ELSEVIER

The role of mitochondrial DNA in breast tumors

Marjolein J.A. Weerts, Stefan Sleijfer and John W.M. Martens

Department of Medical Oncology and Cancer Genomics Netherlands, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, The Netherlands

Somatic variation in mitochondrial DNA (mtDNA) has been described in primary breast tumors, including single-nucleotide variants and variation in the number of mtDNA molecules per cell (mtDNA content). However, there is currently a gap in the knowledge on the link between mitochondrial variation in breast cancer cells and their phenotypic behavior (i.e., tumorigenesis) or outcome. This review focuses on recent findings on mtDNA content and mtDNA somatic mutations in breast cancer and the potential biological impact and clinical relevance.

Introduction

Our knowledge on the genetic makeup of breast cancer has been rapidly expanded by massive parallel sequencing of primary tumor specimens [1]. With this technique, major somatic alterations including single-nucleotide variants, small insertions or deletions, copy number variations and large structural variants have been characterized in the tumor chromosomes. So far, almost 100 tumor-driving genes have been identified [1]. Also, mutational signatures of base substitutions and rearrangements have been identified, pinpointing the processes shaping breast cancer genomes and paving the way toward new diagnostics and treatments [e.g., poly ADP ribose polymerase (PARP) inhibitors for cases carrying tumor genome signatures induced by defective homologous-recombination-based DNA double-strand break repair].

Often forgotten or overlooked in these findings is the mitochondrial DNA (mtDNA). Mitochondria are essential in multiple cellular processes, with energy production and initiation of apoptosis evident in the hallmarks of cancer [2]. mtDNA encodes proteins essential for the oxidative phosphorylation system and thus mitochondrial function. Nearly a century ago, the metabolic switch from oxidative phosphorylation to fermentation of tumor cells, even in the presence of oxygen, was described [3]. Despite the widely recognized importance of mitochondria in cancer and the role of mtDNA in mitochondrial function, so far only a few studies have explored mtDNA in large cohorts of human cancers [4–8]. This review focuses on recent findings on mtDNA content and mtDNA somatic mutations in breast cancer and their potential biological impact and clinical relevance.

mtDNA

Human mtDNA is gene-dense; it is only ~17 000 base pairs in size but encodes 13 proteins, as well as two ribosomal RNAs and 22 transfer RNAs functioning in the mitochondrial protein translation apparatus (gene density of 1 per ~0.45 kilobases). By contrast, the 23 chromosomes of the nuclear DNA (nDNA) comprise ~3 billion base pairs and >20 000 genes (gene density of 1 per ~120 kilobases). Characteristic for mtDNA is that multiple copies of mtDNA can reside in a single mitochondrion, and that multiple mitochondria can reside in a single cell. As a result, the number of mtDNA molecules per cell (mtDNA content) is highly variable between tissue types [9–11]. Generally, the mtDNA content is dependent on the energy demand of a cell; for example, skeletal muscle and liver cells (high energy demand) harbor thousands of mtDNA molecules but blood lymphocytes (lower energy demand) contain only hundreds of mtDNA molecules [12]. This cell-type-specific mtDNA content is assumed to be fairly stable under physiological conditions but can be altered by stress such as exogenous toxins [13] or viral infection [14]. Also, the mutation rate of mtDNA is several orders of magnitude higher than that of nDNA [15,16],

Corresponding author: Weerts, Marjolein J.A. (m.weerts@erasmusmc.nl)

https://doi.org/10.1016/j.drudis.2019.03.019

mainly attributed to the fidelity of the mitochondrial DNA polymerase (POLG) [4,17,18]. The polyploid nature of mtDNA combined with its mutation rate invokes the concept of heteroplasmy – the state where genetically different mtDNA molecules reside within a single cell or even within a single mitochondrion. Heteroplasmy patterns within an individual can differ between tissues [19–22] (Fig. 1), where a heteroplasmic mtDNA variant can either be present in only one tissue within an individual or in multiple tissues but at variable heteroplasmic allele frequencies.

mtDNA changes in cancer

The mtDNA content among human cancers appears to be highly variable (Fig. 2a). Increases and decreases in mtDNA content compared with tumor-adjacent tissue have been described for different tumor types [7]. With respect to mutations, somatic single-nucleotide variants are more common than somatic small insertions or deletions in the tumor mtDNA [4–6,8]. The major process shaping the mutational signature of those single nucleotide variants is replication-coupled [4], owing to the fidelity of POLG, similar to the process shaping the germline mtDNA polymorphic variation. Among different tumor types the number of somatic mtDNA single-nucleotide variants (mutational burden) per tumor does not vary extensively (median between 0 and 3 per tumor) (Fig. 2b), similar to the number of heteroplasmic mtDNA variants in tissues within an individual (median between 1 and 4 per tissue) (Fig. 1). This is different compared with the nuclear somatic mutational burden per tumor, where the median mutational burden varies roughly between 0.5 and 10 mutations per megabase (dependent on tumor type) [23]. The mtDNA mutational burden is thus in the order of 10- to 100-fold greater than the nDNA mutational burden in human cancers (not taking into account the mtDNA content). The somatic mtDNA variants in primary tumors appear across the entire mtDNA. There is some recurrence on certain positions, which can be explained by the underlying mutational process (by POLG); but it cannot be excluded that mutational selection is involved giving selective advantage to certain positions. Besides the mtDNA content and somatic mtDNA mutations, the mtDNA 'common deletion' has also been studied in cancer. This 4977 base-pair deletion occurs at recurring breakpoints within the mtDNA and is observed in several diseases. Although this deletion has been detected in tumor specimens, it is also frequently detected in non-neoplastic specimens such as tumor-adjacent normal tissue or blood [24] and its occurrence has been related to human aging as well [25,26].

