Mitochondrial DNA in Early Cancer Diagnosis and Screening

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ABSTRACT  Biomarkers are used in cancer detection, diagnosis, and prognosis. In cancer, most genetic markers are based on nuclear DNA; mitochondrial DNA (mtDNA) has not been utilized very extensively. This article provides information about using mtDNA alterations as biomarkers to detect different tumor types at early stages of carcinogenesis. Mitochondria play an important role in cellular energy metabolism, free radical generation, and apoptosis. Alterations in respiratory activity and mtDNA alterations are an integral part of carcinogenesis. Mitochondria contain their own genome along with their own transcription, translation, and protein assembly machinery. Because mtDNA lacks introns,
it does not have histones and is more susceptible to oxidative damage and other environmental insults. It has been suggested that most mutations and deletions will occur in coding sequences, and the subsequent accumulation of mutations may lead to tumor formation. Mitochondrial mutations have been reported in bladder, brain, breast, colon, cervical, esophageal, gastric, liver, lung, and prostate cancers. Some of these mutations occur quite early during cancer development and can be used in screening large populations to identify high-risk individuals. Mitochondrial DNA alteration can be detected in biospecimens collected non-invasively. The implications of using mitochondrial information in identifying populations that are at high risk of developing cancer are discussed.

KEY WORDS: cancer, detection, diagnosis, epidemiology, haplogroups, mitochondria, prognosis, risk assessment, screening, survival, treatment.

Abbreviations

- miRNA: microRNA
- mtDNA: mitochondrial DNA
- ROS: reactive oxygen species
- PSA: prostate specific antigen

6.1 Introduction

Genomic, proteomic, metabolomic, epigenomic, and imaging biomarkers are commonly used in cancer risk assessment, early diagnosis, prognosis, therapy, and follow-up survival (Yu, 2011). The advantages of using DNA biomarkers include their stability and the ability to conveniently isolate them from different cells (Chauhan et al., 2007). In epidemiologic studies, in which thousands of samples are analyzed, biomarkers should be assayed using high-throughput methods from conveniently stored samples and assays should not be expensive (Verma and Kumar, 2007; Verma et al., 2003). Proteomic biomarkers represent functional aspects of carcinogenesis and are more informative than genomic markers; because of the low stability of proteins, however, it is inconvenient to utilize proteomic biomarkers in epidemiologic studies (Verma et al., 2003, 2006). In retrospective studies, biomarkers are tested on samples that have been stored for several years; most proteomic biomarkers are degraded if they are not stored properly (Verma et al., 2004). Metabolomic markers are better than proteomic markers because the end products of the assay represent results of biology undergoing disease development. Furthermore, disease-associated metabolites are very stable and can be measured easily with high sensitivity and specificity in biofluids such as urine, blood, nipple aspirate, saliva, cerebrospinal fluid (CSF), and pancreatic juice. Epigenetic biomarkers represent functional aspects against the genomic background. Although DNA methylation, histone modification, microRNA (miRNA) expression, and chromatin modeling biomarkers are the major epigenetic biomarkers, only DNA methylation biomarkers are currently suitable for epidemiologic studies (Banerjee and Verma, 2009; Kumar and Verma, 2009; Verma et al., 2004, 2006). Imaging markers are excellent in most of the cases but are very expensive and time-consuming. Mitochondrial DNA (mtDNA) biomarkers may provide information that is complementary to the information gained from other biomarkers that are used for early cancer detection, diagnosis, prognosis, and survival (Greaves et al., 2009).

6.2 Why Mitochondrial DNA Markers?

Mitochondria are 0.002–0.008 nm long, membrane-bound, semiautonomous organelles that supply energy to the cell through the process known as oxidative phosphorylation (Parr et al., 2006; Valko et al., 2006). Mitochondria are susceptible to environmental insults. They do not have a well-developed repair system, and because of their proximity to the respiratory chain, damage to the mtDNA is greater than the
damage to the nuclear genome (Radjour et al., 2009). The mutational rate of mtDNA is approximately 10-fold higher than that of nuclear DNA (nDNA) (Ebner et al., 2011). For a long time, mitochondria were considered to be simply the powerhouse of the cell that supplies energy in the form of adenosine triphosphate (ATP). Later, reactive oxygen species (ROS) and free radical-based damage to mitochondria were linked with different steps in carcinogenesis (Czarnecka et al., 2006, 2010; Ishikawa et al., 2008; Ralph et al., 2010). Along with other proteins, oncoproteins also are transported to mitochondria and initiate mitochondrial malignant transformation programs (Kulawiec et al., 2006; Verma et al., 2003; Yu, 2011).

Mitochondrial dysfunction is a hallmark of cancer cells (Ospelt and Gay, 2005). Mutations (both germ line and somatic) in mitochondria have been detected in different tumor types (Lievre et al., 2006). However, it is not clear whether the mitochondrial genomic status of human cells affects nuclear genome stability and whether proteins involved in intergenomic cross talk are involved in tumorigenesis. Somatic mitochondrial mutations are common in human cancers and can be used as a tool for early detection of cancer (Parr et al., 2006). Selected mutations and tumor types are discussed later. The majority of these somatic mutations are homoplasmic in nature, indicating that the mutant mtDNA becomes dominant in tumor cells. mtDNA copy numbers were found to be correlated with the advancement of liver cancer (Yin et al., 2004). It has been suggested that the extent of mtDNA mutations might be useful in determining cancer prognosis and/or response to certain therapies (Krishnan and Birch-Machin, 2006).

