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13

OXIDATIVE STRESS-MEDIATED SIGNALING PATHWAYS BY ENVIRONMENTAL STRESSORS

HIDEKO SONE AND HIROMI AKANUMA
National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan

13.1 INTRODUCTION

Oxidative stress in the form of excess reactive oxygen species (ROS) or reactive nitrogen species (RNS) can affect cells deleteriously or beneficially. Such stress might be generated by intracellular or extracellular sources. Furthermore, oxidative stress can cause various biological effects. Environmental stress is a key contributor to human disease. A number of substances such as metals, particulate materials, smoke, pesticides, and physical agents are environmental stressors [1] that contribute to many diseases. Concerns related to environmental stressor-related diseases such as cancer, chronic lung disease, diabetes mellitus, neurodegenerative diseases, and reproductive disorders have been raised recently. Research efforts elucidating the modes by which environmental stressors influence the development and progression of diseases or exploring preventive approaches are expected to engender further improvements in our knowledge. Understanding environmental stressor-induced influences at the molecular level will also provide a wealth of information related to the exploration of biomarkers for environmental stressor-related diseases [2–4].

The mechanisms of redox adaptation in living bodies and cells might involve multiple influences on an active redox-sensitive signaling pathway, such as ROS metabolism and antioxidant defenses, p53 pathway signaling, nitric oxide (NO) signaling pathway, hypoxia signaling, transforming growth factor (TGF)-β-bone morphogenetic protein (BMP) signaling, tumor necrosis factor (TNF) ligand-receptor signaling, and mitochondrial function (Table 13.1). For example, transcription factors such as nuclear factor-κB (NF-κB), nuclear factor erythroid 2-related factor 2 (Nrf2), c-Jun, and hypoxia-inducible factor-1 (HIF-1) engender increased expression of antioxidant molecules such as superoxide dismutase (SOD), catalase, thioredoxin, and the GSH antioxidant system. Metal ions such as arsenic (III/V) or copper (II) directly influence expression levels of those transcription factors and induce various oxidative stress events including thiol molecule perturbation, generation of oxidative DNA adducts, and induction of oxidative molecular biomarkers [5–8]. Nonmetal chemicals such as retinoic acids and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are also known to influence the expression of oxidative stress-related genes and proteins during carcinogenesis and during embryonic development [9–12]. In relation to cancer, a growing tumor might also produce intracellular and extracellular oxidative stress, which can modify its malignant features. Endogenous sources of tumor ROS or RNS include impaired intracellular genomes or proteomes, metabolism pathways, and xenobiotic metabolism. Consequently, the study of transcriptional regulation of gene expression in the research field of oxidative stress has been useful for identifying new transregulatory factors or new biomarkers induced by exposure to environmental stressors.

Microarray technology has been used in environmental toxicology and biology studies and has led to the establishment of gene expression signatures profiling

Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling, First Edition.
Edited by Tahira Farooqui and Akhlaq A. Farooqui.

TABLE 13.1 Summary of oxidative stress-mediated signaling pathways

Categorical pathways	Canonical Pathway (orthology)
Reactive Oxygen Species (ROS) Metabolism and Antioxidant Defenses	Glutathione Peroxidases (GPx)
	Peroxiredoxins (TPx)
	Superoxide Dismutases (SOD)
	Genes Involved in Superoxide Metabolism
	Genes Involved in ROS Metabolism
	Other Peroxidases and Antioxidant-Related Genes
p53 Signaling (including DNA damage)	Apoptosis-Related Genes
	Cell Cycle Arrest and Checkpoint
	Regulation of the Cell Cycle
	Regulation of Cell Proliferation, Cell Growth, and Differentiation
	Damaged DNA Binding
	Mismatch, Base-Excision and Double-Strand Break Repair
Nitric Oxide (NO) Signaling Pathway	Genes with NO Synthase and Regulators of NO Biosynthesis
	Genes regulated by NO and NO Signaling Pathway
	Genes Involved in Superoxide Release
	Antiapoptosis Genes
	Genes with Antioxidant and Superoxide Dismutase Activity
	Genes with Glutathione Peroxidase, Oxidoreductase, or Peroxidase Activity
	Transcription Regulators
Hypoxia Signaling	Response to Hypoxia and Signal Transduction, Oxidative Stress
	Genes Related to Stress and Immune Response
	Hemoglobin Complex-Associated Genes
	Peroxidase, Oxidoreductase-Related Genes
	Transcription Factors and Regulators and Protein Binding
	Antiapoptosis Induction of Apoptosis and Caspase Activity
	Protein Biosynthesis, Phosphorylation, and Metabolism
	Cytoskeleton and Other Extracellular Molecules
	Cell Cycle, Cell Proliferation, and Growth Factors
	Carbohydrate, Lipid, One-Carbon Compound Metabolism
	RNA Metabolism
	Cardiac Excitation-Contraction (E-C) Coupling
TGF-β-BMP Signaling	TGF-β superfamily, bmp (bone morphogenetic protein) family members, gdf (growth differentiation factor), activin, and activin receptors
	Smad family members, TGF-β/activin-responsive genes, bmp-responsive genes, molecules regulating signaling of the TGF-β superfamily, adhesion molecules, extracellular matrix structural constituents, other extracellular molecules, transcription factors and regulators
Tumor Necrosis Factor (TNF) Ligand-Receptor Signaling	Caspase activation, caspase inhibition, anti-apoptosis genes, induction of apoptosis, other apoptosis-related genes, jnk signaling pathway, nfκb signaling pathway, tnf superfamily members, tnfr1 and tnfr2 signaling pathway, inflammatory response, transcription regulators
Mitochondria	Mitochondrial processing, mitochondrial transportation Fatty acid biosynthesis

the toxicity of environmental stressors [13, 14]. Statistical methods used for DNA microarray studies are mostly multivariate approaches. Although basic methods treat genes as traits, which is consistent with the rules of experimental design, several approaches have been developed using expression ratio data sets. Such approaches regard the genes as cases and the array plates as variables. Most well-known methods based on singular value decomposition have used principal component analysis [15, 16]. In alternative approaches, our previous reports have described that a Bayesian network technique, which is a probabilistic graphical model that represents a set of variable identities, is applicable to investigation of the gene expression interaction networks and the detection of differences arising in them from exposure to different doses of chemicals [17, 18]. Bayesian network techniques can provide predictive information related to the relations between agents and gene expression signatures in the life science fields [19–21].

This chapter addresses various environmental stressor-induced toxicities in experimental animals such as

rats and humans to elucidate the molecular mechanisms underlying toxicity-induced oxidative stress.