A definite link between changes in mtDNA content or somatic mtDNA single-nucleotide variants and tumorigenesis or progression has never been established. In preclinical models depletion of mtDNA in tumor cells yields increased and decreased in vitro tumorigenic phenotypes [27–34], as well as gain and loss of tumorigenic potential in in vivo mouse xenografts [33–38]. Only recently has it been shown that the tumorigenic potential of cells depleted of mtDNA is dependent on restoring functional oxidative phosphorylation, via the acquisition of whole mitochondria and their mtDNA from surrounding cells [37,39,40]. However, the downregulation of oxidative phosphorylation has been associated

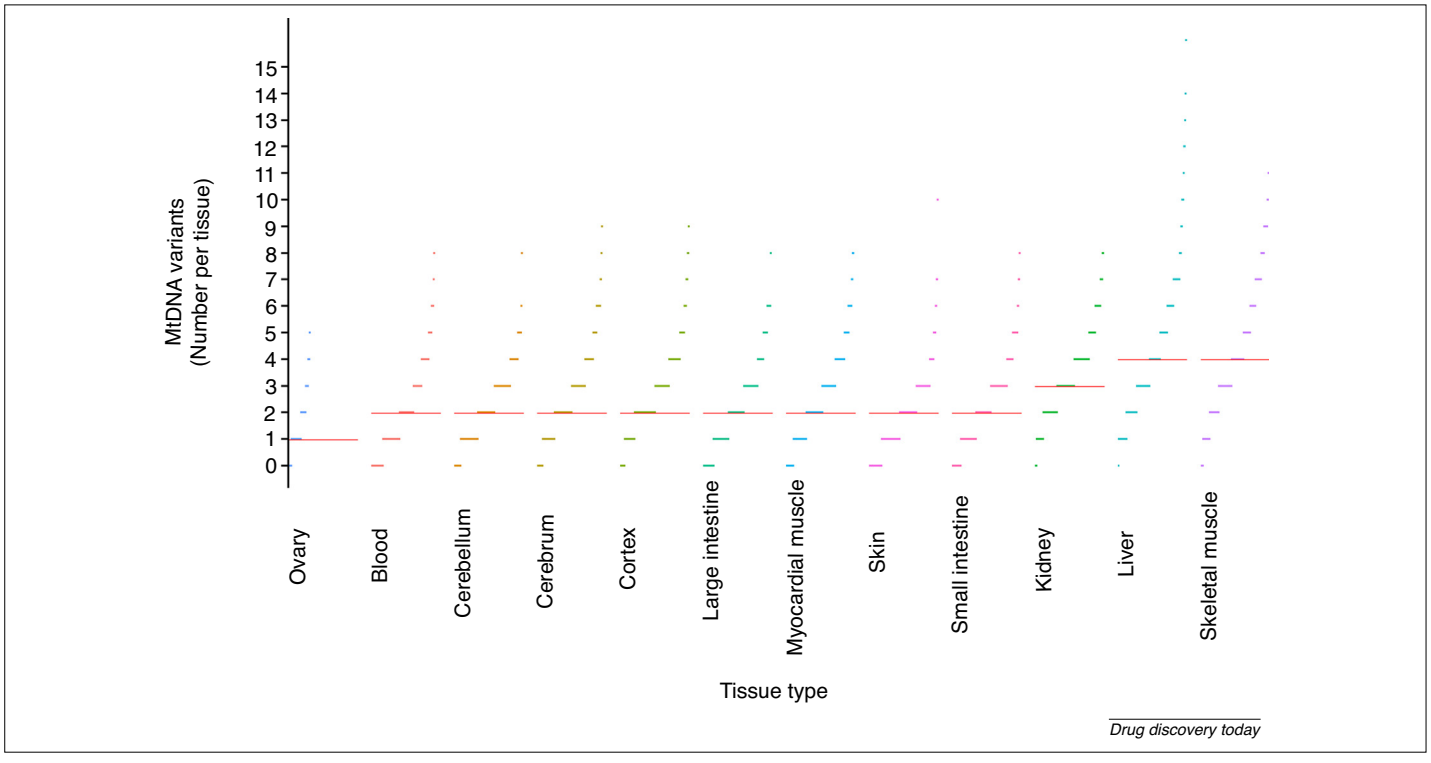

FIGURE 1

Number of somatic heteroplasmic mitochondrial DNA (mtDNA) variants across (noncancerous) human tissue types. Somatic heteroplasmic mtDNA variants (number per tissue per individual) within different tissue types obtained at autopsy (causes of death: 32.2% cardiovascular-related, 23% traumatic injuries, 21.7% natural causes, 10.5% intoxication and 12.5% unclear or other) as published by Li *et al.* [22]. Heteroplasmy levels of variants not shown (between 0.5% and 94.5% allele frequency).

