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Temporal Differential Gene Expression in Explanted Human Retinal Pigment Epithelial Cells at 0.5, 1.0, 3.0, 6.0, 12 and 24 Hours Post-Exposure to 1064 nm, 3.6 ns Pulsed Laser Light.

John W. Obringer
Martin D. Johnson

Laser and Optics Research Center, Department of Physics

United States Air Force Academy
Colorado Springs, Colorado 80840

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DEAN OF THE FACULTY
UNITED STATES AIR FORCE ACADEMY
COLORADO 80840

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This article, "Temporal Differential Gene Expression in Explanted Human Retinal Pigment Epithelial Cells at 0.5, 1.0, 3.0, 6.0, 12 and 24-hours Post-Exposure to 1064 nm, 3.6 ns Pulsed Laser Light," is presented as a competent treatment of the subject, worthy of publication. The United States Air Force Academy vouches for the quality of the research, without necessarily endorsing the opinions and conclusions of the authors. Therefore, the views expressed in this article are those of the authors and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the US Government.

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14. ABSTRACT
The use of laser light for targeting devices and weapons has dramatically increased the likelihood that personnel will be exposed to laser energy during military operations. Expanded medical, research, and industrial laser use may lead to excessive risk of exposure of researchers and technicians and also during commercial applications. Further, the nature and importance of the biophysical mechanisms of photon-tissue interaction at such pulse widths and irradiances are not understood at the fundamental cell and molecular level. A human in vitro model for assessing laser-light damage to tissue at the cell and molecular level is desirable for scientific, political and fiduciary reasons. We assessed the sublethal insult to human retinal pigment epithelial cells using a cadaver organ donor explant system for genes differentially expressed 30 min. and 1, 3, 6, 12, and 24 hours post-exposure using gene expression microarray technology (gene chip). It appears that pulses of laser light are sensed and markedly altered gene expression over time. The 120 pulses of 1064 nm light at 280 mJ per square centimeter appeared to induce the cells into cessation of cell cycling and a series of events that would lead to DNA repair, then DNA replication followed by the preparation for cell cycling. As expected the various genes assayed fluctuated in expression in all conceivable permutations.

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Temporal Differential Gene Expression in Explanted Human Retinal Pigment Epithelial Cells at 0.5, 1.0, 3.0, 6.0, 12 and 24 Hours Post-Exposure to 1064 nm, 3.6 ns Pulsed Laser-Light

ABSTRACT

The use of laser light for military and commercial applications has sharply increased the likelihood of personnel exposure to laser light during operations. The increased potential for human exposure highlights the fact that there is paucity of basic science at the cell and molecular level concerning the effects of laser exposure of human cells. Current safety standards are largely extrapolations of exposure limits using a minimal visible lesion endpoint in the Rhesus monkey retinal model. A non-animal model for assessing laser-light damage to tissue, particularly human, is quite desirous for obvious scientific, political, and fiduciary reasons. We assessed the sublethal insult to human retinal pigment epithelial cells using a cadaver organ donor explant system for genes differentially expressed 30 min. and 1, 3, 6, 12, and 24 hours post-exposure using gene expression microarray technology (gene chip). It appears that pulses of laser light are sensed and markedly altered gene expression over time. The 120 pulses of 1064 nm light at 280 mJ per square centimeter appeared to induce the cells into cessation of cell cycling and a series of events that would lead to DNA repair, then DNA replication followed by the preparation for cell cycling. As was expected the various genes assayed fluctuated in expression in all conceivable permutations. For example, up-regulated genes involved in the elimination of denatured proteins (UPP) provided strong evidence for the implication of intracellular oxygen-related damage indicating oxidative damage. This investigative approach also showcases a global methodology for characterizing environmental stressors on a living system via genetic profiling and marks the first use of human explants as an experimental model for assessing laser-induced bioeffects at the cell and molecular level temporally over a 24 hour time course.

BACKGROUND

The use of laser light for targeting devices, blinders and weapons has sharply increased the likelihood of aircrew and support personnel exposure to laser light during operations. The advent of the airborne and space based laser defense systems potentially threatens inadvertent exposure to noncombatants due to backscatter and angle of attack. Expanded laser research efforts also may lead to excessive exposure of researchers and technicians. The increased potential for human exposure highlights the fact that there are no scientifically based cell and molecular level safety standards for laser exposures at ultrashort pulse lengths. Current ANSI (American National Standards Institute) guidelines (revision in 2002) are extrapolations of exposure limits at longer pulse lengths or are based on a very limited number of data points. The great peak powers achieved at ultrashort pulse lengths suggest that the current standards may not be sufficient for protection. Additionally, safety standards are based on visible damage to tissues, and subsequent treatment of exposed persons ends when healing of such macroscopic damage (e.g., a retinal lesion) is complete. Consideration is not given to more subtle damage or

to potential long-term sequelae, which are yet undefined. This study addresses the possibility of more subtle sub-lethal and long-term effects of irradiation, which may become manifest long after treatment ends. This work will identify cellular and molecular changes in cells, which survive irradiation but are not obviously damaged (lesioned). Earlier work in this laboratory compels us to think that previously unsuspected long-term effects should be considered, to include cancer and delayed loss of visual acuity.

There is a significant void in the current understanding of laser-tissue interaction at the cell and molecular level. Ocular laser-light damage has been primarily assessed by determining the Total Intraocular Energy (TIE) in Joules to cause retinal Minimal Visible Lesions (MVL) in 50% of exposures (ED50) (Cain, et al., 1994; Toth, et al., 1996; Zuchlich, et al., 1994, 1993). This criterion is also used to establish the American National Standards Institute (ANSI) and AF Surgeon General laser safety standard. The ANSI standard is a Maximum Permissible Exposure (MPE) 10-times less than the ED50 for the MVL energy for that laser-light wavelength and pulse width. This standard has obvious problems in that the MVL is based on the resolution power of the ophthalmoscope and operator. Further, the standard completely ignores the sublethal longer-term consequences of laser-light irradiation. This is especially important since the MVLs are scored usually at one or at 24 hours postexposure. Some biochemical studies have investigated free radical formation in the melanosomes of the retinal pigment epithelial (RPE), which are hypothesized to lead to oxidative damage (Glickman, et al., 1996a, 1996b, 1995, 1993, 1992; Lam, et al., 1992). Belkin and Schwartz (review, 1994) state that there is no known mechanism to explain the subthreshold effects of laser irradiation on ophthalmic tissue. More recently, Hall, (2001) et al, demonstrated the production of fragmented DNA in bovine RPE cells exposed to femtosecond pulses or continuous wave of 800 nm laser light using single cell electrophoresis (Hall R.M., et al., 2001). To date there remains a paucity of basic research on the cellular and molecular damage response caused by laser light at any dosage to eye or skin tissue.

Previous work in our laboratory has yielded three significant discoveries. (1) Irradiation with visible (532 nm) laser light was found to be more effective in damaging cultured mammalian cells than an equal irradiance of near-infrared (1064 nm) laser light (unpublished). This finding is consistent with others indicating that shorter wavelengths do more damage at equivalent energies. (2) A tenfold increase in mutation rate in mammalian cells was observed after irradiation with multiple laser pulses at 532 nm (Leavitt, et al., 1997). This finding would indicate that human eyes lased with high energy 532 nm light may subsequently develop cancer. (3) A transient increase in the expression of certain intracellular proteins was observed in irradiated cells (unpublished). The function of most of these proteins in cellular damage, repair, or other responses to laser irradiation is unknown. However, matrix assisted laser-desorption ionization mass spectrometry identification of 7 protein spots recovered from on a 2D-PAGE gel resulted in 8 known proteins and 1 unknown being significantly altered by a sub-acute picosecond (ps) and nanosecond (ns) pulsed laser light exposure (Obringer, unpublished). The indication from this limited snapshot of the cellular protein-level response was that the cells had undergone some major perturbations to the electron transport system (energy production) and the cytoskeleton of the exposed RPE cells.

Using gene expression microarrays (GEM) with genes for DNA damage and repair as one possible cellular system to investigate, we investigated the effect of the 532 nm laser-light by repeating the approximate exposures used by Leavitt, et al. (1997) to attempt to validate the finding of mutagensis in mammalian cells as mentioned above, but by introducing the human RPE cell as the model. This has significance in that mutagenic events are considered the genetic precursors to the development of most cancers. It could also demonstrate the existence in human cells of a mechanism of mutagenesis by green light (532 nm), which currently is hypothesized as being the phenomenon of multiple photon absorption and frequency up-conversion. The next higher harmonic of 532 nm light is 266 nm light which is in the ultra-violet (UV) range. The mutagenic effects of UV-light are well characterized as it causes pyrimidine dimer formation in DNA, the genetic material of life. The physical phenomenon for the laser induced DNA damage was first proposed by Cao, et al. (1993) stating that "exposure of thymine and DNA to high-intensity 532 nm pulsed radiation from a Nd:YAG laser resulted in the cyclobutylpyrimidine dimers, which were measured by the method of high performance liquid chromatography." They went on to suggest that the photochemistry was initiated by two-photon absorption by biomacromolecules. Their observation was further validated by Konig, et al. (1996), who observed pulsed beams at wavelengths less than 800 nm are capable of damaging cells through two-photon absorption. Other investigators have seen more gross forms of DNA damage from laser light such as sister chromatid exchanges (presumably the result of damage repair) from radiation at 632 nm (Quero, et al. 1997) and 810 nm light caused frank breakage of the DNA molecule (Both, et al.1990, Shafirovich, et al. 1999). Previous work in our laboratory has yielded molecular biomarkers for DNA damage in a human liver cell reporter-gene system with the induction of p53 after exposure to mode locked 1064 nm, 37 picosecond (ps) as well as 3 nanosecond (ns) pulsewidth light (Obringer et al. 2000, Obringer, et al. 1999a) and the induction of the proto-oncogene *fos* after 532 nm, 37 ps exposure (Obringer, et al. 1999b). Therefore, we further explored these and other bioeffects of laser exposures of the above and other parameters in human RPE and skin cells using DNA microarray technology.

The recent development of DNA microarray or GEM technology now enables researchers to qualitatively and quantitatively examine the differential expression patterns of thousands of genes in one experiment (Hubin, et al. 2001, Schena, et al., 1996, Schena, et al, 1995). As the literature on laser induced bioeffects on the cells of the retinal pigment epithelium at the cell and molecular level is virtually nonexistent, there is an obvious need for basic science knowledge. DNA microarray methodology opens an entirely new window for examining the perturbation of retinal tissue by laser exposure at the genetic level. By examining the differential gene expression patterns one can gain insight into the physiological state of the cells and, thereby, deduce the type and amount of damage/perturbation the tissue has undergone. A conceptual overview cartoon is presented in Figure 1. This strategy has proven successful in several other applications such as the investigation into human aging where mitotic misregulation seems to play a role (Danith, et al., 2000), *Drosophila* developmental regulation (White, et al., 1999), murine response to caloric restriction and age retardation (Lee, et al., 1999),

transcriptional response of human fibroblasts to serum (Iyer, et al., 1999), and gene expression variation in human gliomas (Zhang, et al., 1997) to illustrate a few examples..

MATERIALS AND METHODS

Explant procurement and processing: General overview (Figure 1)

Tissues were received as a tissue donor gift through the Rocky Mountain Lion's Eye Bank who accomplishes all of the donor consent paperwork. Posterior globes of both eyes were harvested usually no later than 8 hours post time of death and put into a 50 ml vial with approx. 25 ml of buffered saline. The tissue was transported directly to tissue culture lab where the vitreous humor and retina were mechanically removed. Then the RPE still attached to the sclera were cut into 3-5 mm square pieces. The pieces were then placed into 96 well microtiter plates (1 per well) with 150 microliters (ul) of the media (DME/F12 with 10% FBS plus antibiotics) and cultured at 37 degrees C in 5% CO2 until re-plated for exposure. In a fresh 96 well plate the pieces were placed RPE side up centered in the well, in 50 ul media (just covers the explant) to be exposed. Explants were kept at 37 degrees until they were transported in a pre-warmed insulated box to the laser lab and exposed at room temperature in the plates on an X-Y translation stage one well at a time as quickly as possible to minimize temperature fluctuations then returned to the incubator after stereoscopic examination and the additional 100 ul of warm media. At the desired time post exposure, RPE was mechanically removed from the sclera and collected in microcentrifuge tubes, labeled and frozen at -65 degrees C. Samples were shipped frozen to the vendor with approx. 10 lbs of dry ice via overnight delivery. We accepted donors age 65 years or younger, either sex, with no mitigating ocular or retinal pathology such as glaucoma, diabetic retinopathy, retinitis pigmentosa, etc.

Donor:

The RPE tissue donor was a 34 year old Caucasian, brown eyed, male that died of a myocardial infarction. No ocular pathologies were noted. From the time of death until tissue harvest was 6 hrs. and exposure was at 47 hrs.

Explant preparation:

Globes were removed 6 hr., 10 min. after death and stored for transport in neutral saline or media. The globes were removed from the transport liquid and processed for experimentation. Muscle and fatty tissue were removed from the outer globe surface using scissors. The anterior segment was removed using a circumferential cut through the *pars plana* with iridectomy scissors. The globe was then hemisected with scissors passing through the edge of the optic disc. The optic nerve and disc were removed and the retina was peeled away from the RPE using fine forceps. The sclera with retinal pigment epithelium attached was cut into square pieces approximately 3 mm on a side using a number 10 Propper carbon steel surgical blade. Each scleral piece was placed RPE side up, centered, in Falcon 96-well plates with in 100 ul media containing

streptomycin and penicillin. Plates were labeled and wells labeled as to which eye the explant was derived from.

Laser: Equipment Used

Laser (Nd:YAG)	Coherent, model Infinity 40-100, serial number 4363
Power Meter	Molectron OM4001 power meter, serial number 136C
Detector Head	J50 Detector Head, with diffuser, serial number 1518B
Shutter	nmLaser model LS055S3W8
Shutter Controller	nmLaser model CX2450
Velmex XY Stage	model NF90-2

Figure 2 shows how the pulses were directed into each well of a 96 well microtiter culture plate. After a well was exposed to the laser, an x-y translation stage was used to bring the next well into position. A shaping lens was placed in the beam to match the beam diameter with the 6-mm diameter of a well entrance. Burn paper was used to verify that the beam neither under nor overfilled the cell. Prior to testing, a dish filled with water was placed on the x-y stage below the final turning mirror, and the angle of the mirror was adjusted until the reflection of the laser beam off the water's front surface returned along its incoming path. This ensured the pulses entered the wells at a ninety-degree angle with the horizontal.

Pulse energy was determined by placing a power meter on the x-y translation stage (the site of target exposure) and dividing the measured average power by the pulse repetition rate. This method was considered adequate since pulse-to-pulse energy typically varied less than 10%. The beam profile is a "top hat" with less than 5% variation across the wave front.

Laser-light exposure:

The explants were initially plated in flat bottomed 96 well microtiter plates and maintained in RPE growth medium until exposure. The diameter of the wells is 6 mm so the laser beam was focused to fill the well with light. Immediately prior to the experimental treatment the scleral/RPE sections were re-plated one per well in a fresh 96 well plate, RPE side up, centered in each well with 50 microliters of warmed fresh growth medium to reduce the amount of light absorbed by the medium and ensure an equal amount of medium in each well. Plates were kept in a 37 degrees C incubator until just prior to dosing. Then the plates were transported to the laser suite in an insulated warm box and then positioned onto the x-y translation stage and exposed in a sequential fashion to the dose listed below. The measured temperature change of the fluid in the wells during the three minutes required for the exposure process was approximately one degree C. Control explants were treated identically, except they were sham exposed with the laser beam blocked. Explants from each eye were approximately equally represented in each sample. The explant containing plates were then returned to the 37 degree C, 5% CO2 incubator after the addition of 150 microliters of room temperature medium.

Laser-light exposure:

For procedures see USAFA-TR-2004-01. The table below contains the exposure parameters for the experiment reported herein.

Treatment	**N4HX**
Wavelength (nm)	**1064**
Average Power (mW)	**2800**
Pulse Energy (mJ)	**280 ± 1 mJ**
Pulse Length (FWHM)	**3.6 ns**
Total Incident Energy (mJ)	**3360**
Peak Power (W)	**77.8×10^6**
Fluence (mJ/cm^2)	**594**
Exposure Laser Repetition Rate (Hz)	**10**
Beam Diameter ($1/e^2$)	**6 mm**
Irradiance (kW/m^2)	**99.0**

Total incident energy (TIE) is defined as the amount of laser-light energy that was delivered to the 6 mm well containing the RPE explants. Abbreviations: nm-nanometer; m-meter, mm-millimeter, ns-nanosecond; mJ-milliJoule; mW-milliWatt; FWHM-Full Width Half Max; Hz-Hertz; sec-second; W-watt; e-natural log.

Laser exposure of Human RPE Explants

The Nd:YAG laser light exposure regimen was based on empirical data (not shown) that established cell viability after a range of laser exposures. The exposure described above for treatment N4 was calculated to be 9.9 k J/m^2 which is approximately 39% of the MVL value and approximately three times the MPE for the pulse width and wavelength considered (Sliney and Wolbarsht, 1980 and ANSI Z136.1-2000 Table 5a).

