishikawa K, Shimizu A. Mutations in mitochon-
drial DNA regulate mitochondrial diseases and metastasis but do not
27. Berridge MV, Dong L, Neuzil J. Mitochondrial DNA in tumor initiation,
progression, and metastasis: role of horizontal mtDNA transfer. Cancer Res
28. Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ.
Expression of the neu protooncogene in the mammary epithelium of trans-
suppressors in breast cancers: mechanistic insights and clinical potential.
30. Kenny TC, Germain D. mtDNA, metastasis, and the mitochondrial unfold-
missense mutation of mitochondrial ND6 gene promotes cell migration
and invasion in human lung adenocarcinoma. BMC Cancer 2015;
15:346.
neuronal network of mitochondrial dynamics regulates metastasis. Nat
33. Chau NC, Altieri DC. Cancer cells exploit adaptive mitochondrial
dynamics to increase tumor cell invasion. Cell Cycle 2015;14:
3242–7.
34. Saki M, Prakash A. DNA damage related crosstalk between the nucleus and
35. Hsu CC, Tseng LM, Lee HC. Role of mitochondrial dysfunction in cancer
36. Delisle R, Kachhap S, Ambazhagan R, Gabrielson E, Singh KK. Nuclear genes
involved in mitochondria-to-nuclear communication in breast cancer cells.
37. Minczuk M, Papworth DA, Miller JC, Murphy MP, Klug A. Development of
a single-chain, quasi-dimeric zinc-finger nuclease for the selective degra-
dation of mutated human mitochondrial DNA. Nucleic Acids Res 2008;
36:3926–38.
38. Gammage PA, Gaude F, Van HL, Rebelo-Guiomar P, Jackson CR,
Ronbach J, et al. Near-complete elimination of mutant mtDNA by
iterative or dynamic dose-controlled treatment with mtZFNs. Nucleic
40. Bacman SR, Williams SL, Pinto M, Peralta S, Moraes CT. Specific elimina-
tion of mutant mitochondrial genomes in patient-derived cells by mito-
41. Minczuk M. Engineered zinc finger proteins for manipulation of the human mitochondrial genome. Methods Mol Biol 2010;649:
257–70.
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