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A Focused Microarray to Study Human Mitochondrial and Nuclear Gene Expression

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Abstract

A focused microarray (huMITOchip) was developed to study alterations of human mitochondrial and nuclear gene expression in health and disease. The huMITOchip contains 4,774 probe sets identical to the Affymetrix U 133 plus 2.0 chip covering genes affecting mitochondrial, lipid, cytokine, apoptosis, and muscle function transcripts. Unlike other gene chips, the huMITOchip has 51 probe sets that interrogate 37 genes of the mitochondrial genome. The human mitochondrial gene chip was validated against the Affymetrix U133 plus 2.0 array using an in vitro system of CCL136 muscle cell line stimulated with or without interferon gamma (IFN- γ). The 37 genes from the mtDNA demonstrated absolute gene expression levels ranging from 0.1 to 3,182. The comparison of the two gene chips yielded an excellent Pearson's correlation coefficient ($r = 0.98$). At least 17 probe sets were differentially expressed in response to IFN- γ on both chips, with a high degree of concordance. This is the first report on the development of a focused oligonucleotide microarray containing genes of the mitochondrial genome.

Keywords

mitochondria; microarray; gene expression; interferon gamma

Tissue with high energy demand such as skeletal and cardiac muscles and the brain are vulnerable to defects in mitochondrial function that impair cellular respiration, thus reducing the ATP to ADP ratio (Giordano et al., 2003; Latini et al., 2005). The human mitochondrial genome is circular with 16,569 base pairs and encodes 37 genes coding for 22 transport RNAs, 2 ribosomal RNAs, and 13 messenger RNAs. It also contains a highly variable noncoding D-loop region. The mitochondrial genome encodes for only a small fraction of the more than 1,000 proteins needed to form the outer and inner mitochondrial membranes and the complexes of the respiratory function (Gibson, 2005). The existing gene expression microarrays lack representation of genes of the mitochondrial genome and focus exclusively on nuclear genes. The only available microarray with representation of mitochondrial-

relevant genes is a cDNA array on glass slides with a limited number of genes that is not commercially available (Manoli et al., 2005). Smaller focused arrays with fewer genes that target certain pathways or biological functions (e.g., Jak-STAT pathway or cell cycle) provide specific information and are less confounding for analysis and more affordable for investigators (Zeng, 2003). In light of the complexity of microarray technology in terms of reliability and validity (Larkin, Frank, Gavras, Sultana, & Quackenbush, 2005; Sherlock, 2005), the objective of this project was to develop a tool that would encompass known nuclear and mitochondrial genes relevant to mitochondrial structure and function as well as the genes significantly impacted by mitochondrial dysfunction such as those involved in skeletal muscle and lipid metabolism (Bhattacharjee, Venugopal, Wong, Goto, & Bhattacharjee, 2006). The resulting focused microarray, the huMITOchip, is a complement to the already existing oligonucleotide gene sequencing MITO CHIP for rapid and high-throughput analysis of mitochondrial DNA mutations (Maitra et al., 2007).

Materials and Methods

The huMITOchip Design

The mitochondrial sequence information was taken from the universally accepted Cambridge reference sequence (Andrews et al., 1999) and each mitochondrial gene is represented by one or more probe sets. The nuclear genes relevant to mitochondrial structure and function, muscle function and inflammation were adapted from the annotated genes in the NCBI GenBank (www.ncbi.nlm.nih.gov/Genbank/index.html). The huMITO chip is based on the unique Affymetrix methodology of matching and mismatched probe sets. Each gene of interest is represented by 11 25-mer oligonucleotide probe sets with a matching sequence as reported in Genbank, along with 11 control probe sets of the same gene with a single base pair mismatch against the same target transcript that function as a combined unit. A present–marginal–absent call for this particular gene is based on the number of matching sequence probe sets compared to the number of mismatched probe sets for the same gene. This design allows investigators to assess transcript levels and determine the background hybridization for each probe set as part of the analysis, thereby establishing internal controls for the hybridization signals of each gene. Extensive FASTA searches for individual mitochondrial gene sequences that were not cross-hybridizing resulted in a total of 51 probe sets of 37 mitochondrial genes. We selected nuclear and mitochondrial proteins listed on Swiss-Prot, an annotated protein sequence database, and matched them to NCBI Genbank–accessible gene sequences, which assured the best selection of already existing nuclear gene probe sets in the Affymetrix data bank. The final custom-designed huMITOchip includes 4,825 probe sets for 37 mitochondrial, 1,000 nuclear mitochondrial-related, 800 inflammatory, 500 lipid metabolism, 200 muscle function, 100 apoptotic, and 100 housekeeping genes. The Human Genome HU 133 Plus 2.0 Array, used as a control, includes 54,000 human nuclear probe sets representing approximately 38,500 genes (Affymetrix, Santa Clara, CA).

