Nuclear and Mitochondrial Genome Instability in Human Breast Cancer

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ABSTRACT

We analyzed 40 pairs of breast normal/cancer tissues for the presence of mitochondrial (mt) genome instability and nuclear MSI in tumor cells. As mt markers we used a (CA)ₙ mt microsatellite (MS) starting at the 514-bp position of the D loop region and 4 informative MnlI sites located between the 16,108- and 16,420-bp positions of the D loop region. Nuclear microsatellite instability (MSI) was tested with 8 (CA)ₙ MS, syntenic for the 13q chromosome arm. Moreover, we tested the spontaneous frequency of mtMSI and mt-MnlI mutations in 459 mother/descendant events. Mutations of mt-MnlI sites were found in 19 of 40 (47.5%) breast tumors, representing a 216-fold increase over the spontaneous rate in the female germline. Instability of the mtMSI occurred in 17 of 40 (42.5%) breast cancers, which implies a 16-fold increase over the rate of spontaneous mutations. Nuclear MSI was found in 20 of 40 (50%) cases. In 15 of these cases the MSI was restricted to one locus, whereas in 5 instances the change of alleles was detected in 2 or 3 loci. Analysis of the correlation between mt and nuclear mutations showed no significant associations, suggesting that different systems are responsible for mt and nuclear genome instability in tumor cells. We propose that the two main mechanisms producing mtRFLP and mtMSI are by free radicals and error repair by the polymerase γ, the first mechanism being a major cause of MnlI mutations and a secondary cause of mtMSI.

INTRODUCTION

Cancer is the result of a multistep mechanism that ends in malignant cell transformation. Despite the enormous progress made during the last decade in the understanding of the role of mismatch repair genes, oncogenes, and tumor suppressor genes, several aspects in the process of carcinogenesis still remain unclear. In this regard, the finding of mutations and deletions in the mtDNA of tumor cells has focused the attention on these cytoplasmic organelles as concurrent factors in the start or progress of tumors (1).

mtDNA is transmitted from the mother to all children, but only the female descendants pass it on to the next generation. Accordingly, mtDNA molecules are haploid due to the lack of homologous molecules of paternal origin. The number of mt genomes per cell varies from a few (resting lymphocytes) to several thousand (oocytes), giving rise to mtDNA polyhaploidy in all cells. A common form of nuclear genome instability is the appearance of an uninterrupted poly(C) tract due to the T→C transition and the poly(C) tract heteroplasmy in tumor but not in normal cells (23), the T→C transition being the primary event and the poly(C) tract instability the secondary effect.

In normal individuals, starting at 514 bp of the mtDNA control region, there is a (CA)ₙ microsatellite showing 5 haploid alleles differing in size by the number of dinucleotide repeats (Ref; 20; data in this report). Heerdt et al. (11) tested 24 pairs of normal/colorectal cancer tissues and found no allelic change in the mtMSI.

Extending from 16,184 bp to 16,193 bp of the mtDNA control region, there is a poly(C) tract interrupted by a T at the 16,189 bp position (21). One of the mt variants that usually occur in normal individuals is the appearance of an uninterrupted poly(C) tract due to a T→C transition at 16,189 bp; these cases, in addition to the T→C transition, also show by length heteroplasmy of the poly(C) tract that are maternally transmitted from the female ancestor to her descendants (22). Some colorectal cancers carry the T→C transition and the poly(C) tract heteroplasmy in tumor but not in normal cells (23), the T→C transition being the primary event and the poly(C) tract instability the secondary effect.

In this report, we analyze 40 pairs of breast normal/cancer cells. The rate of allelic changes in the mt(CA)ₙ MS of tumor cells determines the frequency of mtMSI. Moreover, we used 4 informative MnlI sites located between 16,108 bp and 16,420 bp of the mt control region (21) to detect RFLP between normal and tumor cells; the T→C transition at 16,189 bp is detected by the loss of one of the above sites. We also tested 8 MS loci to assess the rate of nuclear MSI in tumor cells. Furthermore, we analyzed the frequency of appearance of mtRFLPs and (CA)ₙ mtMS mutations in 459 mother/descendant events to estimate the spontaneous rate of mtMSI and mt-MnlI mutations in the female germline and to compare these rates with the rate of mt mutations in breast tumor tissues.

