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# Development of mitochondria-specific mouse oligonucleotide microarray and validation of data by real-time PCR

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**Abstract**

This study describes the development of a mitochondria-specific microarray, MitoChip, to measure transcripts of mitochondria-associated genes in various diseases and drug-induced toxicities in the mouse. The array consists of 542 oligonucleotides that represent genes from the mitochondrial and nuclear genomes associated with mitochondrial structure and functions. The expression of mitochondrial genes was measured in the liver of both p53 haplodeficient (+/−) and wild-type (+/+) C3B6F$_1$ female mice exposed to antiretroviral agents, Zidovudine (AZT) and Lamivudine (3TC). Among genes whose expression was significantly altered, a set was selected for real-time PCR analysis to verify their differential gene expression. The real-time PCR data confirmed the observations by microarray analysis suggesting that the MitoChip may be an important tool for examining mitochondrial involvement in diseases and drug-induced toxicities.


*Keywords:* Mitochondria; Mouse MitoChip; Microarray; Zidovudine (AZT); Lamivudine (3TC)

## 1. Introduction

Mitochondria are unique cytoplasmic organelles in that they have their own DNA and genetic machinery. However; mitochondrial function is not solely controlled by the mitochondrial DNA, but significantly influenced by nuclear genes. A complex interactive signaling network between the mitochondrial and nuclear genomes is crucial for the maintenance of mitochondrial structure and regulation of a wide range of mitochondrial functions, such as oxidative phosphorylation, fatty acid oxidation, steroid metabolism, biosynthesis of heme, intermediary metabolism, and apoptosis (Petit and Kroemer, 1998; Schatz, 1995; Taanman, 1999). In view of the key role of mitochondria in energy production, an altered mitochondrial function can affect virtually every tissue and organ in the living organism. It is evident that the effect of altered mitochondrial function will be adversely manifested in the most energy-dependent tissues, such as the heart, skeletal muscle, brain, optic nerve, kidney, and liver. There is substantial evidence indicating that mitochondrial dysfunction is involved in aging and in the patho-physiology of a number of diseases and disorders, such as cardiovascular diseases, neurological disorders, cancer, type-2 diabetes mellitus, renal diseases, and liver diseases (Gerbitz et al., 1996; Beal, 1998; Wallace, 1999; Marin-Garcia and Goldenthal, 2002). Mitochondrial impairment can also simultaneously affect multiple tissues in various combinations, commonly referred to as "mitochondrial disorders" that include neuropathies, cardiomyopathies, and myopathies (Scheffler,

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2000). In addition, impaired mitochondrial function has been well documented in various drug- and chemical-induced toxicities, including those caused by chemotherapeutic agents, anti-retroviral drugs, mycotoxins, and neurotoxins (Foli et al., 2001; Colombo et al., 2001).

Mitochondria-mediated diseases and drug- or chemical-induced toxicities may be due to several mechanisms. Among these are, altered expression of mitochondrial and/or nuclear genes encoding mitochondrial proteins, interference with the regulation of expression of the mitochondrial genome, defects in communication between mitochondrial and nuclear genomes, and disruption of mitochondrial membrane potential, or combinations of these factors. While there is an enormous literature available that recognizes an association between mitochondria and degenerative diseases or drug-induced toxicities, knowledge of molecular events underlying these pathophysiologic conditions is still limited. It is unclear whether mitochondrial dysfunction is the underlying cause or consequence of these toxicities and the resulting diseases. Biochemical and genomic techniques have been pursued to explain the link between mitochondria and degenerative diseases, metabolic disorders, and drug-induced toxicities (Desai et al., 1996, 2001; Di Lisa and Bernardi, 2005; Corbucci et al., 2006; Wang et al., 2006). Thus far, the molecular mechanisms of mitochondrial dysfunction that may be responsible for diseases and toxicities are not clearly understood.

