Isoflurane (basolateral amygdalae)	Brca2, Gpx2, Ift172, Mif, Nos2, Pttg1, Rad1, Rad51c, Tpo, Ung	Atrx, Atrx, Gsr, Nox4, Pold3, Prnp, Ptgs2, Scd1, Smc3, Xrcc6	GDS2073
Fe deficiency (jejunum)	Aass, Gadd45a, Gsr, Nqo1, Srxn1, Tdg, Tmod1, Txnrd1, Xrcc1,	Gpx7, Hba-a2, Lpo, Mgmt, Nthl1, Pms2, Rad52, Smc3, Xpc, Xrcc6	GDS2093
Pregnenolone16alpha-carbonitrile (liver)	Dnm2, Gpx6, Lpo, Nqo1, Prdx5, Ptgs2, Scd1, Srxn1, Tpo, Txnrd1	Aass, Als2, Apoe, Hbz, Nos2, Rad51c, Slc38a5, Srd5a2, Tpo	GDS2194
Particulate matter (TPM)/l of cigarette smoke (lung)	Aass, Apc, Brca1, Brca2, Cry2, Gpx2, Hus1, Slc38a4, Tpo, Txnrd1	Chaf1a, Mb, Mutyh, Nos2, Pold3, Ptgs2, Rad50, Tmod1, Tnp1, Tpo	GDS2616
Genistein (mammary epithelial cells)	Atrx, Brca2, Hba-a2, Ngb, Rad23a, Rad52, Smc3, Tpo, Ung, Zmynd17	Apex1, Brca1, Gpx6, Lpo, Pttg1, Slc38a4, Srd5a2, Tnp1, Tpo	GDS2639
Aging (hippocampi)	Atrx, Ehd2, Gadd45a, Gtf2h1, Mgmt, Ncf1, Nthl1, Ptgs2, Pttg1, Srxn1	Ercc6, Mlh1, Pms2, Rad50, Rad52, Slc38a1, Trpc2, Txnip, Wrnip1, Xpc	GDS2774
Depolarization. (midbrain)	Apc, Apoe, Atrx, Brca1, Pold3, Ptgs2, Rad23a Slc38a4, Smc3, Zmynd17	Apex1, Atrx, Chaf1a, Gpx2, Hba-a2, Nos2, Pttg1, Srxn1, Tmod1, Tnp1	GDS2901
Aristolochic acid (kidney)	Apoe, Atrx, Cry2, Ngb, Ppp1r15b, Scd1, Srxn1, Tpo	Apoe, Atrx, Fen1, Gadd45a, Gpx6, Ift172, Pold3, Rad52, Txnip, Zmynd17	GSM1038
Cluster 4			
Pyridine activator (ventricular myocytes)	Aass, Chaf1a, Dhcr24, Nthl1, Pinx1, Pold3, Rad52, Scd1, Slc38a1, Xirp1	Apex1, Brca2, Cry2, Gpx6, Hus1, Lpo, Mutyh, Pold1, Rad51c, Tpo	GDS902
Reinnervation (tibialis anterior muscles)	Apex1, Atrx, Chek1, Gpx6, Mgmt, Ncf1, Nox4, Pold3, Smc3, Tnp1	Atrx, Brca1, Chaf1a, Lpo, Nthl1, Rad50, Slc41a3, Txnrd2, Ung, Zmynd17	GDS2243
Hyperinsulinemia (kidney)	Apoe, Chaf1a, Gpx6, Hbaa2, Lpo, Ngb, Ptgs2, Scd1, Slk, Srd5a2	Apc, Atrx, Duox2, Hbz, Mb, Ncf1, Slc38a4, Tmod1, Tnp1, Txnip	GDS2361
Cluster 5			
Sulfur mustard bis-(2-chloroethyl) sulfide (lung)	Apoe, Gadd45a, Gpx2, Hba-a2, Mif, Prdx5, Ptgs2, Scd1, Smc3, Srxn1	Apc, Atrx, Dnm2, Duox2, Gab1, Gpx6, Mutyh, Nox4, Srd5a2, Tpo	GDS1027
Amoxicillin (intestine)	Apc, Apoe, Atrx, Lpo, Mutyh, Slc38a4, Tnp1, Tpo	Apex1, Chaf1a, Cry2, Gpx2, Ngb, Nox4, Scd1, Tpo, Trpc2, Zmynd17	GDS1273
Ischemia (heart)	Apc, Apoe, Gpx7, Nos2, Nox4, Nxn, Prdx4, Rad52, Scd1, Smc3	Atrx, Brca1, Chaf1a, Hus1, Lpo, Pold1, Prdx5, Rad51c, Slc38a4, Xirp1	GDS1959
Cluster 6			
Carbon tetrachloride (liver)	Chaf1a, Ehd2, Gpx2, Hba-a2, Ncf1, Prnp, Ptgs2, Slc38a4, Vim, Zmynd17	Apoe, Dpagt1, Gab1, Hus1, Nos2, Nxn, Ptgs1, Slk, Trpc2, Txnip	GDS1354
Dexamethasone (marrow-derived stromal cells)	Apoe, Ehd2, Gpx6, Mgmt, Mpp4, Srd5a2, Tmod1, Tpo	Apex1, Apoe, Chaf1a, Dnm2, Nos2, Rad50, Rad51c, Slk, Smc1a, Smc3	GDS2231

