Kidney Toxicogenomics of Chronic Potassium Bromate Exposure in F344 Male Rats

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Abstract

Background: Potassium bromate ($KBrO_3$), used in both the food and cosmetics industry, and a drinking water disinfection by-product, is a nephrotoxic compound and rodent carcinogen. To gain insight into the carcinogenic mechanism of action and provide possible biomarkers of $KBrO_3$ exposure, the gene expression in kidneys from chronically exposed male F344 rats was investigated.

Methods: Male F344 rats were exposed to $KBrO_3$ in drinking water for 52 and 100 wk. Kidneys were removed, frozen, and stored at –80ºC, then used for Affymetrix microarray analysis. Gene expression patterns were examined using a non-carcinogenic (20 ppm) and carcinogenic dose (400 ppm) at 52 wk, and compared to 100 wk high dose (400 ppm) and adenoma gene expression.

Results: Statistical analysis revealed 144, 224, 43, and 994 genes out of 15866 from the 52 wk low, 52 wk high, 100 wk high, and adenomas respectively, were differentially expressed when compared to control kidneys. Gene ontology classification of the 52 wk high dose showed alterations of gene transcripts involved in oxidative stress, lipid metabolism, kidney function/ion transport, and cellular function. In a comparison of kidney development gene expression, alterations were seen in the adenomas but not in the 52 wk bromate-treated kidneys. However, the normal kidney from the high dose group resembled the adenoma expression pattern with early kidney development genes being up-regulated and adult phase genes being down-regulated. Moreover, eight genes were identified which could serve as biomarkers of carcinogenic exposure to bromate. The most promising of these was Pendrin, or Slc26a4, a solute carrier of chloride and iodide active in the kidney, thyroid, and inner ear. All these tissues are targets of $KBrO_3$ toxicity. Expression array results were verified with quantitative real-time rtPCR.

Conclusions: These data demonstrate that the 400 ppm carcinogenic dose of $KBrO_3$ showed marked gene expression differences from the 20 ppm non-carcinogenic dose. Comparison of kidney development gene expression showed that the adenoma patterns were more characteristic of embryonic than adult kidneys, and that the normal kidney from the high dose group resembled the adenoma-like gene expression pattern. Taken together, the analysis from this study identifies potential biomarkers of exposure and illuminates a possible carcinogenic mode of action for $KBrO_3$.

Abbreviations: CD: collecting duct, DBPs: disinfection by-products, DCT: distal convoluted tubules, DEG: differently expressed genes, K: potassium, KBrO3: potassium bromate, LH: loop of Henle, ppm: part per million, PCT: proximal convoluted tubules, QRT-rtPCR: Quantitative Real Time- rtPCR, THMs: trihalomethanes.

Keywords: drinking water, bromate, disinfection by-product, gene expression, biomarker.

Background

The disinfection of public drinking water over the past century has dramatically decreased infectious waterborne diseases and is a hallmark of American public health policy. The benefits of drinking water disinfection are well recognized, however, an undesirable side effect is the production of disinfection by-products (DBPs). These DBPs are formed when disinfectants such as chlorine, chloramine, and ozone react with organic and inorganic matter in water. In the mid 1970's, it was discovered that

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trihalomethanes (THMs) were produced in chlorination of drinking water and that they are carcinogenic in laboratory animals (Rook, 1974; Bellar et al. 1974; NCI, 1976). Because of the potential health risks associated with THMs, in 1979 the U.S. Environmental Protection Agency (U.S. EPA) began the regulation of DBPs in the water supply. Initially, concern focused on the trihalomethanes (THMs), but it is now recognized that a wide variety of DBPs are produced during chlorination.

In an effort to reduce exposure to halogenated DBPs, many utilities have switched to ozonation as an alternative treatment method to chlorination. Ozonation is also preferred because it reduces the turbidity of the water and is effective in treating chlorine resistant organisms. However, if ozonation is performed using surface water high in bromide content, brominated by-products, such as bromate ion (BrO_3^-), can be generated (Fiessinger et al. 1985). Potassium bromate ($KBrO_3$), a salt of the bromate ion, is nephro- and neurotoxic in humans and carcinogenic in rodents (IARC, 1986; Kurokawa et al. 1990). Under the current guidelines for cancer risk assessment (U.S. EPA, 1986), bromate is classified as a probable human carcinogen due to its kidney carcinogenicity in male and female rats following exposure in drinking water (Kurokawa et al. 1983, 1986a, 1986b; DeAngelo et al. 1998; Wolf et al. 1998). A dose-response relationship in rat kidneys was observed in progressive severity from renal dysplastic foci, preneoplastic lesions, through renal adenomas, and finally renal carcinoma (Kurokawa et al. 1986a; DeAngelo et al. 1998; Wolf et al. 1998).

Mechanistic studies by Umemura et al. (2004, 2006) demonstrated dose-dependent changes in oxidative stress and cell proliferation parameters at carcinogenic doses of potassium bromate in male and female rat kidneys. After four weeks of continuous drinking water exposure, 8-oxodeoxyguanosine levels (8-oxodG), an indicator of promutagenic oxidative DNA damage (Wood et al. 1990; Shibutani et al. 1991), were significantly elevated in male and female rats administered potassium bromate at concentrations of 250 ppm and higher. In addition, BrdU labeling, an indicator of cell proliferation, was also increased in the proximal tubule of female rats at similar concentrations and in male rats at concentrations as low as 30 ppm. It was suggested, however, that the susceptibility of the male rat to increased cell proliferation at lower concentrations was probably attributable to increased α2u-globulin in the male rat proximal tubule. These studies provide important information regarding the potential mechanism of action of potassium bromate carcinogenicity.

The primary objective in this study was to examine renal gene expression differences in male F344 rats exposed to a non-carcinogenic and carcinogenic dose (20 and 400 ppm, respectively) of $KBrO_3$ in drinking water for 52 wk. Furthermore, renal gene expression from the high dose (400 ppm) and adenomas from 100 wk exposed animals were examined and compared to the 52 wk exposure groups. This was accomplished by extracting kidney and adenoma RNA from rats exposed in the previous 1998 DeAngelo et al. study. Comparisons of gene expression profiles between these groups were used to identify functional pathways and individual genes that might contribute to the carcinogenic mechanism of action for $KBrO_3$ and provide insight into potential biomarkers of exposure.

Methods

Animal maintenance

Complete study details were published previously (DeAngelo et al. 1998). Briefly, $KBrO_3$ (99%; CAS 7758–01–2) dissolved in deionized water at concentrations of 0, 20, 100, 200, and 400 ppm was administered to male F344 rats as the sole water source for 12, 26, 52, 78, or 100 wk. Rats, 28 to 30-days-old, were allowed to acclimate for 1 wk and then randomly assigned to treatment groups. Treatment rooms were maintained at 20–22ºC and 40–60% humidity with a 12-hr light: dark cycle. Rats were housed 3 per cage on wood chips and provided Purina Rodent Laboratory Chow (St. Louis, MO) and water ad libitum. Animals were observed daily and moribund animals were euthanized and necropsied. Six animals from each group were euthanized by CO_2 asphyxiation and necropsied after 52 wk of treatment. At necropsy, kidneys were removed, washed, flash frozen in liquid nitrogen, and stored at –80ºC.

Microarray experiment

For the microarray experiment, rats exposed to 0, 20, and 400 ppm $KBrO_3$ for 52 wk, were selected because they represent a control, non-carcinogenic,

and carcinogenic dose, respectively (DeAngelo et al. 1998; Wolf et al. 1998). Kidneys from animals exposed to 20 and 400 ppm $KBrO_3$ for 52 wk were compared to kidneys from 52 wk control animals. In addition, kidneys from rats exposed to 0 and 400 ppm $KBrO_3$ and two adenomas from animals exposed for 100 wk were also examined. For this comparison, kidneys from the 100 wk high dose and adenomas were compared to kidneys from 100 wk control animals. Kidney RNA was extracted from three animals per dose group in the 52 wk exposure, and two animals per dose group in the 100 wk samples. RNA from two 100 wk adenomas were also extracted. RNA extraction was performed by acid guanidinium isothiocynate-phenol-bromochloropropane treatment (Tri reagent; Molecular Research Center, Inc., Cincinnati, OH, U.S.A.) and purified on an affinity resin (RNeasy; Qiagen, Valencia, CA) according to manufacturer instructions. Extractions were tested on an Agilent Bioanalyzer to determine RNA quality. Clearly defined 28S and 18S bands were observed on all samples used for microarray analysis. Samples showing degradation were not used for microarray analysis. Microarray procedures were performed as recommended by the manufacturer of the GeneChip system (Affymetrix, Inc, Santa Clara, CA, U.S.A.). The gene expression probe array used was the Rat Expression Array 230A gene chip containing 15,866 probe sets. For each animal, one kidney sample was used for gene expression analysis. Chips were examined by M versus A plots, chip clustering, and principal component analysis to determine outlying chips. Arrays demonstrating poor hybridization were not used for analysis.