Mitochondria have been implicated in the carcinogenesis process because of their role in apoptosis and other aspects of tumor biology. mtDNA is present in high copy numbers per cell in cancer cells (Yu, 2011). The number of copies per cell varies between the normal and disease states. These copy numbers are determined based on nuclear hemoglobin gene copy numbers in the nuclear genome. The mtDNA codes for 13 polypeptides (involved in oxidative phosphorylation), 2 rRNAs, and 22 tRNAs. One noncoding region, the displacement region or D-region, also exists and contains the origins of replication. It is believed that the early mitochondrial genome derived from symbiotic bacteria in proto-eukaryotic cells could have contained other genes that were lost or integrated into the nDNA during evolution. Effect of environment, radiation, and genotoxic substances on the generation of heteroplasmic and homoplasmic mitochondria is shown in Figure 6.1. Effects of free oxygen radicals and oxidative stress and their contribution in alterations of mitochondrial and nuclear genomes are shown in Figure 6.2.

Two approaches are used to understand the utility of mtDNA in cancer epidemiology: one approach is to look for somatic mutations in mitochondria and the other approach is to look for disease-associated haplogroups. The inheritance pattern of mitochondria in patients with cancer has been studied by

![FIGURE 6.1 Effects of environment, radiation, and genotoxic substances on the generation of heteroplasmic and homoplasmic mitochondria.](image-url)
haplogroup analysis. Polymerase chain reaction of key polymorphic sites in the mitochondrial genome was performed in samples from cancer patients and normal individuals to determine if there is an association between mitochondrial genotype and cancer. Such an analysis has been accomplished in prostate and renal cancers.

mtDNA alterations are able to maintain genome independence from the nucleus. However, as a consequence of protomitochondrial genes integrating into the nuclear genome throughout evolution, most mitochondrial proteins are encoded by nDNA and imported into the mitochondria. Quantification of the mitochondrial mutation load is easy to determine (Greaves et al., 2009).

### 6.3 Mitochondrial DNA in Different Cancers

Somatic mutations and germ line alterations in different tumor types are described in the following sections.

#### 6.3.1 Bladder Cancer

According to data from the Surveillance, Epidemiology, and End Results (SEER) Program, in 2011, about 69,300 new bladder cancer cases were expected to be reported and about 15,000 people were expected to die as a result of this cancer (http://seer.cancer.gov/statfacts/html/urinb.html). Based on rates from 2006 to 2008, 2.41% of men and women born today will be diagnosed with cancer of the urinary bladder at some time during their lifetime. For analysis of mtDNA, human bladder cancer tissues were obtained by radical cystectomy and transurethral resection of bladder tumors (Chen et al., 2004; Dasgupta et al., 2008). Mutations were detected in the noncoding D-loop region and in different mtDNA genes. Deletions of variable lengths in mononucleotide repeats in the D-loop region, ND2, ATPase 8, and COIII genes also were observed. The repetitive sequences of mononucleotides within the mitochondria genome are known to be unstable and subject to deletions. The high incidence of mtDNA mutations in bladder cancer suggests that mtDNA and mitochondria could play an important role in the process of carcinogenesis and that mtDNA could be valuable as a marker for early bladder cancer diagnosis (Chen et al., 2004). In another study, urothelial carcinoma-specific mtDNA mutations were observed in 76% of patients (Yoo et al., 2010). Jakupciak et al. (2008) reported finding heteroplasmic mtDNA mutations in samples from bladder cancer patients. When mitochondrial genes ATPase6, CytB, ND1, and D310 were analyzed for bladder cancer-associated mutations in a population study, G8697A, G14905A, C15452A, and A15607G mutations were frequently observed (Guney et al., 2012).
6.3.2 Brain Cancer

SEER data estimated that there would be about 22,300 new cases and about 13,100 deaths from brain cancer in 2011. Mitochondrial pathways and energetics are critical in glioblastoma multiforme (Griguer and Oliva, 2011). Mutations in mtDNA also have been reported for glioblastoma (Krell et al., 2011). When the D-loop region of the mtDNA was analyzed to detect mutations in brain samples, 36% of the samples were found to contain mutations (Montanini et al., 2005). A follow-up study indicated that there was no correlation between these mutations and the aggressiveness of the disease. These mutations could be utilized for follow-up of the disease but not for diagnosis or prognosis (Montanini et al., 2005). In another study, CSF samples from medulloblastoma cases were analyzed to detect mtDNA mutations (Wong et al., 2003). A total of 18 mutations were detected in one-half of the samples analyzed (16); some of the mutations were found when treated samples were followed and the disease relapsed. This shows promise for future research using CSF in follow-up studies. Mutations also were reported in glioma (Montanini et al., 2005).