Cell Culture

CCL136 muscle cells (ATCC, Manassas, VA) were suspended in 10 ml muscle culture medium and cultured in six T25 flasks until the cells reached 80% confluence. The medium

was removed and the cells were washed twice with 1x phosphate-buffered saline, pH 7.4. Cells in three flasks were treated with (500 IU/ml) IFN- γ for 6 hr, while the other three flasks remained in a serum-free medium for 6 hr to serve as controls (Raju, Vasconcelos, Granger, & Dalakas, 2003).

Microarray

Total RNA was isolated using an RNeasy Mini Kit (Quiagen, Valencia, CA). The quality of total RNA was assessed by visualization of intact 18S and 28S ribosomal bands on a 1.2% formaldehyde agarose gel (Ambion, Austin, TX). Reverse transcription, cDNA and cRNA synthesis, and cRNA fragmentation were done according to the manufacturer's instructions (Affymetrix; Ambion, Austin, TX).

The hybridization cocktail was made with 15 μ g of fragmented, biotinylated cRNA along with 0.1 mg/mL herring sperm DNA (Promega, Madison, WI), 0.5 mg/mL bovine serum albumin (Invitrogen, Carlsbad, CA), 50 pM of control oligonucleotide B2, and eukaryotic hybridization controls (bioB, bioC, bioD, and Cre 1.5, 5.25, and 100 pM, respectively) in the presence of dimethylsulfoxide (DSMO), according to the manufacturer's instructions (Affymetrix). The hybridization cocktail was heated at 99°C for 5 min and then at 45°C for 5 min. The hybridization cocktail (200 μ L) was hybridized to the human gold standard (HU 133 Plus 2.0; $n = 3$ control) and to the huMITOchip ($n = 3$ treatment) for 16 hr at 45°C. The microarrays were washed and stained by the Affymetrix Fluidics Station using the standard format as described by Affymetrix. The probe arrays were stained with streptavidin phycoerythrin solution (Molecular Probes, Carlsbad, CA) and enhanced by using an antibody solution containing 0.5 mg/mL of biotinylated anti-streptavidin (Vector Laboratories, Burlingame, CA). An Affymetrix Gene Chip Scanner 3000 was used to scan the probe arrays. Gene expression was calculated using the Gene Chip Operating software 1.2 (GCOS 1.2, Affymetrix).

Data Analysis

Raw data files for both array types (huMITOchip and HU 133 Plus 2.0) were transferred from the Affymetrix GCOS 1.2 software (signal and present call values) to the NIHLIMS database, then retrieved and analyzed using the MSCL Analyst's Toolbox (<http://abs.cit.nih.gov/MSCLtoolbox/>) and the JMP version 5.01 statistics package (SAS, Cary, NC). For comparison of the two array types, the 4,774 probe sets common to both arrays were analyzed as a single data set. Present calls for both were transformed to numeric values ($P = 1/M = .5/A = 0$) to facilitate numeric computations, and signal values were subjected to the median normalized log₁₀ transform, thereby compensating for any multiplicative changes in intensity from chip to chip.

Data Quality Control

The quality of the results was assessed by comparison of parameters (percentage present calls, scaling factors, rawQ, 3'/5' ratios) to historical values for our laboratory. To detect any possible outliers among the 12 chips, the transformed signal values were subject to a principal components analysis, a statistical technique for representing the between-chip variation of all 4,774 probe sets in a small number of "principal components" ordered by the

magnitude. The first three components were plotted and visually inspected. One array departed slightly from the remaining cluster but not enough to be considered an outlier. Subsequent analysis revealed that the automated gridding algorithm of the image file for this chip (dat. file) had failed in a portion of the image.

