Breast cancer as a mitochondrial disorder (Review)

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Abstract. Mitochondria have been implicated in cell transformation since Otto Warburg considered 'respiration damage' to be a pivotal feature of cancer cells. Numerous somatic mitochondrial DNA (mtDNA) mutations have been found in various types of neoplasms, including breast cancer. Establishing the mtDNA mutation pattern in breast cancer cells may enhance the specificity of cancer diagnostics, detection and prediction of cancer growth rate and/or patients' outcomes; and therefore be used as a new molecular cancer bio-marker. The aim of this review is to summarize data on mtDNA mutation involvement in breast cancer and estimate effects of resulting amino acid changes on mitochondrial protein function. In this article published mtDNA mutation analyses are critically evaluated and interpreted in the functional context.

Contents

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Abbreviations: ATPase (6,8), ATPase subunits 6 and 8; COX (1-3), cytochrome c oxidase subunits 1-3; Cyt b, cytochrome b; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species

Key words: breast cancer, mitochondria, protein structure, mtDNA, mutation

1. Introduction

According to research carried out on the United States population, breast cancer is the most common cancer type among females (212,929 cases annually) and is responsible for 40,970 deaths a year, accounting for 15% causes of all cancer deaths among women. In 2007 breast cancer alone accounted for ~26% (178,480) of all new cancer cases among women (1,2). Breast cancer is also the second most commonly diagnosed cancer in the EU, and accounts for 17.9/100,000 deaths (3). Many low-penetrance genes are known to be involved in the process of breast cancer carcinogenesis and their cumulative attributable risk for breast cancer development must be considered substantial. Moreover, 5% of breast cancers are associated with a genetic predisposition, transmitted as an autosomal dominant trait. Mutations in the BRCA1 or BRCA2 genes are associated with a high risk of breast or/and ovarian cancer. Women with these mutations have a 65-85% cumulative lifetime risk of developing invasive breast cancer and a 15-65% cumulative lifetime risk of developing invasive ovarian cancer (4-6). Other genes besides BRCA1 and BRCA2 related to breast cancer susceptibility include the 'guardian of the genome' TP53, and genes of proteins from p53-DNA repair-pathways, the PTEN (phosphatase and tensin homolog-mutated in multiple advanced cancers 1) gene, and the CHEK2 (protein kinase CHK2 isoform c) gene. Other genes mutated in breast cancer patients include the ATM (ataxia telangiectasia mutated) gene, XPD (ERCC2, excision repair cross-complementing rodent repair deficiency, complementation group 2 protein) and HER-2 (human epidermal growth factor receptor) gene (7,8). Recently a new breast cancer marker- PALB2 was discovered. This protein interacts with BRCA2, and is involved in homologous recombination and the repair of DNA double-strand breaks (9). The mitochondrial genome has also been screened for mutations specific for breast cancer (Table I) and subsequently NAF (breast nipple aspirate fluid) with mtDNA mutations at positions 204, 207 and 16293 has been suggested as indicative for breast cancer (10) and mtDNA D-loop mutations have been proposed as an independent prognostic marker (11).

Currently mitochondrial biology is one of the most rapidly growing areas in genetics and medicine. Substantial progress

Table I. Summary of mtDNA research in the field of breast cancers.