6.3.3 Breast Cancer

Breast cancer is the most prevalent cancer among women in the Western world. According to SEER data, in 2011, about 230,500 women were expected to be diagnosed with breast cancer and about 39,500 women were expected to die from breast cancer. Mutations and deletions have been reported in breast cancer by different groups of investigators (Cantar et al., 2005; Gochhait et al., 2008; Singh et al., 2009; Tseng et al., 2011). Mutations in D310 region were reported during early development of breast cancer by Xu et al. (2012). Deletions ranged from 50 nucleotides to 4977 nucleotides (Radpour et al., 2009). The incidence of the 4977 base pair (bp) deletion and somatic mutations in the D-loop region were examined in breast cancer and adjacent tissues. Results indicated mutations in the D-loop region. Level of estrogen and survival data also were collected in this specific population in Taiwan. In another study, Zhu et al. reported several mutations in nipple aspirates collected from breast cancer patients (Zhu et al., 2005). In addition, an mtDNA G10398A polymorphism in breast cancer has been reported in African–American women (Cantar et al., 2005; Darvishi et al., 2007). Two novel polymorphisms in the D-loop region were recently reported for breast cancer detection (Sultana et al., 2012).

6.3.4 Colon Cancer

Shimomura et al. (2011) reported mitochondrial mutations in traditional serrated adenomas of the colon. Other investigators also have reported mtDNA alterations in colorectal cancer (Czarnecka et al., 2006; Habano et al., 1999; Nooteboom et al., 2010). According to SEER data, in 2011, about 141,200 new cases were expected to be reported for colon cancer and about 49,400 deaths from colorectal cancer were expected. Colon cancer is divided into three histopathological categories: nonneoplastic polyps, neoplastic polyps (adenomatous polyps, adenomas), and cancers (Czarnecka et al., 2006). More than 95% of colon cancers are adenocarcinomas (Copeland et al., 2002). Vogelstein’s group sequenced the mtDNA genome of 10 human cancer cell lines and found 12 different mutations in 7 of those 10 cell lines (Polyak et al., 1998). These mutations were localized in the protein-coding genes (ND1, ND4L, ND5, COX subunit II and III, cytochrome b) and in rRNA genes. This observation was confirmed by other investigators (Alonso et al., 1997; Copeland et al., 2002; Taylor et al., 2003). AT/GC transitions were observed in colon cancer samples by Alonso et al. (1997), and polycytidine tract mutations in the D-loop region and polyadenosine tract were observed by Habano et al. (1999). Although the D-loop region is only 1.12 kb long, numerous mutations are found in this region compared to the remaining mtDNA. The largest number of samples in which the D-loop region has been sequenced is 365, and the mutation rate was 38% (Czarnecka et al., 2006). A 3-year survival rate of 53% was observed in patients with mtDNA mutations, as opposed to a rate of 61% in patients without mutations. Thyagarajan et al. (2012) completed the first prospectively designed study in the Singapore Chinese Health Study and observed a U-shaped association between the relative mtDNA copy number and risk of colorectal cancer. Blood samples were used for this study from 422 colorectal cancer cases and 874 controls. Blood collection was from 168 prediagnosed cases and 254 postdiagnosed cases.
6.3.5 Endometrial Cancer

According to SEER data, in 2011, about 46,500 new cases of endometrial cancer were expected to be reported, and about 8,100 women were expected to die as a result of this cancer. Futyma et al. (2008) reported mtDNA4977 deletions in endometrial cancer. Somatic mutation, deletion, and microsatellite instability (MSI) were observed in a small (N = 50) endometrial cancer sample analysis. Mutations were located in the D-loop region and in the 12S and 16S rRNA genes; genomic instability was observed as a result of these mutations (Futyma et al., 2008; Liu et al., 2003). Mutational hot spot regions were located in the D-loop region and 12S rRNA genes. In a separate study, the Cytb gene region was found to be mutated when samples were collected from patients with gynecological malignancies as well as eight patients with benign gynecological tumors (Li et al., 2003). The malignant tumors were squamous cervical carcinomas, endometrial carcinomas, and epithelial ovarian cancers (EOCs); the benign tumors were ovarian epithelial tumors and uterine myomas. A mononucleotide repeat (D310) in mtDNA also has been identified as a mutational hot spot in cervical cancer (Parrella et al., 2003). Because individuals in these studies were not followed, it is not possible to say whether mtDNA is a marker of prognosis or how long the individuals survived.

6.3.6 Esophageal Cancer

According to SEER data, in 2011, about 17,000 new cases of esophageal cancer were expected to be reported, and about 14,700 people were expected to die from esophageal cancer. Somatic mutations of mtDNA have been reported to play an important role in the carcinogenesis of the esophagus and gastrointestinal tract (Gochhait et al., 2008; Tan et al., 2006). Samples of 82 esophageal cancers, 96 gastric cancers, and 138 colorectal cancers were examined to detect mtDNA mutations, and microsatellite assays were performed in the D310 mononucleotide repeat of mtDNA. The frequencies of mtDNA mutations were similar in esophageal, gastric, and colorectal cancers. No significant relationships were found between mtDNA mutations and patient age or sex, tumor location, depth of tumor invasion, and lymph node metastasis in each digestive tract cancer, which suggests that mtDNA mutations play a role in the development but not progression of each digestive tract cancer. The observations made in this study also suggest that the role of mtDNA mutations might be similar among the different digestive tract cancers. Another group of investigators reported frameshift mutations in the ND4L, ATP6 subunit, and ND4 genes region and other mutations in the D-loop region (Tan et al., 2006).