13.2 OXIDATIVE STRESS-MEDIATED SIGNALING PATHWAYS

Cells respond and adapt to environmental signals such as stressors [22–24] through multiple mechanisms that involve communication pathways and signal transduction processes. The impact of oxidative stress on various diseases and aging has been reviewed comprehensively. In particular, free radical-induced oxidative stress plays an important role in cancer development, metabolism-related diseases like diabetes and hypertension, and neurodegenerative disorders [4, 25–36]. Our survey of microarray databases and many other published references has revealed the categorical pathways induced by oxidative stress presented in Table 13.2.

ROS metabolism and antioxidant defenses center upon ROS, which are necessary for biological functions and which regulate many signal transduction pathways by directly reacting with and modifying the structure of proteins, transcription factors, and genes to modulate their functions. Actually, ROS induce expression levels of genes associated with signaling cell growth and differentiation, regulating the activity of enzymes (such as ribonucleotide reductase and peroxidase). Control of ROS levels is achieved by balancing ROS generation with their elimination through ROS-scavenging systems such as superoxide dismutases (SOD1, SOD2, and SOD3), glutathione peroxidase, peroxiredoxins, glutaredoxin, and thioredoxin catalase. The ROS can modulate the activities and expression of many transcription factors and signaling proteins that are involved in stress response and cell survival through multiple mechanisms. Therefore, this category includes glutathione peroxidases (GPx), peroxiredoxins (TPx), superoxide dismutases (SOD), genes involved in superoxide metabolism such as arachidonate 12-lipoxygenase (ALOX12), and copper chaperone for superoxide dismutase (CCS). In fact, p53 signaling plays a central role in coordinating the cellular responses to a broad range of cellular stress factors; 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. Moreover, p53 controls transactivation of target genes, which is an essential feature of stress response pathways [37–39]. In other words, p53 activation leads to a complicated network of responses to the various stress signals encountered by cells [40–44]. The mitochondrial respiratory chain produces NO, which can generate other RNS when cells are under hypoxic conditions. Although excess ROS and RNS can engender oxidative and nitrosative stress, moderate to low levels of both function in cellular signaling pathways. Especially important are the roles of these mitochondrion-generated free radicals in hypoxic signaling pathways, which have important implications for cancer, inflammation, and various other diseases [25, 45]. Hypoxic signaling events include vasodilation, modulation of mitochondrial respiration, and cytoprotection following ischemic insult. These phenomena are attributed to the reduction of nitrite anions to NO if local oxygen levels in tissues decrease [46], which activates the expression of genes through oxygen-sensitive transcription factors including HIF and NF-κB. Hypoxia-dependent gene expression can have important physiological or pathophysiological consequences for an organism, depending upon the cause of the hypoxic insult [47]. These NO signaling and hypoxia signaling pathways are linked to the p53 pathway [48], because recent studies have shown that HIF2α inhibition promotes p53-mediated responses by disrupting cellular redox homeostasis, thereby permitting ROS accumulation and DNA damage [49]. Reportedly, hypoxia activates the tumor suppressor protein p53 by upregulating Sema3E expression [50].

TGF-β-BMP signaling is involved in developmental morphogenesis and cancer morphogenesis. Morphogens such as those of the TGF-β family inhibit and stimulate basic cell proliferation, respectively, at high and low concentrations. A signaling gradient of declining TGF-β concentration regulates the inhibition and stimulation of cell proliferation [51]. ROS can activate TGF-β either directly or indirectly via the activation of proteases. In addition, TGF-β itself induces ROS production as part of its signal-transduction pathway. Pulmonary tissues are vulnerable to the toxic effects of inhaled air. The oxidant pathways are especially relevant in the lung, where TGF-β is known to have a role in tissue repair and connective tissue turnover. In pulmonary fibrosis and renal endothelial cells, TGF-β activation is considered a hallmark of disease progression [52, 53]. In ovarian cancer, overexpression of FOXG1 contributes to TGF-β resistance through inhibition of p21WAF1/CIP1 expression, which is repressed by p53 [54]. Recent studies have revealed some additional novel functions of the p53 pathway. These include the downregulation of two central cell-growth pathways, the IGF/AKT-1 and mTOR pathways, and the upregulation of the activities of the endosomal compartment [55–57]. The mTOR pathway including the IGF-1/AKT pathway plays critical roles in regulation of cell proliferation, survival, and energy metabolism to shut down cell growth and division to avoid the introduction of infidelity into the process of cell growth and division [58, 59]. In response to stress, IGF-BP3, PTEN, TSC2, AMPK beta1, and Sestrin1/2 are transcribed by p53, play a critical