Results were compared between array types by computing the correlation of transformed signal values for the same sample run on each array. Correlations were also computed between chips of the same type (HU 133 Plus 2.0 or huMITOchip) within treatment groups. Differentially expressed genes were identified using a separate ANOVA for each chip type. Fold changes (treatment/control) and *p*-values for each probe set were calculated. Probe sets were marked as absent and discarded from the analysis if they did not receive at least two out of three present calls in the treatment or control group. Genes were declared differentially expressed if the false discovery rate (Hochberg & Benjamini, 1990) was calculated to be 10% or less and they exhibited more than a two-fold change in expression after IFN- γ treatment. A comparison of the fold change for differentially expressed genes was performed using a correlation plot.

Gene Expression Confirmation with Real-Time Polymerase Chain Reaction

The microarray data were selectively corroborated with real-time reverse transcription-polymerase chain reaction (RT-PCR) experiments (Raju et al., 2003). Total RNA was extracted from the six muscle cell cultures using the RNeasy kit from Quiagen according the manufacturer's instructions. In brief, the cells were dissolved in 350 μ L of lysis buffer and homogenized with QIAshredder mini columns. The RNA was eluted in 30 μ L of water and stored at -80°C . RNA purity and integrity were tested by measurement of optical density and by electrophoresis (1% agarose gel) using ethidium bromide staining. The PCR for the reverse transcription was performed in a Peltier Thermal Cycler PTC-200 from MJ Research (Watertown, MA) and optimized in pilot experiments. The cycle protocol consisted of 2 min at 50°C , hot activation for 10 min at 95°C , denaturing for 40 cycles for 15 s at 95°C and annealing for 1 min at 60°C . The cDNA synthesis was performed with SuperScript II Reverse Transcriptase from Invitrogen (Carlsbad, CA), according to the manufacturer's instructions. The resulting cDNA was stored at -20°C . For amplification, we used 0.5 μ L of cDNA in a 20 μ L reaction with 0.5 U of Taq DNA polymerase, 0.5 U of Taq antibody, 1.5 mM MgCl_2 and 0.2 mM dNTP in PCR buffer (all from Invitrogen) with 0.5 μ M primer mix. Taqman primers were obtained from Applied Biosystems (Santa Clara, CA) and the protocol used was the one provided by the manufacturer. The real-time PCR was performed using an Opticon II thermocycler (MJ Research, Waltham, MA) using six-carboxy-fluorescein (FAM)-labeled probes and specific primers (Applied Biosystems, Foster City, CA). Expression levels for six representative genes, STAT 1, HLA E, Toll-like Receptor 3 (TLR3), CXCL 9 (MIG), CXCL 10 (IP-10), and CXCL 11 (I-TAC), were tested by real-time PCR experiments in triplicate on the muscle culture samples prestimulation ($n = 3$) and poststimulation ($n = 3$) with IFN- γ . Glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) was selected to be the control for gene amplification based on previous experiences in our lab and its relatively stable expression in skeletal muscle (Touchberry, Wacker, Richmond, Whitman, & Godard, 2006). Expression of GAPDH on the huMITOchip and the HU 133

Plus 2.0 differed by 1% to 2% in controls and treatment. Fold change of microarray data with real-time PCR was calculated using Microsoft Excel

Results

Nuclear Probe Set Performance

To investigate the sensitivity of the selected nuclear probe sets, we looked at the within-group performance for the huMITOchip ($n = 6$) versus the HU 133 Plus 2.0 ($n = 6$). All the 4,774 nuclear probe sets on the huMITOchip were also represented on the HU 133 Plus 2.0 array, allowing a direct comparison of expression profiles using the two array types. After the log transformation of the genes in both sets of chips, the mean values were correlated for 4,774 genes ($r = 0.98$; Pearson's correlation coefficient), providing excellent concordance between the two types of chips. The wider scatter at the lower end in Figure 1 confirms established knowledge that very low gene expression levels are measured less precisely in microarray experiments than are higher levels of expression (van Haften et al., 2006). To determine specificity, the expression ratios (pre/poststimulation) of 4,774 genes that were represented on both arrays were compared and plotted. Of the 17 probe sets found to be significantly differentially expressed between the arrays, all showed agreement in sign and magnitude of fold change to within 40% error.