In the past decade, gene array technology has advanced genomic research due to its ability to measure simultaneously the expression level of thousands of genes in a single experiment. Using this technology, we have developed a mitochondria-specific mouse MitoChip that was designed to shed light on the complexity of the inter-genomic relationship between the mitochondrial and nuclear genomes. The ultimate objective of this initiative is to gain more knowledge of mitochondrial activity at the mRNA level during development and progression of diseases. This genomic approach can also be applied to detect toxicity-specific novel gene expression profiles, which may prove valuable as biomarkers and in risk assessment. Moreover, information gained by MitoChip analysis can be applied towards the development of new effective therapeutic strategies to combat mitochondria-associated toxicities or disorders through the manipulation of mitochondrial functions.

## 2. Materials and methods

### 2.1. Development of the MitoChip

#### 2.1.1. Design of a library

A library of 542 genes containing both mitochondrial and nuclear genes associated with mitochondrial structure and functions was created using the GenBank and Mouse Genome Informatics (MGI) databases (see Supplementary data). The library represents genes involved in oxidative phosphorylation, beta-oxidation of free fatty acids, tri-carboxylic acid cycle, apoptosis, mitochondrial DNA replication, transcription, and DNA repair. In addition, it includes 9 housekeeping genes and 9 Arabidopsis plant genes to serve as positive and negative control genes, respectively. Oligonucleotides corresponding to each gene in the library are fifty bases long and were designed and synthesized by MWG Biotech, Inc. (Ebersberg, Germany).

#### 2.1.2. Printing of the MitoChip

The mouse oligonucleotides were provided in lyophilized form in 384 well plates by MWG Biotech, Inc. These oligonucleotides were re-suspended in 1× printing buffer A (MWG Biotech, Inc.) to a final concentration of 40 μM. Oligonucleotides were printed on poly-L-lysine coated glass slides (Erie Scientific, Portsmouth, NH) using OmniGrid® 100 microarrayer (GeneMachines, San Carlos, CA) in a printing room maintained at 23 °C and 50% humidity at the Center for Functional Genomics, National Center for Toxicological Research. Two arrays of 560 oligonucleotides each were printed on each slide. Each array consists of 9 housekeeping genes and 9 Arabidopsis genes printed randomly in triplicates. Printed arrays were baked for 2 h at 80 °C followed by cross-linking with 300 mJ of UV. Further, arrays were treated with bovine serum albumin (BSA, fraction V) solution containing sodium dodecyl sulphate (SDS), according to the procedure by Erie Scientific (www.eriesci.com) with minor modifications. The processed arrays were stored dry in Drykeeper desiccator (without desiccant) (Sanplatech Corp, Japan) maintained at 10% humidity.

### 2.2. Animal treatments and tissue collection

Animals used in this study were part of an ongoing National Toxicology Program (NTP)-funded carcinogenesis project at NCTR. This project is investigating transplacental and neonatal exposures of antiretroviral agents, AZT and 3TC, in the new C3B6F$_1$*trp*53(+/−) haplodeficient transgenic mouse model as a short term toxicity screen. In brief, Taconic C3H/HeNTac females were mated with C57BL/6(N5)*trp*53(−/−) males to produce C3B6F$_1$ *trp*53(+/−) mice. In addition, C3H/HeNTac females were mated with Taconic C57BL/6(N5)*trp*53(+/+) males to produce C3B6F$_1$*trp*53(+/+) mice. The pregnant females were treated daily by oral gavage as described below. Exposure of the pups was continued to postnatal day (PND)-28. All animals were raised in a pathogen-free environment at NCTR and treated according to the Institutional Animal Care and Use Committee guidelines. All mice were housed singly in standard polycarbonate cages with hardwood chip bedding and maintained at 23 °C with a relative humidity of 50%. The animals were conditioned to a 12 h light:dark cycle with lights on from 0600 to 1800 h daily and fed *ad libitum* NIH-31 diet.

There is a large body of literature indicating altered mitochondrial function during exposures to AZT and