The cells were exposed to either 1) sham exposed to no laser-light (beam blocked upstream), or 2) 120 pulses of 1064 nm visible laser-light. Each pulse containing 280 mJ (on average) of energy was delivered to a microtiter plate well 6 mm in diameter containing 50 microliters of medium. The 1064 nm wavelength was chosen of the extreme damage possibilities of this common laser wavelength, and, more importantly, so as to provide a basis of comparison to the previous genomic experiments and subsequent genomic, proteomic and lipomic investigations using explants and 1064 nm laser-light exposures. See Figure 1 for a general overview of the experimental procedures.

Exposed RPE collection

After the prescribed time post-exposure the RPE tissue was mechanically separated from the sclera using a sterilized forceps and probe, and collected in a pre-chilled 1.5 ml tube. The tubes were then immediately frozen and stored at –65 degrees C until further processing. 7 samples were collected and designated N control as the unexposed control sample and 6 experimental samples labeled N30 collected 30 minutes post-exposure, N1 collected 1 hour post-exposure, N3 collected 3 hours post-exposure and so on. The tissue was overnight express shipped to Lofstrand Labs Limited, Gaitherburg, MD for mRNA isolation and differential gene expression (Atlas 1.2 Microarray, Clontec) analysis as described immediately below (personal communication, Jerry Dathe)

Oligonucleotide Microarray :

Materials Provided — 12 samples containing human explants of retinal pigment epithelial tissue (N30, N1, N3, N6, N12, N24 and N control for each experimental)

RNA Purification

RNA extraction was carried out on all samples using TRIzol reagent (Invitrogen). Extraction was performed per protocol in a total volume of 8ml of TRIzol with the addition of glycogen (400μg) to compensate for the small amount of starting material. Samples were extracted with 1.6ml of chloroform and then spun at 12K rpm in an HB4 rotor at 4°C for 15 minutes. Organic phases of the extractions were saved and stored at -70°C for possible future study. Samples were precipitated with isopropanol and rinsed with 75% ethanol (both 12K rpm in an HB4 rotor at 4°C for 10 minutes). Approximate concentrations of the samples were 40-50 ng/μl as determined by ethidium bromide agarose plate visualization against known standards. All total RNA samples were then DNase treated with the DNA-free Kit (Ambion) to remove any genomic DNA (gDNA) contamination (per protocol: 2 units DNase I; 37°C for 1 hour). PCR was used to check for presence of gDNA, using primers that were specific for GAPDH (5'-TGCMTCCTGCACCACCAACT-3' and 5'-YGCCTGCTTCACCACCTTC-3') and β-actin (5'-TYGTGATGGACTCCGGWG-AC-3' and 5'-CRCCAGACAGCACTGTGTTG-3'). PCR was performed using Ready-To-Go PCR Beads (Amersham Pharmacia), 1μl RNA and 25pmol of each primer, with an annealing temperature of 50°C for 30 cycles. There was evidence of gDNA contamination after the first DNA-free treatment, so another DNA-free treatment was performed (3 units DNase I; 37°C for 1 hour). Subsequent PCR showed no evidence of gDNA contamination.

Probe Synthesis

Probe synthesis was performed using the Atlas SMART PCR Probe Amplification Kit for the Atlas Human 1.2 Array (both BD Biosciences/Clontech). Due to the number of arrays (4) versus the number of samples (6 + 1 control), the samples were labeled and arrays hybridized in the following groups: 1, 3, 6, control and 12, 24, 30, control. 3.5μl of total RNA was used along with the Human 1.2 CDS primer and the SMART II oligo

for first strand synthesis using Powerscript RT (BD Biosciences/Clontech). PCR was then performed per protocol using 5µl of the first strand reaction, the kit PCR primer and Advantage 2 polymerase mix (BD Biosciences/Clontech). 5µl of the cDNA samples were run on an agarose gel to check yield. The cDNAs were then purified using NucleoSpin columns per the SMART Probe Purification Kit protocol (BD Biosciences/Clontech) with a final elution volume of 50µl.

Probe Labeling

The Atlas SMART PCR Probe Amplification Kit (BD Biosciences/Clontech) was used to label 33µl of each cDNA probe. Labeling was performed using StripEZ dNTP mix (Ambion) and ^{33}P-dATP (PerkinElmer Life Sciences). After the labeling reactions were complete, the radioactive probes were purified using NucleoSpin columns per the SMART Probe Purification Kit protocol (BD Biosciences/Clontech) with a final elution volume of 100µl. 2µl of each probe was then counted in 5ml of scintillation fluid for 1 minute to determine the cpm for each probe.

Array Pre-hybridization, Hybridization and Washing

Pre-hybridization was carried out with UltraHyb solution (Ambion) using 15ml per array in individual heat seal bags. The solution was preheated at 68°C and then 15ml was added in with each array. The bags were sealed and then pre-hybed at 42°C with agitation for a minimum 30 minutes. 4µl of Human Cot-1 DNA was added to each of the probes. The probes were then boiled for 2 minutes and put on ice for 2 minutes. The probes were spun down and added to the heat seal bags. The bags were re-sealed, gently mixed and the arrays then hybridized overnight at 42°C with agitation.

The four arrays were washed together with ~300ml of high stringency wash solution (0.1X SSC + 0.1% SDS) at 42°C with agitation for 15 minutes. The wash solution was discarded and a second was using fresh solution was performed. The arrays were then sealed in plastic wrap and exposed to SR (super resolution) phosphor screens (Packard Instrument) overnight at room temperature. Two arrays were exposed to one screen.

Array Imaging and Analysis

Each screen was imaged using the Cyclone Storage Phosphor System (Packard Instrument). A medium carousel was used with a resolution of 600 DPI. Images were then analyzed using AtlasImage software (BD Biosciences/Clontech).

Array Stripping

The hybridized arrays were stripped using StripEZ Probe Degradation Buffer and StripEZ Blot Reconstitution Buffer per the StripEZ RT Kit protocol (Ambion). 40ml of each buffer were used to strip all 4 arrays simultaneously. Efficiency of stripping was checked by phosphorimaging. If necessary, a second stripping was performed using 60ml of each buffer. Stripped blots were sealed in plastic wrap and stored at -20°C.

RESULTS

The results (Appendix A) of a gene expression microarray are expressed in a ratio of expression for one gene in the control versus the same gene in the experimental samples. For example, if gene YFG is expressed four times greater in the treated cells than in the sham exposed controls, it would show a fold change of positive four (4) in Appendix A that functionally means that gene YFG mRNA was found in 4 times greater concentration in the treated cells than in the controls. Thus, we conclude that the treatment induced the genetic expression of gene YFG four times greater in the experimentally treated cells than in the shame treated cells, presumably in response as the biological effect of the treatment. Conversely, if the YFG mRNA is 4 fold less in the experimental sample that in the control then a value of 0.25 is calculated. In the context of understanding the significance of fold change or fold induction of a gene, the analysis software calculates a 95% confidence level of fold change for each experiment.

Appendix A presents the most pertinent genes listed ordered by gene code found in the far left column. To help clarify the interpretation of this appendix the following heading explanations are offered. Gene code: the code name, listed alpha-numerically, of the gene being probed. Hours Post Exposure Ratio: The adjusted intensity of the control is compared to the adjusted intensity of the experimental to determine the expression ration. The adjusted intensity is determined by subtracting background intensity from the signal from the probe spot intensity. Control probe sets have been deleted from the data set in Appendix A. All signals in Appendix A used to calculate the ratios have passed the quality control standards established by the manufacturer. The internal controls are used by Clontec to calibrate the array and as quality control elements. The experimental adjusted intensity value divided by the control adjusted intensity determines the fold change based on the comparative signal strength of the control RPE sample as compared to the experimental. **This is the ratio of change value that is used as the endpoint value, and for further analysis in the interpretation of the differential gene expression microarray results for the designated genetic elements listed under "Gene code."** The ratios shown in Appendix A are listed in a column indicating the amount of time in hours the sample was collected post-exposure to the laser-light treatment. Protein/gene: a brief description of the gene or protein that is represented in the probe set. The appendix obviously contains only a portion of the total number of elements probed (1176) and only those whose absolute fold change was at least 2.0 or higher that has been calculated to be at or above the statistical significance of 95%.

An arithmetic tabulation of the data in Appendix A yields the observation that the number of RPE mRNA that was at or above 2.0 fold change in the 1176 probe elements on the GEM varied with the time post-exposure as the table below indicates.

TIME (hr)	0.5	1	3	6	12	24
TOTAL # +/- 2 FOLD:	151	222	252	295	503	210

% of TOTAL ASSAYED:	13	19	21	25	43	18
# UP-REGULATED:	99	94	132	131	291	109
# DOWN-REGULATED:	52	128	120	164	212	101
% UP-REGULATED:	66	42	52	44	58	52

In summary, the greatest number of significant changes in gene expression was in the 12 hours post-exposure sample and in the up-regulated direction. The same can be said across all samples in that the trend was in the up-regulated direction, but not dramatically. The greatest magnitude of change for single genes was also up-regulation with several above several orders of magnitude fold change.

Further inspection of the data indicates that the fold change (ratio) varied as widely as several orders of magnitude for certain genes and that the same genes fluctuated in direction of their expression over the 24 hour time course sampled. Several genes were differentially expressed in 5 of the 6 samples, but in different patterns. Almost every conceivable permutation of expression patterns could be found ranging from a single differential expression to multiples varying by several orders of magnitude in the same or opposite directions.

DISCUSSION

Perhaps the most obvious, yet striking, observation is the affected genes' expression patterns fluctuation of the 24 hour, six sample time course in almost all conceivable patterns. This observation is certainly not intuitively surprising, but can be visualized and validated in Appendix A by gene and upon closer inspection by gene clusters. Selected genes from Appendix A will be reviewed as to the physiological function and/or biological marker for which they are known whenever possible. Figure 2 illustrates several of the most differentially expressed genes in both the positive and negative direction respectively. Figure 3 briefly capsulates the functional genomics of the differential gene expression profiles.

Turning our attention to the collection of differentially expressed genetic elements extracted from Appendix A whose function (at least one) and/or association is known, we will attempt to develop a few example analyses of cellular response 30 min. to 24 hours post exposure to the perturbation caused by infrared laser-light exposure. It is obvious that pulses of laser light are sensed and markedly alter gene expression in the model cells. The cellular physiological pattern that is evident from the analysis of the differential gene expression patterns is fundamentally that the treated cells are varying gene expression of the genetic programs toward cellular repair and recovery. In the discussion to follow a few of the more significantly altered physiological systems that appear to be perturbed by the above laser exposure will be discussed in greater detail. Note: The number in [brackets] following a gene name indicates the fold change in that gene's expression and the designation in (parentheses) indicates the alpha-numeric gene code in Appendix A.

Macromolecule Trafficking

As mentioned, several physiological systems appear to be induced at the transcriptional level. Not surprisingly, some processes for protein degradation were up-regulated as compared to the levels of the sham-exposed controls indicating a increase in protein trafficking. Most notably up-regulated were the genes for the enzymes essential in the ubiquitin-proteoasome pathway (UPP) shown to be up-regulated in response to oxidative stress in eye tissue (1). An example was ubiquitin (G11), expressed at 0.5 at 30 min., 3.3 at 1 hr., baseline at 3 and 6 hrs., and below normal levels at 12 and 24 hours. Similarly, ubiquitin-conjugating enzyme E2 (D05a) showed a two-fold up-regulation at 12 hrs. as well as ubiquitin-conjugating enzyme E2H10 (A09k) [15]. Also up-regulated was HSP-70 (F06b) approximately 3 fold at 1, 3, 6 hours and down regulated at 12 and 24 hours. Also, HSP-related, protein 6 (F03a), heat shock-related 70-kDa protein 2 (F04a), heat shock 90-kDa protein A (F04b), and HSP 40 (F02b) showed a similar pattern which is intuitively satisfying considering what is known about the physiology of these genes. In the results presented in USAFA-TR-2004-01 it appeared that the cells at the 12 hour point had indeed up-regulated the protein degradation machinery for cellular debridement; while the snapshot we viewed at 24 hours post exposure in USAFA-TR-2003-03 showed the contrary reflected the transcriptional rebound (return to homeostasis) of a system having possibly previously been induced (as described here) to deal with protein damage. These results are largely concordant with the results of this temporal study. As discussed in the previous publication, this conjecture is corroborated by Lykins, et al. (2002), reporting that when epithelial cells were exposed to a similar laser laser-light treatment and examined 12 hours post-exposure at the protein level, the ubiquitination systems was markedly up-regulated implying that transcription of this system is early in the post-exposure gene-expression cascade. Also note that in USAFA-TR-2004-01, HSP 70 is up-regulated >3-fold as in this report. As HSP 70 facilitates the removal of injured proteins by ubiquitin-mediated proteasomal degradation arguing for the prior activation of the UPP system despite the current state of expression of the above mentioned ubiquitin-activating enzyme E1. Furthermore, HSP 70 acts downstream to ubiquitination and is regarded as a chaperone driving a multi-protein degradation complex (2). This notion is also supported by the up-regulation of a proteasome activator gene (F13k) over two-fold at 1 and 3 hours followed by a two-fold down-regulation at 12 hours as well as a proteasome inhibitor (F14k) with a two-fold decrease at 12 hours also.

Proliferation

In the context of cell cycling, the gene expression profile would indicate that the cells had ceased proliferation prior to 12 hours post-exposure, but showed strong signals for the genetic preamble for its resumption at 12 hours. For example, cyclin A1 (A11h) [7], cyclin E2 (A14g) [5], Cyclin K (A13g) [4], G1/S specific cyclin D1 (A03h) [38], cyclin-dependent protein kinase 2 (A03i) [7], cell division protein kinase 9 (A03j) [12], cell division protein kinase 6 (A05j) [5] and cyclin D binding Myb-like protein (A03m) [4] were all up-regulated at 12 hours only while cyclin-dependent kinase 4 inhibitor (A03k) was down-regulated [0.5] at 1 hour and increased to 7-fold over the control at 12 hours post-exposure. Cyclin-dependent kinase 4 inhibitor (A04k) was also up-regulated [3] at 12 hours. Numerous other cell-cycle genes like cyclin H (A08h) [24], cyclin-G associated kinase (A08j) [11] and proliferating cell nucleolar antigen P120 (A09l) [149] were also

differentially regulated largely consistent with the pattern mentioned above. The gene expression pattern of the lased cells indicates the cells had halted proliferation, but were preparing to resume cycling at least at the 12 hr. point, possibly after recovery of whatever perturbation they may have suffered such as oxygen-related stress.

Oxygen-related Metabolism

Intracellular reactive oxygen species (ROS) has long been known for disruption of cellular function by the oxidative alteration of a whole host of biologically significant macromolecules. The over 2 and 3 fold at 1 and 3 hours respectively induction of the thioredoxin reductase gene (F09b) flagships the lased tissues' response to this laser treatment closely followed by a 2 fold increase in glutathione S-transferase (F13b). Note that both of the above genes were expressed at below basal levels at the 12 hour point as well as glutathione S-transferase A1 (F14b) [3] when cell cycling was apparently resuming as discussed above. Thioredoxin has been definitively shown to be up-regulated by oxygen stress and is a key component in the anti-oxidant defense system as well as glutathione. Other genetic indicators of ROS in the exposed tissue were the expression up-regulation of the following genes: HSP 70 (F06b) [3.0], cytochrome P450 (C06k) up-regulated as much as 8-fold until the 12 hour point [0.4]. Cytochrome P450 IIC9 (F11a) was also upregulated over 4 fold at the 3 and 6 hour points. Ironically, it has been found that cytochrome P450 (CYP) over-expression itself generates large amounts of ROS via the induction of CYP-dependent monooxygenases (3). Sapaone, et al. (3) further hypothesized that long-term CYP induction can have a co-carcinogenic and/or promoting potential. Another corroborating indicator of O stress is the up-regulation of thiol specific antioxidant protein (F07a) 6 plus fold at the 1, 3, 6 hour points and cytosolic superoxide dismutase 1 (F07b) over 2-fold up-regulated at the same time points and down-regulated [0.4] at the 12 hour point. Overall, it appears that it can safely be said that laser-light exposure alters oxygen-metabolism in the context of macromolecule damage due to oxidative damage, most likely due to ROS production. Overall, it appears that the cells were on the mend and had up-regulated protective/restorative genetic responses at the transcriptional level.

Translation Initiation and Ribosome Reconstruction

In USAFA-TR-2004-01 undoubtedly, one of the most striking results of this investigation is the number of genes up-regulated that have to do with the cell's translational machinery. Intriguingly, of the first 100 up-regulated genes 37 or 37% of them had to do with the some aspect of translation and most with the reconstruction of the ribosome and the trend continued throughout the remainder of the up-regulated genes. That finding reflected an amazing metabolic investment in the machinery for protein synthesis, and also indicates that the ribosome may be the primary target for damage from this treatment. From that observation we speculated that the ribosome may constitute a sub-organellular chromophore for this regime of laser-light exposure. Again, in this work we observe an up-regulation of the genes involved in ribosomal reconstruction especially at the 24 hr. point, but not nearly to the levels seen with the 532 nm, 12 hr. post-exposure experiment described in USAFA-TR-2004-01. Some indicators genes are several

ribosomal kinases (B03h, B03l, B04l, B05l) and 40S ribosomal protein S19 (A07l) up-regulated nominally 10-fold at 12 hrs.