Hybridization Signal Intensity for Mitochondrial Genes

For the 37 mitochondrial genes represented in 51 probe sets, the absolute gene expression levels ranged from 0.1 to 3182 (see Table 1). Because of the differences in the expression levels for mitochondrial genes, we classified them into groups of high ($>1,000$), moderate (>100), low (>10), and extremely low (>0.1) expressors. IFN- γ stimulation for 6 hr did not result in statistically significant changes in any particular mitochondrial gene, considering a two-fold change and 10% false discovery rate (FDR), the criterion of significance.

Nuclear Gene Expression in IFN- γ Stimulated CCL-136 Muscle Cells

Gene expression of nuclear probe sets between the huMITOchip and the HU 133 Plus 2.0 array showed excellent concordance. Evaluation of the pre- and poststimulation signal intensity for six nuclear genes by huMITOchip microarray and PCR that were known to be modulated following stimulation with IFN- γ revealed comparable results (Table 2). All genes were screened at an FDR of 10% and with at least a twofold increase in expression after stimulation (Table 3). We identified 17 genes that were differentially expressed on at least one of the two array types. Among these, 6 were differentially expressed on the huMITOchip, 2 on the HU 133 Plus 2.0, and 9 on both. The set level of significance or the twofold cutoff contributed in most cases to a nonsignificant status in one or the other chip. The reduction of gene expression also contributed to lower significance values.

We divided our findings into tumor-related, muscle protein-related, apoptosis-related, and inflammation-related gene expression (see Table 3). Tumor-related genes included tumor necrosis factor receptor super family 14, the signal transducer and activator of transcription 1 (STAT 1), and colony-stimulating factor (CSF). Muscle protein-related genes included chloride intracellular channel 2 (CLIC-2). The altered genes related to apoptosis were

poly(ADP-ribose) polymerase 9 and 14 and apolipoprotein 6 (APOL6). The inflammation-related genes that were significantly altered included IL-18 binding protein (IL-18BP), α 2-macroglobulin (A2M), and the major histocompatibility complex class I E (HLA type I E).

To confirm that IFN- γ triggers the expression of IFN- γ -inducible chemokines in muscle tissue, we conducted quantitative RT-PCR experiments and found CXCL9 (10-fold), CXCL10 (3- to 10-fold), and CXCL11 (1.8- to 3-fold) to be upregulated (see Table 2).

Discussion

The goal in developing the huMITOchip was to design and test a focused array that would allow simultaneous investigations on nuclear and mitochondrial gene expression. This goal was achieved by carefully selecting probe sets that provided the greatest sensitivity in reflecting expression of nuclear and mitochondrial function. Using the mitochondrial Cambridge reference sequence assured access to an original, universally agreed-on mitochondrial sequence database. Selecting Swiss Prot-listed nuclear and mitochondrial proteins and matching them to NCBI Genbank-accessible gene sequences assured the best selection of already existing nuclear and mitochondrial gene probe sets in the Affymetrix data bank. In the final selection, 4,774 gene probe sets overlapped between the huMITOchip and the HU 133 Plus 2.0 array with an excellent correlation (Pearson's $r = 0.98$). All 17 differentially expressed genes behaved coherently on the two chip types. The gene expression profile that we observed was consistent with the reported findings on the effect of IFN- γ on skeletal muscle cells.

Exposure of CCL136 myotubes to IFN- γ reduced tumor necrosis factor- α expression and augmented the expression of poly(ADP-ribose) polymerase (PARP 9 and 14). IFN- γ stimulated the STAT1 pathway (signal transducer and activator of transcription 1) ($p < .0001$) and upregulated ($p < .0002$) the CSF-1. CLIC-2, which belongs to a family of soluble or membrane-bound chloride channel forming proteins, was upregulated ($p < .0001$) in the stimulated CCL136 cells compared to the control. IFN- γ regulates chloride channels and fluid transport in the lung, but the mechanisms involved in these functions are not clear (Chu, Blaisdell, Bamford, & Ferro, 2004). The importance of CLIC-2 in skeletal muscle needs to be further investigated.

APOL 6 expression was fourfold higher in cells treated with IFN- γ as compared with control cells. APOL 6 induces mitochondria-mediated apoptosis. Interleukin-18 binding protein (IL-18BP) was highly expressed after IFN- γ stimulation ($p < .0001$). It is a circulating antagonist of IL-18 and is expressed in type II skeletal muscle fibers but not in type I fibers. Further, we found α 2-macroglobulin, an early inflammatory marker, and HLA class I E, a cell surface marker, to be upregulated by as much as 10-fold ($p < .0001$). Both genes have been previously reported to be highly responsive to stimulation with IFN- γ (Jinbo, Sakamoto, & Yamamoto, 2001; Barrett, Gustafsson, Wang, Wang, & Ginder, 2004).