Reference	(30)	(49)	(10)	(50)
No. of patients	19	10	15	39
Population studied	USA (Georgetown)	P.R. China	USA (Columbia)	USA (Columbia)
Sequence analyzed	Whole mtDNA	Whole mtDNA	98.5% mtDNA-tumor, D-loop-NAF	8412-13650, 8381-13532, 2828-7850, 3304-8310
Control tissue analyzed	Paired normal	I) Paired pre-cancerous II) Distant normal	Paired normal	I) Paired normal II) Normal from healthy woman III) Blood from cancer patients and healthy women
Control sequence used	I) Paired normal II) CRS	I) Paired pre-cancerous II) Distant normal	I) Paired normal III) CRS	I) Paired normal
Control population analyzed	None	None	None	23 healthy women
Patients' haplogroups analyzed	No	Yes	No	No
Affected genes	D-loop, ND2, 16SrRNA, ATPase6	16SrRNA, ATPase6	D-loop, 16SrRNA, ND1, ND2, ATPase, COIII, ND4, ND5, CYTB, tRNA-I, tRNA-T	ATPase8, ATPase6, COII, ND5, ND4, ND3, 16SrRNA, ND2, tRNAs
Amino-acid substitution	ATP6	None	ND2, ATPase, COIII, ND5, CYTB	None
Mutations (somatic) reported	Yes-27, 22/27-D loop, 4/27-coding region	Yes, in cancer + in pre-cancerous tissues	Yes-45	Deletions, found in cancer and normal tissues (3939 bp, 4388 bp, 4977 bp)
Polymorphisms (germline) reported	Yes	Yes	Yes-155; 55/155 in D-loop	No
Homo/heteroplasmy analyzed	Hetero 10/27, Homo 17/27	2/10 Hetero	Homo 24/45, Hetero 21/45	NA
Results/conclusions	14/19 (74%) tumors display at least 1 somatic mutation, 12/19 tumors had mutation in D-loop	No mutations found in haplogroup specific sites or D310 region	One or more somatic mutation in 14/15 (93%) tumors, mutations at positions 204, 207 and 16293 may indicate cancer	4576 bp deletion is frequent in breast cancer
Reference	(51)	(11)	(25)	(24)
No. of patients	51	60	654	124
Population studied	Hong Kong	Taiwan	African-American women	haplogroup N and with sub-lineages
Sequence analyzed	12 microsatellite regions	D-loop	Position 10398	Position 10398
Control tissue analyzed	Cervix, endometrium, ovary or lymphocytes	Paired normal	No	Paired normal
Control sequence used	None	Paired normal	Normal population	Paired normal
Control population analyzed	None	None	605 healthy African-American women	273 healthy women (matched ethnically)
Patient's haplogroups analyzed	No	No	No	Yes (N)
Affected genes	D-loop, 12SrRNa, ND1, X ND2, COI, ND5, ATPase6	D-loop, ATPase8, ATPase6, COII, ND5	ND3	ND3
Amino-acid substitution	No	No	Yes	Yes

Table I. Continued.

Reference	(51)	(11)	(25)	(24)
Mutations (somatic) reported	Yes-mtMSI	Yes,-22; 18/60 (30%) in D-loop 4/60-2 mutation, 14/60-1 mutation	No	No
Polymorphisms (germline) reported	Yes, but not shown	No	G10398A	G10398A
Homo/heteroplasmy analyzed	No	Hetero 19/22	No	No
Results/conclusions	mtMSI is most prevalent in D-loop, 29.4% breast cancer samples carry 1 or more mtMSI, mtMSI is not associated with age, grade, or histologic type	4977 bp deletion is more frequently found in non-tumorous tissues (47%) than in cancer tissues (5%), MtDNA copy number is decreased in 63% of breast cancer, D-loop mutation is associated with older age, lack of ER expression and poor survival, D-loop mutation in prognostic marker	G10398A polymorphism is associated with invasive breast cancer and is an independent risk factor, G10398A is involved in ROS production	G10398A polymorphism belongs to 8701-9540-10398-10873-15301 haplotype (N), Haplotype N is associated with increased rate if breast cancer

Reference	(26)	(52)	(29)	(53)
No. of patients	156	14	17	63
Population studied	Unrelated European American females with familial breast cancer (not Jewish)	USA (Baltimore), known BRCA1 status	Italy (Rome)	Spain (Madrid)
Sequence analyzed	69 SNP selected from Mitomap (mt diseases)	D310 marker	84% of mtDNA	All mtDNA, G6267A
Control tissue analyzed	None	None	I) Paired normal II) Peripheral blood lymphocytes	None
Control sequence used	None	None	Paired normal	None
Control population analyzed	260 age-matched healthy European-American women	No	No	No
Patients' haplogroups analyzed	Yes	No	No	Yes
Affected genes	ATPase6, ND3, D-loop, 16SrRNA, ND5	D-loop	ND5, D-loop, ND1, ND4, CYTB	COI
Amino-acid substitution	ATPase6, ND3, ND5	No	ND5, ND4,	COI
Mutations (somatic) reported	No	Yes	Yes	Yes
Polymorphisms (germline) reported	Yes	No	Yes-110	Yes
Homo/heteroplasmy analyzed	No	No	No	Hetero-1 patient, Homo-1 patient
Results/conclusions	G9055A, A10398G, T16519C mutations increase breast cancer risk, T3197C and G13708A decrease breast cancer risk	No mtDNA mutations were found in wt BRCA1 subjects, The same mutations found in DL and NAF	MtDNA mutations in 61% of patients, Mutations in metastatic lymph nodes the same as in tumor	The mutation Ala/Thr is expected to impair contacts between subunit I and II of cytochrome c oxidase, Mutant has impaired growth on galactose and reduced complex IV activity by 50%

Table II. Amino acid altering mutations in mtDNA from breast cancers.