microarrays capture only transient responses to oxidative stimuli. However, we can predict the underlying mechanism of environmental stressors through oxidative signatures for gene expression. For example, methylprednisolone [80, 81], streptozotocin [82], trimethyltin [83], and octreotide [84] upregulate GPXs, NOS, and NOX, suggesting that environmental stressors in cluster 1 can activate the NO signaling that leads inflammation or other cellular damage. Thioredoxin interacting protein, Txnip, was identified as a unique gene in this category. In cluster 2 (GDS696 [85], GDS880 [86], GDS1518 [87], GDS1626 [88], GDS2107 [89], GDS2372 [90], GDS2457 [91], GDS2688 [92]), Rad23, Rad50, and Rad51c, which are DNA repair and recombination proteins, and the other DNA replication proteins DNA-directed DNA polymerase delta (Pold)1 and Pold3 were classified. This classification suggests that environmental stressors in cluster 2 such as fibronectin, protein restriction, heregulin, kainic acid, hypoxia, and ethanol harmed mitochondria or damaged DNA more than the stressors in cluster 1. In cluster 3 (GDS1363 [93], GDS1452 [94], GDS1922 [95], GDS2037 [96], GDS2073 [97], GDS2093 [98], GDS 2194 [99], GDS2616 [100], GDS2639 [101], GDS2774 [102], GDS2901 [103], GDM1038 [104]), Gadd45a, Nthl1, Mgmt, Mpp4, Chek1 Cry2, and Txnrd1 were observed as upregulated genes. Since these genes interact with DNA repair and are p53 signaling activated, it is possible that environmental stressors in cluster 3 cause DNA damage and remodeling. In cluster 4 (GDS902 [105], GDS2243 [106], GDS2361 [107]), DNA replication proteins Pinx1 and Slk were detected as unique genes. In particular, STE20-like kinase (Slk) appears to influence cell survival and proliferation. In fact, Slk has been suggested to have a central growth-suppressive role for Mst orthologs, with intriguing possible links to other established tumor suppressors through work in model organisms. A part of the genes in cluster 5 (GDS1027 [108], GDS1273 [109], GDS1959 [110]) were overlapped in clusters 1 and 3. In cluster 6 (GDS1354 [111], GDS2231 [112]), a part of the genes were overlapped in clusters 2 and 4. However, Vim was detected as a unique gene in GDS1354, which is an experiment in cirrhotic rats [111], since upregulation of this gene was also observed in renal cell carcinoma [113], cerebral tumors [114], and germ cell and trophoblastic neoplasms [115].

13.2.2 Prediction of Biological Influences from Gene Expression Signatures in Rats Exposed to Environmental Stressors

These clusters were characterized by several biological functions. Data of gene expression signatures in Table 13.3 were analyzed through the use of Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). The Functional Analysis identified the biological functions that were most significant to the data set. Molecules from the data set that met the expression value associated with biological functions and/or diseases in Ingenuity's Knowledge Base were considered for the analysis. Right-tailed Fisher's exact test was used to calculate a *P*-value determining the probability that each biological function and/or disease assigned to that data set is due to chance alone. In Table 13.4, the highest probability predictive function in cluster 1 showed "DNA Replication, Recombination, and Repair"; that in cluster 2 showed "Small Molecule Biochemistry"; that in cluster 3 also showed "Small Molecule Biochemistry"; that in cluster 4 showed "DNA Replication, Recombination, and Repair"; that in cluster 5 showed "Cancer"; and that in cluster 6 showed "Lipid Metabolism." In "Small Molecule Biochemistry," genes related with "degradation or catabolism of hydrogen peroxide" like CAT, GPX3, and GPX4 and peroxidation of lipid were affected in clusters 2, 3 and 6. In "Gene expression," genes related with "binding of p53 consensus binding site" like APEX1, BRCA1, and PTTG1 were affected. For instance, the top-rated network ~~generated~~ by retinoic X receptor ligand LG100268 is shown in Figure 13.1. ~~This network consists of a cluster of 16 molecules meet 44 molecules,~~ which belong to biological functions of DNA replication, recombination and repair, cancer, and cell cycle. AQ

13.2.3 Oxidative Stress-Mediated p53 Pathways in Human Tissues

Among many oxidative responsive pathways, p53 signaling has been studied extensively and has been thought to play a main role in the orchestration of oxidative events in cells. It coordinates the cellular responses to a broad range of cellular stress factors. In fact, p53 functions as a node for organizing whether the cell responds to various types and levels of stress with apoptosis, cell cycle arrest, senescence, DNA repair, cell metabolism, or autophagy, as described above in this chapter [37–39]. To control and fine-tune responses to various stress signals encountered by cells, as a transcription factor that both activates and represses a broad range of target genes, p53 demands an exquisitely complicated regulatory network (Fig. 13.2). The classical model for activation of p53 specifically examines three simple and rate-limiting steps: p53 stabilization induced by ataxia telangiectasia mutated (ATM)/ataxia telangiectasia and Rad3 related (ATR)-mediated phosphorylation, sequence-specific DNA binding, and target gene activation through interaction with the general transcriptional machinery [29]. Recent studies with animal models describe that mouse double minute

TABLE 13.4 Predicted biological functions by gene expression signatures shown in Table 13.3

Cluster	Predictive Biological Functions	*P* Value
1	DNA Replication, Recombination, and Repair	1.02E-07
	Hematological Disease	1.33E-06
	Cardiovascular System Development and Function	3.62E-06
	Lipid Metabolism	3.62E-06
	Organ Morphology	3.62E-06
2	Small Molecule Biochemistry	2.34E-07
	Cell Cycle	3.80E-06
	DNA Replication, Recombination, and Repair	3.80E-06
	Cell-to-Cell Signaling and Interaction	3.22E-05
	Cell Death	6.18E-05
3	Small Molecule Biochemistry	2.41E-07
	Gene Expression	7.30E-07
	Cellular Compromise	1.93E-06
	Cell Cycle	4.02E-06
	DNA Replication, Recombination, and Repair	4.57E-06
4	DNA Replication, Recombination, and Repair	7.14E-11
	Cell Cycle	1.18E-05
	Cell Death	2.52E-05
	Respiratory System Development and Function	2.52E-05
	Reproductive System Development and Function	1.10E-04
5	Cancer	8.09E-09
	Gastrointestinal Disease	8.09E-09
	Cell Death	1.20E-07
	Dermatological Diseases and Conditions	5.01E-07
	Organismal Functions	3.57E-06
6	Lipid Metabolism	1.45E-09
	Small Molecule Biochemistry	1.45E-09
	Cell-to-Cell Signaling and Interaction	1.26E-08
	Nervous System Development and Function	1.26E-08
	Cell Death	3.49E-08