TABLE 13.2 Oxidative stress-mediated signaling pathways and their related genes

Categorical Pathways Canonical Pathway (ontology)	Gene Name (Symbol)
Reactive Oxygen Species (ROS) Metabolism and Antioxidant Defenses	
Glutathione Peroxidases (GPx)	GPX1, GPX2, GPX3, GPX4, GPX5, GPX6, GPX7, GSTZ1
Peroxiredoxins (TPx)	PRDX1, PRDX2, PRDX3, PRDX4, PRDX5, PRDX6
Other Peroxidases	CAT, CSDE1, CYGB, DUOX1, DUOX2, EPX, GPR156, LPO, MGST3, MPO, PIP3-E, PTGS1, PTGS2, PXDN, PXDNL, TPO, TTN
Other Antioxidants	ALB, APOE, GSR, MT3, SELS, SRXN1, TXNDC2, TXNRD1, TXNRD2
Superoxide Dismutases (SOD)	SOD1, SOD2, SOD3
Other Genes Involved in Superoxide Metabolism	ALOX12, CCS, CYBA, DUOX1, DUOX2, GTF2I, MT3, NCF1, NCF2, NOS2A, NOX5, PREX1, PRG3
Genes Involved in ROS Metabolism	AOX1, BNIP3, EPHX2, MPV17, SFTPD
Oxidative Stress-Responsive Genes	ANGPTL7, ATOX1, CAT, CCL5, CSDE1, DGKK, DHCR24, DUSP1, EPX, FOXM1, GLRX2, GPR156, GSS, KRT1, LPO, MBL2, MPO, MSRA, MTL5, NME5, NUDT1, OXR1, OXSR1, PDLIM1, PIP3-E, PNKP, PRDX2, PRDX5, PRDX6, PRNP, RNF7, SCARA3, SELS, SEPP1, SGK2, SIRT2, SRXN1, STK25, TPO, TTN
p53 Signaling Pathway	
Induction of Apoptosis	BAX, BID, CDKN1A, CRADD, EI24, FADD, FASLG (TNFSF6), FOXO3, PCBP4, PRKCA, TNFRSF10B, TP53, TP73, TP73L
Antiapoptosis	BCL2, BCL2A1, BIRC5, CASP2, HDAC1, IGF1R, MCL1, NFKB1, RELA, TNF, TNFRSF10
Other Apoptosis Genes	APAF1, BRCA1, CASP9, E2F1, GADD45A, GML, LRDD, P53AIP1, SIAH1, SIRT1, TP53BP2, TRAF2
Cell Cycle Arrest	CDKN1A, CDKN2A, CHEK1, CHEK2, GADD45A, GML, MYC, PCAF, PCBP4, RPRM, SESN1, SESN2
Cell Cycle Checkpoint	ATR, BRCA1, CCNE2, CCNG2, CDKN2A, RB1, TP53
Negative Regulation of the Cell Cycle	BAX, BRCA1, CDKN2A, MSH2, NF1, PTEN, RB1, TP53, TP73, TP73L, TSC1, WT1
Regulation of the Cell Cycle	BRCA2, CDC2, CDC25A, CDK4, E2F1, E2F3, HK2, IGF1R, KRAS, PPM1D, PRKCA, STAT1, TADA3L, TP53BP2
Other Cell Cycle Genes	BIRC5, CCNH, CCNB2, ESR1, MLH1, PCNA, PRC1
Negative Regulation of Cell Proliferation	BAI1, BCL2, BTG2, CDKN1A, CDKN2A, CHEK1, GML, IFNB1, IL6, MDM2, MDM4, NF1, PCAF, PPM1D, SESN1
Positive Regulation of Cell Proliferation	IGF1R, IL6
Cell Proliferation	BRCA1, CDC25A, CDC25C, CDK4, E2F1, MYC, PCNA, PRKCA
Cell Growth and Differentiation	ESR1, MCL1, MYOD1
Other Genes Related to Cell Growth, Proliferation, and Differentiation	EGR1, FOXO3A, JUN, KRAS, PTTG1
DNA Repair Genes	ATM, ATR, BRCA1, BTG2, CCNH, DNMT1, GADD45A, MSH2, PCNA, PTTG1, TP53, XRCC5
Human Nitric Oxide Signaling Pathway	
Genes with Nitric Oxide Synthase or Oxidoreductase activity	NOS1, NOS2A, NOS3, NQO1
Positive Regulators of Nitric Oxide Biosynthesis	HSP90AB1 (HSPCB), INS
Negative Regulators of Nitric Oxide Biosynthesis	DNCL1, GLA, IL10

Other Genes Involved in NO Biosynthesis	AKT1, ARG2, DDAH2, DNCL1, EGFR, GCH1, GCHFR
Genes Induced by NO	CDKN1A, IL8, JUN, VEGFA
Genes Suppressed by NO	CCNA1, MYB, TROAP
Genes Involved in NO Signaling Pathway	CAMK1, DLG4, GRIN2D, NOS1, PPP3CA, PRKAR1B, PRKCA
Genes Involved in Superoxide Release	ALOX12, DUOX1, DUOX2, NOX5, PRG3
Genes with Oxidoreductase Activity	ALOX12, CYBA, DUOX1, DUOX2, NOS2A, NOX5, SOD1, SOD2, SOD3
Genes with Peroxidase Activity	DUOX1, DUOX2
Genes with Superoxide Dismutase Activity	SOD2
Other Genes Involved in Superoxide Metabolism	CCS, NCF1, NCF2, PREX1
Antiapoptosis Genes	MPO, MTL5, NME5, PRDX2, RNF7
Genes with Antioxidant Activity	APOE, MT3, SELS, SOD1, SOD3, SRXN1 (C20orf139)
Genes with Glutathione Peroxidase Activity	GPX1, GPX2, GPX3, GPX4, GPX5, GPX6, LOC493869
Genes with Oxidoreductase Activity	CAT, EPX, GPX1, GPX2, GPX3, GPX4, GPX5, GPX6, LPO, MPO, MSRA, PRDX2, PRDX6, SOD1, SOD2, SRXN1(C20orf139), TPO, TXNRD2
Genes with Peroxidase Activity	CYGB, EPX, GPR156, LPO, MPO, PRDX2, PRDX5, PRDX6, TPO, TTN, UNR
Transcription Regulators	FOXM1, GLRX2, SCRT2, SIRT2, SOD2, UNR
Other Genes Involved in Oxidative Stress	ATOX1, DUSP1, GSS, KRT1, MBL2, NUDT1, OXR1, PNKP, PRNP, SCARA3, SEPP1, SGK2
DNA Damage Signaling	
Apoptosis	ABL1, BRCA1, CIDEA, GADD45A, GADD45G, GML, IHPK3, PCBP4, AIFM1 (PDCD8), PPP1R15A, RAD21, TP53, TP73
Cell Cycle Arrest	CHEK1, CHEK2, DDIT3 (CHOP), GADD45A, GML, GTSE1, HUS1, MAP2K6, MAPK12, PCBP4, PPP1R15A, RAD17, RAD9A, SESN1, ZAK
Cell Cycle Checkpoint	ATR, BRCA1, FANCG, NBN (NBS1), RAD1, RBBP8, SMC1A (SMC1L1), TP53
Damaged DNA Binding	ANKRD17, BRCA1, DDB1, DMC1, ERCC1, FANCG, FEN1, MPG, MSH2, MSH3, N4BP2, NBN (NBS1), OGG1, PMS2L3 (PMS2L9), PNKP, RAD1, RAD18, RAD51, RAD51L1, REV1 (REV1L), SEMA4A, XPA, XPC, XRCC1, XRCC2, XRCC3
Base-Excision Repair	APEX1, MBD4, MPG, MUTYH, NTHL1, OGG1, UNG
Double-Strand Break Repair	CIB1, FEN1, XRCC6 (G22P1), XRCC6BP1 (KUB3), MRE11A, NBN (NBS1), PRKDC, RAD21, RAD50
Mismatch Repair	ABL1, ANKRD17, EXO1, MLH1, MLH3, MSH2, MSH3, MUTYH, N4BP2, PMS1, PMS2, PMS2L3 (PMS2L9), TP73, TREX1
Other Genes Related to DNA Repair	APEX2, ATM, ATRX, BTG2, CCNH, CDK7, CRY1, ERCC2 (XPD), GTF2H1, GTF2H2, IGHMBP2, LIG1, MNAT1, PCNA, RPA1, SUMO1
Mitochondria	
Membrane Polarization & Potential	BAK1, BCL2, BCL2L1, BNIP3, SOD1, TP53, UCP1, UCP2, UCP3
Mitochondrial Transport	AIP, BAK1, BCL2, BCL2L1, BNIP3, CPT1B, CPT2, DNAJC19, FXC1 (TIMM10B), GRPEL1, HSP90AA1, HSPD1, IMMP2L, MFN2, MIPEP, MTX2, STARD3, TP53, TSPO, UCP1, UCP2, UCP3
Small Molecule Transport	SLC25A1, SLC25A10, SLC25A12, SLC25A13, SLC25A14, SLC25A15, SLC25A16, SLC25A17, SLC25A19, SLC25A2, SLC25A20, SLC25A21, SLC25A22, SLC25A23, SLC25A24, SLC25A25, SLC25A27, SLC25A3, SLC25A30, SLC25A31, SLC25A37, SLC25A4, SLC25A5
Targeting Proteins to Mitochondria	AIP, DNAJC19, FXC1 (TIMM10B), GRPEL1, HSPD1, IMMP2L, MFN2, MIPEP, TSPO
Mitochondrial Protein Import	AIP, COX10, COX18, DNAJC19, FXC1 (TIMM10B), GRPEL1, HSPD1, MIPEP, SH3GLB1