	Nucleotide change	Gene	Amino acid change	Ref.
1	G4665A	ND2	Ala-Thr	(10)
2	A8498G	ATP	Lys -Glu	(10)
3	T9131C	ATP6	Leu-Pro	(30)
4	T9885A	COX3	Phe-Ile	(10)
5	A11768G	ND4	Thr-Ala	(10)
6	G11900A	ND4	Val-Met	(29)
7	T12344A	ND5	Met-Lys	(29)
8	T13397A	ND5	Gln-Leu	(10)
9	T13398A	ND5	Gln-Leu	(10)
10	T13674G	ND5	Asn-Lys	(10)
11	G13708A	ND5	Ala-Thr	(29)
12	G15755T	CytB	Glu-Trp	(10)
13	T15783C	CytB	Leu-Pro	(10)
14	A15824G	CytB	Thr-Ala	(10)

has recently been made in understanding the genetic basis and pathogenic mechanisms in disorders associated with mitochondrial DNA (mtDNA) mutations in tRNA, rRNA and protein-encoding genes. Moreover, some disorders were found to arise from altered mitochondrial DNA stability and/or expression. All these defects that finally result in an impairment of electron transport chain function, include a wide spectrum of rare childhood disorders such as Kearns-Sayre syndrome, NARP, MELAS, or MERRF syndromes (12), but also encompass an increasing number of common aging-related disorders, including Alzheimer's, Parkinson's and Huntington's diseases, also diabetes, heart disease and cancer; however, effective therapies for diseases caused by mitochondrial dysfunction remain elusive (13,14). The term 'mitochondrial medicine' has been proposed to cover this emerging and diverse field that is becoming increasingly important in differential diagnosis and in genetic counseling (15,16).

Mitochondria have been implicated in carcinogenesis since the 1930s when Otto Warburg suggested that 'respiration damage' is a pivotal feature of cancer cells. In his very early experiments Warburg demonstrated that an increased rate of glycolysis was a unique attribute of tumor metabolism (17,18). Today we know that mitochondrial dysfunction is one of the most prominent features of cancer cells, as many studies show strong correlation between this phenomenon and the development and progression of cancer. Alterations of mitochondrial DNA (mtDNA) have been instrumental in studies of human phylogeny, in population genetics, and in molecular medicine to link pathological mutations to a variety of human diseases of complex etiology (19,20). However, evidence for direct linkage of respiratory deficiency in a specific tumor type with a specific mtDNA mutation is still missing (21). Interestingly, haplogroup U is associated with an increased risk of prostate cancer and renal cancer (22) and cytochrome oxidase subunit I (COI) gene mutations that alter conserved amino acids have been reported to increase tumorigenicity in prostate cancer (23). A few mtDNA polymorphisms are associated with sporadic (G10398A) (24,25) and familial (G9055A, A10398G, T16519C) breast cancer (26).

mtDNA mutations that alter Complex I structure and function may alter a cell's ability to respond to oxygen deficit and contribute to resistance to chemotherapeutic agents that require redox cycling for activation (27). It is therefore possible that structural changes in mtDNA-encoded protein subunits cause impaired electron transport function and thereby increase the electron leak and ROS production, which in turn elevate the oxidative stress and oxidative damage to mitochondria in the process of cell transformation and drive the vicious cycle of carcinogenesis. Previous studies seem to favor this hypothesis for breast cancer (10,28,29).

