

# Review: Mitochondrial Defects in Breast Cancer

Josefa Salgado<sup>1</sup> Beatriz Honorato<sup>1</sup> and Jesús García-Foncillas<sup>1,2</sup>

<sup>1</sup>Clinical Genetics Unit, University Clinic of Navarra (CUN), Avda, Pio XII, 36, 31008 Pamplona, Navarra, Spain. <sup>2</sup>Laboratory of Pharmacogenomics, Centre for Applied Medical Research (CIMA), Avda, Pio XII 55, 31008 Pamplona, Navarra, Spain.

**Abstract:** Mitochondria play important roles in cellular energy metabolism, free radical generation, and apoptosis. Mitochondrial DNA has been proposed to be involved in carcinogenesis because of its high susceptibility to mutations and limited repair mechanisms in comparison to nuclear DNA. Breast cancer is the most frequent cancer type among women in the world and, although exhaustive research has been done on nuclear DNA changes, several studies describe a variety of mitochondrial DNA alterations present in breast cancer. In this review article, we provide a summary of the mitochondrial genomic alterations reported in breast cancer and their functional consequences.

**Keywords:** mitochondrial DNA, breast cancer, mutations, heteroplasmy

## Introduction

Mitochondria are cytoplasmic semi-autonomously functioning organelles, which contain a resident genome, and replicate, translate and transcribe their own DNA (mtDNA) (Anderson et al. 1981; Taanman, 1999). Each organelle contains 2–10 copies of mtDNA molecules, and each human cell contains thousands of copies which varies in number with the cell type (Chen and Butow, 2005). Mitochondria are responsible for generating approximately 90% of cellular ATP through the process of oxidative phosphorylation (OXPHOS) and play essential roles in cellular energy metabolism, free radical generation, and programmed cell death (Chan, 2006). Human mtDNA is a circular double-stranded molecule consisting of 16.571bp encoding 13 polypeptides that constitute respiratory chain complexes, as well as 2 rRNAs and a set of 22 tRNAs for mitochondrial protein synthesis (Anderson et al. 1981; Andrews et al. 1999). The mtDNA undergoes replication using a different origin for each of the two strands, the purine (guanine) abundant H-strand, and the pyrimidine (cytosine) abundant L-strand. The displacement (D)-loop encompasses 1124 bp, between nt 16024 and nt576, and is the only major non-coding region of the mitochondrial genome. It contains the origin of replication for H-strand synthesis, both mitochondrial transcription promoters (Lutz et al. 1997; Taanman, 1999), and has a triple-stranded DNA structure that is more sensitive to oxidative damage due to the exposure of the displaced single strand in the arrested replication intermediate (Clayton, 2000). It is also the attachment point of the mtDNA molecule to the mitochondrial inner membrane (Clayton, 1992). Replication is initiated at the H-strand origin, and proceeds to two-thirds around the genome until the L-strand origin is exposed, thus initiating L-strand synthesis (Fig. 1). Unlike nuclear DNA (nDNA), mtDNA may replicate more than once during each cell cycle, or not at all. Moreover, mtDNA may replicate in non-dividing cells. Compared with nDNA, mtDNA is more vulnerable to damages due to the lack of protective histones, limited DNA repair capacity, lack of introns, and is exposed continuously to endogenous reactive oxygen species (ROS) (Chen and Butow, 2005; Higuchi, 2007). Since all of the genes in mtDNA are crucial for biogenesis and bioenergetics function of mitochondria, any mtDNA alteration may result in impairment of OXPHOS and enhanced ROS production, which in turn, may accelerate the rate of mtDNA mutations (Higuchi, 2007; Lee and Wei, 2005). Mutated mtDNA and wild-type mtDNA molecules can coexist in the same cell, tissue or organ in a state called heteroplasmy. Mitochondria containing only mutated DNA (or only wild-type mtDNA) are called homoplasmic. The study of genotype-phenotype relationships in mitochondrial diseases has shown clearly the existence of a “phenotypic threshold effect”, which can be characterized by the

**Correspondence:** Dr. Josefa Salgado, Clinical Genetics Unit, University Clinic of Navarra, Avda, Pio XII, 36, 31008 Pamplona, Navarra (SPAIN). Tel: +34-948-255400, Ext: 1104; Fax: +34-948-296795; Email: jsalgadog@unav.es



Copyright in this article, its metadata, and any supplementary data is held by its author or authors. It is published under the Creative Commons Attribution By licence. For further information go to: <http://creativecommons.org/licenses/by/3.0/>.

following: (i) a low proportion of wild-type mtDNA coexisting with mutated mtDNA allows a normal phenotype to be maintained, but (ii) a small decrease in this proportion below a threshold value alters the phenotype.