(Mdm) 2 and MdmX might determine whether a cell responds to p53 activation with growth arrest or apoptosis, but the molecular mechanism of these differential effects remains unknown. In fact, Mdm2 and MdmX can both be recruited to p53 promoter regions. Via a multitude of mechanisms, they can repress transcription of p53 target genes [116–118]. p53 protein binds sequence-specific regions of DNA of the target gene to process sensing and removal of oxidative damage to nuclear DNA and genetic instability. Furthermore, p53 acts as a transcription factor to regulate the expression of many prooxidant and antioxidant genes. A newly refined model for p53 activation includes three key steps: (1) p53 stabilization, (2) antirepression, and (3) promoter-specific activation. Among the three steps, most environmental stressors contribute mainly to p53 stabilization and promoter-specific activation. Several reports describe that low-weight molecules engender induction of stress-induced genes such as NAD(P)H dehydrogenase, quinone (NQO)1, and NQO2, which stabilize and transiently activate p53 and downstream genes leading to protection against adverse effects of stressors [119–121].

Therefore, to understand how stress-induced genes are downstream within the p53 pathway, we analyzed gene expression of p53 signaling pathways in array data sets GDS2780 [122] and GSE7967 [123] that had been obtained from the GEO database. In the GDS2780 study, six heavy metals and three organic compounds to which liver carcinoma HepG2 cells were exposed responded dramatically to gene expression of CHK1, CHK2, Cyclin B, Cdc2 p21, p53R2, Cop1-1, and Gadd45 [1]. Interestingly, expression levels of p53R2 and Gadd45 responded differently to the heavy metals: p53R2 is likely to associate with mitochondrial DNA and play a critical role in embryogenesis and neurogenesis [124–128]; in contrast, Gadd45 plays a vital role as a cellular stress sensor in the modulation of cell signal transduction in response to stress. Increasing Gadd45 can stabilize p53 activation, leading to cell cycle arrest or progression to apoptosis [129–131]. Consequently, exposure of cultured human cells to heavy metals

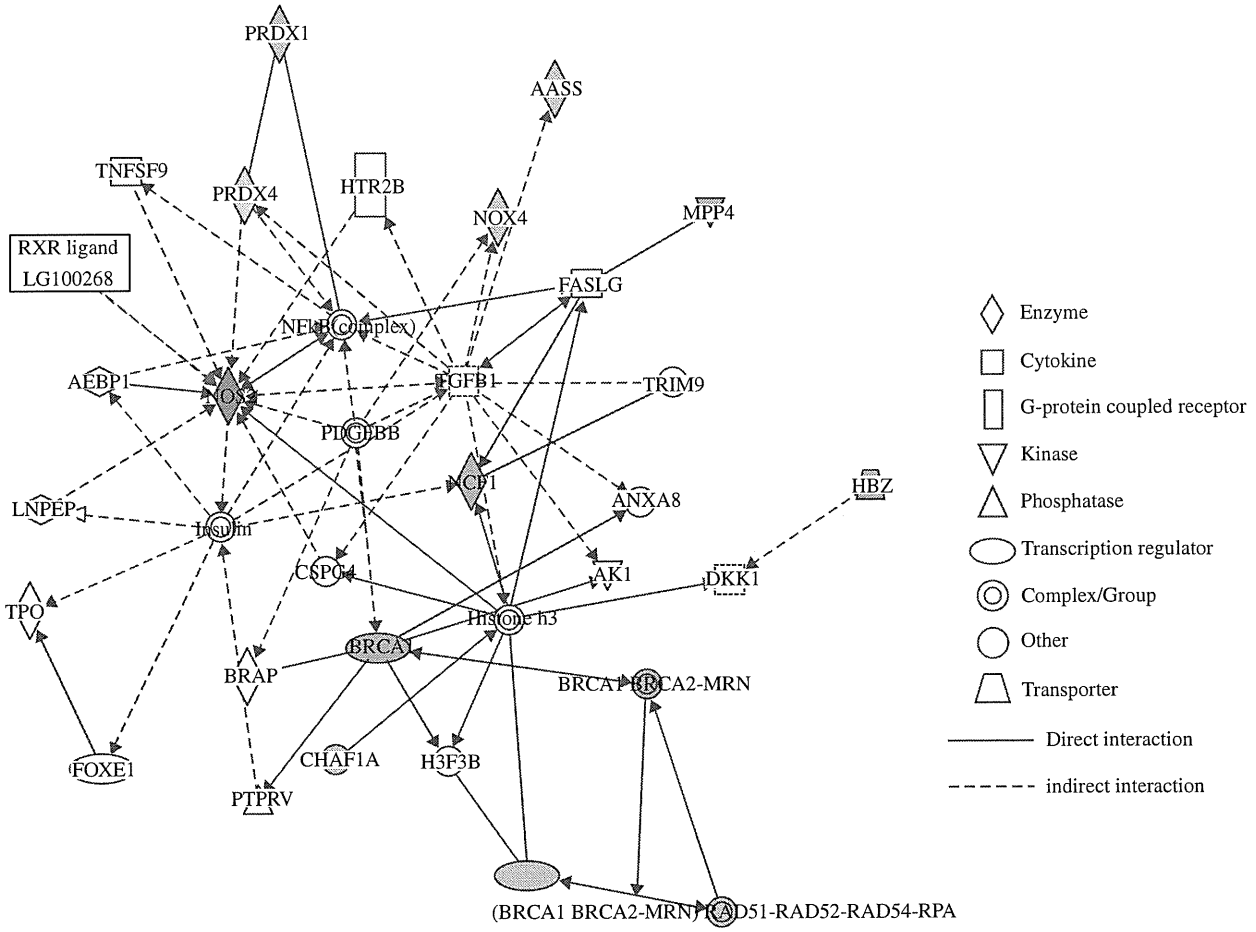

Fig. 13.1 The network generated by retinoic X receptor ligand LG100268 in cluster 3 shown in Tables 13.3 and 13.4: the top-rated network as an example