We conclude that the development of the first mitochondrial oligonucleotide microarray chip (huMITOchip) was highly successful. The observation that IFN- γ inducible chemokines are upregulated in the CCL136 cells after stimulation with IFN- γ confirmed

previous findings. The reproducibility of the model mechanisms between the huMITOchip and the HU 133 Plus 2.0, such as the stimulation of muscle cells with a proinflammatory marker, adds to early reliability and validity of the new gene array. The huMITOchip has great potential as a tool for mitochondria-related investigations in drug toxicity, disease, and symptom etiology studies.

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References

- Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nature Genetics*. 1999; 23:147. [PubMed: 10508508]
- Barrett DM, Gustafson KS, Wang J, Wang SZ, Ginder GD. A GATA factor mediates cell type-restricted induction of HLA-E gene transcription by gamma interferon. *Molecular and Cellular Biology*. 2004; 24:6194–6204. [PubMed: 15226423]
- Bhattacharjee M, Venugopal B, Wong KT, Goto YI, Bhattacharjee MB. Mitochondrial disorder, diabetes mellitus, and findings in three muscles, including the heart. *Ultrastructural Pathology*. 2006; 30:481–487. [PubMed: 17183762]
- Chu S, Blaisdell CJ, Bamford P, Ferro T. Interferon- γ regulates CIC-2 chloride channel in lung epithelial cells. *Biochemical and Biophysical Research Communications*. 2004; 324:31–39. [PubMed: 15464978]
- Gibson BW. The human mitochondrial proteome: Oxidative stress, protein modifications and oxidative phosphorylation. *International Journal of Biochemistry and Cell Biology*. 2005; 37:927–934. [PubMed: 15743667]
- Giordano A, Calvani M, Petillo O, Carteni M, Melone MR, Peluso G. Skeletal muscle metabolism in physiology and in cancer disease. *Journal of Cellular Biochemistry*. 2003; 90:170–186. [PubMed: 12938166]
- Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. *Statistics in Medicine*. 1990; 9:811–818. [PubMed: 2218183]
- Jinbo T, Sakamoto T, Yamamoto S. *Laboratory Animals*. 2001; 36:153–157. [PubMed: 11943079]
- Larkin JE, Frank BC, Gavras H, Sultana R, Quackenbush J. Independence and reproducibility across microarray platforms. *Nature Methods*. 2005; 2:337–344. [PubMed: 15846360]
- Latini A, da Silva CG, Ferreira GC, Schuck PF, Scussiato K, Sarkis JJ, et al. Mitochondrial energy metabolism is markedly impaired by D-2-hydroxyglutaric acid in rat tissues. *Molecular Genetics and Metabolism*. 2005; 86:188–199. [PubMed: 15963747]
- Maitra A, Cohen Y, Gillespie SE, Mambo E, Fukushima N, Hoque MO, et al. The human MitoChip: A high-throughput sequencing microarray for mitochondrial mutation detection. *Genome Research*. 2004; 14:812–819. [PubMed: 15123581]
- Manoli I, Le H, Alesci S, McFann KK, Su YA, Kino T, et al. Monoamine oxidase-A is a major target gene for glucocorticoids in human skeletal muscle cells. *FASEB Journal*. 2005; 19:1359–1361. [PubMed: 15946989]
- Raju R, Vasconcelos O, Granger R, Dalakas MC. Expression of IFN-gamma-inducible chemokines in inclusion body myositis. *Journal of Neuroimmunology*. 2003; 141:125–131. [PubMed: 12965263]
- Sherlock G. Of fish and chips. *Nature Methods*. 2005; 2:329–330. [PubMed: 15846357]
- Touchberry CD, Wacker MJ, Richmond SR, Whitman SA, Godard MP. Age-related changes in relative expression of real-time PCR housekeeping genes in human skeletal muscle. *Journal of Biomolecular Techniques*. 2006; 17:157–162.