Data analysis

The resulting image files (.cel) were normalized using the procedure of Li and Wong [D-chip] (2001a; 2001b) and the determination of significant differences between groups were performed by using the web interface of Cyber-T [http://visitor.ics.uci.edu/genex/cybert/](Baldi and Long, 2001). A tutorial for using both D-chip and Cyber-T, found on the Cyber-T website, was used as the procedure for this analysis. Multiple testing correction and false discovery rate test were conducted by using the method of Benjamini and Hochberg (1995) on P-values: $P = < (i/m)q$, where i = # of genes accepted at selected p, m = total # genes, and q = desired false discovery rate. Determination of false positives was performed by: fp = (Pbh * total # genes). This resulted in a unique P-value for each comparison. Genes expressed at levels different than control such that a corrected P-value less than 0.05 were classified as differentially expressed genes (DEG) and annotated using NetAffx (http://www.affymetrix.com). Individual literature review on each DEG was conducted and used to classify genes into functional groups.

Kidney developmental gene response in $KBrO_3$ induced adenoma

Gene expression analysis has become sufficiently standardized that comparisons between experimental datasets can yield insights into biological processes such as the differentiation of renal cells. In this vein, we compared our dataset of gene expression in whole adult rat kidney to that collected and analyzed by Stuart et al. (2001). This study performed the first high density oligonucleotide microarray investigation of kidney development using the 8,740 gene Affymetrix rat U34A microarray. Multiple developmental stages were examined, including embryonic day 13 (E13), E15, E17, E19, newborn, 1 wk, and adult. Cluster analysis defined five temporal expression groups. Early group consisted of genes with very high expression in the early embryonic kidney, many with roles in protein translation and DNA replication. Prenatal group consisted of genes that peaked in mid-embryogenesis and contained many transcripts specifying proteins of the extracellular matrix. Neonatal group consisted of transcripts that peaked in the neonatal period and contained a number of retrotransposon RNAs. Steady group contained genes that steadily increased in relative expression levels throughout development, including many genes involved in energy metabolism and ion and water transport. Adult group consisted of genes with relatively low levels of expression throughout embryogenesis but with markedly higher levels in the adult kidney; this group included a heterogeneous mix of transporters, detoxification enzymes, and oxidative stress genes.

Lists of the five groups of genes identified in Stuart et al. (2001), were obtained from the supplemental data website (http://organogenesis.ucsd.edu/). These gene-lists used U34A probe set

identifiers. We determined the Rat Expression Array 230A probe set identifiers that corresponded to the U34A probe sets using the best match comparison file from Affymetrix. For the five kidney development groups mentioned above, there were 838, 168, 61, 510, and 193 genes located on the U34 array and 676, 126, 42, 428, and 155 genes located on the Rat Expression Array 230A array. The minimum sequence identity for a given probe set between the U34A and Rat Expression Array 230A array was 93% and the average identity was greater than 99%. For each group, the genes that were identified in the Affymetrix best match comparison file constituted the gene members for that group in subsequent analysis.

Real time PCR determination of gene expression

Quantitative real-time rtPCR (QRT-rtPCR) data was collected from fresh aliquots of the total RNA samples used to obtain array data. On average, 85 ng of total RNA was loaded in a one-step QRT-rtPCR reaction. Multiscribe reverse transcriptase was used to generate the cDNA template followed by amplification with AmpliTaq Gold DNA polymerase (Applied Biosystems). Target specific assays were either custom designed or purchased from Applied Biosystems' TaqMan Assays on Demand. All reactions were performed according to manufacturer's procedures. The QRT-rtPCR cycling parameters were: 48ºC, 45 min cDNA synthesis; 95ºC, 10 min AmpliTaq Gold (Applied Biosystems) activation; and 40 cycles of amplification at 94ºC, 15 sec melting followed by 60ºC, 1 min annealing/extension. Reaction volumes totaled 20 μl and were run in triplicate in 384 well plates on an ABI prism 7900HT. Based on the array data, *Rpl*27 (ribosomal protein L27) was chosen as a reference gene since it exhibited no differential gene expression across treatment groups and showed similar expression levels to the genes of interest. Confirmation that *Rpl*27 was not differentially expressed across treatment groups was confirmed by QRT-rtPCR analysis (data not shown).

Results

Microarray analysis

The results of the statistical comparison among tissues from the 52 wk control, low, and high dose groups, and the 100 wk control, high, and adenoma groups are given in Table 1. This study has been archived in ArrayExpress under accession number E-TOXM-21.

Analysis of gene expression

Gene ontology (GO) analysis of DEG expression in the kidney from male F344 rats exposed to low (20 ppm) and high (400 ppm) $KBrO_3$ for 52 wk are shown in Table 2. The individual functional group information composed of gene symbol and name, Affymetrix and accession numbers, and fold change are given for oxidative stress (Table 3), and kidney function / ion transport (Table 4) genes. In addition to the above mentioned groups, a large amount of lipid metabolism, oxidoreductase, and cellular function genes were observed and are shown as additional files: "lipid metabolism table.pdf, oxidoreductase table.pdf and cellular function table.pdf." A total of 99 and 139 genes were used to provide an interpretive basis for differences in

Table 1. Results of the statistical comparison between 52 wk low, 52 wk high, 100 wk high, and 100 wk adenoma and their corresponding control. Shown are the numbers of genes less than 0.01 that contribute to the p-value calculation as given in the methods. The resultant p-value and the number of false positives are shown, as well as the number of differentially expressed and annotated genes. The gene expression probe array used was the Affymetrix Rat Expression Array 230A gene chip containing 15,866 probe sets.

Treatment	Genes < 0.01	P-value	False Positives	Differentially Expressed	Annotated
52 wk low (3)*	470	0.00128	21	144	99
52 wk high (3)	603	0.00189	30	224	139
100 wk high (2)	244	0.000766	13	43	27
100 wk adenomas (2)	1431	0.0045	72	994	671

* The sample size for the comparison is shown with the treatment group.

Table 2. Gene ontology (GO) analysis of differentially expressed genes (DEG) in kidneys from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk. Results are the number of differentially expressed genes within a functional group that were either up or down regulated, with group total. Functional analyses for all groups were compiled by individual gene review.

	Control versus Low			Control versus High		
Functional Group*	**Down**	**Up**	**Total**	**Down**	**Up**	**Total**
Oxidative Stress	4	0	4	5	5	10
Lipid Metabolism	6	4	10	9	1	10
Kidney Function	10	3	13	20	3	23
Oxidoreductase	10	1	11	13	0	13
Cell Function	30	10	40	20	22	42

*A total of 99 and 139 genes were used for gene ontology analyses from low and high exposure concentrations respectively.

expression that were observed in the kidneys of rats treated with non-carcinogenic and carcinogenic doses, respectively.

Analysis of the low dose showed a general suppression of gene expression with 71% of genes down-regulated relative to control tissues compared to 56% in the high dose. The most notable down-regulated groups in the low dose were oxidative stress and kidney function with 100% and 77% down-regulated, respectively. In the high dose, 87% of genes in kidney function and 90% of genes in lipid metabolism groups were down-regulated. Overall these changes suggest a general suppression of gene expression in the low dose group, especially those genes involved in oxidative stress and kidney function. This is in contrast to the high dose, where kidney function and lipid metabolism genes are suppressed in concert with an increased amount of oxidative stress related genes.

A comparison of genes whose expression was significantly altered in the 52 wk high dose (400 ppm) and the 100 wk adenomas is given as an additional file: "similar 52,100, adenomas.pdf." In this comparison, 35 genes were down-regulated relative to the kidney of control animals, 3 were

Table 3. List of differentially expressed oxidative stress genes in kidney from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk. All comparisons were made between the specific treatment group and their corresponding control.