dramatically altered the gene expression of oxidative stress-responsive genes. However, in human tissues of the GSE7967 study, the p53 signaling pathway differed from that of heavy metals in the GDS2780 study. Overall, the gene expression signals were weaker than those examined in the GDS2780 study. The GSE7967 study examined cord blood collected at birth from infants whose mothers were exposed or unexposed to arsenic (0.1–68.63 mg/g), showing activation of inflammation and NF-kB signaling in infants born to mothers exposed to arsenic at high concentration. Therefore, after downloading the data sets, we selected four subjects according to blood concentrations of 0.1, 1.76, 9.66, and 68.63 mg/g; then gene expression of the arsenic exposure-induced responses were visualized in the p53 signaling pathway map. The highest concentration subject showed Gadd45, p53-inducible ribonucleotide reductase small subunit 2 (p53R2), spermatogenic leucine zipper 1 (TSP1), cyclinB, Cdc2, Fas, Noxa, and ATR that were higher than those of the subject with the low concentration. However, p53 was opposite: high in the low-exposure subject and low in the high-exposure subject, suggesting that the downregulation of p53 facilitates apoptosis and promotes cell proliferation.

Previous works described in our study showed that GSS (glutathione synthetase) and PRDX2 (peroxiredoxin 2) regulated TRADD (TNFRSF1A-associated via death domain), NUDT1 (nucleoside diphosphate linked moiety X-type motif 1), SOD1 (superoxide dismutase 1, soluble), and INSIG1 (Insulin induced gene 1) in the low-exposure group (mean blood concentration 0.142 μg/g) and that NUDT1 regulated TRADD, TXNRD2 (thioredoxin reductase 2), and PRDX2 in the high-exposure group (21.41 μg/g), using the theoretical algorithm for identifying optimal gene expression networks (TAO-Gen), which is a Bayesian network algorithm used to describe gene interaction networks [18, 132–134] (Fig. 13.3). In fact, NUDT1 is a DNA repair and recombination protein. The H_2O_2 treatment significantly increased this gene and other oxidative

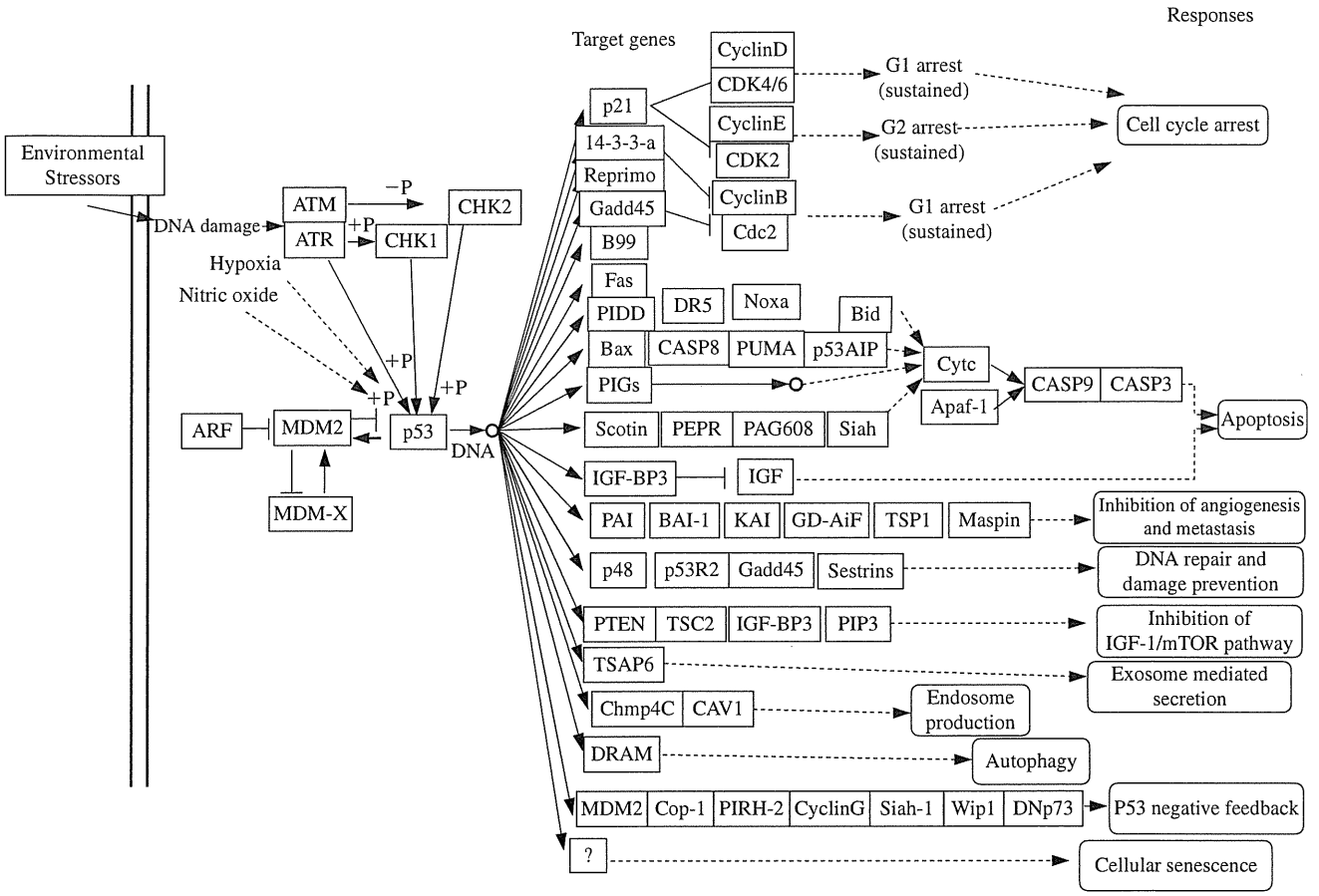

Fig. 13.2 Environmental stressor-mediated p53 signaling pathways. Maps of the p53 signaling pathway partly consulted the KEGG pathway

