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Review

Maternally inherited susceptibility to cancer ☆

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ABSTRACT

Tumor microenvironment promotes mtDNA mutations. A number of these mutations will affect cell metabolism and increase cell survival. These mutations are positively selected and contribute to other tumor features, such as extracellular matrix remodeling and angiogenic processes, thus favoring metastases. Like somatic mutations, although with less marked effects, some mtDNA population polymorphisms will affect OXPHOS function, cell metabolism, and homeostasis. Thus, they could behave as inherited susceptibility factors for cancer. However, in addition to epidemiological evidence, other more direct clues are required. The cybrid approach can help to clarify this issue. This article is part of a Special Issue entitled: Bioenergetics of Cancer.

1. Introduction

Carcinogenesis is a sequence of phenotypical adaptations to distinct microenvironmental proliferation barriers. Multiple strategies can successfully adapt to the same barrier, thus explaining the phenotypical and genotypical heterogeneity of cancer populations [1]. Therefore, the number of cancer genes is large. Thus, the cancer genome is composed of a few commonly mutated genes and many infrequently mutated genes that function in a relatively small number of pathways that cooperate to induce disease. The pathway components that are altered in individual tumors vary widely, suggesting that mutations in many genes in the pathway can have similar effects on tumor cell growth. Thus, the pathways, rather than individual genes, dictate the course of tumorigenesis [2]. Multiple genetic changes seem to be required in carcinogenesis [3], and different tumors can show distinct mutations. This means that no particular mutation in cancer-associated nuclear genes is sufficient or necessary to cause cancer, when cancer is considered as a class of diseases. The same considerations must be taken into account when mutations in mtDNA genes are analyzed in relation with the tumoral phenotype. Therefore, we will not address whether mtDNA mutations and OXPHOS dysfunction are required for tumor development, but we will focus on whether mtDNA mutations can influence the tumor phenotype.

Large families with multiple cases of early-onset cancer affecting several generations provide clear evidence that inherited factors are important causes of cancer. Family history is an important risk factor in almost all cancers, but most family cancers are not caused by mutations in the rare relatively high-risk tumor-associated genes, so lower-risk genes must be present [4]. In this review, because mtDNA is inherited by maternal lineage, we will explore whether or not there is a maternally inherited predisposition to cancer. In other words, is there a mtDNA-inherited susceptibility to cancer?

2. Cytoplasmic suppression of tumor formation

2.1. Hybrids

A possible contribution of extrachromosomal factors to tumorigenicity was suggested after the observation that tumorigenicity was still suppressed in some human (fibroblast)–mouse (malignant melanoma) hybrid clones after segregation of all detectable human chromosomes derived from the nontumorigenic cell [5]. The radiosensitivity of this factor suggested that it was a genetic entity. But, because many previous studies on human–mouse hybrids had shown that those hybrids where the human chromosomes had been preferentially eliminated did not contain human mtDNA, the authors excluded mtDNA as the extrachromosomal factor. However, the fact that these hybrids can retain human mtDNA for many generations [6] and that mtDNA is the only cytoplasmic genetic factor in mammalian cells suggested that mtDNA was the tumorigenicity suppressor.

2.2. Heteroplasmic cybrids

The effect of cytoplasm upon the expression of tumorigenicity can be evaluated by the cybrid approach. Cybrids are cell lines originating

