Published OnlineFirst January 9, 2007; DOI: 10.1158/0008-5472.CAN-06-4119

Meeting Report: Mitochondrial DNA and Cancer Epidemiology

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Introduction

The Analytic Epidemiology Research Branch of the National Cancer Institute (NCI) hosted a meeting on "Mitochondrial DNA and Cancer Epidemiology" in Bethesda, Maryland, on September 7 to 8, 2006 to review progress in the area of mitochondrial DNA (mtDNA) and its use in cancer epidemiology and risk assessment. International leaders in the fields of mitochondrial biology, cancer epidemiology, oncology, and biotechnology participated in the meeting. Mitochondria have been implicated in carcinogenesis because of their vital role in energy production, nuclear-mitochondrial and mitochondria-to-nucleus signal integration, control of apoptosis, and various metabolic pathways. During neoplastic transformation, there is an increase in reactive oxygen species (ROS) that damages the mitochondrial genome accelerating the somatic mutation rate in mtDNA. These mutations may serve as an early indication of potential cancer development and may represent a means for tracking tumor progression and therapeutic response. Workshop participants discussed mtDNA haplotype-associated risk in human population and recommended that the somatic mutations in mtDNA should be analyzed in conjunction with nuclear DNA in future studies of cancer epidemiology.

Mitochondria play important roles in cellular energy metabolism, free radical generation, and apoptosis. Mitochondrial structure is dynamic and varies according to cell type and environmental conditions. In oocytes, they are 300 to 600 nm spheres. In fibroblasts, they are thread-like filaments measuring 0.4 × 2 μm to 0.4 × 8 μm. In other cell types, mitochondria appear as a dynamic, interconnected network, with fission and fusion events occurring throughout the cell cycle. In all cell types, mitochondria display close connections with the cytoskeleton and supply energy to the cell through the process known as oxidative phosphorylation. Mitochondria contain their own DNA, a 16.6-kb circular molecule that encodes 13 proteins, 22 tRNAs, and 2 rRNAs. In addition, ~1,500 other proteins, including all the proteins required for mitochondrial transcription, translation, protein folding, and assembly, are encoded by genes in the nucleus and imported from the cytoplasm. These nucleus-encoded, mitochondrial proteins are tailored by the transcriptional program of cells so that every mitochondrion performs both tissue-specific and housekeeping metabolic functions. Because mtDNA lacks introns, most mutations occur in coding sequences and subsequent accumulation of mutations can lead to the development of tumors.

The goal of the meeting was to get recommendations from experts in mitochondria and cancer epidemiology fields to provide information on cancer epidemiology, which helps to identify high-risk populations and to develop cancer control strategies. The meeting was chaired by Dr. Keshav Singh (Roswell Park Cancer Institute, Buffalo, NY) and cochaired by Dr. Robert K. Naviaux (University of California, San Diego, CA). Thirteen talks addressed the scientific issues related to mtDNA and risk of cancer. These include various tumor types containing mutant mtDNA, use of mtDNA early detection of cancer, factors contributing to alterations in mitochondrial genome, interaction of mitochondrial and nuclear genomes, mitochondrial haplogroups and associated cancer risks, and high-throughput technologies to detect mtDNA mutations and polymorphisms.

Mitochondria and Cancer

Mitochondrial dysfunction is a hallmark of cancer cells. Mitochondrial dysfunction can lead to resistance to apoptosis and to the well-known Warburg effect associated with aerobic glycolysis. Somatic mtDNA mutations have been detected in various tumors and have been suggested as markers for early detection. The majority of these somatic mutations are homoplasmic in nature, indicating that the mutant mtDNA becomes dominant in tumor cells. However, it is not clear whether the status of mtDNA affects nuclear genomic stability or whether the proteins involved in intergenomic cross-talk are involved in tumorigenesis. To understand the use of mtDNA in cancer epidemiology, one approach is to look for the somatic mutations in mitochondria; another approach is to look for disease-associated haplotypes and the single-nucleotide polymorphisms (SNP) associated with those haplotypes. The inheritance pattern of mitochondria in patients with cancer has been studied by haplotype analysis.

The meeting began with an overall presentation, summary of the field of mitochondria and cancer, and discussion by Dr. Singh about the importance of mitochondria-to-nuclear retrograde response in tumorigenesis. Dr. Singh showed that loss of mitochondrial function leads to cell cycle arrest, cellular senescence, and tumorigenic phenotype. In light of these and earlier studies, he hypothesized the existence of a mitochondria damage checkpoint (mitocheckpoint) in human cells. The mitocheckpoint permits cells to arrest in the cell cycle to repair/restore mitochondrial function to the normal level. On overwhelming, persistent, or severe damage to mitochondria, mitocheckpoint machinery may allow cells to undergo senescence. Consequently, cellular senescence may function as another checkpoint before cells initiate programmed cell death resulting in aging of tissues and organs. Alternatively, mutations

Note: The "Mitochondrial DNA and Cancer Epidemiology" meeting was held in Bethesda, MD, September 7–8, 2006.