## Mitochondrial DNA Mutations and Breast Cancer

Mitochondrial defects have long been suspected to play an important role in the development and progression of cancer. Over 70 years ago, Warburg pioneered the research on mitochondrial respiration alterations in the context of cancer and proposed a mechanism to explain how they evolve during the carcinogenic process. He hypothesized that a key event in carcinogenesis involved an “injury” to the respiratory machinery, resulting in compensatory increases in glycolytic ATP production. Due to the inherent inefficiency of glycolytic ATP generation, this represents a unique metabolic state of the malignant cells and would require high consumption of glucose to fulfil cellular energy requirements. Therefore, cancer cells actively metabolize glucose and produce excessive lactic acid while, at the same time, consume oxygen via OXPHOS. Warburg called this phenomenon “aerobic glycosysis” (Warburg, 1930; Warburg, 1956). Warburg’s observation stimulated many investigators to analyse mitochondrial function in tumor cells. Since then, many studies have reported mtDNA abnormalities in colorectal, bladder, head and neck, lung, pancreatic, gastric, hepatic, renal, ovarian and breast cancers and haematological diseases among others (Brandon et al. 2006; Chatterjee et al. 2006). The role of mtDNA mutations in tumor development is still unclear and only a subset of the mutations observed are predicted to have a relevant effect. The mtDNA mutations could arise either in the germ line and predispose to cancer (oncogene germline mutations), or in the mtDNA of the tissues (tumor-specific somatic mutations) and participate in the tumor progression process. Breast cancer is the most commonly diagnosed cancer of women and the second leading cause of cancer deaths among women. On one hand, inherited mutations in the breast cancer susceptibility genes, BRCA1 and BRCA2, predispose to breast, ovarian, and other types of cancer (Thompson and Easton, 2004). On the other hand, according to epidemiological studies, prolonged exposure to estrogen, including an earlier

menarche, and prolonged reproductive stimulation during lifetime, significantly associate with an increased risk of sporadic breast cancer (Oldenburg et al. 2007). Until recently, research has focused on nDNA changes, while alterations in mtDNA were largely ignored. Nowadays, there has become increasing awareness that mtDNA mutations might be more worthy of the kind of scrutiny once applied only to nDNA. Several studies indicate that most breast cancers harboured mtDNA that contains a variety of alterations including point mutations (germline and/or somatic), mtDNA depletion and microsatellite instability (MSI).

## Germline mtDNA mutations in breast cancer

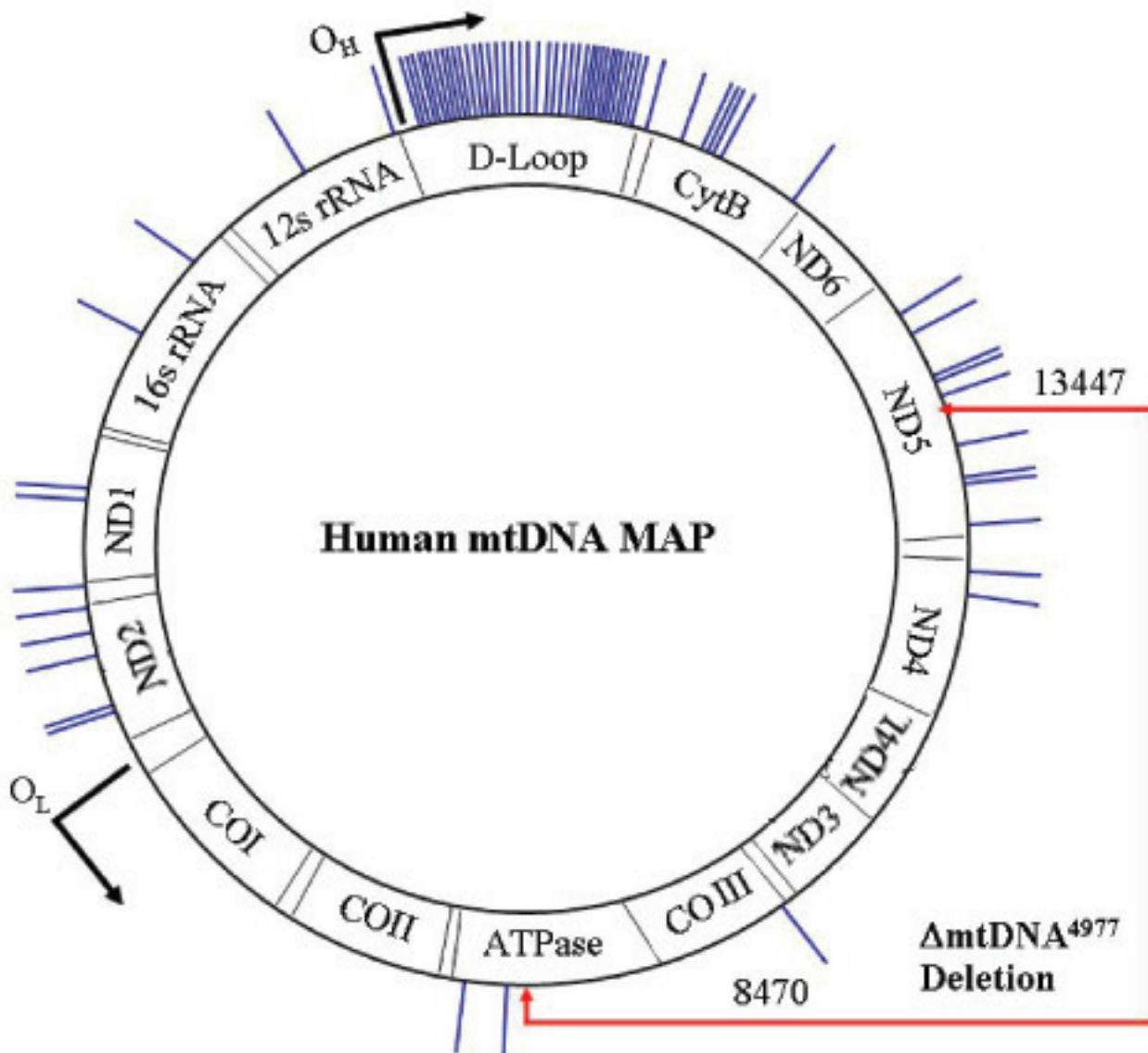
Several mtDNA population polymorphisms have been associated with increased breast cancer risk, stressing the importance of germline mtDNA mutations in the aetiology of certain cancers. One of those mutations is the germline T16189C substitution in one of the poly (C)-T-poly(C) structural motifs within the D-loop region. The T to C change in the wild-type (C<sub>5</sub>TC<sub>4</sub>) generates a long run of C residues considered to be a source of enhanced instability. Some authors have associated this variant with susceptibility to endometrial cancer (Liu et al. 2003) and breast cancer (Wang et al. 2006). It has been proposed as a potential marker for cancers with metabolic risk factor, since it has been associated significantly with type II diabetes (Poulton et al. 2002) and it has long been known that diabetes mellitus is an important epidemiologic risk factor for endometrial and breast cancers (Weiderpass et al. 1997). Another intensively studied change is a G10398A polymorphism (codon 114) which results in a non-conservative amino acid substitution of threonine (encoded by the A allele) for alanine (encoded by the G allele), within the NADH Dehydrogenase (ND3) subunit of Complex I. The 10398A allele was linked to increased risk for invasive breast cancer in both pre- and post-menopausal African-American women, although the mechanism underlying this relationship remains to be elucidated (Canter et al. 2005). Since Complex I is a key component of the mitochondrial electron transport chain, the amino acid change may increase the rate of electron leakage and ROS generation, contributing to mtDNA and nDNA mutations and cumulative mitochondrial dysfunction which could contribute to disease.