MATERIALS AND METHODS

Samples. We studied 40 pairs of normal/cancer breast specimens fixed in 4% formalin and embedded in paraffin. Normal and tumor tissues were identified by microscopy; cancer regions selected for DNA extraction showed...
>90% of abnormal cells. DNA from paraffin slices was extracted according to the method of Mashal et al. (24). We also studied DNA samples from 40 CEPH families comprising 67 ancestral matrilineages and 459 mother/descendant events.

**MitDNA Analysis.** The (CA)$_n$ microsatellite starting at 514 bp (21) was amplified with the primers and PCR conditions reported by Szibor et al. (20). Microsatellite allele identification was performed by electrophoresis in 15% neutral polyacrylamide and Sybr-Green fluorescence. Allele nomenclature in the text and tables was based on the number of repeats (20).

RFLP for *Mnl* sites [CCTC(N)$_n$] was detected in a mtDNA fragment of 312 bp extending from the 16,108 bp position to the 16,420 bp position. Primers used were mtL16,108 5'-CAGCCACCATGAATATTGTAC-3’ (forward) and mtH16,420 5'-TGATTTCACGGATGGTG-3’ (backward). PCR conditions were 30 cycles with 56°C annealing temperature. According to the reference sequence of Anderson et al. (21), the 312-bp fragment comprises five *Mnl* sites producing fragments of 118, 89, 39, 35, 28, and 3 bp (Fig. 1, a). With the exception of the 3-bp fragment, which could not be identified due to its small size, all of the other fragments and their corresponding RFLPs (Fig. 1, a–l) were detected by electrophoresis in 10% neutral polyacrylamide and Sybr-Green fluorescence staining. In the text, tables, and figures, the position of *Mnl* sites is identified by the position of the restriction site located 7 bp downstream of the recognition site.

All pairs of normal/cancer samples and all CEPH cases showing mt marker changes between the ancestor and a given descendant were tested 2–4 times to confirm the consistency of mt marker identifications.

**Autosomal MS.** A set of eight (CA)$_n$ MS syntenic for the 13q chromosome arm were amplified with the primers reported by Deka et al. (25). We used four duplex PCR reactions combining the primers for FLT1 and D13S197 (55°C annealing), D13S118 and D13S193 (55°C annealing), D13S121 and D13S71 (55°C annealing), and D13S122 and D13S124 (49°C annealing). Alleles were detected by electrophoresis in 15% neutral polyacrylamide and Sybr-Green fluorescence. Alleles are identified in the text by the number of repeats. Allele diversities reported in literature for each one of the MS are: FLT1, 19 alleles (156–200 bp); D13S118, 12 alleles (176–200 bp); D13S21, 15 alleles (150–

![Fig. 1. *Mnl* RFLPs patterns. The site at the 16,417 bp position was not informative. Accordingly, the a–l patterns are based on the four informative *Mnl* sites. The a pattern corresponds to the reference sequence of Anderson et al. (21). *Mnl* sites are defined by restriction positions that are located 7 bp downstream of the recognition site of the enzyme.](cancerres.aacrjournals.org)
RESULTS

CEPH Families. The mt (CA)_n alleles observed in the 67 founder maternal lineages are indicated in Table 1. Allele changes between the ancestor and descendant(s) were observed in three families (Fig. 2). In family 35, six of the eight F1 descendants showed allele changes. One case was homoplasmic, two other cases were heteroplasmic for two mtDNA subpopulations, and three were heteroplasmic for three subpopulations, one of which corresponded to the maternal ancestor allele (Fig. 2). In F2 of family 1408 (Fig. 2), the ancestral allele 7 changed into a homoplasmic allele 8 in one male descendant and into a 7/8 heteroplasm in a female descendant. Finally, in family 1413, we observed one homoplasmic change that does not fit the pattern of maternal transmission (Fig. 2). Because the CEPH family repository is stored as Epstein-Barr immortalized lymphoblastoid cell lines (26), we assume that the allele shifting in family 1413 was due to the process of immortalization. Allelic changes caused by lymphocyte immortalization have been reported by Weber and Wong (27) and Bianchi et al. (28). Thus, if in heteroplasms we count as mutations only those alleles differing from the maternal allele, we have a total of 12 novel MS alleles representing a spontaneous mutation rate of 2.6% (12 of 459) and a mutation rate due to lymphoblastoid transformation of 0.2% (1 of 459).