6.3.7 Gastric Cancer

According to SEER data, in 2011, about 21,500 new cases of gastric cancer were expected to be reported, and about 10,300 people were expected to die from this disease. Gastric cancer is detected very late during disease development, and the 5-year survival rate is 10%–15%. Recently, Wei et al. (2011) reported finding a polymorphism in the D-loop region in gastric cancer. Somatic mutations in mtDNA have been detected in only 14% of gastric cancers (Hiyama et al., 2003; Hung et al., 2010; Kose et al., 2005). In another study, mutations in the rRNA gene of the mitochondria were found to be related to the development of gastric cancer (Hiyama et al., 2003). During the carcinogenesis process, heteroplasmic mtDNA was converted to homoplasmic DNA (Han et al., 2005). Mutations also have been reported in the D-loop region, and the number of mutations is higher in advanced stages of cancer compared to the early stages (Lee et al., 2005). ROS, apoptosis, and proliferation in the mutation group all were significantly higher than in the control group (Lee et al., 2005; Zhao et al., 2005). Shen et al. (2003) reported finding deletion of 4977 bp in gastric cancer. In another study, however, no correlation was observed between the presence of mutation in mtDNA and carcinogenesis (Martin et al., 2005). Validation of results from these two studies is needed to find a clear answer. Helicobacter pylori has been correlated with the development of gastric cancer, and Bax was found to be transported to mitochondria during this process (Ashktorab et al., 2004). No significant relationships were observed between mtDNA mutations and clinicopathological features such as patient age or sex, tumor location, depth of tumor invasion, and lymph node metastasis in gastric cancer.
6.3.8 Head and Neck Cancer

According to SEER data, in 2011, about 39,400 new cases of head and neck cancer were expected to be reported, and about 7,900 people were expected to die from this cancer. Parr’s group observed mutations in mtDNA in head and neck cancer; some of these mutations were detected early in the carcinogenesis process (Parr et al., 2006). Head and neck cancer samples also were found to contain mutations in the D-loop region, although these mutations are not associated with the prognosis of head and neck cancer (Challen et al., 2011). These investigators suggested that mtDNA mutations in cancer might represent bystander genotoxic damage as a consequence of tumor development and progression. Mitochondrial mutations have been found in recurrent head and neck cancer tumors (Dasgupta et al., 2010).

6.3.9 Kidney Cancer

Samples from kidney cancer patients showed mitochondrial mutations in the D-loop region and in rRNA and tRNA genes (Meierhofer et al., 2006b; Nagy et al., 2002, 2003). Few groups have explored extensive mutation analysis in samples from kidney cancer patients (Sangkhathat et al., 2005). Germ line mitochondrial mutations in the succinate dehydrogenase gene were detected in kidney cancer samples (Housley et al., 2010). According to SEER data, in 2011, about 60,900 new cases were expected to be reported for kidney cancer, and about 13,100 people were expected to die from kidney cancer. Wada et al. (2006) reported mtDNA mutations and 8-hydroxy-2′ deoxyguanosine contents in the Japanese population in the United States.

6.3.10 Leukemia

According to SEER data, in 2011, about 44,600 new cases were expected to be reported for leukemia, and about 21,800 people were expected to die from this blood cancer. Meierhofer et al. (2006a) and Kwok et al. (2011) reported finding mtDNA mutations in leukemia. Most of the mutations were reported in the D-loop region in Kwok’s study. Other groups also have reported mtDNA mutations in childhood leukemia (Sharawat et al., 2010). Previously somatic mtDNA mutations in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) were reported in brain tumors and in a small proportion of acute myeloid leukemia (AML). This observational study led Chotirat et al. (2012) to conduct a large study with 230 newly diagnosed AML patients and evaluate the presence of mtDNA mutations in IDH1 and IDH2. About 19% of newly diagnosed patients had these mutations.