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from the fusion of a nucleated cell with an enucleated cell (cytoplast) [7]. Cell fragments with mitochondria but without a nucleus, such as platelets [8] or synaptosomes [9] or whole cells treated with nuclear poisons [10], can also be used as cytoplasm donors (Fig. 1). In cybrid clones formed by the fusion of tumorigenic CHEF/16 hamster cells with nontumorigenic CHEF/18 cytoplasts, the tumorigenicity was partially suppressed, as assessed by the transformation rate in cell culture and by the rate and extent of tumor formation in nude mice. Thus, this analysis suggested that suppression of tumor-forming ability might be cytoplasmically transmitted [11]. These studies were performed with cell populations that had undergone 20–30 doublings after the fusion, therefore ruling out the role of nuclear gene products that would have been diluted and thus reinforcing a possible role for mtDNA. The suppression of tumorigenicity was also reported by using tumorigenic rat thyroid cells and cytoplasts from epithelial cells derived from normal rat thyroids [12]. Also, cybrids produced by fusing C2B2 tumorigenic mouse mammary epithelial cells and cytoplasts from THOC nontumorigenic BALB/c 3T3 fibroblasts, after 7–13 postfusion doublings, showed a reduction of the tumorigenic capacity in terms of tumor incidence and latency when tested in newborn (less than 1 day old) BALB/c 3T3 mice [13]. The cybrids derived from the fusion of highly tumorigenic mouse teratocarcinoma lines (984 C1 10–15), which had lost the ability to differentiate into skeletal muscle, and cytoplasts from nontumorigenic mouse cells (AMT) did not retain the tumorigenicity of the original cell line when evaluated in nude mice, and most of the cybrids demonstrated the ability to differentiate into skeletal muscle. A factor was present in the nontumorigenic cell cytoplasm that was able to induce long-term (greater than a year in continuous culture) heritable suppression of tumorigenicity [14,15]. The fusion of normal rat hepatocyte cytoplasm to tumorigenic rat hepatocyte whole cells resulted in suppression of the tumorigenicity in only one of five cybrid clones analyzed when injected into newborn rat pups that were isogenic with those from which the cell culture was initiated. The cybrid clones produced tumors in 51% of the injected animals compared to 92% of the animals injected with the tumorigenic parent. Moreover, those animals that developed tumors from the cybrid cells survived longer than those injected with cells from the tumorigenic parent [15–17]. Tumorigenicity was also suppressed in cybrids formed by a fusion between cells from highly tumorigenic mouse melanoma B16 and cytoplasts from nontumorigenic rat myoblastic cells. At about 3 months after the fusions, three cybrid clones were examined for tumorigenicity by injection into nude mice. None of them produced a tumor within 45 days after the injection, although parental B16 cells produced tumors after only 7 days [18].

2.3. Recons

In the previous experiments, and others with cybrids that did not show suppression of tumorigenicity [11,19–23], the cytoplasm of the tumoral cell was diluted with the one coming from the donor cytoplast, so the contribution of each cytoplasm to the cell after several cell generations is very difficult to evaluate. A way to increase the cytoplasm contribution from the donor nontumorigenic cell is through the reconstituted cell (recons) approach. In this approach, most of the cytoplasm of the receptor cell is removed, and a karyoplast is obtained. A karyoplast is a nucleus surrounded by a thin cytoplasm layer (Fig. 1). Thus, the fusion of normal rat hepatocyte cytoplasm to karyoplasts obtained from tumorigenic hepatocytes resulted in suppression of tumorigenicity in five of the six recons clones analyzed. Out of 68 animals injected with these reconstituted cells, only one tumor was observed [15–17]. Tumorigenicity was also suppressed in reconstituted cells formed by a fusion between karyoplasts from highly tumorigenic mouse melanoma B16 cells and cytoplasts from nontumorigenic rat myoblastic cells. At about 3 months after the fusions, three reconstituted cell clones were examined for tumorigenicity by injection into nude mice. None of them produced a tumor within 45 days after the injection, although parental B16 cells produced tumors after only 7 days [18]. The experiments performed with hamster cell cybrids [11] were repeated using reconstituted cells. As in the cybrids, both tumor-forming and -suppressing clones were obtained [24]. None of the recons from tumorigenic NIH/3T3 BPDE 17 karyoplasts and nontumorigenic NIH/3T3 cytoplasts was tumorigenic. In contrast, those with the same karyoplast but cytoplast from NIH/3T3 BPDE 17 were tumorigenic [25]. Also, no recons from tumorigenic NIH/3T3 BPDE karyoplasts and nontumorigenic B10mtJ cytoplasts were tumorigenic, but all the recons from the same karyoplasts and tumorigenic B10mtJ cytoplasts were tumorigenic [26].

2.4. Homoplasmic cybrids

A mix of mtDNA from the donor and receptor cells will be present in cybrids and, in lesser extension, in recons. This condition is called heteroplasmy. In contrast, a cell with only one type of mtDNA is called homoplasmic. If cytoplasmic suppression is based upon mtDNA, then the success of suppression would depend on relative replication rates of both mtDNA and the impact of selective pressure [24]. Therefore, it is important to remove the parental mtDNA to truly check the effect of the donor mtDNA. This can be performed by using rho0 cells (cells without mtDNA) [27] or by pretreating cells with the toxic mitochondrial dye, rhodamine 6G [28] (Fig. 1). It has been shown that PC3 prostate cancer cells were able to produce big tumors in nude mice after 4 weeks [29]. However, cybrids produced by treating PC3 cells with rhodamine 6G and fusing them to cytoplasm from a cell line without mtDNA mutations barely grew in nude mice after 4 months [30]. Interestingly, the original mtDNA of PC3 cells harbored two mutations. The first, m.11120T>C/p.MT-ND4:F121L, has not been described in more than 3500 human mtDNAs [31], and it has a low