The total number of Mnl restriction patterns detected in normal, tumor, and CEPH samples was 12; 9 of them corresponded to CEPH lineages. In only one of the CEPH lineages (family 2) did we observe that the ancestral restriction pattern b changed to pattern i (Fig. 2; Table 2). Accordingly, the spontaneous rate of Mnl mutations in female germline cells is 1 of 459 or 0.22%. We sequenced three randomly selected samples showing the T→C transition at 16,189 bp, and in all of them we found the poly(C) length heteroplasmy reported by Bendall and Sykes (22). We conclude that in our series of normal/cancer pairs poly(C) instability is a constant event accompanying all cases with a T→C transition at 16,189 bp.

We did not observe any case of heteroplasmy during the analysis of Mnl I RFLPs in CEPH samples. Zischler et al. (29) have reported that human chromosomes 11 carry a recent integration of a segment of the mt control region extending from 16,089 bp to 59 bp. The region we used to analyze Mnl I RFLPs is part of the nuclear pseudomitochondrial gene reported by Zischler et al. (29); moreover, the Mt.16,108 forward primer is homologous to the nuclear sequence, and the MnlI6,420 backward primer differs from the complementary nuclear sequence by a single base located at the second 5'-position. Thus, the PCR reactions with these primers not only amplify the mt sequence but should also amplify the nuclear mt-like sequence. Nuclear mt-like fragments lack the Mnl I recognition site at 16,189 bp due to a T→A transversion (29). In case of equivalent coamplification of mt-like nuclear and genuine mt sequences, all samples having the Mt16,189 mt site would be heteroplasmic due to the coexistence of 89-bp + 35-bp fragments (presence of the site in the mt fragment) with a 124-bp fragment (lack of the site in the mt-like nuclear fragment). Because this is not the case, we conclude that due to the differential amount of targets (thousands of mtDNA targets per cell versus only two chromosome 11 targets per cell) the PCR fragment amplified is predominantly of mt and not of nuclear origin, a conclusion valid not only for CEPH families but also for normal/cancer breast tissue pairs.

mtMS, we detected one heteroplasmic sample (case 4, alleles 5/6; Table 2). The mutant allele changing in family 1413 does not correspond to the pattern of maternal transmission. Numbers in parentheses, percentage.

![Fig. 2. Genealogies of the three families showing spontaneous mutations of the mtMS.](image-url)

Table 1 Allelic frequencies of mtMS

<table>
<thead>
<tr>
<th>Alleles</th>
<th>CEPH lineages</th>
<th>Breast normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>11 (16.4%)</td>
<td>9 (22.0%)</td>
</tr>
<tr>
<td>5</td>
<td>44 (65.6%)</td>
<td>31 (77.6%)</td>
</tr>
<tr>
<td>6</td>
<td>10 (15.0%)</td>
<td>1 (2.4%)</td>
</tr>
<tr>
<td>7</td>
<td>2 (3.0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>41</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Table 2 Frequency of Mnl I RFLPs in CEPH lineages and in normal and tumoral breast tissues

<table>
<thead>
<tr>
<th>Samples</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
<th>j</th>
<th>k</th>
<th>l</th>
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</thead>
<tbody>
<tr>
<td>CEPH</td>
<td>39</td>
<td>7</td>
<td>10</td>
<td>13</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Normal breast cells</td>
<td>27</td>
<td>6</td>
<td>8</td>
<td>20</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Tumoral breast cells</td>
<td>16</td>
<td>4</td>
<td>10</td>
<td>25</td>
<td>1</td>
<td>2.5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Letters correspond to RFLP patterns illustrated in Figures 2 and 3.
* The a pattern corresponds to the reference sequence of Anderson et al. (21).

All tables and figures are included in the article text. The content includes tables and figures illustrating the distribution of alleles and RFLP patterns among normal and tumor tissues, as well as genealogical trees showing the transmission of mtMS mutations through generations. The text provides a detailed analysis of the results, discussing the frequencies and patterns of mtMS mutations, and their implications for the study of genome instability in breast tumors.