Transcription

Among the genes whose expression was altered at or above the 2.0 fold level, those involved in transcription would appear to have been globally up-regulated. Numerous oncogenes and a plethora of other classes of transcriptional factors/regulators were differentially regulated throughout, but especially at the 12 hr. point. These constitute transcriptional factors that are associated with the virtually every aspect of cellular metabolism to include several homeotic genes.

Inflammatory Response

Also prevalent in the gene expression profile of the laser-light treated cells was the dramatic fluctuation of expression of a number of the immune system genes especially having to do with the inflammatory response. A few examples are the expression patterns of the genes coded B04d, B05d, B06d, C05a, D08b, and the list goes on. This reaction may provide an alternate explanation for the appearance of the late (24 hr) onset lesions in the primate MVL studies reviewed in the background section.

DNA Repair

Although it is known that the DNA damage/repair genes are always "on," it is interesting to note that several were significantly up-regulated in the exposed samples. For example DNA lyase (AP endonuclease 1) (C08m) was up-regulated approx. an order of magnitude at 1 hour and stayed turned on until12 hrs where it dropped to 0.2. Photolyase (C06m) was up-regulated 10-fold at 1-3 hrs and –5-fold at 12 hrs. Roughly the same can be said for xeroderma pigmentosum group CC repair complementing protein p58 (C08n). Whereas, the DNA repair protein (D01a) was down-regulated approx. 2-fold at 3 and 6 hrs and up to 8-fold at 12 hours. A similar pattern was seen with GADD 45 (D01b). The GADD 153, growth arrest and DNA-damage inducible (C12j), gene was up-regulated nearly 6 fold at 6 hours and decreased to 0.4 at 12 hours and the DNA mismatch repair protein PMS2 (C10n) showed a greater than 2-fold up-regulation at 6 hours. Overall, it appears that DNA repair was initiated at about 1 hour and continued until some time after the 6 hour point and was followed by DNA replication as the reader will note in the following section.

DNA Replication

In addition to the abovementioned genes functioning in DNA repair, numerous other DNA metabolism genes were turned-on at the 6 to 12 hour points indicating the initiation of DNA replication. For example, replication protein A 70-kDa (C07l) was up-regulated 20-fold at 1 hr and 129-fold at 3 hours. Another few examples are replication factor C 3-kDa (C12l) up-regulated 5-fold at 6 hours and down 5-fold at 12 hours post exposure and DNA ligase (C12m) up-regulated over 2 fold at 1 hour, nearly 3 fold at 6 hours and

down-regulated 5-fold from baseline at 12 hours. Also over expressed are such genes as replication protein A 70-kDa subunit (C07l) up-regulated 21-fold at 1 hour and a whopping 129-fold at 3 hours and then to baseline. This protein is also essential in DNA repair as it binds single-stranded DNA. Various DNA replication-licensing factors (C03m, C01m and C02m) were also up-regulated. Overall, it appears that the cells went into a repair condition then followed by at least the preamble for DNA replication and cell cycling, especially since the apoptotic response was quelled genetically.

Apoptosis

Perhaps the most telling apoptotic gene to be expressed is DAD1 (defender against cell death) (C03k) up-regulated over 5-fold at 3 hours. Despite genetic indications of the induction of cell suicide, DAD1 and other genes appeared to have quelled the apoptotic response of the lased cells. This statement is further supported by the expression of the inhibitor of apoptosis protein 1 gene (C09k) up-regulated 5-fold at 1 hour, as well as the apoptosis regulator bcl-x gene (C09i) and bcl-w (C08i) expressed over 2-fold and 6-fold, respectively, above the control at 1 hour and 6 hrs. Apoptotic protease activating factor 1 (C08j) was turned up 3-fold at 3 and 6 hrs then fell off an order of magnitude at 12 hrs.; whereas, TRRAP protein(C09e), a strong indicator of apoptosis induction, was down-regulated to 0.4 at 6 and 12 hrs.

Summary

Taken together, global perturbations to the cellular metabolism was evidenced to have occurred during the 24 hours post-exposure time-course given the numerous cellular systems affected. The nanosecond pulses of 1064 nm light appeared to induce the cells into cessation of cell cycling and a nearly global up-regulation of transcription include the genes involved in macromolecular trafficking, proliferation, oxygen-related metabolism and various transcription-related regulatory genes. The transcriptional up-regulation pattern of several key genes involved in the UPP system indicates cellular debridement. Additionally, there appears to be little doubt that this laser-light treatment had a major impact on the oxygen-related metabolism of the cells by the production of ROS. Generally, it appears that the cells received and "sensed" damage of several types (e.g. DNA damage, ROS creation/interaction) and began on the path to recovery in the absence of the commitment to apoptosis. DNA repair appears to have been induced between 1 and 6 hrs, DNA replication from 6 to 12 hours and cell cycling commencing post-12 hours with the UPP system functioning in the background especially in the early time periods. Further work will indicate if the cells were capable of restored physiological function without long term detrimental sequelae.

Closing

As a closing comment we offer the following observation: The gene profile seen in this experiment is very reminiscent of those seen in the experiments reported in the last several USAFA-TRs published by us. Therefore, this work represents confirmation of our previous work and validation of differential expression microarray analysis as a

viable approach for understanding the physiological decisions the cell is making at the genetic level in response to laser-light exposure. Also of note was the apparent attempt of the RPE cells to resume their normal physiology, or were progressing to cancer, as evidenced by the following gene expression patterns. The genes retinoblastoma-like protein 2 (A09a) [15], retinoblastoma-associated protein 1 (A11a) [114], retinoblastoma binding protein (D11l) [4], retinal guanylyl cyclase 2 precursor (C03c) [2], recoverin (C07c) [0.3, 0.4, 0.4, 2] and retinoic acid receptor epsilon (C14f) [3] all showed marked differential expression. Additionally, neuropeptide Y receptor type 1 (D12b) was up-regulated 4-fold at 12 hrs. as seen in previous experiments and should strongly be considered as a possible therapeutic agent given it's physiologic role.

Acknowledgements

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BIBLIOGRAPHY

Belkin M, Schwartz M: Ophthalmic effects of low-energy laser irradiation. Survey of Ophthalmology 1994;39:113-121.

Cain CP, Noojin GD, Hammer DX, Thomas RJ, Rockwell BA: Artificial Eye for in vitro experiments of laser light interaction with aqueous media. Biomedical Optier 1996;F-30:1-20.

Cain CP, Dicarlo CD, Rockwell BA, Kennedy PK, Noojin GD, Stolarski DJ, Hammer DX, Toth CA, Roach WP: Retinal damage and laser-induced breakdown produced by ultrashort-pulse lasers. Graefe's Arch Clin Exp Ophthalmol 1996;234:S 28-S 37.

Cain CP, Noojin GD, Stolarski DJ: Ultrashort pulse laser effects in the primate eye. AL/OE-TR 1994;0141:1-115.

Cao E, Wang J, Xin S: Nonlinear biological effects of high-intensity visible laser radiation on DNA. Proc. SPIE Int. Soc. Opt. Eng. 1993;1882:309-313.

Danith H, Lockhart D J, Lerner R A and Schultz P G: Mitotic Misregulation and Human Aging. Science 2000; 287:2486-2492.

Dunn KC, Aotaki-Keen AE, Putkey FR, Hjelmeland LM: ARPE-19, A Human Retinal Pigment Epithelial Cell Line with Differentiated Properties. Exp. Eye Res. 1996;62:155-162.

Glickman RD: A study of oxidative reactions mediated by laser-excited ocular melanin. AL/OEO 1996;12:1-20.

Glickman RD, Jacques SL, Schwartz JA, Rodriques T, Lam KW, Buhr G: Photodisruption increases the free radical reactivity of melanosomes isolated from retinal pigment epithelium. Laser-Tissue Interaction VII 1996;2681:460-467.

Glickman RD, Lam KW: Melanin may promote photooxidation of linoleic acid. Laser-Tissue Interaction VI 1995;2391:254-261.

Glickman RD, Sowell R, Lam KW: Kinetic properties of light-dependent ascorbic acid oxidation by melanin. Free Radical Biology & Medicine 1993;15:453-457.

Glickman RD, Lam KW: Oxidation of ascorbic acid as an indicator of photooxidative stress in the eye. Photochemistry and Photobiology 1992;55:191-196.

Hall RM, Glickman RD, Rockwell BA, Kumar N, Noojin GD: Pulsewidth-dependent nature of laser-induced DNA damage in RPE cells. In Laser-Tissue Interaction: Photochemical, Photothermal, and Photomechanical, (Edited by D. D. Duncan, S. L. Jacques, and P. C. Johnson), Proc. SPIE 2001;4257 :159-166, SPIE, Bellingham, WA.

Huibin Y, Eastman PS, Wang BB, Minor J, Doctolero MH, Nuttall RL, Stack R, Becker JW, Montgomery J R, Vainer M and Johnston R: An evaluation of the performance of cDNA microarrays for detecting changes in glogal mRNA xpression. Nuc. Acids Res. 2001;29(8):e41-51.

Iyer VR, Eiseb MB, Ross DT, Shuler G, Moore T, Lee JCF, Trent JM, Staudt L M, Hudson J, Boguski MS, Lashkari D, Shalon D, Botstein D and Brown PO: The Transcriptional Program in the Response of Human Fibroblasts to Serum. Science 1999; 283:83-88.

Konig K: Cell damage in near-infrared multimode optical traps as a result of multiphoton absorption. Optics Letters 1996;21:1090-1092.

Konig K, Liang H, Berns MW, Tromberg BJ: Cell damage in near-infrared multimode optical traps as a result of multiphoton absorption. Optics Letters 1996;21:1090-1092.

Lam KW, Glickman RD: Prevention of light-induced free radical production from melanin granules by ascorbic acid. Oxygen Radicals 1992;633-636.

Leavitt J, Fatone M, Hestalen C, Obringer J, Tillinghast H S: Mutagenic Activity if High-Energy 532 nm Ultra-Short Laser Pulses. Rad. Res. 1997;147:490-494.

Lee C, Klopp RG, Weindruch R, Prolla T A: Gene Expression Profile of Aging and Its Retardation by Caloric Restriction. Science 1999;285:1390-1393.

Lykins DR, Obringer JW, Johnson MD: Laser Bioeffects: Differential Protein Expression of Cultured Human Melanocytes Treated With 532 nm Picosecond Pulse Laser-Light. Technical Report, USAFA-TR-2002-02; Aug. 2002

Obringer J W, Johnson MD, Lewis DR: Differential Gene Expression in Cultured Human Reitnal Pigment Epithelial Cells 24 Hours Post-Exposure to 532 nm, 27 ps Pulsed Laser Light. Technical Report, USAFA-TR-2003-02, USAF Academy; Feb. 2003.

Obringer J W, Johnson MD, Lewis DR: Differential Gene Expression in Cultured Human Reitnal Pigment Epithelial Cells 24 Hours Post-Exposure to 532 nm, 3.0 ns Pulsed Laser Light. Technical Report, USAFA-TR-2003-01, USAF Academy; Sep. 2002.

Obringer J W, Phipps S, Johnson MD: Genetic Induction of Cultured Human Cells by High Energy, Ultrashort Pulse Laser-Light. Journal of Laser Applications 2000; 12 (1):10-15.

Obringer J W, Phipps S, Johnson MD: High Energy, Ultrashort Green Laser-Light Exposure of Cultured Human Cells Yields Evidence of DNA Damage. Technical Report, USAFA-TR-2000-02, USAF Academy 1999.

Obringer J W, Phipps S, Johnson MD: Near Infrared, High Energy, Ultrashort Pulse Laser-Light Exposure Genetically Induces p53, a Gene in the DNA Repair and Cell Suicide Pathways in Cultured Human Cells. Technical Report, USAFA-TR-2000-01, USAF Academy 1999.

Schena M, Shalon D, Davis R, and Brown P O: Quantitative monitoring of gene expression patterns with a complementary DNA miroarray. Science 1995;270:467-470.

Schena M, Shalon D, Heller R, Chai A, Brown PO, Davis R: Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes. Proc. of the Nat. Acad. of Sciences 1996;93(20):10614-10619.

Toth CA, Narayan DG, Cain CP, Noojin G, Winter KP, Rockwell BA, Roach WP: Pathology of Macular Lesions from subnanasecond pulses of visible laser energy. AL/OE-TR-1996:1-11.

White KP, Rifkin SA, Hurban P, Hogness DS: Microarray analysis of *Drosphila* development during metamorphosis. Science 1999; 286:2179-2184.

Wu GS, Burns TF, McDonald ER, Jiang W: KILLER/DR5 is a DNA damage-inducible p53-regulated death gene. Nature Genetics 1997;17(2):141-143.

Zhang W, Chenchik A, Chen S, Siebert P, Rhee CH: Molecular profiling of human gliomas by cDNA expression array. J. Gene Med 1997;1(1):57-59.

Zuchlich JA, Glickman RD, Menendez AR: In situ measurements of lens fluorescence and its interference with visual function. Investigative Ophthalmology & Visual Science 1994;33:410-415.

Zuchlich JA, Elliott WR, Cain CP, Noojin GD: Ocular damage induced by ultrashort laser pulses. AL/OE-TR 1993;0099:1-29

Gene References

The references below are presented in the NCBI searchable database format.

1. Exp Eye Res 2003 May;76(5):623-31

2. J Biol Chem 2003 May 14; [e pub]

3. Mutat Res 2003 Jun 19;529 (1-2):67-80

Figure 1

MICROARRAY HYBRIDIZATION

Atlas 1.2 Microarray (Clontech)

- Clone Listing –www.atlas.clontech.com
- Out-source:Lofstrand Labs Limited, Gaithersburg, MD
- Methodology:mRNA
- Differential Gene Expression
- Elements – 1,176 (cDNA)
  - Annotated genes
  - Biological relevance

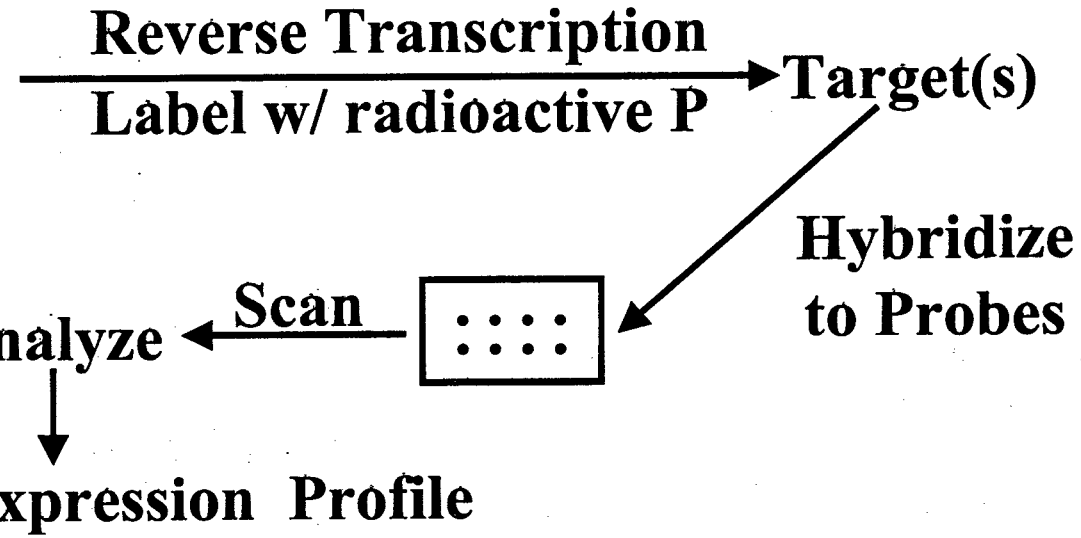

Figure 2

RESULTS:N4HX DIFFERENTIAL EXPRESSION (Ratio = Exp/Cont)

Time(hr)	0.5	1.0	3.0	6.0	12	24
GENES						
Prot. tyrosine phos.	3.0	----	---	---	---	---
Insulin-like GF R	0.5	0.1	<.1	<.1	0.3	---
Ubiquitin	0.5	3.8	---	---	0.2	<.1
HSP70	---	3.3	2.8	3.5	0.4	0.4
DNA excis. rep. (XPG)	---	9.6	10.3	7.5	0.2	---
Nucleobindin precur.	---	8.8	11.4	7.9	0.4	---
GADD-153	---	---	---	5.8	0.5	---
Mito. MP (HSP60)	---	6.9	6.3	5.2	---	---
Cyclin G-assoc. kinase	2.0	---	---	---	10.9	---
Ras-related prot.	---	---	3.1	---	11.9	15.8
Maj. Prion pro. prec.	---	---	---	---	2.1	0.1
BRCA-2 (sus. prot.)	2.2	---	---	---	4.8	6.2
Interleukin-2 rec.	---	10.3	8.5	5.1	---	---

ure 3

Differential Gene Expression

N4HX

• Gene	Function/Marker
Prot. tyrosine phos.	Dephosphorylate proteins
Insulin-like GF R	Growth factor Receptor
Ubiquitin	Marks proteins for destruction
HSP 70	Protein stabilizes proteins
DNA excis. rep. (XPG)	DNA repair-removes damaged bases
Nucleobindin precur.	Binds DNA, Ca sensor, golgi involvement
GADD-153	Growth Arrest and DNA damage inducible
Mito. MP (HSP60)	Chaperonin, HSP 60, mitochondrial matrix
Cyclin G-assoc. kinase	Up-regulates mitosis
Ras-related prot.	Transcription factor-oncogene
Maj. Prion pro. prec.	Neuropathology-KJ disease
BRCA-2	Breast cancer susceptibility protein marker
Interleukin-2 rec.	Inflammatory response receptor