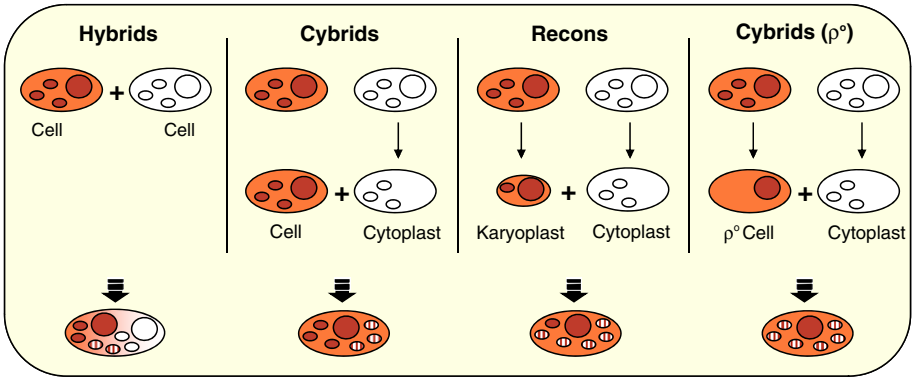

Fig. 1. Cell models to study the influence of mtDNA mutations on cancer. The results of the fusion after several cell generations are represented. Different origins are coded in orange and white. Combined orange and white cytoplasm indicates nuclear gene products from both origins. Striped mitochondria mirror a mixed origin with nuclear gene products of one of the original cells and mtDNA-encoded products from the other. Rho 0 cells contain mitochondria but do not have mtDNA.

conservation index and probably is not functionally significant. However, the second one, m.13802C>T/p.MT-ND5:T489M, could be functionally relevant. It was previously found in an individual, but the age, sex, or health status was not reported. The affected position has a moderate conservation index, and it is possible that removal of this mutation reduced the tumorigenic potential of the PC3 cells [30].

3. Mitochondrial DNA enhancement of tumor formation

Most of the proteins and RNAs in the cybrid cells are nucleus-encoded products. This means that most of the donor cytoplasm proteins and RNAs will be diluted after several culture passages after the cybrid construction. Only 37 RNAs and proteins in the cybrid cells do not have this origin. These are mtDNA-encoded products, and they will remain in the mitochondria cell generation after cell generation. This fact suggests that the donor mtDNA was responsible for the suppression of the tumorigenicity in the previous experiments. If the new mtDNA suppresses the tumorigenicity, the substituted one should favor the tumoral process.

In one study, portions of an immortalized and cloned rat liver-derived epithelial cell culture were sequentially frozen. Nontransformed early and transformed late passage cells were retrieved. Cybrids derived from the fusion of normal whole cells to cytoplasts from malignantly transformed cells produced tumors in 17% of the newborn rat pups injected. Recons derived from the fusion of karyoplasts of normal cells with cytoplasts from malignant cells produced tumors in 97% of the animals injected [32]. New mutations that accumulated in the mtDNA of the transformed late passage cells could explain the previous results. MtDNA can mutate in cultured cells. Thus, HeLa cell sublines, isolated and maintained in culture for decades, have accumulated mtDNA mutations. One of these sublines harbors a premature chain termination codon m.12533G>A/p.MT-ND5:W66TER. Although this mutation was not characterized, it likely has a dramatic phenotypic effect on the OXPHOS function [33]. Cybrids can also accumulate mtDNA mutations during culture. For example, the AL4.3 line showed three new mtDNA mutations (m.1843T>C, m.1940A>G, and m. 2623A>G, all of them in the MT-RNR2 gene) and a negative bioenergetic phenotype [34].

Otto Warburg proposed that tumor cells have a heritable respiration defect. Surprisingly, before the discovery of the mtDNA in animal cells [35], Warburg wrote "…we understand why the respiration connected with the grana remains damaged when it has once been damaged; it is for the same reason that properties linked with genes remain damaged when the genes have been damaged" [36]. Once a mitochondrion was damaged, it remained so, transmitting its defect to progeny (presumably mitochondria), just as would occur, he asserted, in the case of a damaged nuclear gene [37].