(*Continued*)

TABLE 13.2 Continued

Categorical Pathways Canonical Pathway (ontology)	Gene Name (Symbol)
Outer Membrane Translocation	TOMM20, TOMM22, TOMM34, TOMM40, TOMM40L, TOMM70A
Inner Membrane Translocation	FXC1 (TIMM10B), IMMP1L, IMMP2L, OPA1, TAZ, TIMM10, TIMM17A, TIMM17B, TIMM22, TIMM23, TIMM44, TIMM50, TIMM8A, TIMM8B, TIMM9
Mitochondrial Fission & Fusion	COX10, COX18, FIS1, MFN1, MFN2, OPA1
Mitochondrial Localization	DNM1L, LRPPRC, MFN2, MSTO1, NEFL, OPA1, RHOT1, RHOT2, UXT
Apoptotic Genes	AIFM2, BAK1, BBC3, BCL2, BCL2L1, BID, BNIP3, CDKN2A, DNM1L, PMAIP1, SFN, SH3GLB1, SOD2, TP53
Hypoxia Signaling	
Response to Hypoxia	ANGPTL4, ARNT2, CREBBP, EP300, HIF1A, MT3, PRKAA1
Response to Oxidative Stress	CAT, CYGB, GPX1, PIP3-E
Immune Response	GPI, IL1A, IL6, IL6ST, NOS2A, NOTCH1, PTX3, RARA
Other Genes Related to Stress Response	ADM, EPO, HYOU1, VEGFA
Hemoglobin Complex-Associated Genes	CYGB, EPO, HBB, HMOX1, NOS2A, PIP3-E
Peroxidase	CAT, CYGB, GPX1, PIP3-E
Other Oxidoreductase-Related Genes	HIF1AN, HMOX1, MT3, NOS2A, PLOD3, TH
Transcription Cofactors	CREBBP, DR1, ENO1, EP300, EPAS1, HTATIP, RARA
Transcription Factors	ARNT2, BHLHB2, CREBBP, ENO1, EP300, EPAS1, HIF1A, HIF3A, KHSRP, MYBL2, PPARA, RARA
Other Transcription Factors and Regulators	HIF1AN, NOTCH1
Antiapoptosis	BAX, ANGPTL4, BIRC5, IL1A, MYBL2, PEA15, PRKAA1, VEGFA
Caspase Activity	BIRC5, CASP1
Induction of Apoptosis	BAX, DAPK3, NUDT2
Other Apoptosis Genes	EP300
Signal Transduction	ADM, ARNT2, CASP1, CDC42, CREBBP, EP300, EPAS1, EPO, GNA11, HIF1A, HIF3A, HMOX1, IGFBP1, IL1A, IL6, IL6ST, IQGAP1, KIT, LEP, PLAU, RARA, VEGFA
Protein Biosynthesis	EEF1A1, PDIA2 (PDIP), PRKAA1, RPL28, RPL32, RPS2, RPS7
Protein Heterodimerization	ARNT2, HIF1A, RARA, SAE1
Protein Homodimerization	ARNT2, RARA, VEGFA
Protein Amino Acid Phosphorylation	DAPK3, KIT, PRKAA1
Protein Binding	CASP1, CREBBP, ENO1, EP300, IQGAP1, NOS2A, PEA15, PPP2CB, RARA
Other Genes Related to Protein Metabolism	ARD1A, CDC42, GNA11, HYOU1, MAN2B1, PLOD3, PSMB3, SUMO2, TUBA4A (TUBA1)
Protease Inhibitors	BIRC5, CSTB
Protease Molecules	AGTPBP1, CASP1, ECE1, PLAU, PSMB3
Other Extracellular Molecules	ADM, ANGPTL4, CHGA, COL1A1, EPO, IGF2, IGFBP1, IL1A, IL6, LEP, NPY, PTX3, VEGFA
Cytoskeleton	DCTN2, SPTBN1
Cell Cycle	BAX, BIRC5, EP300, HK2, IGF2, IL1A, MYBL2, SSSCA1, VEGFA
Cell Proliferation	DCTN2, IGF2, IL1A, IL6, MT3, NPY, RARA, VEGFA
Growth Factors	GPI, IGF2, IGFBP1, IL1A, IL6, KIT, VEGFA
Other Genes Related to Cell Growth	ENO1