Appendix A

Gene code	Hours Post Exposure Ratio 0.5	1	3	6	12	24	Protein/gene
A01a					68.3	38.6	Von Hippel-Lindau tumor suppressor protein (VHL)
A01c				5.0	12.4		LUCA2; lysosomal hyaluronidase 2 (HYAL2) ; PH-20 homolog
A01d		6.2	8.6	5.2	6.5		N-myc proto-oncogene
A01e					4.4		B-raf proto-oncogene (RAFB1)
A01f	0.1				2.4		vascular endothelial growth factor receptor 1 (VEGFR1); tyrosine-protein kinase receptor flt + s
A01g					9.1		transforming protein rhoA H12 (RHO12; ARH12; ARHA)
A01i					4.0		BUBR1 protein kinase
A01j					4.5		wee1Hu CDK tyrosine 15-kinase; wee-1-like protein kinase
A01k				0.5	5.6		aurora-related kinase 1 (ARK1)
A01m					3.1	0.3	transmembrane 4 superfamily protein; SAS
A01n		0.2			3.5	0.3	calcium-activated potassium channel beta subunit; maxi K channel beta subunit; BK channel be
A02a			2.9		12.5		mothers against dpp homolog 4 (SMAD4); MADR4; pancreatic carcinoma gene 4 (DPC4)
A02b			3.1		11.9	15.9	ras-related protein RAP-1A; C21KG; KREV-1 protein; GTP-binding protein SMG-p21A; G-22K
A02c					6.2	7.2	LUCA15 putative tumor suppressor
A02d	0.4				3.0		erythroblastosis virus oncogene homolog 1 (ETS-1); p54
A02f				4.3	8.0		tyrosine-protein kinase receptor tyro3 precursor; rse; sky; dtk
A02g					11.4		transforming protein p21/K-ras 2B
A02j					4.3		serine/threonine-protein kinase PLK1 (STPK13)
A02k					4.7		aurora- & IPL1-like midbody-associated protein kinase 1 (AIM1); ARK2
A02l					3.7		CDC25C; M-phase inducer phosphatase 3
A02m					5.1		C-1
A02n		0.1			3.9		G protein-activated inward rectifier potassium channel 1 (GIRK1); KIR31
A03b			2.1		55.1	64.0	EB1 protein
A03c					13.9	14.1	neogenin
A03d					9.2	4.4	MAD protein; MAX dimerizer
A03e					4.5		c-raf proto-oncogene
A03f	0.5				8.2	2.9	c-ros-1 tyrosine-protein kinase proto-oncogene
A03g				2.2	10.3		N-ras; transforming p21 protein
A03h					37.5		G1/S-specific cyclin D1 (CCND1); cyclin PRAD1; bcl-1 oncogene
A03i					6.6		cyclin-dependent protein kinase 2 (CDK2); p33 protein kinase
A03j					12.4		cell division protein kinase 9 (CDK9); serine/threonine protein kinase PITALRE
A03k		0.5			7.0		cyclin-dependent kinase 4 inhibitor B (CDKN2B); p14-INK4B; multiple tumor suppressor 2 (MTS

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
A03m					3.7		cyclin-D binding Myb-like protein (hDMP1)
A03n					2.3	0.4	G protein-activated inward rectifier potassium channel 2 (GIRK2); KATP-2; BIR1; KIR32
A04a	2.2				4.8	6.2	breast cancer type 2 susceptibility protein (BRCA2)
A04b		0.3		0.2	7.0	7.9	ezrin; cytovillin 2; villin 2 (VIL2)
A04c		0.3			5.6	4.5	transforming growth factor-beta signaling protein 1 (BSP1); mothers against dpp homolog (MAD
A04d				2.0			jun-D
A04e		0.5			3.2		A-raf proto-oncogene serine/threonine-protein kinase; PKS2
A04f		3.9			10.1		proto-oncogene tyrosine-protein kinase abl; p150; c-abl
A04g			2.3		12.6	7.1	C-cbl proto-oncogene
A04i		0.4					cell division protein kinase 4; cyclin-dependent kinase 4 (CDK4); PSK-J3
A04j					3.4		stem cell tyrosine kinase 1 (STK1); FL cytokine receptor precursor; tyrosine-protein kinase rece
A04k					3.0		cyclin-dependent kinase 4 inhibitor (CDK4I; CDKN2); p16-INK4; multiple tumor suppressor 1 (M
A04l	1.9				5.0		DNA-binding protein inhibitor ID-1; Id-1H
A04m		0.5			3.6		water channel aquaporin 3 (AQP3)
A04n					2.3	0.5	ASIC3 proton gated cation channel
A05a		0.4			3.7	4.1	tumor suppressor protein DCC precursor; colorectal cancer suppressor
A05b				0.5	5.6	3.4	transforming growth factor-beta 3 (TGF-beta3)
A05d					26.5		B-myb
A05e			0.5		5.2		tyrosine-protein kinase receptor UFO precursor; axl oncogene
A05f					5.5	2.6	tyrosine-protein kinase ABL2; tyrosine kinase ARG (ABLL)
A05g	6.3						INT-2 proto-oncogene protein precursor (fibroblast growth factor-3) (FGF-3) (HBGF-3)
A05h				2.7			G1/S-specific cyclin D3 (CCND3)
A05i					4.7		cell division protein kinase 6 (CDK6); serine/threonine protein kinase PLSTIRE
A05j					4.0		serine/threonine-protein kinase KKIALRE
A05k					3.7		cyclin-dependent kinase 4 inhibitor D (CDKN2D); p19-INK4D
A05m		0.4			7.8		sulfate transporter; diastrophic dysplasia protein
A05n					63.7		G protein-activated inward rectifier potassium channel 3 (GIRK3); KIR3.3
A06a		0.5		0.3	5.4	3.1	p53-associated mdm2 protein
A06b					6.8		transforming growth factor beta receptor III precursor (TGF beta receptor III; TGFR3); betaglyca
A06c	2.2				3.9	2.6	C-maf transcription factor
A06d					24.0	15.1	fos-related antigen 2 (FRA2)
A06e					8.4		macrophage colony stimulating factor I receptor precursor (CSF-1-R); fms proto-oncogene (c-fm
A06f					13.8		C-src proto-oncogene (SRC1)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
A06g					8.9		mas proto-oncogene
A06h			3.7	4.5	22.1		G1/S-specific cyclin E (CCNE)
A06i			3.3	3.2	10.6		cell division protein kinase 5 (CDK5); tau protein kinase II catalytic subunit (TPKII catalytic subu
A06j					10.5		CDC2-related protein kinase CHED
A06k			2.4		5.5		cyclin-dependent kinase inhibitor 1C (CDKN1C); p57-KIP2
A06l	2.0				4.7		helix-loop-helix protein HLH 1R21; DNA-binding protein inhibitor Id-3; HEIR-1
A06n		0.5			2.8		ATP-sensitive inward rectifier potassium channel 8 ; UKATP-1; ATP-sensitive inwardly rectifyin
A07a					7.5		neurofibromatosis protein type I (NF1); neurofibromin
A07b				0.3	7.9		prohibitin (PHB)
A07c	2.0				4.6	2.2	elk-1; ets-related proto-oncogene
A07d					6.9		fos-related antigen (FRA1)
A07f					7.5		C-yes proto-oncogene (YES1)
A07g					9.1		thrombopoietin receptor precursor (TPOR); myeloproliferative leukemia protein (MPL)
A07i					29.9		protein serine/threonine kinase STK1; cell division protein kinase 7 (CDK7); CDK-activating kin
A07j					16.2		p35 cyclin-like CAK1-associated protein
A07k	1.9	0.2			3.2	2.1	cyclin-dependent kinase inhibitor 1 (CDKN1A); melanoma differentiation-associated protein 6 (
A07l					9.8		40S ribosomal protein S19 (RPS19)
A07m		0.5			3.2		liver glucose transporter 2
A07n					5.5		calcium-activated potassium channel HSK1
A08a					14.6		moesin-ezrin-radixin-like protein (MERLIN); schwannomin (SCH); neurofibromatosis 2 (NF2)
A08b				0.2	7.0		tight junction protein zonula occludens (ZO-1); tight junction protein 1 (TJP1)
A08c					4.9		A-myb proto-oncogene; myb-related protein A
A08d					2.9		v-erbA related protein (EAR2)
A08e					2.7		met proto-oncogene; hepatocyte growth factor receptor precursor (HGF-SF receptor)
A08f					3.1		C-fes proto-oncogene
A08h					23.8		cyclin H (CCNH); MO15-associated protein
A08i			2.1		######		extracellular signal-regulated kinase 1 (ERK1; p44-ERK1); microtubule-associated protein 2 kin
A08j	2.0				10.9		cyclin G-associated kinase (GAK)
A08k					28.9		cyclin-dependent kinase inhibitor 3 (CDKN3); CDK2-associated dual-specificity phosphatase; k
A08l					25.7		bullous pemphigoid antigen 1 (BPAG1; BPA); hemidesmosomal plaque protein
A08m				2.3	3.8		brain glucose transporter 3 (GTR3)
A08n		0.5			4.2		chloride conductance regulatory protein ICLN; nucleotide-sensitive chloride channel 1A; chlorid
A09a					15.0		retinoblastoma-like protein 2 (RBL2; RB2); 130-kDa retinoblastoma-associated protein

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
A09b					9.5		c-myc purine-binding transcription factor puf; nucleoside diphosphate kinase B (NDP kinase B;
A09d					10.0		ets-related protein tel; ets translocation variant 6 (ETV6)
A09e					9.0		papillary thyroid carcinoma-encoded protein + ret proto-oncogene
A09f			3.2				C-fgr proto-oncogene (p55-FGR); SRC2
A09g					10.7		insulin-like growth factor binding protein 2 (IGFBP2)
A09h					16.9		fte-1; yeast mitochondrial protein import homolog; 40S ribosomal protein S3A (RPS3A)
A09i					13.1		extracellular signal-regulated kinase 2 (ERK2); mitogen-activated protein kinase 2 (MAP kinase
A09j					8.5		serine/threonine-protein kinase NEK3; NIMA-related protein kinase 3; HSPK 36
A09k					15.0		ubiquitin-conjugating enzyme E2 H10; ubiquitin-protein ligase; ubiquitin carrier protein
A09l					149.0		proliferating cell nucleolar antigen P120; NOL1
A09m					3.5		E16 amino acid transporter
A09n					2.2		voltage-gated potassium channel protein KV12; HUKIV; HBK5; RBK2; NGK1
A10b					6.5		nucleoside diphosphate kinase A (NDKA); NDP kinase A; tumor metastatic process-associated
A10c		3.4			5.6		c-jun proto-oncogene; transcription factor AP-1
A10d					3.3		triiodothyronine receptor; thyroid hormone receptor (THRA1); v-erbA-related protein ear-1
A10e	0.1					0.2	epidermal growth factor receptor (EGFR)
A10f				2.6	5.8		shb proto-oncogene
A10g	2.0				5.5		T-lymphoma invasion and metastasis inducing TIAM1
A10h		2.4		2.3	4.2		cation-independent mannose-6-phosphate receptor precursor (CI man-6-P receptor; CI-MPR);
A10i			2.2	2.1			extracellular signal-regulated kinase 3 (ERK3); MAP kinase 3 (MAPK3; p97-MAPK); PRKM5
A10j					18.6		CDC-like kinase 2 (CLK2)
A10k					4.8		geminin
A10l					3.6		NuMA
A10m					2.8		aquaporin 4; WCH4; mercurial-insensitive water channel (MIWC)
A10n					2.4		voltage-gated potassium channel protein KV11; HUKI; HBK1
A11a					113.5		retinoblastoma-associated protein (RB1); PP110; P105-RB
A11b					15.7		TSG101 tumor susceptibility protein
A11c		2.1			3.3		myb proto-oncogene; c-myb
A11d					4.0	3.6	v-erbA related protein (EAR3); COUP transcription factor (COUP-TF)
A11e					4.6		ERBB2 receptor protein-tyrosine kinase; neu proto-oncogene; c-erbB2 + HER2 receptor
A11f	1.9				3.3		ski oncogene
A11g					3.3		matrix metalloproteinase 11 (MMP11); stromelysin 3
A11h					7.2		cyclin A1 (CCNA1)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
A11k					3.6		katanin p80 subunit
A11l					10.1		myeloid cell nuclear differentiation antigen (MNDA)
A11m					2.3		aquaporin 9
A11n					2.6		voltage-gated potassium channel protein KV14; HUKII; HBK4; HPCN2
A12a					10.3		Wilms' tumor protein (WT33; WT1)
A12b					5.0		maguk p55 subfamily member 2; MPP2 protein; discs large homolog 2
A12c					2.3		c-myc oncogene
A12d					3.9		ETS oncogene (PEP1)
A12e					3.5		ERBB-3 receptor protein-tyrosine kinase precursor; epidermal growth factor receptor
A12g				0.4	3.8		cyclin T CDK9-associated
A12i					2.2		extracellular signal-regulated kinase 5 (ERK5); BMK1 kinase
A12j					2.3		serum-inducible kinase (SNK)
A12k				0.4			diaphanous 1 (HDIA1)
A12l					2.8		transducer of erbB2 (TOB)
A12m						2.1	cationic amino acid transporter 3
A13a					4.9		putative protein-tyrosine phosphatase PTEN; mutated in multiple advanced cancers 1
A13b					13.0		tumor suppressor maspin; protease inhibitor 5 (PI5)
A13d					5.2	2.2	cot proto-oncogene
A13e					11.0		ERBB4 receptor protein-tyrosine kinase; Her4 tyrosine kinase-EGF receptor related
A13f					12.3		CBL-B
A13g				0.5	4.1		cyclin K
A13h				0.5	3.5		bub1 mitotic checkpoint kinase
A13i					4.4		cdc2-related protein kinase PISSLRE
A13j				0.4	2.9		cyclin-dependent kinase regulatory subunit 1 (CKS1)
A13k					2.2	2.5	sprouty 2 (SPRY2)
A13l					5.0	5.5	p55CDC
A13m						2.7	putative renal organic anion transporter 1
A13n					2.4		dihydropyridine-sensitive L-type calcium channel beta-3 subunit (CAB3A/CAB3B); CACNLB3
A14a					11.2		colorectal mutant cancer protein (MCC)
A14b					7.2		tumor suppressor LUCA1; hyaluronoglucosaminidase (HYAL1)
A14d	2.7				7.0	3.9	C-mos proto-oncogene serine/threonine-protein kinase
A14e					6.3		platelet-derived growth factor receptor alpha subunit (PDGFRA); CD140A antigen
A14f	2.8				6.8		H-ras proto-oncogene; transforming G protein