Many factors, other than the mtDNA G10398A polymorphism are likely to be important in the development and progression of invasive breast cancer in African-American women (Hall et al. 2005). In a very recent study Bai et al. showed that 10398A > G, 9055G > A and 16519T > C may increase the risk of developing breast cancer or are in linkage disequilibrium with functional variants that increase this risk. However, 3197T > C and 13708G > A have a protective effect or are in linkage disequilibrium with protective variants. In addition, this group studied mtDNA haplogroups and find an important role in disease expression. Clusters of mtDNA polymorphisms constitute haplogroups that have been extensively used to track human migrations and to explore variation in disease phenotypes. Within the 10 European haplogroups observed there was much higher proportion of breast cancer cases compared with controls in haplogroup K (defined by 29 polymorphic sites), whereas opposite result is observed for haplogroup U. Therefore, mitochondrial genetic background would play a very important role in modifying the individual's risk to breast cancer (Bai et al. 2007).

### Somatic mtDNA mutations in breast cancer

While interpretation of linking germline mtDNA mutations to cancer can be controversial due to the high background frequency of functional mtDNA polymorphisms, studies of somatic mtDNA mutations could be easy to handle since the cancer cell should have the neoplastic mtDNA mutation while the normal tissue should not. Parrella et al. determined the frequency and distribution of mtDNA mutations in 18 primary breast tumors by direct sequencing. 42% were deletions or insertions within the D-loop, 58% were single-base substitutions in the coding (ND1, ND4, ND5 and cytochrome b genes) or non-coding regions (D-loop). In 25% of the cases the mutations detected in the coding region led to amino acid substitutions in the protein sequence. Identical changes were found in breast nipple fine-needle aspirate fluid (FNA) and metastatic positive lymph nodes (Parrella et al. 2001). Zhu et al. described 45 somatic mutations in breast cancer tissue and in nipple aspirate fluid, 35 of those were at unique loci. Mutations at nine of the loci (seven in the D-loop and two in non-D loop areas)

have been reported in other cancer types suggesting that these sites were susceptible to mutation in a variety of human malignancies (Bianchi et al. 1995; Polyak et al. 1998; Fliss et al. 2000; Parrella et al. 2001). At the same time, and although not all the FNA had sufficient tumor cells to detect the mutations identified in the tissue, FNA represent a promising non-invasive approach to obtain breast epithelial cells (Zhu et al. 2005). Tan et al. used a combination of temporal temperature gel electrophoresis and direct DNA sequencing to screen the complete mitochondrial genome for mutations in 19 sets of paired normal and breast tumor tissues. Somatic mutations were identified in 74% of the patients. The bulk of the mutations (82%) were restricted to the D-loop region reinforcing, again, the findings that this region is a mutation hot spot (Tan et al. 2002). D-loop alterations may interfere with the sequence in the promoter areas and modify the affinity to the inducers or modulators of mtDNA transcription, thus putatively resulting in changes in the rate of transcription, RNA primer synthesis, and mtDNA replication (Clayton, 2000; Shadel and Clayton, 1997). Indeed, mtDNA content of hepatocellular carcinoma and breast tumors was significantly decreased in 71% and 88% of patients with D-loop region mutations (Lee et al. 2004; Yu et al. 2007). Data suggest a possible contribution of mtDNA D-loop mutation to alterations in mtDNA level. A representation of mtDNA point mutations in breast cancer, described in the literature, is shown in Figure 1. The most frequently observed mtDNA mutation in human tissues is the  $\Delta$ mtDNA<sup>4977</sup> deletion. This mutation affects genes encoding 7 polypeptide components of the mitochondrial respiratory chain, and 5 of the 22 tRNAs necessary for mitochondrial protein synthesis (Porteous et al. 1998). The  $\Delta$ mtDNA<sup>4977</sup> mutation was initially reported as occurring in one out of seven breast cancer samples (Bianchi et al. 1995). Further studies reported a different pattern of  $\Delta$ mtDNA<sup>4977</sup> mutation in tumors compared to normal tissue (Dani et al. 2004; Tseng et al. 2006). Recently, high sensitivity and specificity assays show that this  $\Delta$ mtDNA<sup>4977</sup> mutation is ubiquitous in both tumor and non-tumor tissues of both breast cancer and benign breast disease (Ye et al. 2007). Bibliographic data do not support the notion that the  $\Delta$ mtDNA<sup>4977</sup> mutation plays a major role in breast carcinogenesis (Schwartz et al. 2006; Tseng et al. 2006; Zhu et al. 2004). The amount