Soon after its discovery, mtDNA began to be analyzed in tumoral cells [38]. Since then, many mtDNA mutations have been found in different tumors [39], but their impact on tumorigenicity has not been well established. However, the nature of some of these mutations pointed to a phenotypic effect on mitochondrial respiration. For example, the first tumor likely to be associated with a mtDNA mutation with phenotypic consequences was reported in 1983 [40]. This was the deletion of a C within a tract of three C's (nucleotide positions 5550–5552, human numbering) in the anticodon stem of the MT-TW gene in a rat tumor. Only two insertions/deletions have been reported in this stem in human mt-tRNAs: a deletion of one of three T-A nucleotide pairs in the MT-TL1 gene [41] and a single T insertion at nucleotide position 5537 in the MT-TW [42,43]. Both were associated with a pathological condition, a mitochondrial disease. Another clue that a mtDNA mutation in cancer cells could be functionally significant came from the report of a renal adenocarcinoma in which 50% of the mtDNAs contained a 264-nt in-frame deletion in the MT-ND1 gene, resulting in a truncated mRNA [44]. It should be considered that mtDNA deletions are a relatively frequent cause of mitochondrial disease [45]. In the case of mtDNA mutations causing stop codons, they will truncate the protein and will probably have a phenotypic effect. The first mtDNA stop codon mutation associated with cancer was found in a colorectal tumor. This was a m.6264G>A/p.MT-CO1:G121TER mutation [46]. It has been found that similar changes, such as the m.6930G>A/p.MT-CO1:G343TER mutation, are associated with mitochondrial disorders [47].

Transmitochondrial cell lines (cybrids) can be used to check the effect of cancer-associated mtDNA mutations [48]. For some tumor mtDNA mutations, a biochemical and molecular analysis has been carried out. Osteosarcoma 143B cybrids harboring two mtDNA mutations from the thyroid oncocytic cell line XTC.UC1, m.3571Ci that provokes a frameshift and a stop codon at p.MT-ND1:G101TER (>92.6% mutant) and m.15557G>A/p.MT-CYB:E271K (>27% mutant), showed decreased viability and ATP levels when grown in galactose medium, which forces mammalian cells to rely on mitochondrial OXPHOS [49]. Moreover, a homoplasmic cybrid clone for the m.3571Ci mutation grew more slowly in galactose medium and was defective in complex I assembly and ATP synthesis when compared with another cybrid clone that was 80% mutant. This homoplasmic cybrid also showed a α-ketoglutarate/succinate ratio that was significantly higher [50]. Osteosarcoma 143B cybrids were also built with mitochondria from the VACO 425 colorectal cancer cell line [46]. In addition to the m.6264G>A/p.MT-CO1:G121TER mutation, this mtDNA contained an insertion of a single A nucleotide at position m.12418 (m.12418Ai) that causes a reading frame shift at K28, thereby producing a truncated p.MT-ND5 protein. No p.MT-ND5 or p.MT-CO1 was detected in these cybrids. In consequence, they showed extremely low endogenous respiration and markedly reduced complex I + III and IV activities [51]. Other osteosarcoma 143B cybrids, harboring the m.12418Ai mutation but without the m.6264G>A mutation, had less oxygen consumption, less ATP synthesis, and grew more slowly in galactose medium. They also produced more extracellular lactate, more mitochondrial superoxide and overexpressed some antioxidant enzymes [52]. Moreover, osteosarcoma 143B cybrids harboring mtDNA mutations (m.7458G>A/MT-TS1 and m.15894G>A/MT-TT) from two breast cancer cell lines (MDA-MB-231 and MDA-MB-436) showed lower levels of the two affected tRNAs, lower respiratory complex activities, a reduced oxygen consumption, decreased ATP synthesis, and, finally a significantly reduced cell viability when grown in galactose medium [53].