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
A14g	2.1				4.9		cyclin E2
A14h					4.4		serine/threonine-protein kinase NEK2; NIMA-related protein kinase 2; NIMA-like protein kinase
A14i					3.3		CDC-like kinase 1 (CLK1)
A14j					2.3		cyclin-dependent kinase regulatory subunit (CKS2)
A14l		0.5			2.1	3.1	RCL growth-related c-myc-responsive gene
A14m		0.4					erythrocyte urea transporter (UTE; UT1); SLC14A1; HUT11; RACH1
B01a					5.8	4.7	kidney glomeruli chloride channel; CIC-5
B01b					5.2		monocarboxylate transporter 1 (MCT1)
B01c					4.3	3.0	zinc transporter 4
B01d					3.9	2.2	macrophage-stimulating protein receptor precursor (MSP receptor); p185-RON; CD136 antigen
B01e					8.1		urokinase-type plasminogen activator receptor GPI-anchored form precursor (U-PAR); monocy
B01f	2.5				4.7		related to receptor tyrosine kinase (RYK)
B01g					19.5		proto-oncogene tyrosine-protein kinase lck; p56-lck; lymphocyte-specific protein tyrosine kinase
B01h	2.6				3.5		glycogen synthase kinase 3 beta (GSK3 beta); tau kinase subunit; factor A
B01i	1.9				3.4		dual-specificity mitogen-activated protein kinase kinase 6 (MAP kinase kinase 6; MAPKK 6; MK
B01j		0.3			3.8		cAMP-dependent protein kinase type II alpha regulatory subunit (PRKAR2A; PKR2)
B01k					2.2		lipid-activated protein kinase PRK1;PKN cell morphology-related protein kinase
B01l			2.0			2.5	serine/threonin-protein kinase PAK-beta; p21-activated kinase 3
B01m						2.8	phospholipase C-delta-1 (PLC-delta-1; PLCD1); 1-phosphatidylinositol-4,5-bisphosphate phosp
B01n		0.4					ADP-ribosylation factor 1
B02a					2.6	4.0	cardiac muscle sodium channel alpha subunit; HH1
B02b					2.8	3.5	sodium/hydrogen exchanger 1 (Na+/H+ exchanger 1; NHE1); amiloride-sensitive Na+/H+ antip
B02c					5.1	5.3	Golgi 4-transmembrane spanning transporter; MTP
B02d		0.2					autocrine motility factor receptor (AMF receptor; AMFR)
B02e		0.3					vascular endothelial growth factor receptor 2 precursor (VEGFR2); kinase insert domain recept
B02h					3.0		pyruvate dehydrogenase kinase kinase precursor
B02i	2.3				3.4	2.0	MAPK/ERK kinase kinase 3 (MEK kinase 3; MEKK3)
B02j	3.6				3.9	3.6	Janus kinase 1 (JAK1)
B02k						3.1	serum- & glucocorticoid-regulated serine/threonine protein kinase (SGK)
B02m		0.3				2.4	phosphatidylinositol 3-kinase catalytic subunit delta isoform (PI3-kinase p110 subunit delta; PT
B02n		0.3				2.5	ras-related protein RAP-1B; GTP-binding protein SMG p21B
B03a	2.2	0.5				3.2	KCNQ3 potassium channel
B03b					2.9	4.0	sodium/hydrogen exchanger 3 (Na+/H+ exchanger 3; NHE3)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
B03c	1.9	0.4				4.7	organic cation transporter 1
B03d		0.2		0.5			colon carcinoma kinase 4 precursor (CCK4) + transmembrane receptor PTK7
B03f				0.2			neurotrophic tyrosine kinase receptor-related 3; TKT precursor
B03h	1.8				2.4	2.5	ribosomal protein kinase B (RSKB)
B03i				0.3			protein kinase C alpha polypeptide (PKC-alpha; PKCA)
B03j			0.4	0.5			janus kinase 3 (JAK3); leukocyte janus kinase (L-JAK)
B03l						4.5	ribosomal protein S6 kinase II alpha 1 (S6KII-alpha 1); ribosomal S6 kinase 1 (RSK1)
B03m		0.4		0.4		2.8	phosphatidylinositol 3-kinase regulatory beta subunit (PI3-kinase p85-beta subunit; PTDINS-3-
B04a	3.1				3.9	6.2	voltage-gated potassium channel
B04b	2.5				3.4	4.9	small intestine oligopeptide transporter; peptide transporter 1; intestinal H+/peptide cotransport
B04c						10.5	apolipoprotein E precursor (APOE)
B04d				0.5	4.1	5.7	T-lymphocyte activation CD86 antigen precursor; activation B7-2 antigen; B70; fun-1; BU63; CT
B04e	1.8			0.4	2.0	4.0	high-affinity nerve growth factor receptor precursor; trk-1 transforming tyrosine kinase protein; p
B04f				0.4			tyrosine kinase receptor tie-1 precursor
B04h				0.4			tyrosine-protein kinase ack
B04i				0.3			protein kinase C beta I (PKC-beta-1)
B04j			0.4	0.4			c-jun N-terminal kinase 1 (JNK1); JNK46
B04k			0.4	0.4		3.3	protein kinase MLK-3; sprk
B04l						2.4	ribosomal protein S6 kinase II alpha 2 (S6KII-alpha 2); ribosomal S6 kinase 3 (RSK3)
B04m		0.4		0.4		3.0	68-kDa type I phosphatidylinositol-4-phosphate 5-kinase alpha (PTDINS(4)P-5-kinase); 1-phos
B04n				0.3			ras-related protein RAB3B
B05d	2.6				3.3	5.5	CC chemokine receptor type 1 (CC CKR1; CCR 1); macrophage inflammatory protein 1 alpha r
B05i				0.4			protein kinase C delta (NPKC-delta)
B05j	1.9		0.4			2.7	c-jun N-terminal kinase 2 (JNK2); JNK55
B05k			0.5				tyrosine kinase tnk1
B05l						4.1	ribosomal protein S6 kinase II alpha 3 (S6KII-alpha 3); ribosomal S6 kinase 2 (RSK2); insulin-s
B06a						4.0	sodium-dependent dopamine transporter; DA transporter (DAT)
B06b						4.0	plasma membrane calcium-transporting ATPase isoform 2 (PMCA2); ATP2B2; calcium pump;
B06c						9.5	lecithin-cholesterol acyltransferase (LCAT); phosphatidylcholine-sterol acyltransferase precurso
B06d				0.3		10.5	thrombin receptor (TR); F2R; PAR1
B06e						7.0	NT-3 growth factor receptor precursor (NTRK3); C-trk tyrosine kinase (TRKC)
B06f						4.2	leukocyte tyrosine kinase receptor precursor (LTK)
B06g						3.5	NCK melanoma cytoplasmic src homolog (HSNCK)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
B06i						2.3	protein kinase C epsilon type (NPKC-epsilon)
B06k						4.0	serine kinase
B07b	2.1	0.4	0.5	0.4		3.0	copper-transporting ATPase 2; copper pump 2; Wilson disease-associated protein
B07e	2.2					4.4	G protein-coupled receptor kinase GRK5
B07g	2.1					2.9	Ink adaptor protein
B07i						2.3	protein kinase C eta type (NPKC-eta); PKC-L
B07j						4.0	focal adhesion kinase (FADK); proline-rich tyrosine kinase 2 (PYK2)
B07k						2.7	calcium/calmodulin-dependent protein kinase I (CAMKI)
B07l						4.4	ephrin A3 precursor (EFNA3); EPH-related receptor tyrosine kinase ligand 3 (EPLG3); LERK3;
B07m						3.0	Ral A; GTP-binding protein
B08a	3.5					4.2	sodium-dependent serotonin transporter; 5HT transporter (5HTT)
B08b		0.5					sodium/potassium-transporting ATPase beta 3 subunit (ATPB3); sodium/potassium-dependent
B08c	3.3					5.7	thyroxine-binding globulin precursor; T4-binding globulin
B08e						7.9	transferrin receptor (TFRC); CD71 antigen
B08i						2.3	protein kinase C gamma type (PKC-gamma)
B08k			0.4				phosphorylase B kinase gamma catalytic chain testis isoform (PHK-gamma-T; PHKGT); PSK-C
B08l			0.5			2.2	phosphatidylinositol-4-phosphate 5-kinase II beta; 1-phosphatidylinositol-4-phosphate kinase (
B08m			0.5				transforming protein rhoB; ARHB; ARH6
B09a	2.1					3.1	sodium-dependent noradrenaline transporter; norepinephrine transporter (NET)
B09b						2.6	synaptic vesicle amine transporter (SVAT); monoamine transporter; vesicular amine transporte
B09d	4.8					4.9	frizzled
B09e			2.1			4.2	vascular endothelial growth factor receptor 3 precursor (VEGFR3); tyrosine-protein kinase rece
B09f			2.2			3.2	guanine nucleotide release/exchange factor (GNRP); ras-GRF; sos
B09h	1.8					3.3	MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MAPKAPK-2)
B09i						2.1	protein kinase C zeta type (NPKC-zeta)
B09l						3.3	phospholipase C beta 3 (PLC beta 3); 1-phosphatidylinositol-4,5-bisphosphate phosphodiestera
B10a						2.7	sodium- & chloride-dependent taurine transporter
B10d		3.8	3.0			4.6	ephrin type-B receptor 3 precursor; tyrosine-protein kinase receptor HEK-2
B10e				0.5			ephrin A receptor 4 precursor; tyrosine-protein kinase receptor sek; hek8
B10f			3.0				c-src kinase (CSK); protein-tyrosine kinase cyl
B10m	0.5	0.4	0.2		0.3		ras-related protein RAB-7
B10n			0.3	0.5			guanine nucleotide-binding protein G-i/G-s/G-t beta subunit 2; transducin beta 2 subunit 2
B11e				0.5			ephrin type-A receptor 2 precursor; epithelial cell kinase (ECK); tyrosine-protein kinase recepto

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
B11f	0.2				0.2		tyrosine-protein kinase HCK; P59-HCK & P60-HCK; hemopoietic cell kinase
B11g	0.5						mitogen-activated protein kinase kinase kinase 5 (MAP/ERK kinase kinase 5; MAPKKK5; MEK
B11i					0.5		calcium/calmodulin-dependent protein kinase type II beta subunit (CAM-kinase II beta; CAMK-I
B11k			0.4		0.4		cAMP-dependent protein kinase gamma-catalytic subunit (PKA C-gamma)
B11l	0.6	0.5	0.5		0.3		phosphatidylinositol 4-kinase alpha (PI4-kinase; PTDINS-4-kinase; PI4K-alpha)
B11m	0.4				0.4		guanine nucleotide regulatory protein alpha-13 subunit; G13
B11n	0.4				0.2		transducin beta 5 subunit; GTP-binding protein G(i)/G(s)/G(t) beta subunit 3
B12a	1.8			0.4	0.5		kidney oligopeptide transporter; kidney H+/peptide cotransporter
B12b	2.3						ATP-binding casette 8 (ABC8); Drosophila white homolog
B12c				0.5	0.4		lactotransferrin precursor; lactoferrin
B12e					0.2		serine/threonine-protein kinase receptor R4 precursor (SKR4); activin receptor-like kinase 5 (A
B12g			0.5		0.3	0.5	myosin light chain kinase (MLCK) smooth muscle & non-muscle isozymes
B12h	8.5						dual specificity mitogen-activated protein kinase kinase 3 (MAP kinase kinase 3; MAPKK 3; MK
B12j					0.2		tyrosine-protein kinase tec
B12k					0.2		cAMP-dependent protein kinase type I beta regulatory subunit (PRKAR1B)
B12l		0.5			0.2		phospholipase C beta 2 (PLC-beta 2; PLCB2); 1-phosphatidylinositol 4,5-bisphosphate phosph
B12m					0.2		guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 1 (GNB1); transducin beta-1 su
B12n					0.1		GTP-binding protein ras associated with diabetes (RAD1)
B13a	2.0			0.5	0.5		sodium-dependent proline transporter
B13b	2.2						canalicular multispecific organic anion transporter; multidrug resistance-associated protein 2 (M
B13c			0.5				melanotransferrin precursor; melanoma-associated antigen p97
B13d						2.8	stromal cell derived factor 1 receptor (SDF1 receptor); fusin; CXCR4; leukocyte-derived seven
B13g			0.1		0.4		titin
B13h	4.0						c-jun N-terminal kinase kinase 1 (JNKK); JNK activating kinase 1 (JNKK1); MAP kinase kinase
B13i			0.4		0.4		casein kinase II alpha subunit (CK II); CSNK2A1
B13k					0.2		cAMP-dependent protein kinase type II beta regulatory subunit (PRKAR2B; PKR2)
B13l					0.1		phospholipase C gamma 1 (PLC-gamma 1; PLCG1); 1-phosphatidylinositol 4,5-bisphosphate p
B13n					0.3		RalB GTP-binding protein
B14a	2.3	0.4					neutral amino acid transporter A (SATT); alanine/serine/cysteine/threonine transporter (ASCT1
B14b	1.9						cystic fibrosis transmembrane conductance regulator (CFTR); cAMP- dependent chloride chan
B14c					0.5		Insulin receptor-related protein precursor (IR-related receptor; IRR)
B14e					0.4		fibroblast growth factor receptor 3 precursor (FGFR3); JTK4 + fibroblast growth factor receptor
B14g	1.8						cAMP-dependent protein kinase I alpha regulatory subunit; tissue-specific extinguisher 1 (TSE1

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
B14i					0.4		cAMP-dependent protein kinase alpha-catalytic subunit (PKA C-alpha)
B14k					0.2		focal adhesion kinase 2 (FADK2; FAK2); cell adhesion kinase beta (CAKbeta); proline-rich tyro
B14l					0.3	0.2	phospholipase C-gamma-2 (PLC-gamma-2; PLCG2); 1-phosphatidylinositol-4,5-bisphosphate p
B14h			4.7	5.9			dual-specificity protein phosphatase 9; mitogen-activated protein kinase phosphatase 4 (MAP k
C01b	1.8				0.5		PTPCAAX1 nuclear tyrosine phosphatase (PRL-1)
C01c	1.8				0.4		adenylyl cyclase IX
C01e	1.9						14-3-3 protein beta/alpha; protein kinase C inhibitor protein-1 (KCIP-1); protein 1054
C01k		4.8	7.7	3.5			poly(ADP-ribose) polymerase (PARP; PPOL); ADPRT; NAD+ ADP-ribosyltransferase; poly(AD
C01l					0.3		DNA polymerase beta subunit (DPOB)
C01m			2.1		0.2		MCM2 DNA replication licensing factor; nuclear protein BM28; KIAA0030
C01n			3.2	2.7			DNA excision repair protein ERCC1
C02a	2.8						leukocyte common antigen precursor (L-CA); CD45 antigen; PTPRC
C02b	2.5					2.1	cGMP-inhibited 3',5'-cyclic phosphodiesterase B (CGI-PDE B; CGIPDE1)
C02c	2.4						retinal guanylyl cyclase 1 precursor (RETGC-1); retinal guanylate cyclase 2D; rod outer segme
C02e	2.3						protein kinase C substrate 80-kDa protein heavy chain (PKCSH); 80K-H protein
C02f			0.4				SH3P17 SH3 domain-containing protein
C02g				3.1			WSL protein + TRAMP + Apo-3 + death domain receptor 3 (DDR3)
C02i		2.2	2.0		0.5		calcium-dependent protease small (regulatory) subunit; calpain; calcium-activated neutral prote
C02j		3.1	3.6		0.3	0.5	CAD; DNA fragmentation factor 40-kDa subunit (DFF40)
C02k		5.0	5.0	4.2	0.2		inducible nitric oxide synthase (INOS); type II NOS; hepatocyte NOS (HEP-NOS)
C02l					0.4		DNA polymerase gamma (POLG); mitochondrial DNA polymerase catalytic subunit (MDP1)
C02m					0.3		MCM4 DNA replication licensing factor; CDC21 homolog
C02n				2.9			xeroderma pigmentosum group D complementing protein (XPD); DNA excision repair protein E
C03a	4.4	0.2					protein-tyrosine phosphatase 1B (PTP-1B)
C03b					0.3		cGMP-inhibited 3',5'-cyclic phosphodiesterase A (CGI-PDE A)
C03c	2.1						retinal guanylyl cyclase 2 precursor (RETGC-2); retinal guanylate cyclase 2F; rod outer segme
C03d	3.8						cAMP-response element binding protein (CREB)
C03g	2.2						CD27L antigen receptor precursor; T-cell activation CD27 antigen
C03i		2.2			0.5		BAD protein; bcl-2 binding component 6 (BBC6); bcl-2L8
C03i							BAD protein; bcl-2 binding component 6 (BBC6); bcl-2L8
C03j			2.9	2.0			DNA fragmentation factor 45 (DFF45)
C03k			5.4				defender against cell death 1 (DAD1)
C03l		3.6	2.4	3.1	0.3		DNA polymerase delta catalytic subunit

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
C03m				2.2	0.3		MCM5 DNA replication licensing factor; CDC46 homolog
C03n		3.1	3.0	2.9	0.2		xeroderma pigmentosum group B complementing protein (XPB); DNA excision repair protein E
C04a	3.0						protein-tyrosine phosphatase 2C (PTP-2C); SH-PTP2
C04b					0.2		3'5'-cAMP phosphodiesterase HPDE4A6
C04c					0.2		soluble guanylyl cyclase beta 2 subunit; guanylate cyclase; guanyl cyclase
C04d	2.2			2.1	0.3		RalGDSB; GTP/GDP dissociation stimulator for a ras-related GTPase (RALGEF)
C04e	1.8						hint protein; protein kinase C inhibitor 1 (PKCI1)
C04h		2.6					caspase-2 precursor (CASP2); ICH-1L protease + ICH-1S protease
C04i					0.2		BCL-2 binding athanogene-1 (BAG-1); glucocorticoid receptor-associated protein RAP46
C04k		2.5	3.1				inhibitor of apoptosis protein 3 (API3; IAP3); X-linked inhibitor of apotosis protein (X-linked IAP
C04l	0.4	3.6	3.0	2.8	0.1	0.2	DNA topoisomerase I (TOP1)
C04m					0.2		MCM6 DNA replication licensing factor ; p105MCM
C05a	3.2						leukocyte antigen-related protein precursor (LAR) ; PTPRF + leukocyte surface CD47 antigen p
C05b	2.4				0.4		adenylate cyclase type I; ATP pyrophosphate-lyase; Ca2+/calmodulin-activated adenylyl cyclas
C05c					0.3		cGMP-dependent 3',5'-cyclic phosphodiesterase (CGS-PDE)
C05d			0.5		0.4		oligophrenin 1
C05e			0.4		0.3		macMARCKS; MARCKS-related protein (MRP); MLP
C05f					0.3		connector enhancer of KSR-like protein (CNK)
C05g					0.4		fasL receptor; apoptosis-mediating surface antigen fas; APO-1 antigen; CD95 antigen
C05h		4.8					caspase-3 (CASP3); apopain precursor; cysteine protease CPP32; YAMA protein; SREBP clea
C05k		8.4	11.0	5.3	0.4		cytoplasmic dynein light chain 1 (HDLC1); protein inihibitor of neuronal nitric oxide synthase (P
C05l		2.7	3.0	2.1	0.2		DNA topoisomerase II alpha (TOP2A)
C05m		2.5	2.7		0.2		MCM7 DNA replication licensing factor; CDC47 homolog; p1.1-MCM3
C05n	2.4	5.7	4.9	4.7		2.4	6-O-methylguanine-DNA methyltransferase (MGMT); methylated-DNA-protein-cysteine methylt
C06a	2.0	0.4	0.5	0.5			serine/threonine protein phosphatase PP2A-alpha catalytic subunit
C06b	2.4						adenylate cyclase type II; ATP pyrophosphate-lyase; adenylyl cyclase
C06c	2.2						neurogranin (NRGN); RC3
C06d			0.3	0.4	0.3		ran GTPase activating protein 1 (RANGAP1)
C06e	2.0						14-3-3 protein sigma; stratifin; epithelial cell marker protein 1
C06f	2.0		0.4	3.1	0.4		CD40 ligand (CD40-L); tumor necrosis factor (TNF)-related activation protein (TRAP); T-cell an
C06g		2.1			0.4		tumor necrosis factor receptor 1 (TNFR1); tumor necrosis factor binding protein 1 (TBP1); CD1
C06h					0.3	0.4	caspase-4 precursor (CASP4); ICH-2 protease; TX protease; ICE(REL)-II + caspase-5 precurs
C06i					0.2		apoptosis regulator bax