- van Haaften RI, Schroen B, Janssen BJ, van Erk A, Debets JJ, Smeets HJ, et al. Biologically relevant effects of mRNA amplification on gene expression profiles. *BMC Bioinformatics*. 2006; 7:200. [PubMed: 16608515]
- Zeng X. The making of a portrait: Bringing it into focus. *Current Pharmacological Biotechnology*. 2003; 4:397–399.

Figure 1.

Mean differences in expression for 4774 probe sets represented on both the HU 133 Plus 2.0 (x-axis) and the huMITOchip (y-axis) were log-transformed and the linear regression was plotted (Pearson's correlation coefficient, $r = 0.98$).

Table 1

Mitochondrial Gene Expression as Measured by the huMitoChip

Gene ID	Gene Name (Replication Strand)	Mean Expression Pretreatment	Present/ Marginal/Absent Call	Mean Expression Posttreatment	Present/ Marginal/Absent Call	Percentage Change
MTTF_at	tRNA Phenylalanine (H)	1423.77	P/P/P	1527.5	P/P/P	7
MTRNR1A_s_at	12 S ribosomal RNA (H)	2066.37	P/P/P	2176.6	P/P/P	5
MTTV_at	tRNA valine (H)	2386.67	P/P/P	2636.93	P/P/P	9.5
MTRNR2A_at	16 S ribosomal RNA (H)	2944.67	P/P/P	3182.3	P/P/P	7
MTTL1_at	tRNA Leucine 1 (H)	1785.27	P/P/P	1849.25	P/P/P	4.5
MTTL_at	tRNA Isoleucine (H)	0.87	A/A/A	0.73	A/A/A	-22
MTTQ_at	tRNA Glutamine (L)	141.93	P/P/P	151.57	P/P/P	6
MTTM_at	tRNA Methionine (H)	67.73	P/P/P	77.97	P/P/P	14
MTTW_x_at	tRNA Tryptophan (H)	81.1	P/P/P	83.8	P/P/P	3
MTTA_at	tRNA Alanine (L)	2539.38	P/P/P	2586.43	P/P/P	2
MTTN_x_at	tRNA Asparagine (L)	71.87	P/P/P	90.3	P/P/P	20.5
MTTC_at	tRNA Cysteine (L)	10.33	P/P/P	16.33	P/P/P	37
MTTY_at	tRNA Tyrosine(L)	88.7	P/P/P	99.6	P/P/P	11
MTTS1_x_at	tRNA Serine 1 (L)	55.13	P/P/P	63.73	P/P/P	13.5
MTTD_at	tRNA Aspartic acid (H)	0.07	A/A/A	0.3	A/A/A	77*
MTTK_x_at	tRNA Lysine (H)	110.9	P/P/P	116.7	P/P/P	5
MTTG_at	tRNA Glycine (H)	1.07	A/A/P	2.47	P/P/P	43*
MTTR_at	tRNA Arginine (H)	1.93	A/A/P	3	P/P/P	36
MTTH_at	tRNA Histidine (H)	178.77	P/P/P	189.93	P/P/P	6
MTTS2_at	tRNA Serine 2 (H)	369.23	P/P/P	400.2	P/P/P	8
MTTL2_at	tRNA Leucine 2 (H)	22.3	P/P/P	26.43	P/P/P	16
MTTE_at	tRNA Glutamic acid (L)	343.23	P/P/P	405.5	P/P/P	16
MTT_x_at	tRNA Threonine (H)	164.13	P/P/P	187.13	P/P/P	12
MTTP_s_at	tRNA Proline (L)	125.2	P/P/P	152.3	P/P/P	18
MTND1_at	NADH Dehydrogenase subunit 1 (H)	1818.02	P/P/P	1942.65	P/P/P	6.5
MTND2_s_at	NADH Dehydrogenase subunit 2 (H)	1666.33	P/P/P	1725.87	P/P/P	3.5
MTCO1_at	Cytochrome c oxidase subunit 1 (H)	2393.05	P/P/P	2410.13	P/P/P	1