**Figure 1.** mtDNA point mutations in breast cancer described in the literature. Every tick represents a mutation locus detected.  
**Abbreviations:** 12S: 12S ribosomal RNA; 16S: 16S ribosomal RNA; ND1, 2, 4, 5 and 6: NADH dehydrogenase subunit 1, 2, 4, 5 and 6; ATPase: ATP Synthase F0 subunit 8; COI, II and III: cytochrome c oxidase subunit I, II and III; Cyt B: cytochrome b; O<sub>H</sub>: H-strand replication origin; O<sub>L</sub>: L-strand replication origin.

of  $\Delta\text{mtDNA}^{4977}$  mutation that accumulates may not be sufficient, by itself, to cause a significant defect or dysfunction in breast cancer cells (Ye et al. 2007). Other large deletions ( $\Delta\text{mtDNA}^{3938}$ ,  $\Delta\text{mtDNA}^{4388}$  and  $\Delta\text{mtDNA}^{4576}$ ) require further investigations. The first two have been found to be more frequent in cancerous than in adjacent histologically normal breast specimens, but were also identified in normal tissue from women without breast cancer. The last one, was detected in 13% of histologically normal specimens from a breast with cancer but in 77% of cancer specimens from the same breast (Zhu et al. 2004).

### Somatic mtDNA mutations could not be what they look like

To learn more about the nature of somatic mtDNA mutations in tumors, Brandon et al. recruited all data on somatic mtDNA, found in different types of cancers, and compared them with the mtDNA variant sites contained in their global human mtDNA database of 1920 complete mtDNA sequences plus 532 coding region mtDNA sequences (Brandon et al. 2006; Brandon et al. 2005). Surprisingly, 72% of the somatic mtDNA mutations were also sequence variants in population specific haplogroups database. A clear example is the nt13708

G to A missense mutation in ND5 (A458T), reported in breast cancer tumor (Parrella et al. 2001). In African L0-L3, Asian M, and at the base of Eurasian N mtDNAs, the nt13708 is a G (458A). However, at the root of European haplogroup J, and in important sublineages of the European X and Asian B haplogroups, the nt13708 is an A (458T). Therefore, the tumor-specific mutation results in the conversion of the mtDNA sequence to a Eurasian-like allele. Authors suggest that cancer cells acquire some of the same functional mtDNA mutations that confer adaptive advantages in population haplotypes, as cells migrate into new environments. They consider two main classes of mtDNA mutations: *tumorigenic mutations* and *adaptive mutations*. The first ones are found only in tumors and lead to a relatively severer deleterious effects on mitochondrial metabolism and ROS production. The second ones, alter the same nucleotides as population variants and may have a milder impact on mitochondrial OXPHOS, and help the tumors to adapt to adverse environments. Moreover, these two different classes of somatic mutations might arise at different times, being tumorigenic mutations with advantage in the initial phases of tumor growth. In this early stage, tumor is hypoxic and thus can tolerate OXPHOS deficiency since its generating ATP by glycolysis. By contrast, adaptive mtDNA mutations may be more advantageous when the tumor becomes vascularized and/or invade new environments as it metastasizes (Brandon et al. 2006). Therefore, understanding mtDNA variations in cancer cells provide new major insights into the aetiology of solid tumors.

### Mitochondrial DNA Copy Number in Breast Cancer

The evidence regarding the role of mtDNA content in mammary tumorigenesis, and its relationship with clinico-pathological factors and patient outcome, is limited. It has been shown that a reduction of mtDNA content occurred in breast tumors, but this proportion was greater for papillary thyroid carcinomas, suggesting that changes in mtDNA content during carcinogenesis may be regulated in a tumor-specific manner (Mambo et al. 2005). In a study of 60 pairs of tumors, and corresponding no tumorous breast tissues, results indicated that 63% of the tumors had a significant lower mtDNA content compared to their corresponding

non-tumorous breast tissue (Tseng et al. 2006). The analysis of 59 pairs of breast tumors and adjacent non-tumorous tissues has shown recently that 78% of the cases carried a reduction of mtDNA content compared to the corresponding non-tumorous cells. It has been well documented that ER-negative breast tumors have significantly more aggressive clinical behaviour and are unresponsive to anti-estrogenic therapy ((EBCTCG). 2005). A reduced mtDNA copy number tends to have a significant correlation with ER status (Yu et al. 2007). Several lines of evidence have demonstrated that altered mtDNA content may lead to a functional defect in oxidative phosphorylation and affect the respiratory activity of the cells, ultimately changing their growth behaviour (Isidoro et al. 2004). The mtDNA level reduction can be due to: (i) mtDNA D-loop mutations and (ii) dysfunction of p53 gene, among other causes. In the first case, and as it has been mentioned before, there is a possible contribution of mtDNA D-loop mutations to alterations in mtDNA level. In the second case, it has been reported that the p53 plays an important role in maintaining mitochondrial genetic stability via the interaction with mtDNA polymerase  $\gamma$  (Achanta et al. 2005; Heyne et al. 2004). Loss of p53 could lead to increased mtDNA vulnerability to exogenous and endogenous damage, subsequently elevating frequency of mtDNA mutations (as for example D-loop mutations), and therefore mtDNA depletion.