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
C06k	1.9	7.8	5.8	4.5	0.4		cytochrome P450 reductase
C06l	1.9	14.1	25.3	10.3	0.3		proliferating cyclic nuclear antigen (PCNA); cyclin
C06m		10.8	13.3	8.1	0.2		photolyase/blue-light receptor homolog
C06n				6.4			mutL protein homolog; DNA mismatch repair protein MLH1; COCA2
C07a		0.3	0.5	0.3			protein phosphatase PP2A 55-kDa regulatory subunit neuronal isoform; protein phosphatase P
C07b	1.8		0.5	0.5	0.5		guanylate cyclase soluble alpha 2 subunit
C07c	0.3			0.4	0.4	2.2	recoverin; cancer-associated retinopathy protein (CAR protein)
C07e					0.4		GAP-associated protein
C07g	2.3			2.4			tumor necrosis factor receptor (TNFR) + tumor necrosis factor receptor 2 (TNFR2); tumor nec
C07h				2.3			caspase-6 precursor (CASP6); cysteine protease MCH2 isoforms alpha + beta
C07j		8.2		6.1			Fas-activated serine/threonine (FAST) kinase
C07k		24.7	12.4	11.0			cytoplasmic antiproteinase 3 (CAP3); protease inhibitor 19 (PI9)
C07l		20.5	128.5				replication protein A 70-kDa subunit (RPA70; REPA1; RF-A); single-stranded DNA-binding pro
C07m		15.0	18.4	9.4	0.3		nibrin (NBS1)
C07n		9.6	10.3	7.5	0.2		xeroderma pigmentosum group G complementing protein (XPG); DNA excision repair protein E
C08a		0.3	0.5	0.3	0.2		protein phosphatase PP2A 55-kDa regulatory subunit alpha isoform; protein phosphatase PP2A
C08b		0.2	0.3	0.2	0.3		guanylate cyclase soluble beta-1 subunit; guanylate cyclase 70-kDa subunit
C08c		0.3	0.2	0.2	0.2		S100 calcium-binding protein A7; psoriasin
C08d				0.2			rap1 GTPase-GDP dissociation stimulator 1; SMG p21 stimulatory GDP/GTP exchange protein
C08f	2.3						tumor necrosis factor precursor (TNF-alpha; TNFA); cachectin
C08g				2.9			protein-tyrosine phosphatase zeta precursor (R-PTP-zeta)
C08h				4.9			cysteine protease ICE-LAP3
C08i		5.9		4.2			apoptosis regulator bclw; KIAA0271; BCL2L2
C08j		2.8		2.8	0.3		apoptotic protease activating factor 1 (APAF1)
C08k		2.6			0.4		ionizing radiation resistance-conferring protein + death-associated protein 3 (DAP3)
C08m		9.6	8.1	5.0	0.2	0.4	DNA-(apurinic or apyrimidinic site) lyase; AP endonuclease 1; APEX nuclease (APEN; APE1);
C08n		7.0	7.5	5.9	0.3		xeroderma pigmentosum group C repair complementing protein p58/HHR23B
C09a				0.3			protein phosphatase 2B regulatory subunit; calcineurin B subunit isoform 1
C09b		0.4	0.4	0.3			bone marrow stromal antigen 1 (BST-1); ADP-ribosyl cyclase 2 precursor; cyclic ADP-ribose hy
C09c		0.2	0.3	0.2	0.1		S100 calcium-binding protein A1; S-100 protein alpha chain
C09d			2.2		0.1		rho GDP dissociation inihibitor 1 (RHO-GDI 1); RHO-GDI alpha (GDIA1); ARHGDIA
C09e				0.4	0.4		TRRAP protein
C09f			0.4		0.4		lymphotoxin-alpha precursor (LT-alpha); tumor necrosis factor-beta (TNF-beta; TNFB)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
C09g			0.5	2.6	0.4		adenosine A1 receptor (ADORA1)
C09h					0.5		caspase-8 precursor (CASP8); ICE-like apoptotic protease 5 (ICE-LAP5); MORT1-associated C
C09i		2.2					apoptosis regulator bcl-x
C09j					0.4		IEX-1L anti-death protein; PRG-1; DIF-2
C09k		4.9					inhibitor of apoptosis protein1 (HIAP1; API1) + IAP homolog C; TNFR2-TRAF signaling comple
C09m		2.8	2.6		0.2	0.4	ataxia telangiectasia (ATM)
C10a	2.1				0.2		serine/threonine protein phosphatase 2B catalytic subunit alpha isoform; calmodulin-dependen
C10b				0.3			calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1A (CAM-PDE1A); HC
C10c		0.2		0.3			interferon regulatory factor 1 (IRF1)
C10e				0.5	0.4		leucine-rich repeat protein SHOC-2; ras-binding protein SUR-8
C10g					0.3		adenosine A2A receptor (ADORA2A)
C10i					0.5		induced myeloid leukemia cell differentiation protein MCL-1
C10l		2.9	2.0		0.3		activator 1 40-kDa subunit; replication factor C 40-kDa subunit (RFC40); RFC2
C10m			2.3	2.9	0.2	0.5	Ku 70-kDa subunit; ATP-dependent DNA helicase II 70-kDa subunit; lupus ku autoantigen prote
C10n				2.4			DNA mismatch repair protein PMS2 (PMS1 protein homolog 2)
C11a	2.3	0.5			0.3		protein phosphatase 2C alpha isoform (PP2C-alpha)
C11b		0.5		0.5	0.3		calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1B (CAM-PDE1B); HC
C11c		0.1		0.2			cAMP response element binding protein (CRE-BP1); transcription factor ATF2; HB16
C11d					0.4		58-kDa inhibitor of the RNA-activated protein kinase
C11e					0.5		IkappaB kinase complex-associated protein (IKAP)
C11g	0.4						adenosine A3 receptor (ADORA3)
C11k	0.5				0.3	0.5	ALG-2 calcium-binding protein
C11l					0.3	0.4	DNA polymerase epsilon subunit B; DNA polymerase II subunit B
C11m		5.2		5.1			Ku (p70/p80) subunit; ATP-dependent DNA helicase II 86-kDa subunit; lupus ku autoantigen pr
C11n				3.3			Rad50
C12a		0.1	0.5		0.3		serine/threonine protein phosphatase PP1-alpha 1 catalytic subunit (PP-1A)
C12d				0.3	0.5		cortactin; amplaxin; ems-1 oncogene
C12e				0.3			zyxin + zyxin-2
C12f		0.2	0.1	0.1			CD27 ligand (CD27LG); CD70 antigen
C12g		0.4	0.4	0.3			receptor interacting protein; serine/threonine protein kinase RIP transferase; serine/threonine-p
C12h				0.2			interleukin-1 beta convertase precursor (IL-1BC); IL-1 beta converting enzyme (ICE); p45; casp
C12j				5.8	0.4		growth arrest & DNA-damage-inducible protein 153 (GADD153); DNA-damage-inducible transc
C12k				3.3	0.4		DNA polymerase epsilon catalytic subunit A; DNA polymerase II subunit A

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
C12l				4.6	0.2		replication factor C 36-kDa subunit (RFC36); activator 1 36-kDa subunit
C12m		2.2		2.8	0.2		DNA ligase I; polydeoxyribonucleotide synthase (ATP) (DNL1) (LIG1)
C13a		0.1			0.4		dual-specificity protein phosphatase 2; PAC-1
C13b					0.3		cAMP-dependent 3',5'-cyclic phosphodiesterase 4D (PDE43); DPDE3
C13c				0.5			signal transducer and activator of transcription 1 alpha/beta (STAT1); transcription factor ISGF-
C13d			0.5	0.3			muscle/brain cAMP-dependent protein kinase inhibitor (PKI-alpha)
C13e		0.2	0.2	0.1	0.5		leukemia inhibitory factor receptor precursor (LIF-R)
C13f	0.5	0.0	0.0	0.0	0.3		insulin-like growth factor I receptor (IGF1R)
C13g				0.5			DAXX
C13i		0.2	0.2	0.2			NIP1 (NIP1)
C13j	0.4	0.5	0.4		0.4		clusterin precursor (CLU); complement-associated protein SP-40,40; complement cytolysis inh
C13k			2.1	3.7	0.4		MCM3 DNA replication licensing factor; DNA polymerase alpha holoenzyme-associated protein
C13l					0.3		replication factor C 38-kDa subunit (RFC38); activator 1 38-kDa subunit
C13n					0.3		DNA-repair protein complementing XP-C cells; xeroderma pigmentosum group C complementin
C14a		0.2	0.4	0.5	0.2		myotubularin
C14b			0.3		0.4		adenylate cyclase VII; ATP pyrophosphate-lyase; adenylyl cyclase; KIAA0037
C14c			0.2		0.2		signal transducer and activator of transcription 2 (STAT2); p113
C14d			0.3				14-3-3n protein eta; protein AS1; YWHAH; YWHA1
C14e					0.4		junction plakoglobin (JUP); desmoplakin III (DP3)
C14f				2.5			retinoic acid receptor epsilon (RAR-epsilon); retinoic acid receptor beta 2 (RAR-beta2; RARB);
C14g					0.5		tumor necrosis factor receptor 1-associated death domain protein (TNFR1-associated death do
C14h						2.2	calpain 2 large (catalytic) subunit; M-type calcium-activated neutral proteinase (CANP)
C14k					0.1		DNA polymerase alpha catalytic subunit (POLA)
C14l		0.4		0.5	0.4		activator 1 37-kDa subunit; replication factor C 37-kDa subunit (RFC37); RFC4
C14m		0.2	0.5	0.5	0.3		DNA ligase IV (LIG4); polydeoxyribonucleotide synthase
C14n		0.3	0.5	0.5	0.3		uracil-DNA glycosylase precursor (UNG1)
D01a			0.4	0.4	7.7		DNA-repair protein XRCC1
D01b			0.3	0.4	4.0		growth arrest & DNA-damage-inducible protein (GADD45); DNA-damage-inducible transcript 1
D01c	0.2		0.4		4.3	0.2	galanin receptor type 1 (GALNR1; GALR1)
D01d				0.5	7.3		neuronal acetylcholine receptor protein beta-2 subunit precursor (CHRNB2; NACHRB2)
D01e			0.3		7.1		low-affinity nerve growth factor receptor (NGF receptor; NGFR); GP80-LNGFR
D01f			0.3		3.1		dopamine beta-hydroxylase (DBH); dopamine-beta-monooxygenase precursor
D01g					2.8	0.0	neuroendocrine protein 7B2 precursor; secretory granule endocrine protein I; secretogranin V

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
D01h		0.5			2.9		achaete-scute homolog 1 (ASH1)
D01i		0.5			3.2		myelin proteolipid protein (PLP); lipophilin
D01j					2.1		ataxia-telangiectasia group D-associated protein
D01m				5.3	2.1		E2F-3
D02a				0.5	3.6		DNA-dependent protein kinase (DNA-PK) + DNA-PK catalytic subunit (DNA-PKCS)
D02b				0.4	3.0		muscle-specific DNase I-like precursor (DNase1L1; DNL1L); DNase X
D02c					3.1		somatostatin receptor type 2 (SS2R); SRIF-1
D02d	0.4		0.4	0.3	3.0	0.2	5-hydroxytryptamine 3 receptor precursor (5-HT-3); serotonin-gated ion channel receptor
D02e	0.3		0.5		3.2	0.1	aromatic-L-amino-acid decarboxylase; DOPA decarboxylase (DDC)
D02f			0.4		3.1		phenylethanolamine N-methyltransferase (PNMTase); noradrenaline N-methyltransferase
D02h					2.4	0.0	brain-specific polypeptide PEP-19; brain-specific antigen PCP-4
D02i	0.2					0.0	peripheral myelin protein 22 (PMP22); CD25 protein; SR13 myelin protein
D02j					2.7		cyclic-AMP-dependent transcription factor atr-1; TREB36 protein
D02k	1.9				2.1	0.0	transcription intermediary factor 1 (TIF1)
D02m	0.1					0.0	E2F dimerization partner 1; DRTF1-polypeptide 1 (DP1)
D03b					5.5		melatonin receptor type 1A (MEL-1A-R)
D03c					3.2		prostaglandin E2 (PGE) receptor EP4 subtype (PTGER4; PTGER2); prostanoid EP4 receptor
D03d				0.4	3.1		gamma-aminobutyric-acid receptor epsilon subunit precursor (GABA(A) receptor)
D03e	0.5			0.4	3.2	0.1	acetylcholinesterase precursor (ACHE)
D03f	0.3			0.5	3.5	0.0	secretogranin II precursor (SGII); chromogranin C
D03g					3.6		nociceptin precursor; orphanin FQ; PPNOC
D03h		0.5		0.4		0.0	neuronatin; brain-specific mammalian developmental gene
D03i	0.5			0.5		0.1	myelin-oligodendrocyte glycoprotein precursor (MOG)
D03j				0.4			CCAAT transcription binding factor gamma subunit
D03n	0.4					0.0	cellular nucleic acid binding protein (CNBP); sterol regulatory element-binding protein
D04a					2.1	0.5	HHR23A; UV excision repair protein protein RAD23A
D04c					2.2		metabotropic glutamate receptor 5 precursor (GRM5; MGLUR5)
D04e				0.4	3.3	0.2	choline O-acetyltransferase (CHAT); choactase; choline acetylase
D04f	0.5			0.4	2.6	0.0	neurotensin/neuromedin N precursor (NT/NMN)
D04g				0.4	2.6	0.0	leptin precursor; obesity factor; obese protein
D04h		0.4	0.5	0.3	2.4	0.1	roundabout 2 (ROBO2)
D04i		0.4	0.3	0.2		0.1	myelin basic protein (MBP)
D04j		0.4	0.4	0.2		0.0	CCAAT/enhancer binding protein alpha (C/EBP alpha)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
D04k				0.4		0.0	metal-regulatory transcription factor
D04l				0.5	2.2	0.0	human immunodeficiency virus type I enhancer-binding protein 2 (HIV-EP2)
D04n						0.0	basic transcription factor 2 44-kDa subunit (BTF2p44)
D05a					2.1	0.4	ubiquitin-conjugating enzyme E2 17-kDa (UBE2A); ubiquitin-protein ligase; ubiquitin carrier pro
D05c						0.2	orexin receptor 2
D05d						0.0	GABA-B receptor 1A subunit (GABA-BR1A)
D05e					3.6		glutamate decarboxylase 67-kDa isoform; 67-kDa glutamic acid decarboxylase (GAD-67); GAD
D05f					2.6		neuromedin B precursor
D05g			0.4	0.4			neuronal pentraxin II precursor (NP2)
D05h			0.3	0.4	2.1	0.0	veli-1
D05i		0.4	0.1	0.2		0.0	neuroglycan C precursor
D05j			0.4	0.4			hepatocyte nuclear factor 4 (HNF4); transcription factor 14
D05l					2.7		ets-related gene transforming protein (ERG1)
D05n		0.4					estrogen receptor hSNF2b; global transcription activator SNF2L4; brg-1 protein; mitotic growth
D06a					5.2		translin; recombination hotspot binding protein
D06b					4.7		mu-type opioid receptor (MOR-1)
D06c				0.4	2.1	0.4	P2X purinoceptor 1; ATP receptor P2X1
D06d						0.2	GABA-B receptor 2 subunit (GABA-BR2)
D06e					3.9		glutamate decarboxylase 65-kDa isoform; 65-kDa glutamic acid decarboxylase (GAD-65); GAD
D06g					3.1		survival of motor neuron (hSMN)
D06h			0.5	0.5	3.8		43-kDa postsynaptic protein; acetylcholine receptor-associated 43-kDa protein; RAPSYN
D06i		0.4	0.3	0.3		0.0	parkin
D06j			0.3			0.0	TIS11B protein; EGF response factor 1 (ERF1)
D06k			0.4			0.0	transcription repressor protein PRDI-BF1; beta-interferon gene positive regulatory domain I bin
D06l			0.4				transcription factor GATA-4; GATA binding factor-4
D06m		0.5	0.4	0.3	3.3		octamer-binding transcription factor 1 (oct-1; OTF1); octamer binding protein NF-A1; POU2F1
D06n		0.1	0.2	0.2			transcriptional repressor NF-X1
D07b	2.1				6.3		nociceptin receptor; orphanin FQ receptor; kappa-type 3 opioid receptor (KOR-3)
D07e					4.2		neuroendocrine convertase 1 precursor (NEC 1); prohormone convertase 1 (PC1); proprotein c
D07f					3.3	0.2	proenkephalin A precursor
D07g	0.2				2.6	0.3	lissencephalin X; doublecortin (DCX)
D07h	0.5				3.0	0.4	synaptosomal-associated protein 25 (SNAP-25); super protein (SUP)
D07i			0.5			0.2	huntingtin; Huntington's disease protein (HD protein)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
D07j				0.5		0.0	HIV-1 TATA element modulatory factor
D07k			0.3			0.0	PCAF-associated factor 65 beta
D07m			0.4		4.3	0.0	pre-B-cell leukemia transcription factor-1; homeobox protein pbx1; Homeobox protein prl
D07n		0.5			3.5		cAMP-responsive element-binding protein (CREB1)
D08b	2.7				6.4		prostaglandin E2 receptor EP3 subtype (PGE receptor EP3 subtype; PTGER3); prostanoid EP
D08c				2.4	12.9		gamma-aminobutyric-acid receptor beta-1 subunit precursor (GABA(A) receptor)
D08d			2.1	2.1	10.2		neuronal acetylcholine receptor protein alpha 6 subunit precursor
D08e					4.7		neuroendocrine convertase 2 precursor (NEC 2); prohormone convertase 2 (PC2); proprotein c
D08f					8.7		beta-neoendorphin-dynorphin precursor; proenkephalin B precursor; preprodynorphin
D08g					3.1	0.3	roundabout 1 (ROBO1)
D08i					2.1	0.1	major prion protein precursor (PRP); PRP27-30; PRP33-35C; ASCR
D08j					6.2		hypoxia-inducible factor 1 alpha (HIF1 alpha); ARNT-interacting protein; member of PAS protei
D08k	0.4				2.7	0.1	PCAF-associated factor 65 alpha
D08l					3.6	0.1	homeobox protein HOX-A5; HOX-1C
D08m					3.6		endothelial transcription factor GATA2
D08n					4.3	0.0	GA-binding protein alpha subunit (GABP-alpha); transcription factor E4TF1-47; nuclear respira
D09b					8.0		substance-P receptor (SPR); NK-1 receptor (NK-1R)
D09c					7.5		gamma-aminobutyric-acid receptor gamma-2 subunit precursor (GABA(A) receptor)
D09d				2.1	4.7		neuronal acetylcholine receptor protein beta 4 subunit precursor (CHRNB4; NACHRB4)
D09e		2.2		3.4	11.8		membrane-bound & soluble catechol-O-methyltransferase (COMT)
D09g					3.0	0.2	CASK
D09i					3.6		Alzheimer's disease amyloid A4 protein precursor; protease nexin-II (PN-II); APPI
D09j					3.2	0.0	jun activation domain binding protein
D09k					3.5	0.0	SPT3-like protein
D09m	0.5					0.0	basic transcription element-binding protein 2 (BTEB2); GC-box binding protein 2
D09n				0.4			GA-binding protein beta-2 subunit (GABP-beta2); transcription factor E4TF1-60
D10a		0.3	0.4		2.6		telomerase reverse transcriptase (hTRT)
D10b		0.4	0.5		3.1		substance-K receptor (SKR); neurokinin A receptor; NK-2 receptor (NK-2R)
D10d				2.2			glutamate (NMDA) receptor subunit epsilon 2 precursor; N-methyl D-aspartate receptor subtype
D10e					5.8		tryptophan 5-hydroxylase (TRPH); tryptophan 5-monooxygenase
D10f	0.5					0.2	neurotrophin-3 precursor (NT-3); neurotrophic factor (HDNF); nerve growth factor 2 (NGF-2)
D10g		0.5			3.6		neuromodulin; axonal membrane protein GAP-43; PP46; protein F1; calmodulin-binding protein
D10h			0.4			0.3	synapsin IIIA