Carbohydrate Metabolism	GPI, HK2, LCT, MAN2B1, PEA15, PRKAA1, SLC2A1, SLC2A4
Lipid Metabolism	AGPAT2, ANGPTL4, PPARA, PRKAA1
One-Carbon Compound Metabolism	CA1
Superoxide Metabolism	MT3, NOS2A
RNA Metabolism	PRPF40A (FNBP3), KHSRP, RARA, RPL28, RPS2, SNRP70
Other Genes Related to Metabolism	ADM, AGPAT2, MOCS3, NUDT2, TH, TST, UCP2
Cardiac Excitation-Contraction (E-C) Coupling	ARNT2, CHGA, DAPK3, GNA11, IQGAP1, KIT, NOS2A, NOTCH1, NPY, PRKAA1, SPTBN1
TGF-β BMP Signaling	
TGF-β	TGFB1, TGFB2, TGFB3
BMP	BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7
GDF	AMH, GDF2 (BMP9), GDF3 (Vgr-2), GDF5 (CDMP-1), GDF6, GDF7, IGF1, IGFBP3, IL6, INHA (inhibin a), INHBA (inhibin BA), LEFTY1, LTBP1, LTBP2, LTBP4, NODAL, PDGFB
Activin	INHA (inhibin a), INHBA (inhibin BA), INHBB (inhibin BB), LEFTY1, NODAL
Receptors	ACVR1 (ALK2), ACVR2A, ACVRL1 (ALK1), AMHR2, BMPR1A (ALK3), BMPR1B (ALK6), BMPR2, ITGB5 (integrin B5), ITGB7 (integrin B7), LTBP1, NR0B1, STAT1, TGFB1I1, TGFBR1, (ALK5) TGFBR2, TGFBR3, TGFBRAP1
SMAD	SMAD1 (MADH1), SMAD2 (MADH2), SMAD3 (MADH3), SMAD4 (MADH4), SMAD5 (MADH5)
TGF-β/Activin-Responsive	CDC25A, CDKN1A (p21WAF1 / p21CIP1), CDKN2B (p15LNK2B), COL1A1, COL1A2, COL3A1, FOS, GSC (goosecoid), IGF1, IGFBP3, IL6, ITGB5 (integrin B5), ITGB7 (integrin B7), JUN, JUNB, MYC, PDGFB, SERPINE 1 (PAI-1), TGFB1I1, TSC22D1 (TGFB1I4), TGFBI, TGIF1
BMP-Responsive	BGLAP (osteocalcin), DLX2, ID1, ID2, JUNB, SOX4, STAT1
Molecules Regulating Signaling of the TGF-β Superfamily	BAMBI, BMPER, CDKN2B (p15LNK2B), CER1 (cerberus), CHRD (chordin), CST3, ENG (Evi-1), EVI1, FKBP1B, FST (follistatin), HIPK2, NBL1 (DAN), NOG, PLAU (uPA), RUNX1 (AML1), SMURF1
Adhesion Molecules	BGLAP (osteocalcin), ENG (Evi-1), ITGB5 (integrin B5), ITGB7 (integrin B7), TGFB1I1, TGFBI
Extracellular Matrix Structural Constituents	BGLAP (osteocalcin), COL1A1, COL1A2, COL3A1, LTBP1, LTBP2, LTBP4, TGFBI
Other Extracellular Molecules	AMH, BMP1, BMP2, FST (follistatin), GDF2 (BMP9), GDF3 (Vgr-2), IGF1, IGFBP3, IL6, INHA (inhibin a), INHBA (inhibin BA), INHBB (inhibin BB), PDGFB, PLAU (uPA), SERPINE1
Transcription Factors and Regulators	DLX2, EVI1, FOS, GSC (goosecoid), HIPK2, ID1, JUN, JUNB, MYC, NR0B1, RUNX1 (AML1), SMAD1 (MADH1), SMAD2 (MADH2), SMAD3 (MADH3), SMAD4 (MADH4), SMAD5 (MADH5), SOX4, STAT1, TGFB1I1, TSC22D1 (TGFB1I4), TGIF1
Tumor Necrosis Factor (TNF) Ligand and Receptor	
Induction of Apoptosis	CASP2, CASP3, CASP8, CRADD, FADD, FAS, FASLG, LTA, TNFSF8, TNFRSF10, TNFRSF10A, TNFRSF10B, TNFSF14, TNFRSF19, TNFRSF25, CD27 (TNFRSF7), TNFRSF9, TRADD
Caspase Activation	TNFRSF10A, TNFRSF10B, TNFSF15
Caspase Inhibition	CD27 (TNFRSF7), TNFSF14
Anti-apoptosis Genes	CD40LG, FAS, TNF, TNFRSF10D, TNFRSF6B, CD27 (TNFRSF7), TNFSF18
Other Apoptosis-Related Genes	CD40, CD70 (TNFSF7), TNFSF9, LTBR, NGFR, TNFRSF10C, TNFRSF11B, TNFRSF12A, TNFRSF14, TNFRSF1A, TNFRSF1B, TNFRSF21, DFFA, PAK1, TRAF2
Inflammatory Response	CD40LG, TNF, TNFRSF1A
NF-κB Signaling Pathway	CASP8, FADD, TNF, TNFRSF1A, FASLG, TNF, TNFSF10, TNFSF14, TNFSF15, CD40, EDA2R, LTBR, TNFRSF10A, TNFRSF10B, CD27 (TNFRSF7), TRADD

(*Continued*)

TABLE 13.2 Continued

Categorical Pathways Canonical Pathway (ontology)	Gene Name (Symbol)
JNK Signaling Pathway	EDA2R, TNFRSF19, CD27 (TNFRSF7), MAP2K4, MAPK8, PAK1
Other TNF Superfamily Members and ligands	LTB, PGLYRP1, TNFSF11, TNFSF12, TNFSF13, TNFSF13B, TNFSF4, TNFSF5IP1, TNFRSF11A, TNFRSF13B, TNFRSF13C, TNFRSF17, TNFRSF19L, TNFRSF4, TNFRSF8
Transcription Regulators	JUN, PARP1, RB1, TNF, TNFRSF1A, TNFRSF25, CD27 (TNFRSF7), TNFRSF9
TNFR1 Signaling Pathway	
FAS signaling pathway	ARHGDIB, CAD, HRB, LMNA, LMNB1, LMNB2, MADD, MAP3K1, MAP3K7, PAK2, PRKDC, SPTAN1
Induction of Apoptosis	IKBKG, LTA, TRAF3, TNFRSF14, TNFRSF1A, TNFRSF1B
Anti-apoptosis Genes	NFKB1, TNFAIP3
Other Apoptosis Genes	NFKBIA, TNFRSF1B, TRAF1, TRAF2
Inflammatory Response	NFKB1
NF-κB Signaling Pathway	CHUK, IKBKB, IKBKG, NFKBIA, TNFAIP3
Transcription Regulators	IKBKB, IKBKG, NFKB1, NFKBIA
TNFR2 Signaling Pathway	DUSP1, HRB, IKBKAP, MAP3K1, MAP3K14, TANK

role as negative regulators, and lead to the reduction in the activities of these two pathways. Furthermore, p53 transcriptionally regulates TSAP6, Chmp4C, Caveolin-1, and DRAM, which are critical genes in the endosomal compartment, increases exosome secretion and the rate of endosomal removal of growth factor receptors from cell surface, and enhances autophagy [60–63]. It is thought that these p53-mediated activities slow down cell growth and division, conserve and recycle cellular resources, communicate with adjacent cells and dendritic cells of the immune system, and inform other tissues of the stress signals [55, 64, 65].