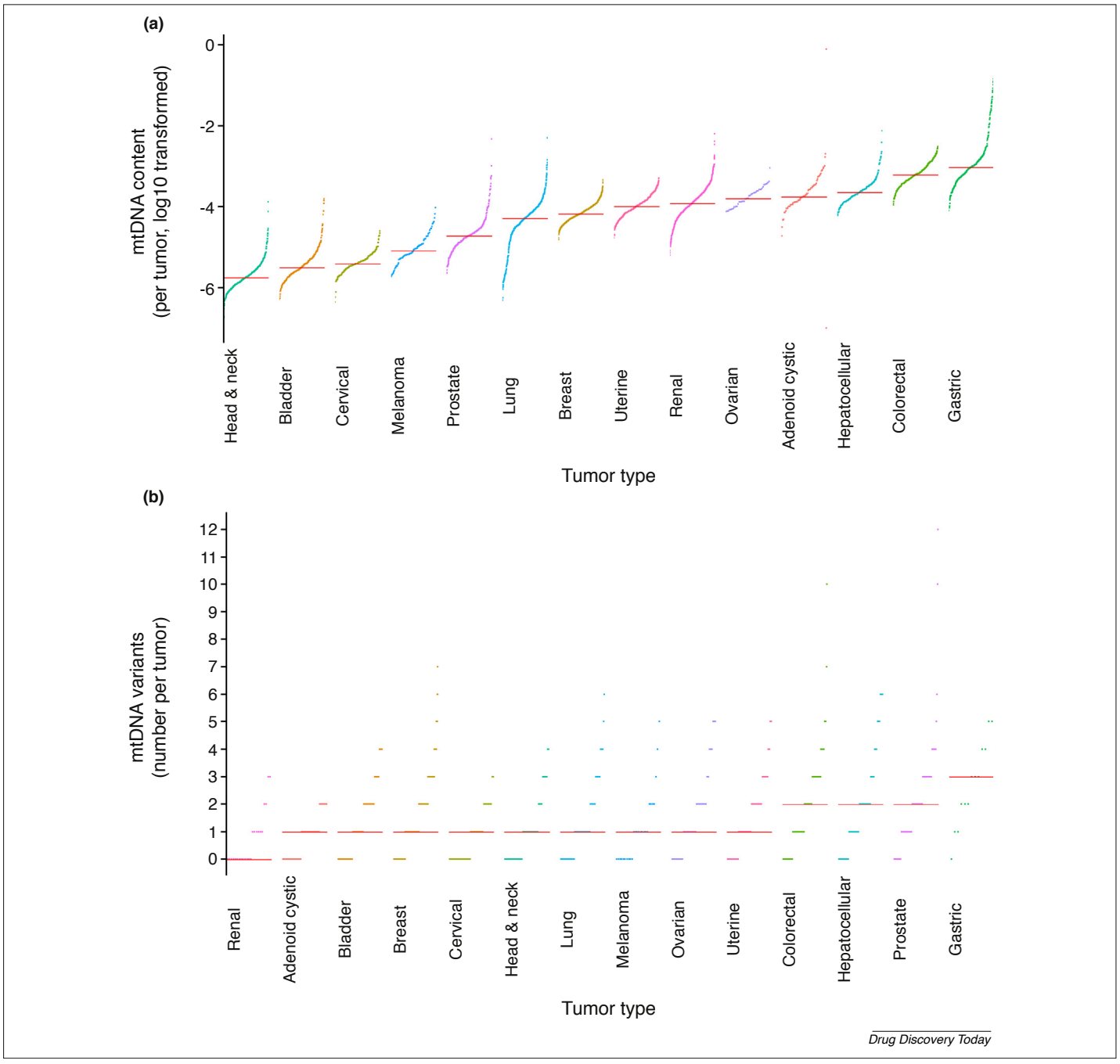

FIGURE 2

Mitochondrial DNA (mtDNA) content and number of somatic mtDNA variants across human cancer types. **(a)** mtDNA content (log10 transformed) within primary tumors as published by Reznik *et al*. [7] and **(b)** (heteroplasmic) somatic mtDNA variants (number per tumor per individual) within primary tumors as published by Ju *et al*. [4]. Heteroplasmy levels of variants not shown (between 3% and 100% allele frequency).

with poor survival in multiple cancer types [41]. Regarding somatic mutations in the mtDNA of primary tumors, only a minor proportion (<1%) overlap with mutations associated with mitochondrial disease and are thus known to affect mitochondrial function [4]. Examples exist in the literature where specific mtDNA variants (mainly at homoplasmy) or specific haplotypes have been shown to have an effect on tumorigenesis or metastatic potential in model systems by disrupting oxidative phosphorylation [42–44] (and reviewed in [45]). The occurrence of variants that disrupt oxidative phosphorylation (present either at hetero- or homoplasmy) has to date not been evaluated in large cohorts of human tumor specimens. It appears that mtDNA changes can act as a modifier in tumor cells. Heteroplasmy levels complicate interpretation of mtDNA changes detected in human tumor specimens. Negative selection on mtDNA mutations that potentially damage mitochondrial activity (e.g., protein truncating) has been described – nearly always present at heteroplasmy and seldom at homoplasmy – whereas mutations that appear to have no effect on

oxidative phosphorylation can drift toward homoplasmy [4,5]. Nevertheless, positive selection has also been described for somatic variants in the tumor [8]. Thus, there currently remains a gap in knowledge on somatic mtDNA variants and their biological significance in tumor formation and/or progression.