### Mitochondrial DNA and Microsatellite Instability (MSI) in Breast Cancer

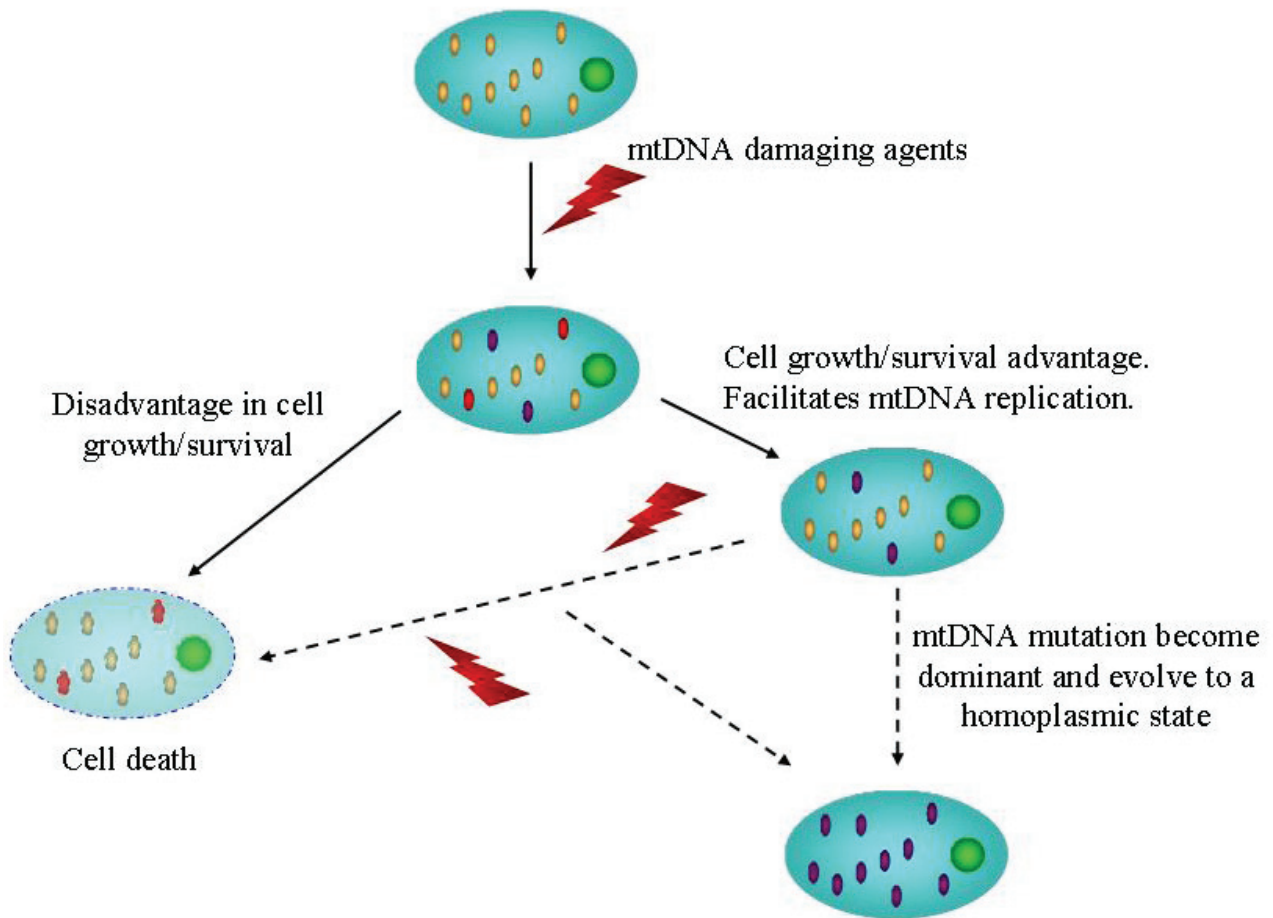
Microsatellites are short tandem repeats of sequence motifs, usually ranging from one to five DNA bases. MSI is the change of length in any such stretch, due to either insertions or deletions of repeating units in a microsatellite, within tumor DNA compared to that of normal tissue. Nuclear MSI is thought to result from a defect in the mismatch repair system, which leads to failure in correction of the slippage errors made by DNA polymerases during replication. Nuclear MSI was first described in colorectal cancer (Thibodeau et al. 1993) and subsequently observed in most cancers (Lawes et al. 2003). However, instability in these terms of the mitochondrial genome, which is also subject to genetic alterations in cancer cells, (Bianchi et al. 2001; Richard et al. 2000) is worse