However, an OXPHOS defect due to these mutations does not mean that the mutations affect the tumorigenicity. To check this important point, several studies used mice to analyze the tumorigenicity of cybrids with different mtDNA mutations. Thus, nude mice were injected with cybrids derived from a rhodamine 6G-treated PC3 prostate cancer cell line and cytoplasts harboring a m.8993T>G/p.MT-ATP6:L156R mutation or isogenic cybrids without this mutation. Patients with this mutation suffered a mitochondrial disease [54], and osteosarcoma 143B cybrids with the mutation showed an OXPHOS defect [55]. The mutant PC3 cybrids were found to generate tumors that were seven times larger than the wild-type cybrids. The mutant tumors also generated significantly more ROS. Therefore, these results showed that mtDNA mutations could play an important role in cancer [30,56]. In the same year, HeLa cybrids with or without the pathologic m.8993T>G and m.9176T>C/p.MT-ATP6:L217P [57] mutations were injected into nude mice. These mutations conferred an advantage in the early stage of tumor growth. Because apoptosis occurred less frequently in mutant cybrids, the mtDNA mutation seemed to promote tumors by preventing apoptosis [58]. A higher metastatic potential of cells with mtDNA mutations was suggested after the observation that osteosarcoma cybrids harboring the m.3243A>G/MT-TL1 mutation were more invasive in a matrigel invasion assay than those built with a wild-type mtDNA [59]. This issue was confirmed in another experiment in which mitochondria from poorly and highly metastatic mouse tumor cell lines were interchanged. The highly metastatic cell line contained the m.13997G>A/p.MT-ND6:P25L and m.13885Ci/p.MT-ND6 (this insertion

generates a truncated protein) mutations that produce a deficiency in respiratory complex I activity associated with the overproduction of ROS. By using metastasis assays in mice, it was found that the recipient tumor cells acquired the metastatic potential of the transferred mtDNA. Thus, these results indicated that mtDNA mutations could contribute to tumor progression by enhancing the metastatic potential of these cells [60]. The first evidence that a mtDNA mutation found in a human tumor was able to affect tumorigenicity was recently obtained. Cybrids harboring the heteroplasmic m.12418Ai mutation (there is a tract of 8 As beginning at 12418, therefore this mutation should be called m.12425Ai), the same mutation found in a colorectal tumor [46], produced tumors that grew faster and were larger in volume when injected into nude mice that those from wild type cybrids [52].

4. MtDNA mutations and tumorigenicity

Cell proliferation is controlled by an interaction with other cells and the extracellular matrix and by the levels of growth factors. Therefore, the earliest steps in carcinogenesis require nuclear genetic alterations to surmount these tissue constraints. These requirements probably explain why the cytoplasm from tumoral cells is unable to provoke tumorigenicity in normal cells. As proliferation pushes cells further away from their blood supply and oxygen concentrations decrease, premalignant lesions will face hypoxic regions near the oxygen diffusion limit. Near-zero partial pressures of oxygen are observed at short distances from a blood vessel, where cells are exposed to an unstable environment due to hypoxia–reoxygenation cycles. In this hypoxic microenvironment, selection forces will favor phenotypes that adapt to anaerobic metabolism [61]. When oxygen levels are very low, the electron carriers of the mitochondrial respiratory chain will remain more reduced [62], more mitochondrial ROS will be generated and hypoxia-inducible factors (HIFs) will be activated [63]. HIF1α acts a transcriptional factor triggering the expression of many genes, among them, those for glycolytic enzymes. In this way, tumoral cells enhance their glycolytic metabolism and their survival in this hypoxic environment. However, cells derived from tumors typically maintain their metabolic phenotypes in culture under normoxic conditions, indicating that aerobic glycolysis is constitutively upregulated through stable genetic or epigenetic changes.

In hypoxia–reoxygenation cycles, higher ROS amounts can be produced, and these are mutagenic compounds. Hypoxic conditions are sufficient to generate mutations in nuclear [64] and mitochondrial [65] genes. Thus, the metabolic phenotype, as another proliferation barrier [1], might be acquired by different nDNA and mtDNA mutations. Therefore, despite the fact that mtDNA mutations are not necessary for some tumors, they might contribute to many others. In fact, mtDNA accumulates mutations much faster than nDNA. MtDNA is located in the mitochondrial matrix and is bound to the inner membrane where the electron transport chain (ETC), the main source of ROS in the cell, is embedded. Moreover, the mutational process induced by ROS is facilitated because repair systems for mtDNA are not as abundant as those for nDNA [66]. Interestingly, DNA repair pathways may be compromised in cells exposed to hypoxic conditions [67]. Together, these facts can explain the high frequency of mtDNA mutations found in cancer cells [68].