Clinical relevance of mtDNA content in breast cancer

In breast cancer, current clinical practice applies traditional prognostic markers [46] including age at diagnosis, tumor size, lymph node status and tumor grade to classify individual primary breast cancer patients for their risk of metastasizing and/or death [47–49], and they are used to determine whether or not an individual patient is advised to receive perioperative treatment. Moreover, presence of the estrogen receptor (ER) and/or progesterone receptor (PR), and amplification of *ERBB2* (HER2) in the primary tumor, classify patients in a certain risk group, but this particularly provides indication for respectively endocrine and anti-HER2 therapy [50]. Primary breast cancer patients who classify as high-risk based on the traditional clinicopathological markers receive perioperative chemotherapy and/or endocrine treatment, intended to eliminate potential micrometastases (curative setting). Recently, for patients with an inconclusive risk score the indication for perioperative systemic treatment can be based on gene-expression profiling [51]. In metastatic breast cancer patients, chemotherapy is also a major line of systemic treatment intended to prolong life (palliative setting). Here, anthracycline-based chemotherapy regimens are frequently given, although taxane-based regimens are also commonly used [52]. In addition to regimens consisting of traditional cytotoxic agents, also in the metastatic setting, several lines of endocrine (combination) and/or anti-HER2 treatment can be applied based on the presence of ER and/or PR or *ERBB2* amplification in the (metastatic) tumor [53–55].

Numerous studies have explored new biomarkers that might add to the currently available prognostic and predictive models in breast cancer, whereas only a few addressed whether alterations in mtDNA could be used for this purpose. A decrease in mtDNA content is frequently observed in primary breast tumors when compared with tumor-adjacent normal mammary epithelium (±70% of cases) [7,56–60]. No recurrent association has been described between the mtDNA content in the primary tumor and any of the traditional prognostic or predictive clinicopathological markers [7,56–64]. However, we have recently evaluated the association between the expression of mtDNA in the primary tumor and ER status and observed a positive association in two independent (*ERBB2* balanced) cohorts [65], indicating that metabolic differences might be present across breast cancer subtypes. In line with this, downregulation of nuclear-encoded genes involved in oxidative phosphorylation has been described for triple-negative breast cancer [66]. Nuclear effects of estrogen through ER on mtDNA transcription have been described (as reviewed in [67]), where ER indirectly upregulates expression of the mitochondrial transcription factor (TFAM). Our work has also shown that patients with the lowest mtDNA content in the primary tumor had a worse prognosis (10-year distant metastasis-free survival) in a retrospective cohort of primary breast cancer patients with lymph-node-negative disease who did not receive any perioperative systemic therapy [62]. Additionally, patients with low mtDNA content showed an improved outcome to adjuvant anthracycline-based chemotherapy in a retrospective cohort of lymph-node-positive disease (distant metastasis-free survival in the curative setting) as well as to first-line anthracycline-based chemotherapy in a retrospective cohort of patients with advanced disease (progression-free survival in the palliative setting) [63]. This did not apply to the patients treated with methotrexate-based chemotherapy in both settings [63]. These associations with outcome were independent of the above-mentioned clinicopathological markers [62,63]. The mechanisms underlying these associations remain to be established. A possible explanation might be based on the fact that anthracyclines induce severe oxidative stress [68] and are known to accumulate in mitochondria, where they can intercalate and damage mtDNA [69], whereas methotrexate is an antimetabolite, ultimately leading to inhibition of DNA synthesis, and induces only low levels of oxidative stress [68]. Elimination of damaged mtDNA has been described in response to oxidative stress [70]. Hypothetically tumor cells with low mtDNA content are more susceptible to stress induced to the mitochondria or mtDNA, such as those induced by anthracyclines, than cells with high mtDNA content. Other studies have also reported on breast cancer patient disease-free or overall survival in relation to tumorous mtDNA content [57,58,71,72], but those studies contained rather heterogeneous groups with either relatively small sample sizes or no information about systemic treatments administered, rendering interpretation and comparison between studies difficult. Larger cohorts of uniformly treated patients are necessary to validate these findings and to further unravel the clinical relevance of mtDNA content – or expression – quantification in breast cancer.

Nevertheless, the putative link between low mtDNA content and susceptibility to regimens that induce severe oxidative stress in mitochondria is interesting. This phenomenon probably does not typically apply to breast cancer but could also be of significance to other human cancers treated with similar regimens. Anthracyclines are also frequently used in the treatment of sarcoma and hematological malignancies, and these cancer types are therefore of interest to evaluate the link between mtDNA content in the tumor and outcome after chemotherapy. In addition, platinum-based chemotherapy also induces oxidative stress and changes to mtDNA [73,74], whereas bleomycin has been shown to damage mtDNA more extensively than it does nDNA [73,75], and thus also for these regimens mtDNA content is potentially a predictive marker. To take this even a little further, we could speculate that not only the tumor cells but also nontumor cells with low mtDNA content are more susceptible to anthracyclines or other regimens that induce severe oxidative stress. Interestingly, the endocrine agent tamoxifen has been shown to inhibit mtDNA replication and decrease the mtDNA content in the liver in in vivo model systems [76], similar to antiretroviral therapy which is known to decrease mtDNA content in peripheral blood cells of HIV-infected patients [77,78]. This could mean that heterogeneity in mtDNA content in nontumor cells might result in (i) intraindividual specific side-effects because certain tissues with low mtDNA content are affected to a larger extent and (ii) interindividual differences in toxicities experienced because some individuals might be more susceptible to treatment side-effects because of endogenous lower mtDNA content in their healthy tissues. Elaborating on this, one can imagine that also nuclear effects on mtDNA

content and/or expression can exert such differences on response and/or toxicity. Perhaps somatic variants in *ESR1* that alter the ER to be constitutively active also influence the nuclear effects on mitochondrial expression.