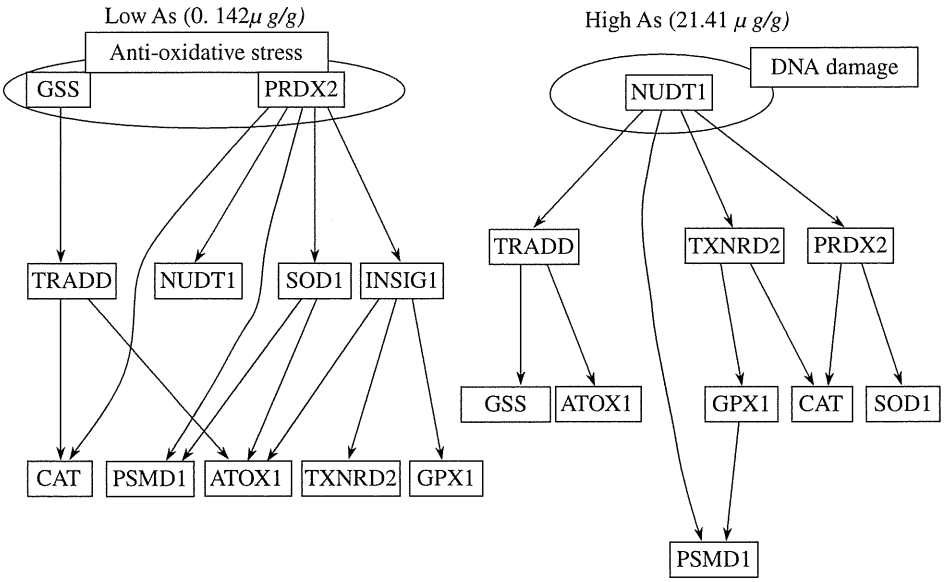

Fig. 13.3 Networks for 11 oxidative stress-related genes selected from the GSE7967 study. TAO-Gen algorithms can predict different mechanisms in low and high exposure to arsenic

stress genes involved in cell cycle arrest [135]. Results of our analyses suggest that anti-oxidative stress-related genes play key roles in protection against cellular damage in the low-exposure group, but a DNA damage-related gene was dominant in the high-exposure group, in which cell damage would progress. Data sets used in this chapter are from fundamental exposure to environmental stressors in normal tissues and cell lines, Therefore, this discrepancy indicates that gene expression signatures in human clinical tissues or epidemiological studies apparently reflect more inflammation than those of experimental materials, which show acute toxicity in animals after short exposure to oxidants in cell cultures.

13.3 CONCLUSION

In this chapter, we have reviewed gene expression signatures of oxidative stress-mediated signaling pathways by environmental stressors and proposed categorical pathways and canonical pathways of oxidative stress in rat and human systems. Analyses of gene expression signatures in environment-related disease such as neuronal disorders, cancer, and diabetes is an important approach in etiology and risk assessment for human health to elucidate the underlying mechanisms of induced health effects. This will take many more genetic and reverse genetic analyses, combined with functional analysis studies. Furthermore, we have shown that oxidative stress has been associated with many signaling pathways and different environmental stressors impacting different molecules, but they are all connected to the same goals like apoptosis or cell cycle. From a therapeutic point of view, researchers must consider that the best biomarker and/or therapy for oxidative stress-related disease may rely on a combination of several different agents, each specifically targeting one aspect of the oxidative stress machinery.

ACKNOWLEDGMENTS

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Gene Expression Signatures of Environmental Chemical in Cancer and Developmental Disorders

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Summary

The determination of gene expression signatures as a means to explore cellular responses to chemical exposures offers a new approach for predicting such responses and to investigate chemical agent-gene interactions. Here, we focus on genes responsive to oxidative stress in metal-induced toxicity in rats and humans, and non-metal-induced toxicity in embryonic stem cells. We apply a statistical clustering and a Bayesian network analysis technique, which is a probabilistic graphical model that represents a set of variable identities. We found that the gene expression signature of copper accumulation in the LEC rat liver was unique for this toxic agent. Next, we showed differential gene expression patterns following arsenic exposure in the human liver cell line HepG2 and in human cord blood. Finally, we showed that the gene expression signature of retinoic acid exposure in embryonic stem (ES) cells could be useful for to predicting neuronal disease.

Introduction

The field of molecular genetics has provided a wealth of information regarding the mechanisms underlying environmental chemical (EC)-related carcinogenesis and developmental disorders[1, 2]. Microarray technology has now been used in environmental toxicology studies and has resulted in the establishment of gene expression signatures profiling the toxicity of ECs[3, 4]. Metal ions such as arsenic and copper II induce a variety of oxidative stresses including thiol molecule perturbation, the generation of oxidative DNA adducts and the induction of molecular biomarkers[5-8]. Non-metal chemicals such as the retinoic acids and 2,3,7,8-tetrachlorodebenzo-*p*-dioxin (TCDD) are known to influence oxidative stress-related gene and protein expression during carcinogenesis and in embryonic development[9-12]. Our previous studies have shown that a Bayesian network

technique, which is a probabilistic graphical model that represents a set of variable identities, can be applied to the detection of differences in the gene expression interaction networks that result from exposure to different doses of chemicals[13-16]. Here, we focused on metal-induced toxicity and carcinogenicity, and non-metal-induced toxicity in embryonic stem cells to provide information predicting outcomes, and to better understand the molecular mechanisms underlying these toxicities.