TNF ligand-receptor signaling occurs because TNF, as a multifunctional cytokine, can induce cell death through receptor-mediated caspase activation and mitochondrial dysfunction by a trigger of oxidative stress induced in cardiovascular disease, neuronal disease, and cancer [66]. Opposing these cell death-promoting signals, binding of TNF receptors can also trigger survival signal activation. A critical balance among various intracellular signaling pathways determines the predominant in vivo bioactivity of TNF, as best exemplified by the differential responses of various organs.

A major source of ROS in cells is the mitochondria. Electron leakage from the mitochondrial respiratory chain can react with molecular oxygen, resulting in the formation of the superoxide anion radical, which can subsequently be converted to other ROS. In phagocytes and some cancer cells, ROS can be produced through a reaction that is catalyzed by NADPH oxidase complexes. When attackers from the outside, such as environmental stressors, damage mitochondria, electron leakage is also induced; this dysfunction induces severe problems in tissues [67–70]. Mitochondrial dysfunction causes the onset of some diseases [71–74]. Recent evidence has shown that mitochondrial dysfunction is related closely to insulin resistance and metabolic syndrome. The underlying mechanism of mitochondrial dysfunction is very complex, including genetic factors from both the nucleus and mitochondrial genome, with numerous environmental factors also impacting [75].

Exposure to air pollution, including particles, metals, and other organic compounds as environmental stressors, is associated with pulmonary diseases and cancer. The mechanisms of induced health effects are believed to involve oxidative stress. Oxidative stress mediated by airborne particles and/or fibers might arise from direct generation of ROS from the surfaces of particles and fibers, soluble compounds such as transition metals or organic compounds, and activation of inflammatory cells capable of generating ROS and RNS. Generation of ROS/RNS can cause covalent modifications to DNA directly, or they can initiate the formation of genotoxic lipid hydroperoxides. The resulting oxidative DNA damage can engender changed gene expression such as upregulation of tumor promoters and downregulation of tumor suppressor genes; the DNA damage might therefore be implicated in cancer development. This chapter describes the important role of free radicals in particle- and fiber-induced cellular damage, the interaction of ROS with target molecules, especially with DNA, and the modulation of specific genes and transcription factor caused by oxidative stress. Consequently, various environmental stressors cause cellular damage through oxidative stress induction and many signaling pathways. However, what environmental stressor is dominant in which signaling pathway is not always clear. Therefore, identifying gene expression signatures extracted from microarray data can clarify how environmental stressors may damage cells and engender diseases.

13.2.1 Case Studies in Tissues or Organs from Rats Exposed to Environmental Stressors

Many animal models have been studies to elucidate mechanisms of action of oxidants or antioxidants. Research on oxidative stress and its defense has expanded dramatically because of its potential benefit in disease prevention and health promotion. In particular, rats exposed to stress-induced chemicals have been extensively studied in biological systems such as cell cultures, animal models, and clinical trials [76–79]. Therefore, 33 independent studies in rats are focused on in this chapter because these studies used microarrays for which gene expression data are publicly available from the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/gds). Those microarray data with the same platform GPL341 (Affymetrix) sets in rats were downloaded for this study. All data sets were normalized across all arrays by Z-score transformation methods after combination with respect to probe IDs. The normalized values were filtered with oxidative-related genes listed in this work (Table 13.2), and then the top 10 genes from the upregulated and downregulated genes were chosen to analyze gene expression signatures (Table 13.3). The selected genes were classified by using principal component analysis to create gene expression signatures of oxidative stress and were divided into six groups. Most selected genes could be assigned to gene ontology (GO) categories: DNA repair, oxygen and reactive oxygen species metabolism, and response to stress, but cyclins and cyclin dependent kinase contained in "Apoptosis related genes, Cell Cycle Arrest and Checkpoint, Regulation of the Cell Cycle, Regulation of Cell Proliferation, Cell Growth and Differentiation" of "p53 signaling" and "TGF-beta signaling" were not observed. Experimental conditions selected from GPL341 data sets in this work were almost all of short-period exposure using in vivo and in vitro culture systems of rats. It is noteworthy that