Gene Symbol[a]	**Gene Name[a]**	**Affymetrix No.[a]**	**Accession No.[a]**	**Fold Change**
Low dose				
*Dscr*1	Down syndrome critical region homolog 1	1388686_at	NM_153724	–1.4
*Hspb*1	Heat shock protein	1367577_at	NM_031970	–1.5
*Txnrd*1	Thioredoxin reductase 1	1386958_at	NM_031614	–1.4
Xdh	Xanthine dehydrogenase	1369973_at	NM_017154	–1.4
High dose				
*Dscr*1	Down syndrome critical region homolog 1	1388686_at	NM_153724	–1.3
Gnmt	Glycine N-methyltransferase	1387672_at	NM_017084	–1.3
*Gsta*2	Glutathione S-transferase A2	1368180_s_at	NM_017013	–1.2
*Hspbap*1	Heat shock associated protein	1368195_at	NM_134419	–1.8
*Pex*11*a*	Peroxisomal biogenesis factor 11a	1379361_at	NM_053487	–1.3
*Ccng*1	Cyclin G1	1367764_at	NM_012923	1.3
Cp	Ceruloplasmin (ferroxidase)	1368418_a_at	NM_012532	1.3
Gclm	Glutamate-cysteine ligase, modifier subunit	1370030_at	NM_017305	1.3
*Gstm*1	Glutathione S-transferase M 1	1386985_at	NM_017014	1.4
*Gstp*1	Glutathione S-transferase Pi 1	1388122_at	NM_012577	1.3

[a] Gene symbols and accession numbers from Affymetrix Netaffx (http://www.affymetrix.com/analysis/index.affx).

Table 4. List of differentially expressed kidney function/ion transport genes in kidney from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk. All comparisons were made between the specific treatment group and their corresponding control.

Gene Symbol[a]	Gene Name[a]	Affymetrix No.[a]	Accession No.[a]	Fold Change
Low dose				
*Aqp*1	Aquaporin 1	1387651_at	NM_012778	–1.3
*Atp*1*a*1	ATPase, Na+/K+ transporting, alpha 1	1371108_a_at	NM_012504	–1.2
*Dscr*1	Down syndrome critical region homolog 1	1388686_at	NM_153724	–1.4
G6pc	Glucose-6-phosphatase,	1386944_a_at	NM_013098	–1.7
*Ramp*3	Receptor (calcitonin) activity modifying protein 3	1387389_at	NM_020100	–1.9
Sgk	Serum/glucocorticoid regulated kinase	1367802_at	NM_019232	–1.6
*Slc*15*a*1	Solute carrier family 15, member 1	1369381_a_at	NM_057121	–1.5
*Slc*16*a*1	Solute carrier family 16, member 1	1386981_at	NM_012716	–2.8
*Slc*22*a*1	Solute carrier family 22, member 1	1368191_a_at	NM_012697	–1.5
Xdh	Xanthine dehydrogenase	1369973_at	NM_017154	–1.4
Calca	Calcitonin/calcitonin-related polypeptide, alpha	1370775_a_at	NM_017338	1.4
*Cldn*16	Claudin 16	1369184_at	NM_131905	1.4
*Slc*21*a*4	Kidney specific organic anion, scf 21, member 4	1368498_a_at	NM_030837	1.4
High dose				
*Aqp*2	Aquaporin 2	1368568_at	NM_012909	–1.4
*Aqp*3	Aquaporin 3	1387100_at	NM_031703	–1.4
*Calb*1	Calbindin 1	1370201_at	NM_031984	–1.3
*Clcnk*11	Chloride channel K1-like	1388175_at	NM_173103	–1.4
*Dscr*1	Down syndrome critical region homolog 1	1388686_at	NM_153724	–1.4
*Edn*1	Endothelin 1	1369519_at	NM_012548	–1.4
*Kcnj*16	Potassium inwardly-rectifying channel, J16	1373991_at	AI411366	–1.2
*Kcnq*1	Potassium voltage-gated channel, KQT-like 1	1368371_at	NM_032073	–1.4
Ngfg	Nerve growth factor, gamma subunit	1367961_at	NM_031523	–1.9
*Prkwnk*4	Protein kinase, lysine deficient 4	1389662_at	NM_175579	–1.6
*Scnn*1*a*	Sodium channel nonvoltage-gated 1A	1387104_at	NM_031548	–1.3
*Scnn*1*g*	Sodium channel nonvoltage-gated 1G	1370481_at	NM_017046	–1.5
*Slc*5*a*2	Solute carrier family 5, member 2	1368414_at	NM_022590	–1.4
*Slc*9*a*3	Solute carrier family 9 (Na/H exchanger), isoform 3	1387542_at	NM_012654	–1.3
*Slc*12*a*3	Solute carrier family 12 (Na/Cl transporters), 3	1387230_at	NM_019345	–1.4
*Slc*13*a*3	Solute carrier family 13 Na+ transport, member 3	1368047_at	NM_022866	–1.3
*Slc*22*a*5	Solute carrier family 22, member 5	1367950_at	NM_019269	–1.3
*Slc*22*a*8	Solute carrier family 22, member 8	1368461_at	NM_031332	–1.3
*Slc*26*a*4	Pendrin, solute carrier family 26, member 4	1368193_at	NM_019214	–4.6
*Slc*37*a*4	Solute carrier family 37, member 4	1386960_at	NM_031589	–1.3
*Agtr*2	Angiotensin II receptor, type 2	1369711_at	NM_012494	1.5
*Cldn*16	Claudin 16	1369184_at	NM_131905	1.6
Cp	Ceruloplasmin (ferroxidase)	1368418_a_at	NM_012532	1.3
*Slc*12*a*1	Solute carrier family 12 (Na/K/Cl), member 1	1368548_at	NM_019134	1.5

[a] Gene symbols and accession numbers from Affymetrix Netaffx (http://www.affymetrix.com/analysis/index.affx).

up-regulated, and 9 genes did not show the same direction of change. The majority of these down-regulated genes were associated with kidney function.

A common list of genes whose expression was significantly altered relative to control tissue in the 52 wk high (400 ppm), the 100 wk high (400 ppm), and the 100 wk adenoma groups is given in Table 5. In most cases, the magnitude of fold change increases with duration of exposure and/or in tumor tissue. These genes may represent potential biomarkers of bromate exposure and effect

because the magnitude of their alteration is time-dependent and/or a larger number of renal cells incorporate these transcript changes with continued exposure. Since tumor tissue is theoretically the product of clonal expansion of target cells, these eight genes might also aid in the development of useful tumor markers of $KBrO_3$ carcinogenicity. These genes were: *Calb*1 (Calbindin 1), *Gp*2 (Glycoprotein 2), *Klk*7 (Kallikrein 7), an EST (LOC362802), *Ngfg* (Nerve growth factor, gamma), *Prps*2 (Phosphoribosyl pyrophosphate synthetase 2), *Slc*12*a*3 (Solute carrier family 12, member 3), and *Slc*26*a*4 (Solute carrier family 26, member 4).

Kidney developmental gene response in $KBrO_3$ induced adenoma

The processes involved in organ development, including cell proliferation, apoptosis, cell adhesion, and differentiation, are processes that are disregulated during carcinogenesis. Therefore, a comparison was made between a previous gene expression profile observed in rat kidney development to that of the kidney adenoma profile from this study. Stuart et al. (2001) identified five groups of genes whose expression characterized stages of rat kidney development. As described in the Methods, genes were identified in the current study that matched specific development groups. Figure 1A shows the average fold change of each group compared to control for low bromate, high bromate and adenoma. Early and prenatal genes, associated with cell proliferation and laying down the extracellular matrix, are up-regulated in the adenoma. Steady and adult genes, associated with energy metabolism, transport, detoxification, and oxidative stress response, are down-regulated in the adenoma. This adenoma expression profile shows the up-regulation of genes prevalent in early kidney development and down-regulation of adult stage genes. This observation is in agreement with the proliferation and de-differentiation profiles seen in classical tumor development.

Adenoma expression profile resemble high dose bromate kidney

The adenoma demonstrated an expression profile for kidney development gene groups early, prenatal, steady, and adult that was distinct from control and bromate treated kidneys. In order to determine if the adenoma profile, as determined above, was present in part, in either the low or high $KBrO_3$ exposed animals, the top ten DEGs were selected from each of the four groups mentioned above. Figure 1B shows the average fold change of each group compared to control for low and high dose bromate. This figure illustrates that the high dose kidney, but not the low, did resemble the adenoma expression pattern with the early and prenatal phase genes being up-regulated and the steady and adult phase genes being down-regulated.

Real time PCR determination of gene expression

Quantitative Real Time rtPCR assays were conducted to verify gene expression of genes deemed biologically relevant based on pathway

Table 5. List of similar genes between 52 wk high dose potassium bromate (400 ppm), 100 wk high dose, and adenomas (n = 2) that occurred at 100 wk. This list of genes may be usable as tumor marker genes. All comparisons were made between the specific treatment group and their corresponding control.