A high frequency of homoplasmic mtDNA mutations has been reported in human tumors. It has been estimated that only approximately 70 cell generations are required for a mutation to become homoplasmic by chance [69]. However, in the earliest steps of carcinogenesis, most cells die by necrosis when exposed to hypoxia [61]. Therefore, they do not have an unlimited possibility of proliferation. After a very small number of cell divisions, they will face this hypoxic environment and be removed. However, a mtDNA mutation that increases ROS production will provide selective advantages. Higher percentages of the mutation will offer higher survival possibilities. Thus, it is possible that many mtDNA mutations will appear and be enriched in this hypermutagenic environment because they contribute to the tumoral phenotype, and not just by chance.

In addition to survival in a particular microenvironment, other tumor-related phenotypes can be associated with mtDNA mutations. Thus, OXPHOS-deficient cybrids due to a mtDNA deletion upregulate and downregulate the expression of matrix metalloproteinase 1 (MMP1) and its natural inhibitors (tissue inhibitors of the MMP family, TMP1 and TMP2), respectively. Moreover, HIF1α stimulates the production of angiogenic factors, such as VEGF, and this promotes increased vascularity and metastases. These results suggest that OXPHOS defects also participate in tumor progression by modulating extracellular matrix remodeling and angiogenic processes [59].

5. Mitochondrial DNA inherited susceptibility to cancer phenotypes

Mutations in the p.MT-CO1 subunit have been found in many different tumors, although population polymorphisms and pathological mutations in this gene are relatively uncommon. Thus, MT-CO1 mutations were present in 11% of 180 prostate cancer specimens from European American patients, 0% of 46 European American noncancer controls and 6.5% of a population of 898 samples from European ancestors without information about their sex, age, and health status. Therefore, MT-CO1 mutations are significantly increased in prostate cancer samples over the noncancer controls and the general population. Moreover, the interspecific conservation of the altered p.MT-CO1 amino acids in prostate cancer was significantly higher than that in the general population. These results suggest that p.MT-CO1 mutations must be functionally significant. Due to most of the MT-CO1 mutations being homoplasmic in different tissues of the patients, they must have arisen in the female germ line. Therefore, inherited mtDNA mutations could be a risk factor for cancer [30].

Several types of cancers show geographic or ethnic clustering. As an example, the incidence of prostate cancer is much higher among men of African descent compared to Europeans. In addition to environmental factors that likely contribute to the regional differences in prevalence, genetic susceptibility could be another risk factor. Thus, different alleles of nuclear genes have been proposed to explain some of these geographic or ethnic differences [70,71]. In contrast to the nuclear genome, mtDNA shows considerable geographic and ethnic diversity [72]. MtDNA is inherited by maternal lineage. When a mutation in the mtDNA appears and survives in the population, a new maternal lineage is formed. With time, new mutations will create new branches, producing a mtDNA phylogenetic tree. The groups of phylogenetically related mtDNA genotypes are known as mtDNA haplogroups. Phylogeographic studies of human mtDNAs have revealed a remarkable correlation between mtDNA lineages and the geographic origins of indigenous populations. The human mtDNA tree is rooted in Africa, and it has specific branches radiating into different geographical regions [72]. African mtDNAs are the most diverse and ancient and fall into several major haplogroups known as L lineages. In northeastern Africa, two main mtDNA lineages, M and N, arose and were successful in leaving sub-Saharan Africa and radiating into Eurasia to give rise to all of the Eurasian mtDNA haplogroups (Fig. 2). Therefore, this geographical mtDNA variability could contribute to the geographical clustering of some cancers.

Carcinogenesis is a multistep process often described as somatic evolution. As we have previously mentioned, somatic evolution of cancer cells can be viewed as a sequence of phenotypical adaptations to distinct microenvironmental barriers. Models of carcinogenesis are typically based on the Darwinian principle that evolution requires genetic changes that generate new phenotypes [1]. Interestingly, many tumor-specific somatic mtDNA mutations have also been found as haplogroup-defining population genetic variants. These mutations may facilitate the tumor survival under new environments with altered or reduced energetic substrates, reduced oxygen tension,