2. Structural mitochondrial DNA (mtDNA) mutations in breast cancer.

Scarce data are currently available on mtDNA mutations in breast cancer and only a few structural mutations have been reported. Parrella and co-workers who analyzed invasive ductal breast carcinomas found somatic mtDNA mutations in 11 out of 18 examined tumor samples (29). In the study carried out by Tan *et al* 19 cancer cases were examined of which 14 were found to contain somatic mutations, but only 4 mutations occurred in the polypeptide encoding genes and only one of these was a missense mutation (30). Zhu and co-workers investigated 15 breast cancer samples and reported 45 mutations 15 of which were missense (10) (Table II). The first mutation reported - T9131C results in a substitution of leucine to proline in position 202 of the ATPase 6 protein. This position is highly conserved among *Eucaryota* (Fig. 1). Leucine-202 is located on the surface of the α-helix chain, which in native protein is in contact with subunit c of ATPaseV. Therefore, this mutation possibly disturbs the interactions between two subunits of ATPaseV as the structure of the α-helix is disturbed by proline (28). Mutation T12344A in subunit ND5 substituting methionine with lysine in the third position of ND5 polypeptide is located in poorly conserved protein region, thus presumably has no impact on protein structure. Another mutation in subunit ND5 reported by Parrella and co-workers-G13708A-substituting alanine to threonine, is known as a common polymorphism in one of the European haplogroups-J (31). In order to define whether this polymorphism can lead to an increased risk of cancer development, population studies are necessary, but have not been carried out so far. Nevertheless G13708A has been found in sporadic parathyroid adenoma and acute leukemia (31,32).

Breast cancer patients have also been reported to harbor G11900A mutation which changes valine to methionine in subunit ND5 in the polypeptide region highly variable among phyla, with different hydrophobic amino acids (including 'mutant' methionine) found in this position. Similarly A8498G mutation causing substitution of lysine by glutamate is unlikely to affect protein function as it is also located in a variable region of the peptide, as is the A15824G mutation