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
D10i					3.2	0.0	atrophin-1; dentatorubral-pallidoluysian atrophy protein (DRPLA)
D10j				2.2	3.1	0.1	ets domain protein elk-3; NET; SRF accessory protein 2 (SAP2)
D10k					2.9	0.0	ADA3-like protein
D10l				4.5			interleukin enhancer-binding factor (ILF) ILF + interleukin enhancer binding factor 2 (ILF2) + int
D10m			6.5	7.7			basic transcription factor 62-kDa subunit (BTF2)
D11a		0.4					TRF1-interacting ankyrin-related ADP-ribose polymerase tankyrase
D11b		0.3	0.3	0.4	2.6		neuromedin K receptor (NKR); neurokinin B receptor; NK-3 receptor (NK-3R)
D11c					4.2		glutamate receptor 2 precursor (GLUR2); GLUR-B; GLUR-K2
D11d				3.2			glutamate receptor subunit epsilon 3 precursor (GRIN2C); N-methyl D-aspartate receptor subty
D11f		0.3			2.4		neurotrophin-4 (NT-4)
D11g					3.1		axonin-1 precursor; transient axonal glycoprotein 1 (TAG-1)
D11i			0.4			0.3	paraneoplastic encephalomyelitis antigen HUD; HU-antigen D
D11j			0.5	0.5			histone acetyltransferase B subunit 2; retinoblastoma-binding protein p46; retinoblastoma-bindi
D11k					3.0	0.0	ADA2-like protein
D11l		0.3		0.4	4.3		RBP2 retinoblastoma binding protein
D11m			0.5			0.0	helix-loop-helix protein; DNA-binding protein inhibitor Id-2
D12a		0.3		0.3			delta lactoferrin
D12b				0.4	2.8		neuropeptide Y receptor type 1 (NPY1R)
D12c					4.2		glycine receptor alpha-1 subunit precursor (GLRA1); strychnine binding subunit
D12d		0.4	0.4		2.1		P2X purinoceptor 5 (P2X5)
D12e		0.3					histidine decarboxylase (HDC)
D12f		0.3	0.5			0.3	neuropeptide Y precursor (NPY)
D12g		0.4					glia maturation factor beta (GMF-beta)
D12h		0.3	0.2				amphiphysin (AMPH)
D12i		0.3					Machado-Joseph disease protein 1 (MJD1)
D12j		0.4				0.4	BRCA1-associated ring domain protein
D12k		0.5		0.4		0.2	B-cell lymphoma 3-encoded protein (bcl-3)
D12l						0.0	BRCA1-associated ring domain protein (BARD1)
D12m			0.4	0.5			DNA-binding protein SMBP-2; glial factor-1 (GF-1)
D12n	0.3					0.1	ZFM1 protein alternatively spliced product
D13a					2.2		deoxyribonuclease I (DNase I)
D13b				0.5	3.3		metabotropic glutamate receptor 1 precursor (GRM1; MGLUR1)
D13c					6.0		glycine receptor beta subunit precursor (GLRB)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
D13d			0.5		2.4		P2X purinoceptor 6 (P2X6); P2XM
D13e		0.3	0.3			0.4	phenylalanine-4-hydroxylase (PAH); phe-4-monooxygenase
D13f		0.3	0.3				5-hydroxytryptamine 1D receptor (5-HT-1D; HTR1D); serotonin receptor
D13g		0.5	0.3	0.5			C-jun N-terminal kinase kinase 2 (JNKK2); mitogen-activated protein kinase kinase 7 (MAP kin
D13h		0.4	0.2	0.4			neurexin III alpha
D13i			0.4	0.5			Kallmann syndrome protein precursor (KAL); adhesion molecule-like X-linked
D13j						0.5	serum response factor (SRF)
D13k		0.4		0.3	0.5	0.3	B-cell lymphoma 6 protein (bcl-6); zinc finger protein 51 (ZNF51); LAZ-3 protein
D13m		0.4		0.5	2.5		global transcription activator SNF2L1
D13n		0.3					transcription factor RZR-alpha (RZRA); RAR-related orphan receptor alpha 1 (ROR-alpha1; RO
D14a					2.2		deoxyribonuclease II (DNase II); acid DNase; lysosomal DNase II
D14b					3.2		D2 dopamine receptor (DRD2)
D14c					4.4		neuronal acetylcholine receptor protein alpha-3 subunit precursor (NACHRA3); cholinergic rece
D14d				0.5	3.0		leptin receptor precursor; obese receptor (OB receptor; OB-R)
D14e		0.4					tyrosine 3-hydroxylase (TYH); tyrosine 3-monooxygenase isozymes
D14f		0.3	0.5				glial cell line-derived neurotropic factor precursor (GDNF)
D14g		0.3	0.2	0.5			myelin-associated glycoprotein precursor (MAG)
D14h	0.1	0.5	0.1	0.2	0.1	0.2	synapse-associated protein 97 (SAP97); homolog of Drosophila discs large protein isoform 1 (H
D14i		0.4	0.5	0.5			FCMD; fukutin
D14k			0.5	0.4			CYCLIC-AMP-DEPENDENT TRANSCRIPTION FACTOR ATF-3 (ACTIVATING FACTOR 3)
D14m		0.5	0.5			0.0	interferon consensus sequence-binding protein (ICSBP)
D14n		0.4					paired box homeotic protein (PAX8) isoforms 8A/8B + isoforms 8C/8D
E01a					2.5		brain-specific homeobox/POU domain protein 3A (brn-3A); RDC-1; octamer binding transcriptio
E01b					3.0		transcription factor TFIIB; GTF2B
E01c					3.4		transcription factor NF-ATc
E01d				0.4	2.4		ets transcription factor; NERF2
E01e				0.4	4.0		CCAAT displacement protein; CUTL1; CASP
E01i				3.1			integrin beta 5 subunit precursor (ITGB5)
E01k		19.7	21.2	13.8			granulocyte colony stimulating factor receptor precursor (GCSF-R); CD114 antigen
E01l		10.3	8.5	5.1			interleukin-2 receptor alpha subunit precursor (IL-2 receptor alpha subunit; IL2RA); p55; TAC a
E01n					2.8		growth arrest & DNA-damage-inducible protein 45 gamma (GADD45 gamma)
E02c				0.4	2.3	2.4	R kappa B DNA-binding protein
E02g				0.2			cadherin 3 (CDH3); placental cadherin precursor (P-cadherin; CDHP)

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
E02i	0.3						integrin alpha 4 precursor (ITGA4); VLA4; CD49D antigen
E02j		0.3					semaphorin; CD100
E02k		0.4	0.5	0.5			C5a anaphylatoxin receptor (C5AR); CD88 antigen
E02l			0.5	0.5	2.7		interleukin-6 receptor alpha subunit precursor (IL-6R-alpha; IL6R); CD126 antigen
E02n		0.4				0.3	growth arrest & DNA-damage-inducible protein 45 beta (GADD45 beta)
E03c					2.3		transcription factor 11 (TCF11); HBZ17; locus control region-factor 1 (LCR-F1)
E03e	0.4						heat shock factor protein 1 (HSF1); heat shock transcription factor 1 (HSTF1); TCF5
E03j	0.2				0.5		T-cell surface glycoprotein T4/leu-3; CD4 antigen
E03k	0.3	0.4					neuromedin B receptor (NMBR); neuromedin-B-preferring bombesin receptor
E03l	0.2		0.4			0.3	interferon-alpha/beta receptor alpha subunit precursor (IFN-alpha receptor; IFNAR)
E03n		0.4		0.4			14.5-kDa translational inhibitor protein (p14.5); UK114 antigen homolog
E04b		3.5	2.9			3.8	nuclease-sensitive element DNA-binding protein (NSEP)
E04d				2.1			AP4 basic helix-loop-helix DNA-binding protein
E04e				2.3			transcriptional activator hSNF2-alpha
E04g				3.6			cadherin 11 precursor (CDH11); osteoblast-cadherin (OB-cadherin); OSF4
E04h				0.5			E-selectin precursor; endothelial leukocyte adhesion molecule 1 (ELAM-1); leukocyte-endothel
E04i				2.1			platelet membrane glycoprotein IIB precursor (GP2B); integrin alpha 2B (ITGA2B); CD41 antige
E04k	0.2						Duffy blood group antigen; FY glycoprotein (GPFY); glycoprotein D (GPD)
E04m				2.0			androgen receptor (AR)
E04n		0.3					beta-defensin 2 precursor (hBD2); skin-antimicrobial peptide 1 (SAP1)
E05e			2.4				putative transcription activator DB1
E05h		0.5	0.4	0.3			NADH-ubiquinone oxidoreductase B18 subunit; complex I-B18 (CI-B18); cell adhesion protein S
E05l						0.5	interleukin-3 receptor alpha subunit precursor (IL-3R-alpha; IL3R); CD123 antigen
E05m				0.4			angiotensin II type 1A receptor (AT1AR)
E05n		0.2					defensin 6 precursor
E06c	2.2						homeobox A1 protein (HOXA1); HOX1F
E06d	0.5						raf-responsive zinc finger protein
E06g		0.2	0.2	0.1			cadherin 8 (CDH8)
E06h				0.4			neural-cadherin precursor (N-cadherin; NCAD); cadherin 2 (CDH2)
E06l		0.5					interleukin-4 receptor alpha subunit precursor (IL-4R-alpha; IL4R); CD124 antigen
E06n			0.4	0.5			cytochrome P450 IA2 (P450-P3) (P450-4)
E07c	0.4				0.4		homeobox protein hLim1; LHX1
E07e	0.5				0.5		zinc finger protein 91 (ZNF92); HPF7; HTF10

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
E07f				0.4			RPD3 protein; histone deacetylase 1 (HD1)
E07g		0.4	0.3	0.2			intercellular adhesion molecule 2 precursor (ICAM2); CD102 antigen
E07h			0.2	0.4			B-cell differentiation CD72 antigen; Lyb-2
E07l			0.5				interleukin-5 receptor alpha subunit precursor (IL-5R-alpha; IL5RA); CD125 antigen
E07m		0.4	0.4	0.5			calcitonin receptor (CTR; CALCR)
E07n		0.4	0.3				cytochrome P450 IVB1 (EC 1.14.14.1) (P450-HP)
E08a						4.5	transcription factor AP-2 (TFAP2; AP2TF)
E08b					0.5		paired box protein PAX-5; B-cell specific transcription factor; BSAP
E08c				0.4			trans-acting T-cell specific transcription factor GATA3
E08d	0.5				0.5		nuclear factor kappa-B DNA binding subunit (NF-kappaB; NFKB)
E08e	0.4				0.2		guanine nucleotide-binding protein G-s alpha subunit (GNAS); adenylate cyclase-stimulating G
E08f			0.5	0.4			high mobility group protein (HMG-I)
E08g				0.2			integrin alpha E precursor (ITGAE); mucosal lymphocyte-1 antigen; hml-1 antigen; CD103 antig
E08n		0.3	0.3				soluble epoxide hydrolase (SEH); epoxide hydratase; cytosolic epoxide hydrolase (CEH); EPH
E09a				0.5			mitochondrial transcription factor 1 (MTTF1); TCF6L1
E09b				0.4			special AT-rich sequence binding protein 1 (SATB1); MAR/SAR DNA-binding protein
E09d				0.2			zinc-finger DNA-binding protein
E09f		0.5		0.5			procollagen alpha 2(IV) subunit precursor
E09g		0.4	0.4	0.2			integrin beta 8 precursor (ITGB8)
E09i				0.4			fibronectin receptor beta subunit (FNRB); integrin beta 1 (ITGB1); VLA4 beta subunit; CD29 an
E09l				0.5			interleukin-2 receptor gamma subunit (IL-2R gamma; IL2RG); cytokine receptor common gamm
E09m				0.4			alpha 1A adrenergic receptor (ADRA1A); alpha 1D adrenergic receptor (ADRA1D); alpha 1D-a
E09n		0.4	0.3	0.3			dimethylaniline monooxygenase (N-oxide forming) 1 (EC 1.14.13.8); fetal hepatic flavin-contain
E10a	0.2	0.5	0.2	0.2	0.4		early growth response protein 1 (hEGR1); transcription factor ETR103; KROX24; zinc finger pr
E10b	0.4	0.3	0.3	0.1	0.2		MSX-1 homeobox protein; HOX7
E10c		0.4		0.4			I-rel (RELB)
E10g		0.4					thrombospondin 2 precursor (THBS2; TSP2)
E10h		0.4	0.4	0.5			contactin precursor (CNTN1); glycoprotein gp135
E10i				0.4			integrin alpha 6 precursor (ITGA6); VLA6; CD49F antigen
E10k	0.2		0.2	0.5	0.4		corticotropin releasing factor receptor 1 precursor (CRF-R; CRF1)
E10m	0.1	0.0			0.2	0.1	ferrochelatase precursor; protoheme ferro-lyase; heme synthetase
E10n	0.1				0.1	0.1	glutathione reductase (GRase; GSR; GR)
E11a	0.4		0.5	0.3	0.2		transcription factor ETR101