Gene ID	Gene Name (Replication Strand)	Mean Expression Pretreatment	Present/ Marginal/ Absent Call	Mean Expression Posttreatment	Present/ Marginal/ Absent Call	Percentage Change
MTCO2_at	Cytochrome c oxidase subunit 2 (H)	2754.7	P/P/P	2942.17	P/P/P	6
MTATP8_at	ATP synthase F0 subunit 8 (H)	2439.87	P/P/P	2642.033	P/P/P	7
MTATP6_at	ATP synthase F0 subunit 6 (H)	2378.63	P/P/P	2563.6	P/P/P	6
MTCO3_at	Cytochrome c oxidase subunit 3 (H)	2384.3	P/P/P	2612.33	P/P/P	8
MTND3_at	NADH dehydrogenase subunit 3 (H)	2488.65	P/P/P	2841.07	P/P/P	12.5
MTND4L_at	NADH dehydrogenase subunit 4L (H)	2597.7	P/P/P	2784.63	P/P/P	7
MTND4_at	NADH dehydrogenase subunit 4 (H)	2336.53	P/P/P	2452.87	P/P/P	5
MTND5_at	NADH dehydrogenase subunit 5 (H)	403.73	P/P/P	405.27	P/P/P	0.4
MTND6_at	NADH dehydrogenase subunit 6 (L)	45.83	P/P/P	58.3	P/P/P	21
MTCYB_at	Cytochrome b (H)	36.77	P/P/P	43	P/P/P	14.5

NOTE: Replication strand on mitochondrial genome: H = H-strand; L = L-strand.

* Statistically significant at the .01 level.

Confirmation of huMITOchip Results by Reverse Transcription-Polymerase Chain Reaction

Table 2

Probe Set ID	Gene Title	Inflammatory- Related Genes	Fold Change Pre-Post Stimulation	Fold Change by PCR	p-Value ^a	FDR ^b at 10%
209969_s_at	Signal transducer and activator of transcription 1, 91 kDa	STAT 1	10.1	4.2	.00001	0.0009
200904_at	Major histocompatibility complex, class I, E	MHC I, E	7.7	11.4	.00001	0.0107
206271_at	Toll-like receptor 3	TLR3	3.0	2.65	.00141	0.1380
203915_at	Chemokine (C-X-C motif) ligand 9	CXCL 9 MIG	9.0	10.4	.00581	0.2671
204533_at	Chemokine (C-X-C motif) ligand 10	CXCL 10 IP-10	3.5	9.15	.00097	0.1225
211122_s_at	Chemokine (C-X-C motif) ligand 11	CXCL 11 I-TAC	2.8	1.8	.02066	0.4060

^a p-value = level of significance for pre-post treatment group measured with the huMITOchip.

^b FDR = false discovery rate for huMITOchip.

Table 3

Genes significantly altered in CCL136 cell line following stimulation with IFN-gamma, found by huMitochip or HU133 Plus 2.0 array.

Probe Set ID	Gene Title	Gene Symbol	huMitochip	HU 133 Plus 2.0 Array
Tumor related				
209716_at	Colony stimulating factor 1 (macrophage)	CSF1	X	.
200887_s_at	Signal transducer and activator of transcription 1, 91kDa	STAT1	X	X
209969_s_at	Signal transducer and activator of transcription 1, 91kDa	STAT1	X	X
AFFX-HUMISGF3A/ M97935_3_at	Signal transducer and activator of transcription 1, 91kDa	STAT1	X	X
AFFX-HUMISGF3A/ M97935_5_at	Signal transducer and activator of transcription 1, 91kDa	STAT1	X	.
AFFX-HUMISGF3A/ M97935_MA_at	Signal transducer and activator of transcription 1, 91kDa	STAT1	X	X
AFFX-HUMISGF3A/ M97935_MB_at	Signal transducer and activator of transcription 1, 91kDa	STAT1	X	X
209354_at	Tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator)	TNFRSF14	.	X
Muscle related				
213415_at	Chloride intracellular channel 2	CLIC2	X	.
Apoptosis-related				
219716_at	Apolipoprotein L, 6	APOL6	X	.
241869_at	Apolipoprotein L, 6	APOL6	X	X
224701_at	Poly (ADP-ribose) polymerase family, member 14	PARP14	X	X
223220_s_at	Poly (ADP-ribose) polymerase family, member 9	PARP9	X	X
Inflammation related				
217757_at	Alpha-2-macroglobulin	A2M	X	.
200904_at	Mmajor histocompatibility complex, class I, E	HLA-E	X	X
222868_s_at	Interleukin 18 binding protein	IL18BP	X	.
Other				
231769_at	F-box protein 6	FBXO6	.	X