Off note, the mtDNA content in peripheral blood of breast cancer patients is higher compared with controls [79–82] and decreased mtDNA content in peripheral blood was associated with increased cancer-related fatigue in breast cancer patients [83]. However, because mtDNA content varies between cell types and blood composition was not analyzed, it should be evaluated whether the observed changes in mtDNA content reflect an alteration in blood composition in patients versus controls or patients suffering from cancer-related fatigue. Summarizing, we propose that the frequently observed decrease in mtDNA content in breast tumors, potentially mtDNA expression, but also mtDNA content and/or expression in nontumor tissues, could be exploited to guide chemotherapeutic regimen decision-making.

Somatic mtDNA variants in breast cancer

Somatic mtDNA variants are frequently observed in primary breast tumors (±70% of cases) [4,5,61,65]. Most of these variants are single-nucleotide variants and not small insertions or deletions. These variants are distributed along the entire mitochondrial genome, showing large heterogeneity among cases, and are acquired independently of the three major mutational processes shaping the nDNA within breast tumors [65]. So far, no somatic mtDNA mutations that clearly affect breast cancer tumorigenesis or progression have been described; however, some mtDNA haplogroups have been described as modifiers for metastatic potential [43,44].

No recurrent association has been described between the number of somatic mtDNA variants in the primary tumor and the traditional prognostic or predictive clinicopathological markers tumor size, lymph node status, tumor grade, ER and/or PR status, or *ERBB2* amplification status. However, an association between the number of somatic variants within the primary breast tumor and the age of diagnosis has been described [4,65,84]. An explanation for this association is the presence of allele-specific variation among tissues within an individual [19–22], which has also been associated with age [12]. Because the major process generating somatic variants as detected in tumor tissue also appears to be caused by the fidelity of the mitochondrial polymerase [4,65], the association with an older age implies that a large amount of the somatic variants detected in the tumor specimen are probably not tumor-acquired but already present or acquired in the normal mammary epithelial cell from which the tumor originated. Often no matched mammary epithelial tissue is available, so the distinction between somatic variants acquired in the mammary epithelial cells before cancer initiation and those truly acquired in the tumor cells cannot be made. This is a limitation frequently overlooked. Matched normal material commonly used in studies is blood, but (somatic) variants in blood are unlikely to adequately reflect the somatic mtDNA status in the mammary epithelial tissue from which the cancer arose. In addition, the tumor specimen commonly contains not only tumor cells but also other cells such as surrounding epithelial cells or immune cells infiltrating the tumor. In line with the allele-specific variation among tissues within an individual, we have shown extensive mtDNA heterogeneity between tumor and tumor-adjacent tissue in a small patient cohort of breast and colorectal cancer patients [85].

Importantly, the effect of somatic mtDNA variants in the tumor is based on the actual position and consequence of the variant(s) combined with their heteroplasmy level within the tumor cells, rather than the number of variants present. Only if variants have an effect on the mitochondrial oxidative phosphorylation system or another physiological process do they have the potential to influence a patient's cancer. Somatic variants that have no effect on a physiological process are merely bystanders. Here, somatic variants in the tumor might exert effects on tumor formation and/or progression but might also induce the above-mentioned susceptibility to a regimen that induces severe oxidative stress in mitochondria. Elaborating on this, not only somatic but also germline mtDNA variants can exert effects on the oxidative phosphorylation system [86–88]. An illustrative example is that of mtDNA haplogroups (containing certain mtDNA variants) with decreased mitochondrial respiration system coupling efficiency selected for in populations inhabiting colder climates, leading to decreased ATP generation in favor of increased heat production. Perhaps also certain mtDNA haplogroups exert increased or decreased response and/or risk of toxicity after certain anticancer treatments.

Although it could be attractive at first sight because of the multiple mtDNA copies and high mutation rate, the use of somatic mtDNA variants in cancer lineage tracing or as a blood-circulating biomarker is complicated given the large heterogeneity of mtDNA variants within an individual. First, it is difficult (if not impossible) to pinpoint the truly tumor-specific mtDNA variants, because of the potential 'founder effect'. Second, the dynamics of mtDNA content can exert bottleneck effects on somatic mtDNA variants when there is a (large) reduction in mtDNA content. Obviously, such founder- or bottleneck-effects not only apply to differences between the normal mammary epithelium but also to differences between primary tumor and metastases or among metastases. For now, it remains to be elucidated whether somatic mtDNA variants have biological significance in tumor formation and progression, or perhaps also in susceptibility to certain systemic treatment regimens and can be of added value in the clinic.

Concluding remarks

Patients diagnosed at a higher age harbor more somatic mtDNA variants in their primary tumor. Also, there is large heterogeneity in somatic mtDNA variants within primary breast tumors. Although mtDNA content in the primary breast tumor is not associated with any of the traditional clinicopathological markers, low mtDNA levels in the primary breast tumor indicate a more aggressive cancer, which appears more susceptible to anthracycline-based regimens. The question remains how mtDNA genotype (somatic and germline haplotype), heteroplasmy, expression and content are affected in metastatic disease. Also, it remains to be evaluated whether certain tumor-specific mtDNA variants, or mtRNA expression associated with the subtype, are connected with patient outcome and have any clinical importance.