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occur in the mitochondrial and/or nuclear DNA, resulting in tumorigenesis. Mitochondrial dysfunction resulting from changes in mtDNA invokes mitochondria-to-nucleus retrograde response in human cells. Dr. Singh's group carried out a comparative proteomic analysis using a cell line in which the mitochondrial genome was completely depleted (ρ^0, cells lacking all mtDNA-encoded protein subunits), a cybrid cell line in which mtDNA was restored, and the parental cell line. His studies showed that retrograde proteins function as tumor suppressors or oncogenes.

Claudio Franceschi (University of Bologna, Bologna, Italy) also described cross-talk between mitochondrial and nuclear genomes. He showed the effect of *p53* codon 72 alleles on tumorigenesis, oxidative stress-induced apoptosis in dermal fibroblasts, and peripheral blood lymphocytes that increases with the age of the donor.

Lee-Jun Wong (Baylor College of Medicine, Houston, TX) shared her experience in studying mtDNA mutations and germ-line mtDNA polymorphisms in neurofibroma, medulloblastoma, breast, liver, oral, and esophageal cancers. She discussed mtDNA mutations, including point mutations, large deletions, mtDNA depletion or amplification, and microsatellite instability in the control region hypervariable and coding regions.

William Copeland (National Institute of Environmental Health Sciences, Research Triangle Park, NC) presented background on the origins of mtDNA mutations, which include DNA damage from environmental factors and oxidative stress as well as from spontaneous errors in DNA replication, translesion synthesis, and repair resynthesis.

Masahiro Higuchi (University of Arkansas, Little Rock, AR) presented recent results on mtDNA differences between androgen-dependent and androgen-independent prostate cancer cell lines. He showed deletions and depletion of mtDNA in androgen-dependent LNCAP prostate cancer cell lines on inoculation in castrated mice, suggesting that mtDNA determines the androgen status of prostate cells; this may be a key in determining the progression of disease during and after prostate cancer treatment.

Peng Huang (M. D. Anderson Cancer Center, Houston, TX) recounted that the two most prominent metabolic abnormalities in cancer cells are the Warburg effect, characterized by increased glycolysis for ATP generation even in the presence of oxygen, higher acidity relative to normal surrounding tissues, and redox imbalance, which causes increased ROS generation and oxidative stress. He described the mechanisms of mtDNA mutations resulting in increase in oxidative stress and lack of response to chemotherapy.

Mitochondrial Haplogroups and Cancer Risk

mtDNA haplotypes have been used in characterizing admixture population. Dr. Masashi Tanaka (Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan), John Petros (Emory University, Atlanta, GA), and Dr. Jeffery Canter (Vanderbilt University, Nashville, TN) provided evidence that mitochondrial haplotypes, and in some cases specific nonsynonymous SNPs, could be correlated with cancer development. To understand the inheritance pattern of the mitochondrial genome in cancer, prostate and renal cell cancer samples were analyzed. Among nine European mtDNA haplogroups studied in this American population, haplogroup U was associated with an increased risk for these two cancers, with an odds ratio (OR) value of 1.95 for prostate cancer and 2.52 for renal cell cancer. About 20 million Caucasian individuals have this haplogroup. Over two dozen mtDNA haplogroups are known among human populations around the world. Haplogroup J, for example, contained populations with high risk of developing cancer. The population that was studied by Dr. Tanaka included aged individuals, and he proposed that the mtDNA of these individuals might be protected against damage and apoptosis. DNA analysis from such individuals might serve as control for comparison with individuals who develop the disease. Among these mtSNPs, the 12358A>G (*ND5*: Thr8Ala) would be one of the potentially functional polymorphisms.

The *ND5* subunit seems to be essential for the function of complex I because various missense mutations in the *ND5* gene have been reported in patients with various mitochondrial diseases, including Leber's hereditary optical neuropathy. Therefore, it seems possible that this AA replacement might also affect the function of the ND5 subunit and complex I. In a cancer-related study, Dr. Tanaka studied a population of 1,503 autopsied cases (696 male, 807 female) at Tokyo Metropolitan Geriatric Hospital registered in JG-SNP database. The haplotypes were classified into 30 haplogroups (i.e., F, B5, B4a, B4b, B4c, A, N9a, N9b, Y, M10+M11+M12, M7a, M7b2, M7c, M8+Z+C, G1, G2, M9, D5, D4a, D4b, D4d, D4e, D4g, D4h, D4j, D4k, D4k, D4l, D4m, and D4n). Among 1,363 subjects whose data (including age, gender, history of alcohol intake, history of smoking, and mitochondrial haplogroups) were available for the presence or absence of cancer, 819 subjects carried a pathologically confirmed cancer(s) at the time of autopsy. Subjects with the haplogroup M7b2 tended to have an increased risk for hemopoietic cancer (P = 0.037), having an OR of 2.46 (95% confidence interval, 1.06–5.73). Dr. Tanaka suggested that epidemiologic studies are possible in Japanese populations as most of the SNPs in mtDNA are characterized, but he noted that a similar fluorescent beads system for Caucasians, Africans, and Hispanics would need to be constructed before studying these populations.