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3). Accordingly, the total number of alleles detected in the 40 samples of normal breast tissues was 41. Table 1 depicts the allele frequency in normal tissues. The analysis of tumor sample counterparts showed allele changes in 17 of 40 cases (42.5%; Table 3). Eight of these cases exhibited a shifting from allele 5 in the normal tissue to allele 4 in the tumor tissue; 7 cases showed a 4- to 5-allele change; one case had allele 5 in normal tissue and a 6/8 heteroplasmy in cancer cells (case 11; Table 3), whereas the remaining case was heteroplasmic for alleles 5/6 in normal tissue and homoplasmic for allele 5 in tumor cells (case 11; Table 3).

The appearance of MnlI mutations was observed in 19 of 40 cases of breast tumor cells (47.5%; Table 3). Nine cases gained 1 and 10 cases lost 1 MnlI site with regard to normal tissues. If we combine MS and MnlI data, the mtGI observed in cancer tissues was 27 of 40 (65%).

Nuclear MSI. To define MSI, we followed the recommendations and guidelines reported for colorectal cancers (30). Thus, we identified as MSI any change in length produced by the insertion or deletion of one or more repeat units in tumor tissues MS as compared with MS alleles in normal counterpart tissues. MSI-L and MSI-H were characterized, respectively, by allelic changes in one locus (MSI-L) or in two or more loci (MSI-H) of the eight loci used. Cases in which the normal tissues showed different alleles and tumor tissues showed one of the alleles in normal cells (i.e., cases 1, 2, 20, and 23; Table 3) were not counted as MSI because we considered it difficult to clearly discern between loss of heterozygosity or MSI (30). Using these criteria, we found 15 of 40 (37.5%) cases of MSI-L (cases 5, 6, 8, 11, 14, 15, 16, 18, 20, and 23–28; Table 3) and 5 cases (12.5%) of MSI-H (cases 17, 19, 21, 22, and 30; Table 3). Approximately one-half of the mutated alleles showed length contraction and half-length expansion.
**DISCUSSION**

**Frequency and Characterization of Nuclear MSI.** Data in the literature on the frequency of nuclear MSI in breast cancer range from 0% [7 loci analyzed (13)] to 80% [8 loci tested (31)]. Between these minimum and maximum rates, the frequencies reported are: 1.3% [8 loci tested (19)]; 8% [11 loci tested (32)]; 10.8% [12 loci tested (33)]; 26% [7 loci tested (34)]; 28.2% [7 loci tested (35)]; 39.3% [8 loci tested (18)]; 40% [11 loci tested (36)]. Although the causes for these extreme ranges of frequencies are not totally clear, it seems obvious that the instability of the markers used probably plays an important role, mainly when we take into account that the type of MS tested by different authors varies from di- to tetranucleotides, the loci locations comprise a large number of chromosome regions, and the spontaneous rate of germline MS mutation reported in the literature varies from $2.3 \times 10^{-5}$ (37) to $1.2 \times 10^{-3}$ (27, 37–39) per locus per gamete per generation for autosomal MS; $1.2 \times 10^{-3}$ for Y-specific MS (28); and 1.5% per gamete per generation for the X-linked DXS981 MS (40). Thus, results from different laboratories are not comparable.

We had no special reason to select the eight MS markers used in this report except that they are routinely used by our and other groups for population studies. One of the main peculiarities of this set of markers is that they do not follow the predictions of the single-step mutation model (25). Thus, length contractions and expansions comprising more than one repeat are frequent (Table 3).

We found MSI in 20 of 40 (50%) of tumor cases. Liu et al. (41) have defined three different groups of cancer with MSI: group 1, cancers with a family history of malignancy, which usually show two or more loci exhibiting MSI (MSI-H); group b, tumors with MSI-H but with no family history of cancer; and group c, tumors with no family history of cancer and with a lower prevalence of MSI (MSI-L). According to these criteria, 15 of our cases correspond to the third group and 5 to the second group.

Malfunction of the two alleles in at least one locus of the MMR genes loci (hMSH2, hMLH1, hPMS2, or hPMS1) has been documented in colorectal cancers of groups a and b (15, 41), and also, although at lower frequency, in endometrial and gastric cancers with MSI-H (42–44). Therefore, by extrapolation it has been assumed that breast cancers showing MSI-H have also defective MMR genes (18). On the other hand, no defective MMR genes have been detected in colorectal, endometrial, and gastric cancers with MSI-L (42, 44, 45). MSI-L in these tumors has been explained by endogenous damage by ROS, exogenous factors including smoking and diet, and deficiencies in repair mechanisms not controlled by the major cohort of MMR genes (30).