The role of mtDNA variation in (breast) tumors has been largely neglected. A complete picture should be obtained by studying primary tumor and metastatic sites for their (somatic) mtDNA variation. The effects these variations have on mitochondrial

activity should be assessed by functional assays, such as measurement of the activity of the complexes of the oxidative phosphorylation system, generation of ATP or ability to induce the apoptotic pathway. When assessing such effects, one should also take into account germline and cell-lineage-specific somatic variation, tissue distribution, the interactions between the nuclear genome and the mitochondrial genome, and also tissue dependence on oxidative phosphorylation. Also, more insight is needed into the role of mtDNA variations in healthy tissue on toxicities and to what extent they might be associated with specific toxicities and interindividual differences. Although the pivotal work from Warburg describing the crucial role of mitochondria in cancer already dates from a century ago, mitochondria and their mtDNA deserve more attention now.

References

1 Nik-Zainal, S. *et al.* (2016) Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* 534, 47–54
2 Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell* 144, 646–674
3 Warburg, O. *et al.* (1927) The metabolism of tumors in the body. *J. Gen. Physiol.* 8, 519–530
4 Ju, Y.S. *et al.* (2014) Origins and functional consequences of somatic mitochondrial DNA mutations in human cancer. *Elife* 3, e02935
5 Stewart, J.B. *et al.* (2015) Simultaneous DNA and RNA mapping of somatic mitochondrial mutations across diverse human cancers. *PLoS Genet.* 11, e1005333
6 Larman, T.C. *et al.* (2012) Spectrum of somatic mitochondrial mutations in five cancers. *Proc. Natl. Acad. Sci. U. S. A.* 109, 14087–14091
7 Reznik, E. *et al.* (2016) Mitochondrial DNA copy number variation across human cancers. *Elife* 5, e10769
8 Grandhi, S. *et al.* (2017) Heteroplasmic shifts in tumor mitochondrial genomes reveal tissue-specific signals of relaxed and positive selection. *Hum. Mol. Genet.* 26, 2912–2922
9 Robin, E.D. and Wong, R. (1988) Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. *J. Cell Physiol.* 136, 507–513
10 Wiesner, R.J. *et al.* (1992) Counting target molecules by exponential polymerase chain reaction: copy number of mitochondrial DNA in rat tissues. *Biochem. Biophys. Res. Commun.* 183, 553–559
11 Legros, F. *et al.* (2004) Organization and dynamics of human mitochondrial DNA. *J. Cell Sci.* 117, 2653–2662
12 Wachsmuth, M. *et al.* (2016) Age-related and heteroplasmy-related variation in human mtDNA copy number. *PLoS Genet.* 12, e1005939
13 Mansouri, A. *et al.* (1999) An alcoholic binge causes massive degradation of hepatic mitochondrial DNA in mice. *Gastroenterology* 117, 181–190
14 Casula, M. *et al.* (2005) Infection with HIV-1 induces a decrease in mtDNA. *J. Infect. Dis.* 191, 1468–1471
15 Brown, W.M. *et al.* (1979) Rapid evolution of animal mitochondrial DNA. *Proc. Natl. Acad. Sci. U. S. A.* 76, 1967–1971
16 Lynch, M. *et al.* (2006) Mutation pressure and the evolution of organelle genomic architecture. *Science* 311, 1727–1730
17 Johnson, A.A. and Johnson, K.A. (2001) Exonuclease proofreading by human mitochondrial DNA polymerase. *J. Biol. Chem.* 276, 38097–38107
18 Nikolaou, C. and Almirantis, Y. (2006) Deviations from Chargaff's second parity rule in organellar DNA Insights into the evolution of organellar genomes. *Gene* 381, 34–41
19 Calloway, C.D. *et al.* (2000) The frequency of heteroplasmy in the HVII region of mtDNA differs across tissue types and increases with age. *Am. J. Hum. Genet.* 66, 1384–1397
20 He, Y. *et al.* (2010) Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. *Nature* 464, 610–614
21 Samuels, D.C. *et al.* (2013) Recurrent tissue-specific mtDNA mutations are common in humans. *PLoS Genet.* 9, e1003929
22 Li, M.K. *et al.* (2015) Extensive tissue-related and allele-related mtDNA heteroplasmy suggests positive selection for somatic mutations. *Proc. Natl. Acad. Sci. U. S. A.* 112, 2491–2496
23 Alexandrov, L.B. *et al.* (2013) Signatures of mutational processes in human cancer. *Nature* 500, 415–421