Materials and Methods

We adopted three different approaches in this study: 1) To determine the effects of copper accumulation on oxidative stress marker genes, we performed oligonucleotide array analysis (Affymetrix) in the LEC rat strain (*Atp7b m/m*), which accumulates copper in the liver, and compared the results obtained for LEC rats with those of a sibling line of the *Atp7b w/w* genotype[17-21]. 2) Array data for exposure to arsenic trioxide and other arsenic compounds were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). For this purpose we performed gene network analysis with data selected from ChemToxGen, which is software that can extract multiple types of information for chemicals. 3) An embryonic stem cell line, B6-G2 (RIKEN), which ubiquitously expresses GFP was exposed to retinoic acids (1 - 100 nM). The differentiation process from embryonic stem to embryonic body formation and neuronal lineage diffretiation was then characterized by gene expression profiling using bead array analysis (Illumina), and the effects of exposure to these compounds were examined. Gene expression signatures of each oxidative stress gene set were established using TAO-Gen (Theoretical algorithm for identifying optimal gene expression networks) Analysis, which is a Bayesian network algorithm (a directed acyclic graph) to describe gene interaction networks

Results and Conclusions

1) Copper-induced gene expression signature. The LEC model showed a unique gene expression profile in which *Npdc1*, *Tradd*, *Med17*, *Psmd1*, *Insig11*, *Abcc2*, and *G6pc* were differentially expressed in the copper-exposed LEC liver compared to wild-type siblings (Table 1). Expression of these genes was significantly increased or decreased in comparison with the expression of known oxidative stress-responsive genes. To establish whether copper-induced signatures in this model were unique, 40 GEO datasets with in the search term Affymetrix GeneChip Rat Expression Set (GPL341) were scrutinized (http://www.ncbi.nlm.nih.gov/sites/entrez) and subjected to hierarchical clustering analysis (Fig. 1). Within these 40 datasets, four clusters similar

to the LEC liver signature were identified, namely "Lung response to cigarette smoke (GDS2194)", "High Fe/low Fe at 12 weeks (GDS1027, GDS1054, GDS2073)", "Aged marrow-derived stromal cell response to dexamethasone (GDS1280, GDS2231)" and "Liver response to skin burn (GDS1273, 964, 1393, 1959, 2237)". This analysis suggested that gene expression signatures resulting from copper accumulation in the LEC rat liver were infrequent, although similar patterns could be detected under certain other stress conditions. The combination of the 7 genes identified here may be useful biomarkers for oxidative stress-related cancer.

2) Heavy metals in the Public Database. We further analyzed arsenic-induced gene expression profiles in order to compare them with the gene expression signature of copper exposure. Networks of oxidative stress-related genes were classified according to chemical exposure dosage and also by the types of arsenic used in different experiments. Array datasets (GDS2780 and GSE7967) for heavy metals were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). To predict common biomarkers and understand molecular mechanisms, we performed gene network analysis with Bayesian algorithms on liver carcinoma HepG2 cells exposed to different heavy metals in the GDS 2780 study and on human cord blood exposed to arsenic at different concentrations in the GSE7967 study. The results provide insight into the molecular basis of heavy metal cytotoxicity in the HepG2 study. In the GSE7967 study, the gene expression pattern suggested activation of Inflammation and NFκB signaling in infants born to arsenic-exposed mothers. Cord blood was collected at birth from infants whose mothers were exposed or unexposed to arsenic (0.1 - 68.63 mg/g) in the study. Therefore, we divided the subjects into 4 groups according to blood as concentrations (0.1, 1.76, 9.66, 68.63 mg/g). Expression array data are shown for the MAP kinase pathway, which is an important inflammation pathway (Fig. 2). Eleven oxidative stress-related genes were then selected and analyzed in two groups (mean blood concentration 0.142 μg/g in the low exposure group and 21.41 μg/g in the high exposure group) by the Bayesian network algorithm. The results of this analysis showed that *GSS* and *PRDX* regulate *TRDD*, *NUDT1*, *SOD1* and *INSIG1* in the low exposure group, and that *NUD1* regulates *TRDD*, *TXNRD2* and *PRDX2* in the high exposure group, suggesting that anti-oxidative stress-related genes play a key role in to protection against cellular damage in the low exposure group, but a DNA damage-related gene is dominant in the high exposure group, in which cell damage would progress.

3) *Retinoic acid-induced gene expression profiles reflecting developmental differentiation and disease.* In the early stage of neuronal development, the ES to EB

neuronal lineages differentiation period, it is known that exposure to RA activates anti-oxidative stress systems. Oxidative stress-responsive genes play roles in neuronal differentiation and development of disease such as Alzheimer's disease. Therefore, we analyzed gene signatures during neuronal development. Gene expression profiling and gene-network analysis, in terms of oxidative stress-related genes, revealed different signatures during neuronal lineage differentiation induced by retinoic acids. For the neuronal disease-related gene set including oxidative stress-responsive genes (Table 2), the gene-interaction network indicated that ubiquitination was activated and apoptosis was inhibited due to exposure to RA. This was despite the finding that the Sod system was activated with copper from App and ApoE and apoptosis was promoted under control conditions in differentiated neuronal cells (Fig. 4). Our current approaches could thus provide a useful way to obtain information on the properties of specific hazardous chemicals. Gene expression profiling and gene-network analysis revealed signatures for retinoic acid treatment in terms of oxidative stress-related genes, suggesting that they play different roles in early embryonic toxicity.

In conclusion, Gene expression signatures for oxidative stress associated with copper accumulation in the rat liver, heavy metals and RA exposure in mouse embryonic stem cells could effectively facilitate biomarker discovery and the improved understanding of molecular mechanisms of environmental chemical effects on cancer and developmental disorders.[22]

Table 1. List of genes changed remarkably in the LEC rat liver

Gene Name	Fold change*	Annotation, Function
Npdc1	12.1	Neural proliferation, differentiation and control,1
Tradd	11.5	TNFRSF1A-associated via death
Med17	10.5	Mediator complex subunit 17
Psmd1	8.8	Proteasome 26S subunit, non-ATPase, 1
Insig1	-7.7	Insulin induced gene 1
Abcc2	-6.2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2
G6pc	-6.1	Glucose-6-phosphatase, catalytic