Gene Symbol[a]	Affymetrix No.[a]	Accession No.[a]	52 wk High Dose Fold Change	100 wk High Dose Fold Change	Adenoma Fold Change
*Calb*1	1370201_at	NM_031984	–1.3	–11.4	–153.4
*Gp*2	1386933_at	NM_134418	–2.2	–11.9	–18.6
Klk7	1387820_at	NM_012593	–1.4	–12.1	–57.3
*LOC*362802	1376239_at	NM_001014199	–1.9	–3.6	–2.6
Ngfg	1367961_at	NM_031523	–1.9	–20.5	–77.3
*Prps*2	1375932_at	NM_012634	–1.4	–2.9	–2.6
*Slc*12*a*3	1387230_at	NM_019345	–1.4	–4.8	–6.6
*Slc*26*a*4	1368193_at	NM_019214	–4.6	–6.0	–6.3

[a] Gene symbols and accession numbers from Affymetrix Netaffx (http://www.affymetrix.com/analysis/index.affx).

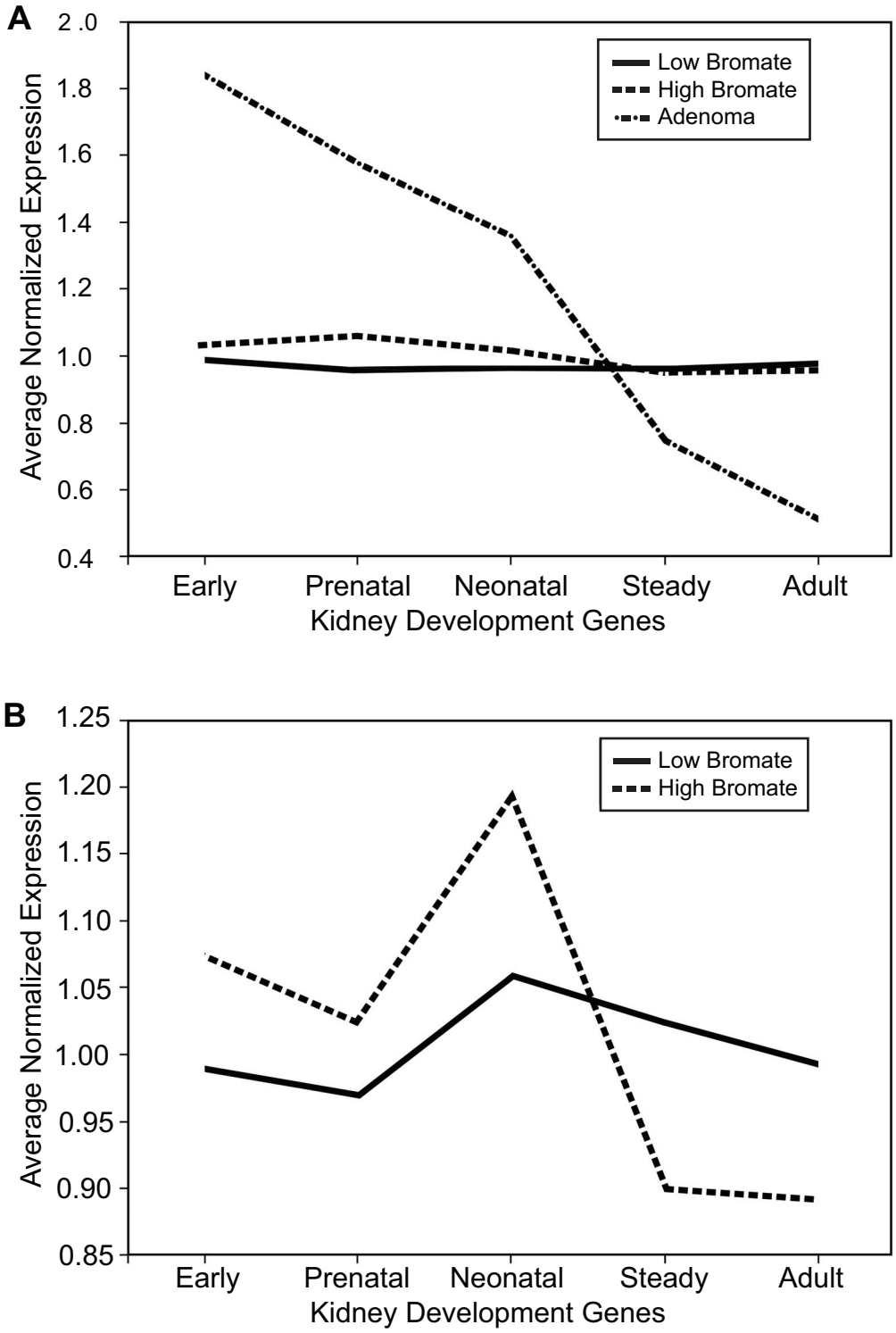

Figure 1A. Kidney development gene response in F344 male rats exposed to $KBrO_3$ in drinking water. Kidney development gene groups were defined in Stuart et al. (2003) and applied to our data as described in the methods section. For each group of genes, the expression was normalized to control and an average response was calculated for: 52 wk low (20 ppm) $KBrO_3$, 52 wk high (400 ppm) $KBrO_3$, and 100 wk $KBrO_3$-induced adenomas. For each group, the average fold change normalized to control was plotted on the y-axis.

Figure 1B. Gene expression pattern in F344 male rats exposed to high $KBrO_3$ mimics adenoma expression pattern. Kidney development gene groups were defined in Stuart et al. (2003) and applied to our data as described in the methods section. The differentially expressed genes in the 100 wk $KBrO_3$-induced adenomas were categorized into kidney development groups. The top ten genes from each developmental group were then used in the 52 wk low (20 ppm) and high (400 ppm) $KBrO_3$ exposed animals. For these 50 genes, the high exposure group showed a similar expression pattern to the adenomas across kidney development groups.

analysis and genes that could possibly be used as biomarkers of exposure. Figure 2 shows that QRT-rtPCR results confirmed the expression patterns of the genes tested. However, differences in magnitude of change were observed, for example *Aqp*2 shows a –1.4 microarray fold change, but an –18.5 QRT-rtPCR fold change. This discrepancy in the magnitude of fold change between microarray data and rtPCR data has been noted in other genomics studies and may be attributable to the superior utility of the rtPCR procedure in quantifying transcript abundance (Crosby et al. 2000). The specific genes selected, including target sequence are given in additional file: "QRT-rtPCR_table.pdf."

Discussion

In the DeAngelo et al. (1998) and Wolf et al. (1998) studies, male F344 rats exposed to 400 ppm $KBrO_3$ for 52 wk developed kidney cancer, while those exposed to 20 ppm did not. This study used the same animal kidneys from that study to determine if a difference in gene expression could be discerned between the two doses. Examining global gene expression for each dose allowed categorizing of genes into the following groups; oxidative stress, lipid metabolism, kidney function/ion transport, cellular function, and oxidoreductase function.

Oxidative stress response

Reactive oxygen species (ROS) are natural by-products produced by the metabolism of O_2 during aerobic respiration. Oxidative stress occurs when there are increases in ROS production, and/or depressed antioxidant defense. Glutathione (GSH) is an important component of the antioxidant and detoxification systems in most tissues. However, under normal cellular conditions, $KBrO_3$ induces oxidative DNA damage in the form of 8-oxodeoxyguanosine (8-oxodG) that is dependent on GSH and other sulfhydryls (Ballmaier and Epe, 1995; Murata et al. 2001). Bromate-induced oxidative DNA damage is caused by the reduction of bromate to bromine oxides and bromine radicals generated by sulfhydryls like GSH. *In vivo*, it has been suggested that sulfhydryls, present at the brush borders of the proximal convoluted tubule (Zager and Burkhart, 1998), are important in the generation of bromine oxides and bromine radicals (Murata et al. 2001). The relative reduction of extracellular and intracellular bromate and its metabolites also