TABLE 13.3 Environmental stressors induce different gene expression signatures

Environmental Stressors (target organ or tissues)	Up-gene	Down-gene	GEO ID
Cluster 1			
Methylprednisolone (kidney)	Apoe, Gpx2, Ngb, Nos2, Prdx6, Tmod1, Tnp1, Tpo	Brca2, Cry2, Fen1, Hus1, Ptgs2, Pttg1, Rad50, Srxn1, Xrcc6	GDS964
Methylprednisolone (liver)	Aass, Atrx, Ncf1, Nqo1, Scd1, Slc41a3, Srd5a2, Tmod1, Tnp1	Chek1, Cry2, Lig1, Mgmt, Pold1, Pold3, Rad50, Rad52, Smc3, Xrcc6	GDS972
Streptozotocin (penile cavernosal)	Apc, Cat, Duox2, Gpx2, Gpx6, Gsr, Lpo, Slc38a1, Smc3, Tpo	Atrx, Gpx7, Nos2, Park7, Ptgs2, Scd1, Slc38a4, Slc41a3, Srxn1, Zmynd17	GDS1393
Trimethyltin (hippocampus)	Apex1, Dnm2, Fancc, Gpx7, Lpo, Mgmt, Park7, Prnp, Txnip, Ucp3	Apc, Apoe, Hbz, Mpp4, Ptgs2, Smc3, Srd5a2, Tnp1, Tpo	GDS2555
Octreotide (gastric ECL)	Brca1, Brca2, Dnm2, Duox2, Msh2, Nox4, Tmod1, Tpo, Xirp1	Apex1, Atrx, Cry2, Gpx6, Nos2, Slc38a1, Slc38a4, Slk, Tmod1, Tpo	GDS2558
Cluster 2			
Fibronectin (ventricular myocytes)	Apoe, Atrx, Chaf1a, Ngb, Rad51c, Smc3, Srxn1, Tpo, Zmynd17	Actb, Atrx, Gsr, Mutyh, Ngb, Prdx6, Rad52, Smc3, Tpo, Txnrd1	GDS696
Protein restriction (visceral adipose tissue)	Aass, Apc, Gpx6, Gstk1, Ngb, Prnp, Rad51c, Scd1, Tmod1, Tnp1	Brca2, Chaf1a, Lpo, Mutyh, Nos2, Pttg1, Slc38a1, Slc38a4, Tpo, Ung	GDS880
Heregulin (ureteric buds)	Dhcr24, Hus1, Ldha, Mif, Park7, Rad1, Rad50, Scd1, Tdg, Ung	Actb, Atrx, Nos2, Nox4, Nqo1, Ptgs1, Rad23a, Srxn1, Txnrd1	GDS1518
Kainic acid (hippocampi)	Apoe, Brca2, Ncf1, Nox4, Pold1, Rad23a, Rad50, Rad51c, Srd5a2, Tmod1	Chaf1a, Hbz, Lpo, Mb, Pold3, Tnp1, Tpo, Ucp3, Ung, Zmynd17	GDS1626
Ethanol (pancreas)	Apoe, Atrx, Hbz, Ogg1, Ptgs2, Scd1, Srxn1, Tmod1, Txnrd2, Zmynd17	Cry2, Hus1, Mb, Msh2, Nox4, Nthl1, Prdx6, Rad52, Slk, Srd5a2	GDS2107
Sulfur dioxide (lung)	Aass, Brca1, Cry2, Hus1, Nos2, Ptgs2, Pttg1, Rad50, Tpo, Zmynd17	Apex1, Brca2, Gpx6, Nos2, Nox4, Rad23a, Rad51c, Srd5a2, Tnp1, Tpo	GDS2372
Hypoxia (adrenal gland)	Chaf1a, Duox2, Ldha, Ngb, Pold3, Rad23a, Slc41a3, Tpo, Txnrd2	Aass, Apc, Apoe, Atrx, Cry2, Lpo, Nox4, Rad52, Srd5a2, Tnp1	GDS2457
Methylprednisolone (skeletal muscles)	Aass, Atrx, Hbz, Ngb, Rad1, Scd1, Slc38a5, Tmod1, Tpo, Xirp1	Als2, Atrx, Brca2, Cat, Gsr, Ncf1, Nox4, Nqo1, Slc41a3, Trpc2	GDS2688
Cluster 3			
Forskolin (pheochromocytoma cell)	Aass, Apex1, Brca1, Chek1, Duox2, Gpx2, Hbz, Nxn, Ptgs1, Pttg1	Atrx, Cat, Cygb, Ehd2, Gpx3, Gpx4, Gpx7, Scd1, Sod3, Vim	GDS1363
N-methyl-*N*-nitrosourea (mammary tumors)	Cat, Ehd2, Gadd45a, Gstk1, Mgmt, Prdx3, Prdx6, Scd1, Srxn1, Ube2a	Dpagt1, Gab1, Gpx3, Lpo, Mpg, Nxn, Prdx4, Prnp, Rad52, Txnip	GDS1452
Retinoic X receptor ligand LG100268 (mammary gland)	Brca1, Dnm2, Gpx6, Hbz, Mpp4, Ncf1, Nos2, Slc38a1, Tpo	Aass, Atrx, Chaf1a, Gsr, Idh1, Nox4, Prdx1, Rad23a, Xrcc1, Zmynd17	GDS1922
Angiopoietin-1 (aortic rings)	Apex1, Dnm2, Mgmt, Ngb, Pold3, Rad50, Slc38a1, Srd5a2, Srxn1, Ucp3	Atrx, Brca2, Chaf1a, Gpx6, Mb, Nox4, Rad23a, Slk, Tpo, Zmynd17	GDS2037

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.

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, Copl-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

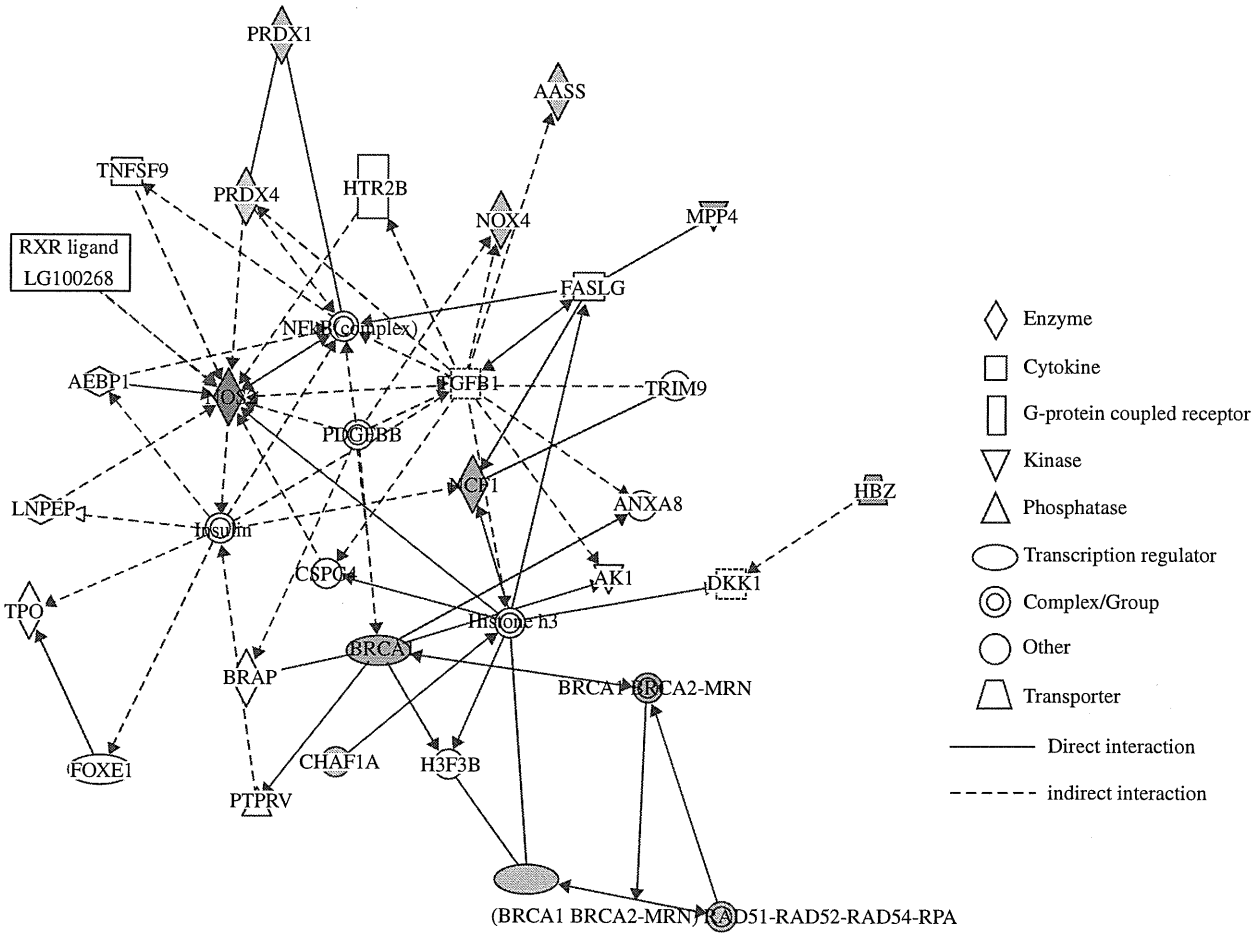

Fig. 13.1 The network generated by retinoic X receptor ligand LG100268 in cluster 3 shown in Tables 13.3 and 13.4: the top-rated network as an example