**mt GI.** The presence of mtDNA mutations in tumor cells has been investigated in gastric (46), breast (10), and colorectal cancers (11, 12, 23). A deletion of 50 bp extending from 298 to 348 bp was detected in 12.5% of gastric adenocarcinomas of the gastroesophageal junction (4 of 32 cases tested) but in none of the 45 cases having more distal gastric cancers (46). Furthermore, of a total of seven cases of breast cancer in which the whole mt genome was tested for RFLP with the use of nine different restriction enzymes, five cases exhibited mutations in tumor but not in normal cells (10). Heerdt et al. (11) sequenced a fragment of the mt-D loop (371–570 bp) and analyzed the appearance of allele changes in the (CA)n mtMS in 24 pairs of normal/tumor tissues of colorectal cancers, finding no tumor mutations. Polvak et al. (12) amplified by PCR and sequenced the entire mt genome in seven cell lines derived from colorectal tumors. Of the 88 mutations detected (4–31 per tumor cell line), 8 were present in protein coding genes and 4 in tRNA genes. To confirm whether these variants were specific of cancer cells or the result of the in vitro conditions, the authors tested the primary cancer cells from which the cell lines were derived and also the normal colon tissue counterparts. They found that 12 of the 88 mutations were specific of tumor cells. Eleven of these mutations were T→C or G→A transitions and one was a base insertion; 10 mutations were homoplasmic, and 2 were heteroplasmic for 2 mt genome subpopulations. Habano et al. (23) analyzed the (CA)n mtMS and the D loop poly(C) tract in 45 pairs of normal/tumor tissues from sporadic colorectal cancers. No mtMS was detected in tumor tissues, whereas 20 cases (44%) of tumor cells showed instability of the poly(C) tract very likely produced by T→C transitions at the 16,189 bp position.

In the series reported here, we found 17 of 40 (42.5%) tumors showing mtMSI. Because the spontaneous rate mutation rate for this MS in female gametes was 2.6%, we conclude that breast tumor tissues had a 16-fold increase in the rate of mtMSI. Mutations of Mnth sites appeared in 47.5% (19 of 40) of breast cancer tissues and 0.22% of CEPH pedigrees; this represents a 216-fold increase in the mutation rate of cancer cells with regard to the spontaneous rate detected in female gametes.

Table 4 shows the lack of significance between the observed and the expected frequencies of nuclear MSI, mtMSI, and mtRFLP associations. Data in this table indicate that nuclear GI and the two forms of mtGI analyzed in this report are independent phenomena caused by abnormalities at different stages of the pathway leading to cell transformation.

**Origin of mtGI.** Increased damage by ROS and defective DNA repair are the two causes proposed to explain the mtGI in cancer cells (5, 10–12, 23, 46). Moreover, the same causes have been assumed to play a role in the induction of nuclear MSI in colorectal cancers and other tumors (30). Although these factors are surely involved in the mt damage found in our series of cancer breast tissues, the importance of these mechanisms is different for each one of the mutations analyzed.

The mtDNA fragment tested for Mnth RFLPs is part of the D loop segment. This region has a triple-stranded DNA conformation and is the point of attachment of the mtDNA to the mt membrane (47). All cases of breast cancers included in our series correspond to postmenopausal women whose normal breast cells were in a metabolic resting state due to the lack of hormonal stimulus. Breast cancer cells, on the other hand, exhibit an active OXPHOS pathway producing a marked increase of ROS (10). These DNA breakdown products (lipid peroxides, alkylxyl radicals, peroxyl radicals, and aldehydes) mainly occur at the mt membrane level and preferentially damage the single chain of the triple-stranded DNA structure of the D loop (48). The base repair process used to correct the damage in the mtDNA molecule is mediated by polymerase $\gamma$ (Pol $\gamma$), an enzyme with decreased proof reading efficiency (49, 50). Thus, the combination of increased DNA damage by free radicals and deficient base repair by Pol $\gamma$ are
the causes explaining the 216-fold increase of MnlI mutations detected in the D loop region of breast cancer cells.