6.3.11 Liver Cancer

According to SEER data, in 2011, about 26,200 new cases of liver cancer were expected to be reported, and about 19,600 people were expected to die from this cancer. The mtDNA copy number was found to be correlated with advancement of liver cancer, but specific mutations in the D-loop region and specific deletions were not observed in liver cancer samples as compared to normal liver samples (Gwak et al., 2011; Lee et al., 2004; Wang et al., 2011; Wheelhouse et al., 2005; Yamada et al., 2006; Yin et al., 2004). A correlation was observed between tumor size and time of survival after the onset of disease. In another study, when a small number (n = 26) of samples from liver cancer patients who were undergoing interferon therapy after infection with the hepatitis C virus (HCV) was compared with biospecimens from liver cancer patients without HCV infection, the number of mutations in the mtDNA was found to be higher in the samples from the infected patients (Nishikawa et al., 2005). How the viral infection induces mtDNA mutation is not completely understood. A possible correlation between hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC) also was studied, and the number of mutations in the D-loop region was found to be higher in HBV-infected liver biopsy samples compared to age-matched normal tissues (Wheelhouse et al., 2005). In another study, fewer mtDNA mutations were observed compared to mutations in adjacent normal liver tissues (Tamori et al., 2004). Gwak et al. (2011) did not observe increased mutation rates in mtDNA or common 4977 bp deletion as a result of HBV infection or mutations in the HBV genome. Mitochondrial mutations also might indicate treatment outcomes in liver cancer (Wang et al., 2011).
6.3.12 Lung Cancer

Cigarette smoking in heavy smokers has been identified as a risk factor for lung cancer. ROS generated by cigarette smoke damage mtDNA. To compensate for the damage, cells produce a large number of mitochondrial copies. A case–control study (422 lung cancer patients and 504 controls) by Zheng et al. (2012) evaluated the role of mtDNA copy number and 822 bp deletions in cancer development. Multivariate logistic regression analysis indicated that haplogroups G and M7 might be risk factors for lung cancer, whereas haplogroups D and F were found to be related to individual lung cancer resistance. Lung cancer is a complex disease that has been divided into two categories: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). SCLC is further subtyped into small-cell, mixed small-cell/large-cell, and combined small-cell carcinoma. NSCLC is comprised of squamous cell carcinoma, adenocarcinoma, and large-cell carcinoma. D-loop mutations also have been reported in lung cancer patients (Dasgupta et al., 2012; Suzuki et al., 2003). In a high-throughput MitoChip assay, 18% of lung cancer samples had mutations in the mtDNA. Some of the mutations correlated with the stage of progression and prognosis in NSCLC (Matsuyama et al., 2003). In one report, most of the mtDNA lung cancer mutations that were investigated occurred randomly and were thought to have no impact on carcinogenesis, whereas the homoplasmic mutations might provide a potential diagnostic marker for lung cancer. Mutations in other regions also have been reported in lung cancer (Dai et al., 2006). According to SEER data, in 2011, about 221,100 new lung cancer cases were expected to be reported, and about 156,900 people were expected to die from lung cancer.

6.3.13 Oral Cancer

Each year in the United States, more than 21,000 men and 9,000 women are diagnosed with oral cancer. Oral cancer can develop in any part of the oral cavity (mouth and lips) or the part of the throat at the back of the mouth. Liu et al. (2012) reported mitochondrial mutations in the D-loop region of oral squamous carcinoma cells of 38 patients. This group also followed the effects of tobacco and betel chewing on the rate of mutations and correlated with overall survival. The focus of the mutation analysis was D-loop because this is a crucial site for replication and expression of mitochondrial genome as it holds the leading strand origin of replication and the main promoter for transcription of polycistronic message. Due to its triplet nature, this portion of the mtDNA is very variable and sensitive to external insults resulting in polymorphisms and mutations (Thyagarajan et al., 2012; Verma et al., 2003). The percentage of homoplasmic mutations in oral cancer was very high (68%) and 71% mutations were mononucleotide repeats located in the polycytidine stretch over 303nt of the mtDNA (Liu et al., 2012). Mitochondrial pathways are disturbed in oral cancer resulting in structure of genes (Lin et al., 2009).

6.3.14 Ovarian Cancer

According to SEER data, in 2011, about 22,000 new ovarian cancer cases were expected to be reported, and about 15,500 women were expected to die from this cancer. Histologically, three categories of ovarian cancer have been reported: ovarian epithelial cancer, sex cord–stromal tumors, and steroid cell tumors. The majority of tumors belong to the EOC category. Few reports have been published on mtDNA and ovarian cancer. In one study in which the D-loop region of 25 ovarian tumors was analyzed, 26 mutations were identified, resulting in a mutation rate of 32% (Shi et al., 2002). Another group screened several ovarian cancer samples to locate cancer-associated mutations but the mutations that were found had little clinical relevance (Bragoszewski et al., 2008). When multiple samples from the same patients were analyzed to detect mtDNA mutations, several mutations located in different regions of the genome were reported (Van Trappen et al., 2007). The 5-year survival rate for ovarian cancer is 25%.

6.3.15 Pancreatic Cancer

According to SEER data, in 2011, about 44,000 new pancreatic cancer cases were expected to be reported, and about 37,700 people were expected to die from this cancer. Pancreatic cancer mutations
were identified in several genes, including 12S rRNA, 16S rRNA, NDI, ND2, COXI, COXII, ATPase 6, COXIII, ND4, ND4L, ND5, ND6, and Cyt b, as well as in the noncoding D-loop region (Jones et al., 2001). Analysis of other genes in the mtDNA molecule might demonstrate an even higher incidence of mtDNA somatic variants in patients (Kassauei et al., 2006). Germ line mtDNA variations exhibit associations with metabolism and outcome (Navaglia et al., 2006). In a recent epidemiologic study, haplogroup H contained the most mutations (Lam et al., 2012). This was a population-based, case–control study of 532 pancreatic cancer cases and 1701 controls that was conducted between 1994 and 2001 in the San Francisco Bay Area.