*Values were confirmed by semi-quantitative RT-PCR

Table 2. List of genes related oxidative stress and neuronal disease

Gene name	Discription	Summary of biological function
Apbb	amyloid beta (A4) precursor protein-binding	binds to the intracellular domain of the Alzheimer's disease beta-amyloid precursor protein
App	amyloid beta (A4) precursor protein	This protein may relate in absorption of Cu.
Bace1	Beta-site APP-cleaving enzyme 1	Amyloid beta peptide is generated by proteolytic cleavage of amyloid precursor protein (APP) by g-secretase and this protease. Also called b-secretase.
Bcl2	B-cell CLL/lymphoma 2	This gene encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death of cells.
Ccs	Copper chaperone for superoxide dismutase	Copper chaperone for superoxide dismutase specifically delivers Cu to superoxide dismutase 1 and may activate superoxide dismutase 1 through direct insertion of the Cu cofactor.
Cdk5	Cyclin-dependent kinase 5	The product of this gene binds to APP and transfer it to b-secretase under the oxidative stress.
Gpx1	Glutathione peroxidase 1	Glutathione peroxidase functions in the detoxification of hydrogen peroxide, and is one of the most important antioxidant enzymes
Ide	Insulin-degrading enzyme	belong to a protease family responsible for intercellular peptide signalling. It degrades the intracellular domain of the amyloid precursor
Psen1	Presenilin 1	A subunit of g-secretase. It plays essential role in noramal cleavage of the amyloid precursor protein (APP).
Sod1	Superoxide dismutase 1, soluble	The protein binds copper and zinc ions and is one of two isozymes responsible for destroying free superoxide radicals in the cells. Mutations in this gene have been implicated as causes of familial amyotrophic lateral sclerosis.
Ubb	Ubiquitin B	Polyubiquitin precursor with a final amino acid after the last repeat. Aberrant form of this protein has been noticed in patients with Alzheimer's and Down syndrome.
Ube2l3	Ubiquitin-conjugating enzyme E2L 3	A member of the E2 ubiquitin-conjugating enzyme family.
Ubqln1	Ubiquilin 1	This ubiquilin has been shown to modulate accumulation of presenilin proteins, and it is found in lesions associated with Alzheimer's and
Uchl1	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	UCHL1 is a member of a gene family whose products hydrolyze small C-terminal adducts of ubiquitin to generate the ubiquitin monomer. It is present in all neurons.

Figure 1. Differential gene expression of copper accumulation-response genes with a genome informatics approach

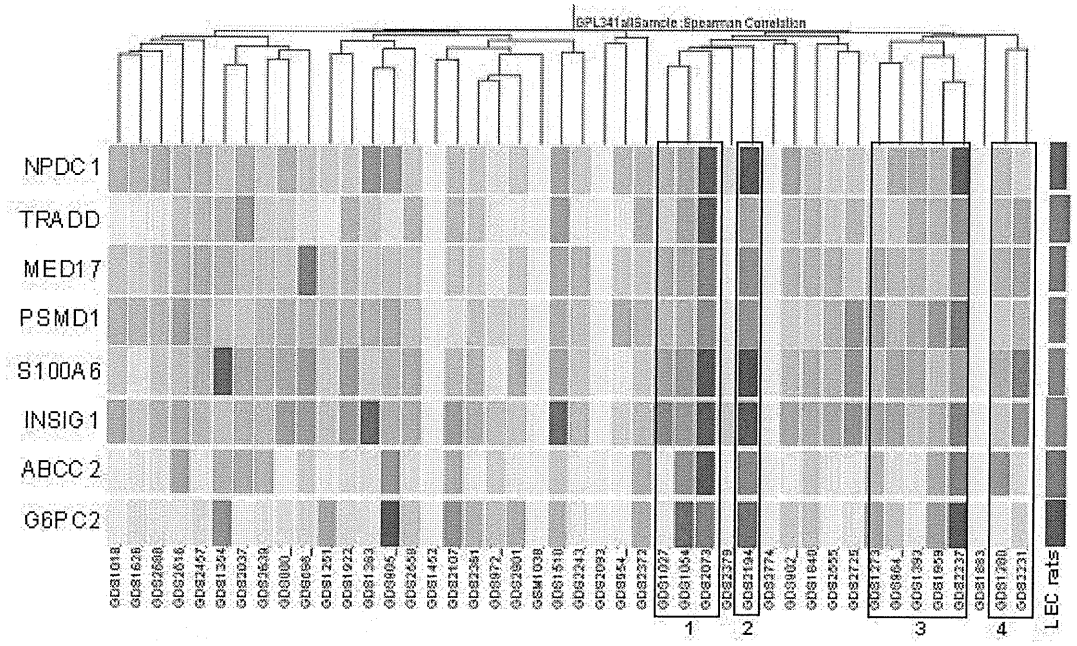


Figure 2. Prediction of gene interaction network for oxidative stress regulation in the heavy metal exposure in HepG2 cells