Nine main European haplotypes (H, I, J, K, T, U, V, W, and X) were observed in a series of patients with prostate and renal cancers studied by John Petros and colleagues.

Dr. Canter reported on his recent epidemiologic studies in breast cancer that showed the G10398A substitution in ND3 was associated with an increased risk (OR = 1.6) in African-American women. Dr. Canter also reported one of the first cases of synergy among SNPs. The T4216C substitution in ND1 conferred no increased risk in isolation. However, when the T4216C substitution was present with G10398A, the risk of breast cancer was increased (OR = 3.1).

Factors Contributing to Mitochondrial Genome Alterations

Edward R. Sauter (University of Missouri, Columbia, MO) proposed that an increase in the mtDNA copy number is associated with transfer of mtDNA to nucleus (often seen in gliomas). Dr. William Copeland (NIEHS, Research Triangle Park, NC) analyzed mitochondrial mutational spectra in human cells, tissues, and derived tumors for 10,030 to 10,130 bp by constant denaturing capillary electrophoresis, indicating that 83% of mutations detected *in vivo* were reproduced *in vitro* with recombinant DNA polymerase γ. Furthermore, the endogenous error mediated by DNA polymerase γ constitutes the primary source of mitochondrial point mutations in human tissues.

High-Throughput Technologies in Detecting Mitochondrial Dysfunction, Mutations, and Polymorphisms

In epidemiologic studies, hundreds of samples are analyzed simultaneously, which require high-throughput assays. Paul

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Gourley (Sandia National Laboratories, Albuquerque, NM) discussed how bioenergetic disturbances resulting from structural and functional changes in mitochondria in cancer cells can be measured by biocavity laser spectroscopy and related BioMicro-Nanotechnologies. These technologies have the potential to provide accurate, real-time, high-throughput screening of tumor cells without the need for time-consuming sample preparation. In normal cells, mitochondria are highly organized within the cytoplasm and highly scattering, yielding a highly correlated signal. In cancer cells, mitochondria are more chaotically organized and poorly scattering, leading to reduced correlation between mitochondria and light scattering centers imaged by confocal laser scanning microscopy.

Dr. Naviaux discussed the use of biocavity laser spectroscopy of cancer cells and genetic forms of mitochondrial disease. He proposed that a diffused cytoplasmic distribution of mitochondria may be associated with the mitotic potential of the cell. As a single test, the diagnostic resolution of the biocavity laser surpassed that of any single gold standard biochemical assay of mitochondrial function. By analyzing a population of hundreds to thousands of organelles derived from single cells, a distribution of optical spectra is collected in real time that constitutes an optical fingerprint of normal and cancer cells.

Konstantin Khrapko (Harvard Medical School, Boston, MA) presented information on newer, cost-effective approaches to mitochondrial genome sequencing.

Yan Su (George Washington University, Washington, DC) described the use of hMitoChip3 in ascertaining expression profiles and molecular pathways related to the mitochondrial transciptome in melanoma.

Types of Samples Suitable to Isolate mtDNA and Mutation Analysis

A variety of clinical samples have been used for mtDNA mutation detection. For example, nipple aspirate and paraffin-embedded specimens for breast cancer, urine for bladder cancer, buccal cells for head and neck cancer, cerebrospinal fluid for meduloblastoma, and sputum for lung cancer have been used. For epidemiologic studies where thousands of samples are collected and analyzed, it is very important to select which clinical samples should be collected. The procedure should be noninvasive and nonexpensive.

Future Research and Recommendations

Many advances in our knowledge of mtDNA and cancer epidemiology were highlighted at this meeting. Compelling data exist to support use of specific somatic mutations in different tumor types. 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 number, mitochondrial mutations may provide a powerful molecular marker for noninvasive detection of cancer. It has also been suggested that the nature and extent of mtDNA mutations might be useful in the prognosis of cancer outcome and/or the 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 leading to a continuous increase in the production of ROS (persistent oxidative stress), a condition that probably favors tumor development.

No significant relationships between mtDNA mutations and clinicopathologic features, such as patient age or sex, tumor location, depth of tumor invasion, and lymph node metastasis, have yet been identified. Research in this direction is urgently needed.

The participants suggested that three specific tissue types, blood, tumor, and normal tissue adjacent to the tumor, should be collected in future studies to help refine the role of mtDNA in cancer because mtDNA mutations may occur in homoplasmic and heteroplasmic forms in these different specimens. Because the NCI maintains several cohorts, nested case-control studies that take advantage of these ongoing studies should be designed for evaluating mtDNA in conjunction with nuclear DNA markers of cancer risk.

Acknowledgments

Received 11/7/2006; accepted 11/15/2006.

Grant support: Studies in K.K. Singh's laboratory were supported by NIH grants CA 121904 and CA113655.

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Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

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Cancer Res 2007;67:437-439. Published OnlineFirst January 9, 2007.