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O05097          KI-----------------LLPVPLHGFFDMFDGVLQVYVFVLLTMIFTKLGIEH---
P0AB98          ---PWWSQWILNV-----------PWAIFHILIITLQAFIFMVLTIVYLSMASEEH--
P43719          SANMAIA-------AL--GIPLHLAWAIFHILVITLQAFIFMMLTVVYLSIAYNKADH
P00846          AM----STINLPST--LIIFTILILLTILEIAVALIQAYVFTLLVSLYLHDNT-----
Q9T9Y7          AM----STTNLPST--LIIFTVLILLTMLEIAVALIQAYVFTLLVSLYLHENT-----
P00847          AL----MSISTTTA--LITFTILILLTILEFAVAMIQAYVFTLLVSLYLHDNT-----
P14092          AL----LPMMPSIS--ALTALILFLLTILEVAVAMIQAYVFVLLLSLYLQENI-----
Q9MIY5          VL----LPMMPAVA--ILTASVLFLLTILEVAVAMIQAYVFILLLSLYLQENI-----
P00850          SM----S-YMLVTFL----LMAQIALLVLESAVAMIQSYVFAVLSTLYSSEVN-----
P34834          MA----SNYLILSLI----LTTQIALLVLESAVAIIQSYVFAVLSTLYSSEVN-----
Q37385          NVFKKYALIS------FLPLLFIVFIIVLEFCIAIVQAYIFSILTCIYLNDIYNTSH-
P50363          SACMAVSSILLKGITIGLPLAVLVVLYGLELLVALLQSYVFTLLTCSYLADIVNMGDH
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Figure 1. Alignment of ATPase 6 subunit sequences. Sequences are derived from UniProtKB database, from organisms: *Clostridium acetobutyllicum* (O05097), *Escherichia coli* (P0AB98), *Haemophilus influenzae* (P43719), *Homo sapiens* (P00846), *Gorilla gorilla* (Q9T9Y7), *Bos taurus* (P00847), *Gallus gallus* (P14092), *Brachydanio rerio* (Q9MIY5), *Drosophila melanogaster* (P00850), *Anopheles gambiae* (P34834), *Acanthamoeba castellanii* (Q37385) and *Allomyces arbuscula* (P50363). Amino acid substitution region is shown in frame. All alignments were generated with the Tcoffe program (45).

```
P92514      GFHVIIGTLFLIICGIRQYLGHLIKEHHVGFEAAAWYWHFVDVVWLFLFVSIYWWGGI--
Q37374      GLHVIIGTLFLIVCFFRLIDLHFIYNHHFGYEAAIWYWHFVDVVWIFLFLSIYCWG-S-G
P24012      GAHVAFGLMWISTLMIRNAKRGLNLYTAPKFYVASLYWHFIDVVWVFIFTVVYLMG-MVG
Q9AEL8      ALHVIAGVMAFVVVLMRIHKSKFTPAQATAAMVVSYYWHFVDVVWIGLFITIYFI----Q
P24891      GIHVLCGGLFLAFNFLRLLKNHFNYNHHLGLEFAILYWHFVDVVWLFLFVFVYWWS-Y--
P00421      GLHVIIGTAFLAVGLWRLAAYHLIDHHHLGYESGILYWHFVDVVWLFLYISVYYWG-Y--
P48891      GLHVMVGTLFLFVNLVRTYYYHFSTTHHVGFLAAAWYWHFVDVVWLFLYISIYWWG-S--
Q9MIY4      GLHVIIGSTFLAVCLLRQVLFHFTSDHHFGFEAAAWYWHFVDVVWLFLYVSIYWWG-S--
P00414      GLHVIIGSTFLTICFIRQLMFHFISKHHFGFEAAAWYWHFVDVVWLFLYVSIYWWG-S--
P00415      GLHVIIGSTFLIVCFFRQLKFHFISNHHFGFEAAAWYWHFVDVVWLFLYVSIYWWG-S--
P18945      GLHVIIGSSFLTVCLLRLIKFHFTPNHHFGFEAAAWYWHFVDIIWLFLYMSMYWWG-S--
P00417      GIHVLIGTTFLLVCLLRHLNNHFSKNHHFGFEAAAWYWHFVDVVWLFLYITIYWWG-G--
            . **  *   :     *     :             ****:*::*: ::  :*
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Figure 2. Alignment of COX3 subunit sequences. Sequences are derived from UniProtKB database from: *Arabidopsis thaliana* (P92514), *Acanthamoeba castellanii* (Q37374), *Bacillus subtilis* (P24012), *Corynebacterium glutamicum* (Q9AEL8), *Caenorhabditis elegans* (P24891), *Emericella nidulans* (P00421), *Albinaria coerulea* (P48891), *Brachydanio rerio* (Q9MIY4), *Homo sapiens* (P00414), *Bos taurus* (P00415), *Gallus gallus* (P18945), and *Drosophila melanogaster* (P00417). Amino acid substitution region is shown in frame.

that may be considered silent because of poor conservation of the protein sequence in the affected region (10,33). In contrast, the G4665A mutation reported by Zhu and co-workers (10) in subunit ND2 replacing alanine by threonine may lead to improper protein folding. Alanine is conserved among vertebrates, and alanine or serine is present in lower organisms. Serine is a polar amino acid, while alanine is hydrophobic, both of them, however, belong to the class of the smallest amino acids, with a short side chain (serine, alanine and glycine). It seems that the presence of small amino acids in this position is crucial for the maintenance of protein structure whereas the threonine branched side chain causes steric collisions destabilize protein structure.

Mutation T9885A reported by Zhu in mtDNA of breast cancer patients results in the replacement of phenylalanine by isoleucine, at the C terminus of COX3 polypeptide (10). This region is highly conserved within the animal kingdom, and amino acid different than phenylalanine, tyrosine is found only in *Branchiostoma sp*., but only the presence of a hydroxyl group in the *ortho* position of the ring distinguishes tyrosine from phenylalanine (Fig. 2), therefore it seems that phenylalanine in this position is necessary for maintenance of protein function. Analysis of the structure of cytochrome c oxidase from *Bos taurus* shows that phenylalanine 227 is located in a loop between two α helical chains of the COX3 subunit (Fig. 3). It is located only 3.5A˚ from amino acids

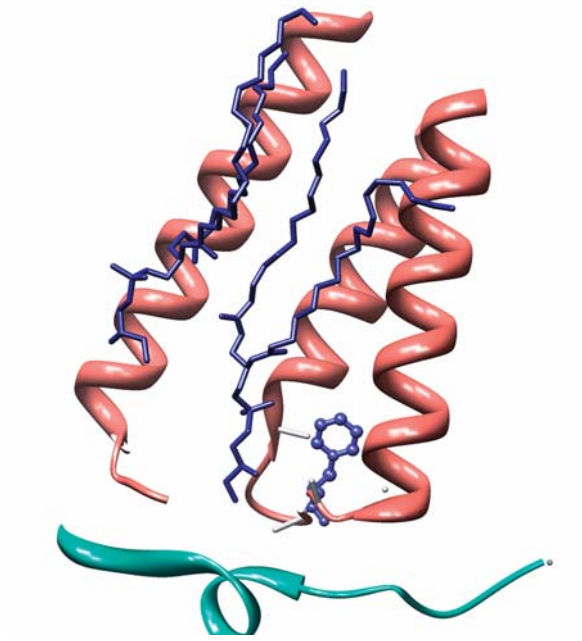

Figure 3. Fragment of the structure of cytochrome c oxidase subunit from *Bos taurus* from the PDB database (46), PDB ID 1v54 (47). Fragment of COX3 subunit is colored pink, subunit COX5b light blue and phospholipid chain dark blue. Phenylalanine 227 is shown in ball model and colored dark blue. Structure visualization performed by CHIMERA program (48).