characterized, and the responsible mechanisms of generation are still unclear. Wang et al. studied 12 candidate microsatellite regions within the entire mtDNA genome of a series of primary cervical (71), endometrial (62), ovarian (73) and breast cancer (51) patients. One relevant finding of this study was that the poly (C)-T-poly(C) structural motifs in the D-loop seem to be prone to MSI (Wang et al. 2006). The wild-type sequences (according to revised Cambridge sequence, <http://www.mitomap.org/>) of these markers are C<sub>7</sub>TC<sub>5</sub> at nt303, C<sub>5</sub>TC<sub>4</sub> at nt956 and C<sub>5</sub>TC<sub>4</sub> at nt16184 and their instability patterns are different. At nt303, alterations were detected upstream to the T residue, the mtMSI manifestation being (C)<sub>7-11</sub> TC<sub>5</sub>. At nt16184, mtMSI presents a particular pattern, consequential to a T to C germline substitution at nt16189, which has been discussed before. The nt956 showed both types of mtMSI. Authors found that mtMSI had different profiles and frequency in the four types of cancer, suggesting that different tumors may have generated different extents of mtMSI during carcinogenesis (Wang et al. 2006). On the contrary, no microsatellites in the coding region containing poly (A), poly (T) and poly (C) were unstable. It has been suggested that polyC- instability might be related to a highly mitogenic history and to progressive tumors, in particular, tumors that had already metastasized at the time of diagnosis. Nevertheless, mutations in the (C)<sub>n</sub> non-coding repeats could not be selected during tumor progression, but associated with higher replication rates of mtDNA in tumors because of their inherent mutability (Schwartz et al. 2006). Although mtMSI hot spots have been identified, it remains a challenge to address their functional impact.

### Homoplasmy/Heteroplasmy

Published data show that the majority of somatic mtDNA mutations are homoplasmic, indicating that mutant mtDNA had become dominant in the cancer cells, although the mechanism(s) for the development of mtDNA homoplasmy in cancer tissue is controversial. The lack of histones protection, limited repair ability, and close proximity to the electron transport chain make mtDNA far more susceptible to DNA damaging agents (ROS and/or certain anticancer drugs and radiation). Since such induced mutations do not involve all copies of mtDNA, an initial mutation is heteroplasmic with

the mutated mtDNA being the minority. Two models, *classic selection* and *random genetic drift*, have been developed to explain the expansion of homoplasmy in tumors (Cavelier et al. 2000; Collier et al. 2001). The first interpretation is that expansion of the mutation was driven by strong selection for the mutant phenotype (Fig. 2). In this context, if a mutation leads to a disadvantage in cell growth or survival, such mutation is deemed to diminish. Conversely, if a mutation confers cell growth/survival advantage or facilitates mtDNA replication, such a mutation is likely to survive the selection and emerge as a heteroplasmic mutation. Depending on the degree of growth/survival advantage, cells carrying the mtDNA mutation may eventually become dominant and evolve to establish a homoplasmic mutant state. It is possible that mtDNA mutations cause a functional change that compromises the efficiency of the electron transport chain, leading to electron flow bifurcation and increased generation of ROS. A moderate increase of ROS has been found to stimulate cellular proliferation and mitochondrial biogenesis (Liu et al. 2002). The increase in ROS production may cause further damage to both mtDNA and nDNA, leading to cancer development, genetic instability, and disease progression. The second model, published by Collier et al. conclude that human tumors are expected to contain homoplasmic mitochondrial mutations based only on random mutagenesis and segregation of mtDNA (Collier et al. 2001). By using extensive computer modelling programs, authors show that when the mtDNA mutation occurs in a tumor progenitor cell, homoplasmy can be archived entirely by chance through unbiased mtDNA replication and sorting during cell division, without selection for physiological advantage. They estimate the mtDNA mutation rate as 7–10 mutations per base pair per generation, and the number of generations of cell growth experienced by a carcinoma founder cell as 600. Model predicts that 58% of tumors contain at least one homoplasmic point mutation. This fraction is close to the experimental observation that 48% of tumors from which essentially the entire mitochondrial genome was sequenced, contain at least one homoplasmic mtDNA mutation (Fliss et al. 2000; Polyak et al. 1998). Moreover, this model also correctly predicts the distribution of the number of mutations per tumor, and it is relatively stable with respect to changes in parameters such as the number of mitochondria per cell, the mutation rate and the number



**Figure 2.** The *classic selection* method used to explain the expansion of mtDNA homoplasmy in tumors (in the *random genetic drift*, human tumors are expected to contain homoplasmic mitochondrial mutations based only on random mutagenesis and segregation of mtDNA).

of generations. Therefore it could be argued that, some mitochondrial mutations may provide a selective advantage for the carrier mtDNA copy of the host cell. However, although selection may occur in some instances, it is not necessarily the only cause. A large fraction of human tumors are expected to be homoplasmic for mtDNA point mutations based solely on reported mitochondrial mutant fractions and cell growth kinetics of tumors. Whereas it cannot be excluded the possibility that selection occurs in a subset of tumors, physiological advantage or a role for mitochondrial mutations in tumorigenesis, may not be the only cause to explain the existence of homoplasmy or its observed frequency. Finally, although the mitochondrial and nuclear genomes are physically distinct, there is constant communication between the two genomes to carry out many of the mitochondrial functions. The identification of a large number of genes whose expression is influenced

by mitochondrial function provides pathways affected by the impairment of mitochondria in pathological conditions (Delsite et al. 2002). And this impairment would be, at the same time, related to the grade of mitochondrial heteroplasmy. In this context we have to consider that phenotypic manifestations of mitochondrial defects occur when a threshold level is exceeded. Depending on the gene affected by the mutation in mtDNA, and the grade of heteroplasmy, complementation can occur at different levels: transcription, translation, enzyme assembly and activity, mitochondrial ATP synthesis, cell activity and, finally, organ function (Rossignol et al. 2003).