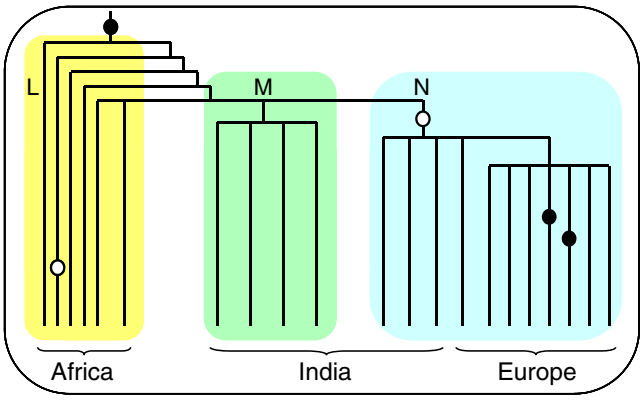

Fig. 2. Mitochondrial DNA phylogenetic tree. The major mtDNA genetic lineages are represented along with their geographic distribution according to the studies discussed in the text. Mutations affecting the m.10398 position are marked (black circle is m.10398G allele and white circle is m.10398A allele).

variations in environmental temperature or increased ROS toxicity. Because humans in their expansion across the planet also faced similar environmental challenges, the same mtDNA mutations might be adaptive in both tumors and people [39]. Therefore, it would be possible that the mitochondrial haplogroup behaves as a susceptibility factor for carcinogenesis.

A transition in the m.10398 position has been found at 12 internal nodes of a mtDNA phylogenetic tree including more than 3500 sequences [31] (Fig. 2). The m.10398G (p.MT-ND3:A114) allele is probably from the most recent common ancestor of humans (mitochondrial Eve), and it is prevalent in individuals of African heritage. The m.10398A allele (p.MT-ND3:T114), along with other polymorphisms, defines the mtDNA N macrolineage. This allele is prevalent in individuals of European heritage. Positive selection has been invoked to explain the high number of times that an m.10398 mutation has occurred in the human phylogeny and the fact that the human-derived allele predominates in the mammalian consensus sequence [73]. In a study including 1259 African-American women (654 patients and 605 controls), it was found that the A allele was associated with a significantly increased risk of invasive breast cancer [74]. This allele was not found to be associated with breast cancer in another study of African-American women that included 1456 patients and 978 controls [75], but it was found to be overrepresented in African-American men with organ-confined prostate cancer [76]. This allele was also significantly associated with an increased risk for sporadic breast carcinoma in India. In this population, the m.10398A along with the m.10400C polymorphisms define macrohaplogroup N. The m.10398G and m.10400T polymorphisms define macrohaplogroup M. Interestingly, there is an apparent worldwide correlation between the increased incidence rate of breast cancer and mtDNA macrohaplogroup N distribution. In India, those states with the highest prevalence of major macrohaplogroup N correlated with the highest breast cancer incidence rate. A comparison between sporadic breast cancer cases and controls with mtDNA genetic backgrounds M and N showed that the macrohaplogroup N was overrepresented in the cases. Moreover, in another cancer type, the squamous cell carcinoma of esophagus, macrohaplogroup N was also overrepresented [77]. However, in another study from India including 308 oral cancer patients and 383 controls, the M macrolineage was found to be associated with cancer [78]. By using cybrids, mitochondrial functional differences were found between macrohaplogroups N and non-N. It was shown that cybrids from the macrohaplogroup N had a lower mitochondrial matrix pH and higher mitochondrial calcium levels when compared with that of the macrohaplogroup non-N [79].

Curiously, in studies of individuals with European heritage, results are different. Thus, it was found that the m.10398G allele was associated with breast cancer in a study including 156 European-American breast cancer patients with a family history of breast cancer and 260 controls [80]. In another study from Poland, the m.10398G allele was present in 23% of 44 sporadic breast cancer patients but only in 3% of 100 controls [81]. However, the m.10398G allele was not found to be associated with breast cancer in two other studies (1561 breast cancer patients and 2209 controls; 678 breast cancer patients and 669 controls) [82].

These contradictory results from epidemiological studies are difficult to interpret. For example, due to the absence of recombination in human mtDNA, it is possible that the m.10398G mutation was in linkage disequilibrium with a more important causative polymorphism. A highly significant interaction was identified between this variant and the m.4216C or m.12308G mutations in patients suffering from breast cancer [83]. The first combination defines haplogroup J, and the second one defines haplogroup Uk1. Uk1, along with J1c, one of most frequent subhaplogroups J in Europe, share another mtDNA polymorphism with a potential phenotypic effect, m.14798C [84]. Different combinations of diverse genetic and environmental factors may be involved in cancer. If mtDNA population polymorphisms are involved in the tumorigenicity process, their contributions can be important, although they will not be dramatic. It is expected that they would have small effects on OXPHOS function, and these effects would be highly dependent on the “context” in which the genetic variant is acting. To solve this issue, epidemiological studies will not be very useful and other approaches are required. Cybrids can, again, help to elucidate the role of mtDNA population polymorphisms in the susceptibility to cancer. A cybrid with the m.10398A polymorphism showed a slower proliferation rate and altered progression through the cell cycle, as well as increased complex I activity and levels of reactive oxygen species and depolarized mitochondria, compared to another cybrid with the m.10398G allele. It also showed resistance to apoptosis triggered by etoposide, increased Akt phosphorylation in the S473 residue, and an increased number of anchorage-independent colonies in vitro and metastases in mice [85].