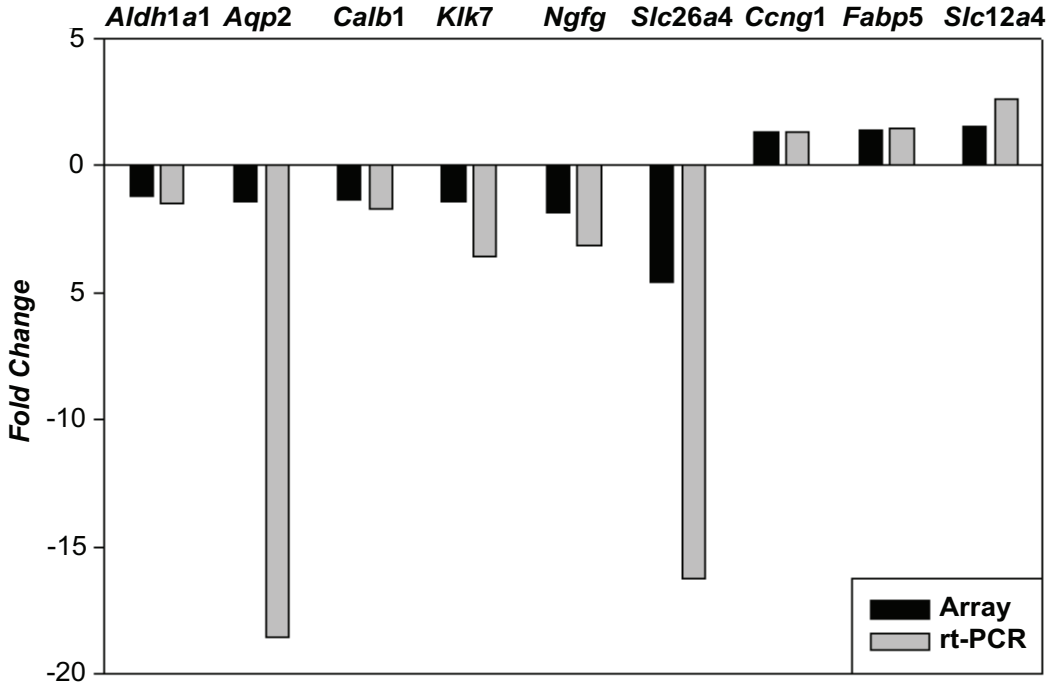

Figure 2. Affymetrix array and QRT-rtPCR fold-change results of select kidney genes of interest from male F344 rats exposed to 400 ppm $KBrO_3$ for 52 wk.

affect the delivery of bromate ions to target macromolecules. Under high dose conditions, more bromate may be delivered to target cells for intracellular activation due to reduced systemic metabolism and/or antioxidant defenses (Delker et al. 2006).

Altered gene expression associated with oxidative stress in the low dose gave only four down-regulated genes *Dscr*1*, Hspb*1*, Txnrd*1*, and Xdh.* It has been reported that in oxidative stress conditions, *Dscr*1*, Hspb*1, and *Txnrd*1 are elevated (Ermak et al. 2004; Dunlop and Muggli, 2000; Yamawaki and Berk, 2005), and increased levels of *Xdh* are associated with free radical generation (Sanhueza et al. 1992). *Dscr*1 is regulated by both oxidative stress and Ca+ levels (Lin et al. 2003), thus it may be influenced more by Ca+ ion concentration than oxidative stress. Although these genes are associated with oxidative stress, they are all down-regulated and appear to reflect inhibition, chronic adaptation, or simply no induction of oxidative stress in animals exposed to 20 ppm $KBrO_3$.

A larger involvement of proposed oxidative stress genes are seen in the high dose group with 10 DEG; 5 down- and 5 up-regulated. In the high dose response, the *Dscr*1 and a heat-shock gene *Hspbap*1, which are up-regulated in response to ROS, are down-regulated in this study. This is an unexpected response following exposure to a known producer of ROS. However, the expression observed in this experiment is that of a kidney chronically exposed to a carcinogenic dose of $KBrO_3$, and may represent an adaptive gene response. Two additional down-regulated genes, *Gnmt* and *Gsta*2, are involved with GSH homeostasis and GSH conjugation, respectively.

The up-regulated oxidative stress genes include *Ccng*1*, Cp, Gclm*, and two glutathione transferases, *Gstm*1 and *Gstp*1. Studies have demonstrated that cyclin G1 (*Ccng*1) plays roles in G2/M arrest, damage recovery, and growth promotion after cellular stress (Kimura et al. 2001). Cyclin G1 accomplishes this by regulating the activity of p53 (Jensen et al. 2003). This may indicate possible oxidative stress induced DNA damage followed by increased cellular proliferation. *Cp* is a plasma protein that is up-regulated in response to oxidative stress and functions as a copper transporter and antioxidant. *Gclm* is involved in glutathione biosynthesis and is up-regulated as a defensive response to oxidative stress (Mathers et al. 2004). Two glutathione S-transferases are up-regulated, *Gstm*1 and *Gstp*1. Both are involved in the conjugation of reduced GSH to a wide number of exogenous and endogenous hydrophobic electrophiles. Taken together, these data support the finding that $KBrO_3$ does produce an oxidative stress response in the kidney at high doses, however the magnitude of expression was not large, resulting in ten genes with relatively small fold-change. This may be characteristic of a kidney that has developed a resistant morphological phenotype to $KBrO_3$-induced oxidative stress due to long-term chronic exposure.

Lipid metabolism

Altered lipid metabolism and lipid peroxidation have been associated with $KBrO_3$ exposure (Chipman et al. 1998). Cellular fatty acids are readily oxidized by reactive oxygen species to produce lipid peroxyl radicals and lipid hydroperoxides (Rice-Evans and Burdon, 1993). It is generally accepted that oxidative stress can lead to the oxidative degradation of lipids (Moller and Wallin, 1998), which can disrupt normal lipid metabolism. Examination of genes associated with lipid metabolism in the low dose exposure showed 6 down-regulated (*Apoe, Cebpb, Chk, Cyp*2*e*1*, Cyp*4*a*10*, and Fads*1) and 4 up-regulated genes (*Abcg*1, *Calca, Gpd*2*, and Nr*1*d*1). The down-regulated genes dealt primarily with fatty acid metabolism and lipid biosynthesis, while the up-regulated genes were associated with lipid homeostasis and metabolism. In the high dose, nine genes were down-regulated (*Acaa*2*, Acsl*3*, Apoc*3*, Apom, Cyp*2*e*1*, Cyp*4*a*10*, Edn*1*, Ehhadh, Hadhsc*), and one up-regulated (*Fabp*5). These genes were primarily involved in fatty acid and lipid metabolism. Overall, gene expression for lipid metabolism is suppressed, with only one up-regulated gene observed in the high dose, contrary to expectations of $KBrO_3$ induced lipid peroxidation. This is consistent with the work of Umemura et al. (2004) which contained no evidence of lipid peroxidation by chronic treatment of rats with carcinogenic doses of $KBrO_3$.

Kidney function and ion transport

The location of DEG genes involved with kidney function and ion transport within the nephron of the kidney is shown in Figure 3. For the low dose (Fig. 3A), of the 9 genes where specific locations could be identified, 6 were in the proximal convo-

luted tubules (PCT), 2 in the ascending loop of Henle (LH), and 1 in the distal convoluted tubules (DCT). Within the PCT, only the kidney specific organic anion transporter was up-regulated. For the high dose (Fig. 3B), 7 genes were found in the PCT, 4 in the ascending LH, 5 in the DCT, and 6 genes in the collecting duct (CD). All genes in the PCT and CD were down-regulated, whereas *Slc*12*a*1 and *Cldn*16 were up-regulated in the ascending loop and *Agtr*2 was up-regulated in the DCT. When comparing the low and high figures, the high dose has more altered gene expression in the PCT and significantly more gene involvement down-stream of the PCT with 14 altered genes compared to only 3 in the low. The localized gene expression changes depicted in this figure reinforce the hypothesis that as the $KBrO_3$ dose increases, additional cellular alterations occur in the PCT. As these changes occur, altered gene expression within and down-stream of the PCT is initiated as an attempt to maintain kidney function.

Kidney function and ion transport gene expression in the kidney from the 20 ppm exposure group showed a total of 10 genes down- and 3 up-regulated. Within the down-regulated group were genes mainly dealing with transport of water (*Aqp*1), sodium (*Atp*1*a*1 and *Skg*), potassium (*Atp*1*a*1), glucose (*G*6*pc*), and organic cations (*Slc*22*a*1). Although *Aqp*1 was down-regulated, no increase in water consumption was observed. In the up-regulated group were genes involved in calcium homeostasis (*Calca*), magnesium and calcium transport (*Cldn*16), and organic anion transport (Slc21*a*4). These gene expression changes suggest a physiological response that helps maintain electrolyte balance in light of the chronic, low dose $KBrO_3$ exposure. Although the low concentration of $KBrO_3$ used in this study (20 ppm) was not carcinogenic, results from a similar study using a dose of 60 ppm in the drinking water found increased proliferation and mild degeneration of the proximal convoluted tubules (Umemura et al. 2004). However, data collected in this paper indicate that physiological adaptation to $KBrO_3$ exposure is beginning to occur even at a non-carcinogenic dose.