belonging to subunit COX5b amino acids and >4 A˚ from the phospholipid chain bound to the enzyme. This phospholipid most likely stabilizes this mitochondrial complex, and may also be involved in complex assembly (34). Substitution of the aromatic phenylalanine with the aliphatic isoleucine may disturb the loop structure, and impair the interactions with subunit COX5b and/or the phospholipid chain.

Two mutations, T13397A and T13398A found in breast cancer samples of two patients both cause the substitution of glutamine by leucine in the ND5 subunit in region that is strongly conserved in evolution. The position in which the amino acid substitution takes place is located in a short stretch of polar amino acids (*Homo sapiens* HNLNNEQDIRK) and this position can be expected to be important in protein folding and maintaining the enzyme function (10).

As the result of the A13674G mutation in ND5 polypeptide asparagine is substituted by lysine. The two amino acids belong to the group of polar amino acids and therefore no substantial conformational changes in polypeptide structure are expected (10). The A11768G mutation results in a change of threonine to alanine in the ND4 protein. ND4 sequence alignment shows poor evolutionary conservation in the mutated region, suggesting that also this mutation has no evident impact on protein structure or function.

The G15755T mutation in breast cancer cells replaces glycine by tryptophan in cytochrome b subunit. In matched healthy tissue from the same patient at position 15755 an atypical nucleotide G was reported (in CRS and 2703 other known mtDNA genomes 15755 is T) (35). It seems that the patient inherited an atypical mtDNA sequence variant and the mutation that occurred in the tumor was actually a reverse mutation and no functional impairment of the 'mutant' should be expected (36). Similarly the T15783C is also a reversion to a common nucleotide (in CRS and 2703 other known mtDNA genomes 15783 is C), and should not be pathogenic (35). Nevertheless more extensive biochemical and molecular studies will be necessary to determine the pathological significance of all reported somatic mutations, as some mutations/polymorphisms including T14487C (Complex I) (37), T8993G (ATPase 6) (38) or A3243G (tRNA Leu) and A8344G (tRNA Lys) (39) have been shown to cause an over-production of ROS leading to an increase in the oxidation of lipids and mtDNA; whereas other polymorphisms including 15257A or 14798C-reduce proton pumping and thus coupling efficiency (40) and these possibilities cannot be excluded for other sequence variants unless evaluated.

3. Conclusions

We believe that defining mtDNA polymorphisms and/or mutation patterns in selected types of cancer, including breast cancer may help to understand the basic biochemical mechanisms involved in the induction of cell transformation and indicate the potential role of mtDNA in cancer progression. Moreover, it may offer opportunity to develop bio-markers providing additional information supplementing currently available clinical and pathological tests and screening procedures that will be of prime importance to assess individual risk posed by inherited mtDNA polymorphisms. Nevertheless it is worth pointing out that most of the somatic mtDNA mutations are actually mtDNA sequence variants found in the general population (20,36). If these mutations are not just sequencing errors in which population variants were overlooked in either the tumor tissue or else the normal tissue was not sequenced (41-43), it is possible that cells undergoing neoplastic transformation are prone to mutations in mtDNA hot-spots analogous to those that mutated in the process of evolution (20). Such mutations might influence function of mRNA and/or mtDNA regulatory regions by yet unidentified mechanisms and provide a functional advantage for the cancer cell. Recently it has been suggested that electron transport chain proteins encoded by mutated mtDNA generate an excess of ROS, which acts as nuclear genome (nDNA) mutagen and as cellular mitogen and thus promotes genome instability and cell proliferation (20,36).

If a mtDNA mutation pattern would be established for breast cancer, it could enhance the specificity of cancer detection and prediction of the biological behavior and outcome of these tumors. OXPHOS activity in cancer cells could serve as one of the biomarkers in tumor staging, determining prognosis and planning adjuvant therapeutic strategies. The recent discovery that ND6 mutations can regulate tumor cell metastasis (44) indicates that similar mutations could also arise in breast cancer and be used as a prognostic marker. The success of mtDNA research in 'mitochondrial oncology' would be to develop new markers that provide information useful to physicians and patients in designing the course of cancer treatment and markers enabling to select a population that should undergo regular medical examinations, as having increased cancer-development risk.

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