Slipped strand mispairing followed by deficient repair are the mechanisms accounting for the origin of MS repeat variants (51). Accordingly, in breast tumors, the nuclear and mtDNA replication resulting from the continuous cell cycling of transformed cells gives the basis for the appearance of slipped strand mispairing. In nuclear MSI-H, the homozygous malfunction in one locus of the cohort of MMR genes seems to play a fundamental role in the appearance of facultative MS allele changes. On the other hand, besides ROS damage, dietary components and smoking have been assumed to enhance MSI-L (30), although the role played by these factors in the induction of slippage mispairing is not clear. The moderate increase of mtMSI (21) and the causes explaining the 216-fold detected in MnlI mtRFLPs) emphasizes that mtMS allele changes in breast cancer are mainly due to Pol γ errors and, to a lesser extent, to ROS-induced DNA damage.

Homoplasmic of mt Mutations. In human ovary carcinoma, the average of mitochondrial per cell was 4.6, with 10 being the average of DNA molecules per mitochondria (52). Although we do not have equivalent estimations for breast cancer cells, it seems reasonable to assume that the number of mtDNA molecules per cell probably ranges from a few hundreds to a few thousands. Damage to mtDNA by ROS and error repair by Pol γ should occur at random, giving rise to cell heteroplasmies comprising multiple subpopulations of mutated mtDNA molecules. Yet, in our series, most tumor cells exhibiting mt MnlI variants or MSI were homoplasmic for the mutated DNA, Polyak et al. (12) also found that 10 of 12 cases of colorectal cancers showed mtDNA homoplasmic mutations. Two opposite hypotheses have been formulated to explain the predominance of homoplasmic versus heteroplasmic mutated mtDNA in cancer cells. According to the report of Shay and Werbin (1), under particular environmental conditions, some of the mt mutations may result in selective advantage, enabling the cell(s) harboring the mutation to outgrow other cells in the population and also allowing the mtDNA with the advantageous mutation to outgrow other mt genomes in the cell. Conversely, Polyak et al. (12) proposed that mt genomes with mutations affecting their functional efficiency overreplicate to maintain a normal OXPHOS pathway by an increase in number. Over the thousands of generations required for the tumor to progress and expand, the most overreplicated mtDNA molecule(s) easily replace other mt subpopulations, giving rise to homoplasy. Because all mt genes and markers are in linkage disequilibrium, the overreplicated mtDNA gives rise to homoplasy, not only for the advantageous or disadvantageous mutations but also for all other mt markers associated with it.

Final Remarks. By using eight autosomal MS, one mtMS, and four polymorphic mt MnlI sites, we were able to detect nuclear and/or mtGI in 35 of 40 (87.5%) breast cancers; a figure high enough to have importance for the diagnosis of malignancy. Damage by ROS and lipid peroxides combined with Pol γ error repair seem to be the mechanisms subjacent for MnlI mtRFLPs. On the other hand, mtMSI seems to depend more on the Pol γ mismatch repair than on damage by ROS. Although we did not find any correlation between the type of nuclear or mt damage detected and the pathology of the tumor cases analyzed (Table 3), in the future it would be advisable to extend these studies to explore whether the distinct types of mt and nuclear GI found in breast cancer cells may be correlated with different clinical forms or with different therapeutic responses and survival.

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REFERENCES


4. De Marchis, L., Contegiacomo, A., D’Amico, C., Palmirotta, R., Pizzi, C., Ottini, L., Frati, L., Cama, A., and Mariani-Costantini, R. Microsatellite instability is correlated with homoplasmic for the mutated DNA. Polyak et al. (1), under particular environmental conditions, some of the mt mutations may result in selective advantage, enabling the cell(s) harboring the mutation to outgrow other cells in the population and also allowing the mtDNA with the advantageous mutation to outgrow other mt genomes in the cell. Conversely, Polyak et al. (12) proposed that mt genomes with mutations affecting their functional efficiency overreplicate to maintain a normal OXPHOS pathway by an increase in number. Over the thousands of generations required for the tumor to progress and expand, the most overreplicated mtDNA molecule(s) easily replace other mt subpopulations, giving rise to homoplasy. Because all mt genes and markers are in linkage disequilibrium, the overreplicated mtDNA gives rise to homoplasy, not only for the advantageous or disadvantageous mutations but also for all other mt markers associated with it.

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GENOME INSTABILITY IN BREAST TUMORS


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