Kidney function and ion transport gene expression in the kidney from the 400 ppm exposure group showed 20 down- and 4 up-regulated genes. Within the down-regulated group were genes involved with transport of water (*Aqp*2, and *Aqp*3), calcium (*Calb*1), chloride (*Clcnk*11 and *Prkwnk*4, *Slc*12*a*3, and *Slc*26*a*4), potassium (*Kcnj*16, *Kcnq*1, and *Prkwnk*4), sodium (*Ngfg, Prkwnk*4, *Scnn*1*a, Scnn*1*g, Slc*5*a*2, *Slc*9*a*3, *Slc*12*a*3, *and Slc*13*a*3), organic ions (*Slc*22*a*5 and *Slc*22*a*8), glucose (*Slc*5*a*2 and *Slc*37*a*4), and regulation of blood pressure (*End*1). In the up-regulated group were *Agtr*2, an angiotensin II receptor; *Cldn*16, a magnesium and calcium transporter (also up-regulated in the low dose); *Cp*, an iron transporter, and *Slc*12*a*1, a sodium, potassium, chloride co-transporter. These results imply significant alterations in the expression of genes involved in kidney function, and possibly decreased organ function.

In this light, when examining the DeAngelo et al. (1998) and Wolf et al. (1998) study, a dose-dependent increase in water consumption was observed starting at wk 4 and continuing through the duration of the study. Overall, it was determined that rats from the high dose (400 ppm) group drank over 30% more than controls. Taken together with the large number of down-regulated kidney function and ion transport genes from the high dose $KBrO_3$ exposure, these data possibly indicate a kidney in chronic renal insufficiency/failure. This condition occurs when the kidneys are unable to conserve water as they perform their blood filtering function. The amount of water conserved is controlled by antidiuretic hormone (ADH), also known as vasopressin. ADH controls kidney osmosis by inserting water pores into the collecting ducts. The water pores, *Aqp*2 and 3, are responsible for the final adjustment of urine concentration. In the high dose animals, *Aqp*2 and 3 transcripts are down-regulated. This would result in fewer water pores in the collecting duct leading to increased amounts of dilute urine. With the increase in urine production, the kidney may respond to maintain ionic balance and blood volume by down-regulating the genes contributing to loss of nutrients and electrolytes and up-regulating transporter genes necessary for reabsorption, such as *Slc*12*a*1 and *Cldn*16.

Within the high dose PCT and CD is a down-regulated gene called pendrin, or Slc26a4. Pendrin functions as a sodium-independent transporter of chloride and iodide where it is expressed in kidney, thyroid, and inner ear (Scott et al. 1999; Soleimani et al. 2001). It should be noted that $KBrO_3$, in addition to being a kidney carcinogen, is also a thyroid carcinogen, and can cause deafness (Yoshino et al. 2004). Moreover, pendrin expression was lower in thyroid carcinomas than in normal thyroid tissue

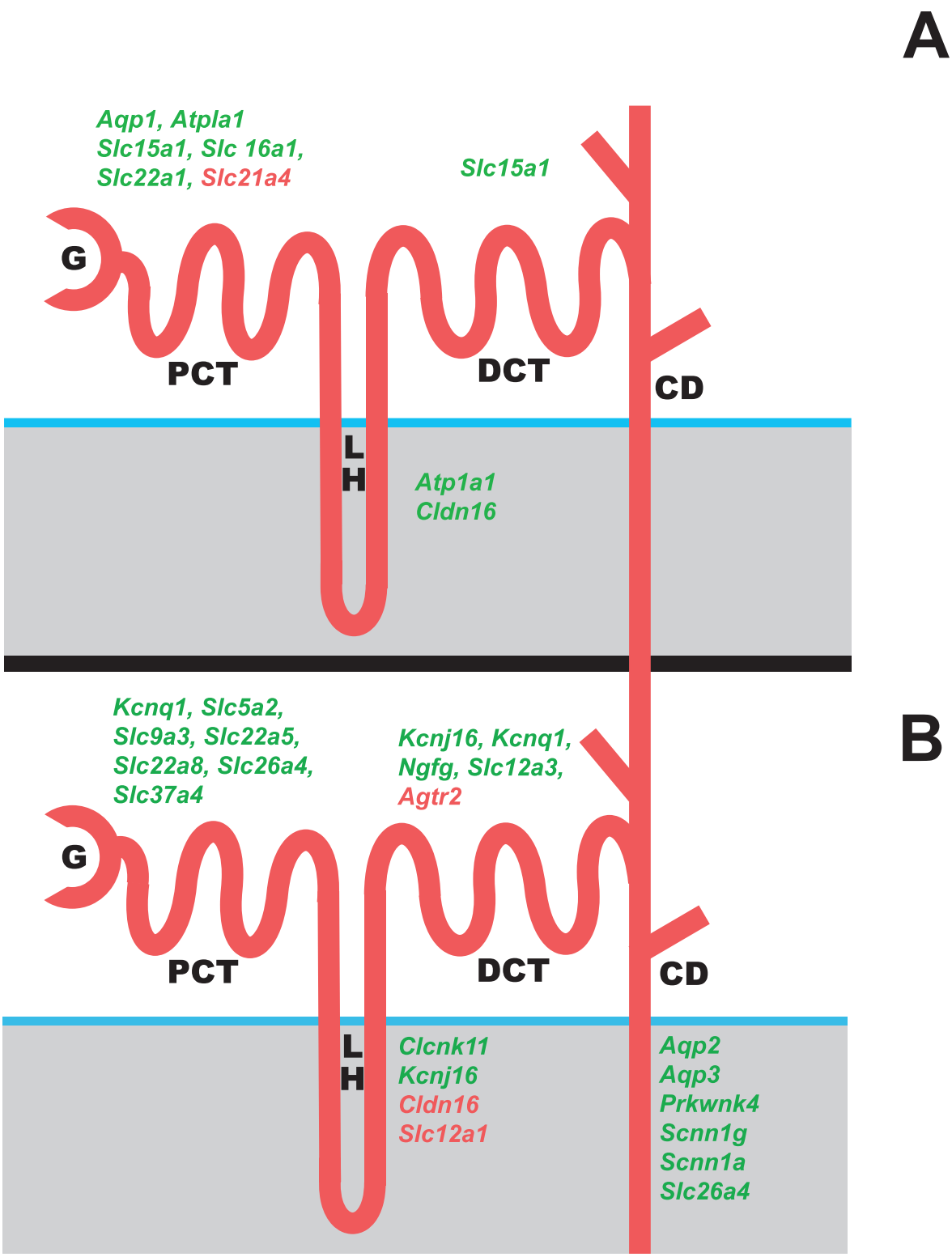

Figure 3A and 3B. Figure 3A (20 ppm) and 3B (400 ppm) show the location within the kidney nephron of specific genes associated with kidney function from male rats exposed to $KBrO_3$ for 52 wk. Genes in green and red font are down-regulated and up-regulated respectively. G: glomerulus, PCT: proximal convoluted tubules, LH: loop of Henle, DCT: distal convoluted tubules, and CD: collecting duct.

(Skubis-Zegadlo et al. 2005; Kondo et al. 2003). Pendrin transcripts examined by QT-rtPCR in this study were not altered in the low dose (20 ppm), suppressed in high dose (400 ppm), and virtually absent in kidney adenomas, mirroring the results seen in the thyroid carcinomas. Due to pendrin already being identified as a biomarker in thyroid carcinogenesis, and its involvement in kidney function following $KBrO_3$ exposure, we propose this gene as a possible biomarker of carcinogenic $KBrO_3$ exposure.

Cellular function

The primary objective of this study was to determine if a discernable difference in kidney gene expression could be observed between a non-carcinogenic and carcinogenic dose of $KBrO_3$. Gene ontology analysis of altered gene expression revealed an accumulation of changes in the following functionally-related categories: cancer, cell cycle, cell death, cell-to-cell signaling and interaction, cellular development, and cellular growth and proliferation. Thus, the genes that fell into these categories were placed into a general cellular functional group for analysis. Within this group were 30 down- and 11 up-regulated genes in the low $KBrO_3$ exposure group, and 20 down- and 22 up-regulated genes in the high exposure group.