24 Nie, H. *et al.* (2013) Mitochondrial common deletion, a potential biomarker for cancer occurrence, is selected against in cancer background: a meta-analysis of 38 studies. *PLoS One* 8, e67953
25 Wei, Y.H. and Lee, H.C. (2002) Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp. Biol. Med.* 227, 671–682
26 Pavicic, W.H. and Richard, S.M. (2009) Correlation analysis between mtDNA 4977-bp deletion and ageing. *Mutat. Res.* 670, 99–102
27 Kulawiec, M. *et al.* (2008) Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. *Cancer Biol. Ther.* 7, 1732–1743
28 Singh, K.K. *et al.* (2005) Inter-genomic cross talk between mitochondria and the nucleus plays an important role in tumorigenesis. *Gene* 354, 140–146
29 Naito, A. *et al.* (2008) Progressive tumor features accompany epithelial-mesenchymal transition induced in mitochondrial DNA-depleted cells. *Cancer Sci.* 99, 1584–1588
30 Moro, L. *et al.* (2009) Mitochondrial DNA depletion in prostate epithelial cells promotes anoikis resistance and invasion through activation of PI3K/Akt2. *Cell Death Differ.* 16, 571–583
31 Guha, M. *et al.* (2014) Mitochondrial retrograde signaling induces epithelial-mesenchymal transition and generates breast cancer stem cells. *Oncogene* 33, 5238–5250
32 Pelicano, H. *et al.* (2006) Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. *J. Cell Biol.* 175, 913–923
33 Yu, M. *et al.* (2007) Depletion of mitochondrial DNA by ethidium bromide treatment inhibits the proliferation and tumorigenesis of T47D human breast cancer cells. *Toxicol. Lett.* 170, 83–93
34 Cavalli, L.R. *et al.* (1997) Diminished tumorigenic phenotype after depletion of mitochondrial DNA. *Cell Growth Differ.* 8, 1189–1198
35 Morais, R. *et al.* (1994) Tumor-forming ability in athymic nude mice of human cell lines devoid of mitochondrial DNA. *Cancer Res.* 54, 3889–3896
36 Magda, D. *et al.* (2008) mtDNA depletion confers specific gene expression profiles in human cells grown in culture and in xenograft. *BMC Genomics* 9, 521
37 Tan, A.S. *et al.* (2015) Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. *Cell Metab.* 21, 81–94
38 Guo, J. *et al.* (2011) Frequent truncating mutation of TFAM induces mitochondrial DNA depletion and apoptotic resistance in microsatellite-unstable colorectal cancer. *Cancer Res.* 71, 2978–2987
39 Bajzikova, M. *et al.* (2019) Reactivation of dihydroorotate dehydrogenase-driven pyrimidine biosynthesis restores tumor growth of respiration-deficient cancer cells. *Cell Metab.* 29, 399–416
40 Dong, L.F. *et al.* (2017) Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. *Elife* 6 . http://dx.doi.org/10.7554/eLife.22187
41 Gaude, E. and Frezza, C. (2016) Tissue-specific and convergent metabolic transformation of cancer correlates with metastatic potential and patient survival. *Nat. Commun.* 7, 13041
42 Ishikawa, K. *et al.* (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. *Science* 320, 661–664
43 Feeley, K.P. *et al.* (2015) Mitochondrial genetics regulate breast cancer tumorigenicity and metastatic potential. *Cancer Res.* 75, 4429–4436
44 Brinker, A.E. *et al.* (2017) Mitochondrial haplotype alters mammary cancer tumorigenicity and metastasis in an oncogenic driver-dependent manner. *Cancer Res.* 77, 6941–6949
45 Garcia-Heredia, J.M. and Carnero, A. (2015) Decoding Warburg's hypothesis: tumor-related mutations in the mitochondrial respiratory chain. *Oncotarget* 6, 41582–41599
46 Goldhirsch, A. *et al.* (2009) Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. *Ann. Oncol.* 20, 1319–1329
47 Olivotto, I.A. *et al.* (2005) Population-based validation of the prognostic model ADJUVANT! for early breast cancer *J. Clin. Oncol.* 23, 2716–2725
48 Ravdin, P.M. *et al.* (2001) Computer program to assist in making decisions about adjuvant therapy for women with early breast cancer. *J. Clin. Oncol.* 19, 980–991
49 Lundin, J. *et al.* (2006) Generalisability of survival estimates for patients with breast cancer–a comparison across two population-based series. *Eur. J. Cancer* 42, 3228–3235
50 Goldhirsch, A. *et al.* (2006) First—select the target: better choice of adjuvant treatments for breast cancer patients. *Ann. Oncol.* 17, 1772–1776