6.3.16 Prostate Cancer

Mitochondrial mutations have been reported in prostate cancer. For example, 6267G > A is a recurring mutation that introduces the Ala122Thr substitution in the mitochondrially encoded cytochrome c oxidase I (MT-CO1) (Gallardo et al., 2006; Petros et al., 2005). Most of the sequence variants were present in the D-loop region (52%), RNR2 (14%), and ND4 (13%) (Gomez-Zaera et al., 2006). Older age and a positive family history of prostate cancer are important risk factors (Jessie et al., 2001; Singh, 2006). Increased electron transport chain activity, increased oxygen consumption, and perhaps excess ROS production compared with normal prostate epithelial cells also might contribute to the development of prostate cancer (Feng et al., 2005; Schalken et al., 2005). Results from prostate-specific antigen (PSA) tests were compared with results from mtDNA alterations in prostate cancer samples, and the correlation between mutation rate and PSA level was observed (Kloss-Brandstätter et al., 2010). According to SEER data, about 240,900 new prostate cancer cases were expected to be reported in 2011, and about 33,700 men were expected to die from this cancer. Prostate cancer is the second most frequent cancer among men in the European Union and the most common cancer among men in the United States (Dakubo et al., 2006; Jeronimo et al., 2001).

6.3.17 Skin Cancer

According to SEER data, about 76,300 new skin cancer cases (excluding basal and squamous) were expected to be reported in 2011, and about 12,000 people were expected to die from this cancer. Multiple mtDNA deletions and tandem duplications have been reported in skin cancer (Hubbard et al., 2008; Krishnan and Birch-Machin, 2006; Mithani et al., 2008). These mutations have been used as biomarkers of photoaging in skin (Eshaghian et al., 2006; Krishnan and Birch-Machin, 2006). The most common deletions were 3715, 4977, and 6278 bp. Studies are being conducted in nonmelanoma skin cancer and photodamaged skin; it appears that more mutations are present in photodamaged skin. In another study, mtDNA instability in malignant melanoma of the skin was found to be restricted mostly to the nodular and metastatic stages (Poetsch et al., 2004). Induction of the common deletion was paralleled by a measurable decrease of oxygen consumption, mitochondrial membrane potential, and ATP content, as well as an increase of matrix metalloproteinase-1 (Berneburg et al., 2005). A high-throughput mitochondrial genome screening method also has been developed using multiplexed temperature gradient capillary electrophoresis (Girald-Rosa et al., 2005).

6.3.18 Thyroid Cancer

According to SEER data, in 2011, about 48,000 new thyroid cancer cases were expected to be reported, and about 1,700 people were expected to die from this cancer. Gasparre et al. (2007) demonstrated that disrupted mtDNA mutations can be markers of oncocytic phenotype in thyroid tumors. Other investigators have evaluated the presence of mtDNA mutations in thyroid cancer (Ding et al., 2010; Maximo et al., 2005; Tong et al., 2003). Witte et al. (2007) reported mtDNA mutations in differentiated thyroid cancer in older patients. Oncocytic tumors are proliferative lesions comprised of a high degree of mitochondrial hyperplasia that is frequent in the thyroid gland (Bonora et al., 2006). A literature survey did not show evidence of somatic mutations in mtDNA in other cancers such as leukemia and lymphoma.
6.4 Types of Samples Suitable for Isolating mtDNA and Mutation Analysis

A variety of clinical samples have been utilized to detect mtDNA mutations (Table 6.1). For example, tissues were used for thyroid cancer (Bonora et al., 2006; Maximo et al., 2005; Tong et al., 2003), nipple aspirate and paraffin-embedded specimens for breast cancer, urine for bladder cancer, buccal cells for head and neck cancer (Pai et al., 2006), CSF for medulloblastoma, and sputum for lung cancer (Pai et al., 2006). For epidemiologic studies in which thousands of samples are collected and analyzed, it is very important to determine which clinical samples should be collected. The procedure should be noninvasive and inexpensive. The characteristics of ideal biomarkers are shown in Figure 6.3.

<table>
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<th>Patent Application Reference</th>
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<tr>
<td>US6649144</td>
<td>Zila, Inc., Phoenix, AZ</td>
<td>Methods for detecting and killing epithelial cells</td>
<td>A diagnostic method for detection of cancerous epithelial cells by selective marking of the mitochondria</td>
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<tr>
<td>US6605433</td>
<td>The Johns Hopkins University, Baltimore, MD</td>
<td>Mitochondria dosimeter</td>
<td>Determining mutation in D-loop region by PCR method Can be used for lung, head and neck, bladder, prostate, and pancreatic cancer detection</td>
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<td>US8008008</td>
<td>Mitomics, Inc. Thunder Bay, Ontario, Canada</td>
<td>Mitochondrial mutations and rearrangements as a diagnostic tools for the detection of sun exposure, prostate cancer, and other cancers</td>
<td>Mutation detection in biological samples Deletion in regions covering genes encoding NADH dehydrogenase, tRNA histidine, tRNA serine2, and tRNA leucine 2</td>
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FIGURE 6.3 Characteristics of an ideal biomarker.
6.5 Technological Advancements