In the low $KBrO_3$ concentration, the majority of genes associated with cellular function were down-regulated (30 versus 11 up-regulated). Several low dose genes when down-regulated are associated with decreased cell proliferation and apoptosis (*Akap*12, *Arhb*, *Cebpb*, *Csf*2*rb*, *Dusp*6, *Epim*, *Gadd*45*a*, *Id*2, *Id*3, *Igfbp*3, *Igfbp*6, *Jun*, *Pim*1, *Rgc*32, *Tieg*, and *Vegfb*). This is countered by only one up-regulated gene *Ccnd*1 (Cyclin d1), which is associated with increased cell proliferation and apoptosis. From this observation, it appears that the gene expression from the low dose kidney does not support increased levels of cell proliferation similar to the BrdU findings of Umemura et al. (2004) whose no-effect level was 15 mg/L.

The gene expression in the high $KBrO_3$ concentration was more equally distributed with 22 down- and 20 up-regulated genes. There were 10 genes shared in the cellular function category between the low and high $KBrO_3$ concentration. These were *Cyp*2*e*1, *Dscr*1, *Dsipi*, *F*3, *Id*2, *Igfbp*1, *Ms*4*a*2, *Rgc*32, *Ptgds*, and *Vipr*1. All were directionally concordant except for *Igfbp*1.

In contrast to the low dose, fewer genes were associated with cell proliferation and apoptosis (*Ccng*1, *Eno*1, *Hrasls*3, *Id*2, *Igfbp*1, *Madh*7, and *Nupr*1). *Eno*1 and *Hrasls*3, when down-regulated (Subramanian and Miller, 2000; Feo et al. 2000; Sers et al. 2002), and the up-regulated genes, *Nupr*1, and *Ccng*1 promote cellular growth and proliferation. However, *Id*2 and *Madh*7 when down-regulated support decreased proliferation (Lasorella et al. 1996; Lallemand et al. 2001). Although there are only a few genes in this group, the majority suggest increased cell proliferation and apoptosis.

Adenoma comparison

Stuart et al. (2001) established a benchmark expression profile for normal kidney development. By comparing the gene expression changes in the present study to this profile, expression changes that deviated from normal kidney were detected. In four defined kidney development groups, the 52 wk bromate-treated kidneys did not deviate from normal developmental patterns whereas the adenoma samples did. In these samples, the adenoma expression patterns were more characteristic of embryonic than adult kidneys. Although the bromate-treated kidneys did not show a strong expression pattern that matched the adenoma, the high dose kidney, but not the low, did resemble the adenoma expression pattern with genes prevalent in early kidney development being up-regulated and adult phase genes being down-regulated. This observation is in agreement with the proliferation and de-differentiation profiles seen in classical tumor development. Furthermore, the method of comparing developmental gene expression patterns between tumor and exposed animals could serve as a mechanism to identify biomarkers of tumor initiation.

Conclusion

These data suggest the 400 ppm carcinogenic dose of $KBrO_3$ showed marked gene expression differences from the non-carcinogenic dose. These include gene expression changes in oxidative stress and kidney function/ ion transport genes. Comparison of kidney development gene expression showed that the adenoma patterns were more characteristic of embryonic than adult kidneys, and that

the high dose kidney gene expression resembled an adenoma-like expression pattern. Taken together, these analyses from this study identify potential biomarkers of exposure and illuminate a possible carcinogenic mode of action for $KBrO_3$.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

DG aided in study design, carried out Affymetrix experiments, analyzed data, and wrote the manuscript. WW analyzed adenoma comparison with kidney development genes and aided in manuscript and figure preparation. GK performed QRT-rtPCR experiments and data analysis, and aided in manuscript and figure preparation. JR aided in manuscript and figure preparation. AD, RO, and JA were involved in study design with RO assisting in data analysis. DD was involved in study design, carried out Affymetrix experiments, analyzed data, and aided in manuscript preparation.

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Additional Files

File name: Oxidoreductase_table.pdf
File format: .pdf
Title of data: Oxidoreductase table
Description of data: List of differentially expressed oxidoreductase gene expression in kidney from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk.

File name: Lipid metabolism_table.pdf
File format: .pdf
Title of data: Lipid metabolism table
Description of data: List of differentially expressed lipid metabolism gene expression in kidney from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk.

File name: Cellular function_table.pdf
File format: .pdf
Title of data: Cellular function table
Description of data: List of differentially expressed cellular function gene expression in kidney from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk.

List of differentially expressed lipid metabolism genes in kidney from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk. All comparisons were made between the specific treatment group and their corresponding control.

Gene Symbol[a]	Gene Name[a]	Affymetrix No.[a]	Accession No.[a]	Fold Change
Low dose				
Apoe	Apolipoprotein E	1370862_at	NM_138828	-1.4
Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	1387087_at	NM_024125	-1.5
Chk	Choline kinase alpha	1368692_a_at	NM_017127	-1.7
Cyp2e1	Cytochrome P450, family 2, subfamily e1	1367871_at	NM_031543	-1.4
Cyp4a10	Cytochrome P450, family 4, subfamily a10	1368934_at	NM_016999	-1.4
Fads1	Fatty acid desaturase 1	1367857_at	NM_053445	-1.3
Abcg1	ATP-binding cassette, sub-family G, member 1	1368103_at	NM_053502	1.6
Calca	Calcitonin/calcitonin-related polypeptide, alpha	1370775_a_at	NM_017338	1.4
Gpd2	Glycerol-3-phosphate dehydrogenase 2	1369666_at	NM_012736	1.5
Nr1d1	Nuclear receptor subfamily 1, group D, member 1	1370816_at	NM_145775	1.7
High dose				
Acaa2	Acetyl-Coenzyme A acyltransferase 2	1386880_at	NM_130433	-1.2
Acsl3	Acyl-CoA synthetase long-chain family member 3	1368177_at	NM_057107	-1.3
Apoc3	Apolipoprotein C-III	1370009_at	NM_012501	-1.6
Apom	Apolipoprotein M	1386980_at	NM_019373	-1.2
Cyp2e1	Cytochrome P450, family 2, subfamily e1	1367871_at	NM_031543	-1.3
Cyp4a10	Cytochrome P450, family 4, subfamily a10	1368934_at	NM_016999	-1.3
Edn1	Endothelin 1	1369519_at	NM_012548	-1.4
Ehhadh	3-hydroxyacyl coenzyme A dehydrogenase	1368283_at	NM_133606	-1.4
Hadhsc	L-3-hydroxyacyl-Coenzyme A dehydrogenase	1370237_at	NM_057186	-1.3
Fabp5	Fatty acid binding protein 5, epidermal	1370281_at	NM_145878	1.4

[a] Gene symbols and accession numbers from Affymetrix Netaffx (http://www.affymetrix.com/analysis/index.affx)

List of differentially expressed cellular function genes in kidney from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk. All comparisons were made between the specific treatment group and their corresponding control.

Gene Symbol[a]	Gene Name[a]	Affymetrix No.[a]	Accession No.[a]	Fold Change
Low dose				
Akap12	A kinase (PRKA) anchor protein (gravin) 12	1368869_at	NM_057103	-1.8
Arhb	Ras homolog gene family, member B	1369958_at	NM_004040	-1.5
Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	1387087_at	NM_024125	-1.5
Csf2rb	Colony stimulating factor 2 receptor, beta 1	1369828_at	NM_133555	-1.3
Cyp2e1	Cytochrome P450 family 2, subfamily e1	1367871_at	NM_031543	-1.4
Dscr1	Down syndrome critical region homolog 1	1388686_at	NM_153724	-1.4
Dsipi	Delta sleep inducing peptide, immunoreactor	1367771_at	NM_031345	-1.7
Dusp6	Dual specificity phosphatase 6	1387024_at	NM_053883	-1.4
Epim	Epimorphin	1372832_at	NM_012748	-1.4
F3	Coagulation factor 3	1369182_at	NM_013057	-1.6
Gadd45a	Growth arrest and DNA-damage-inducible 45 alpha	1368947_at	NM_024127	-1.4
Hba-a1	Hemoglobin alpha, adult chain 1	1375519_at	NM_013096	-1.3
Hbb	Hemoglobin beta chain complex	1371102_x_at	NM_033234	-1.6
Id2	Inhibitor of DNA binding 2	1368870_at	NM_013060	-1.5
Id3	Inhibitor of DNA binding 3	1387769_a_at	NM_013058	-1.5
Igfbp3	Insulin-like growth factor binding protein 3	1367652_at	NM_012588	-1.5
Igfbp6	Insulin-like growth factor binding protein 6	1372168_s_at	NM_013104	-2.3
Jun	v-jun sarcoma virus 17 oncogene homolog	1389528_s_at	NM_021835	-1.6
Lgmn	Legumain	1368430_at	NM_022226	-1.3
Mcl1	Myeloid cell leukemia sequence 1	1370141_at	NM_021846	-1.6
Pim1	Serine threonine protein kinase	1374429_at	NM_017034	-1.6
Ptgds	Prostaglandin D2 synthase	1367851_at	NM_013015	-1.4
Rgc32	Response gene to complement 32	1368080_at	NM_054008	-1.8
Rhob	rhoB gene	1369958_at	NM_022542	-1.4
Sgk	Serum/glucocorticoid regulated kinase	1367802_at	NM_019232	-1.6
Tieg	Tgfb inducible early growth response	1368650_at	NM_031135	-1.4
Txnrd1	Thioredoxin reductase 1	1386958_at	NM_031614	-1.3
Vegfb	Vascular endothelial growth factor B	1380854_at	AF022952	-1.3
Xdh	Xanthine dehydrogenase	1369973_at	NM_017154	-1.4
Calca	Calcitonin/calcitonin-related polypeptide, alpha	1370775_a_at	NM_017338	1.4
Ccnd1	Cyclin D1	1383075_at	NM_171992	1.3
Dlgh4	Discs, large homolog 4	1371183_a_at	NM_019621	2.0
Hand1	Heart and neural crest derivatives transcript 1	1370128_at	NM_021592	1.6
Ifng	Interferon gamma	1370790_at	NM_138880	1.9
Ms4a2	Membrane-spanning 4-domains, A2	1369399_at	NM_012845	1.6
Nr1d1	Nuclear receptor subfamily 1, group D, member 1	1370816_at	NM_145775	1.7
Prlr	Prolactin receptor	1370384_a_at	NM_012630	1.4
Siat1	Sialyltransferase 1	1370907_at	NM_147205	1.4
Vipr1	Vasoactive intestinal peptide receptor 1	1387561_at	NM_012685	1.6