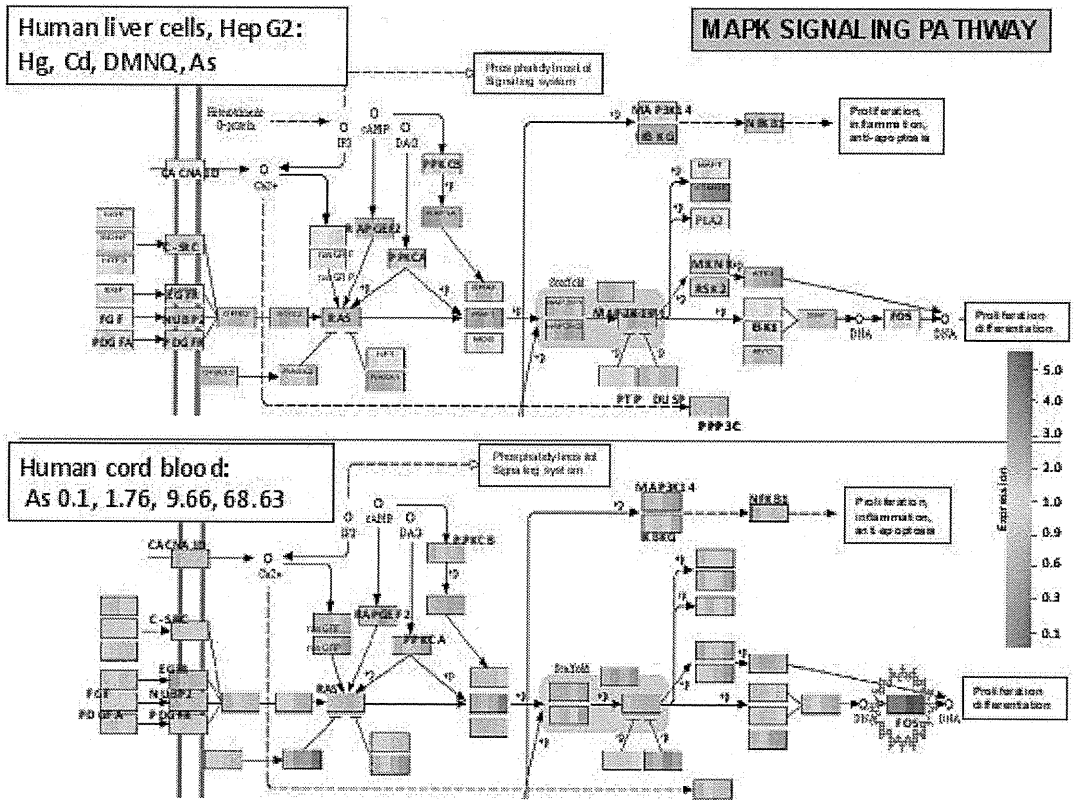


Figure 3. Genetic networks of oxidative stress 11 genes in data selected from the human cord blood study

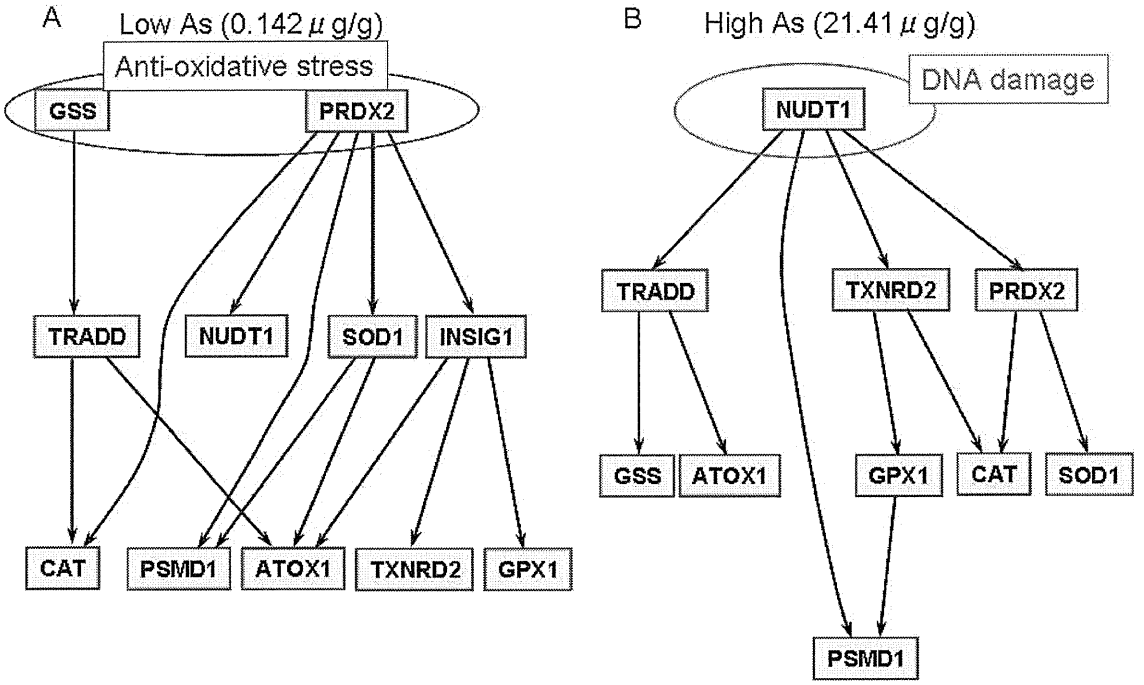
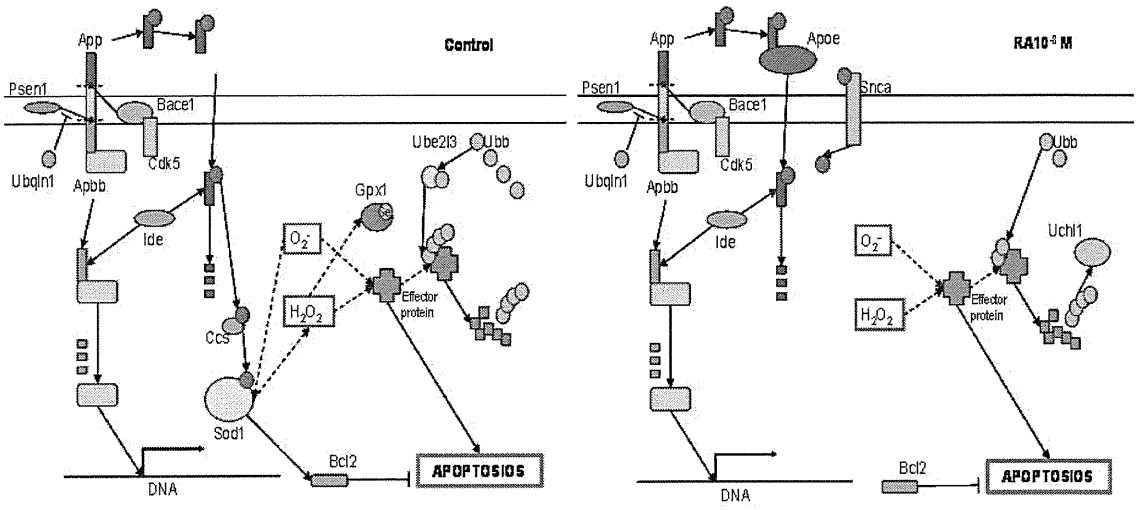


Figure 4. Gene Expression Signature influenced with RA exposure during development of ES to neuronal line ages



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