Because of its small size, mtDNA is coprecipitated with nDNA and amplified using proper primers specific for mitochondria. However, microdissected samples have been used successfully to identify mtDNA mutations in clinical samples (Goebel et al., 2005). Pure populations of cells could be isolated from heterogeneous cells in the surgically isolated samples (Aldridge et al., 2003). The specificity of the mutational assays was 90%, whereas the sensitivity was 95% (Beck et al., 2010; Cai et al., 2011). Qiu et al. (2012) reported the sensitivity of the approach permitted detection of less than 5% mtDNA heteroplasmic levels. To understand the etiology of the disease and perform mitochondrial genotyping, a pure population of cells is needed. High-throughput microarray technologies (MitoChip) have been developed for somatic mutation detection (Kassauei et al., 2006; Maitra et al., 2004). Improved methods are needed to detect the integration of mtDNA in nDNA. Mitochondrial mutation detection assays have been patented (Table 6.2). Furukawa et al. (2012) developed MITO-Porter device that can be used to deliver chemicals and proteins in mitochondrial membrane to study the function of specific genes or study microenvironment inside the mitochondria. For complete genome of mitochondria, the term motogenome has been used (Meng et al., 2012).

6.6 Haplogroups in Mitochondria

Similar to nuclear genomic haplogroups, mitochondrial haplogroups have been reported (Darvishi et al., 2007; Ebner et al., 2011; Fang et al., 2010). mtDNA haplogroups have been used in characterizing admixed populations (Haber et al., 2012; Tofanelli et al., 2009). To understand the inheritance pattern of the mitochondrial genome in cancer, samples have been analyzed from different tissues (Darvishi et al., 2007). Haplogroup U, with an OR value of 1.95, also has been identified in prostate cancer samples. Inheritance of the U haplogroup is associated with a high risk of developing prostate cancer; about 20 million white individuals have this haplogroup. Thus far, nine mitochondrial haplogroups—H, I, J, K, T, U, V, W, and X—have been identified (Ebner et al., 2011; Li et al., 2011). The presence of a specific haplogroup predisposes an individual to a risk of prostate cancer. Haplogroup N contains the G10398A polymorphism, which is common in breast and esophageal cancer. Similar observations have been made for renal cancer. Haplotyping is performed by restriction analysis, whereas the whole mitochondrial genome is sequenced in mutation analysis.
6.7 Analytical and Clinical Validation of Mitochondrial Biomarkers

A number of biomarkers (SNPs, mutations, deletions, and copy number variances) have been reported by different investigators in different tumor types. However, results have not been verified or validated independently by other groups. As is true for other biomarkers, mitochondrial biomarkers should be validated: first by analytic techniques and then clinically. These processes are time-consuming and expensive but should be completed. Ideally, one national center should be created that can oversee the validation of biomarkers and coordinate all of the data generated from this research. The clinical implications of mitochondrial biomarker are shown in Figure 6.4. The implication of mitochondrial information in cancer epidemiology is summarized in Table 6.3. A few selected databases of mitochondrial information are presented in Table 6.4. The National Cancer Institute (NCI), National Institutes of Health, supports mitochondrial research in cancer etiology and cancer epidemiology where mtDNA biomarkers play a significant role. A few selected projects supported in this area are presented in Table 6.5. The Mitochondrial Medicine Society (http://mitosoc.org/blogs/about-us/) is an excellent resource for clinical application of mitochondrial-related information and getting updated about the criteria for mitochondrial biomarkers in different diseases and about other information related with mitochondrial diseases. The broad impact that Mitochondrial Medicine is now having on the biomedical sciences stems from the fact that traditional biomedical science has emphasized the tissue specificity of disease and the quantized genetics of Mendelian genes, while Mitochondrial Medicine also takes into account the systemic importance of energy and quantitative genetics of the mtDNA. Since the mitochondrial genome encompasses not only the energy genes of the mtDNA but also over 1500 nDNA genes that impact on mitochondrial structure and function, mitochondrial principles are providing new insights into the inheritance, development, and pathophysiology of a broad spectrum of clinical problems.