Another example that shows the utility of the cybrid approach comes from the CFPAC-1 and CAPAN-2 human pancreatic cancer cell lines. These contain never before described nonsynonymous mutations, m.8696T>C/p.MT-ATP6:M57T and m.10176G>A/p.MT-ND3:G40S, respectively [86]. The phenotypic effects of these mtDNA genotypes were not very dramatic. Cybrids prepared using mitochondria derived from these cells did not show significant differences in mitochondrial inner membrane potential and oxygen consumption, although they tended to be different, and they grew slower than wild-type cybrids [87]. Interestingly, one of these cell lines (CAPAN-2) also contained the m.6267G>A/p.MT-CO1:A122T mutation. This mutation was also found in 1 of 260 patients with prostate cancer [30], 1 of 63 patients with breast cancer, 1 of 64 patients with colon cancer, and 2 of 13 cancer cell lines [88]. Altogether, it was found in 6 out of 415 samples from patients with cancer. Biochemical analysis of cybrids with this mutation indicated that growth in galactose medium, respiration, and complex IV activity were impaired [88]. This mutation has also been found five times in more than 3500 “control” individuals, whose age and health status were unknown [31]. Interestingly, two of them belonged to the same mitochondrial haplogroup, M38 [89]. The complete sequences of these two individuals allowed characterization of this haplogroup-defining mutation as an ancient one, being present in the population for thousands of years. Since the appearance of this mutation, at least five and seven other mutations accumulated in the mtDNA lineages that produced the mtDNA genotypes harbored by these two individuals. The high frequency of this mutation in cancer samples and its rareness in the normal population, along with its effect on the OXPHOS function of transmitochondrial cell lines, raise the possibility that this transition might influence the tumoral phenotype. Confirming this suggestion, cybrids harboring mitochondria from the CAPAN-2 human pancreatic cancer cell line and from healthy individuals were transplanted into

nude mice to generate tumors. Tumors derived from cybrids with mtDNA from cancer lines were more resistant than those with mtDNA from healthy individuals in suppressing tumor growth and inducing massive apoptosis when anticancer drugs were administered [87].

Mutations in mtDNA can be responsible for the anticancer drug tolerance of tumoral cells, but mtDNA population polymorphisms can also affect the tumoral phenotype in a different way. Thus, the mitochondrial genetic background may affect the appearance of side effects after anticancer therapy. Cisplatin is a highly effective chemotherapeutic agent, but its use is limited by its nephrotoxicity, neurotoxicity, and ototoxicity. It was recently shown that 5 of 20 patients with hearing impairment under therapeutic doses of cisplatin belonged to the mtDNA haplogroup J, but only 1 of 19 patients without hearing impairment belonged to this haplogroup [90].

6. Conclusions

The tumoral microenvironment favors the appearance of mtDNA mutations. Some of these mutations can affect the cancer phenotype by altering tumor metabolism. The OXPHOS system is important for adapting to the environment. In fact, external signals, such as nutrients and oxygen, interact at the OXPHOS level and modify the levels of second messengers, such as ATP, ROS, and Ca^{2+}, or the cell red–ox state, then triggering intracellular retrograde responses important for surmounting the microenvironmental barriers to proliferation.

Along human evolution, mtDNA has accumulated mutations. Many of these surely did not have any phenotypic effect, but some of them probably assisted individual survival by modifying the OXPHOS balances. Thus, individuals from diverse haplogroups would have small differences in their metabolism and, therefore, distinct susceptibilities to cancer. Because cancer is a multifactorial disorder, small metabolic differences due to mtDNA SNPs can be difficult to analyze in epidemiological studies, and other approaches are required. As we have seen in this review, cybrids and nude mice can be interesting tools to study the influence of population polymorphisms and mtDNA-inherited susceptibility to cancer.

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