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

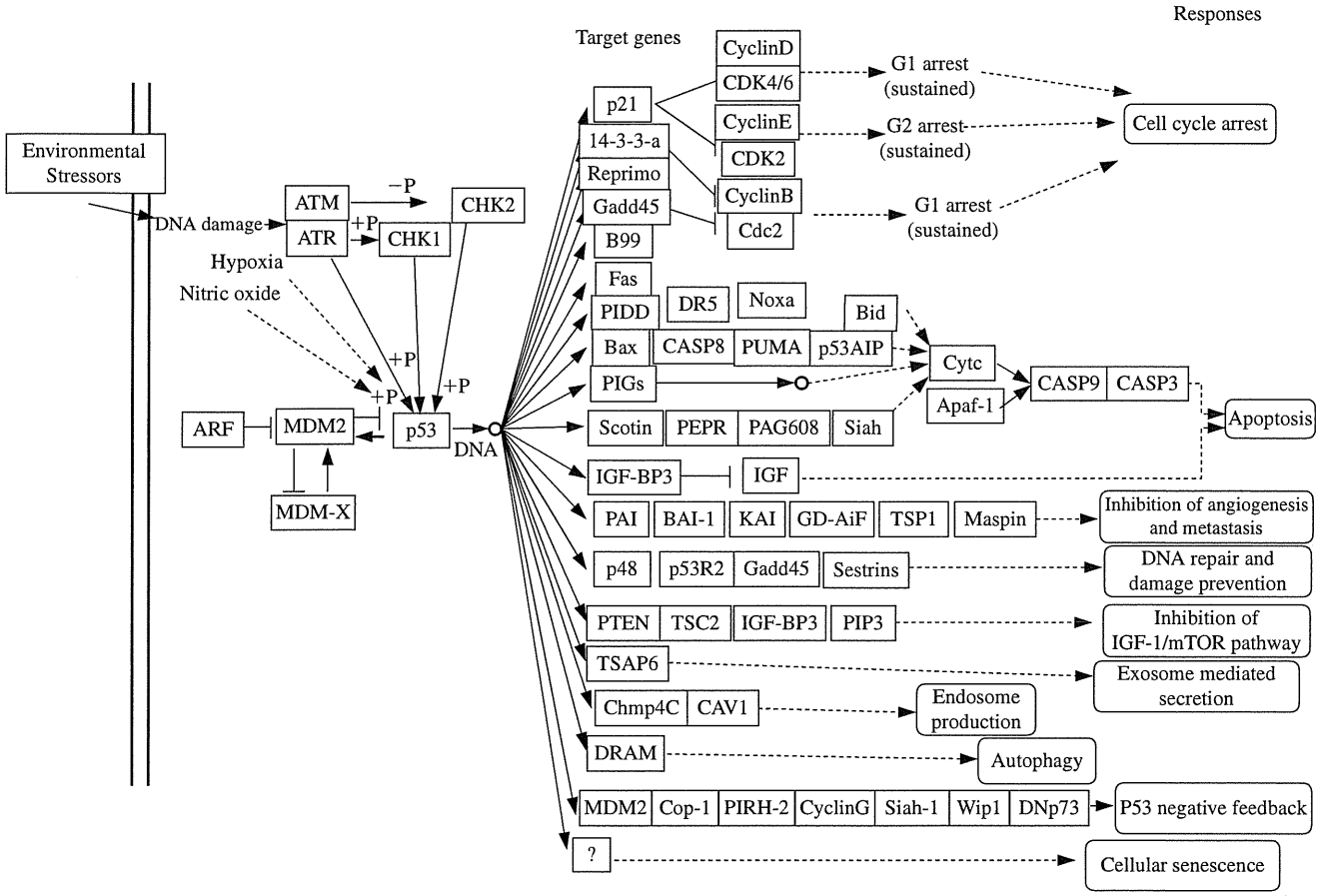

Fig. 13.2 Environmental stressor-mediated p53 signaling pathways. Maps of the p53 signaling pathway partly consulted the KEGG pathway

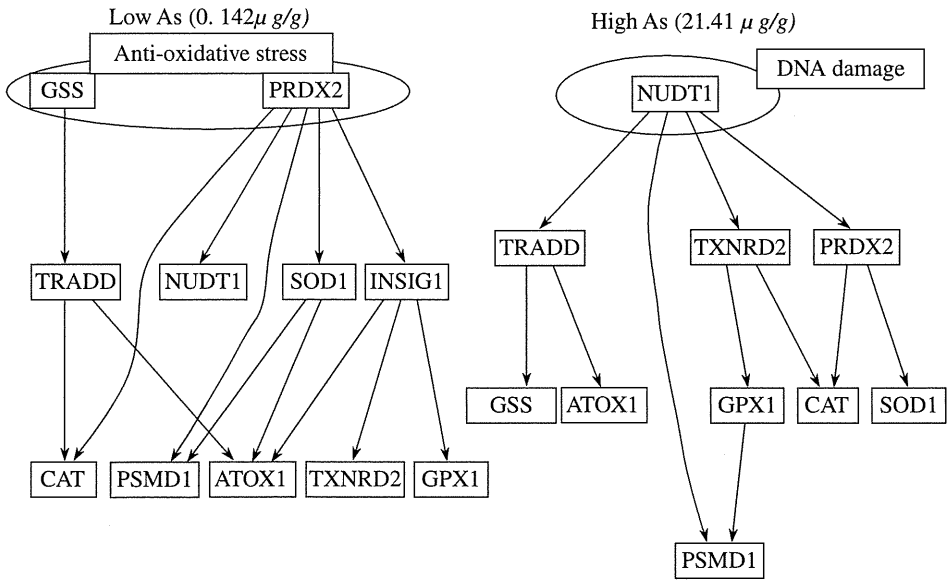

Fig. 13.3 Networks for 11 oxidative stress-related genes selected from the GSE7967 study. TAO-Gen algorithms can predict different mechanisms in low and high exposure to arsenic

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.

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