![Flowchart](chart.jpg)

**FIGURE 6.4** Clinical implication of mitochondrial markers.

**TABLE 6.3**

Implication of Mitochondrial Biomarker Information in Cancer Epidemiology

**Implications**

- Can we utilize mitochondrial haplogroup information to identify high-risk population?
- How can we utilize mitochondrial proteomic information to understand gene–gene and gene–environment studies and cancer etiology?
- Are different mitochondrial markers associated with development of cancer and are they mediated by specific nongenetic risk factors?
- Are mitochondrial markers useful for identification of high-risk groups before clinical onset of disease, and are they associated with recurrence of tumors?
- Can we utilize mitochondrial information to predict prognosis and better survival?
6.8 Concluding Remarks and Future Perspectives

Although problems still exist in the mtDNA field, mtDNA has potential to be used in the clinic, especially for risk assessment purposes. Areas requiring improvement include establishing a causative relationship between mitochondrial mutations and cancer development (an association with disease does not mean causation), identifying pathophysiological consequences of mtDNA alterations, validating already identified mtDNA mutation markers and polymorphisms in different populations, and assessing accidental amplification of nuclear mitochondrial pseudogenes (NUMTs) (Ospelt and Gay, 2005). Critical evaluation of mitochondrial primers is needed for high-quality results. Validating results in large numbers of population samples is recommended and has been adopted by a few investigators (Glazer et al., 2008). Correlating patient information (exposure history, lifestyle, BMI, alcohol consumption, smoking history, genetic background, and family history) with mitochondrial genetic alterations (copy number changes and mutations) should be accomplished before deriving any clinical inferences. No significant relationships have been identified between mtDNA mutations and clinicopathological features such as patient age or sex, tumor location, depth of tumor invasion, and lymph node metastasis in digestive tract cancers. Research in these directions is urgently needed. Some progress has been made in mitochondrial proteomics to identify new diagnostic and prognostic biomarkers of different tumor types (Bottoni et al., 2012).

The recent surge in mitochondrial research has been driven by the identification of mitochondria-associated diseases and the role of mitochondria in apoptosis. By virtue of their clonal nature and high copy numbers, mitochondrial mutations may provide a powerful molecular marker for the noninvasive detection of cancer. It has been suggested that the extent of mtDNA mutations might be useful in the prognosis of cancer outcome and/or response to certain therapies.

Although most cancer cells harbor somatic mutations in mtDNA, the question of whether such mutations contribute to the promotion of carcinomas remains unsolved. mtDNA mutations can initiate...
a cascade of events that leads to a continuous increase in the production of ROS (persistent oxidative stress), a condition that probably favors tumor development.

Mitochondria have been implicated in the process of carcinogenesis because of their vital role in energy production, nuclear–cytoplasmic signal integration, and control of metabolic pathways. Interestingly, at some point during neoplastic transformation, there is an increase in ROS, which damage the mitochondrial genome (Valko et al., 2006). This accelerates the somatic mutation rate of mtDNA. It has been proposed that these mutations may serve as an early indication of potential cancer development and may represent a means for tracking tumor progression.

The mechanisms responsible for the initiation and evolution of mtDNA mutations and their roles in the development of cancer, drug resistance, and disease progression are not completely understood. The mitochondrial genome is dependent upon the nuclear genome for transcription, translation, replication, and repair, but precise mechanisms for how the two genomes interact and integrate with each other are poorly understood. The relatively small size of the genome (16.5 kb) and the use of automated DNA sequencing make it possible to sequence the entire genome from clinical specimens in days.

The biggest challenge in this area is determining an accurate mtDNA copy number, because in some situations mtDNA becomes integrated into the nuclear genome at nonspecific sites. Another challenge is the simultaneous characterization of nuclear and mtDNA in cases and controls. Although this is technically possible, such studies have not yet been conducted within epidemiologic studies. Selection of sample is another problem. When mutations in blood DNA were compared with mutations in breast cancer tissues from the same patient, the mutations did not match. This suggests that blood might not be the appropriate biospecimen to use in such studies. Challenges exist in storing and mining data. There is general consensus that biology is transforming into an information science, and biomedical informatics is no longer an option but an integral component of all biomedical research. Traditional research methods must be complemented by comprehensive web and database searches for hypothesis generation. New computational methods and tools are constantly required to manage new types of data that often are unstructured, complex, massive, and nonintuitive. Peer-reviewed publications no longer provide the only source of fresh knowledge. Often, new discoveries are made by individuals who have the tools and skills to integrate and perform in-depth analyses of data from not only their own laboratories but, more importantly, from all accessible sources.

Replication of several studies mentioned in this chapter has not been completed. In epidemiology, replication of studies in different sets of participants with diverse exposure is very essential to reduce bias and confounding. Validation of findings also helps in identifying genetic variations that are influenced by environmental factors. Guidelines developed for proper reporting of observations and validation of biomarkers in molecular epidemiology should be followed.

Many challenges remain to be addressed before the use of information technology in everyday research is broadly accepted and adopted. These include the lack of user-friendly tools and training for bench biologists, especially in areas of emerging technologies, disengagement of tool builders and potential users, high overhead for data storage and dissemination, lack of common standards for data exchange, lack of computational infrastructure to accommodate new types of high-throughput data, and ineffective programs to attract computational scientists to the field of biology.

A coordination center (informatics forum) is needed that is responsible for (1) informing the research community about existing computational tools and resources, (2) disseminating technology from central institute supported tools, (3) gathering user feedback, and (4) identifying deficiencies in technology and critical needs through community consultation.

In summary, mtDNA information is promising and may lead to the identification of novel biomarkers for cancer detection, diagnosis, and follow-up of survival and treatment. It also might make it possible to identify populations that would be responsive to treatment.

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References


Mitochondrial DNA in Early Cancer Diagnosis and Screening


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