51 Krop, I. *et al.* (2017) Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology Clinical Practice Guideline focused update. *J. Clin. Oncol.* 35, 2838–2847
52 Piccart-Gebhart, M.J. *et al.* (2008) Taxanes alone or in combination with anthracyclines as first-line therapy of patients with metastatic breast cancer. *J. Clin. Oncol.* 26, 1980–1986
53 Giordano, S.H. *et al.* (2018) Systemic therapy for patients with advanced human epidermal growth factor receptor 2-positive breast cancer: ASCO Clinical Practice Guideline update. *J. Clin. Oncol.* 36, 2736–2740
54 Rugo, H.S. *et al.* (2016) Endocrine therapy for hormone receptor-positive metastatic breast cancer: American Society of Clinical Oncology Guideline. *J. Clin. Oncol.* 34, 3069–3103
55 Van Poznak, C. *et al.* (2015) Use of biomarkers to guide decisions on systemic therapy for women with metastatic breast cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J. Clin. Oncol.* 33, 2695–2704
56 Mambo, E. *et al.* (2005) Tumor-specific changes in mtDNA content in human cancer. *Int. J. Cancer* 116, 920–924
57 Yu, M. *et al.* (2007) Reduced mitochondrial DNA copy number is correlated with tumor progression and prognosis in Chinese breast cancer patients. *IUBMB Life* 59, 450–457
58 Tseng, L.M. *et al.* (2006) Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. *Genes Chromosomes Cancer* 45, 629–638
59 Fan, A.X. *et al.* (2009) Mitochondrial DNA content in paired normal and cancerous breast tissue samples from patients with breast cancer. *J. Cancer Res. Clin. Oncol.* 135, 983–989
60 Barekati, Z. *et al.* (2010) Methylation profile of TP53 regulatory pathway and mtDNA alterations in breast cancer patients lacking TP53 mutations. *Hum. Mol. Genet.* 19, 2936–2946
61 McMahon, S. and LaFramboise, T. (2014) Mutational patterns in the breast cancer mitochondrial genome, with clinical correlates. *Carcinogenesis* 35, 1046–1054
62 Weerts, M.J. *et al.* (2016) Mitochondrial DNA content in breast cancer: impact on in vitro and in vivo phenotype and patient prognosis. *Oncotarget* 7, 29166–29176
63 Weerts, M.J.A. *et al.* (2017) Low tumor mitochondrial DNA content is associated with better outcome in breast cancer patients receiving anthracycline-based chemotherapy. *Clin. Cancer Res.* 23, 4735–4743
64 Ebrahimi, E. *et al.* (2018) Mitochondrial DNA copy number instability in ERBB2-amplified breast cancer tumors. *EXCLI J.* 17, 149–158
65 Weerts, M.J.A. *et al.* (2018) Mitochondrial RNA expression and single nucleotide variants in association with clinical parameters in primary breast cancers. *Cancers* 10, 12
66 Guha, M. *et al.* (2018) Aggressive triple negative breast cancers have unique molecular signature on the basis of mitochondrial genetic and functional defects. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864, 1060–1071
67 Klinge, C.M. (2008) Estrogenic control of mitochondrial function and biogenesis. *J. Cell Biochem.* 105, 1342–1351
68 Conklin, K.A. (2004) Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. *Integr. Cancer Ther.* 3, 294–300
69 Ashley, N. and Poulton, J. (2009) Mitochondrial DNA is a direct target of anti-cancer anthracycline drugs. *Biochem. Biophys. Res. Commun.* 378, 450–455
70 Shokolenko, I. *et al.* (2009) Oxidative stress induces degradation of mitochondrial DNA. *Nucleic Acids Res.* 37, 2539–2548
71 Hsu, C.W. *et al.* (2010) Mitochondrial DNA content as a potential marker to predict response to anthracycline in breast cancer patients. *Breast J.* 16, 264–270
72 Bai, R.K. *et al.* (2011) Mitochondrial DNA content varies with pathological characteristics of breast cancer. *J. Oncol.* 496189
73 Lehle, S. *et al.* (2014) LORD-Q: a long-run real-time PCR-based DNA-damage quantification method for nuclear and mitochondrial genome analysis. *Nucleic Acids Res.* 42, e41
74 Podratz, J.L. *et al.* (2011) Cisplatin induced mitochondrial DNA damage in dorsal root ganglion neurons. *Neurobiol. Dis.* 41, 661–668
75 Lim, L.O. and Neims, A.H. (1987) Mitochondrial DNA damage by bleomycin. *Biochem. Pharmacol.* 36, 2769–2774
76 Larosche, I. *et al.* (2007) Tamoxifen inhibits topoisomerases, depletes mitochondrial DNA, and triggers steatosis in mouse liver. *J. Pharmacol. Exp. Ther.* 321, 526–535
77 Subashini, D. *et al.* (2018) Mitochondrial DNA content of peripheral blood mononuclear cells in ART untreated & stavudine/zidovudine treated HIV-1-infected patients. *Indian J. Med. Res.* 148, 207–214
78 Masyeni, S. *et al.* (2018) Evaluation of antiretroviral effect on mitochondrial DNA depletion among HIV-infected patients in Bali. *HIV AIDS* 10, 145–150
79 Lemnrau, A. *et al.* (2015) Mitochondrial DNA copy number in peripheral blood cells and risk of developing breast cancer. *Cancer Res.* 75, 2844–2850
80 Shen, J. *et al.* (2015) Peripheral blood mitochondrial DNA copy number, length heteroplasmy and breast cancer risk: a replication study. *Carcinogenesis* 36, 1307–1313
81 Campa, D. *et al.* (2018) Mitochondrial DNA copy number variation, leukocyte telomere length, and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Breast Cancer Res.* 20, 29
82 Hu, L. *et al.* (2016) Altered mitochondrial DNA copy number contributes to human cancer risk: evidence from an updated meta-analysis. *Sci. Rep.* 6, 35859
83 Chae, J.W. *et al.* (2018) Association of mitochondrial DNA content in peripheral blood with cancer-related fatigue and chemotherapy-related cognitive impairment in early-stage breast cancer patients: a prospective cohort study. *Breast Cancer Res. Treat.* 168, 713–721
84 Tseng, L.M. *et al.* (2011) Somatic mutations of the mitochondrial genome in human breast cancers. *Genes Chromosomes Cancer* 50, 800–811
85 Weerts, M.J.A. *et al.* (2018) Tumor-specific mitochondrial DNA variants are rarely detected in cell-free DNA. *Neoplasia* 20, 687–696
86 Wallace, D.C. and Chalkia, D. (2013) Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. *Cold Spring Harb. Perspect. Biol.* 5, a021220
87 Ruiz-Pesini, E. *et al.* (2004) Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* 303, 223–226
88 Mishmar, D. *et al.* (2003) Natural selection shaped regional mtDNA variation in humans. *Proc. Natl. Acad. Sci. U. S. A.* 100, 171–176