High dose				
Aldh1a1	Aldehyde dehydrogenase family 1, member A1	1387022_at	NM_022407	-1.2
Calb1	Calbindin 1	1370201_at	NM_031984	-1.3
Cyp2e1	Cytochrome P450, family 2, subfamily e1	1367871_at	NM_031543	-1.3
Ddc	Dopa decarboxylase	1368064_a_at	NM_012545	-1.5
Dscr1	Down syndrome critical region homolog 1	1388686_at	NM_153724	-1.3
Dsipi	Delta sleep inducing peptide, immunoreactor	1367771_at	NM_031345	-1.7
Edn1	Endothelin 1	1369519_at	NM_012548	-1.4
Eno1	Enolase 1, alpha	1367575_at	NM_012554	-1.3
F3	Coagulation factor 3	1369182_at	NM_013057	-1.4
Folh1	Folate hydrolase	1387363_at	NM_057185	-1.6
Hrasls3	HRAS like suppressor	1370202_at	NM_017060	-1.3
Hrg	Histidine-rich glycoprotein	1368583_a_at	NM_133428	-2.0
Id2	Inhibitor of DNA binding 2	1368870_at	NM_013060	-1.5
Madh7	MAD homolog 7	1368896_at	NM_030858	-1.5
Ptgds	Prostaglandin D2 synthase	1367851_at	NM_013015	-1.5
Rgc32	Response gene to complement 32	1368080_at	NM_054008	-1.7
Sdc4	Syndecan 4	1367721_at	NM_012649	-1.2
Serpinf1	Serine (or cysteine) proteinase inhibitor, clade F1	1388569_at	NM_177927	-1.3
Sp3	Sp3 transcription factor	1375384_at	AI715114	-1.6
Ubtf	Upstream binding transcription factor, RNA poly I	1389830_at	AI105117	-1.3
Yc2	Glutathione S-transferase Yc2 subunit	1371089_at	NM_001009920	-1.2
Adamts1	A disintegrin-like and metalloprotease	1368223_at	NM_024400	1.5
Adcyap1r1	Adenylate cyclase activating polypeptide 1 receptor 1	1387302_at	NM_133511	1.6
Agtr2	Angiotensin II receptor, type 2	1369711_at	NM_012494	1.5
Atf5	Activating transcription factor 5	1372601_at	NM_172336	1.7
Ccng1	Cyclin G1	1367764_at	NM_012923	1.3
Clu	Clusterin	1367784_a_at	NM_053021	1.5
Ctsl	Cathepsin L	1370244_at	NM_013156	1.2
Fabp5	Fatty acid binding protein 5, epidermal	1370281_at	NM_145878	1.4
Ghrl	Ghrelin precursor	1387254_at	NM_021669	1.7
Grm1	Glutamate receptor, metabotropic 1	1371180_a_at	NM_017011	1.7
Gstp1	Glutathione-S-transferase, pi 1	1388122_at	NM_012577	1.3
Lta	Lymphotoxin A	1368722_at	NM_080769	1.3
Ms4a2	Membrane-spanning 4-domains, subfamily A2	1369399_at	NM_012845	1.6
Nupr1	Nuclear protein 1 "P8"	1367847_at	NM_053611	1.8
Plau	Plasminogen activator, urokinase	1387675_at	NM_013085	1.3
Ptprc	Protein tyrosine phosphatase, receptor type, C	1390798_at	BF288130	1.5
Th	Tyrosine hydroxylase	1387075_at	NM_012740	1.4
Tpm3	Tropomyosin isoform 6	1376929_at	NM_057208	1.7
Ubd	Ubiquitin D	1368762_at	NM_053299	1.7
Unc13a	Unc-13 homolog A	1369330_at	NM_022861	2.3
Vipr1	Vasoactive intestinal peptide receptor 1	1387561_at	NM_012685	1.8

[a] Gene symbols and accession numbers from Affymetrix Netaffx (http://www.affymetrix.com/analysis/index.affx)

List of significantly altered oxidoreductase gene expression in kidney from male rats exposed to 20 ppm (low) and 400 ppm (high) potassium bromate in drinking water for 52 wk.

Gene Symbol[a]	Gene Name[a]	Affymetrix No.[a]	Accession No.[a]	Fold Change
Control versus Low				
Adh1	Alcoholdehydrogenasel	1368021_at	NM_130780	-1.298
Cox8h	Cytochrom c oxidase subunit VIII-H	1367739_at	NM_012786	-1.888
Cyp2e1	Cytochrome P450,family 2, subfamilye1	1367871_at	NM_031543	-1.347
Cyp4a10	Cytochrome P450,family 4, subfamily a10	1368934_at	NM_016999	-1.338
Fads1	Fattyacid desaturase 1	1367857_at	NM_053445	-1.333
P4ha1	Procollagen-proline,alpha1 polypeptide	1370954_at	NM_172062	-1.365
Phgdh	3-phosphoglycerate dehydrogenase	1367811_at	NM_031620	-1.525
Sord	Sorbitol dehydrogenase	1369635_at	NM_017052	-1.437
Txnrd1	Thioredoxin reductase 1	1386958_at	NM_031614	-1.319
Xdh	Xanthine dehydrogenase	1369973_at	NM_017154	-1.386
Gpd2	Glycerol-3-phosphate dehydrogenase 2	1369666_at	NM_012736	1.504
Control versus High				
Adh1	Alcohol dehydrogenase 1	1368021_at	NM_130780	-1.396
Aldh1a1	Aldehyde dehydrogenase family 1, member A1	1387022_at	NM_022407	-1.235
Cyp2e1	Cytochrome P450,family2, subfamily e1	1367871_at	NM_031543	-1.341
Cyp4a10	Cytochrome P450,family 4, subfamily a10	1368934_at	NM_016999	-1.341
Ehhadh	3-hydroxyacyl coenzyme A dehydrogenase	1368283_at	NM_133606	-1.389
Fmol1	Flavin containing monooxygenase 1	1387053_at	NM_012792	-1.240
Hadhsc	L-3-hydroxyacyl-Coenzyme A dehydrogenase	1370237_at	NM_057186	-1.280
Hpd	4-hydroxyphenylpyruvic acid dioxygenase	1368188_at	NM_017233	-1.288
P5	Thioredoxin domain containing 7	1376239_at	NM_001004442	-1.910
Phgdh	3-phosphoglycerate dehydrogenase	1367811_at	NM_031620	-1.426
Ptgds	Prostaglandin D2 synthase	1367851_at	NM_013015	-1.464
Sord	Sorbitol dehydrogenase	1369635_at	NM_017052	-1.320
Suox	Sulfite oxidase	1370036_at	NM_031127	-1.387

[a]Gene symbols and accession numbers from Affymetrix Netaffx (http:/www.affymetrix.com/analysis/index