## Summary

The presence of mtDNA mutations in cancer is consistent with the intrinsic susceptibility of mtDNA to damage and constitutive oxidative stress.

The central role of the mitochondria in energy production, ROS generation and apoptosis regulation, combined with a variety of mtDNA alterations (germline and/or somatic point mutations/deletions, mtDNA depletion and MSI) could provide rational explanation for many of the metabolic features of solid tumor biology. At the same time genetic background (mtDNA haplogroups and variations) is important in modifying the individual's risk of cancer. Therefore, the aetiology of the protective or detrimental effect of variants/mutations should be considered in the context of mtDNA haplogroups. Moreover, the grade of heteroplasmy and the phenotypic threshold effect may play a major role in the impairment of mitochondria in pathological conditions. Finally, mtDNA is not under the stringent control of the cell cycle and intergenomic signalling pathways in carcinogenesis could be exploited in cancer therapeutics.

## References

- Achanta, G., Sasaki, R., Feng, L. et al. 2005. Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA Pol gamma. *Embo. J.*, 24:3482–3492.
- Anderson, S., Bankier, A.T., Barrell, B.G. et al. 1981. Sequence and organization of the human mitochondrial genome. *Nature*, 290:457–465.
- Andrews, R.M., Kubacka, I., Chinnery, P.F. et al. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat. Genet.*, 23:147.
- Bai, R.K., Leal, S.M., Covarrubias, D. et al. 2007. Mitochondrial genetic background modifies breast cancer risk. *Cancer Res.*, 67:4687–4694.
- Bianchi, M.S., Bianchi, N.O. and Bailliet, G. 1995. Mitochondrial DNA mutations in normal and tumor tissues from breast cancer patients. *Cytogenet. Cell. Genet.*, 71:99–103.
- Bianchi, N.O., Bianchi, M.S. and Richard, S.M. 2001. Mitochondrial genome instability in human cancers. *Mutat. Res.*, 488:9–23.
- Brandon, M., Baldi, P. and Wallace, D.C. 2006. Mitochondrial mutations in cancer. *Oncogene*, 25:4647–4662.
- Brandon, M.C., Lott, M.T., Nguyen, K.C. et al. 2005. MITOMAP: a human mitochondrial genome database—2004 update. *Nucleic Acids Res.*, 33:D611–613.
- Canter, J.A., Kallianpur, A.R., Parl, F.F. et al. 2005. Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. *Cancer Res.*, 65:8028–8033.
- Cavelier, L., Jazin, E., Jalonen, P. et al. 2000. mtDNA substitution rate and segregation of heteroplasmy in coding and noncoding regions. *Hum. Genet.*, 107:45–50.
- Clayton, D.A. 1992. Structure and function of the mitochondrial genome. *J. Inherit. Metab. Dis.*, 15:439–447.
- Clayton, D.A. 2000. Transcription and replication of mitochondrial DNA. *Hum. Reprod.*, 15(2):11–17.
- Coller, H.A., Khrapko, K., Bodyak, N.D. et al. 2001. High frequency of homoplasmic mitochondrial DNA mutations in human tumors can be explained without selection. *Nat. Genet.*, 28:147–150.
- Chan, D.C. 2006. Mitochondria: dynamic organelles in disease, aging, and development. *Cell.*, 125:1241–1252.
- Chatterjee, A., Mambo, E. and Sidransky, D. 2006. Mitochondrial DNA mutations in human cancer. *Oncogene*, 25:4663–4674.
- Chen, X.J. and Butow, R.A. 2005. The organization and inheritance of the mitochondrial genome. *Nat. Rev. Genet.*, 6:815–825.
- Dani, M.A., Dani, S.U., Lima, S.P. et al. 2004. Less DeltamtDNA4977 than normal in various types of tumors suggests that cancer cells are essentially free of this mutation. *Genet. Mol. Res.*, 3:395–409.
- Delsite, R., Kachhap, S., Anbazhagan, R. et al. 2002. Nuclear genes involved in mitochondria-to-nucleus communication in breast cancer cells. *Mol. Cancer*, 1:6.
- EBCTCG, E. B. C. T. C. G. 2005. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet*, 365:1687–1717.
- Fliss, M.S., Usadel, H., Caballero, O.L. et al. 2000. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. *Science*, 287:2017–2019.
- Hall, I.J., Moorman, P.G., Millikan, R.C. et al. 2005. Comparative analysis of breast cancer risk factors among African-American women and White women. *Am. J. Epidemiol.*, 161:40–51.
- Heyne, K., Mannebach, S., Wuertz, E. et al. 2004. Identification of a putative p53 binding sequence within the human mitochondrial genome. *FEBS Lett.*, 578:198–202.
- Higuchi, M. 2007. Regulation of mitochondrial DNA content and cancer. *Mitochondrion*, 7:53–57.
- Isidoro, A., Martinez, M., Fernandez, P.L. et al. 2004. Alteration of the bioenergetic phenotype of mitochondria is a hallmark of breast, gastric, lung and oesophageal cancer. *Biochem. J.*, 378:17–20.
- Lawes, D.A., SenGupta, S. and Boulos, P.B. 2003. The clinical importance and prognostic implications of microsatellite instability in sporadic cancer. *Eur. J. Surg. Oncol.*, 29:201–212.
- Lee, H.C., Li, S.H., Lin, J.C. et al. 2004. Somatic mutations in the D-loop and decrease in the copy number of mitochondrial DNA in human hepatocellular carcinoma. *Mutat. Res.*, 547:71–78.
- Lee, H.C. and Wei, Y.H. 2005. Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. *Int. J. Biochem. Cell. Biol.*, 37:822–834.
- Liu, S.L., Lin, X., Shi, D.Y. et al. 2002. Reactive oxygen species stimulated human hepatoma cell proliferation via cross-talk between PI3-K/PKB. and JNK signaling pathways. *Arch. Biochem. Biophys.*, 406:173–182.
- Liu, V.W., Wang, Y., Yang, H.J. et al. 2003. Mitochondrial DNA variant 16189T > C is associated with susceptibility to endometrial cancer. *Hum. Mutat.*, 22:173–174.
- Lutz, S., Weisser, H.J., Heizmann, J. et al. 1997. A third hypervariable region in the human mitochondrial D-loop. *Hum. Genet.*, 101:384.
- Mambo, E., Chatterjee, A., Xing, M. et al. 2005. Tumor-specific changes in mtDNA content in human cancer. *Int. J. Cancer*, 116:920–924.
- Oldenburg, R.A., Meijers-Heijboer, H., Cornelisse, C.J. et al. 2007. Genetic susceptibility for breast cancer: how many more genes to be found?. *Crit. Rev. Oncol. Hematol.*, 63:125–149.
- Parrella, P., Xiao, Y., Fliss, M. et al. 2001. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. *Cancer Res.*, 61:7623–7626.
- Polyak, K., Li, Y., Zhu, H. et al. 1998. Somatic mutations of the mitochondrial genome in human colorectal tumours. *Nat. Genet.*, 20:291–293.
- Porteous, W.K., James, A.M., Sheard, P.W. et al. 1998. Bioenergetic consequences of accumulating the common 4977-bp mitochondrial DNA deletion. *Eur. J. Biochem.*, 257:192–201.
- Poulton, J., Luan, J., Macaulay, V. et al. 2002. Type 2 diabetes is associated with a common mitochondrial variant: evidence from a population-based case-control study. *Hum. Mol. Genet.*, 11:1581–1583.
- Richard, S.M., Bailliet, G., Paez, G.L. et al. 2000. Nuclear and mitochondrial genome instability in human breast cancer. *Cancer Res.*, 60:4231–4237.
- Rosignol, R., Faustin, B., Rocher, C. et al. 2003. Mitochondrial threshold effects. *Biochem. J.*, 370:751–762.