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
E11b	0.3		0.4	0.3	0.2		PAX3/forkhead transcription factor fusion
E11d	0.4						purine-rich single-stranded DNA-binding protein alpha (PURA)
E11e		8.5	5.8	6.1			tristetraproline (TTP); TIS11; ZFP36; growth factor-inducible nuclear protein 475 (NUP475)
E11h			0.4	0.4			neural cell adhesion molecule phosphatidylinositol-linked isoform precursor (NCAM120); CD56
E11i		0.3	0.5	0.5			integrin beta 4 (ITGB4); CD104 antigen
E11j			0.4				endothelin receptor type B (EDNRB; ETB); endothelin receptor non-selective type
E11k			0.5				cytokine receptor EBI3
E11n	0.1				0.2	0.3	microsomal glutathione S-transferase 12 (GST12; MGST1)
E12a					0.2		transcriptional enhancer factor (TEF1); protein GT-IIC; transcription factor 13 (TCF13)
E12b			2.2				transcription factor IIIC box B-binding subunit
E12d		5.8	5.8				transcription initiation factor 250-kDa subunit (TAFII250); TBP-associated factor 250-kDa subun
E12e		8.8	11.4	8.0	0.4		nucleobindin precursor (NUC)
E12f		12.8	13.1	13.5	0.3		tastin
E12g		5.8		3.5			vitronectin receptor alpha subunit (VNRA); integrin alpha 5 subunit (ITGA5); CD51 antigen
E12h		7.3	7.7	7.2			desmoglein 2 precursor (DSG2); HDGC
E12j		2.2	2.4	3.3	0.4		insulin receptor precursor (INSR)
E12k		0.4					CC chemokine receptor type 2 (CC CKR2; CCR2); monocyte chemoattractant protein 1 recepto
E12l		0.5					interleukin 10 receptor (IL-10R)
E12m					0.4	0.4	selenium-binding protein
E12n					0.3	0.3	glutathione S-transferase pi (GSTP1; GST3)
E13a			2.7				homeobox protein HOX-11; tcl-3 proto-oncogene
E13c			4.5				homeobox protein HOX-D3; HOX-4A
E13d		3.1	4.0	2.3			CCAAT-binding transcription factor subunit B (CBF-B); NF-Y protein subunit A (NF-YA); Hap2;
E13f		4.1	3.6	3.4	0.4		trophinin
E13g	2.3	9.8	8.5	7.1			alpha1 catenin (CTNNA1); cadherin-associated protein; alpha E-catenin
E13h		3.6	2.8	3.3			platelet membrane glycoprotein IIIA precursor (GP3A); integrin beta 3 (ITGB3); CD61 antigen
E13i		4.8	5.7	4.8			integrin alpha 7B precursor (IGA7B)
E13j			2.8	3.5			platelet-derived growth factor receptor beta subunit (PDGFRB); CD140B antigen
E13k				4.5			N-sam; fibroblast growth factor receptor1 precursor (FGFR1); basic fibroblast growth factor rec
E13n					0.5		glutathione S-transferase theta 1 (GSTT1)
E14c			2.1				transcription factor TFIIIB 90 kDa subunit (HTFIIIB90)
E14d					0.4		DNA-binding protein HIP116; ATPase; SNF2/SWI2-related protein
E14e			2.7				transcription factor LSF

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
E14f	2.0		2.5				Herpes virus entry protein C (HVEC)
E14g	7.4			7.7			intercellular adhesion molecule-1 precursor (ICAM1); major group rhinovirus receptor; CD54 an
E14h	2.8	7.6	8.2	11.6			cell surface glycoprotein mac-1 alpha subunit precursor; CD11B antigen; leukocyte adhesion re
E14i	2.4	3.0	3.0	2.4			leukocyte adhesion glycoprotein LFA-1 alpha subunit precursor; leukocyte function-associated
E14j				9.2			tumor necrosis factor-inducible protein TSG-6; hyaluronate-binding protein
F01a			2.5				heme oxygenase 2 (HO2)
F01c				0.4	0.3		MPV17 protein
F01d	2.3					2.1	bone morphogenetic protein 4 (BMP4) + bone morphogenetic protein 2B (BMP2B)
F01e	4.6		2.4			4.4	thrombomodulin precursor (THBD; THRM); fetomodulin; CD141 antigen
F01f	2.3						insulin-like growth factor binding protein 1 (IGFBP1); placental protein 12 (PP12)
F01g			2.2				teratocarcinoma-derived growth factor 1 (TDGF1); epidermal growth factor-like CRIPTO protein
F01j			2.8	2.7	0.4		interferon-beta (IFN-beta; IFNB); fibroblast interferon
F01k		2.2					follistatin-related protein precursor
F02b		3.6	3.5	2.0			heat-shock protein 40 (HSP40)
F02f	2.7	2.2	4.5			2.8	vascular endothelial growth factor precursor (VEGF); vascular permeability factor (VPF)
F02g			2.4				endothelial-monocyte activating polypeptide II (EMAP II)
F02h			3.1				delta-like protein precursor (DLK)
F02i			2.6		0.3		alpha calcitonin precursor
F02j			3.3		0.4		interferon-alpha2 precursor (IFN-alpha; IFNA); leukocyte interferon-alphaA (LEIF A); roferon +
F02k					0.5		complement component 5 (C5)
F02l					0.1		proteasome component C3; macropain subunit C3; multicatalytic endopeptidase complex subu
F02n			2.0	2.2			protein C inhibitor (PROCI; PCI); plasma serine protease inhibitor precursor; plasminogen activ
F03a		3.3	2.8	2.4	0.4		heat shock 70-kDa protein 6 (heat shock 70-kDa protein B)
F03b		7.0	6.3	5.2			mitochondrial matrix protein P1 precursor; p60 lymphocyte protein; chaperonin homolog; HUCH
F03e					0.3		macrophage-specific colony-stimulating factor (CSF-1; MCSF)
F03h			2.6		0.3		macrophage inflammatory protein 1 beta precursor (MIP1-beta); T-cell activation protein 2 (AT2
F03i			3.1		0.2		parathyroid hormone-related protein precursor (PTH-RP)
F03j			2.9		0.3		interleukin-10 precursor (IL-10); cytokine synthesis inhibitory factor (CSIF)
F03k			2.1		0.3		puromycin-sensitive aminopeptidase (PSA)
F03l					0.2		proteasome component C5; macropain subunit C5; proteasome gamma subunit; multicatalytic
F04a		9.9	9.7	16.0		2.1	heat shock-related 70-kDa protein 2
F04b		2.4	2.3	2.1	0.3		heat shock 90-kDa protein A (HSP90A; HSPCA); HSP86
F04c		7.9		8.2	0.2		B94 protein

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
F04e					0.2		hepatocyte growth factor activator (HGF activator)
F04f					0.1		T-cell-secreted protein I-309 precursor; small inducible cytokine A1 (SCYA1)
F04h			3.0		0.1		ribonuclease/angiogenin inhibitor (RAI); placental ribonuclease inhibitor; RNH
F04i			2.5		0.3		interleukin-16 (IL-16); lymphocyte chemoattractant factor (LCF)
F04j			2.6		0.1	0.4	interleukin-13 precursor (IL-13); NC30
F04l					0.1	0.2	proteasome component C8; macropain subunit C8; multicatalytic endopeptidase complex subu
F04n					0.1		placental plasminogen activator inhibitor 2 (PAI-2; PLANH2); monocyte ARG-serpin; urokinase
F05a		12.3	8.2	15.0	0.4		glutathione peroxidase (GSHPX1; GPX1)
F05b		7.0	4.1	6.6	0.4	2.1	27-kDa heat-shock protein (HSP27); stress-responsive protein 27 (SRP27); estrogen-regulated
F05c	0.5				0.3		C-reactive protein precursor
F05e					0.2		hepatoma-derived growth factor (HDGF)
F05g					0.1		migration inhibitory factor-related protein 14 (MRP14); calgranulin B; leukocyte L1 complex hea
F05i			2.0		0.2		interleukin-18 precursor (IL-18); interferon-gamma-inducing factor (IFN-gamma-inducing factor)
F05l					0.1		proteasome component C9; macropain subunit C9; multicatalytic endopeptidase complex subu
F05m			2.1				matrix metalloproteinase 7 (MMP7); matrilysin
F05n					0.1		metalloproteinase inhibitor 1 precursor (TIMP1); erythroid potentiating activity (EPA); fibroblast
F06a		7.3		6.7			glutathione peroxidase-gastrointestinal (GSHPX-GI); glutathione peroxidase-related protein 2 (
F06b		3.4	2.8	3.5	0.4		70-kDa heat shock protein 1 (HSP70.1; HSPA1)
F06c		2.6	2.4		0.4		eosinophil granule major basic protein precursor (MBP); pregnancy-associated major basic pro
F06d				3.0			insulin-like growth factor II (IGF2); somatomedin A
F06e					0.2	0.4	endothelin 3 (EDN3; ET3)
F06g				0.3			migration inhibitory factor-related protein 8 (MRP8); calgranulin A; leukocyte L1 complex light s
F06h			2.3				angiotensin-converting enzyme (ACE)
F06i					0.2		interferon gamma precursor (IFN-gamma; IFNG); immune interferon
F06j					0.1	0.4	interleukin-11 (IL-11); adipogenesis inhibitory factor (AGIF)
F06k					0.3	0.5	carboxypeptidase H precursor (CPH); carboxypeptidase E (CPE); enkephalin convertase; proh
F06l					0.4		acrosin precursor
F06m					0.4		matrix metalloproteinase 8 (MMP8); neutrophil collagenase precursor (CLG1); PMNL collagena
F07a		7.2	5.8	5.8			natural killer cell enhancing factor (NKEFB) + thiol-specific antioxidant protein (TSA); thioredox
F07b		2.4	3.0	2.1	0.4		cytosolic superoxide dismutase 1 (SOD1)
F07c			3.0		0.4		monocyte chemotactic protein 4 precursor (MCP4); monocyte chemoattractant protein 4; CK-b
F07d			2.4		0.4		platelet-derived growth factor B subunit precursor (PDGFB; PDGF2); bacaplermin; c-sis
F07e			2.9		0.4		neuroleukin (NLK); glucose-6-phosphate isomerase (GPI); phosphoglucose isomerase (PGI); p

	Hours Post Exposure Ratio						
Gene code	0.5	1	3	6	12	24	Protein/gene
F07f					0.5	0.5	hepatocyte growth factor (HGF); scatter factor (SF); hepatopoeitin A
F07g		2.4			0.4		platelet-derived growth factor A subunit precursor (PDGFA; PDGF1)
F07i					0.4		interleukin-7 (IL-7)
F07j					0.2		interleukin-12 beta subunit precursor (IL-12B); cytotoxic lymphocyte maturation factor 40-kDa s
F07k				0.4	0.3		dipeptidyl-peptidase I precursor (DPP-I); cathepsin C; cathepsin J; dipeptidyl transferase
F07l				0.5	0.3		acrosin-trypsin inhibtor II precursor; HUSI II
F07m				0.5			matrix metalloproteinase 9 (MMP9); gelatinase B; 92-kDa type IV collagenase precursor (CLG4
F08b			5.8	3.7			glutaredoxin
F08d			4.6	3.3			granulocyte-macrophage colony stimulating factor (GM-CSF); CSF2
F08g				2.5			leukemia inhibitory factor precursor (LIF); differentiation-stimulating factor (D factor); melanoma
F08j				0.3			interleukin-12 alpha subunit precursor (IL-12A); cytotoxic lymphocyte maturation factor 35-kDa
F08k				0.3	0.3		cathepsin H precursor
F08l				0.5			leukocyte elastase inhibitor (LEI); monocyte/neutrophil elastase inhibitor) (EI)
F09a				5.2			cytochrome P450 IIF1 (CYP2F1)
F09b		2.2	3.3		0.2		thioredoxin reductase
F09c			9.2		0.4		osteoclast stimulating factor
F09d			7.7	5.0			transforming growth factor-alpha (TGF-alpha; TGFA); EGF-like TGF (ETGF)
F09k					0.4		cystatin-related epididymal spermatogenic protein
F09n		0.5					tripeptidyl-peptidase I precursor; tripeptidyl aminopeptidase; lysosomal peptstatin-insensitive pr
F10a			2.4		0.2		dioxin-inducible cytochrome P450 1B1 (CYP1B1)
F10b			2.2		0.2		NAD(P)H dehydrogenase; quinone reductase; DT-diaphorase; azoreductase; phylloquinone red
F10e	2.3	4.1	5.8	5.6		2.6	T-cell-specific rantes protein precursor; sis delta; small inducible cytokine A5 (SCYA5); rantes p
F10f				4.9			embryonic growth/differentiation factor 1 (GDF1) + UOG-1
F10g				4.4			macrophage inflammatory protein 2 alpha (MIP2-alpha); growth-regulated protein beta (GRO-b
F10h		2.1	2.3	2.8			inhibin alpha subunit precursor (INHA)
F10i	1.8	2.2		2.0			interleukin-1 beta precursor (IL-1 ; IL1B); catabolin
F10l					0.3		inter-alpha-trypsin inhibitor heavy chain H3 precursor (ITI heavy chain H3)
F11a			4.0	4.2			S-mephenytoin 4 hydroxylase; cytochrome P450 IIC9 (CYP2C9) + CYP2C10 + CYP2C17 + CY
F11b			2.3		0.3		25-hydroxy vitamin D3 1-alpha hydroxylase mitochondrial precursor (VD3 1A hydroxylase); 25-
F11c					0.4		bone morphogenetic protein 3B precursor (BMP3B); growth differentiation factor 10 (GDF10); b
F11d			2.3	2.9			granulocyte colony-stimulating factor precursor (G-CSF); pluripoietin; CSF3
F11e			3.1	5.2			macrophage inflammatory protein 1 alpha precursor (MIP1-alpha); tonsillar lymphocyte LD78 a
F11f				3.6			endothelin 2 (ET2)

	Hours Post Exposure Ratio						
Gene code	**0.5**	**1**	**3**	**6**	**12**	**24**	**Protein/gene**
F11g				3.0			placenta growth factors 1 + 2 (PLGF1 + PLGF2)
F11h				5.9			estrogen sulfotransferase (STE; EST1)
F11j		2.2					interleukin-17 precursor (IL-17); cytotoxic T-lymphocyte-associted antigen 8 (CTLA8)
F11k	2.7						insulin-degrading enzyme; insulysin; insulinase; insulin protease
F11n		2.4					myeloblastin precursor (MBN); leukocyte proteinase 3 (PRTN3; PR3); AGP7; Wegener's autoa
F12d			2.0		0.3		transforming growth factor beta2 precursor (TGF-beta2; TGFB2); glioblastoma -derived T-cell s
F12f				3.0			hepatocyte growth factor-like protein; macrophage-stimulating protein (MSP)
F12g		3.2		4.3			granulocyte chemotactic protein 2 (GCP 2); neutrophil-activating peptide ENA-78
F12h				2.8		2.3	insulin-like growth factor-binding protein 3 precursor (IGF-binding protein 3; IGFBP3; IBP3)
F12i		2.2					interleukin-4 precursor (IL-4); B-cell stimulatory factor 1 (BSF-1); lymphocyte stimulatory factor
F12j		2.3	2.0				parathymosin
F12k	2.1						methionine aminopeptidase 2 (METAP2); peptidase M2; initiation factor 2-associated 67-kDa g
F12l	2.0						neuroserpin precursor; protease inhibitor 12
F12n		2.4	2.5				cathepsin L precursor; major excreted protein (MEP)
F13b			2.1		0.4		glutathione S-transferase mu1 (GSTM1; GST1); HB subunit 4; GTH4
F13c		4.4	5.4		0.4		bone morphogenetic protein 2A (BMP2A)
F13e	2.6		3.1	2.0			oncostatin M (OSM)
F13f	2.9		3.4	2.7			thymosin beta-10 (TMSB10; THYB10); PTMB10
F13g				3.5			OX40 ligand (OX40L); GP34; tax-transcriptionally activated glycoprotein 1 (TXGP1)
F13h		4.5		3.7			cellular retinoic acid-binding protein II (CRABP2)
F13i		2.3			0.2	0.3	interleukin-6 precursor (IL-6); B-cell stimulatory factor 2 (BSF2); interferon beta-2 (IFNB2); hybr
F13j			2.1		0.4		thymosin beta 4; FX
F13k		2.1	2.5		0.4		proteasome activator HPA28 subunit beta
F13l	1.9			0.5	0.5		cytoplasmic antiproteinase 2 (CAP2); protease inhibitor 8
F13m	2.3					0.4	cathepsin D precursor (CTSD)
F14b					0.3		glutathione S-transferase A1 (GTH1; GSTA1); HA subunit 1; GST-epsilon
F14f		3.7	3.4				connective tissue growth factor precursor (CTGF)
F14h	2.0	2.4					corticotropin-releasing factor-binding protein
F14j			2.9				Wnt-13
F14k					0.4		proteasome inhibitor HPI31 subunit
F14l		2.8	5.4	2.7	0.2	0.4	bikunin; hepatocyte growth factor activator inhibitor 2
F14m						0.4	metalloprotease/disintegrin/cysteine-rich protein precursor (MDC9)
F14n				4.1			zinc finger X-chromosomal protein (ZFX)

	Hours Post Exposure Ratio						
Gene code	**0.5**	**1**	**3**	**6**	**12**	**24**	**Protein/gene**
G11	0.5	3.8			0.2	0.0	ubiquitin
G13		2.3					phospholipase A2
G27	0.2					0.3	liver glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
G29						0.4	brain-specific tubulin alpha 1 subunit (TUBA1)
G31				0.5			HLA class I histocompatibility antigen C-4 alpha subunit (HLAC)
G47		0.0				0.3	40S ribosomal protein S9