- Schwartz, S.Jr., Alazzouzi, H. and Perucho, M. 2006. Mutational dynamics in human tumors confirm the neutral intrinsic instability of the mitochondrial D-loop poly-cytidine repeat. *Genes Chromosomes Cancer*, 45:770–780.
- Shadel, G.S. and Clayton, D.A. 1997. Mitochondrial DNA maintenance in vertebrates. *Annu. Rev. Biochem.*, 66:409–435.
- Taanman, J.W. 1999. The mitochondrial genome: structure, transcription, translation and replication. *Biochim. Biophys. Acta.*, 1410:103–123.
- Tan, D.J., Bai, R.K. and Wong, L.J. 2002. Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. *Cancer Res.*, 62:972–976.
- Thibodeau, S.N., Bren, G. and Schaid, D. 1993. Microsatellite instability in cancer of the proximal colon. *Science*, 260:816–819.
- Thompson, D. and Easton, D. 2004. The genetic epidemiology of breast cancer genes. *J. Mammary Gland Biol. Neoplasia*, 9:221–236.
- Tseng, L.M., Yin, P.H., Chi, C.W. et al. 2006. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. *Genes Chromosomes Cancer*, 45:629–638.
- Wang, Y., Liu, V.W., Tsang, P.C. et al. 2006. Microsatellite instability in mitochondrial genome of common female cancers. *Int. J. Gynecol. Cancer*, 16 Suppl 1:259–266.
- Warburg, O. 1930. The metabolism of tumors. London Constable Co Ltd.
- Warburg, O. 1956. On the origin of cancer cells. *Science*, 123:309–314.
- Weiderpass, E., Gridley, G., Persson, I. et al. 1997. Risk of endometrial and breast cancer in patients with diabetes mellitus. *Int. J. Cancer*, 71:360–363.
- Ye, C., Shu, X.O., Wen, W. et al. 2007. Quantitative analysis of mitochondrial DNA 4977-bp deletion in sporadic breast cancer and benign breast diseases. *Breast Cancer Res. Treat.*, May 31 [Epub ahead of print].
- Yu, M., Zhou, Y., Shi, Y. 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.
- Zhu, W., Qin, W., Bradley, P. et al. 2005. Mitochondrial DNA mutations in breast cancer tissue and in matched nipple aspirate fluid. *Carcinogenesis*, 26:145–152.
- Zhu, W., Qin, W. and Sauter, E.R. 2004. Large-scale mitochondrial DNA deletion mutations and nuclear genome instability in human breast cancer. *Cancer Detect. Prev.*, 28:119–126.